# Apolipoprotein A-I and cholesterol in synovial fluid of patients with rheumatoid arthritis, psoriatic arthritis and osteoarthritis

F. Oliviero[1], P. Sfriso[1], G. Baldo[2], J.-M. Dayer[4], S. Giunco[2], A. Scanu[1], D. Bernardi[3], R. Ramonda[1], M. Plebani[3], L. Punzi[1]

*[1]Rheumatology Unit, Department of Clinical and Experimental Medicine, [2]Department of Medical and Surgical Sciences, and [3]Department of Laboratory Medicine, University of Padova, Italy; [4]Centre Médical Universitaire, Faculty of Medicine, University of Geneva, Switzerland.*

## Abstract

### Objective

*To investigate lipid and apolipoprotein (Apo) levels in synovial fluid (SF) and serum of patients with rheumatoid arthritis (RA), psoriatic arthritis (PsA) and osteoarthritis (OA).*

### Methods

*SF of 44 patients (14 RA, 14 PsA, 16 OA) was tested for Apo A-I, HDL-C, total cholesterol (TC), IL-1β, TNF-α, white blood cell count (WBC) and polymorphonucleate (PMN) percentage. Blood samples, collected simultaneously to the SF, were examined for Apo A-I, HDL-C, TC, TNF-α, serum amyloid A (SAA) and C-reactive protein (CRP). Thirty-three healthy donors served as a control group.*

### Results

*Serum levels of Apo A-I, HDL-C and TC were higher in OA as compared with RA, PsA and the control group. The patients with inflammatory arthritis had lower serum levels of Apo A-I and HDL-C than did the controls. Apo A-I concentrations were higher in SF of RA patients, while PsA showed the highest concentration of TC, though not reaching statistical significance. A negative correlation was found between serum Apo A-I and synovial WBC ($r=-0.48$ $p=0.002$) and IL-1β ($r=-0.42$ $p=0.016$). There was a strong positive correlation between the Apo A-I SF/serum ratio and synovial WBC ($r=0.73$ $p<0.001$), IL-1β ($r=0.68$ $p<0.001$) and a weak, yet significant, correlation with serum CRP ($r=0.49$ $p=0.002$) and SAA ($r=0.41$ $p=0.008$).*

### Conclusion

*Our study confirms that in RA Apo A-I and TC levels are decreased in plasma and increased in SF, thus suggesting infiltration of HDL particles in the inflamed joint with inhibition of the local production of proinflammatory cytokines. On the other hand, it can be hypothesized that the sequestration of Apo A-I in the inflamed tissue may, in part, account for the reduction of circulating HDL and the excess cardiovascular risk in RA and PsA patients.*

**Key words**

Apolipoproteins, synovial fluid, cytokines, inflammation, arthritis.

*Francesca Oliviero, PhD*
*Paolo Sfriso, MD, PhD*
*Goretta Baldo, MD*
*Jean-Michel Dayer, MD, PhD*
*Silvia Giunco, PhD*
*Anna Scanu, PhD*
*Daniela Bernardi, MD*
*Roberta Ramonda, MD, PhD*
*Mario Plebani, MD*
*Leonardo Punzi, MD, PhD*

*Please address correspondence and reprint requests to:*
*Leonardo Punzi, MD, PhD,*
*Rheumatology Unit, Department of Clinical and Experimental Medicine,*
*University of Padova,*
*Via Giustiniani 2,*
*35128 Padova, Italy.*
*E-mail: punzireu@unipd.it*

