

EXTENDED REPORT

Impaired serum cholesterol efflux capacity in rheumatoid arthritis and systemic lupus erythematosus

Nicoletta Ronda,¹ Elda Favari,¹ Maria Orietta Borghi,² Francesca Ingegnoli,³ Maria Gerosa,³ Cecilia Chighizola,³ Francesca Zimetti,¹ Maria Pia Adorni,¹ Franco Bernini,¹ Pier Luigi Meroni^{2,3}

Handling editor Tore K Kvien

► Additional material is published online only. To view please visit the journal online (<http://dx.doi.org/10.1136/annrheumdis-2012-202914>).

¹Department of Pharmacy, University of Parma, Parma, Italy

²Department of Clinical Sciences and Community Health, University of Milan & IRCCS Istituto Auxologico Italiano, Cusano Milanino, Italy

³Department of Clinical Sciences and Community Health, University of Milan & Division of Rheumatology, Istituto G. Pini, Milan, Italy

Correspondence to

Professor Pier Luigi Meroni, Division of Rheumatology, Istituto Gaetano Pini, P.zza Ferrari 1, Milan 20122, Italy; pierluigi.meroni@unimi.it

Accepted 17 March 2013

ABSTRACT

Objectives The marked cardiovascular risk in autoimmune diseases is only partly explained. The capacity of high-density lipoproteins (HDL) to promote cell cholesterol efflux is a property with a well-known anti-atherogenic significance, but is also involved in functional modulation of endothelial and immune cells. The aim of this work was to evaluate HDL functionality with respect to cell cholesterol efflux in rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) patients.

Methods We evaluated serum cholesterol efflux capacity (CEC) of apoB-depleted serum, which mainly reflects HDL activity, from 30 RA and 30 SLE patients, and from 30 healthy controls by radioisotopic ex-vivo systems discriminating between the specific pathways of cholesterol efflux.

Results RA patients presented impairment of ATP-binding cassette G1-mediated CEC that correlated with disease activity. SLE patients showed a more complex pattern of modifications unrelated to disease activity, with marked reduction of ATP-binding cassette G1-mediated CEC and impairment of ATP-binding cassette A1-mediated CEC. The relationship between specific pathways of CEC values and serum total HDL differed between groups and there was no relationship with autoantibody profile or current therapy.

Conclusions CEC is impaired in RA and SLE, with a specific mechanism pattern in each disease not depending on serum HDL levels. These findings provide a new mechanism for the increased atherosclerotic risk in RA and SLE patients.

Autoimmune rheumatic diseases (AIRD) are associated with accelerated atherosclerosis and an increased risk of cardiovascular morbidity and mortality,¹ not fully explained by traditional risk factors. Systemic inflammation and autoimmune reactions leading to monocyte and lymphocyte recruitment and activation, increased lipid deposition and augmented inflammation in vascular intima have been suggested to underlie accelerated atherosclerosis in AIRD. Indeed, the association between dyslipidaemia and cardiovascular risk in AIRD appears to be more complex than in the general population.² Lipid profiles are variable and modifications in composition and function of

lipoproteins have been extensively reported, especially in the case of high-density lipoproteins (HDL).^{3,4}

Atheroprotective properties of HDL are mediated by several mechanisms, including their activity as cell cholesterol acceptors⁵ and their anti-inflammatory and anti-oxidant effects,⁶ and depend not only on their circulating levels but also on their composition and functionality.⁷ Several studies have shown that anti-inflammatory and anti-oxidant properties of HDL are impaired in AIRD,^{4,8} but little is known on HDL functions with respect to their ability to promote cholesterol efflux from macrophages in these conditions.

Macrophage cholesterol efflux is the first step of reverse cholesterol transport, whereby excess cholesterol from peripheral cells is transported to the liver for excretion,⁵ and occurs through various pathways: aqueous diffusion (AD), scavenger receptor BI (SR-BI)-mediated efflux, ATP-binding cassette A1 (ABCA1)- and ATP-binding cassette G1 (ABCG1)-mediated efflux. Cholesterol transporter expression differs among cell types and species, but SR-BI, ABCA1 and ABCG1 are all expressed in human macrophages and foam cells (for further details see supplementary material, available online only). The function of HDL as cholesterol acceptor depends at least partly on their degree of maturity.^{9,10}

An efficient cell cholesterol efflux leads to the inhibition of foam cell formation and to regulatory effects on several cell functions: the interaction between HDL and SR-BI, ABCA1 and ABCG1 is followed by the activation of distinct intracellular signalling pathways both in macrophages and in endothelial cells, resulting in preservation of vessel health.^{11–15} Indeed, cholesterol efflux capacity (CEC) has recently been demonstrated to be inversely related to intima-media thickness and to arterial stiffness in healthy individuals, independently of serum HDL cholesterol levels.^{16,17}

On the other hand, the activation of the immune system itself may lead to modifications in cell cholesterol handling. For example, tumour necrosis factor α is able to reduce the expression of cholesterol transporters,¹⁸ myeloperoxidase may alter apoA-I and impair cholesterol efflux,^{19,20} the activation of Toll-like receptors increases cholesterol

To cite: Ronda N, Favari E, Borghi MO, et al. *Ann Rheum Dis* Published Online First: [please include Day Month Year] doi:10.1136/annrheumdis-2012-202914

Basic and translational research

uptake,²¹ and proliferative stimuli increase cholesterol synthesis and reduce cholesterol efflux.²²

In this work we asked the question whether disturbances of cholesterol trafficking occur in patients with autoimmune diseases and whether they are attributable to inflammation. We analysed serum CEC, that is the ability of a single serum to promote cell cholesterol efflux, mainly dependent on its content in HDL effectively functioning as cholesterol acceptors.²³ CEC can be measured with standardised methods, discriminating between the specific cholesterol efflux pathways.²⁴ We have thus verified whether serum CEC is altered in patients with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), and looked for the possible underlying mechanisms comparing the specific cholesterol efflux pathways relative to SR-BI, ABCG1, ABCA1 and AD. We have also compared RA and SLE CEC patterns looking for disease-specific modifications in HDL function as cholesterol acceptor.

