Intracytoplasmic expression of IL-6 and IL-17A in circulating CD4+ T cells are strongly associated with and predict disease activity in rheumatoid arthritis: A case-control study in Ghana

Samuel Asamoah Sakyi (✉ samasamoahsakyi@yahoo.co.uk)

Kwame Nkrumah University of Science and Technology   https://orcid.org/0000-0001-5168-4762

Tonnies Abeku Buckman

Kwame Nkrumah University of Science and Technology

Daniel Antwi-Berko

University of Energy and Natural Resources

Kwame Yeboah-Mensah

Komfo Anokye Teaching Hospital

Dzifa Dey

Korle Bu Teaching Hospital

Eddie-Williams Owiredu

Kwame Nkrumah University of Science and Technology

Benjamin Amoani

University of Cape Coast

Richard Mantey

Kwame Nkrumah University of Science and Technology

Abstract

Background

T cell cytokines play important roles in the development and progression of rheumatoid arthritis (RA). Loss of Th1/Th2 and Th17/Treg balance has been reported in several inflammatory autoimmune diseases. However, their role in RA within the Ghanaian context has not been explored. Here, we evaluated the intracytoplasmic CD4 + T cell cytokine patterns in rheumatoid arthritis patients in Ghana and determined their relationship with disease activity.

Methods

This case-control study included 48 newly-diagnosed RA patients and 30 healthy controls from two major hospitals in Ghana. Validated structured questionnaires were administered to obtain demographic data; blood samples were collected and processed for flow cytometric analysis.

Results

IFN-γ, TNF-α, IL-4, IL-6, IL-10, IL-17A, IL-6/IL-4 and IL-17/IL-10 expression were significantly higher in RA cases compared to the healthy controls. The expression of IL-6 (0.00 (0.00-0.98) vs 0.82 (0.34−1.10) vs 1.56 (1.39−1.68), $p < 0.0001$), IL-17A (0.00 (0.00-0.02) vs 0.19 (0.09−0.30) vs 0.99 (0.64−1.25), $p < 0.0001$) and IL-17A/IL-10 (0.00 (0.00-0.39) vs 0.15 (0.09−0.26) vs 0.88 (0.41−1.47), $p < 0.0001$) increased significantly from the healthy controls through RA patients with low DAS scores to RA patients with moderate DAS scores. IL-6 ($\beta = 0.681$, $r^2 = 0.527$, $p < 0.0001$), IL-17A ($\beta = 0.770$, $r^2 = 0.593$, $p < 0.0001$) and IL-17A/IL-10 ($\beta = 0.677$, $r^2 = 0.452$, $p < 0.0001$) expression were significantly directly associated with DAS28 scores. IL-6 (Cutoff = 1.32, Sensitivity = 100.0%, Specificity = 100.0%, Accuracy = 100.0%, AUC = 1.000) and IL-17A (Cutoff = 0.58, Sensitivity = 100.0%, Specificity = 100.0%, Accuracy = 100.0%, AUC = 1.000) presented with the best discriminatory power in predicting moderate DAS scores from low DAS scores.

Conclusion

Th1 and Th17 related cytokines predominate in the pathophysiology of RA; with IL-6 and IL-17 being principally and differentially expressed based on the severity of the disease. IL-6 and IL-17A could serve as useful prognostic and disease-monitoring markers in RA in the African context.

Introduction

Rheumatoid arthritis (RA) is the most common chronic, systemic, inflammatory autoimmune disorder with an estimated global prevalence of 1% [1]. The peak incidence usually occurs in individuals between the ages of 30 and 50 years and is characterized by persistent synovitis, pain, swelling and progressive deterioration of the small joints of the hands and feet, accompanied by functional disability [2].

The mechanisms underlying RA development and progression is complex, with T cells principally implicated in the pathophysiology of the disease [3, 4]. In RA, T helper (Th) cells facilitate B cells antibodies production, induce macrophages development, recruit other leukocytes to the sites of inflammation through their production of cytokines and chemokines [5]. Thus, Th cells play crucial roles in RA pathogenesis.