*Received on January 9, 2008; accepted in revised form on July 24, 2008.*



*Competing interests: none declared.*

## Introduction

Rheumatoid arthritis (RA) is associated with an increased risk of atherosclerosis and cardiovascular mortality (1-4). The factors mainly responsible for this risk have not been completely identified. Inflammation experienced in RA may contribute to accelerated atherosclerosis. Multiple alterations in lipid and lipoprotein metabolism occur during the acute-phase response (APR) of inflammation. Pro-inflammatory cytokines, in particular tumour necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6, may contribute to serum changes in atherogenic lipoproteins during APR (5). On the other hand, the production of TNF-α and IL-1β by monocyte-macrophages may in turn be decreased by HDL-apolipoprotein (Apo) A-I during T lymphocyte-monocyte contact (6). It is possible that these reciprocal effects are part of an attempt of the organism to protect the host from further injury. However, in chronic diseases such as RA, the long-term changes in structure and function of lipoproteins might contribute to atherogenesis. In keeping with such a hypothesis, it has been reported that the chronicity and severity of inflammatory process associated with RA may constitute an independent risk of atherosclerosis (7).

How lipoproteins and apolipoproteins relate to inflammatory stimuli at the sites of joint lesions and whether they reflect the local inflammation or result from systemic inflammation remain unknown. Normal synovial fluid (SF) contains low concentrations of lipoproteins and apolipoproteins, in contrast to those found in plasma. Although increased amounts of lipids and apolipoproteins have been found in RA SF (8), little is known about these substances in psoriatic arthritis (PsA) and osteoarthritis (OA) SF.

The aim of the present study was to investigate lipid and apolipoprotein levels in SF and serum of patients with RA, PsA and OA.

## Materials and methods

*Patients*

Forty-four patients with SF effusions were included in the study, 14 affected by RA, 14 by PsA and 16 by OA. All patients fulfilled established diagnostic criteria (9-11). All participants gave written informed consent. Patients with thyroid dysfunction, diabetes, renal disease, impaired hepatic function (aminotransferase levels >2 x upper normal limits) and history of alcohol abuse or smoking were excluded from the study. Subjects on lipid-lowering agents therapy, or who received oral steroids during the previous two weeks or intra-articular steroids during the previous three months, were also excluded. In patients with RA and PsA disease activity was measured using the Disease Activity Score based on the evaluation of 28 joints (DAS28) (12). Patients' characteristics are summarized in Table I.

Thirty-three healthy donors, comparable for age, gender and body mass index, served as a control group.

*Synovial fluid and serum samples*

SF from knee joints was examined for total and differential white blood cell (WBC) count; the surplus SF was centrifuged at 3000 g for 10 min and stored at -80° before being assayed. Blood samples were collected simultaneously to the SF aspiration.

*Quantification of Apo A-I, HDL-C and TC in serum and SF*

From each patient and control subject blood samples were taken in the morning after an overnight fast (12-14 hrs) in no-additive vacutainer tubes to obtain serum after low-speed centrifugation at 4°C. Fresh serum was tested for total cholesterol (TC) levels by standard enzymatic assay (Sigma Chemical). HDL cholesterol (HDL-C) was determined after precipitation of Apo B-containing lipoproteins with phosphotungstic acid (Boehringer-Mannheim). Serum levels of Apo A-I were determined by immunonephelemetric method on a Behring nephelometer (BNA) using specific antibodies and standards (DADE Behring, Germany).

Fresh SF was treated with hyaluronidase type IV-S from bovine testes (Sigma, USA) at a final concentration of 10μg/mL for 15 minutes at 37°C. After incubation, SF was centrifuged for 45' at 4°C and the supernatants concentrat-