MATERIALS AND METHODS

Patients and controls

Thirty patients with an established diagnosis of RA and 30 patients with an established diagnosis of SLE were recruited at the Department of Rheumatology, Istituto G. Pini, Milan. Diagnoses were in accordance with the American College of Rheumatology for SLE²⁵ and the American College of Rheumatology/European League Against Rheumatism for RA.²⁶ Thirty age and sex-matched healthy subjects were recruited on a voluntary basis. For further details about patients and controls and the timing of serum collection see the supplementary material (available online only). In particular, none of the patients or the controls were taking statins. Informed consent was obtained from the participants; the study fulfils the Helsinki criteria and was approved by the local ethical committee (Istituto Auxologico Italiano, Milan; project 20C101-2011/12-10-2010). Serum samples for the determination of lipid profile and CEC measurements were obtained and stored in aliquots at -80°C , a procedure that does not influence CEC values.²⁷

Patient data are reported in table 1. RA disease activity was evaluated as the disease activity score in 28 joints (DAS28) according to Anderson *et al.*²⁸ Lupus disease activity was scored according to the systemic lupus erythematosus disease activity index (SLEDAI).²⁹ None of the patients was under treatment with biological agents. Overall, the disease appeared under control with SLEDAI of 4 or less in all SLE patients except for two cases with 8, one with 14 and one with 16. Serum autoantibodies were measured as previously reported.³⁰

The age (mean \pm SE) of healthy controls was 42.8 ± 0.8 years and gender distribution (female/male) was 26/4; C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) (mean \pm SE) were 0.19 ± 0.03 and 7.53 ± 0.8 , respectively. The lipid profile of control subjects and of RA and SLE patients is reported in supplementary table S1 (available online only).

CEC measurement

Precipitation of serum apoB-containing lipoproteins was obtained as previously described.³¹ Details are given in the supplementary material (available online only). SR-BI, ABCG1 and ABCA1-specific contributions to cholesterol efflux and AD were measured as previously reported.^{23 32 33} Details are given in the supplementary material (available online only).

Statistical analysis

Statistical analyses were performed using Prism (V.5.0) (GraphPad Inc., San Diego, California, USA). Each experiment

Table 1 Clinical data of patients with RA and SLE

	RA N (%)	SLE N (%)
Female	26 (83.8)	29 (96.7)
Age (years, mean \pm SE)	47.1 \pm 2.4	45.8 \pm 2.2
ESR (mm 1st hour, mean \pm SE)	26.9 \pm 3.5	20.5 \pm 3.7
CRP (mg/dl, mean \pm SE)	2.5 \pm 0.9	0.36 \pm 0.2
SLEDAI (mean \pm SE)	–	2.6 \pm 4
DAS28 (mean \pm SE)	4.36 \pm 0.33	–
RF	21 (67.7)	2 (6.6)
ACPA	16 (51.6)	0
RF and ACPA	12 (38.7)	0
ANA	2 (6.4)	30 (100)
aPL	0	6 (20)
Anti-dsDNA antibody	0	15 (50)
Anti-ENA antibody	1 (3.2)	13 (43)
Hypertension	3 (6.4)	4 (13.2)
Diabetes	0	2 (6.6)
Smoking	1 (3.2)	2 (6.6)
Prednisone	24 (77.4)	27 (90)
Methotrexate	17	1 (3.3)
Hydroxychloroquine	11 (35.5)	23 (76.6)
Leflunomide	8	0
Azathioprine	0	6 (20)
Cyclophosphamide	0	2 (6.6)
Micophenolate	0	2 (6.6)
Aspirin	1 (3.2)	5 (16.6)

Hypertension if values greater than 140 mm Hg or 90 mm Hg for Pmax and Pmin, respectively.
ACPA, anti-citrullinated protein antibodies; ANA, antinuclear antibodies; aPL, anti-phospholipid antibodies; CRP, C-reactive protein; DAS28, disease activity score in 28 joints; dsDNA, double-stranded DNA; ENA, extractable nuclear antigens; ESR, erythrocyte sedimentation rate; RA, rheumatoid arthritis; RF, rheumatoid factor; SLE, systemic lupus erythematosus; SLEDAI, systemic lupus erythematosus disease activity index.

was run in triplicate and data were expressed as mean \pm SE. Specific pathway-mediated CEC values of the three groups were compared using one-way analysis of variance (ANOVA) and a post-hoc Tukey multiple comparison test. The same procedure was applied to compare the serum lipid profiles of the three groups. Correlation between specific pathway-mediated CEC values and disease activity scores or inflammation parameters in each group was evaluated with a bivariate two-tailed Pearson test. The relationship between specific pathway-mediated CEC values and total serum HDL levels was evaluated by linear regression.

RESULTS

Patient and control serum CEC

SR-BI-mediated CEC

Significant differences in SR-BI-mediated CEC were found comparing the three groups studied (ANOVA $F=6.95$, $p<0.005$). RA SR-BI-mediated CEC (mean \pm SEM 2.78 ± 0.17) was not significantly different from that of control subjects (mean \pm SEM 3.42 ± 0.24). SLE SR-BI-mediated CEC did not differ from that of controls, but was significantly higher than that of RA patients (mean \pm SEM 3.84 ± 0.19 , $p<0.01$) (figure 1A).

