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Serum soluble interleukin-2 receptor predicts early remission in patients with recent-onset rheumatoid arthritis treated with a single disease-modifying antirheumatic drug

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ABSTRACT

Objective. *To study the value of baseline serum levels of circulating soluble interleukin-2 receptor (sIL-2R) and soluble E-selectin as predictors of early remission in patients with recent-onset rheumatoid arthritis (RA) receiving a single disease-modifying antirheumatic drug (DMARD) (SINGLE) or therapy with a combination of DMARDs (COMBI).*

Methods. *Baseline (n = 157) serum samples originate from the FIN-RACo (FINnish Rheumatoid Arthritis Combination therapy) trial, in which 195 patients with early and clinically active RA were randomly assigned to receive either SINGLE (initially sulfasalazine) with or without prednisolone, or COMBI therapy (sulfasalazine, methotrexate, hydroxychloroquine, and prednisolone). Of the samples, 76 were from SINGLE patients and 81 from COMBI patients. sIL-2R was measured by automated immunoassay analyzer and sE-selectin by enzyme-linked immunosorbent assay.*

Results. A*t six months, 7 (9% [95% CI: 4 to 18]) SINGLE and 19 (23% [95% CI: 15 to 34]) COMBI patients were in remission. In multivariate logistic regression analysis, sIL-2R < 442 U/ml and COMBI therapy were the only predictors of remission. The area under receiver operating characteristic curve for sIL-2R level was 0.86 (95% CI: 0.62 to 0.95) in SINGLE and 0.57 (95% CI: 0.42 to 0.71) in COMBI (p = 0.006). In SINGLE, the optimal cut off-point was 442 U/ml, lower levels predicting remission with sensitivity of 83% (95% CI: 73% to 91%) and specificity of 86% (95% CI: 42% to 100%). Likelihood ratio for positive test was 5.9 (95% CI: 1.6 to 32.8).*

In multivariate logistic regression analysis, sIL-2R < 442 U/ml and COMBI therapy were the only predictors of remission.

Conclusion. *Low baseline serum sIL-2R level predicts early remission of patients with active early RA treated with a single DMARD.*

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease, which manifests as chronic polyarthritis with diverse extra-articular manifestations. The clinical course of RA varies from spontaneous remission to tissue-damaging inflammatory reaction unresponsive to disease-modifying antirheumatic drugs (DMARDs). At present, it is not possible to identify with reasonable accuracy the patients with early RA who will enter remission [reviewed in (1)]. Accordingly, in the FINnish Rheumatoid Arthritis Combination Therapy (FIN-RACo) trial, therapy with a combination of DMARDs was the only variable predicting remission (2). Such therapy, however, renders many patients unduly susceptible to over-treatment, which may increase the risk of adverse effects, costs, and non-compliance with the treatment. Therefore, markers are needed that identify the patients who may enter early remission with less intensive initial therapies.

The pathogenesis of RA is considered to involve activation of T-cells, B-cells, and mononuclear phagocytes, and production of proinflammatory cytokines such as tumor necrosis factor and interleukin-1.(3) The severity of the immune activation can be evaluated by measuring the circulating level of soluble interleukin-2 receptor (sIL-2R). Although sIL-2R is an activation marker for T- and B-lymphocytes as well as monocytes (4), in patients with RA it may reflect activation of T-lymphocytes, which is considered to have a pivotal role in the pathogenesis of RA.(5) Immune activation induces systemic inflammation. Its severity can be evaluated by measuring the blood level of soluble E-selectin (sE-selectin), a marker of activation of microvascular endothelium (6).

We reasoned that RA patients who present with weak immune activation may achieve remission with less intensive initial therapy than do patients with strong immune activation. To address this question we studied whether baseline levels of sIL-2R and/or sE-selectin predict remission at 6 months in the patients enclosed in the FIN-RACo trial, treated either with a single DMARD (SINGLE) or with a combination of DMARDs (COMBI).

