Original Research Article

Natural Killer and Natural Killer T Cells as a Prognostic Factor for Rheumatoid Arthritis and Ankylosing Spondylitis

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Abstract

Object: We aimed to show the relationship between natural killer T and natural killer cells with the diseases activity and functional capacity, in the patient with rheumatoid arthritis and ankylosing spondylitis.

Materials and Methods: All patients included in the study were evaluated to the disease activity and functional capacity and radiographic view and also; their' blood count and C- reactive protein, erythrocyte sedimentation rate and rheumatoid factor and natural killer T and natural killer cells were measured, simultaneously.

Results: It was shown to be the positive correlation between the disease activity with total Sharp score and functional capacity. Significant positive correlation was found the between BASDAI score with natural killer T cells. The ratio of natural killer T and natural killer cells was found higher, using of the methotrexate treatments with rheumatoid arthritis patients. No correlation was found the between percentage of natural killer T and natural killer cells with using of the methotrexate treatment, in the patients with ankylosing spondylitis.

Conclusion: Determination of the levels of natural killer T and natural killer cells can contribute to estimation of the disease course and treatment response and the development of functional disorders, in the patients with rheumatoid arthritis and ankylosing spondylitis.

Keywords: Ankylosing spondylitis, diseases activity, functional capacity, natural killer cells, natural killer T cells, rheumatoid arthritis.

1.Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory and autoimmune disease. Joint inflammation and bone destruction are the important features of RA. It is known, the development of rheumatoid arthritis disease[1] can contribute to the interaction between genetic and environmental factors. Generally; it is demonstrated; RA is the more frequency prevalent among female than men.

The results of the some studies were shown that macrophages, T cells, dendritic cells and natural killer cells (NK), natural killer T cells (NKT) can contribute to the initiate immune response and continues immune response, RA disease.[2]-[4]

NK and NKT cells [3]constitute of lymphocytes population and they have the immune regulatory role. NK and NKT cells contribute to the cytokines production and development of antibody-dependent cellular cytotoxicity (ADCC).Currently; it is suggested to that NK and NKT cells [4] have

protective and pathogenic role as a direct and indirect, the development of rheumatoid arthritis.

The results of several studies[3]-[5] were demonstrated to be decrease of the number NKT and NK cells in the peripheral blood, Th1-type autoimmune disease like RA. Recent years; it has been tried to show the relationship between decrease of the number of NKT and NK cells with complication of disease in RA. Obtaining data is suggested that decrease of the number of NKT and NK cells can cause to the increase of disease activity and joint damage and bone destruction, in RA.

Ankylosing spondylitis (AS) is a chronic inflammatory disease and its effects on the sacroiliac joints and axial skeleton, especially. Presence of AS disease[6] can detect to the positive for human leukocyte antigen (HLA)-B27 and this condition may suggest to the abnormal autoimmune stimulation and

effects of genetic in the patients with AS. Results of the studies were found that CD4+, CD8+, NK and NKT cells can be effective on the development of the disease complications. The relationship between was demonstrated to, the resistance to AS- associated uveitis and radiographic changes with NK and NKT cells in AS. Results of the studies[7][8] were found that the number of NK and NKT cells in the peripheral blood might increase by the disease complication such as the severity of radiographic changes and existence of the resistance uveitis etc, in AS.

We aimed to show the existence of the association between percentage of NK and NKT cells in the peripheral blood with the disease activity and functional capacity and using treatment, under the regularly follow-up and treatment in the patients with RA and AS.

2. Materials and Methods

This study was performed in the Trakya University Medical School Rheumatology Clinic after approved by Ethic Committee of Trakya University Medical School (Grand number: 525; Date: 12- February- 2005). Each of all participants was given information about the study and each of all participants was approved.