ABCG1-mediated CEC

Significant differences in ABCG1-mediated CEC were found comparing the three groups studied (ANOVA $F=23.95$, $p<0.0001$). ABCG1-mediated CEC was significantly impaired

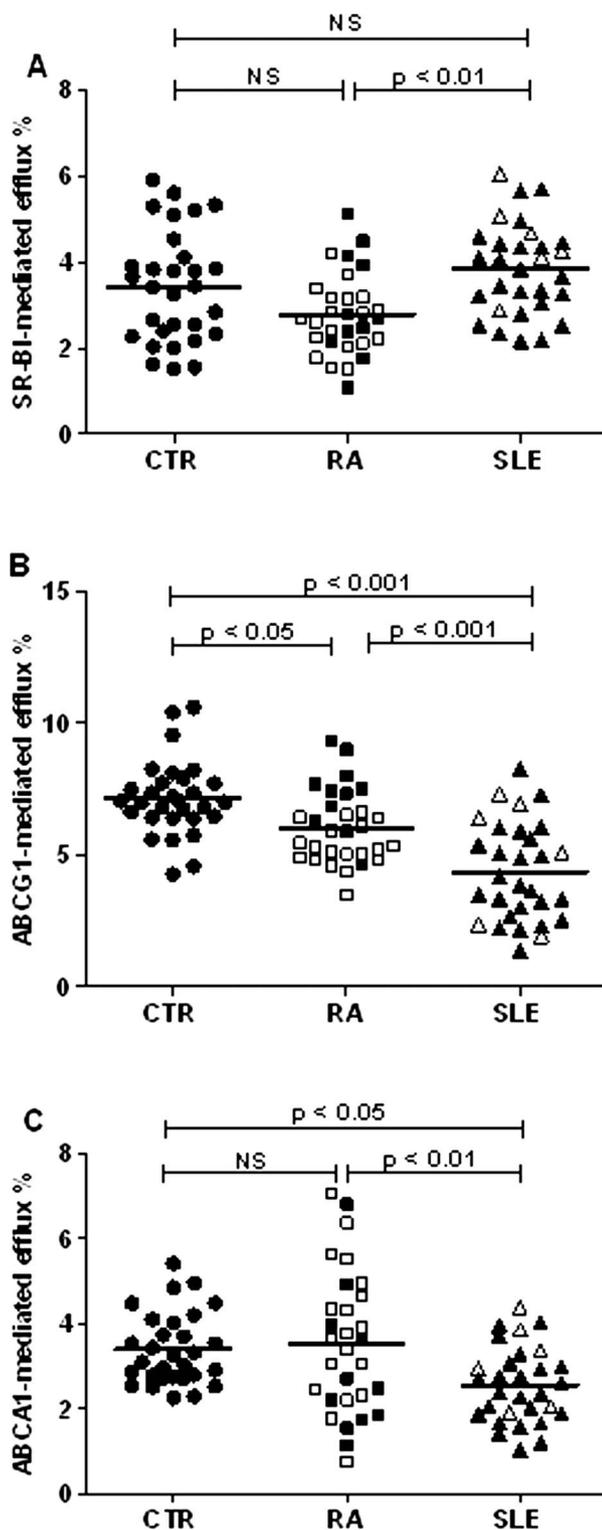


Figure 1 Patient and control serum scavenger receptor BI (SR-BI), ATP-binding cassette G1 (ABCG1) and ATP-binding cassette A1 (ABCA1)-mediated cholesterol efflux capacity (CEC) are shown in panels A, B and C, respectively. Values are expressed as the percentage cholesterol efflux of total cell cholesterol. CEC values relative to rheumatoid arthritis (RA) patients with disease activity score in 28 joints lower or higher than 2.6 or relative to systemic lupus erythematosus (SLE) patients with systemic lupus erythematosus disease activity index lower or higher than 7 have been represented with black or white symbols, respectively. Statistical significance is relative to the post-hoc Tukey multiple comparison test between groups. NS, not significant.

in both patient groups compared to that of controls (mean \pm SEM 7.14 ± 0.26), being reduced by 15% in RA (mean \pm SEM 6.04 ± 0.25 , $p < 0.05$) and by almost 40% in SLE (mean \pm SEM 4.36 ± 0.34 , $p < 0.001$). Comparing ABCG1-mediated CEC of the two patient groups, a significant difference was found ($p < 0.001$) (figure 1B).

ABCA1-mediated CEC

Significant differences in ABCA1-mediated CEC were found comparing the three groups studied (ANOVA $F = 5.90$, $p < 0.01$). RA ABCA1-mediated CEC (mean \pm SEM 3.54 ± 0.31) was not significantly different from that of control subjects (mean \pm SEM 3.39 ± 0.15). ABCA1-mediated CEC was significantly reduced in SLE compared both to controls and to RA (mean \pm SEM 2.56 ± 0.16 , $p < 0.05$ and $p < 0.01$ vs controls and RA patients, respectively) (figure 1C).

AD-mediated CEC

No significant differences in AD-mediated CEC were found comparing the three groups studied (mean \pm SE 5.15 ± 0.19 , 5.5 ± 0.30 and 5.4 ± 0.24 for control subjects, RA patients and SLE patients, respectively, $F = 0.56$, not significant (NS)).

Serum SR-BI, ABCG1 and ABCA1-mediated CEC/serum HDL ratios

The SR-BI-mediated CEC/serum HDL ratio of both RA and SLE patients was significantly lower than that of controls ($p < 0.05$) (see supplementary figure S1, panel A, available online only). The ABCG1-mediated CEC/serum HDL ratio of both RA and SLE patients was significantly lower than that of controls ($p < 0.05$ and $p < 0.001$, respectively) (see supplementary figure S1, panel B, available online only); the difference between the values of the two patient groups was not statistically significant (see supplementary figure S1, panel B, available online only). The ABCA1-mediated CEC/serum HDL ratio did not differ between RA and controls (see supplementary figure S1, panel C, available online only); values relative to SLE were significantly lower than those of controls and RA ($p < 0.001$ and $p < 0.01$, respectively) (see supplementary figure S1, panel C, available online only).

