Upsala J Med Sci 100: 233–242, 1995

Do Changes in Clinical Improvement in Rheumatoid Arthritis Patients Treated with Immunosuppressive Drugs Reflect the Changes in acute Phase Proteins?

Two years follow up study

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ABSTRACT

We sought to investigate whether clinical improvement after immunosuppressive treatment reflects changes in acute phase response (APR) in rheumatoid arthritis (RA). Fifty-eight patients (pts) were treated with methotrexate (MTX), nineteen with intravenous cyclophosphamide (CTX), and fifteen with cyclosporin A (CSA). C-reactive protein (CRP), alpha-1-acid glycoprotein (AGP), alpha-1 antichymotrypsin (ACT), and alpha-1 antitrypsin (AT) serum levels were measured by nephelometry or rocket immunoelectrophoresis.

Clinical improvement was observed in 67% MTX pts, 53% CTX pts, and 47% CSA pts. Baseline serum levels of CRP, AGP, ACT, and AT were significantly higher as compared to healthy controls. After MTX and CTX therapy CRP level significantly decreased. The decrease in serum level of ACT and AT in CTX treated patients was also observed. All analyzed acute phase proteins remained substantially elevated after CSA therapy despite a clear reduction in disease activity. We established a correlation between changes in disease activity and all acute phase proteins (APP) in MTX and CTX pts.

From our study we can conclude that clinical improvement after immunosuppressive treatment correlated with quantitative changes in all APR markers in MTX and CTX treated pts, and none in CSA pts. Although measurement of APP remains the best marker for monitoring RA pts, not always they properly reflect changes in disease activity.

INTRODUCTION

Rheumatoid arthritis (RA) a widespread illness is the most common autoimmune disease. The etiology, perhaps multifactorial still now remains unknown. The outcome of the disease is more severe than, it was previously considered (9, 13). Mortality among RA patients is higher than in control population matched by age and sex. However, for most of the patients, conservative therapy provides substantial benefit. In those patients who suffer from progressively destructive disease more aggressive treatment is necessary. Therefore, cytotoxic agents become more popular in recent years for the treatment of refractory RA, although these drugs should not be considered as routine therapy (2, 7, 8, 23, 24).

Acute phase proteins (APP) are well known markers of inflammatory processes. The elevation of APP strongly correlates with disease activity in some rheumatic diseases such as RA. Therefore they are proposed for monitoring patients suffering from RA (1, 11). Since elevated levels of some APP remains a major symptom of inflammatory response in RA and they have been shown to correlate with disease activity, we observed in the present paper the effect of methotrexate (MTX), cyclophosphamide (CTX), and cyclosporin A (CSA) on serum concentration of C-reactive protein (CRP), alpha-1-acid glycoprotein (AGP), alpha-1 antichymotrypsin (ACT), and alpha-1 antitrypsin (AT).

MATERIALS AND METHODS

Ninety-two patients (75 women and 17 men) fulfilling the American College of Rheumatology criteria (1) were observed for two years. Data concerning age, disease duration, and seropositivity are given in Table 1. Fifty-eight patients received methotrexate (MTX) in a single oral dose (7.5-15 mg weekly). Nineteen patients received an intravenous dose of CTX (0.4g /m^2 body surface area /4-8 weeks). Fifteen patients were treated with cyclosporin A (CSA) which was given twice a day in an average initial dose 2.5 mg/kg/day. After 2 months the dosage was increased up to 4 mg/kg/day depending on the clinical improvement. Nonsteroidal antiinflammatory drugs (NSAID) were administered to all of the subjects. Patients' trial included to the study was not randomized. Patients with severe concomitant diseases (i.e. hypertension, kidney or liver dysfunction) were excluded from the

study. The control group consisted of 39 healthy subjects (24 females, 15 males) with the age ranged from 19 to 49 years (mean of 32.5 ± 7.3 years).

