Association of soluble apoptotic markers with impaired left ventricular deformation in patients with rheumatoid arthritis. Effects of inhibition of interleukin-1 activity by anakinra

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Summary
Myocardial function is impaired in rheumatoid arthritis (RA). Inhibition of interleukin (IL)-1 activity reduces experimental myocardial infarction by limiting apoptosis. We investigated whether a) soluble apoptotic markers are related with impaired left ventricular (LV) performance and b) treatment with anakinra, an IL-1 receptor antagonist, reduces apoptotic markers leading to improved LV performance in RA. We studied 46 RA patients. In an acute, double-blind cross-over trial, 23 patients were randomised to a single injection of anakinra or placebo and after 48 hours (h) to the alternative treatment. In a chronic trial, 23 patients who received anakinra for 30 days were compared with 23 patients who received prednisolone. At baseline, increased apoptotic markers were related with reduced myocardial deformation in patients with rheumatoid arthritis. Effects of inhibition of IL-1 activity by anakinra reduces apoptotic markers leading to improved LV performance in RA.

Key words
Interleukin-1, apoptosis, oxidative stress, myocardial deformation, anakinra, rheumatoid arthritis

Introduction
Cell apoptosis is a highly regulated program of cell death and is mediated by specific receptors in the cytoplasma membrane, namely Fas and Fas ligand (FasL), as well as by the mitochondria via activation of caspase-9 (1). The cell death program is activated in cardiac myocytes by various stressors including cytokine production (1, 2), increased oxidative stress (3) and DNA damage (1, 4).

In autoimmune diseases, apoptosis is enhanced to counterbalance the excess activation of immune cell lines (5, 6). Studies have shown that circulating Fas and FasL (7–9) levels are increased in patients with rheumatoid arthritis (RA) likely after their release from monocytes and T lymphocytes. However, the soluble FasL induces systemic apoptosis per se irrespective of its cellular source of origin (10). Moreover, the behaviour of soluble FasL is considered to reflect the status of activation of the Fas/FasL system within the myocardium in congestive heart failure (11–13). The inflammatory processes observed in patients with RA are strongly linked to enhanced interleukin (IL)-1 activity (14–16). Increased IL-1 activity causes myocardial cell damage and endothelial dysfunction (17). The adverse effects of IL-1 on myocardial and endothelial cells are mediated by an enhanced nitrooxidative stress (14, 18, 19) and production of other inflammatory mediators including tumour necrosis factor (TNF)-α, a proapoptotic cytokine mediating activation of Fas/FasL system (20–22). Thus, in RA, enhanced IL-1 activity may play a key role in the promotion of apoptotic cardiomyocyte death (1, 21).

Anakinra, a recombinant form of human IL-1 receptor antagonist, is commonly used for the treatment of RA. Experimental data indicates that administration of anakinra after acute myocardial infarction ameliorates cardiac remodelling by reducing cardiomyocyte apoptosis (23). However, it has not been defined whether inhibition of IL-1 activity by anakinra reduces apoptosis.
and thus contributes to improvement of left ventricle (LV) performance in humans.

In the present study, we hypothesised that inflammatory mediators such as IL-1β and TNF-α increase apoptosis and thus, contribute to impairment of myocardial performance in RA patients. Additionally, we hypothesised that inhibition of IL-1 activity by anakinra reduces apoptosis leading to improvement in myocardial performance. Therefore, we investigated a) whether IL-1β and TNF-α levels are related with increased circulating apoptotic markers, namely Fas, Fas ligand and caspase-9, b) whether myocardial dysfunction in RA is related with enhanced apoptosis as assessed by circulating apoptotic markers, c) the effects of acute and chronic treatment with anakinra on apoptosis, and d) whether reduction of apoptotic markers is associated with improvement of LV performance as assessed by tissue Doppler and speckle tracking-derived echocardiographic indices in patients with RA.