Patients and methods

Patients

The FIN-RACo (FINnish Rheumatoid Arthritis Combination therapy) trial (2) is a nation-wide multi-center, randomized, open parallel-group trial comparing the efficacy and tolerability of treatment with either a combination of the DMARDs sulfasalazine, methotrexate, hydroxychloroquine, and prednisolone (COMBI) or sulfasalazine with or without prednisolone (SINGLE). The study protocol was approved by the ethics committees in all 18 participating hospitals.

The inclusion criteria were: 1) fulfillment of American College of Rheumatology (ACR) criteria for RA (7), 2) age 18-65 years, 3) duration of symptoms < 2 years, and 4) active disease with ≥ 3 swollen joints, and at least 3 of the following: (a) erythrocyte sedimentation rate (ESR) of ≥ 28 mm/hour or C-reactive protein (CRP) level of > 19 mg/liter, (b) morning stiffness of ≥ 29 minutes, (c) > 5 swollen joints, or (d) >10 tender joints. Of the 199 patients randomized, a total of 195 patients (97 COMBI and 98 SINGLE) started the treatment. The present study included 157 patients (81 COMBI and 76 SINGLE) whose baseline serum samples were available. A total of three SINGLE patients received prednisolone (up to 10 mg daily) during follow-up.

The end point of the study was the achieved remission at six months, as evaluated using the ACR preliminary criteria for remission (8), with the exception of fatigue and duration criteria.

Serum sIL-2R and sE-selectin

The serum samples were stored at -20º C. The levels of sIL-2R were measured by the Immulite automated immunoassay analyzer (DPC, Los Angeles, CA). The detection limit was 10 IU/ml. The levels of sE-selectin were measured using an enzyme-linked immunosorbent assay kit (Bender MedSystems, Vienna, Austria). The detection limit was 0.5 ng/ml.

Statistics

The results were expressed as mean and 95 percent confidence interval (CI) or standard deviation (SD), or median and interquartile range (IQR). The confidence intervals of mean sIL-2R levels were calculated by bootstrapping. Equality of sIL-2R levels in patients in or out of remission were tested using the permutation test. Receiver operating characteristic (ROC) curves were calculated to define the optimal cut-off point for the sIL-2R test in predicting remission. Confidence intervals of area under ROC curves (AUC) were estimated using the bootstrap bias-corrected accelerated method. Equality of area under ROC curves were tested using an algorithm suggested by DeLong, DeLong, and Clarke-Pearson. (9) The optimal cut-off value was defined as the level with the greatest sum of sensitivity and specificity. Odds ratios (OR) with 95% CI for prediction of remission by baseline demographic, clinical, and laboratory variables were calculated using multivariate logistic regression analysis.

Results

Patients

The baseline demographic, clinical, and laboratory characteristics of SINGLE and COMBI patients were comparable (Table I). At six months, 19 COMBI patients (23% [95% CI: 15 to 34]) and 7 SINGLE patients (9% [95% CI: 4 to 18]) (2 on prednisolone) were in remission.

sIL-2R

Among the SINGLE patients, the baseline mean sIL-2R levels were 429 U/ml (95% CI: 366 to 614) in patients who entered remission and 737 U/ml (95% CI: 668 to 829) in patients who did not enter remission ($p < 0.001$) (Fig. 1). Among COMBI patients, the respective figures were 660 U/ml (95% CI: 570 to 802) and 739 U/ml (95% CI: 672 to 824) ($p = 0.35$) (Fig. 1).

COMBI therapy and baseline sIL-2R

Table I. Baseline demographic, clinical, and laboratory characteristics of the patients according to treatment group.

Variable	Treatment group			
	COMBI (n = 81)		SINGLE (n = 76)	
Women, no. (%)	59	(61%)	51	(67%)
Age in years, mean (range)	47	(23 - 65)	48	(20 - 65)
Duration of disease in months, mean (range)	7.2	(2 - 22)	9.4	(3 - 23)
Rheumatoid factor present, no. (%)	56	(69%)	51	(67%)
Swollen joint count, median (IQR)	13	(9, 17)	13	(10, 16)
Tender joint count, median (IQR)	16	(12, 22)	17	(13, 25)
HAQ score, median (IQR)	0.85	(0.38, 1.07)	0.88	(0.38, 1.25)
ESR in mm/hour, median (IQR)	31	(20, 53)	34	(23, 52)
Serum sIL-2R in U/ml, median (IQR)	674	(489, 829)	504	(475, 870)
Serum sE-selectin in ng/ml, median (IQR)	39.2	(27.9, 51.7)	39.1	(26.4, 54.3)