Th cell can differentiate into at least four distinct sub-populations comprising Th1, Th2, Th17, and T regulatory (Treg) cells. The Th cell sub-populations, and the cytokines and chemokines they express/produce act through diverse mechanisms that underpin the development and progression of RA. Evidently, the imbalance of Th1/Th2 and Th17/Treg cells have been implicated in RA development and progression [6−8]. However, the interplay of their cytokine pattern is minimally explored. Through their cytokine production, Th1 and Th17 cells play pro-inflammatory roles whereas Th2 and Treg cells play anti-inflammatory roles. Th1 cells produce interleukin (IL)-2, interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) whereas Th2 cells produce mainly IL-4 and IL-5. Th2 cells also secrete IL-6, IL-10 and IL-13. Th17 cells secrete predominantly interleukin IL-17A and have recently been shown to be increased in the peripheral blood and synovial fluid of patients with RA [9−11]. Tregs produce IL-10 and transforming growth factor-beta (TGF- β) [12]. These cytokines play differential roles in RA [6, 8] and underscore the significance of cytokine patterns in RA development and the progression of the disease.

The outcomes of patients with RA has been related with the disease activity. Disease activity measures have thus been very valuable to reflect patient outcomes and response to therapy in clinical care and in clinical trials [13]. Although several measures exist to provide information about the various dimensions of outcomes, the American College of Rheumatology recommends the Disease Activity Score with 28-joint counts (DAS28) as one of the paramount as it accurately reflects the disease activity; is sensitive to change; discriminates well between low, moderate, and high disease activity states; has remission criteria; and is feasible to perform in clinical settings [14].

RA was believed to be more common in developed countries; however, the disease is increasingly becoming more prevalent in developing countries, primarily due to improved diagnosis, adoption of westernized lifestyle and increased access to health care [15−17]. Additionally, the burden of the disease in low and middle income countries has previously been under reported. In meta-analyses, Usenbo et al. [15] and Rudan et al. [16] indicated paucity of data on RA among the African population; highlighting the need for more RA-related research in the African context.

There is thus a dire need for more RA-related studies, especially within the African population. Against this background, we evaluated the intracytoplasmic CD4 + T cell cytokine patterns in rheumatoid arthritis patients in Ghana and determined their relationship with disease activity.

Materials And Methods

Study design and setting

This was a case-control study conducted between November 2015 and August, 2017. Patient recruitment was done at the orthopedic units of Komfo Anokye Teaching Hospital (KATH), Kumasi and Korle-Bu Teaching Hospital (KBTH), Accra, Ghana. KATH, the second largest hospital in Ghana, is a 1200-bed facility in the Kumasi Metropolis. Kumasi is the second major city in Ghana and has a projected population of 4,780,380. KBTH is the largest and third largest health facility in Ghana and Africa, respectively, with over 2000-bed capacity. The orthopedic units of both hospitals provides health care services to both in- and out-patients with rheumatologic and autoimmune conditions.

Participants recruitment

A total of 48 consecutive consenting newly-diagnosed RA patients, 29 from KATH and 19 from KBTH, were included as cases in this study. Diagnosis of RA at both clinics was based on the American College of Rheumatology/ European League Against Rheumatism (ACR/EULAR) 2010 rheumatoid arthritis classification criteria [18]. All patients were prednisolone-naive. Thirty healthy participants with no chronic pain, cardiovascular complaints, or other chronic inflammatory diseases were included as controls.

Questionnaire administration, blood pressure and anthropometric evaluation

Questionnaires were administered to obtain socio-demographic data from the participants. Data collected include age, sex, marital, educational and employment status. Additional clinical data relevant to the study were retrieved from the hospital's archives. Weight was measured in the upright position to the nearest 0.1 kg using a calibrated balance beam scale. Height was measured (subjects stood erect, barefoot, with feet together, looking forward) to the nearest 0.1 m using a measuring tape. Body Mass Index (BMI) was calculated using the equation: [BMI (kg/m2) = weight/height^2]. Blood pressure was measured with an automated blood pressure apparatus (Omron MX3-Omron Matsusaka Co., Ltd. Japan) from the right arm after the subject had been made to sit for at least five minutes. The average of the two readings taken five minutes apart was recorded.