**Methods**

**Study population**

We examined 46 patients (mean age 56 ± 16 years, 31 females) with RA (American Rheumatism Association criteria who had an inadequate response to disease modifying antirheumatic drugs (DMARDs) and corticosteroids. Our patient cohort included patients also described in our previous studies (14, 15). All patients were on methotrexate 7.5 mg once per week, leflunamide 20 mg once daily (o.d.) and prednisolone 5 mg o.d. None of our patients were on treatment with non-steroidal anti-inflammatory drugs (NSAIDS) within the last year (14, 15). Four (9%) and 11 (24%) patients out of 46 RA patients were on stable treatment with statins and cardioactive medications, respectively, for the last six months (Table 1).

**Table 1: Clinical characteristics, conventional echocardiographic, vascular and biological markers of the study population.**

<table>
<thead>
<tr>
<th>Disease activity score</th>
<th>RA patients (n=46)</th>
<th>Patients treated with anakinra (n=23)</th>
<th>Patients treated with prednisolone (n=23)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>56 (16)</td>
<td>57 (17)</td>
<td>56 (16)</td>
<td>0.4</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>28.6 (7)</td>
<td>29.3 (7)</td>
<td>28.2 (7)</td>
<td>0.6</td>
</tr>
<tr>
<td>Sex (female)</td>
<td>31 (67%)</td>
<td>17 (73%)</td>
<td>14 (61%)</td>
<td>0.3</td>
</tr>
<tr>
<td>Hypertension</td>
<td>20 (43%)</td>
<td>11 (47%)</td>
<td>9 (39%)</td>
<td>0.4</td>
</tr>
<tr>
<td>Smoking</td>
<td>13 (28%)</td>
<td>7 (30%)</td>
<td>6 (26%)</td>
<td>0.7</td>
</tr>
<tr>
<td>Dyslipidaemia</td>
<td>13 (28%)</td>
<td>7 (30%)</td>
<td>6 (26%)</td>
<td>0.5</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>9 (19%)</td>
<td>5 (22%)</td>
<td>4 (17%)</td>
<td>0.7</td>
</tr>
<tr>
<td>ACE-I</td>
<td>11 (24%)</td>
<td>6 (27%)</td>
<td>5 (22%)</td>
<td>0.7</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>5 (11%)</td>
<td>3 (13%)</td>
<td>2 (9%)</td>
<td>0.6</td>
</tr>
<tr>
<td>Ca²⁺ channel blockers</td>
<td>10 (22%)</td>
<td>5 (22%)</td>
<td>5 (22%)</td>
<td>0.7</td>
</tr>
<tr>
<td>Diuretics</td>
<td>11 (24%)</td>
<td>6 (27%)</td>
<td>6 (26%)</td>
<td>0.7</td>
</tr>
<tr>
<td>Statins</td>
<td>4 (9%)</td>
<td>2 (9%)</td>
<td>2 (9%)</td>
<td>0.9</td>
</tr>
<tr>
<td>Antidiabetics</td>
<td>2 (4%)</td>
<td>1 (4%)</td>
<td>1 (4%)</td>
<td>0.9</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>128.3 (17.8)</td>
<td>127.3 (19.8)</td>
<td>129.0 (14.6)</td>
<td>0.5</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>80.2 (11.5)</td>
<td>79 (11.9)</td>
<td>82 (11)</td>
<td>0.4</td>
</tr>
<tr>
<td>EF (%)</td>
<td>65.1 (14.0)</td>
<td>65.5 (12.1)</td>
<td>64.6 (16.9)</td>
<td>0.9</td>
</tr>
<tr>
<td>LVEDV (ml)</td>
<td>90.0 (16.9)</td>
<td>90.9 (16.9)</td>
<td>88.8 (18.1)</td>
<td>0.7</td>
</tr>
<tr>
<td>LVESV (ml)</td>
<td>30.5 (14.5)</td>
<td>30.2 (13.2)</td>
<td>31.0 (15.9)</td>
<td>0.8</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>11.00 (1.19–45)</td>
<td>11.6 (6.5–35)</td>
<td>10.6 (6.4–38)</td>
<td>0.4</td>
</tr>
<tr>
<td>RF (U/ml)</td>
<td>76 (26–224)</td>
<td>79 (20–236)</td>
<td>72 (26–210)</td>
<td>0.6</td>
</tr>
<tr>
<td>Anti-CCP (U/ml)</td>
<td>40.5 (4.8–171.0)</td>
<td>41.4 (4.9–173.2)</td>
<td>39.8 (4.6–168.1)</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Values for biomarkers are median and interquartile range, ACE-I: angiotensin converting enzyme inhibitors, SBP: systolic blood pressure, DBP: diastolic blood pressure, LVEDV: LV end diastolic volume, LVESV: LV end systolic volume, EF: ejection fraction, CRP: C-reactive protein, RF: Rheumatoid factor, Anti-CCP: antibodies to cyclic citrullinated peptide. p: p value for comparisons between anakinra-treated and prednisolone-treated patients.
We used the following equation to calculate the composite inflammatory disease activity score (DAS), which utilises C-reactive protein (CRP), the visual analogue score (VAS) of well-being, and the number of tender and swollen joints (from a total of 28 joints assessed): DAS = $\sqrt{(0.56 \times \text{number of tender joints}) + \sqrt{(0.28 \times \text{number of swollen joints})} + [0.70 \times \ln(\text{CRP})] + (0.014 \times \text{VAS})}$ (14, 15).