**Table I.** Demographic and inflammatory characteristics of studied patients.

| | OA (n=16) | RA (n=14) | PsA (n=14) | HC (n=33) |
|---|---|---|---|---|
| Gender (female/male) | 11/5 | 8/6 | 6/8 | 19/14 |
| Mean age (years±SD) | 54.7 ± 11.5 | 53.5 ± 16.0 | 45.2 ± 14.9 | 51.9 ± 13.6 |
| Mean BMI (±SD) | 26.9 ± 4.1 | 26.3 ± 3.3 | 26.6 ± 4.3 | 25.9 ± 3.2 |
| Mean disease duration (years±SD) | 3.8 ± 7.1 | 6.8 ± 5.4 | 7.8 ± 7.2 | – |
| DAS28 | – | 3.8 ± 0.9 | 3.4 ± 1.3 | – |
| CRP (mg/L) | 3.7 (4.4) | 23.5 (32.2)* | 13.6 (18.0)* | 0.5 (0.7) |
| SAA (mg/L) | 2.8 (4.5) | 15.1 (42.2)* | 12.0 (22.4)* | 3.8 (2.9) |
| SF WBC (cells /mm$^3$) | 450 (450) | 11000 (12500)*** | 6100 (1775)** | |
| SF PMN (%) | 2.0 (3.5) | 62.6 (25.5)*** | 43.5 (62.2)*** | |
| SF IL-1β (pg/ml) | 5.0 (0.0) | 16.5 (26.7)* | 7.4 (11.1) | |
| SF TNF-α (pg/ml) | 14.3 (15.6) | 34.6 (20.2)* | 20.1 (37.4) | |

OA: osteoarthritis, RA: rheumatoid arthritis, PsA: psoriatic arthritis, HC: healthy controls, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, SAA: serum amyloid A.
Values are medians (IQR) unless otherwise stated.
*$p$<0.05 **$p$<0.01 ***$p$<0.001 *vs.* OA (Bonferroni test).

**Table II.** Serum levels of Apo A-I, TC and HDL-C of patients with osteoarthritis (OA), rheumatoid arthritis (RA), psoriatic arthritis (PsA) and healthy controls (HC).

| mg/dl | OA (n = 16) | PsA (n = 14) | RA (n = 14) | HC (n = 33) |
|---|---|---|---|---|
| Apo A-I | 170.0 (38.8) | 149.6 (47.9) | 142.5 (36.4)* | 166.0 (30.5) |
| TC | 239.7 (32.7) | 208.5 (75.7) | 202.5 (58.3)** | 191.0 (32.5) |
| HDL-C | 56.7 (30.3) | 43.1 (16.4) | 47.5 (20.4) | 59.0 (14.5) |

Values are medians (IQR). *$p$<0.05 **$p$<0.01 *vs.* OA (Bonferroni test).

ed utilizing Centricon YM-10 (Millipore, USA) at 4800 g, at 8°C, for 1 hour. Concentrated SF was tested for TC, HDL-C and Apo A-I as described above.

*Determination of cytokines*
Quantitative measurements of serum and SF TNF-α and SF IL-1β were performed by immunometric assays using specific murine monoclonal antibody (TNF-α DPC and IL-1β DPC, Medical Systems, Italy) on Immulite One Analyzer. Analytical sensitivity was 1.7 and 1.5 pg/ml for TNF-α and IL-1β, respectively.

*Determination of serum CRP and SAA*
C-reactive protein (CRP) levels were assessed by a highly sensitive immunonephelemetric method (DADE Behring, Italy) on BN II Analyzer. Serum amyloid A (SAA) levels were determined by means of particle-enhanced immunonephelometry using BN II System. The analytical sensitivity of the assays was 0.03 and 0.007 mg/l for CRP and SAA, respectively.

*Determination of total proteins*
Total protein concentrations in serum and SF were determined by colorimetric assay (Roche, Milano, Italy) on Hitachi 747 Analyzer.

*Statistics*
Statistical analysis was performed by SPSS software, version 14.0 for Windows (SPSS Inc, USA). Differences in levels of apolipoprotein, lipids and inflammatory indices between independent groups were calculated by ANOVA analysis followed by the Bonferroni post-test. Correlations between different variables were assessed with the Spearman rank correlation test. All values are expressed as the median and the interquartilic range (IQR). Patients' age and disease duration are reported as the mean ± standard deviation. A $p$-value <0.05 is considered significant.

