Concise report

Cathepsin S and cathepsin L in serum and synovial fluid in rheumatoid arthritis with and without autoantibodies

Tomas Weitoft¹, Anders Larsson², Vivek A. Manivel³, Jörgen Lysholm⁴, Ann Knight⁵ and Johan Rönnelid³

Abstract

Objectives. Cathepsin S and cathepsin L are endosomal proteolytic enzymes involved in the degradation of extracellular matrixes, angiogenesis and antigen presentation. Cathepsins could thus play several roles in the disease process of RA. The aim of this study was to examine differences in cathepsin S and cathepsin L levels in serum and SF of RA patients with and without ACPA and RF.

Methods. In this study 121 patients with RA and clinical signs of knee synovitis were recruited. Patient characteristics were collected and matched samples of serum and SF were analysed for cathepsin S, cathepsin L, ACPA, IgA and IgM RF, CRP and MMP3.

Results. SF levels of cathepsin L, cathepsin S and MMP3 were significantly higher than in serum. Serum levels of both cathepsins were significantly higher in patients with ACPA, IgM-RF and IgA-RF compared with patients without these antibodies. SF levels of both cathepsins correlated with DAS28 and CRP in ACPA- and RF-positive but not in seronegative patients.

Conclusion. The differences in cathepsin S and cathepsin L between RA patients with and without autoantibodies indicate that these cathepsins have a specific role in the disease process of seropositive RA. In this phenotype, cathepsin serum levels may reflect the autoimmune activity, whereas the levels in SF may reflect the local inflammatory and matrix degrading process in the joint.

Key words: rheumatoid arthritis, cathepsin S, cathepsin L, ACPA, rheumatoid factor.

Introduction

In the adaptive immune system, foreign antigens, recognized as non-self, are processed and presented on the surface of antigen-presenting cells (APC). Antigen-derived peptides are displayed in association with an MHC class II molecule and recognized by CD4⁺ T cells, which in turn activate other immune cells to produce antigen-specific antibodies and pro-inflammatory cytokines [1].

Cysteine cathepsins are a group of endosomal proteases involved in antigen processing and preparation of MHC class II molecules for antigen presentation. The invariant chain, which blocks the antigen-presenting site during maturation of MHC molecules, is replaced by the antigenic peptides after being cleaved by cathepsins. This process is of key importance for the development of an adaptive immune response [2].

Although cathepsin L is ubiquitously expressed in human tissue, its expression is especially prominent in

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thymic cortical cells. Therefore cathepsin L is assumed to be important for the positive selection of T cells able to recognize non-self structures [3]. Cathepsin S has been found in professional APC: dendritic cells, macrophages and B cells. It is also expressed in synovial macrophages, has potent proteoglycan-degrading activity and is extremely efficient in hydrolysing aggrecans at neutral and acidic pH [4].

Animal studies have shown that cathepsin L-deficient mice have reduced arthritis severity [5] and that cathepsin S knockout mice have decreased susceptibility to collagen-induced arthritis [6]. Cathepsin S inhibitors are discussed as future treatment options in chronic arthritis, and clinical trials have been initiated [4].

RA is an inflammatory autoimmune disease that may lead to progressive joint destruction. The expression of proteases, especially MMPs and cysteine cathepsins, is stimulated by pro-inflammatory cytokines and is involved in the joint destruction process [1]. Both cathepsin L and cathepsin S are detected in the synovial membrane and SF of patients with RA and OA [7, 8]. However, the levels are significantly higher in RA, suggesting a specific role for cathepsins in the inflammatory and destructive process of this disease [8].

MMP3 is a powerful collagenolytic enzyme with great importance for the development of joint erosions in RA, but without known immunological effects [9]. MMP3 levels are high in SF, reflecting the ongoing local destructive processes. Serum levels are much lower and correlate with markers for inflammation [10].