None of the patients had cardiovascular or renal disease or ischaemia during thallium scintigraphy or dobutamine stress.

The study protocol was approved by the Institute’s Ethics Committee, and written informed consents were obtained from all patients.

**Echocardiography**

Studies were performed using a Vivid 7 (GE Medical Systems, Horten, Norway) ultrasound system. All studies were digitally stored in a computerised station (Echopac, GE Medical systems) and were analysed by two observers blinded to clinical and laboratory data. All patients had adequate images for analysis.

Two-dimensional echocardiography

From cross-sectional echocardiographic images of the LV, were measured end-diastolic (LVEDV) and end-systolic volume (LVESV), as well as ejection fraction (EF) (%) using the Simpson’s method of discs.

Two-dimensional strain measurements

Using a dedicated software package (Echopac, GE Medical systems), two-dimensional strain and strain rate was measured. In our previous study (15) we observed that there was a greater increase after inhibition of IL-1 activity in longitudinal systolic deformation parameters. Furthermore, longitudinal strain rate is well correlated with invasively determined parameters of global function, and is currently suggested for use in clinical practice as an adjunctive to LV ejection fraction for the quantification of global systolic performance (15). Thus, in the present study, we studied the chronic effects of anakinra only in longitudinal deformation parameters. Apical four-chamber views of the LV were obtained at end-expiratory apnea and three cardiac cycles were stored from each view in cineloop format for subsequent offline analysis. Sectors were adjusted to achieve frame rates $\geq 50$ frames/second (sec) (50–82 frames/sec). By tracing the endocardiac contour from an end diastolic frame the software automatically tracked the contour on subsequent frames. Adequate tracking can be verified in real time and corrected by adjusting the region of interest or manually correcting the contour to ensure optimal tracking. After having defined by anatomical M-mode mitral and aortic valve opening and closure, were measured average longitudinal peak systolic strain (LongS) and longitudinal peak systolic strain rate (LongSRS) (15). The inter- and intra-observer variability of these measurements were $\leq 8\%$ and $\leq 10\%$, respectively.

**Laboratory assays**

CRP was measured by a high-sensitivity particle-enhanced immunonephelometry (Dade Behring, Marburg, Germany, measurement range: 0.175–30 mg/l) (24). Rheumatoid factor (RF, U/ml) was determined by nephelometry (Siemens Healthcare Diagnostics, Munich, Germany, positivity $\geq 15$ U/ml). Antibodies to cyclic citrullinated peptide (anti-CCP, U/ml) were tested using a second generation ELISA (Diostat, Axis-Shield Diagnostics Ltd., Dundee, Scotland, UK, positivity $\geq 5$ U/ml).

Concentrations of IL-1β and TNF-α as well as Fas-L, Fas and caspase-9 were measured using commercially available enzyme-linked immunosorbent assay kits according to the manufacturer’s specifications (for IL-1β: high sensitivity, R&D Systems; method sensitivity 0.023–0.140 pg/ml, for TNF-α: Invitrogen; method sensitivity <0.09 pg/ml, for Fas-L: Diaclone, Cedex; method sensitivity <12 pg/ml, for Fas: Diaclone, Cedex; method sensitivity <47 pg/ml and for caspase-9: R&D Systems, UK; method sensitivity <0.1 ng/ml).