## Results

*Serum levels of Apo A-I, TC and HDL-C in patients and controls*
Patients with OA showed the highest concentrations of Apo A-I and TC (Table II). Levels of Apo A-I were similar in RA and PsA. The analysis of variance revealed significant differences for Apo A-I ($p$=0.006), HDL-C ($p$=0.003) and TC ($p$=0.002) in RA, PsA, OA and healthy controls. Figure 1 shows medians and IR of the concentrations of Apo A-I, TC and HDL-C found in the four groups. The patients with inflammatory arthritis had lower serum levels of Apo A-I and HDL-C than did the controls.

*Synovial fluid levels of Apo A-I and TC*
Apo A-I concentrations were highest in SF of RA patients, while PsA showed the highest concentration of TC (Table III).The analysis of variance did not reveal any significant differences between groups except for the Apo A-I SF/serum ratio (Table III).

*Correlations*
SF Apo A-I and TC did not show any correlation with local and systemic inflammatory indices, whereas a negative correlation was found between serum Apo A-I and synovial WBC (r=-0.48 $p$=0.002) and IL-1β (r=-0.42 $p$=0.016). There was a strong positive correlation between the Apo A-I SF/serum ratio and synovial WBC (r=0.73 $p$<0.001), IL-1β (r=0.68 $p$<0.001) and a weak, yet significant, correlation with serum CRP (r=0.49 $p$=0.002) and SAA (r=0.41 $p$=0.008) (Table IV).

## Discussion

Patients affected by autoimmune rheumatic diseases have an increased predisposition to atherosclerosis as compared with OA patients and the general population (13-15).
Inflammation is an established risk factor for cardiovascular diseases in patients with RA, and lipoprotein abnormalities may also contribute to the increased risk. A pattern of dyslipoproteinaemia, similar to that seen in RA has been reported in PsA, which normalises with a reduction in disease activity.
Few data have been published to date on the SF to plasma concentration ratios for lipid and apolipoprotein in patients with RA, PsA and OA.