Table 1. Clinical data of 86 RA patients included in the study.
MTX - methotrexate treated patients
CTX - cyclophosphamide treated patients
CSA - cyclosporin A treated patients

	MTX	CTX	CSA
No. of patients:	58	19	15
Sex: (female/male)	46/12	16/3	13/2
Age: years (SD)	53 (SD 12)	46 (SD 7)	41 (SD 5)
Disease Duration: years (SD)	13 (SD 9)	5 (SD 4)	4 (SD 3)
Seropositive:	50 (86%)	18 (95%)	14 (93%)

Clinical assessments. Clinical evaluation of disease activity was performed at the onset of the study and after the 6th, the 12th, and the 24th month of the observation. Clinical assessment was performed according to the method proposed by Mallya and Mace (17), including: time of the duration of morning stiffness, grip strength, Ritche index (19), the number of painful and swollen joints, hemoglobin level (Hb), and erythrocyte sedimentation rate (ESR). Clinical improvement was defined as at least one-third decrease in the activity score. CRP was measured by nephelometry. AGP, ACT, and AT were measured by rocket immunoelectrophoresis according to Laurell (16).

Statistical analysis: The Wilcoxon signed rank test or Mann-Whitney rank order tests were used to evaluate statistical significance. Relationship between variables was examined using Spearman's correlation coefficient for continuous variables.

RESULTS

Thirty-nine RA patients treated with MTX (67%) showed clinical improvement. The clinical improvement was observed also in 10 CTX (53%) and 7 CSA (47%) treated

patients. Ten patients had to discontinue the therapy either because of adverse effects or unresponsiveness. Amelioration of the disease appeared sooner in patients receiving MTX as compared to patients treated with CTX or CSA .

Table 2. The Changes in acute phase proteins (APP) serum levels and disease activity according to Mallya-Mace in 86 patients treated with methotrexate, cyclophosphamide, and cyclosporin A.

APP [mg/l]	healthy controls	before treatment	after 6 months of treatment	after 12 months of treatment	after 24 months of treatment
		Mrthotrexate treated patients [n=58]			
CRP	3 (SD 2)	35 (SD14)##	20 (SD 11)	18 (SD 10)	17 (SD11) **
AGP	484 (SD 133)	1060 (SD 407)#	828 (SD 280)	789 (SD 296)	726 (SD 308)
ACT	457 (SD 161)	779 (SD 264)#	686 (SD 219)	638 (SD 216)	626 (SD 222)
AT	3212 (SD 611)	3744 (SD 1024)#	3521 (SD 983)	3584 (SD 1081)	3374 (SD 847)
MM		2.8	2.1	1.9	1.8
		Cyclophosphamide treated patients [n=19]			
CRP	3 (SD 2)	39 (SD 10)##	28 (SD 13)	25 (SD 13)	27 (SD 14)*
AGP	484 (SD 133)	1117 (SD 289)##	842 (SD 199)	836 (SD 208)	812 (SD 256)
ACT	457 (SD 161)	866 (SD 234)#	651 (SD 245)	722 (SD 201)	663 (SD 216)
AT	3212 (SD 611)	4025 (SD 862)#	3812 (SD 546)	3447 (SD 627)	3168 (SD 492)*
MM		3.1	2.4	2.3	2.4
		Cyclosporin A treated patients [n=15]			
CRP	3 (SD 2)	40 (SD13)##	35 (SD14)	36 (SD 13)	33 (SD 12)
AGP	484 (SD 133)	1294 (SD 486)#	1411 (SD 513)	1396 (SD 487)	1186 (SD 426)
ACT	457 (SD 161)	906 (SD 188)#	912 (SD 203)	836 (SD 247)	905 (SD 206)
AT	3212 (SD 611)	3567 (SD 1088)	3324 (SD 934)	3619 (SD 867)	3705 (SD 891)
MM		3.0	2.3	2.5	2.4

CRP C-reactive protein
AGP alpha-1-acid glycoprotein
ACT alpha-1 antichymotrypsin
AT alpha-1 antitrypsin
MM disease activity according to Mallya-Mace

Statistical significance:
(#, ##) before treatment vs healthy controls ($p<0.05$, $p<0.01$)
(*, **) after 24 months of treatment vs before treatment ($p<0.05$, $p<0.01$)

