Synovial fibronectin fragmentation and domain expressions in relation to rheumatoid arthritis progression

M. Przybysz, K. Borysewicz, J. Szechiński and I. Kaźnik-Prastowska

Objective. To determine whether the expression of some fibronectin (FN) domains and a degree of FN degradation are associated with the progression of rheumatoid arthritis (RA).

Methods. Based on radiographs of the hands of RA patients, three groups of synovial fluid and plasma samples were distinguished: (i) those with early radiological changes, (ii) established and (iii) late progressive radiological changes. The expressions of FN domains were determined by ELISA using appropriate domain-specific monoclonal antibodies. FN fragmentation was analysed by immunoblotting.

Results. In the early RA group, synovial FN was found to be totally degraded to a mixture of FN fragments. In the established group, it consisted of a portion of intact FN molecules and a smaller part of FN fragments, whereas in the late group the synovial FN immunoblotting pattern was similar to that of intact FN. The FN fragmentation was accompanied by decreases in FN immune reactivity with monoclonal antibodies specific to the collagen, fibrin and C-terminal FN domains. In the blood plasma of all studied groups of RA patients, the FN immunopattern was analogous to that in normal plasma. However, the expressions of the plasma FN domains were higher than those of healthy individuals.

Conclusions. Profound degradation of FN and low collagen, fibrin and C-terminal domain expressions in FN were only associated with early destructive changes observed in radiographs of the RA patients’ hands.

KEY WORDS: Fibronectin, Fibronectin fragments, Fibronectin domains, Rheumatoid arthritis, Synovial fluid.

Introduction

Fibronectin (FN) is a macromolecular adhesion glycoprotein abundant in the extracellular matrix (ECM) of connective tissues as well as in body fluids. It generally originates from various cells (e.g. fibroblasts, chondrocytes), but plasma FN is synthesized by liver hepatocytes. The FN found in the extracellular matrix and on the surface of cells is as an insoluble multimeric fibrillar component, whereas that present in body fluids is a soluble, compact globular dimer. There is some evidence that plasma FN diffuses from the blood or body fluids into connective tissues, where it is incorporated into fibrillar aggregates as multimeric FN (reviewed in [1]). The conformational equilibrium between both forms is suggested to play a key role in maintaining of the normal balance of ECM component synthesis and degradation [2, 5].

Structurally, FN is a homo- or heterodimer of two 200–250-kDa subunits cross-linked at their C-termini by disulphide bonds. Each FN monomer is composed of repeating modules which are organized into several independently folded globular and functional domains as well as extra, alternatively spliced ED-A, ED-B and III-CS domains (reviewed in [1, 6]) and three cartilage specific segments [7]. The FN domains are known to form binding sites for a number of ligands, such as cell-surface receptors [8], collagen [9], fibrin [10], heparin and other glycosaminoglycans [11]. Because of the presence of so many domains, FN is an important functional component of the ECM and is largely responsible for a variety of cellular processes, including adhesion, migration, proliferation, transformation, matrix remodelling, tissue repair, wound healing and homoeostasis [6, 12].

Traditionally, the diagnosis of the rheumatoid arthritis (RA) and the degree of disease activity are evaluated by measuring subjective clinical variables, laboratory tests and radiographic findings [13, 14]. FN, which facilitates a wide range of processes vital to homoeostasis and tissue repair is a candidate as a biomarker for the arthritic disease process and its analysis could apparently be important for patient management. In the synovial fluid and plasma of osteoarthritis and RA patients, the FN level has been found to increase 3- to 4-fold [15]. Moreover, FN degradation products, identified in RA, osteoarthritis and traumatic and septic arthritis synovial fluids, have been reported either to enhance catabolic damage activities or possibly to be involved in normal cartilage homoeostasis (reviewed in [16–18]).

Because of reported experimental evidence [2–4, 19, 20] for a very large elasticity of the FN molecule and in view of the hypothesis that conformational changes precede its function, we were interested in analysing the expressions of the cellular (CellFN), collagen (CollagenFN), fibrin (FibrinFN) and C-terminal (cTFN) FN domains (Fig. 1) in the synovial fluid and blood plasma of RA patients. The FN domain concentrations were studied in relation to the degree of synovial FN degradation as well as RA progression. The FN domain concentrations were determined by enzyme-linked immunoassays (ELISA), which was performed based on FN epitope accessibility for immune reaction with domain-specific monoclonal antibodies. Western immunoblotting of FN using of a monoclonal antibody directed to its central cellular domain allowed us to analyse the range and degree of FN fragmentation in synovial fluid.

