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Neutrophil/Lymphocyte Ratio in Patients with Rheumatoid Arthritis Treated with Biological Agents

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Background: Disease activity of rheumatoid arthritis (RA) is evaluated by composite measures, such as Disease Activity Score (DAS). Recently, much attention has been paid to a neutrophil-lymphocyte (N/L) ratio to evaluate the prognosis and the efficacy of intervention in various diseases. To determine whether the N/L ratio is a prognostic marker or a surrogate marker of response to biologics, this study investigated the N/L ratio in RA patients treated with biological agents.

Methods: The medical records were reviewed of 358 patients with RA in routine care who were treated with infliximab (144 patients), etanercept (120 patients), adalimumab (25 patients), tocilizumab (41 patients), or abatacept (28 patients). The 28-joint DAS (DAS28), a hemogram, erythrocyte sedimentation rate (ESR), and serum levels of C-reactive protein and matrix metalloproteinase 3 were assessed at baseline and 6 months after the treatment.

Results: The average N/L ratio significantly decreased from 5.9 at baseline to 4.5 6 months after the treatment. The N/L ratio and the DAS28-ESR, both at baseline and 6 months after the treatment, were modestly but significantly correlated. The N/L ratio was greater in patients with high disease activity than in patients with low disease sactivity. The change of the N/L ratio (ΔN/L) and the change of the DAS28-ESR were modestly but significantly correlated. Regarding the therapeutic response, the N/L ratio at baseline showed no significant difference between the response criteria; however, the N/L ratio after 6 months of treatment and the ΔN/L ratio differed significantly. The ΔN/L was also significantly correlated with the change of the serum level of C-reactive protein and the change of the DAS28-ESR.

Conclusion: The N/L ratio is a marker of disease activity in RA. The ΔN/L ratio reflects the efficacy of biological agents but does not predict the response to biological agents.

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Key words: rheumatoid arthritis, biologics, DAS28, neutrophil-lymphocyte ratio

Introduction

Rheumatoid arthritis (RA) is an inflammatory disease that causes devastating joint destruction if patients are not appropriately treated. Because no single measure is available to correctly estimate the disease activity of RA, composite measures that have been used include the 28-joint Disease Activity Score (DAS28), the Simplified Disease Activity Index, and the Clinical Disease Activity Index[1,2]. Since the start of this century, RA and other rheumatic diseases have treated with biological agents. Although such biologics show effects on RA superior to those of conventional synthetic disease-modifying antirheumatic drugs, their high-cost and adverse events have been significant concerns[3]. To administer expensive biologics in clinical practice, measuring disease activity and then evaluating drug efficacy are important. The DAS28-

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Table 1 Demographic and baseline disease characteristics in patients with RA

Patients	358 patients (309 men and 49 women)
Biologics received	
Infliximab	144 patients
Etanercept	120patients
Adalimumab	25patients
Tocilizumab	41patients
Abatacept	28patients
Naïve cases	288cases
Age (range)	56.9years (20.3–85.8 years)
Disease Duration (range)	18.0years (1.0–76.0 years)
Prednisolone	5.9mg (72.1%) *
Methotrexate	7.0mg/week (68.7%) *
C-reactive protein	2.5±2.4mg/dL
MMP-3	249.6±214.5ng/mL
DAS28-ESR	4.8±1.4

*The percentage of patients receiving prednisolone or methotrexate

erythrocyte sedimentation rate (ESR) is calculated with 4 indices—a 28-swollen joint count, a 28-tender joint count, ESR, and a 100-mm visual analogue scale for general health—and has been a standard measure to evaluate the disease activity of RA. However, because the DAS28 and other composite measures include subjective indices, such as a visual analogue scale for general health or a visual analogue scale for pain evaluated by patients, an objective measure would be more helpful.