This prospective study was included 31 patients with RA and 28 patients with AS and 41 healthy subject. Diagnoses of the patients with RA and AS were determined according to the criteria of The American College of Rheumatology (ACR)[9] and The European Spondylarthropathy Study Group[10]. Patients had other chronic inflammatory diseases (other collagen tissue diseases and autoimmune disease; psoriasis, systemic lupus erythematosus, familial mediterranean fever (etcetera) and the patients with acute inflammation (pneumonia, diabetic foot etcetera) were excluded from the study, because they can effective on the acute phase response. The healthy subjects were not determined to the clinic evidence of rheumatoid arthritis and ankylosing spondylitis and other chronic and acute inflammatory diseases.

All of the subjects were recorded age, gender and general demographic features and using of the methotrexate treatment also; their' physical examinations were made and the disease activity and functional capacity were evaluated.

Also; venous blood samples were taken following a fasting period of 12 hours from all of the participants, simultaneously. All of the participants were measured blood count, C- reactive protein (CRP), erythrocyte sedimentation rate(ESR) and rheumatoid factor (RF) and the numbers of NK and

NKT cell in the peripheral blood and however their' joint X- ray were viewed including the hand X- ray, wrist X- ray and foot X- ray.

Disease Activity Score 28[11] was used to evaluation of the disease activity of patients with RA. The patients with RA were questioned to the presence of the joint tenderness, joint pain and joint swelling and each of the information was recorded. Obtaining data was used to measurement of DAS-28.

DAS 28 Formula = 0.56 X $\sqrt{}$ (number of joint tenderness) + 0.28 X $\sqrt{}$ (number of joint swelling) + 0.70 ln (erythrocyte sedimentation rate) + 0,014 X (overview of scoring methods)

Classification of Health Assessment Questionnaire (HAQ) and ACR were used to show the functional capacity of patients with RA.

According to the criteria of the Sharp- van der Heijde method [12]was evaluated to the radiographic results, in all the patients.

The detection of functional capacity was used to the criteria of Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)[13] in AS patients.

The levels of hemoglobin, hematocrit and thrombocyte were measured using a Coulter LH750 Autoanalyzer device (Coulter, USA).

Using of the nephelometric method was measured the levels of CRP by Beckman Coulter device (normal range < 5 mg/L) (Coulter, USA).

Electa Lab. Device was used to the measurement of ESR levels (normal range: 20- 30mm/h) (Electa Lab.;Italy).

Using of the turbidimetric immunoassay was measured the levels of RF by Beckman Coulter/ Synchron Autoanalyzer LX- 20 device (Coulter, USA).

The number of $CD56^+ CD3^-T$ and $CD3^+ CD161^+ T$ cells in the peripheral blood lymphocytes were measured by Coulter Epics XL-MCL flow-cytometry device (Coulter, USA).

2.1 Statistical Evaluations

NCSS (Number Cruncher Statistical System) 2007& PASS (Power Analysis and Sample Size) 2008 Statistical Software (Utah, USA) program was used for the statistical analysis. During the evaluation of the study data, regarding the comparisons of descriptive statistical methods (mean, standard deviation and median) as well as quantitative data, One-way ANOVA test was used for the intergroup comparisons of three groups. Kruskal Wallis test was used for the intergroup comparisons of three groups without normal distribution and Mann Whitney U test was used for determination of group causing difference. Pearson

Chi-Square test was used for the comparison of qualitative data. Spearman's Correlation Analysis was used for evaluation of the correlations between the parameters. Significance was evaluated at the levels of $p < 0.05$.

3. Results

Two patients groups and control group were included to this study. According to the distribution of diagnosis were included 31 patients with RA and 28 patients with AS and 41 subjects with the healthy control group, in the study.

Patients with RA whose mean age and gender distribution were determined to 49.29 ± 13.27 years (31- 77 years) and rate of male and female patients (5/26) respectively. Mean of the disease duration was found the 6.86 ± 1.96 years, in the patients with RA (Table 1).

Twenty eight patients with AS whose mean age and gender distribution were determined to be 36.36 ± 11.06 years (22-66 years) and rate of male and female patients (19/9), respectively. Mean of the disease duration was found the 7.71 ± 4.28 years, in the patients with AS (Table 1).