Material and methods

Patients and samples

Fifty eight patients (21–78-yrs-old, mean age 52 ± 14), who were attending the Rheumatology Clinic of the Wrocław Medical University and who fulfilled the 1987 American Rheumatism Association criteria for RA [21] were included in the study after their informed consent had been given. The study was approved by the local ethics committee (approval no 222/2002). All patients were evaluated from the results of clinical examination, plain
X-rays of the hands and routine laboratory blood plasma parameters. The duration of disease was from 2 months to 20 yrs. Patients who had traumatic, septic or microcrystalline arthritis, previous joint surgery or isotopic synovectomy within the 12 months before the study were excluded.

Synovial fluid and blood were drawn from RA patients into plastic syringes and placed directly into tubes containing 1 mM phenylmethylsulphonyl fluoride (PMSF, Sigma Chemical Co., St Louis, MO, USA). The samples were centrifuged, and the synovial fluid supernatants and blood plasma were stored in small aliquots at −78°C until used.

With respect to the radiographic outcome by scoring the X-rays of the patients’ hands [22], the RA synovial fluid and blood plasma samples were divided into three groups:

(i) Early group (15 synovial fluids and 15 plasmas): the patients had early radiological changes, described as soft-tissue swelling symmetrically around the joints involved, juxta-articular, subtle osteoporosis and small erosions of the 'bare' areas of bone. All patients suffered from 2 months up to 2 yrs and 60% of them had CRP concentration <5 mg/l and 47% were RF positive;

(ii) Established group (16 synovial fluids and 25 plasmas): the radiological changes were as in early RA, but the erosions were established. The patients suffered from 4 months to 20 yrs and 70% of patients suffered >2 yrs. CRP concentration was <5 mg/l in 66% of samples, and 34% of samples were RF positive;

(iii) Late group (11 synovial fluids and 18 plasmas): the patients had progressive radiological changes such as joint-space loss, decrease in soft-tissue swelling, diffuse osteoporosis, large subchondral erosions, subluxation and fibrous ankylosis of the digits. All patients suffered >2 yrs. CRP concentration was <5 mg/l in 34% of samples and 66% were RF positive.

A normal group was formed by the blood plasma collected from 46 healthy individuals, Wroclaw Medical University research workers, 20–57 yrs-old, mean age 45 ± 9, median age 45 yrs.

**Quantification of FN domain expression**

The expressions of the cellular (CellFN), collagen (CollagenFN), second fibrin (FibrinFN) and C-terminal region (CTFN) in the FN molecule (Fig. 1) were determined based on the immunoreactivity of conformationally accessible epitopes on FN with the four specific well-defined monoclonal antibodies (TkAkaRa, Shuzo Co., Shiga, Japan), anti-CellFN (FN 30-8; M010), anti-CollagenFN (FN 30-8; M114), anti-Second FibrinFN (FN 8-12; M013) and anti-CTFN (FN 1-1; M001), by the use of four kinds of antibodies (TaKaRa, Shuzo Co., Shiga, Japan), anti-CellFN (FN 30-8; M010), anti- CollagenFN (FNC4-4; M114), anti-FibrinFN (FN 8-12; M001) and anti-CTFN (FN 1-1; M013), by the use of four antibodies (Awareness Technology Inc, USA) at 450 nm with 630 nm as a reference filter. All ELISA immunobinding and washing steps were carried out in TBS containing 0.1% Tween 20, pH 7.3. A human plasma FN preparation (Sigma Chemical Co., from 10 to 50 ng/well) was used as a standard for determining CellFN, CollagenFN, FibrinFN and CTFN, and the results were given in microgram per millilitre.