Blood sample processing, cell preparation and flow cytometric analysis

Eight milliliters (8 ml) of venous blood was drawn from RA patients and healthy controls. Four milliliters (4 ml) was dispensed into EDTA tubes for the estimation of erythrocyte sedimentation rate (ESR) using the Westergren method. The remaining 4 ml was dispensed into tubes containing heparin for the isolation of peripheral mononuclear cells (PBMCs) using Ficoll-Paque density gradient centrifugation (Biochrom, Berlin, Germany). Briefly, whole blood was poured into the ficoll-containing tubes and centrifuged at 1500 rpm for 30 min at 4°C with no brakes. Cells were suspended in RPMI 1640 medium, supplemented with 0.5% DMSO and 0.5 ml Fetal Bovine Serum (Biochrom, Berlin, Germany) at a density of 0.5×10^4

cells /ml. Collected PBMCs were stored at -80°C until further analysis. Prior to flow cytometric assessment of intracellular cytokine expression, frozen PBMCs were thawed in 37°C water bath for 15 min. The cells were washed in phosphate-buffered saline (PBS) and suspended in a small amount of PBS. Hundred microliters (100 μl) of the resultant cell suspension was pipetted into 96 well plates, followed by centrifugation of plates at 1500 rpm for 5 min. About 180 μl supernatant was pipetted and discarded after which cells were incubated for 10 min at room temperature in the dark. Cells were fixed and permeabilized using fixation/permeabilzation reagent from BioLegend (San Diego,CA) followed by intracellular staining for CD4 + T-cell expression of IFN-γ, TNF-α, IL-2, IL-4, IL-6, IL-10 and IL-17A using IFN-γ PE, TNF-α APC, IL-2 PerCP-Cy5.5, IL-4 APC, IL-6 PE, IL-10 APC and IL-17A APC human antibodies. Cells were gated on lymphocyte population and CD4 + T cells and analysis of percentage of cells expressing markers were done on the gated population using the Accuri C6 Flow Cytometer (Accuri Cytometers Inc, Ann Arbor, USA). Representative dot plots of T cell cytokines from RA patient is shown in Fig. 1.

Assessment of disease activity

Disease activity was assessed based on the Disease Activity Score in 28 joints. Estimation was based on clinical parameters (tender and swollen joint counts), visual assessment scale and laboratory markers of inflammation (erythrocyte sedimentation rate (ESR)). RA patients were grouped based on DAS28 scores; low disease activity (DAS28 ≤ 3.2), moderate disease activity (3.2 < DAS28 ≤ 5.1) and high disease activity (DAS28 > 5.1) [19].

Data analysis

Flow cytometry data was analyzed with FlowJo 10.1.5 (FlowJo, LLC, USA). Statistical analysis was performed using the R Language for Statistical Computing version 3.6.0 [20]. Categorical data were presented as frequencies (percentages) and Chi square and Fisher's exact test statistic were used to test for association where applicable. For continuous data, normality was checked using Shapiro-Wilk's test, as well as visual inspection with Q-Q plots. Normally distributed data were presented as mean ± SD and significance of differences was assessed using independent t-tests. Nonparametric data were presented as median (interquartile ranges) and significance of differences were evaluated using Mann-Whitney U tests and Kruskal-Wallis W with Dunn's multiple comparison tests, where applicable. Linear relationship between T cell cytokines (and their ratios) and DAS28 scores were assessed using linear regression models. Regression analysis was limited to cytokines with significantly different expression based on DAS28 subgroups. To determine the capacity of the cytokines to discriminate low and moderate DAS28 scores in RA, receiver operating characteristic (ROC) curve analysis was performed. The ROC curve analysis was based on binary logistic regression and discriminant classification analysis for low and moderate DAS28 groups. Analysis was restricted to controls, low and moderate DAS28 groups (we do not report data for high DAS28) because the relatively low number of RA patients with high DAS28 scores limited statistical reliability. All tests were two-sided and p-value < 0.05 was considered statistically significant.