Twenty six female subjects and 15 male subjects were included, in the healthy control group and mean age of the healthy control group was found the 40.1 ± 13.24 years (21-68 years) (Table 1).

Patients with RA whose mean age were found to be higher than other the groups (p: 0.000). No different significant statistical was found to the comparison of gender distribution, between the groups (p> 0.05). No different significant statistical was found to the disease duration, in the patients with RA and AS (p<0.05) (Table 2).

The patients with RA were determined the decrease of hemoglobin and hematocrit levels than the other groups and the levels of platelet were higher than the other groups, different significant statistical, respectively (p= 0.001; p= 0.031) (Table 2).

Evaluations of the disease activity and radiographic imaging were shown into Table 3. Significant positive correlation was found the

between disease duration with the total Sharp score and functional capacity in the patients with RA, respectively (r: 0.490, p= 0.009; r, 0,394, p= 0.028) (Table 3).

Significant positive correlation was found the between mean age of the disease groups with CD56+ CD3- T cells (r: 0.20, p= 0.047) (Table 4).

Significant negative correlation was found the between DAS28 score with the levels of hemoglobin and hematocrit (r: -0.56, p= 0.001; r: -0,472, p= 0.007).

Significant positive correlation was found the between DAS28 score with the numbers of platelet (r: 0.50; p= 0.004) (Table 4).

Significant positive correlation was found the between BASDAI score with CD3+ CD161 + T cells (r: 0.478; p= 0.010) (Table 4).

The number of CD3+ CD161 + T and CD 56 + CD3 -T cells were higher giving of the methotrexate treatment in the patients with RA, respectively (p= 0.032; p= 0.048) (Table 5).

According to the persistence of the methotrexate treatments was evaluated to the mean rate of CD3+ CD161 + T cells were higher using of the methotrexate treatment than not using of the methotrexate treatments, in the patients with RA, respectively (mean rate: 36.23/26,30, p= 0.032) (Table 5).

Similarly; considering to the persistence of the methotrexate treatments was evaluated to the mean rate of CD56 + CD3 -T cells and using of the methotrexate treatments were higher than not using of the methotrexate treatments in the patients with RA, respectively (mean rate: 18.92/ 11.58, p= 0.048) (Table 5).

No correlation was determined between using of the methotrexate treatment with CD3+ CD161 +T and CD56 + CD3- T cells, in the patients with AS.

Mean standard deviation and the distribution rate of parameters of flow- cytometry were shown to the subjects, into Table 6.

Table 1: Demographic Features and the Patients with Methotrexate Usage.

Healthy Group		Rheumatoid Arthritis Group				Ankylosing Spondylitis Group			
Age	Gender	Age	Gender	Disease Duration	MTX Treatment	Age	Gender	Disease Duration	MTX Treatment
32	Female	61	Female	3		25	Male	7	
49	Female	44	Female	1		36	Male	9	
36	Female	38	Female	5	+	33	Female	2	
47	Female	49	Female	10	+	3	Female	4	
35	Female	60	Female	6	+	33	Male	2	
46	Female	54	Female	12	+	38	Male	15	+
27	Male	65	Female	20	+	23	Male	2	
32	Male	47	Female	7	+	30	Male	3	
40	Female	77	Male	10	+	27	Female	10	+
60	Female	32	Female	1		29	Male	2	
43	Female	35	Female	1		62	Male	1	
24	Female	44	Female	4		28	Male	4	
31	Female	44	Female	4		33	Female	16	+
68	Female	54	Female	3		30	Male	7	
55	Female	35	Male	1		34	Male	2	
53	Female	55	Female	11	+	66	Male	10	+
30	Female	32	Female	1		41	Male	6	
49	Female	67	Male	9	+	33	Female	12	+
37	Female	51	Female	2		42	Male	5	
49	Male	76	Female	1		36	Female	20	+
39	Male	63	Female	25	+	47	Male	3	
36	Male	36	Female	12	+	28	Female	8	
34	Male	42	Male	12	+	46	Male	7	
56	Male	31	Male	1		41	Female	20	+
42	Male	32	Female	8	+	53	Female	2	
48	Male	34	Female	5		23	Male	2	
31	Female	62	Female	7	+	22	Male	2	
34	Male	44	Female	10	+	46	Male	15	+
27	Male	63	Female	5					
26	Female	54	Female	7	+				
48	Female	47	Female	3					
41	Male								
65	Female								
56	Female								
22	Female								
26	Female								
24	Male								
25	Male								
28	Female								
21	Female								
25	Male								