The results did not change if data analysis was repeated excluding patients with a smoking habit, diabetes or hypertension (see supplementary material, available online only).

Correlation between CEC, disease activity scores and autoantibodies

A significant inverse correlation was found between ABCG1-mediated CEC values and DAS28 in RA patients (Pearson $r = -0.468$, $p < 0.01$) (figure 2).

No correlation was found between any other specific pathway-mediated CEC and DAS28 in RA patients nor between any specific pathway-mediated CEC and the activity score SLEDAI in SLE patients. No correlation was found between any of the specific pathway-mediated CEC and CRP nor ESR. No differences were found comparing specific pathway-mediated CEC of subgroups of patients, divided according to the serum positivity or negativity for rheumatoid factor or anti-citrullinated protein antibodies in the case of RA, and for anti-DNA and anti-extractable nuclear antigen antibodies in the case of SLE. No correlation was found between the specific pathway-mediated CEC and any of the pharmacological treatments in RA and SLE (data not shown).

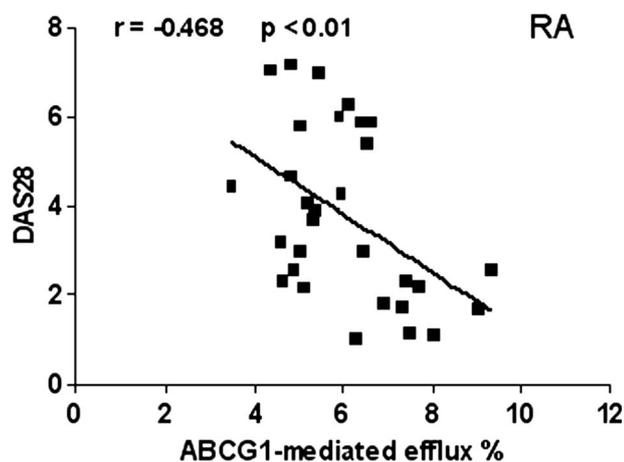


Figure 2 Inverse correlation between ATP-binding cassette G1 (ABCG1)-mediated cholesterol efflux capacity (CEC) values and disease activity score in 28 joints (DAS28) in rheumatoid arthritis (RA) patients. CEC is expressed as the percentage cholesterol efflux of total cell cholesterol.

Relationship between specific pathway-mediated CEC and total serum HDL in patients and controls

A positive linear regression between SR-BI-mediated CEC and total serum HDL was found in all groups, but was stronger in SLE patients (controls: regression coefficient 4.54 ± 1.73 , $p < 0.05$, $r^2 = 0.197$; RA patients: regression coefficient 9.35 ± 3.40 , $p < 0.05$, $r^2 = 0.213$; SLE patients: regression coefficient 16.12 ± 1.79 , $p < 0.0001$, $r^2 = 0.742$) (figure 3, left panels).

A weak positive linear regression between ABCG1-mediated CEC and total serum HDL was found in controls (regression coefficient 3.07 ± 1.63 , $p < 0.05$, $r^2 = 0.156$) and in SLE patients (regression coefficient 4.21 ± 1.84 , $p < 0.05$, $r^2 = 0.158$), but not in the RA group (regression coefficient 4.79 ± 2.43 , NS, $r^2 = 0.122$) (figure 3, right panels).

No significant linear regression was found between ABCA1-mediated efflux and total serum HDL in patients and in controls (controls: regression coefficient -3.40 ± 2.37 , NS, $r^2 = 0.068$; RA patients: regression coefficient -1.78 ± 2.13 , NS, $r^2 = 0.024$; SLE patients: regression coefficient 6.40 ± 4.04 , NS, $r^2 = 0.082$).

Relationship between ABCG1-mediated and SR-BI-mediated CEC

Significant differences in the sum and ratio of ABCG1 and SR-BI-mediated CEC were found comparing the three groups studied (sum: ANOVA $F = 10.23$, $p < 0.001$; ratio: ANOVA $F = 21.45$, $p < 0.001$). A significant deficit in the cumulative contribution (sum) of ABCG1-mediated and SR-BI-mediated CEC was found in RA and in SLE patients with respect to controls ($p < 0.01$ and $p < 0.001$, respectively), while the ratio between the two pathway-mediated CEC was significantly altered in the SLE group only ($p < 0.001$ vs both controls and RA) (table 2).

DISCUSSION

This study shows for the first time that the capacity of serum to promote cell cholesterol efflux is differentially impaired in RA and SLE patients, providing new insights into the mechanisms of accelerated atherosclerosis in these autoimmune diseases. Our data on apoB-depleted serum CEC, mainly reflecting HDL activity as cholesterol acceptor,³⁴ indicate that HDL dysfunction in autoimmune patients, in addition to the known impairment of their anti-inflammatory properties,^{4–8} also involves their capacity to counteract cell cholesterol

accumulation. This finding might have a great impact because HDL promotion of cell cholesterol efflux not only opposes lipid deposition in vessels, but is also crucial for macrophage and endothelial cell inflammatory and immune function modulation.¹¹ Moreover, the data presented strengthen the emerging concept that the simple evaluation of the serum lipid profile does not provide information on the functional characteristics of circulating lipoproteins, especially in selected patient populations.