Results

A total of 48 RA cases (mean age = 51.00 ± 13.01 years old) and 30 healthy controls (mean age = 47.47 ± 3.88 years old) were recruited for this study. There were more females than males and a higher proportion of the participants were married and employed. The average ESR and DAS28 score among the RA cases were 35.50 (29.25–55.25) mm/hr and 3.17 ± 1.07, respectively. The prevalence of low, moderate and high DAS28 scores were 68.7%, 25.0% and 6.3%, respectively (Table 1).

Table 1
Demographic and clinical characteristics of the study population

Variables	RA cases (n = 48)	Control (n = 30)	P-value
Age (years)†	51.00 ± 13.01	47.47 ± 3.88	0.083
Sex			0.062
Male	8 (17.0)	11 (36.7)	
Female	39 (83.0)	19 (63.3)	
Marital status			0.641
Single	18 (37.5)	13 (43.3)	
Married	30 (62.5)	17 (56.7)	
Educational status			0.021
No education	2 (4.2)	3 (10.0)	
Primary	13 (27.1)	9 (30.0)	
Secondary	25 (52.1)	6 (20.0)	
Tertiary	8 (16.7)	12 (40.0)	
Employment status			0.043
Unemployed	10 (20.8)	1 (3.3)	
Employed	38 (79.2)	29 (96.7)	
BMI (kg/m^2)†	30.23 ± 7.24	27.65 ± 5.12	0.093†
SBP (mmHg)†	129.35 ± 13.63	128.90 ± 6.88	0.846†
DBP (mmHg)†	82.60 ± 9.74	82.83 ± 5.17	0.893†
ESR (mm/hr)†	35.50 (29.25−55.25)	na	na
DAS score†	3.17 ± 1.07	na	na
Low	*33 (68.7)*	*na*	*na*
Moderate	*12 (25.0)*	*na*	*na*
High	*3 (6.3)*	*na*	*na*

†; Presented as mean ± SD and compared with independent t-test where applicable; BMI; Body mass index, SBP; Systolic blood pressure, DBP; Diastolic blood pressure, ESR; Erythrocyte sedimentation rate.

IFN-γ (0.30 (0.13−0.55) vs 0.00 (0.00-0.62), p = 0.032), TNF-α (0.20 (0.00-0.43) vs 0.00 (0.00-0.02), p < 0.0001), IL-4 (0.49 (0.17−0.80) vs 0.00 (0.00-0.64), p = 0.006), IL-6 (1.06 (0.63−1.43) vs 0.00 (0.00-0.98),

p < 0.0001), IL-10 (1.10 (0.58−1.49) vs 0.01 (0.00-1.44), p = 0.045), IL-17A (0.29 (0.14−0.65) vs 0.00 (0.00-0.02), p < 0.0001), IL-6/IL-4 (1.80 (0.67−4.84) vs 1.00 (0.49−1.99), p = 0.018), IL-17/IL-10 (0.23 (0.11−0.71) vs 0.00 (0.00-0.39), p = 0.007) were significantly higher in RA cases compared to the healthy controls (Fig. 2).

IL-6 (0.00 (0.00-0.98) vs 0.82 (0.34−1.10) vs 1.56 (1.39−1.68), p < 0.0001), IL-17A (0.00 (0.00-0.02) vs 0.19 (0.09−0.30) vs 0.99 (0.64−1.25), p < 0.0001) and IL-17A/IL-10 (0.00 (0.00-0.39) vs 0.15 (0.09−0.26) vs 0.88 (0.41−1.47), p < 0.0001) increased significantly from the healthy controls through RA patients with low DAS scores to RA patients with moderate DAS scores. RA patients with moderate DAS scores presented with significantly higher IL-6/IL-4 compared to RA patients with low DAS scores and the healthy controls, respectively. TNF-α and IL-4 were significantly higher in RA patients with low DAS scores compared to the healthy controls (Fig. 3).