COMBI: combination therapy with disease-modifying antirheumatic drugs; SINGLE: single drug therapy; IQR: interquartile range; HAQ: Health Assessment Questionnaire; ESR: erythrocyte sedimentation rate; sIL-2R: soluble interleukin-2 receptor; sE-selectin: soluble E-selectin.

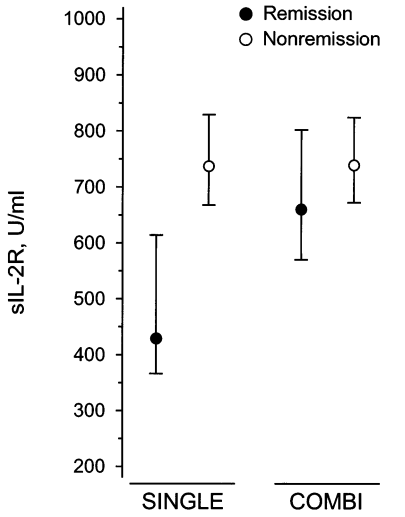

Fig. 1. Baseline sIL-2R levels in single disease-modifying antirheumatic drug group (SINGLE) and combination drug group (COMBI) according to remission after 6 months. Dots denote group means and whiskers their 95% confidence intervals.

level < 442 U/ml were the only significant predictors of remission after six months in logistic regression analysis (Table II). Figure 2 shows the ROC curves of serum sIL-2R level for prediction of remission. Only SINGLE values differed significantly from the reference line i.e., AUC 0.50, p = 0.003. The difference between AUC for SINGLE [0.86 (95% CI: 0.62 to 0.95)] and that for COMBI [0.57 (95% CI: 0.42 to 0.71)] was significant, p = 0.006.

The optimal cut-off value for the sIL-2R level in SINGLE was 442 U/ml, predicting remission with 84% (95% CI: 73% to 91%) sensitivity and 86% (95% CI: 42% to 100%) specificity. The likelihood ratio for positive test was 5.9 (95% CI: 1.6 to 32.8). The optimal cut-off in COMBI was 830 U/ml, with 89% (95% CI: 67% to 99%) sensitivity, 27% (95% CI: 17% to 40%) specificity, and 1.23 (95% CI: 0.92 to 1.52) likelihood ratio for positive test.

sE-selectin

The respective median (IQR) sE-selectin levels in SINGLE for remission and for non-remission subgroups were 33.7 ng/ml (26.9, 52.2) and 40.8 ng/ml (26.2, 55.2). The corresponding values in COMBI were 43.2 ng/ml (32.2, 56.4) and 37.9 ng/ml (27.8, 50.7), respectively. The AUC values for SINGLE and COMBI were comparable, 0.58 (95% CI: 0.34 to 0.73) vs. 0.45 (95% CI: 0.31 to 0.61). Neither group differed significantly from the reference line (AUC 0.50).

Table II. Logistic regression analysis for the odds to reach remission after 6 months.

Variable at beginning of study	OR (95% CI)*
Combination strategy	4.4 (1.6 to 12.2)
Serum sIL-2R level < 442 U/ml	4.7 (1.4 to 15.2)
Female sex	1.0 (0.3 to 3.1)
Age in years	1.0 (0.9 to 1.1)
Disease duration in months	1.0 (0.9 to 1.1)
Rheumatoid factor present	1.2 (0.4 to 3.7)
Erythrocyte sedimentation rate	1.0 (0.9 to 1.1)
Swollen joint count	1.0 (0.9 to 1.1)
Tender joint count	0.9 (0.9 to 1.0)

*Odds ratio and 95% confidence interval. Odds ratios are adjusted for all other variables in the model.