**Study protocols**

**Acute study**

In an acute, double-blind trial, a group of 23 patients with RA (mean age 57 ± 17 years, 17 females) were randomised to receive a single injection of anakinra, a recombinant IL-1 receptor antagonist (150 mg subcutaneously [s.c.]) (n=12) or placebo (n=11). After 48 hours (h) patients were crossed over to the alternate treatment (placebo or anakinra) and measurement of the examined markers was repeated, following a previously published methodology (14). The 48 h interval between the two consecutive studies was decided to secure a sufficient wash-out period of anakinra in accordance to the drug’s half-life time. At baseline and 3 h after the single injection, we assessed IL-1β, TNF-α, as well as Fas, Fas ligand and caspase-9 serum levels, as apoptotic markers.

**Chronic study**

After completion of the acute study, the 23 patients with RA participated in a chronic, non-randomised study with anakinra treatment (150 mg s.c. once daily) for 30 days.
A second group of 23 RA patients was selected on 1:1 basis to have similar age, sex, and inflammatory disease activity as assessed by DAS with the anakinra-treated group and served as control group for the chronic study with anakinra. This group of RA patients was treated with an increase of their initial dose of prednisolone by 5 mg for 30 days according to standard clinical practice (14).

At baseline and after 30 days of treatment we assessed LongS, LongSRS and E/Em ratio as well as IL-1β, TNF-α and all apoptotic markers.

To examine the patients’ compliance in therapy, we asked our patients to provide the used ampoules of anakinra and the used containers of prednisolone tablets at each visit.

**Statistical analysis**

To examine whether patients included in two treatment groups (anakinra- and prednisolone-treated patients) were adequately balanced for atherosclerotic risk factors, we calculated logit propensity score in each patient. We used logistic regression model adjusting for age, sex, hypertension, hyperlipidaemia, smoking status, as atherosclerotic markers and medication. Propensity scores were compared using two-tailed t-test between anakinra- and prednisone-treated patients.

Categorical data were compared between patients treated with anakinra and prednisolone by contingency tables (p-value in Table 1). Continuous variables were tested for normality using

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**Figure 1:** Representation of immunochemical data through the study. Diagrammatic representation of the median values and interquartile range changes in Fas (A), FasL (B) and caspase-9 (C) serum levels, respectively at baseline, 3 h and 30 days after anakinra treatment (grey line), as well as at baseline and 30 days after cortisone treatment (black line). Apoptotic markers were similar between patients treated with anakinra or cortisone at baseline (p=ns). Anakinra treatment reduced apoptotic markers at all time points compared to baseline (p<0.05) while cortisone had no significant effect (p=ns). After 30 days of treatment patients on anikira had lower levels of apoptotic markers than patients on cortisone (p<0.01).
the Kolmogorov-Smirnov test. Normally distributed variables are given as mean (standard deviation). Spearman correlation analysis was used to determine bivariate correlations. Because biomarkers had a non-normal distribution, data are expressed as median (interquartile range) and were analysed after transformation into ranks.

Analysis of variance (ANOVA) (General linear model, SPSS 13, SPSS Inc., Chicago, IL, USA) for repeated measurements was applied to compare a) acute study: measurements of the examined markers at baseline, 3 h post-placebo and 3 h post-anakinra, and b) chronic study: the effects of anakinra versus prednisolone, with measurements at baseline and 30 days post-treatment used as a within-subject factor and type of treatment as between-subject factor. The F and p values of the interaction between time of measurement of the examined markers and type of treatment were calculated. The Greenhouse-Geisser correction was used when the sphericity assumption, as assessed by Mauchly’s test, was not met. Post-hoc comparisons were performed with Bonferroni’s correction.

Comparisons between each treatment group at baseline or at 30 days, were performed using unpaired t-test (LV systolic deformation, LV diastolic function indices) and Mann-Whitney test (biomarkers), as well as the percent changes of the examined indices between baseline and 30 days in the anakinra group versus changes in the prednisolone group. Statistical significance was considered as p<0.05.