**Western immunoblotting**

Synovial fluid FN (300 ng), determined by CellFN-ELISA as described above, was subjected to SDS polyacrylamide gel electrophoresis (SDS-PAGE) on a 7.5% gel under reducing conditions. After electrophoresis, the separated proteins were blotted onto nitrocellulose (Serva Electrophoresis GmbH, Heidelberg, Germany) as described earlier [24]. The blots were developed with mouse monoclonal antibody anti-CellFN (FN 30-8, code M010, TaKaRa, Japan), diluted 1:10 000 in 5% casein in TBS and then with rabbit anti-mouse immunoglobulins conjugated to horseradish peroxidase (Sigma, 1:10 000 dilution in 5% casein in TBS). The colour reaction was developed with dianisobenzidine. Then the blots were dried and analysed. Bands corresponding to the particular proteins were scanned and analysed by densitometry using Sigma Gel (Gel Analysis Software version 1.0, Jandel Scientific Software, San Rafael, Canada). The molecular weights of the FN bands were determined using BioRad molecular-weight protein standards and intact human FN (MW 230 kDa; Sigma Chemical Co.).

The relative amounts of the particular FN fragments were expressed as the percentage of the total number of pixels in a lane. For this purpose, the blots were scanned and analysed by densitometric Sigma Gel Spots mode Measurements (Jandel Scientific Software).

**Statistics**

Data are presented as means ± standard deviations (s.d.). Comparisons between groups were performed by means of the Mann–Whitney U-test. The P-values <0.05 were regarded as significant.

**Results**

The expression of FN domains

The mean value of the CellFN, CollagenFN, FibrinFN and CTFN concentrations in the synovial fluid and blood plasma samples of the RA patients in relation to RA progression are given in Tables 1 and 2, respectively. The expression of synovial CellFN did not show any differences among the early (1081 ± 160 μg/ml) and late (1009 ± 117 μg/ml) RA groups. In contrast, the concentrations of CollagenFN (40 ± 23 μg/ml), FibrinFN (22 ± 10 μg/ml) and CTFN (36 ± 64 μg/ml) were significantly lower in the early RA synovial group than those in the established (198 ± 96 μg/ml; 40 ± 12 μg/ml; 393 ± 266 μg/ml, respectively) and late (304 ± 21 μg/ml; 49 ± 7 μg/ml; 500 ± 257 μg/ml, respectively) groups. Moreover, the concentration of CollagenFN was significantly lower in the established group than that in the late group (Table 1).
In the blood plasma of the RA patients of the early, established and late RA groups, the concentrations of CollagenFN (341 ± 70 μg/ml, 344 ± 120 μg/ml and 331 ± 160 μg/ml, respectively), FibrinFN (335 ± 98 μg/ml, 374 ± 149 μg/ml and 334 ± 174 μg/ml, respectively) and CTFN (696 ± 205 μg/ml, 760 ± 175 μg/ml and 692 ± 247 μg/ml, respectively) did not show any fluctuation when the data were analysed with respect to RA progression (Table 2). However, the FN domain expressions were significantly higher in the RA groups than those in the normal plasma group (CollagenFN: 240 ± 84 μg/ml; FibrinFN: 200 ± 69 μg/ml and CTFN: 517 ± 141 μg/ml). Only the plasma CollFN concentration was at the same level in the RA samples and in normal plasma.

The degree of FN fragmentation

Figure 2 shows representative immunoblot patterns of plasma (lanes 1 and 8) and synovial FN (lanes 2–7), and the mean values of the relative amounts of particular FN bands are given in Table 3. All plasma samples derived from healthy individuals (Fig. 2, lane 1) and RA patients (Fig. 2, lane 8) showed wide FN bands (pattern D), whose molecular weights ranged from 230 to 240 kDa. In contrast, synovial FN underwent extensive degradation and the degree of FN fragmentation was associated with RA progression (Fig. 2, lanes 2–7). In the early RA group, 80% of the synovial fluid samples (Fig. 2, lane 2, Table 3, pattern A) contained FN degraded up to eight FN fragments having molecular weights from 65 to 150 kDa. We called this subgroup A, or totally degraded, because no intact FN band was visible. The remaining 20% of early synovial samples (Fig. 2, lane 3 and Table 3, pattern B) was partly degraded: half of the molecules were intact and the remainder consisted of five FN fragments, with molecular weights ranging from 100 to 200 kDa.

In the established group, most of the synovial samples (94%) contained a portion (68%) of intact FN (bands 240 and 230 kDa) and a portion of degraded FN up to five fragments showing molecular weights from 100 kDa to 200 kDa (Fig. 2, lane 4 and Table 3, pattern B). Only one synovial sample from the established group (Fig. 2, lane 5, Table 3, pattern A) had totally degraded FN, and its immunopattern as well as the relative amounts of fragments were similar to those demonstrated for early RA subgroup A.