RA patients showed a reduction of ABCG1-mediated CEC with respect to control subjects, and CEC values were inversely correlated with disease activity. These findings may be attributed to a selective alteration of the circulating population of HDL specific for ABCG1-mediated efflux⁹ occurring in association with disease activation. Our work adds complementarily to a previous report of a reduced capacity of HDL isolated from RA patients with active disease to induce total efflux from macrophages pre-loaded with cholesterol,³⁵ as we describe the specific pathway-mediated CEC from apoB-depleted RA serum in cells not pre-loaded with cholesterol. Our finding is in line with the profound modifications of HDL composition, especially with respect to protein content, occurring in patients with active RA, which have been detailed in a recent work³ and include increased amounts of serum amyloid A, apo J, fibrinogen, haptoglobin and reduced paraoxonase-1. However, the precise nature of HDL alteration leading to impairment of ABCG1-mediated CEC in RA is worth investigating. The significant inverse correlation between ABCG1-mediated CEC and disease activity in RA can be interpreted as the impact of inflammation and autoimmunity on function rather than serum levels of HDL, as in our patients the total serum HDL concentration did not differ from that of our control subjects, and ABCG1-mediated CEC did not correlate with serum HDL levels. Although we did not find any correlation with acute phase proteins (ie, CRP) we cannot exclude the possibility that inflammatory mediators, cytokines in particular, may themselves play a role in affecting CEC results in RA patients. On the other hand, as ABCG1 is the cholesterol transporter most implicated in macrophage and endothelial cell anti-inflammatory phenotype maintenance through cholesterol and 7-ketocholesterol release,^{36–38} it can be speculated that, taking into account the extension of the vessel tree, the reduced ABCG1-mediated CEC itself might contribute to vessel inflammation and immune reaction promotion in RA patients.

In SLE patients, ABCG1 and ABCA1-mediated CEC were reduced compared to both controls and RA patients, despite the fact that the disease was clinically under control in the large majority of SLE patients. This finding is consistent with a possible reduction/dysfunction of the small HDL populations specific for these transporters⁹ and with a shift to larger HDL, typical acceptors for SR-BI-presented cholesterol, supported also by the much stronger correlation between SR-BI-mediated CEC and total serum HDL levels that we found in SLE compared to controls and RA patients. Indeed, HDL structural alterations described in SLE include size increase.^{39–40} So, while a similar deficit in the cumulative contribution of ABCG1 and SR-BI-mediated CEC was found in RA and SLE with respect to controls, the profoundly altered ratio between these two pathway-mediated CEC in SLE further supports a complex derangement in the HDL functional pattern specific for this disease, even in presence of normal serum HDL levels. Decreased circulating HDL levels have often been reported, although not unanimously, in SLE patients with active disease. The fact that in the large majority of our SLE patients the disease was clinically under control might explain the finding of their normal