Table 2: The Blood Count and Distribution of Acute Phase Response of All Subject

	Hemoglobin (g/dl)	Hematocrit (%)	Leukocyte (/mm^3)	Platelet (/mm^3)	Erythrocyte sedimentation ratio (mm/hour)	C- reactive protein (mg/dl)
Rheumatoid Arthritis (n=31)	12.10 ± 1.90	36.10± 5.50	7863± 2267	297.70± 94.40	35.50 ± 32.20	1.61±1.50
Ankylosing Spondylitis (n=28)	12.76 ± 1.40	40.10±10.50	7560± 1766	274.57± 48.48	22.10± 19.40	1.44± 1.40
Healthy Population (n=41)	13.70± 1.70	40.17 ± 4.50	7329± 1823	254.82± 52.33	-	-
p	0.001	0.031	0.526	0.034	0.063	0.64

Table 3: The Disease Activity and Radiographic Imagistic of All Patients.

	DAS28 (n= 31)	HAQScore (n=31)	SHARP Score (n=31)	Functional Capacity (n=31)	BASDAI (n=28)
Rheumatoid Arthritis	2.91 ± 1.14 (0.68- 5.3)	0.18 ± 0.63 (0-59)	46.74 ± 73.05 (0-255)	0.18±0.635 (0- 3)	-
Ankylosing Spondylitis	-	-	-	-	3.00± 1.90 (0.18 - 8.17)

DAS 28: Disease Activity Score 28, **HAQ Score:** Health Assessment Questionnaire, **SHARP Score:** Sharp- van der Heijde, **BASDAI:** Bath Ankylosing Spondylitis Disease Activity Index.

Table 4: The Correlation of Clinic and Laboratory Parameters in All the Patients.

	HAQ Score	DAS-28 Score	SHARP Score	BASDAI	ESR	CRP	RF	CD3+CD161+ T Cells	CD56+CD3-T Cells
Age									r:0.20 p=0.047
MTX treatment HAQ Score			r:0.583 p: 0.001		r:0.5 p: 0.04			p= 0.032	p=0.048
DAS-28 Score	r:0.511 p: 0.003		r:0.643 p: 0.000		r:0.737 p: 0.000	r:0.471 p: 0.007	r:0.361 p:0.046	r:0.369 p: 0.041	
SHARP Score	r:0.583 p: 0.001	r:0.643 p: 0.000			r:0.647 p: 0.000	r:0.603 p: 0.001			
BASDAI								r:0.478 p: 0.010	
ESR	r:0.5 p: 0.04	r:0.737 p: 0.000	r:0.647 p: 0.000			r:0.563 p: 0.000	r:0.595 p: 0.000		
CRP		r:0.471 p: 0.007	r:0.603 p: 0.001		r:0.563 p: 0.000				
RF		r:0.361 p: 0.046			r:0.595 p: 0.000				
CD3+ CD161+ T Cells									
CD56+CD3- T Cells									

HAQ Score: Health Assessment Questionnaire, **DAS 28:** Disease Activity Score 28, **SHARP Score:** Sharp- van der Heijde, **BASDAI:** Bath Ankylosing Spondylitis Disease Activity Index, **ESR:** Erythrocyte Sedimentation Rate, **CRP:** C- reactive protein, **RF:** Rheumatoid Factor, **MTX**: Methotrexate.