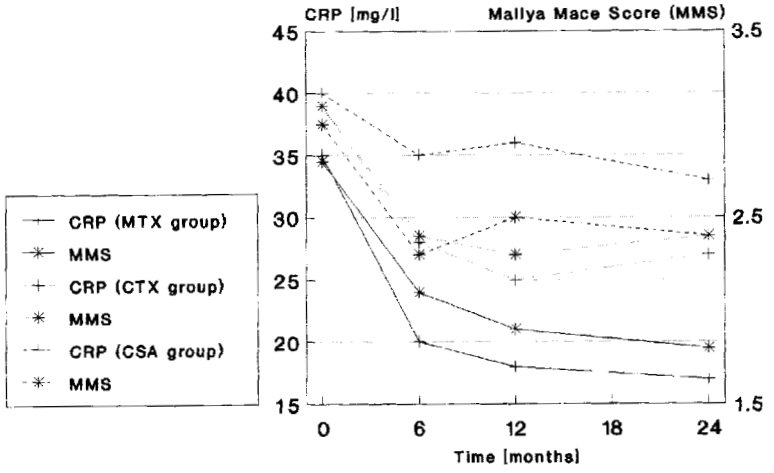

Figure 1.
The changes in CRP serum level and disease activity (MMS) in RA patients treated with methotrexate (MTX), cyclophosphamide (CTX), or cyclosporin A (CSA).

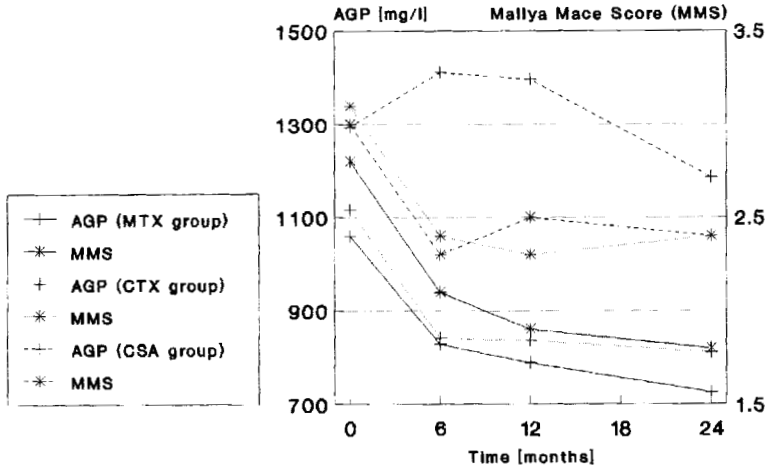

Figure 2.
The changes in AGP serum level and disease activity (MMS) in RA patients treated with methotrexate (MTX), cyclophosphamide (CTX), or cyclosporin A (CSA).

CRP, AGP, ACT, and AT serum levels were increased in all groups of RA patients as compared to healthy controls (Table 2). CRP level significantly decreased in the group of patients treated with MTX and CTX but not CSA (Figure 1, Table 2). The changes in AGP and ACT serum levels were statistically insignificant in all groups of patients; however they followed the changes in disease activity in MTX and CTX group (Figure 2 and 3, Table 2). AT serum levels were significantly lower as compared to baseline ones only in CTX treated patients (Figure 4, Table 2). Moreover, changes in APP's serum level corresponded to changes in disease activity in MTX and CTX groups but not in CSA one (Figure 1, 2, 3, and 4).

For further analysis we calculated the differences between the first and the last measurement in disease activity and APP levels. The correlation analysis between the new variables showed a good association between changes in disease activity and changes in serum levels of all analyzed APR markers in MTX and CTX treated patients (Table 3). We found no relationship between changes in disease activity and APR in CSA treated patients (Table 3).

Table 3. Correlation (r) between changes in disease activity (according to Mallya & Mace) and changes in the level of acute phase proteins in RA patients treated with methotrexate (MTX), cyclophosphamide (CTX), and cyclosporin A (CSA).

	Changes in disease activity		
	MTX (N=58)	CTX (N=19)	CSA (N=16)
Critical value r ($p<0.05$):	+ or - 0.26	+ or - 0.45	+ or - 0.51
Changes in CRP	0.71*	0.64*	-0.24^{NS}
Changes in AGP	0.59*	0.61*	0.43^{NS}
Changes in ACT	0.57*	0.80*	-0.25^{NS}
Changes in AT	0.48*	0.51*	0.19^{NS}