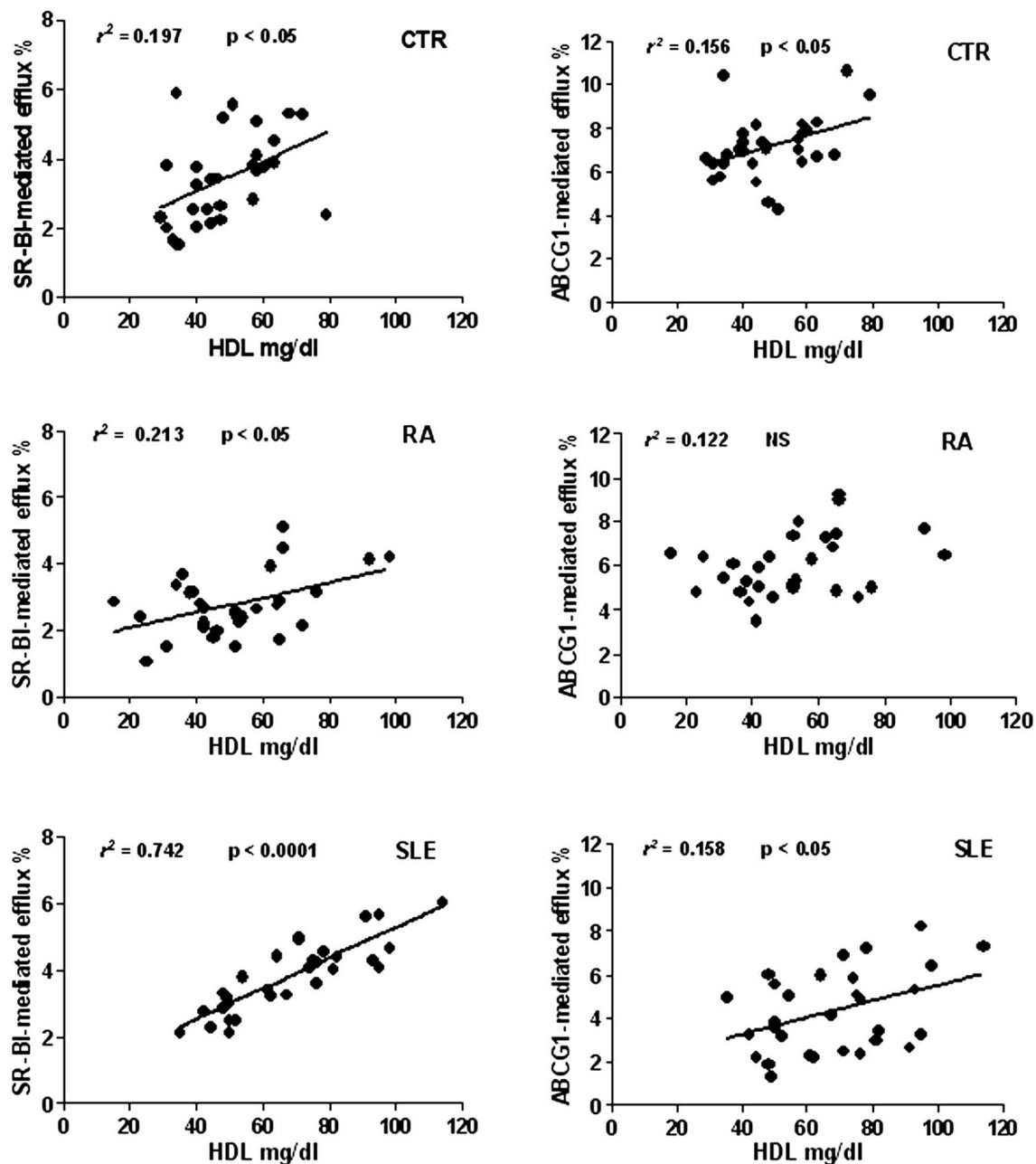


Figure 3 Results of linear regression analysis between total serum high-density lipoproteins (HDL) and scavenger receptor BI (SR-BI) or ATP-binding cassette G1 (ABCG1)-mediated cholesterol efflux capacity (CEC) in patients and controls (CTR). r^2 values and significance of the regression coefficient are given in each graph. In the case of SR-BI-mediated CEC (left panels) a positive linear regression is present in all groups, but is much stronger in systemic lupus erythematosus (SLE) patients. In the case of ABCG1-mediated CEC (right panels) a positive linear regression is present in control subjects and in SLE patients, but not in rheumatoid arthritis (RA) patients. NS, not significant.

HDL serum levels, which were higher than those of RA patients and of our control subjects, selected for having relatively low serum total cholesterol and consequently possibly restrained HDL.

The concurrent impairment of ABCG1 and ABCA1-mediated CEC in SLE might have a great impact on foam cell formation and on inflammatory activation of macrophages and endothelial

Table 2 Sum and ratio of ABCG1 and SR-BI-mediated CEC in patients and controls

	CTR	RA	SLE
ABCG1+SRBI (mean±SEM)	10.56±0.37** vs RA *** vs SLE	8.74±0.33	8.20±0.45
ABCG1/SRBI (mean±SEM)	2.40±0.17*** vs SLE	2.49±0.19*** vs SLE	1.18±0.09

* $p < 0.005$; ** $p < 0.001$; *** $p < 0.0001$.

ABCG1, ATP-binding cassette G1; CEC, cholesterol efflux capacity; CTR, control group; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SR-BI, scavenger receptor BI.

cells.^{11 14} Furthermore, CEC impairment might modulate immune functions critical for disease pathogenesis, such those depending on T cells.¹⁴ Interestingly, a very recent report describes the modulating effect of ABCA1-mediated cholesterol efflux on T-cell function, possibly through lipid raft rearrangement and subsequent intracellular signalling modulation.⁴¹

ApoB-depleted serum CEC is a parameter reflecting the overall HDL function in terms of cholesterol acceptance from cells, as the result of both the quality and quantity of circulating HDL particles. However, after normalisation of CEC values for serum HDL levels, the differences between patients and controls were the same as those found with not normalised values, indicating that our serum CEC results do not merely reflect total serum HDL levels. On the contrary, a modest impairment of normalised SR-BI-mediated CEC emerged in both RA and SLE patients compared to controls; this finding points to a functional disturbance in the SR-BI-specific HDL that is offset by their circulating amount, especially in SLE.

None of the alterations of the specific pathway-mediated CEC that we have shown in RA and SLE were correlated to the ESR or to the CRP values alone. This finding together with the marked CEC alterations found in SLE patients, in which disease clinical manifestations were substantially controlled by therapy (only two patients had a SLEDAI score >8), suggest that HDL function modifications with respect to cholesterol metabolism in autoimmune diseases are not simply linked to an active inflammatory status. It also stands against the possibility that serum cytokines, as the major inflammatory mediators, play a key role in affecting CEC. It can be speculated that specific mediators of immune responses or auto-antibodies⁴² or even genetic factors might influence HDL functions, and that these yet unknown mechanisms are effective underneath therapy-induced clinical disease control.

A limitation of the present study is its cross-sectional design, which does not allow conclusions to be drawn on causality with respect to the relationship between inflammation or autoimmunity and CEC derangement, which should be the object of future longitudinal studies.

In conclusion, the impairment of serum CEC in RA and SLE patients that we have reported provides a new mechanism for the increased atherosclerotic risk in these patients, and supports the development of future studies to clarify the relationship between cell cholesterol metabolism derangement and autoimmune disease mechanisms.

Contributors All authors have given a substantial contribution to the conception and design, or analysis and interpretation of data, have drafted the article or revised it critically for important intellectual content, and have given final approval of the version to be published. There is no one else who fulfils the criteria for authorship but has not been included as an author.

Funding The authors declare financial support for the submitted work from the Gruppo Italiano per la Lotta contro il Lupus Eritematoso Sistemico and by Ricerca Corrente, IRCCS Istituto Auxologico Italiano 2011. CC has received a research grant from the University of Milan and Dote Ricerca: FSE, Regione Lombardia.

Competing interests None.

Ethics approval The study fulfils the Helsinki criteria and was approved by the local ethical committee (Istituto Auxologico Italiano, Milan; project 20C101-2011/12-10-2010).