IL-6 (β = 0.681, r^2 = 0.527, p < 0.0001), IL-17A (β = 0.770, r^2 = 0.593, p < 0.0001), IL-6/IL-4 (β = 0.791, r^2 = 0.526, p < 0.0001) and IL-17A/IL-10 (β = 0.677, r^2 = 0.452, p < 0.0001) were significantly directly associated with DAS28 scores whereas IL-4 (β= -0.418, r^2 = 0.320, p < 0.0001) was inversely related with DAS28 scores (Fig. 4).

IL-6 (Cutoff = 1.32, Sensitivity = 1.00 (0.71-1.00), Specificity = 1.00 (0.87-1.00), Accuracy = 100.0%, AUC = 1.000), IL-17A (Cutoff = 0.58, Sensitivity = 1.00 (0.71-1.00), Specificity = 1.00 (0.87-1.00), Accuracy = 100, AUC = 1.000), IL-6/IL-4 (Cutoff = 2.54, Sensitivity = 1.00 (0.71-1.00), Specificity = 0.92 (0.75−0.99), Accuracy = 94.74, AUC = 0.968) and IL-17A/IL-10 (Cutoff = 299, Sensitivity = 1.00 (0.69-1.00), Specificity = 0.80 (0.62−0.91), Accuracy = 85.37, AUC = 0.952) presented with the best discriminatory power in predicting moderate DAS scores from low DAS scores (Fig. 5).

Discussion

In this comprehensive analysis of intracytoplasmic cytokine profile of circulating CD4 + T cells, we found that, with the exception of IL-2, all other cytokine expressions were significantly higher in RA compared to healthy controls. Of importance, IFN-γ and TNF-α expression were significantly higher in RA, as expected. IFN-γ and TNF-α are pro-inflammatory cytokines produced by Th1 cells. IFN-γ stimulate inflammation in RA by facilitating macrophage activation and enhancing the activity of natural killer cells [1] and TNF-α exacerbate tissue damage by promoting other inflammatory cytokines such as IL-1 and IL-8 produced by macrophages, fibroblast and synovial cells [21]. Our findings thus corroborate previous reports [1, 8] on the role of Th1 and the interplay of their cytokines in RA development.

Interestingly, IL-6 was also higher in RA compared to the healthy controls. Conventionally, Th2 cytokines are considered to have anti-inflammatory effector functions and it was earlier thought, based on studies in murine models, that IL-6 was produced by Th2 cell; hence, should exhibit anti-inflammatory roles [22]. However, evidence suggests that, as opposed to murine models, in humans, IL-6 can be expressed by Th1 cells, hence are not limited to the Th2 cells [8, 22]. Thus, the high IL-6 in RA suggests that its expression in

CD4 + T cell in RA could be skewed towards the Th1 phenotype and hence, more associated with pro-inflammatory than anti-inflammatory effects. Indeed, evidence suggests that IL-6 is a pleiotropic cytokine with broad-ranging effects and acts in a context-dependent manner [23]. A study by Nakahara et al. also reported that IL-6 may not have direct effect on synovial fibroblast and chondrocyte, but improve the efficacy of TNF-α in RA [24].

We also observed an imbalance of Th17/Treg cytokines. IL-17A and IL-17/IL-10 expression was higher in RA compared to the controls. Evidence suggests that IL-17A induces inflammation by mediating the secretion of other cytokines and chemokines [25]. In a study by van Hamburg et al. [26], Th17-producing cells induced secretion of IL-6, IL-8 and tissue-destructive enzymes, such as MMP-1 and MMP-3 by synovial fibroblasts. In another study, Niu et al. found an increase in peripheral Th17-related cytokines levels in RA compared with healthy controls, as consistent with our study findings. Interestingly, we also found the expression of IL-4 and IL-10 which are anti-inflammatory cytokines produced by Th2 and Treg cells, respectively, to be higher in RA than the controls. The higher expression of IL-10 in RA could be due to possible compensatory mechanism initiated to ameliorate the inflammation in the early phases of the disease, as evidenced by the higher IL-4 and IL-10 expression in RA patients with low DAS28 compared to controls, but not in patients with high DAS28 scores. Together, these findings substantiate the predominance of Th1 and Th17 related cytokines in the pathophysiology of RA.