In Caucasian RA populations ~70% of patients have serum antibodies against specific antigens such as citrullinated proteins/peptides (ACPA) and the Fc part of the IgG molecule (RF). RA patients with high serum levels of ACPA and RF of the IgM or IgA subclass have a more severe disease course and a higher risk of joint destruction and disability [11]. In fact, the presence of ACPA is one of the strongest predictors of erosive joint disease. ACPA-positive RA patients show a distinct genetic association with MHC and other genes, and also show dependence on smoking as the strongest environmental risk factor, not seen for ACPA-negative patients. Therefore, it has been suggested that seronegative and seropositive RA patients represent different disease entities [1].

In this study we have related clinical features to cathepsin S, cathepsin L and MMP3 levels in sera and SF obtained in parallel with the occurrence of ACPA, IgM RF and IgA RF. We report that serum levels of both cathepsins show associations with the presence of autoantibodies, arguing for a specific role of cathepsins S and L in the autoimmune process in seropositive RA.

Patients and methods

In the outpatient rheumatology departments at the hospitals in Gävle, Falun and Uppsala, 121 patients with RA [12] and clinical signs of knee synovitis were invited to participate in the study. Patients receiving oral CS treatment corresponding to 10 mg or more of prednisolone and patients in function class 4 according to Steinbrocker [13], were excluded.

Information on patient characteristics (age, sex, disease duration and smoking habit) was collected and serum samples were drawn for CRP, ACPA (measured as anti-CCP2), IgM RF, IgA RF, cathepsin S, cathepsin L and MMP3. Functional disability was evaluated using the Swedish version of the HAQ [14]. The number of tender and swollen joints was counted, and the DAS28 [15] calculated using ESR as laboratory inflammation marker. SF was aspirated from the knee in parallel with serum sampling. A radiographic examination of the knee was performed, and joint destruction was graded between 0 and 5 according to Larsen–Dale [16] by an independent radiologist.

Serum and synovial samples were centrifuged for 20 min at 1800g within 1 h and stored at −70 °C until analysis. The study was approved by the Regional Ethical Review Board in Uppsala, and all participating patients gave informed consent in accordance with the Declaration of Helsinki.

Laboratory methods

Cathepsin S, cathepsin L and MMP3 were analysed by commercial sandwich ELISAs (DY952, DY1183 and DY513, R&D Systems, Minneapolis, MN, USA), according to the recommendations of the manufacturer. The cathepsin S kit detects total cathepsin S, including the pro-, mature and cystatin-complexed forms. The cathepsin L kit recognizes the pro-form of recombinant human cathepsin L and the mature form of recombinant human cathepsin L by itself or when complexed to recombinant human cystatin SA. The MMP3 kit recognizes the pro-, mature and tissue inhibitor of metalloproteinase (TIMP)-complexed forms of recombinant human MMP3. In healthy individuals, the mean in plasma for cathepsin S was 18.6 (s.d. 3.5) µg/l and for CL 2.9 (s.d. 1.5) µg/l [17], but no such data are available for SF. To avoid the effects of batch differences, all samples in the study were run simultaneously with a single reagent batch.

ACPA, IgA RF and IgM RF were investigated with an enzyme immunoassay using a Phadia 250 system (Uppsala, Sweden). Reference ranges for ACPA, IgA RF and IgM RF were <7 arbitrary units/ml, <5 i.u. and <9 i.u./ml, respectively. When investigating 100 healthy blood donors, all individuals were ACPA-negative whereas 4% were IgA and IgM RF positive. CRP (reagent: 6K2601) was analysed on an Architect Ci8200 Analyzer (Abbott Laboratories, Abbott Park, IL, USA).