To evaluate the prognosis and the effectiveness of treatment in various diseases, the use of the neutrophil-lymphocyte (N/L) ratio has been reported[4–11]. Patients with RA have a higher N/L ratio than do subjects without RA[12]. To determine whether the N/L ratio is a prognostic marker or a surrogate marker of the response to biologics, in the present study we investigated the N/L ratio and its change in patients with RA treated with biologics.

Patients and Methods

This retrospective study reviewed the medical records of patients with RA who had received routine care at Nippon Medical School. The patients had been treated with a single biologic agent for at least 6 months (**Table 1**). Infliximab, etanercept, and adalimumab are classified as inhibitors of tumor necrosis factor, tocilizumab is an interleukin 6 blocker, and abatacept is a fusion protein of cytotoxic T-lymphocyte antigen 4 (CTLA-4) that inactivates T cells. Before the biologics were administered, the patients were confirmed to be free of infectious or malignant diseases. Clinical findings, the ESR, a hemogram, and serum levels of C-reactive protein (CRP) and matrix metalloproteinase 3 (MMP-3) were assessed before treatment (baseline) and 6 months after the treatment. Disease activity was evaluated with the DAS28-ESR (continuous scale ranging from 0 to 9.4) and the interrupted scale (low: DAS28-ESR≤3.2; moderate: 3.2<DAS28-ESR≤5.1; and high: DAS28-ESR>5.1). The response to the biologics was also classified according to the European League Against Rheumatism response criteria (good, moderate, and no response)[13].

Neutrophil counts, lymphocyte counts and the N/L ratios in patients with and without prednisolone or in patients with and without methotrexate were explored. The correlation of the N/L ratio and indices of disease activity, and then the correlation of the change of N/L ratio (ΔN/L ratio) and the change of other parameters (ΔESR, ΔCRP, ΔMMP-3, ΔDAS28-CRP) were investigated. In addition, the correlation of the change of the DAS28-ESR (ΔDAS28-ESR) and the change of other parameters were investigated.

Finally, the implication of the N/L ratio in the response to treatment were examined.

Statistical Analysis

Values are expressed as means and standard deviations. The correlations between DAS28 and N/L ratio, and between the changes of theses variables were calculated with Spearman's rank correlation test. Comparisons between groups were assessed with Student's *t*-test, and paired data were evaluated with paired t-tests. Compari-

Table 2 Neutrophil count, lymphocyte count, and N/L ratio at baseline and 6 months with or without prednisolone

Patients groups	n	Neutrophil count		Lymphocyte count		N/L ratio	
		Baseline	6 Months	Baseline	6 Months	Baseline	6 Months
All patients	358	6,680±2,526	5,504±2,516 ‡‡	1,351±553	1,490±577 ‡‡	5.9±3.5	4.5±3.4 ‡‡
Prednisolone-treated	258	7,011±2,476	5,831±2,516 ‡‡	1,309±555	1,452±594 ‡‡	6.3±3.5	4.9±3.6 ‡‡
Non-prednisolone-treated	100	5,757±2,431 **	4,642±2,304 ‡‡ **	1,470±531 *	1,590±517 ‡‡	4.7±3.2 **	3.4±4.9 ‡‡ **
Prednisolone tapered	60	7,518±2,373	5,545±2,299 ‡‡	1,316±485	1,528±554 ‡	6.5±3.1	4.1±2.1 ‡‡