Table 5: Distribution and Mean Standard Deviation of CD3+ CD161+ T Cells and CD56+CD3 - T Cells.

Parameters	Patient and Control Groups	Number of the Subject	Mean Standard Deviation (%)	Distribution of Parameters (%)	p
CD3+ CD161+ T Cells	Healthy Group	41	9.16 ± 5.16	0.78- 16.00	
	Rheumatoid Arthritis Group	31	7.77± 4.09	0.91- 18.30	0.262
	Ankylosing Spondylitis Group	28	6.88 ± 4.33	0.49- 21.80	
CD56+CD3- T Cells	Healthy Group	41	14.98 ± 6.74	2.49- 37.78	
	Rheumatoid Arthritis Group	31	16.44 ± 8.61	1.47- 39.87	0.682
	Ankylosing Spondylitis Group	28	15.21 ± 6.45	1.01- 33.00	

Table 6: The Correlation between CD3+ CD161+ T Cells and CD56+CD3- T Cells with the Treatment of Methotrexate in Rheumatoid Arthritis Patients.

	Methotrexate Treatments (+) (n=16)	Methotrexate Treatments (-) (n=15)	p
CD3+ CD161+ T Cells (ratio of cells)	36.23	26.30	0.032
CD56+CD3 -T Cells (ratio of cells)	18.92	11.58	0.048

4. Discussion

RA is a chronic inflammatory and autoimmune disease. Joint damage and functional disability are the most important features of RA, mainly. Joint damage and disability are frequency seen increasing of the disease duration in rheumatoid arthritis. Joint damage and disability and rheumatoid factor are important to predictive the disease progression and treatment response. However; joint damage accounts are the association with functional disability in RA.

HAQ score and DAS28 score are used to the assessment of functional capacity and disease activity in RA, respectively. Currently, affecting factors on the long-term functional disability and disease activity are investigated by using HAQ score and DAS28 score in many study [14][15]. Especially; it is suggested to that DAS28 level of 2.6[14] may be useful, prevention of the disease progression in rheumatoid arthritis. But; the relationship between radiological damage with functional disability is weak, in early RA. It is established to that maximal radiological damage score[15] is 1.9% per year, the first 20 years of RA. The rate of radiological damages increases over the first 20 years, in RA.

AS is chronic inflammatory disease and it effects on the sacroiliac joint, the spin and extra-spinal and extra-articular areas, especially. In the clinical practice; BASDAI classification is used to the functional evaluation of disease activity and it containing the subjective disease activity results, in AS. According to BASDAI score can be evaluated to, suggesting the inactive disease (BASDAI< 1.3), moderate disease activity (BASDAI< 2,1) and high

disease activity (BASDAI> 2.1- 3.5), the functional activity of patients with AS.

NK cells[16] are large, granular lymphocytes and they cause of the innate immunity and acquired immunity. NKT cells[17] regulate autoimmunity and they contribute to secreting of some cytokines such as T helper type 1 (Th), Th2 and Th17 cytokines. Cytolytic effects of NK and NKT cells have the important role, in RA. Also; some studies[3][4] were shown that immunobiologic of NK and NKT cells correlated with DAS28, in RA.

Results of the some studies [6][7] were shown that the T-cell compartment (especially CD56+ T-cells, NK and NKT cells) might change, in AS. Also; decrease of the number of NK and NKT cells in the peripheral blood lymphocyte might be the association with the radiographic changes, in AS.

We aimed to investigate the presence of association between the number of NK and NKT cells in the peripheral blood lymphocyte with the disease activity and methotrexate usage in the patients with RA and AS.