Results

Patient’s clinical characteristics including disease activity markers (DAS, CRP, RF, anti-CCP) are shown in Table 1. 87% of RA patients were RF positive and 69% were anti-CCP positive.

Twenty RA patients complained of mild erythema at the injection site, and no patient was withdrawn from the study because of adverse effects or inadequate response to treatment.

Interrelations between biomarkers and echocardiographic parameters at baseline

Interrelation of biomarkers

At baseline TNF-α levels were related to sFas and sFas ligand levels (r=0.47, p=0.01 and r=0.38, p=0.04). Additionally, IL-1β levels were related to TNF-α levels (r=0.51, p=0.03) and sFas ligand (r=0.43, p=0.02).

Correlation of biomarkers with LV deformation parameters

E/Em was related with TNF-α, Fas and Fas ligand (r=0.36, p=0.04, r=0.63, p=0.001 and r=0.53, p=0.03, respectively). Moreover, caspase-9 was related to LongS (r=0.61, p=0.03) and LongSRS (r=0.48, p=0.04) while Fas was related with LongSRS (r=0.45, p=0.04). Furthermore, Fas ligand and TNF-α were related with LongS (r=0.39, p=0.04 and r=0.58, p=0.013, respectively) and LongSRS (r=0.37, p=0.05 and r=0.50, p=0.04, respectively). Finally, IL-1β was modestly related to LongSRS (r=0.36, p=0.04).
**Effect of acute administration of anakinra in apoptotic markers**

Compared to baseline, there was a statistically significant improvement in the serum levels of Fas, Fas ligand and caspase-9, after acute administration of anakinra (p<0.05, Table 2, Fig. 1).

In patients who were initially treated with anakinra and then cross-over to placebo, the values of the examined markers post-placebo were similar to the respective values of the markers at baseline (p=0.1, Table 2, Fig. 1). This finding suggests that any potential carry-over effect of anakinra during the acute study was minimal.

Compared to baseline, we observed a significant reduction in IL-1β and TNF-α levels after a single dose of anakinra (IL-1β: 0.35 (0.22–0.92) to 0.20 (0.16–0.56) pg/ml, p for change=0.009 and TNF-α: 0.81 (0.60–1.15) pg/ml to 0.65 (0.37–0.82) pg/ml, p=0.002). In contrary, after placebo, there were no significant changes in IL-1β and TNF-α levels (IL-1β: 0.34 (0.23–0.95) pg/ml, p for change=0.52 and TNF-α: 0.80 (0.62–1.11) pg/ml, p=0.71).

**Effect of chronic treatment with anakinra on apoptotic markers in comparison to prednisolone treatment**

Baseline apoptotic markers were similar between anakinra- and prednisolone-treated patients (Table 3, Fig. 1). Additionally the two treatment groups had similar age, sex, body mass index (BMI), atherosclerotic risk factors, disease activity markers and medications as well as FS, EF, systolic and diastolic blood pressure (Table 1). By logistic regression analysis including age, sex, hypertension, hyperlipidaemia, smoking, cardioactive medication, and statins, the calculated logit propensity scores were similar between the two treatment groups (anakinra vs. prednisolone, p=0.54).

After 30 days of anakinra treatment there was an improvement in apoptotic markers (p<0.05 for all comparisons, Table 3, Fig. 1). Conversely, in the prednisolone-treated group, no statistically significant changes in apoptotic markers were observed after treatment compared to baseline. Therefore, apoptotic markers were lower in patients post-anakinra than post-prednisolone treatment (p<0.05 for all comparisons).

By ANOVA, there was a significant effect of the type of medication (anakinra vs. prednisolone) on the circulating levels of TNF-α and IL-1β after 30 days of treatment (F for interaction F=5.341, p=0.029 and F=6.822, p=0.014, respectively).

In post-hoc analysis, in anakinra treated group, there was a significant reduction in IL-1β and TNF-α levels (IL-1β: from 0.35 (0.22–0.92) to 0.09 (0.07–0.20) pg/ml, p for change = 0.001 and TNF-α: from 0.81 (0.60–1.15) to 0.42 (0.31–0.77) pg/ml, p<0.001). Conversely, in the prednisolone-treated group we observed a borderline significant reduction in IL-1β and TNF-α levels (IL-1β: from 0.31 (0.23–0.94) to 0.24 (0.18–0.42) pg/ml, p for change=0.046 and TNF-α: from 0.80 (0.51–1.02) to 0.70 (0.49–0.91) pg/ml, p=0.043).