In contrast with a study by Chen et al. [1], we found no significant association between DAS28 and IL-2; however, IL-6, IL-17A and IL-17A/IL-10 increased from the healthy controls to RA patients with low DAS28 to patients with moderate DAS28 scores, suggesting that IL-6, IL-17A and the Th17/Treg axis could be the key drivers of the progression of RA among the study population. We could not comment on the dynamics of these markers in patients with high DAS28 because only three RA patients had high DAS28 scores. It is possible that Africans could be prone to less severe disease; however, this is only a supposition given the limited data on RA in the context of Africa, and warrants further research. On the other hand, the potential roles of IL-6, IL-17A and the Th17/Treg axis as drivers of of RA progression is confirmed by the strong linear association between IL-6, IL-17A and IL-17A/IL-10 with DAS28 scores. This finding is partly consistent with a study by Li et al. [27] who found a direct relationship between IL-17A and DAS28 scores in rheumatoid arthritis patients. Indeed, high IL-6 and IL-17 has been implicated in higher DAS28 scores, which together correlate with radiographical progression of RA patients [28–31]. Nonetheless, contrary to our study, Chung et al. [32], using enzyme-linked immunosorbent assay, reported no significant correlation between IL-6 and DAS28 scores. We attribute this discrepancy to the limited scale of RA subjects and differences in methods used in assessing cytokines.

The strong linear relationships between IL-6, IL-17A, IL-6/IL-4, IL-17A/IL-10 and DAS28 scores suggest that these cytokines could be useful in discerning the severity of the disease. To test this, we assessed the capabilities of the cytokine patterns to discriminate between disease activities by using ROC curve analysis with reference to low DAS28 score. Among these, IL-6 and IL-17A presented with the best discriminatory power with excellent sensitivity, specificity and accuracy in predicting moderate DAS28 scores from low DAS28 scores, followed by IL-6/IL-4 and IL-17A/IL-10. In a study by Baillet et al., IL-6 was

identified as a surrogate marker of synovial inflammation at baseline and repeated measurements was a factor for structural progression in early RA among French early arthritis cohort [29]. In another study by Boyapati et al., high baseline IL-6 adequately identified a subgroup of RA patients with rapid joint damage and clinical progression [33]. In a Prospective Study by Kirkham et al., IL-17 mRNA expression was found to be predictive of joint damage progression in RA [34]. A study by Moran et al. also found that IL-17A is highly expressed in the inflammatory joint and drives disease activity in RA [35]. Collectively, our findings, together with previous reports, confirm the pivotal roles played by IL-6 and IL-17A in the progression of the disease and highlight the potential prognostic and disease-monitoring applicability of IL-6 and IL-17A in RA.

Conclusion

Th1 and Th17 related cytokines predominate in the pathophysiology of RA; with IL-6 and IL-17 being principally and differentially expressed based on the severity of the disease. IL-6 and IL-17A could serve as useful prognostic and disease-monitoring markers in RA in the African context.

Abbreviations

RA
rheumatoid arthritis
PBMC
peripheral mononuclear cell
Th
T helper cell
Treg
T regulatory cell
IL
interleukin
IFN-γ
interferon-gamma
TNF-α
tumor necrosis factor-alpha
TGF-β
transforming growth factor-beta
BMI
body mass index
ESR
erythrocyte sedimentation rate
DAS28
disease activity score with 28-joint counts

Declarations

Ethics approval and consent to participate

Ethical approval for this study was obtained from the Committee on Human Research, Publication and Ethics (CHRPE) of the School of Medical Sciences, Kwame Nkrumah University of Science and Technology (CHRPE/AP/003/16) and the institutional review board of KATH and KBTH. Written informed consent was obtained from all participants who opted to participate after the aims and objectives of the study had been explained to them. Participation was voluntary, and respondents were assured that the information obtained was strictly for research and academic purposes only and were guaranteed the liberty to opt out from the study at their own convenience.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Authors' contribution

SAS designed the study, supervised the research and laboratory analysis. TA was involved in the design of the study, collection of data and laboratory analysis. DAB, KYM, DD, EWO, BA and RM were involved in the laboratory analysis. EWO analyzed and interpreted the data. SAS, TA, DAB, DD and EWO drafted and revised the manuscript. All authors read and approved the final manuscript.