In the late RA group (Fig. 2, lanes 6 and 7 and Table 3, patterns C and D), all 11 synovial samples contained wide FN bands of 230–240 kDa corresponding to the intact FN molecule and three of them revealed additionally one FN fragment having a molecular weight of 200 kDa (Fig. 2, lane 6, pattern C). The FN domain expressions were also compared in relation to the degree of the synovial FN degradation i.e. subgroup A vs subgroup B. As is shown in Fig. 3, the CTFN expression was strongly associated with the degree of FN degradation. Its concentration was significantly lower (P < 0.00003) in samples of early and established synovial groups showing pattern A (10 ± 3 μg/ml) when compared with those showing pattern B (373 ± 255 μg/ml). In contrast, the concentrations of CollagenFN, FibrinFN and CellFN in both subgroups A and B of early and established groups were nearly at the same level (data not shown).
collagen, fibrin and C-terminal FN domains. Particularly, CTFN therefore they react weakly with single determinant on degraded the epitopes on repeating domains of a non-reduced FN and the collagen, fibrin and C-terminal domain concentrations, the FN domain. The degradation of FN resulted in the decreases in fragments of 65–85 kDa represented a partly degraded cellular FN domain seems to be independent of the degree of FN degradation and seems to be a promising marker of matrix destruction and the release of degradation products of FN and other ECM proteins [26–30].

In RA, before the development of clinical signs and symptoms, infiltration of the synovium by macrophages and T cells as well as of the regional lymph nodes by a number of CD8+ and B cells has been reported (reviewed in [25]). Highly activated inflammatory cells are able to produce and transiently release proinflammatory cytokines, nitric oxide, arachidonic acid metabolites, as well as matrix metalloproteinases. All these factors penetrate cartilage leading to a cascade of events promoting inflammation as well as matrix destruction and the release of degradation products of FN and other ECM proteins [26–30].

In the present study, the appearance of FN fragments was monitored by a highly specific monoclonal antibody directed towards the largest of others, central and relatively conforma-
tionally stable, cellular domain of FN [31]. Moreover, we chose this monoclonal antibody, because the concentration of the cellular FN domain seems to be independent of the degree of FN degradation and of RA phase. Our results indicate that the synovial FN in the early RA group mainly consisted of FN fragments with molecular weights in the range of 65–200 kDa and a portion of native FN only in some cases (Fig. 2 and Table 3, lanes 2 and 3). Since the molecular weight of the cellular FN domain is reported to be 95–105 kDa [32], the FN fragments of 130–200 kDa (Fig. 2, Table 3) were probably partly degraded FN, although with an intact cellular domain. In contrast, the FN fragments of 65–85 kDa represented a partly degraded cellular FN domain. The degradation of FN resulted in the decreases in the collagen, fibrin and C-terminal FN domain concentrations, but not in cellular FN domain (Table 1). In our opinion, these differences are not only resulted from a degree of FN degradation, but also of the specificity of used monoclonal antibody. Anti-CTFN is able to react with epitopes built from repeating modules type III, located on reduced and non-reduced FN. Thus, its level remained relatively constant and did not change, even when the FN was degraded. In contrast, anti-Collagen/FN, anti-Fibrin/FN and anti-CTFN antibodies are known to react with the epitopes on repeating domains of a non-reduced FN and therefore they react weakly with single determinant on degraded collagen, fibrin and C-terminal FN domains. Particularly, CTFN concentration decreases along with higher degradation of a molecule (Fig. 3). Monoclonal antibody anti-CTFN is known to recognize the epitope, sensitive for conformational changes, located at the C-terminal region of FN, where two polypeptide chains of FN are bound together by disulphide bonds. In our opinion, the CTFN level reflects the degree of FN degradation and seems to be a promising marker of matrix degradation.