Discussion

The results show that low baseline levels of sIL-2R (< 442 U/ml), denoting a low degree of T-cell activation, predict remission at 6 months in patients with active early RA treated with a single DMARD. The finding is novel and suggests that sIL-2R provides a marker, which with reasonable accuracy identifies the patients with active early RA who may enter early remission. The present finding is not in accordance with two previous studies in which the baseline sIL-2R levels failed to predict the clinical outcome of RA patients treated either with methotrexate (10), or with sulfasalazine or parenteral gold. (11) Unlike the present study, however, both studies included patients with longstanding RA, with mean disease duration more than 10 and 5 years, respectively. Such patients with a long disease duration may be less prone to spontaneous or drug induced remissions, which occurs in 10-30% of patients with early RA. (1) It is possible that initially low sIL-2R identifies the patients bound to enter spontaneous remission. Another possibility is that in some patients with a low degree of T-cell activation, even a single DMARD is able to induce remission.

Unlike the patients in SINGLE group, several of our COMBI patients with high baseline sIL-2R levels achieved remission, indicating that the combination of DMARDs used induces remission also in some patients with a high degree of T-cell activation. In COMBI, however, sIL-2R did not predict remission. An explanation for the poor predictive value of sIL-2R may be that neither sIL-2R nor the DMARDs used are specific for the subsets of T-cells, i.e., T-helper 1 cells exacerbating, and T-helper 2 cells suppressing inflammation in patients with RA [reviewed in (12)]. Thus, the activation of either of the T-cell subtypes increases sIL-2R levels. On the other hand, the DMARDs may exert anti-lymphocyte activity by promoting T-cell apoptosis, as shown *in vitro* for hydroxychloroquine (13), methotrexate (14), and sulfasalazine.(15) Glucocorticoids also induce T-cell apoptosis and, in addition, depress inflammation by interfering with expression of a wide variety of activation-induced gene products.(16) Taken together, the findings above indicate that interpretation of the clinical significance of an increased sIL-2R level in patients with early RA is difficult. It appears, however, that an ag-

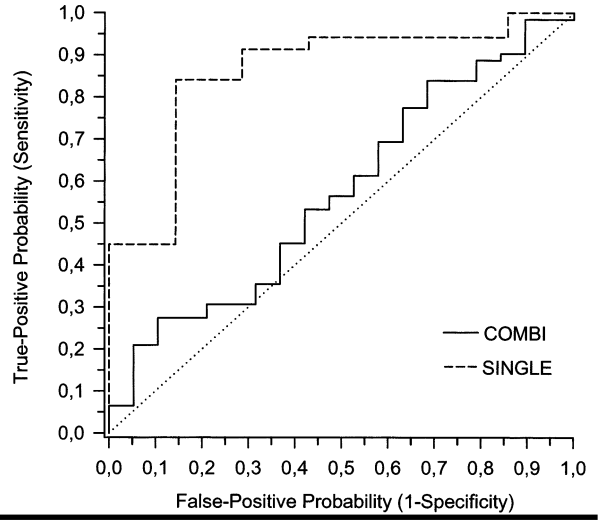

Fig 2. Receiver operating characteristic curves of baseline sIL-2R level for prediction of remission at six months in single disease-modifying antirheumatic drug group (SINGLE) and combination drug group (COMBI). Diagonal dotted line represents reference line ("line of no information").

gressive therapy, such as COMBI, is effective even in severe forms of early RA, such as with elevated levels of sIL-2R.

If the high sIL-2R level derives from proinflammatory T-helper 1 cells, the patient might develop systemic inflammation. In the present study, however, baseline sE-selectin used as marker of systemic inflammation did not predict early remission.

Our findings, if reproduced in larger patient cohorts, indicate that sIL-2R may provide a marker to identify the patients with clinically active early RA inducible into early remission with a single DMARD regimen. The alternative explanation is that these patients enter early remission spontaneously, which is of interest in terms of the role of T-cells in the pathogenesis of RA. Predicting early remission permits, for the first time, an opportunity to select the patients with active early RA in whom a single DMARD instead of the use of more aggressive combination therapies would be adequate initial therapy.

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