We found to be decrease of hemoglobin and hematocrit levels and increase of platelet levels,, in RA. This condition may be explained with the clinical features of RA, because it is a chronic inflammatory disease and disease activity effects on the acute phase response such as increase of the platelet levels and CRP levels, sedimentation levels etcetera. Especially; some studies[15]-[18] were shown that increase of the disease activity might cause to the decrease of hemoglobin and hematocrit levels, in RA. Apart from; using drugs can cause to the decrease of hemoglobin and hematocrit levels. In this study; sedimentation and CRP levels of the patients with RA found as some as the patients with AS and the healthy group. Also; negative correlation was found the between hemoglobin and hematocrit levels with DAS28 score .However; there was shown to be positive correlation the between levels of platelet with DAS28 sore. Thereby; we thought that the decrease of the levels of blood count and hematocrit and increase of platelet levels suggested to presence of the active disease in the patients with RA, in the study. But; there was not demonstrated to the drugs effect on the decrease of blood count and hematocrit levels in the patients with RA. We also found to be the association between Sharp score with disease duration in RA. The results were suggested by some studies[15]-[18]. It is known; radiographic imagistic of joint damages are the relationship with disease activity and diseases duration. Especially; it was shown[15] that can be more to the relationship between X-ray imagistic of joint damages with disease duration, over first the 20 years in RA.

Recent years; it was suggested[3][4]to the decrease of number of NK and NKT cells, in RA but currently, effects of this condition are controversial. It is thought that increase of the percentage of NK and NKT cells[19]effect on the disease activity, positively and immunosuppressive drugs usage can cause the increase of number of NK and NKT cells. In this study; no decrease was found the number of NK and NKT cells and this condition might be the association with methotrexate usage. Some studies[20][21] were shown that increase of the mean ratio of $CD3^+ CD161^+$ T cells and $CD56^+ CD3^-$ T cells were determined to methotrexate usage, in the patients with RA. We thought that methotrexate usage might effect on the number of NK and NKT cells, in the study.

In this study; the between disease activity score with NKT cells were determined to be positive correlation and no correlation was found the between NK and NKT cells with methotrexate usage, in AS. Some studies[6]-[8][20] were shown that the increase of the ratio of NK cells in the peripheral blood lymphocytes and they contribute to the intracellular cytokines production, in the pathogenesis of AS. Especially; it was suggested to be the relationship between increase of the ratio of NK and NKT cells in the peripheral blood with radiographic images and disease duration and disease activity and age, in the patients with AS. However; it was shown[7] that the estimation of disease progression and radiographic images of joint damages are the most important, increasing of the ratio of NK and NKT cells in the peripheral blood over the first three years in AS, mainly.

We found to be the mean disease duration was over the three years,in the patients with AS. However; we didn't show to be lower the ratio of NK and NKT cells. We thought that positive correlation in the between percentage of NK cells with BASDAI score might contribute to show the clinical features of disease, in AS. However; we found that the percentage of NK and NKT cells didn't change by methotrexate usage, in AS. Methotrexate is widely used a disease-modifying antirheumatic drugs (DMARDs), in RA. But, the effects of methotrexate treatment have not been defined to clearly, in the treatment of AS. Results of the some studies[20][21] were shown that no different between was placebo with methotrexate usage and the increase of side effects frequency were determined by methotrexate usage, in the patients with AS. Results of this study were found that using of the methotrexate treatment didn't effect on the disease activity and clinical features, in the patients with AS.

5. Summary

This study was suggested that the T cell compartment might change, in RA and AS. The changes of T cell compartment may be the relationship with the disease activity and disease progression and disease duration. Also; we found the existence of association between methotrexate usage with the percentage of NK and NKT cells, in RA. We thought; the better understanding of the pathophysiologic mechanism of NK and NKT cells might cause to the development of the new treatment strategies.

6. Conclusion

NK and NKT cells may contribute to the development of the new treatment strategies. We believe that the change of the percentage of NK and NKT cells can cause to estimation of the disease prognosis, treatment response and the risk of developing complications. Thereby; measurement of the percentage NK and NKT cells in the peripheral blood cell are used as a biomarker, predictive of the diseases prognosis and treatment response, in the autoimmune and chronic inflammatory disease.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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