Acknowledgements

References

1. Chen J, Li J, Gao H, Wang C, Luo J, Lv Z, et al. Comprehensive evaluation of different T-helper cell subsets differentiation and function in rheumatoid arthritis. BioMed Research International. 2012;2012.

2. Majithia V, Geraci SA. Rheumatoid arthritis: diagnosis and management. Am J Med. 2007;120(11):936–9.

3. Smolen JS, Steiner G. Therapeutic strategies for rheumatoid arthritis. Nature reviews Drug discovery. 2003;2(6):473.

4. Smolen JS, Aletaha D, Koeller M, Weisman MH, Emery P. New therapies for treatment of rheumatoid arthritis. The Lancet. 2007;370(9602):1861–74.

5. Zhu J, Paul WE. CD4 T cells: fates, functions, and faults. Blood. The Journal of the American Society of Hematology. 2008;112(5):1557–69.

6. Wang W, Shao S, Jiao Z, Guo M, Xu H, Wang S. The Th17/Treg imbalance and cytokine environment in peripheral blood of patients with rheumatoid arthritis. Rheumatol Int. 2012;32(4):887–93.

7. Astry B, Venkatesha SH, Moudgil KD. Involvement of the IL-23/IL-17 axis and the Th17/Treg balance in the pathogenesis and control of autoimmune arthritis. Cytokine. 2015;74(1):54–61.

8. Schulze-Koops H, Kalden JR. The balance of Th1/Th2 cytokines in rheumatoid arthritis. Best practice research Clinical rheumatology. 2001;15(5):677–91.

9. Leipe J, Grunke M, Dechant C, Reindl C, Kerzendorf U, Schulze-Koops H, et al. Role of Th17 cells in human autoimmune arthritis. Arthr Rhuem. 2010;62(10):2876–85.

10. Zheng Y, Sun L, Jiang T, Zhang D, He D, Nie H. TNFα promotes Th17 cell differentiation through IL-6 and IL-1β produced by monocytes in rheumatoid arthritis. Journal of immunology research. 2014;2014.

11. Zhang Y, Li Y, Lv TT, Yin ZJ, Wang XB. Elevated circulating Th17 and follicular helper CD4 + T cells in patients with rheumatoid arthritis. Apmis. 2015;123(8):659–66.

12. Zhang X, Zhang X, Zhuang L, Xu C, Li T, Zhang G, et al. Decreased regulatory T–cell frequency and interleukin–35 levels in patients with rheumatoid arthritis. Experimental therapeutic medicine. 2018;16(6):5366–72.

13. Taylor A, Bagga H. Measures of rheumatoid arthritis disease activity in Australian clinical practice. ISRN rheumatology. 2011;2011.

14. Anderson J, Caplan L, Yazdany J, Robbins ML, Neogi T, Michaud K, et al. Rheumatoid arthritis disease activity measures: American College of Rheumatology recommendations for use in clinical practice. Arthritis Care Res. 2012;64(5):640–7.

15. Usenbo A, Kramer V, Young T, Musekiwa A. Prevalence of arthritis in Africa: A systematic review and meta-analysis. PloS one. 2015;10(8).

16. Rudan I, Sidhu S, Papana A, Meng SJ, Xin−Wei Y, Wang W, et al. Prevalence of rheumatoid arthritis in low−and middle−income countries: A systematic review and analysis. Journal of global health. 2015;5(1).

17. Mody GM. Rheumatology in Africa—challenges and opportunities. Arthritis research therapy. 2017;19(1):49.

18. Kay J, Upchurch KS. ACR/EULAR 2010 rheumatoid arthritis classification criteria. Rheumatology. 2012;51(suppl_6):vi5−9.

19. Nell V, Machold K, Eberl G, Stamm T, Uffmann M, Smolen J. Benefit of very early referral and very early therapy with disease-modifying anti-rheumatic drugs in patients with early rheumatoid arthritis. Rheumatology. 2004;43(7):906−14.