Baseline vs 6 Months: ‡ P<0.05 ‡‡ P<0.05

Prednisolone-treated vs non-prednisolone-treated patients: * P<0.05 ** P<0.01

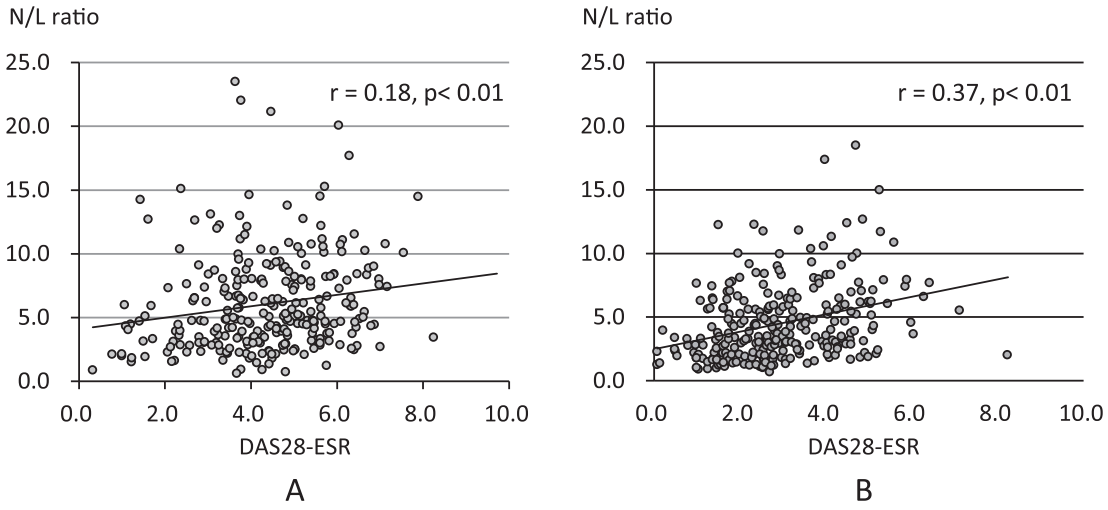

Fig. 1 Correlation between the neutrophil-lymphocyte (N/L) ratio and 28-joint Disease Activity Score—erythrocyte sedimentation rate (DAS28-ESR). **A**: baseline, **B**: at 6 months

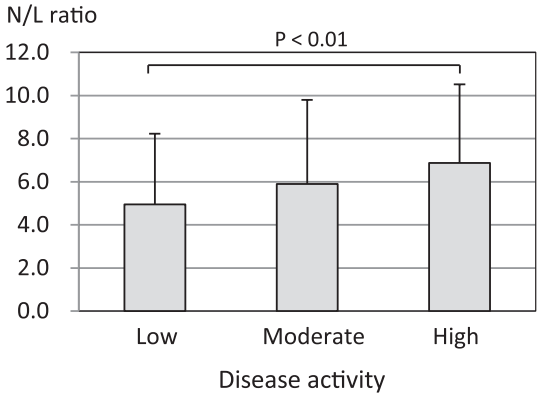

Fig. 2 Neutrophil-lymphocyte (N/L) ratio in each disease activity at baseline. Low: 28-joint Disease Activity Score in 28 joints—erythrocyte sedimentation rate (DAS28-ESR). ≤3.2; Moderate: 3.2<DAS28-ESR≤5.1; High: DAS28-ESR>5.1.

sons between classifications were analyzed with analysis of variance (ANOVA) followed by Turkey's post-hoc analysis. A P values less than 0.05 was considered significant.

Results

Of the 358 patients, 288 were naïve to biologics (**Table 1**). Mean age was 56.1 years, and mean disease duration was 18.0 years. Of the patients, 72% received prednisolone, at a mean dosage of 5.9 mg/day, and 68.7% received methotrexate, at a mean dosage of 7.0 mg/week. The mean serum level of CRP was 2.5 mg/dL and that of MMP-3 was 249.6 ng/mL (normal range of MMP-3: 36.9–121 ng/mL in men; 17.3–59.7 ng/mL in women). The mean DAS28-ESR was 4.8.

From baseline to after 6 months of treatment, the mean neutrophil count and the mean N/L ratio among all patients decreased significantly (P<0.01) and that of the lymphocyte count increased significantly (P<0.01) (**Table 2**). The N/L ratio and the DAS28-ESR were modestly but significantly correlated (r=0.18, P<0.01) at baseline (**Fig. 1 A**). The N/L ratio in patients with high disease activity

was greater than that in patients with low disease activity (**Fig. 2**). The N/L ratio and the DAS28-ESR after 6 months of treatment were also significantly correlated (r= 0.37, P<0.01) (**Fig. 1B**).