Baseline IL-1β, TNF-α were similar between anakinra- and prednisolone-treated patients (p=0.9 and p=0.8). However, TNF-α and IL-1β were lower in patients post-anakinra than post-prednisolone treatment (p=0.019 and p=0.025, respectively). Thus, anakinra treatment resulted in a greater reduction in IL-1β and TNF-α than prednisolone after 30 days of treatment.

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**Table 3: Effects of chronic treatment with anakinra versus prednisolone on apoptotic markers.**

<table>
<thead>
<tr>
<th>Anakinra (n=23)</th>
<th>Prednisolone (n=23)</th>
<th>F</th>
<th>p†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td><strong>30-days</strong></td>
<td><strong>Baseline</strong></td>
<td><strong>30-days</strong></td>
</tr>
<tr>
<td>Fas (pg/ml)</td>
<td>481 (267–567)†</td>
<td>301 (264–332)</td>
<td>0.003</td>
</tr>
<tr>
<td>Fasl. (pg/ml)</td>
<td>289 (187–437)†</td>
<td>190 (134–232)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Caspase-9 (ng/ml)</td>
<td>1.90 (1.42–3.52)†</td>
<td>1.07 (0.90–1.67)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Values are expressed as median (interquartile range). †: non statistically significant differences between two study groups. p*: p value for comparisons between baseline and 30 days among anakinra treated patients. p†: p value for comparisons between baseline and 30 days among prednisolone treated patients. F and p† indicate the interaction between the biomarkers and treatment with anakinra versus treatment with prednisolone at baseline and post-month.
LV performance parameters after chronic treatment

Baseline LV performance parameters were similar between the two treatment groups. Compared to baseline, there were an improvement in LongS (-17.8 ± 3.7% to -22.1 ± 3.5%, p=0.001) LongSRS (-1.02 ± 0.23% to -1.25 ± 0.23%, p=0.008) and E/Em (10.2 ± 4.0 to 8.1 ± 2.0, p<0.001) after 30 days of anakinra treatment.

In the prednisolone-treated group, there were no significant changes in LongS (-19.3 ± 4.2% to -20.3 ± 3.4%, p=0.6), LongSRS (-1.04 ± 0.20% to -1.11 ± 0.17%, p=0.3) and E/Em (9.1 ± 3.3 to 9.5 ± 4.1, p=0.7) after 30 days of treatment compared to baseline.

Thus, anakinra treatment resulted in a greater improvement in LongS (F=13.585, p=0.001 for the overall effect of treatment), LongSRS (F=4.979, p=0.035), E/Em (F=6.296, p=0.005) compared to prednisolone. The LV performance markers were higher in patients post-anakinra than post-prednisolone treatment (p<0.05 for all comparisons).

Correlation between changes in cytokines and apoptotic markers after 30 days of treatment

After 30 days of anakinra treatment, changes in TNF-α were related to changes in Fas and Fas ligand (r=0.48, p=0.04 and r=0.57, p=0.02, respectively), as well as IL-1β (r=0.50, p=0.03). Furthermore, the percent changes in IL-1β were related with the percent changes in Fas and Fas ligand (r=0.51, p=0.034 and r=0.57, p=0.042, respectively).

Correlation of changes in apoptotic markers with LV performance parameters after 30 days of treatment

After 30 days of anakinra treatment, baseline Fas levels predicted the absolute and percent changes of LongSRS (r=-0.52, p=0.03, r=-0.59, p=0.02, respectively). The percent changes in Fas levels were related to the percent changes in E/Em (r=0.45, p=0.03) and LongSRS (r=0.49, p=0.04). Furthermore, changes in TNF-α were related to changes in LongS (r=0.518, p=0.04). Finally, percent changes in caspase-9 were related to the percent changes in LongSRS (r=0.54, p=0.02) and in E/Em (r=0.46, p=0.03).