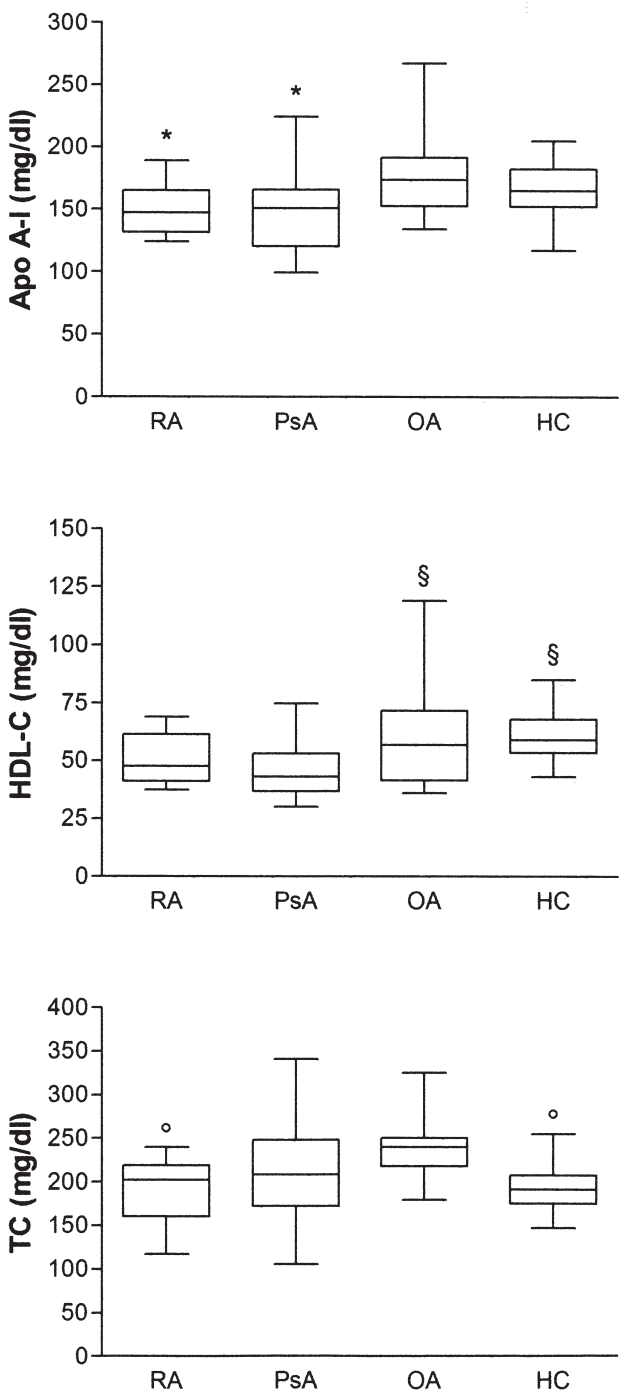


**Fig. 1.** Serum Apo A-I, HDL-C and TC levels in patients compared with healthy controls (HC). The boxes illustrate the 25th-75th percentiles, the horizontal lines the medians and the vertical lines the 10th and 90th percentiles. ANOVA showed the following significant differences: Apo A-I $p$=0.006, HDL-C $p$=0.003, TC $p$=0.002. Bonferroni post test: $^{*}p<0.05$ *vs.* OA; $^{\circ}p<0.01$ *vs.* OA; $^{\S}p<0.05$ *vs.* PsA.

Almost all proteins and solutes present in plasma can be found, at least qualitatively, in SF. However, the low permeability of the blood synovium barrier restricts the entrance of circulating plasma lipoproteins into the SF. Therefore, SF composition reflects the metabolic status of synovial tissue and offers a unique opportunity to study lipoproteins pattern at the site of inflammation.

In this study we confirm previous observations that in RA Apo A-I and total cholesterol levels are decreased in plasma and increased in SF, thus suggesting infiltration of HDL particles in the inflamed joint (16).

We extended these findings further by investigating the Apo A-I and total cholesterol in patients with PsA and found no statistically significant difference with controls, though PsA patients tended to have lower plasma and increased SF levels of these substances.

In addition we found a correlation between circulating Apo A-I and inflammatory markers in SF. SF WBC count, considered to be the most reliable index of SF inflammation correlated inversely with serum levels of Apo A-I, TC and HDL-C.

These associations suggest that substances produced during SF inflammation, mainly pro-inflammatory cytokines IL-1β, TNF-α and IL-6 may also mediate the production of lipoproteins, in analogy to other substances involved in APR (17). On the other hand, the correlation between SF IL-1β and Apo A-I SF/serum ratio may reflect the increased leakage at synovium membrane site due to local inflammation.

To our knowledge, this is the first study to investigate Apo A-I and TC levels in SF from patients with OA. We found higher plasma and decreased SF levels of these substances in OA with respect to RA and PsA. This may be explained by the relatively low degree of inflammation in OA. Therefore, the entrance of circulating plasma lipoproteins into the SF may be restricted by the low permeability of the intact blood synovium barrier. Alternatively, the higher lipoprotein levels might be due to growth factors produced by OA joints and involved in its pathogenesis (18).

It is known that Apo A-I and cytokines, in particular IL-1β and TNF-α, have many relevant reciprocal effects. The two cytokines, whether administered separately or together, have been shown to suppress hepatic Apo A-I mRNA and protein levels (19). In keeping with this effect, HDL-associated Apo A-I is considered a negative acute-phase protein (20). Furthermore, it is a specific inhibitor of cytokine production in monocyte-macrophages upon contact with stimulated T cells (6). So far, very few investigators have tested SF for Apo A-I. Ananth L *et al.* reported enhanced Apo A-I levels in SF of RA patients, even though they were 10 times lower in SF than in plasma (21). In our study, the Apo A-I levels in RA patients were only 3 times lower in SF than in serum. This may be explained by the different degree of the inflammation between the two cohorts of patients.