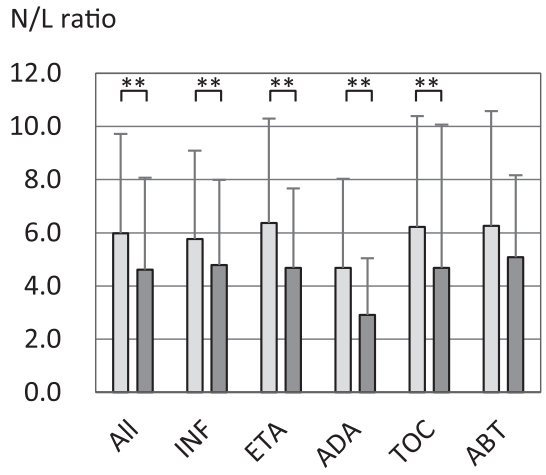

Fig. 3 Neutrophil-lymphocyte (N/L) ratio at baseline and after 6 months of treatment with biological agents. IFX: infliximab; ETA: etanercept; ADA: adalimumab TCZ: tocilizumab; ABT: abatacept. **P<0.01 using paired-*t* test.

The N/L ratio decreased after treatment with biological agents and the decrease was significant, except for abatacept (**Fig. 3**). ΔN/L ratio and ΔDAS28-ESR showed modest but significant correlation (r=0.23, P<0.01) (**Fig. 4**). The ΔN/L ratio was found to be modestly but significantly correlated with the ΔCRP (r=0.17, P<0.01) and the ΔDAS28-CRP (r=0.25, P<0.01) (**Table 3**). The ΔESR strongly correlated with ΔDAS28-ESR. In addition to ΔESR, other variables, such as ΔCRP, ΔMMP-3, the change of the white blood count and the change of the neutrophil count, correlated modestly but significantly with the ΔDAS28-ESR (**Table 4**).

The N/L ratio at baseline showed no significant difference with regard to the response criteria, but the N/L ratio after 6 months of treatment and the ΔN/L ratio showed significant differences between patients with a good response and those with no response (**Fig. 5**). Thus, the pretreatment N/L ratio was not a prognostic marker of response to biologics at baseline but did reflect the re-

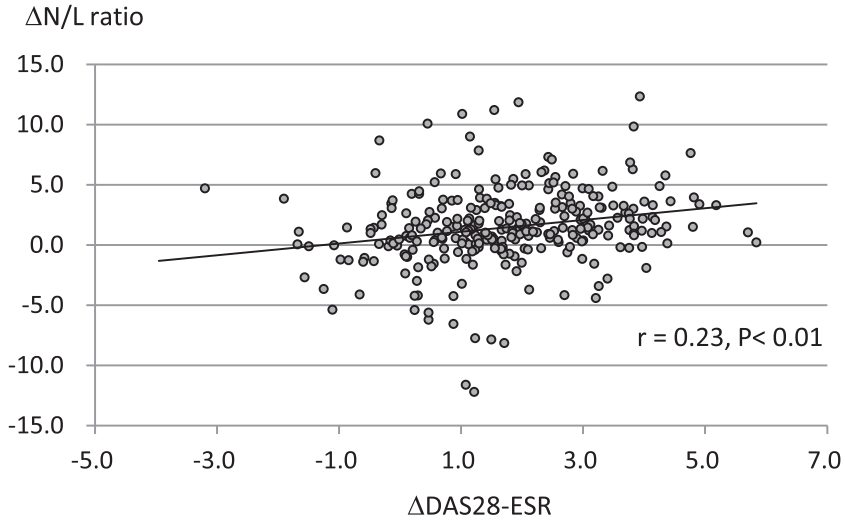

Fig. 4 Correlation between the change of neutrophil-lymphocyte (ΔN/L) ratio and the change of 28-joint Disease Activity Score in 28 joints—erythrocyte sedimentation rate (ΔDAS28-ESR).