Discussion

In the present study, we have shown that soluble apoptotic markers are related to impaired LV myocardial deformation and LV diastolic dysfunction in RA patients. Moreover, in our study apoptotic markers were reduced 3 h and 30 days after inhibition of IL-1 activity by anakinra, while this effect was not observed after 30 days of treatment with corticosteroids in a matched group of RA patients. Furthermore, the percent reduction of apoptotic markers was associated with the percent improvement of LV myocardial deformation and LV diastolic function markers. Interestingly, the percent reduction of apoptotic markers after anakinra was associated to percent reduction of circulating TNF-α and IL-1β after treatment. To our knowledge, this is the first study to report that IL-1 inhibition by anakinra results in the reduction of circulating apoptotic markers in RA patients and that reduction of these markers is linked with a concomitant improvement of LV performance.

Recent data support the hypothesis that cell apoptosis is a common process in cardiovascular disease leading to impairment of LV performance (11–13, 25, 26). In apoptotic cardiomyocyte death, there are two major apoptotic signalling pathways; the intrinsic pathway via mitochondria and the extrinsic pathway via Fas ligand and TNF-α (27). Finally, both pathways converge to activate a sequence of cysteine protease enzymes (caspases), with caspase-8, 9 and 3 being pivotal to the death process (28). Apoptosis in patients with autoimmune disease is considered an essential mechanism to counterbalance the abnormal immune response and to maintain immunologic tolerance (5, 6). Although cell proliferation is exaggerated in the synovial membrane of patients with RA, studies have shown increased levels of synovial and serum Fas and FasL in these patients likely due to the enhanced inflammatory and oxidative burden on infiltrating mononuclear cells and circulating T lymphocytes (7–9).

In the present study, we have shown that soluble apoptotic markers reflecting both the intrinsic (caspase 9) and extrinsic (TNF-α, Fas, Fas ligand) pathways are related to abnormal myocardial deformation as assessed by tissue Doppler and speckle tracking-derived echocardiographic indices in patients with RA. Indeed, studies support that the soluble FasL (sFasL) induces cell apoptosis per se irrespective of its cellular source of origin (10).

Studies have shown that soluble Fas and FasL (7–9) levels are increased in RA patients. In the present study, we showed that treatment with anakinra reduced IL-1β and TNF-α, as well as Fas, Fas ligand and caspase-9 serum levels in RA patients 3 h and 30 days after treatment. Furthermore, there was a close relation between the reduction of IL-1β and TNF-α with the respective reduction in soluble Fas and FasL levels after anakinra treatment. These findings indicate that inhibition of IL-1 receptors reduces apoptotic process through intrinsic, as well as extrinsic pathways and that this reduced apoptosis may be at least partly related to the reduction of the inflammatory process.

The antiapoptotic effects of the IL-1Ra have already been reported in experimental studies (29–32). Furthermore, Abbate et al. (23) showed that administration of anakinra after acute myocardial infarction ameliorates cardiac remodelling by reducing cardiomyocyte apoptosis in two different animal models as well as in patients with ST-elevation myocardial infarction (33). However, the investigators did not examine the mechanisms mediating the beneficial effect of IL-1 inhibition on LV remodelling in humans.

Both cytokines and oxidative stress play a key role in apoptotic cardiomyocyte death (1). In particular, IL-1 a) increases gene and protein expression of caspases in animal cardiomyocytes (34), b) has a direct detrimental effect on cell mitochondria function (35), c) promotes the release of superoxide anion (17) contributing to an enhanced nitrooxidative stress, d) increases intracellular iNOS and TNF-α production (20, 21) facilitating cardiac dysfunction and apoptosis (21, 36, 37), and e) increases expression of nuclear
factor kappa B (20) and thus regulates NADPH oxidase (Nox) activity, a major source of intracellular reactive oxygen species (ROS) (38). ROS react indiscriminately with the majority of biological molecules causing the irreversible damage of DNA, proteins, carbohydrates, and lipids constituents, thereby altering cell function and facilitating cell apoptosis (38, 39). Through the above pathways, increased IL-1 activity may contribute to excessive myocardial cell apoptosis leading to cardiac dysfunction.