20. Team RC. R: A language and environment for statistical computing. 2013.

21. Dayer J-M. Interleukin 1 or tumor necrosis factor-alpha: which is the real target in rheumatoid arthritis? The Journal of rheumatology Supplement. 2002;65:10−5.

22. Abbas AK, Murphy KM, Sher A. Functional diversity of helper T lymphocytes. Nature. 1996;383(6603):787−93.

23. Hunter CA, Jones SA. IL-6 as a keystone cytokine in health and disease. Nature immunology. 2015;16(5):448−57.

24. Nakahara H, Song J, Sugimoto M, Hagihara K, Kishimoto T, Yoshizaki K, et al. Anti−interleukin-6 receptor antibody therapy reduces vascular endothelial growth factor production in rheumatoid arthritis. Arthritis Rheumatism: Official Journal of the American College of Rheumatology. 2003;48(6):1521−9.

25. Hemdan NY, Birkenmeier G, Wichmann G, El-Saad AMA, Krieger T, Conrad K, et al. Interleukin-17-producing T helper cells in autoimmunity. Autoimmun rev. 2010;9(11):785−92.

26. van Hamburg JP, Asmawidjaja P, Davelaar N, Mus A, Colin E, Hazes J, et al. Th17 cells, but not Th1 cells, from patients with early rheumatoid arthritis are potent inducers of matrix metalloproteinases and proinflammatory cytokines upon synovial fibroblast interaction, including autocrine interleukin-17A production. Arthr Rhuem. 2011;63(1):73−83.

27. Li S, Yin H, Zhang K, Wang T, Yang Y, Liu X, et al. Effector T helper cell populations are elevated in the bone marrow of rheumatoid arthritis patients and correlate with disease severity. Scientific reports. 2017;7(1):1−11.

28. Álvarez JLP. Interleukin 6 in the physiopathology of rheumatoid arthritis. Reumatología Clínica (English Edition). 2009;5(1):34−9.

29. Baillet A, Gossec L, Paternotte S, Etcheto A, Combe B, Meyer O, et al. Evaluation of serum interleukin-6 level as a surrogate marker of synovial inflammation and as a factor of structural progression in early rheumatoid arthritis: results from a French national multicenter cohort. Arthritis Care Res. 2015;67(7):905−12.

30. Kellner H. Targeting interleukin-17 in patients with active rheumatoid arthritis: rationale and clinical potential. Therapeutic advances in musculoskeletal disease. 2013;5(3):141–52.

31. Le Goff B, Bouvard B, Lequerre T, Lespessailles E, Marotte H, Pers Y-M, et al. Implication of IL-17 in Bone Loss and Structural Damage in Inflammatory Rheumatic Diseases. Mediators of inflammation. 2019;2019.

32. Chung S-J, Kwon Y-J, Park M-C, Park Y-B, Lee S-K. The correlation between increased serum concentrations of interleukin-6 family cytokines and disease activity in rheumatoid arthritis patients. Yonsei Med J. 2011;52(1):113–20.

33. Boyapati A, Msihid J, Schwartzman S, Choy E, Burmester GR, Lam G, et al. 069 High baseline serum IL-6 identifies a subgroup of rheumatoid arthritis patients with rapid joint damage and clinical progression and predicts increased sarilumab treatment response. Rheumatology. 2019;58(Supplement_3):kez106. 068.

34. Kirkham BW, Lassere MN, Edmonds JP, Juhasz KM, Bird PA, Lee CS, et al. Synovial membrane cytokine expression is predictive of joint damage progression in rheumatoid arthritis: a two-year prospective study (the DAMAGE study cohort). Arthr Rhuem. 2006;54(4):1122–31.

35. Moran EM, Mullan R, McCormick J, Connolly M, Sullivan O, FitzGerald O, et al. Human rheumatoid arthritis tissue production of IL-17A drives matrix and cartilage degradation: synergy with tumour necrosis factor-α, Oncostatin M and response to biologic therapies. Arthritis research therapy. 2009;11(4):R113.