**Table III.** Synovial fluid levels of Apo A-I and TC, and SF/serum ratio of Apo A-I and TC in patients with osteoarthritis (OA), rheumatoid arthritis (RA) and psoriatic arthritis (PsA).

| mg/dl | OA (n=16) | PsA (n=14) | RA (n=14) |
|---|---|---|---|
| Apo A-I | 52.1 (21.0) | 55.0 (26.3) | 60.23 (12.1) |
| TC | 80.5 (35.5) | 94.5 (50.5) | 90.79 (37.8) |
| Apo A-I SF/serum | 0.29 (0.11) | 0.39 (0.14)* | 0.40 (0.09)** |
| TC SF/serum | 0.35 (0.21) | 0.47 (0.21) | 0.46 (0.11) |

Values are medians (IQR). *$p$<0.01 **$p$<0.001 *vs*. OA (Bonferroni test)

**Table IV.** Spearman correlation coefficients between serum, SF and SF/serum ratio of Apo A-I and TC.

| | | serum | | synovial fluid | |
|---|---|---|---|---|---|
| | | CRP | SAA | WBC | IL-1β |
| serum | Apo A-I | -0.171 | -0.142 | -0.447*** | -0.423* |
| | TC | -0.143 | -0.292 | -0.575**** | -0.413* |
| synovial fluid | Apo A-I | 0.309 | 0.255 | 0.325* | 0.258 |
| | TC | 0.227 | 0.171 | 0.203 | 0.252 |
| SF/serum ratio | Apo A-I | 0.489*** | 0.407*** | 0.733**** | 0.680**** |
| | TC | 0.263 | 0.314* | 0.530*** | 0.508*** |

*$p$<0.05, **$p$<0.02, ***$p$<0.01, ****$p$<0.001.

The lipid profile in PsA has rarely been investigated. Jones *et al*. found significantly reduced levels of HDL-C and its third subfraction HDL3, while LDL3 was significantly increased (22). Furthermore, they showed that patients with active synovitis had significantly lower total cholesterol, LDL cholesterol, and HDL3 cholesterol than their controls.

In our study, a similar trend to reduced serum TC and HDL-C was observed. In addition, we found high TC level in SF of patients with PsA though it did not reach statistical significance.

In conclusion, levels of Apo A-I and TC are increased in SF and decreased, along with HDL-C, in the serum of inflammatory arthropathies.

As reported by Bresnihan *et al*., the localization of Apo A-I in inflamed synovium suggests that it can locally inhibit the production of proinflammatory cytokines (16). On the other hand, it cannot be excluded that the sequestration of Apo A-I in the inflamed tissue due to the enhanced vascular permeability may account, at least in part, for the reduction of circulating HDL and the excess cardiovascular risk in RA and PsA patients.