Statistical methods

The groups were compared using statistical analysis with the Mann–Whitney U-test, Wilcoxon signed rank test or Chi-square test when appropriate. Associations between parameters were analysed using the Spearman rank correlation test. P-values <0.05 were considered significant. The statistical calculations were made using the computer software programs IBM SPSS Statistics version 21.
are many sources of possible error. We found no
studies should be done very cautiously because there
cathepsin levels with selected healthy controls in other
cathepsins or for MMP3. Comparing the present serum
appears in healthy subjects is not known, either for these
cesses going on in this environment. How this relationship
reflect the proteolytic and bone matrix-degrading pro-
L and MMP3 in SF compared with those in sera probably
positive patients, but not in seronegative patients.
IgA RF, and SF levels of both cathepsins showed strong
psins were higher in patients with ACPA, IgM RF and
associated autoantibodies. Serum levels of both cathe-
seropositive RA patients, further emphasize that seroposi-
This cathepsin pattern differed from the MMP3 pattern,
in which CRP (but not DAS 28) correlated with both
levels (Table 2), and where serum
correlations were independent of autoantibody status
(data not shown). Serum levels of cathepsins S and L
did not correlate with age, disease duration, HAQ or
a weak correlation was found between DAS28 and
cathepsin S in serum. Severity of radiographic joint
damage did not correlate with cathepsin levels in serum
or in SF (Table 2), and was similar in RA patients with and
without autoantibodies (Table 1).

Discussion

The main findings in the present study were that levels of
cathepsins S and L showed striking associations with RA-
associated autoantibodies. Serum levels of both cathe-
psins were higher in patients with ACPA, IgM RF and
IgA RF, and SF levels of both cathepsins showed strong
correlations with DAS28 and CRP in autoantibody-
positive patients, but not in seronegative patients.

The significantly higher levels of cathepsin S, cathepsin
L and MMP3 in SF compared with those in sera probably
reflect the proteolytic and bone matrix-degrading pro-
cesses going on in this environment. How this relationship
appears in healthy subjects is not known, either for these
cathepsins or for MMP3. Comparing the present serum
cathepsin levels with selected healthy controls in other
studies should be done very cautiously because there
are many sources of possible error. We found no
difference in SF levels of MMP3 or cathepsins between
patients with and without ACPA or RF, indicating similar
proteolytic activity in the knees.

The correlation between cathepsins in SF and mea-
ures of systemic inflammation (DAS28, CRP) in seroposi-
tive patients only suggests that the local processes
involving cathepsins within joints are different from those
in seronegative patients. Proinflammatory cytokines such
as IL6 promote systemic inflammation and stimulate the
proteolytic effects of cathepsins (and MMP3 as well),
causing local matrix destruction and explaining the
increased risk of more erosions and joint destruction in
patients with ACPA and RF [18].

Recently Ruge et al. [17] found no association between
DAS28 and serum levels of cathepsin S and cathepsin L in
RA patients, which is confirmed in the present study.
Thus, it seems as if serum cathepsins do not reflect the
inflammatory activity within joints.

The circulating levels of cathepsin S and cathepsin L
differed significantly between ACPA-positive and ACPA-
negative patients, as well as between IgA and IgM-
positive and -negative patients. This was not found for
MMP3. We hypothesize that these differences in cathe-
psin serum levels between the two major RA phenotypes
(with and without RA-associated autoantibodies, respect-
ively) reflect higher autoimmune disease activity, rather
than more matrix destruction or inflammation, among
the seropositive patients.

Cathepsin K is another collagenolytic protease released
from osteoclasts and important for bone resorption in the
RA joint destructive process. In accordance with our
findings for cathepsin S and cathepsin L, others have
reported higher serum levels of cathepsin K in ACPA-
positive RA patients [19]. This was support for the hypo-
thesis that ACPA antibodies induced osteoclastogenesis
and were a direct link between autoimmunity and joint
destruction. However, in contrast to the cathepsins S
and L investigated in our study, the authors reported
that serum levels of cathepsin K were similar in patients
with and without RF. The differences in serum cathepsin
levels between RA patients with and those without ACPA/
RF antibodies, as well as the strong correlation between
SF cathepsin levels and DAS28 and CRP found only in
seropositive RA patients, further emphasize that seroposi-
tive and seronegative RA represent different disease enti-
ties, probably with different aetiopathogenesis.