Table 3 Correlation between ΔN/L ratio and the change of other serum inflammatory markers and disease activity

	ΔESR	ΔCRP	ΔMMP-3	ΔDAS28-ESR	ΔDAS28-CRP
r	0.11	0.17	0.05	0.23	0.25
P	NS	<0.01	NS	<0.01	<0.01

ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; MMP-3: matrix metaroproteinase 3; DAS28: Disease Activity Score in 28 joints.

Table 4 Correlation between ΔDAS28-ESR and other inflammatory parameters

	ΔESR	ΔCRP	ΔMMP-3	ΔWBC	ΔNEU	ΔLYM	ΔN/L ratio
r	0.62	0.17	0.25	0.18	0.22	–0.01	0.23
P	<0.01	<0.01	<0.01	<0.01	<0.01	NS	<0.01

ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; MMP-3: matrix metaroproteinase-3; WBC: white blood count; NEU: neutrophil; LYM: lymphocyte; N/L: neutrophil-lymphocyte ratio

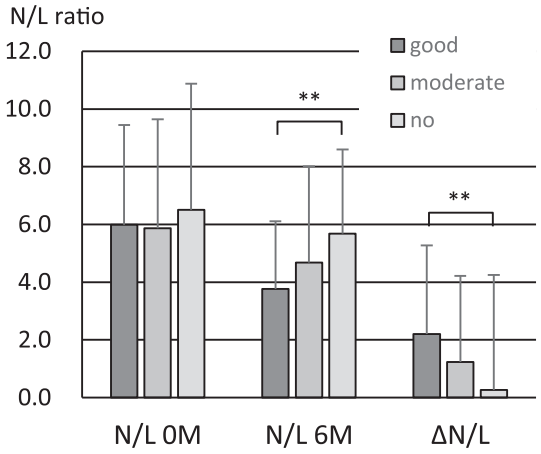

Fig. 5 Neutrophil-lymphocyte (N/L) ratio in each therapeutic response (good response, moderate response, and no response). N/L 0M: baseline, N/L 6M: after 6 months of treatment, ΔN/L: change of N/L ratio. **P<0.01 using analysis of variance followed Turkey's post-hoc analysis.

sponse to biologics.

At baseline the mean neutrophil count and the mean N/L ratio were significantly greater (both P<0.01) and the mean lymphocyte count was significantly lower (P< 0.05) in patients who received prednisolone than in patients who did not (**Table 2**). However, from baseline to after 6 months of treatment in both groups of patients, the neutrophil count and the N/L ratio decreased significantly (both P<0.01) and the lymphocyte count increased significantly (P<0.01) (**Table 2**). The dosage of prednisolone was tapered in 60 of 258 patients because disease activity was found to have decreased. Between baseline and after 6 months of treatment, both the neutrophil count and the N/L ratio decreased significantly and the lymphocyte count increased significantly in these 60 patients (**Table 2**). Furthermore, ΔN/L and ΔDAS28-ESR were significantly correlation both in patients who had received prednisolone (r=0.23, P<0.01) and in patients who had not (r=0.22, P<0.01). Prednisolone did not affect the prognostic role of the N/L ratio (data not shown).

In patients who received methotrexate, the N/L ratio at baseline was significantly lower (6.0 ± 3.2) than in patients who did not receive methotrexate (7.0 ± 3.7, P< 0.05). However, treatment with methotrexate did not affect the correlation between ΔN/L and ΔDAS28-ESR or the prognostic value of the pretreatment N/L ratio.