The FN fragments are thought to have diverse activities in various types of cells and they could have properties not shared by the native molecule. FN fragments comprising an intact cellular domain are able to mediate cell adhesion processes through transmembrane integrins [33], whereas those which are partly degraded could mimic integrin-binding activity and/or inhibit the activity of the intact FN molecule [12]. FN fragments are suggested to act as inducers of cartilage destruction in osteoarthritis and RA [26, 28, 32, 34, 35]; however, they also take part in the regulation processes of some matrix metalloproteinase syntheses [9, 36–38], monocyte migration and stimulation of tumour necrosis factor secretion [18]. It was shown in in vitro experiments that a high concentration of FN fragments initially

### Table 3. Relative amounts of FN fragments in synovial fluid of RA patients

<table>
<thead>
<tr>
<th>Mw of FN bands (kDa)</th>
<th>Early n = 15</th>
<th>Established n = 16</th>
<th>Late n = 11</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lane 2 A: n=12 (80%)</td>
<td>Lane 3 B: n=3 (20%)</td>
<td>Lane 4 B: n=15 (94%)</td>
</tr>
<tr>
<td>240</td>
<td>28.5 ± 1.9</td>
<td>36.9 ± 9.1</td>
<td>33.6 ± 0.4</td>
</tr>
<tr>
<td>230</td>
<td>23.9 ± 0.2</td>
<td>30.8 ± 5.0</td>
<td>34.5 ± 0.3</td>
</tr>
<tr>
<td>200</td>
<td>20.4 ± 0.9</td>
<td>15.0 ± 4.0</td>
<td>31.9 ± 0.2</td>
</tr>
<tr>
<td>170</td>
<td>11.4 ± 1.2</td>
<td>8.0 ± 4.3</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>16.8 ± 2.1</td>
<td>1.6 ± 0.9</td>
<td>13.1</td>
</tr>
<tr>
<td>130</td>
<td>8.0 ± 1.5</td>
<td>5.1 ± 5.1</td>
<td>1.1</td>
</tr>
<tr>
<td>100</td>
<td>22.5 ± 1.3</td>
<td>2.3 ± 2.4</td>
<td>18.4</td>
</tr>
<tr>
<td>90</td>
<td>16.8 ± 1.4</td>
<td></td>
<td>14.5</td>
</tr>
<tr>
<td>85</td>
<td>16.7 ± 0.5</td>
<td></td>
<td>15.2</td>
</tr>
<tr>
<td>80</td>
<td>9.1 ± 2.1</td>
<td></td>
<td>14.1</td>
</tr>
<tr>
<td>73</td>
<td>5.2 ± 0.9</td>
<td></td>
<td>3.4</td>
</tr>
<tr>
<td>65</td>
<td>11.0 ± 3.4</td>
<td></td>
<td>20.2</td>
</tr>
</tbody>
</table>

FN bands shown in Fig. 2 (lanes 1–7) were scanned and analysed by densitometry SigmaGel Spots Mode Measurements (Jandel Scientific Software). The relative amount of particular FN fragments was expressed as the percentage of the total number of pixels in a lane. ‘n’ indicates the number (in parenthesis, its percentage) of synovial samples showing a similar FN immunopattern.

### Discussion

The most important results emerging from this study are the differences found in the expressions of some FN domains with respect to the degree of FN degradation and in relation to RA progression. Profound degradation of FN and low collagen, fibrin and C-terminal domain expressions on FN were mainly associated with early degenerative changes observed in radiographs of the RA patients’ hands.

In RA, before the development of clinical signs and symptoms, infiltration of the synovium by macrophages and T cells as well as of the regional lymph nodes by a number of CD8+ and B cells has been reported (reviewed in [25]). Highly activated inflammatory cells are able to produce and transiently release proinflammatory cytokines, nitric oxide, arachidonic acid metabolites, as well as matrix metalloproteinases. All these factors penetrate cartilage leading to a cascade of events promoting inflammation as well as matrix destruction and the release of degradation products of FN and other ECM proteins [26–30].