Patient consent Obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

- Shoenfeld Y, Gerli R, Doria A, *et al.* Accelerated atherosclerosis in autoimmune rheumatic diseases. *Circulation* 2005;112:3337–47.
- Symmons DP, Gabriel SE. Epidemiology of CVD in rheumatic disease, with a focus on RA and SLE. *Nat Rev Rheumatol* 2011;7:399–408.
- Watanabe J, Charles-Schoeman C, Miao Y, *et al.* Proteomic profiling following immunoaffinity capture of high-density lipoprotein: association of acute-phase proteins and complement factors with proinflammatory high-density lipoprotein in rheumatoid arthritis. *Arthritis Rheum* 2012;64:1828–37.
- McMahon M, Grossman J, FitzGerald J, *et al.* Proinflammatory high-density lipoprotein as a biomarker for atherosclerosis in patients with systemic lupus erythematosus and rheumatoid arthritis. *Arthritis Rheum* 2006;54:2541–9.
- Cuchel M, Rader DJ. Macrophage reverse cholesterol transport: key to the regression of atherosclerosis? *Circulation* 2006;113:2548–55.
- Ansell BJ, Navab M, Hama S, *et al.* Inflammatory/antiinflammatory properties of high-density lipoprotein distinguish patients from control subjects better than high-density lipoprotein cholesterol levels and are favorably affected by simvastatin treatment. *Circulation* 2003;108:2751–6.
- Duffy D, Rader DJ. Update on strategies to increase HDL quantity and function. *Nat Rev Cardiol* 2009;6:455–63.
- Charakida M, Besler C, Batuca JR, *et al.* Vascular abnormalities, paraoxonase activity, and dysfunctional HDL in primary antiphospholipid syndrome. *JAMA* 2009;302:1210–17.
- Favari E, Calabresi L, Adorni MP, *et al.* Small discoidal pre-beta1 HDL particles are efficient acceptors of cell cholesterol via ABCA1 and ABCG1. *Biochemistry* 2009;48:11067–74.
- Rosenson RS, Brewer HB Jr, Davidson WS, *et al.* Cholesterol efflux and atheroprotection: advancing the concept of reverse cholesterol transport. *Circulation* 2012;125:1905–19.
- Prosser HC, Ng MK, Bursill CA. The role of cholesterol efflux in mechanisms of endothelial protection by HDL. *Curr Opin Lipidol* 2012;23:182–9.
- Yvan-Charvet L, Welch C, Pagler TA, *et al.* Increased inflammatory gene expression in ABC transporter-deficient macrophages: free cholesterol accumulation, increased signaling via toll-like receptors, and neutrophil infiltration of atherosclerotic lesions. *Circulation* 2008;118:1837–47.
- Ye D, Lammers B, Zhao Y, *et al.* ATP-binding cassette transporters A1 and G1, HDL metabolism, cholesterol efflux, and inflammation: important targets for the treatment of atherosclerosis. *Curr Drug Targets* 2011;12:647–60.
- Yvan-Charvet L, Wang N, Tall AR. Role of HDL, ABCA1, and ABCG1 transporters in cholesterol efflux and immune responses. *Arterioscler Thromb Vasc Biol* 2010;30:139–43.
- Terasaka N, Westertep M, Koetsveld J, *et al.* ATP-binding cassette transporter G1 and high-density lipoprotein promote endothelial NO synthesis through a decrease in the interaction of caveolin-1 and endothelial NO synthase. *Arterioscler Thromb Vasc Biol* 2010;30:2219–25.
- Khera AV, Cuchel M, de la Llera-Moya M, *et al.* Cholesterol efflux capacity, high-density lipoprotein function, and atherosclerosis. *N Engl J Med* 2011;364:127–35.
- Favari E, Ronda N, Adorni M, *et al.* ABCA1-dependent serum cholesterol efflux capacity inversely correlates with pulse wave velocity in healthy subjects. *J Lipid Res* 2013;54:238–43.
- Zarubica A, Trompieri D, Chimini G. ABCA1, from pathology to membrane function. *Pflugers Arch* 2007;453:569–79.
- Shao B, Bergt C, Fu X, *et al.* Tyrosine 192 in apolipoprotein A-I is the major site of nitration and chlorination by myeloperoxidase, but only chlorination markedly impairs ABCA1-dependent cholesterol transport. *J Biol Chem* 2005;280:5983–93.
- Zheng L, Settle M, Brubaker G, *et al.* Localization of nitration and chlorination sites on apolipoprotein A-I catalyzed by myeloperoxidase in human atheroma and associated oxidative impairment in ABCA1-dependent cholesterol efflux from macrophages. *J Biol Chem* 2005;280:38–47.
- Miller YI, Choi SH, Fang L, *et al.* Toll-like receptor-4 and lipoprotein accumulation in macrophages. *Trends Cardiovasc Med* 2009;19:227–32.
- Yvan-Charvet L, Kling J, Pagler T, *et al.* Cholesterol efflux potential and antiinflammatory properties of high-density lipoprotein after treatment with niacin or anacetrapib. *Arterioscler Thromb Vasc Biol* 2010;30:1430–8.
- Zanotti I, Favari E, Bernini F. Cellular cholesterol efflux pathways: impact on intracellular lipid trafficking and methodological considerations. *Curr Pharm Biotechnol* 2012;13:292–302.
- Calabresi L, Favari E, Molieri E, *et al.* Functional LCAT is not required for macrophage cholesterol efflux to human serum. *Atherosclerosis* 2009;204:141–6.
- Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997;40:1725.
- Aletaha D, Neogi T, Silman AJ, *et al.* Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann Rheum Dis* 2010;69:1580–8.
- de la Llera-Moya M, Drazul-Schrader D, Asztalos BF, *et al.* The ability to promote efflux via ABCA1 determines the capacity of serum specimens with similar high-density lipoprotein cholesterol to remove cholesterol from macrophages. *Arterioscler Thromb Vasc Biol* 2010;30:796–801.
- Anderson JK, Zimmerman L, Caplan L, *et al.* Measures of rheumatoid arthritis disease activity: Patient (PtGA) and Provider (PrGA) Global Assessment of Disease