Our results suggest an important role for cathepsins S
and L in the disease process of autoantibody-positive RA.
More knowledge about the clinical role of cathepsins is,
however, needed. Whether or not cathepsin levels predict
the clinical effects of cathepsin S inhibitors [4] remains to
be elucidated. Our results imply that if such a therapeutic
approach is feasible, it might be especially beneficial in
seropositive RA.

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institute, Cary, NC, USA).
### TABLE 1  Patient characteristics and levels of proteases (cathepsin L, cathepsin S and MMP3) in serum and SF of RA patients with and without autoantibodies

<table>
<thead>
<tr>
<th></th>
<th>All, n = 121</th>
<th>ACPA⁺, n = 87</th>
<th>ACPA⁻, n = 34</th>
<th>IgMRF⁺, n = 90</th>
<th>IgMRF⁻, n = 31</th>
<th>IgARF⁺, n = 79</th>
<th>IgARF⁻, n = 41*</th>
<th>ACPA⁻ and RF, n = 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>62 (24-87)</td>
<td>62 (24-87)</td>
<td>63.5 (24-86)</td>
<td>64 (24-87) 60 (24-86)</td>
<td>63 (24-87) 59 (24-79)</td>
<td>59 (24-77)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>31/90</td>
<td>20/67</td>
<td>11/23</td>
<td>20/70 11/20</td>
<td>21/58 10/31</td>
<td>4/17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RA duration, years</td>
<td>10 (0-60)</td>
<td>10 (0-60)</td>
<td>8.5 (0-46)</td>
<td>11 (0-60) 6 (0-35)</td>
<td>10 (0-60) 6 (0-39)</td>
<td>9 (1-35)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoking, n (%)</td>
<td>19/121 (16)</td>
<td>13/87 (15)</td>
<td>6/34 (18)</td>
<td>13/90 (14) 6/31 (19)</td>
<td>10/79 (13) 9/41 (22)</td>
<td>6/21 (29)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAQ</td>
<td>1.00 (0.00-2.63)</td>
<td>1.13 (0.00-2.63)</td>
<td>0.82 (0.00-2.50)</td>
<td>1.13 (0.00-2.63) 0.75 (0.00-2.50)</td>
<td>1.13 (0.00-2.63) 0.75 (0.00-2.63)</td>
<td>0.75 (0.00-2.63)</td>
<td></td>
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<tr>
<td>DAS28</td>
<td>4.33 (2.00-7.64)</td>
<td>4.50 (2.01-7.64)</td>
<td>3.96 (2.00-6.23)</td>
<td>4.52 (2.01-7.64) 3.96 (2.00-6.23)</td>
<td>4.60 (2.01-7.64) 3.97 (2.00-6.33)</td>
<td>3.86 (2.00-5.60)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP, μg/l</td>
<td>11 (0-159)</td>
<td>11 (1-159)</td>
<td>9.5 (0-80)</td>
<td>11.5 (1-159) 8 (0-107)</td>
<td>16 (1-159) 6* (0-85)</td>
<td>7 (0-71)</td>
<td></td>
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</tr>
<tr>
<td>Larsen-Dale index</td>
<td>1 (0-5)</td>
<td>1 (0-5)</td>
<td>1 (0-5)</td>
<td>1 (0-5) 1 (0-5)</td>
<td>1 (0-5) 1 (0-5)</td>
<td>1 (0-5)</td>
<td></td>
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</tr>
<tr>
<td>S-cathepsin L, μg/l</td>
<td>7.32 (1.54-102.82)</td>
<td>7.54 (1.54-102.82)</td>
<td>5.21 (1.58-47.10)</td>
<td>8.31 (1.54-102.82) 4.50 (1.58-47.10)</td>
<td>8.18 (1.54-102.82) 5.06 (1.58-22.46)</td>
<td>4.36 (1.58-19.02)</td>
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<tr>
<td>SF-cathepsin L, μg/l</td>
<td>12.35 (1.24-97.66)</td>
<td>14.28 (2.32-97.66)</td>
<td>10.16 (1.24-42.14)</td>
<td>13.80 (2.32-97.66) 10.02 (1.24-55.82)</td>
<td>13.56 (2.32-97.66) 10.09 (1.24-42.14)</td>
<td>8.77 (1.24-42.14)</td>
<td></td>
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</tr>
<tr>
<td>S-cathepsin S, μg/l</td>
<td>12.60 (5.08-68.52)</td>
<td>13.24 (5.08-68.52)</td>
<td>11.02 (7.08-61.32)</td>
<td>13.46 (5.08-68.52) 10.16 (7.08-22.16)</td>
<td>14.00 (6.32-68.52) 11.24 (5.08-23.68)</td>
<td>10.44 (7.12-18.24)</td>
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<tr>
<td>SF-cathepsin S, μg/l</td>
<td>29.40 (5.70-93.05)</td>
<td>28.05 (5.70-93.05)</td>
<td>29.80 (10.40-71.95)</td>
<td>29.35 (5.70-93.05) 29.45 (10.40-71.95)</td>
<td>32.80 (5.70-93.05) 26.05 (7.07-51.80)</td>
<td>28.88 (10.40-51.80)</td>
<td></td>
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<tr>
<td>S-MMP3, μg/l</td>
<td>0.14 (0.02-116.71)</td>
<td>0.15 (0.02-116.71)</td>
<td>0.12 (0.03-52.03)</td>
<td>0.14 (0.02-116.71) 0.12 (0.03-116.4)</td>
<td>0.15 (0.02-69.53) 0.11 (0.03-116.71)</td>
<td>0.12 (0.03-106)</td>
<td></td>
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<tr>
<td>SF-MMP3, μg/l</td>
<td>5.37 (0.06-143.90)</td>
<td>4.67 (0.06-143.90)</td>
<td>8.06 (0.06-51.86)</td>
<td>5.06 (0.06-143.90) 7.96 (0.25-52.93)</td>
<td>5.34 (0.06-143.60) 7.14 (0.25-51.86)</td>
<td>8.85 (0.25-51.86)</td>
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</tbody>
</table>