In the present study, the appearance of FN fragments was monitored by a highly specific monoclonal antibody directed towards the largest of others, central and relatively conformationally stable, cellular domain of FN [31]. Moreover, we chose this monoclonal antibody, because the concentration of the cellular FN domain seems to be independent of the degree of FN degradation and of RA phase. Our results indicate that the synovial FN in the early RA group mainly consisted of FN fragments with molecular weights in the range of 65–200 kDa and a portion of native FN only in some cases (Fig. 2 and Table 3, lanes 2 and 3). Since the molecular weight of the cellular FN domain is reported to be 95–105 kDa [32], the FN fragments of 130–200 kDa (Fig. 2, Table 3) were probably partly degraded FN, although with an intact cellular domain. In contrast, the FN fragments of 65–85 kDa represented a partly degraded cellular FN domain. The degradation of FN resulted in the decreases in the collagen, fibrin and C-terminal FN domain concentrations, but not in cellular FN domain (Table 1). In our opinion, these differences are not only resulted from a degree of FN degradation, but also of the specificity of used monoclonal antibody. Anti-CTFN is able to react with epitopes built from repeating modules type III, located on reduced and non-reduced FN. Thus, its level remained relatively constant and did not change, even when the FN was degraded. In contrast, anti-Collagen/FN, anti-Fibrin/FN and anti-CTFN antibodies are known to react with the epitopes on repeating domains of a non-reduced FN and therefore they react weakly with single determinant on degraded collagen, fibrin and C-terminal FN domains. Particularly, CTFN concentration decreases along with higher degradation of a molecule (Fig. 3). Monoclonal antibody anti-CTFN is known to recognize the epitope, sensitive for conformational changes, located at the C-terminal region of FN, where two polypeptide chains of FN are bound together by disulphide bonds. In our opinion, the CTFN level reflects the degree of FN degradation and seems to be a promising marker of matrix degradation.

The FN fragments are thought to have diverse activities in various types of cells and they could have properties not shared by the native molecule. FN fragments comprising an intact cellular domain are able to mediate cell adhesion processes through transmembrane integrins [33], whereas those which are partly degraded could mimic integrin-binding activity and/or inhibit the activity of the intact FN molecule [12]. FN fragments are suggested to act as inducers of cartilage destruction in osteoarthritis and RA [26, 28, 32, 34, 35]; however, they also take part in the regulation processes of some matrix metalloproteinase syntheses [9, 36–38], monocyte migration and stimulation of tumour necrosis factor secretion [18]. It was shown in in vitro experiments that a high concentration of FN fragments initially...
suppressed the cartilage proteoglycans synthesis, while a low concentration enhanced it [17].

After the damage phase, profound metabolic changes have been described, e.g. a decreasing cytokine profile, an increase in proteoglycan synthesis and enhanced anabolic factor release. The enhanced anabolic reparative phase triggered by the damage makes cartilage more refractory to subsequent damage [25, 29]. With progression of disease, we observed that the majority of the synovial samples of established group of RA patients represented a mixture of FN forms consisting of a major portion of intact FN molecules and a smaller portion of FN fragments (Fig. 2, lanes 4 and 5: Table 3). Finally, in the late RA, in contrast to the early and established RA groups, the FN fragments disappeared and the immunoblotting pattern of synovial FN (Fig. 2, lane 7) was similar to that of native FN (Fig. 2, lane 1). That was accompanied by several times higher (5–7 times, 2–2.5 times and 11–14 times, respectively) collagen, fibrin and C-terminal FN domain immuno-reactivities with the respective specific monoclonal antibodies than those found in the early group (Table 1). At the longstanding stage of disease, with persist inflammation, apart from the still active destructive process, FN seems to participate in repair mechanisms. It has been postulated that fibrillogenesis occurs in the presence of cell. The cells assemble a FN molecule in unfolded, stretched form. The cell-derived extension of FN molecule could expose cryptic binding sites for other molecules, including itself, dimer and fragments. As assembly progresses, cell-associated FN fibrils are able to interact together with a fibrin, forming fibrillar aggregates [1, 3, 6]. They are subsequently incorporated into matrices cross-linked with fibrin, where they are known to form a multimeric FN-fibrin network adhering to the synovial surface [39]. FN polymerization is claimed to regulate the composition and stability of ECM fibrils and cell–matrix adhesion [40].

In conclusion, the alterations described here in the degree of synovial FN degradation and some of its domain expressions occurred only locally in joint tissues, the primary site of the pathology. In the blood plasma of RA patients, FN did not undergo fragmentation, and its collagen, fibrin and C-terminal FN domain expressions, although higher than those of normal plasma FN, were not associated with RA progression. The increases in plasma FN domain expressions in RA probably reflected the increased total FN concentration resulting from enhanced FN synthesis by liver cells as a response to inflammation. Our data suggest that synovial FN undergoes dynamic conformational changes associated with the activity of RA. In consequence, this may lead to different FN roles throughout the development and stabilization of disease. The analysis of a degree of synovial FN fragmentation and its collagen, fibrin and C-terminal FN domain expressions can provide clinically applicable data in rheumatological investigations.

The authors have declared no conflicts of interest.

## References