Previous studies suggest that myocardial cell function is impaired per se in RA (14, 15, 40). Pathological processes contributing to myocardial dysfunction in RA include increased nitrooxidative stress production (14, 18, 19) of IL-1 and IL-6 (14, 15, 20, 22, 41, 42). Products of nitrooxidative stress and inflammatory cytokines have a direct negative inotropic action (14, 15, 41). Moreover IL-1 and TNF-α facilitate the expression of myocardial death receptors, namely FasL, promoting the extrinsic pathway of apoptosis (18).

In the present study, we have shown that increased circulating TNF-α, Fas, FasL and caspase-9 were related with impaired LV longitudinal strain, strain rate and E/Em. These associations suggest that the apoptotic process may result in abnormal LV performance through extrinsic, as well as intrinsic apoptotic signaling pathways in patients with RA. In our previous studies, we have shown that treatment with anakinra improves endothelial function, aortic distensibility and coronary flow reserve through reduction of nitro-oxidative stress (14) and further more that lowering IL-1 activity is associated with improved myocardial deformation in RA patients (15). In the present study, we extend our previous findings by showing for the first time that the percent reduction of circulating IL-1β and TNF-α after anakinra treatment are related to the percent reduction of apoptotic markers. Moreover, we have shown that the percent reduction of Fas, FasL and caspase-9 at 3 h and 30 days after administration of anakinra was related with the percent improvement of markers of LV deformation and diastolic function. On the contrary there was no change in apoptotic and echocardiographic markers of LV performance in a matched group of RA patients treated with an increased dose of steroids for 30 days. This finding supports the hypothesis that the inhibition of IL-1 activity may improve LV systolic and diastolic performance through reduction of IL-1-induced apoptosis, suggesting a novel protective action of IL-1 inhibition by anakinra on cardiomyocytes. Indeed, experimental studies have shown that caspase inhibition reduces cardiac myocyte dyshomeostasis and improves cardiac contractile function (43).

In our previous studies, we have suggested that treatment with anakinra improves vascular and LV function through reduction of nitro-oxidative stress (14, 15). In the present study we have shown that the improvement in myocardial deformation and LV diastolic function is mediated by the concomitant reduction of the apoptotic process which was closely linked with elevated TNF-α and IL-1 levels. Thus the reduction of the inflammation-driven apoptosis is a major mechanism determining the improvement in LV performance after anakinra treatment.

Conclusions

In conclusion, in the present study of RA patients, we have shown that increased circulating IL-1β and TNF-α are related with increased levels of soluble apoptotic markers which in turn are linked with impaired LV myocardial deformation and diastolic function. Moreover, IL-1β, TNF-α and apoptotic markers were reduced after inhibition of IL-1 activity by anakinra and the reduction of circulating apoptotic markers was associated with the improvement of LV myocardial deformation and diastolic function. Anakinra treatment was more effective than corticosteroids in the reduction of apoptotic markers and the concomitant improvement of LV deformation.

Study limitations

The study design does not permit to explore the causality between the changes of the apoptosis and LV performance post-anakinra treatment. As RA patients with CAD were excluded, the effects of anakinra in the presence of CAD were not explored in our study. The circulating levels of apoptotic markers may not exclusively reflect the apoptotic process within myocardium. However, the close association between soluble apoptotic markers and tissue Doppler and speckle tracking-derived echocardiographic indices at baseline as well as the association of changes of measured biomarkers with the respective changes of echocardiographic markers of LV performance after treatment suggest that circulating apoptotic markers may be involved in an apoptotic process within the heart muscle leading to impaired LV performance. Indeed, studies support that the sFasL induces cell apoptosis per se irrespective of its cellular source of origin (10–13). Furthermore, the behaviour of sFasL is considered to reflect the status of activation of the Fas/FasL.
In this study, a novel association between soluble apoptotic markers and LV performance was observed under conditions of exaggerated inflammation characterising patients with RA. Furthermore, reduction of apoptotic markers was associated with improvement in LV performance after anakinra. Further studies should be undertaken to elucidate the intracellular mechanisms linking inhibition of IL-1 activity and limitation of apoptosis in the heart muscle.

**Conflict of interest**
None declared.

**References**