The values represent median (range). The significance levels are shown as *P < 0.05, **P < 0.01, ***P < 0.001 when comparing patients with autoantibodies and those without and when comparing serum and SF shown as ¹P < 0.05, ²P < 0.01, ³P < 0.001. Data concerning IgA RF was missing for one patient. S: serum.
<table>
<thead>
<tr>
<th>Age</th>
<th>Disease duration</th>
<th>Larsen score</th>
<th>HAQ</th>
<th>DAS28</th>
<th>CRP</th>
<th>s-Cath S</th>
<th>s-Cath L</th>
<th>s-MMP3</th>
<th>SF-Cath S</th>
<th>SF-Cath L</th>
<th>SF-MMP3</th>
<th>ACPA</th>
<th>IgM-RF</th>
<th>IgA-RF</th>
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<tr>
<td>0.110</td>
<td>0.854</td>
<td>0.277**</td>
<td>0.220*</td>
<td>0.266**</td>
<td>0.286**</td>
<td>0.333**</td>
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<td>0.278</td>
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</table>

Results of Spearman rank correlation test are shown as correlation coefficient ($r$). For each pair of variables (except for the autoantibodies themselves), the upper value represent all patients, the middle value ACPA-positive patients, and the lower value ACPA-negative patients. For autoantibodies, only the correlation values for all patients are shown. The significance level is indicated as *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. 

Cathepsin S and cathepsin L in serum and SF in RA
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