* statistically significant ($p<0.05$)
^{NS} - non significant

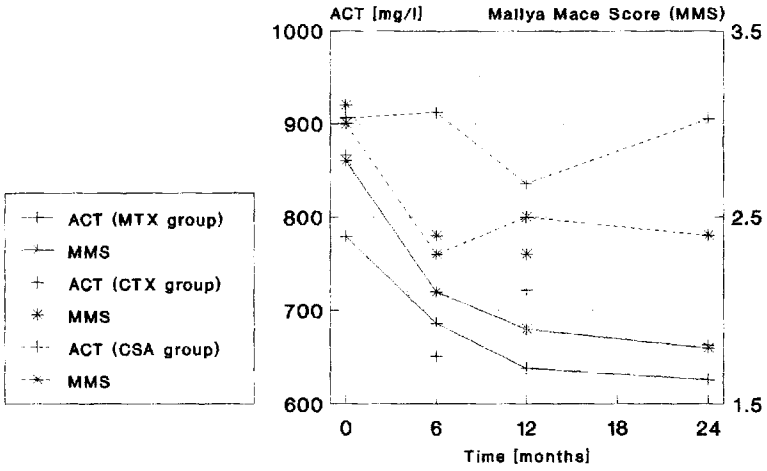

Figure 3.
The changes in ACT serum level and disease activity (MMS) in RA patients treated with methotrexate (MTX), cyclophosphamide (CTX), or cyclosporin A (CSA).

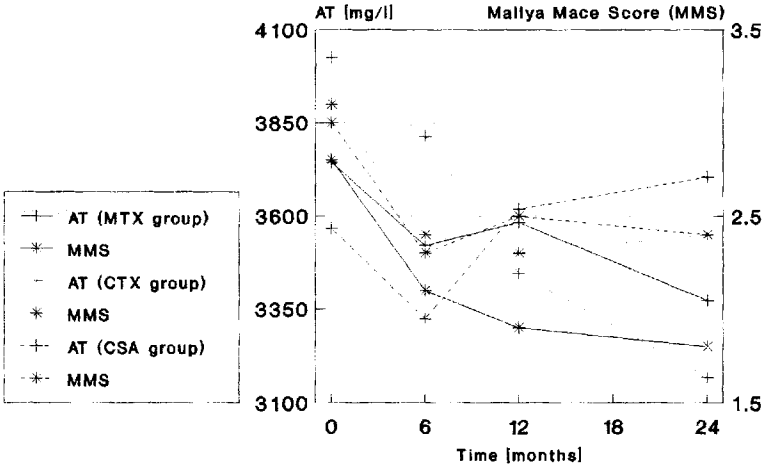

Figure 4.
The changes in AT serum level and disease activity (MMS) in RA patients treated with methotrexate (MTX), cyclophosphamide (CTX), or cyclosporin A (CSA).

DISCUSSION

A statistical decrease in CRP was observed in MTX and CTX but not in CSA treated patients. Although, the changes in AGP, ACT, and AT in MTX and CTX treated patients followed changes in disease activity, they were not significant. Clinical improvement correlated with changes in APP in MTX and CTX treated patients, which agrees with other observations (15, 17, 20, 21). We found no relationship between APR and disease activity in CSA patients.

The utility of MTX in the treatment of RA was shown in many studies (7, 18, 20, 21). Clinical improvement during MTX therapy appears in a few weeks after the onset of the treatment (12, 24), but symptoms of the disease relapse soon after the drug has been discontinued. Therefore, the positive result is rather a consequence of antiinflammatory action of the drug than immunosuppressive activity (10). A good correlation between changes in disease activity and APPs serum levels established in the present study and other studies (22) confirm this statement. Positive effect on the outcome of RA after CTX administration has been observed (2, 8). It seems, that CTX induced remission is long lived. In opposite to MTX, CTX operates as an immunosuppressive agent especially in terms of lymphocyte reactivity (3, 4). APP changes reflected CTX induced clinical improvement. However, the risk/benefit ratio may make this treatment hazardous. CSA was recently introduced to the RA treatment. It selectively inhibits the production of cytokines regulating T-cell activation (5, 6, 23). Observed discrepancies between the clinical course of the disease and the acute phase response could be explained in two ways. Firstly, the low dose of CSA does not affect the gene transcription for increased production of APP. Secondly, the increaseed levels of APP and changes in glycosylation do not always reflect the intensity of inflammation.

Taken together, it seems that the mode of action of MTX depends on its antiinflammatory effect whereas CTX and CSA display mainly immunosuppressive effects. There is an agreement that the changes in APR are the best marker for monitoring RA. Essentially it is true. However, it seems that in some conditions, especially after immunosuppressive treatment, they failed. This is particularly true

in the CSA group (14). Although MTX belongs to the immunosuppressive drugs, it displays rather an antiinflammatory effect, mainly because of the very low doses. So, the good correlation between clinical improvement and APR in the MTX group rather confirms our conclusion than denies it.

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