## References


1. VAN DOORNUM S, McCOLL G, WICKS IP: Accelerated atherosclerosis: an extraarticular feature of rheumatoid arthritis? *Arthritis Rheum* 2002; 46: 862-73.
2. BACON PA, STEVENS RJ, CARRUTHERS DM *et al*.: Accelerated atherogenesis in autoimmune rheumatic diseases. *Autoimmun Rev* 2002; 1: 338-47.
3. GONZALEZ-JUANATEY C, LLORCA J, TESTA A *et al*.: Increased prevalence of severe subclinical atherosclerotic findings in long-term treated rheumatoid arthritis patients without clinically evident atherosclerotic disease. *Medicine* (Baltimore) 2003; 82: 407-13.
4. NAGATA-SAKURAI M, INABA M, GOTO H *et al*.: Inflammation and bone resorption as independent factors of accelerated arterial wall thickening in patients with rheumatoid arthritis. *Arthritis Rheum* 2003; 48: 3061-7.
5. KHOVIDHUNKIT W, KIM MS, MEMON RA *et al*.: Effects of infection and inflammation on lipid and lipoprotein metabolism: mechanisms and consequence to the host. *J Lip Res* 2004; 45: 1169-96.
6. HYKA N, DAYER J-M, MODOUX C *et al*.: Apolipoprotein (APO)-I inhibits the production of interleukin-1β and tumor necrosis factor-α by blocking contact-mediated activation of monocytes by T lymphocytes. *Blood* 2001; 97: 2381-9.
7. GAZI IF, BOUMPAS DT, MIKHAILIDIS DP *et al*.: Clustering of cardiovascular risk factors in rheumatoid arthritis: the rationale for using statins. *Clin Exp Rheumatol* 2007; 25: 102-11.
8. PRETE PE, GURAKAR-OSBORNE A, KASHYAP ML: Synovial fluid lipids and apolipoproteins: a contemporary perspective. *Biorheology* 1995; 32: 1-16.
9. ARNETT FC, EDWORTHY SM, BLOCH DA *et al*.: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; 31: 315-24.
10. MOLL JM, WRIGHT V: Psoriatic arthritis. *Semin Arthritis Rheum* 1973; 33: 55-78.
11. ALTMAN R, ASCH E, BLOCH D *et al*.: Development of criteria for the classification and reporting of osteoarthritis of the knee. *Arthritis Rheum* 1986; 29: 1039-49.
12. PREVOO ML, VAN 'T HOF MA, KUPER HH *et al*.: Modified disease activity scores that include twenty-eight-joint counts: development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum* 1995; 38: 44-8.
13. HAHN BH, GROSSMAN J, CHEN W *et al*.: The pathogenesis of atherosclerosis in autoimmune rheumatic diseases: roles of inflammation and dyslipidemia. *J Autoimmun* 2007; 28: 69-75.
14. DEL RINCON I, WILLIAMS K, STERN MP *et al*.: High incidence of cardiovascular events in a rheumatoid arthritis cohort not explained by traditional cardiac risk factors. *Arthritis Rheum* 2001; 44: 2737-45.
15. WALLBERG-JONSSON S, BACKMAN C, JOHNSON O *et al*.: Increased prevalence of atherosclerosis in patients with medium term rheumatoid arthritis. *J Rheumatol* 2001; 28: 2597-602.
16. BRESNIHAN B, GOGARTY M, FITZGERALD O, DAYER JM, BURGER D: Apolipoprotein A-I infiltration in rheumatoid arthritis synovial tissue: a control mechanism of cytokine production? *Arthritis Res Ther* 2004; 6: R563-6.
17. GABAY C, KUSHNER I: Acute phase proteins and other systemic responses to inflammation. *N Engl J Med* 1999; 340: 448-54.
18. PUNZI L, OLIVIERO F, PLEBANI M: New biochemical insights in the pathogenesis of osteoarthritis and role of laboratory investigations in clinical assessment. *Crit Rev Lab Science* 2005; 42: 279-309.
19. HARDARDOTTIR I, MOSER AH, MEMON R *et al*.: Effects of IL-1 and TNF, and the combination of both cytokines on cholesterol metabolism in Syrian hamsters. *Lymphokine Cytokine Res* 1994; 13: 161-6.
20. BURGER D, DAYER J-M: High-density lipoprotein-associated apolipoprotein A-I: the missing link between infection and chronic inflammation? *Autoimmunity Rev* 2002; 1: 111-7.
21. ANANTH L, PRETE PE, KASHYAP ML: Apolipoproteins A-I and B and cholesterol in synovial fluid of patients with rheumatoid arthritis. *Metabolism* 1993; 42: 803-6.
22. JONES SM, HARRIS CP, LLOYD J *et al*.: Lipoproteins and their subfractions in psoriatic arthritis: identification of an atherogenic profile with active joint disease. *Ann Rheum Dis* 2000; 59: 904-9.