- Activity, Disease Activity Score (DAS) and Disease Activity Score With 28-Joint Counts (DAS28), Simplified Disease Activity Index (SDAI), Clinical Disease Activity Index (CDAI), Patient Activity Score (PAS) and Patient Activity Score-II (PASII), Routine Assessment of Patient Index Data (RAPID), Rheumatoid Arthritis Disease Activity Index (RADAI) and Rheumatoid Arthritis Disease Activity Index-5 (RADAI-5), Chronic Arthritis Systemic Index (CASI), Patient-Based Disease Activity Score With ESR (PDAS1) and Patient-Based Disease Activity Score Without ESR (PDAS2), and Mean Overall Index for Rheumatoid Arthritis (MOI-RA). *Arthritis Care Res* 2011;63: S14–36.
- 29 Petri M, Kim MY, Kalunian KC, *et al*. Combined oral contraceptives in women with systemic lupus erythematosus. *N Engl J Med* 2005;353:2550–8.
- 30 Gerosa M, Cimaz R, Stramba-Badiale M, *et al*. Electrocardiographic abnormalities in infants born from mothers with autoimmune diseases—a multicentre prospective study. *Rheumatology* 2007;46:1285–9.
- 31 Asztalos BF, de la Llera-Moya M, Dallal GE, *et al*. Differential effects of HDL subpopulations on cellular ABCA1- and SR-BI-mediated cholesterol efflux. *J Lipid Res* 2005;46:2246–53.
- 32 Yancey PG, Bortnick AE, Kellner-Weibel G, *et al*. Importance of different pathways of cellular cholesterol efflux. *Arterioscler Thromb Vasc Biol* 2003;23:712–19.
- 33 Rothblat GH, de la Llera-Moya M, Favari E, *et al*. Cellular cholesterol flux studies: methodological considerations. *Atherosclerosis* 2002;163:1–8.
- 34 Pisciotta L, Favari E, Magnolo L, *et al*. Characterization of three kindreds with familial combined hypolipidemia caused by loss-of-function mutations of ANGPTL3. *Circ Cardiovasc Genet* 2012;5:42–50.
- 35 Charles-Schoeman C, Lee YY, Grijalva V, *et al*. Cholesterol efflux by high density lipoproteins is impaired in patients with active rheumatoid arthritis. *Ann Rheum Dis* 2012;71:1157–62.
- 36 Terasaka N, Wang N, Yvan-Charvet L, *et al*. High-density lipoprotein protects macrophages from oxidized low-density lipoprotein-induced apoptosis by promoting efflux of 7-ketocholesterol via ABCG1. *Proc Natl Acad Sci U S A* 2007;104:15093–8.
- 37 Hassan HH, Denis M, Krimbou L, *et al*. Cellular cholesterol homeostasis in vascular endothelial cells. *Can J Cardiol* 2006;22:35B–40B.
- 38 O'Connell BJ, Denis M, Genest J. Cellular physiology of cholesterol efflux in vascular endothelial cells. *Circulation* 2004;110:2881–8.
- 39 Hua X, Su J, Svenungsson E, *et al*. Dyslipidaemia and lipoprotein pattern in systemic lupus erythematosus (SLE) and SLE-related cardiovascular disease. *Scand J Rheumatol* 2009;38:184–9.
- 40 Juarez-Rojas J, Medina-Urrutia A, Posadas-Sanchez R, *et al*. High-density lipoproteins are abnormal in young women with uncomplicated systemic lupus erythematosus. *Lupus* 2008;17:981–7.
- 41 Jiang H, Badralmaa Y, Yang J, *et al*. Retinoic acid and liver X receptor agonist synergistically inhibit HIV infection in CD4+ T cells by up-regulating ABCA1-mediated cholesterol efflux. *Lipids Health Dis* 2012;11:69.
- 42 O'Neill SG, Giles I, Lambrianides A, *et al*. Antibodies to apolipoprotein A-I, high-density lipoprotein, and C-reactive protein are associated with disease activity in patients with systemic lupus erythematosus. *Arthritis Rheum* 2010; 62:845–54.



Impaired serum cholesterol efflux capacity in rheumatoid arthritis and systemic lupus erythematosus

Nicoletta Ronda, Elda Favari, Maria Orietta Borghi, et al.

Ann Rheum Dis published online April 5, 2013
doi: 10.1136/annrheumdis-2012-202914

Updated information and services can be found at:
<http://ard.bmj.com/content/early/2013/04/04/annrheumdis-2012-202914.full.html>

These include:

Data Supplement

"Supplementary Data"

<http://ard.bmj.com/content/suppl/2013/04/04/annrheumdis-2012-202914.DC1.html>

References

This article cites 42 articles, 21 of which can be accessed free at:

<http://ard.bmj.com/content/early/2013/04/04/annrheumdis-2012-202914.full.html#ref-list-1>

P<P

Published online April 5, 2013 in advance of the print journal.

Email alerting service

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Advance online articles have been peer reviewed, accepted for publication, edited and typeset, but have not yet appeared in the paper journal. Advance online articles are citable and establish publication priority; they are indexed by PubMed from initial publication. Citations to Advance online articles must include the digital object identifier (DOIs) and date of initial publication.

To request permissions go to:

<http://group.bmj.com/group/rights-licensing/permissions>

To order reprints go to:

<http://journals.bmj.com/cgi/reprintform>

To subscribe to BMJ go to:

<http://group.bmj.com/subscribe/>

Topic Collections

Articles on similar topics can be found in the following collections

[Immunology \(including allergy\)](#) (3754 articles)
[Connective tissue disease](#) (3174 articles)
[Degenerative joint disease](#) (3434 articles)
[Musculoskeletal syndromes](#) (3684 articles)
[Rheumatoid arthritis](#) (2403 articles)
[Systemic lupus erythematosus](#) (446 articles)

Notes

Advance online articles have been peer reviewed, accepted for publication, edited and typeset, but have not yet appeared in the paper journal. Advance online articles are citable and establish publication priority; they are indexed by PubMed from initial publication. Citations to Advance online articles must include the digital object identifier (DOIs) and date of initial publication.

To request permissions go to:

<http://group.bmj.com/group/rights-licensing/permissions>

To order reprints go to:

<http://journals.bmj.com/cgi/reprintform>

To subscribe to BMJ go to:

<http://group.bmj.com/subscribe/>