Discussion

In the last decade, systemic inflammation has been shown to be a key determinant of outcome, and the N/L ratio has been identified as a useful marker in patients with cancer[4,5]. Attempts have been made with the N/L ratio to predict the therapeutic response in patients with cancer and other diseases. For example, the N/L ratio is reported to be a useful predictor in patients with advanced hepatocellular carcinoma treated with hepatic arterial infusion chemotherapy, and patients with a lower N/L ratio (cut-off level, 2.87) have shown a better response[6]. The N/L ratio before treatment is a prognostic indicator of survival in patients with early-stage non-small cell lung carcinoma treated with stereotactic radiation[7]. Furthermore, in-stent restenosis after percutaneous coronary intervention can be predicted with the ΔN/L ratio, but not with pretreatment N/L ratio[8]. The N/L ratio has been used to stratify the risk of coronary aneurysm and intravenous immunoglobulin resistance in Kawasaki disease[9]. The N/L ratio has also been used to predict blood stream infection in the emergency room and to detect *Helicobacter pylori* infection[10,11].

Patients with RA have frequently shown neutrophilia and lymphopenia. Although neutrophilia is caused by systemic inflammation, the pathogenesis of lymphopenia is unclear. One study has found that lymphopenia is present in 15% of patients with RA despite changes in disease activity and is due to fewer circulating T-cell despite a normal number of circulating B-cells[14]. Fewer T-cells might be circulating because they infiltrate the synovial membrane, which is the main pathogenic focus of RA. Furthermore, the N/L ratio is positively correlated with

the expression of CD10 and CD35 as a result of neutrophil activation[15].

A recently study has found that the N/L ratio is higher in patients with RA (2.12) than in healthy subjects (0.83), is correlated with DAS28 (r=0.345, P<0.0001), and is higher in patients with active disease[12]. In the present study, the N/L ratio was positively and significantly correlated with DAS28-ESR both at baseline and after 6 months of treatment. The N/L ratio was also higher in patients with high disease activity than in patients with low disease activity.

All patients of the present study were treated with biological agents and showed decreased measures of disease activity, including DAS28, ESR, CRP, and MMP-3 (data not shown). Elevated levels of MMP-3 and other markers have been reported to reflect the disease activity of RA[16]. We also found that the N/L ratio decreased after treatment with each biological agent, except for abatacept. Abatacept is a fusion protein of CTLA-4 which modulates the T-cell co-stimulatory signal, and the decrease of the N/L ratio after treatment with abatacept did not reach statistical significance, probably because of a small number of patients treated with abatacept or the depression of T cells by abatacept or both. In patients treated with abatacept, the lymphocyte count did not change significantly from baseline (1,315 ± 608/μL) to after 6 months of treatment (1,472 ± 657/μL), whereas the mean lymphocyte count of all patients had increased significantly after 6 months. The neutrophil count also changed significantly, decreasing from baseline (6,626 ± 1,931/μL) to after 6 months of treatment (5,899 ± 2,055/μL) in patients treated with abatacept.

In the present study we also examined whether the N/L ratio can predict a response to biological agents. Disappointingly, the pretreatment N/L ratio did not predict the response to treatment. Several biomarkers, such as baseline tumor necrosis factor levels[17], circulating T helper 17 cells and interleukin 17[18], and interleukin 1β levels from stimulated WBC, have been studied as predictors of tumor necrosis factor inhibitor[19]; however, no robust biomarker has been identified. In the present study we observed that the ΔN/L ratio was larger and the N/L ratio after 6 months of treatment was lower in patients with a good response. This result indicates again that the N/L ratio reflects disease activity and decreases when disease activity decreases. Moreover, ΔN/L ratio correlated with CRP-related measures (CRP and DAS28-CRP) rather than with ESR (**Table 3**). Although ΔDAS28-ESR correlated strongly with ΔESR, it also correlated modestly with ΔCRP, ΔMMP-3, ΔWBC, the change of the neutrophil count, and ΔN/L ratio (**Table 4**).

In conclusion, the present study has found that the N/L ratio is a marker of disease activity in RA and that the ΔN/L ratio reflects the efficacy of biological agents. However, the pretreatment N/L ratio does not predict the response to biological agents.

Conflict of Interest: None.

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