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CARACTERITZACIÓ DE LA SINOVITIS MITJANÇANT MÈTODES ARTROSCÒPICS I IMMUNOHISTOQUÍMICS. VALOR DIAGNÒSTIC I PRONÒSTIC

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Synovial vascular patterns and angiogenic factors expression in synovial tissue and serum of patients with rheumatoid arthritis

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***Objective*. To determine whether subgroups of rheumatoid arthritis (RA) patients classified according to their synovial vascular pattern have a different expression of angiogenic mediators or exhibit distinct clinical or biological characteristics.**
***Methods*. Arthroscopies were performed in 27 patients with RA and synovial samples were obtained. Vascular morphology was classified in three patterns: straight (S), tortuous (T) and mixed (M). Immunostaining was performed with anti-vascular endothelial growth factor (anti-VEGF), anti-vascular endothelial growth factor receptor (VEGFR)-1, anti-VEGFR-2, anti-IL-8 and anti-TGF-β, and measured by digital image analysis. Serum levels of VEGF, TGF-β and IL-8, and clinical, radiographic and serological data were also analysed.**
***Results*. Eleven (41%) patients had the S pattern, nine (33%) the M pattern and seven (26%) the T pattern. The S and M groups had a higher prevalence of rheumatoid factor positivity and erosive disease, and higher levels of markers of systemic inflammation compared with the T group. Synovial expression of VEGF was higher in the S and T groups compared with the M group, whereas TGF-β was higher in the T compared with the S and M groups. Distinct synovial distribution of VEGF and TGF-β between groups was also observed.**
***Conclusions*. This preliminary study suggests that RA patients with the S and M patterns share different clinical, biological and serological characteristics compared with those with the T pattern, which may constitute a group with less severe disease. Differences in the intensity and distribution of synovial expression of VEGF and TGF-β observed between groups could have pathophysiological relevance. However, larger, prospective multicentre studies would be need to determine the clinical relevance of vascular patterns in RA.**

KEY WORDS: Synovial tissue, Arthroscopy, Vascular pattern, Angiogenesis, Rheumatoid arthritis.

Rheumatoid arthritis (RA) is a chronic immuno-inflammatory disease that primarily involves synovium. Thus, the study of synovial tissue samples may provide insight into the pathogenesis of the disease and the efficacy of treatments [1].

RA is a clinically, genetically and histologically heterogeneous disease, and recent data also show pathophysiological differences between patients [2]. Therefore, identification of new variants of RA may have prognostic and therapeutic implications.

The systematic study of rheumatoid synovitis has revealed that, although there is increased cellularity in the synovial lining layer and an over-representation of plasma cells and macrophages in the sublining [3], these changes are not specific for RA, some studies finding no differences in these synovial components between RA and psoriatic arthritis (PsA) [4, 5].

RA synovitis is characterized by overexpression of Th1-derived cytokines, whereas in spondyloarthropathies (SpA) Th2 or Th0 patterns predominate [6]. Furthermore, the macroscopic appearance of rheumatoid synovium displays some differences compared with SpA. Systematic descriptions of synovial vascular morphology have been performed using rheumatological arthroscopy and show that RA patients predominantly exhibit a straight (S) pattern, whereas in SpA patients a tortuous (T) pattern predominates [7, 8]. However, the T and mixed (M) patterns have also been described in RA patients [9, 10]. These distinct synovial vascular patterns could reflect different pathogenic mechanisms of angiogenesis among RA and SpA synovitis [9], but possibly also among RA patients with different vascular patterns.

Angiogenesis or vascular proliferation has a pivotal role in the pathogenesis of RA, determining both the hyperplasic and the destructive character of synovium [11]. Angiogenesis is regulated by the imbalance between pro-angiogenic and anti-angiogenic factors [12], and occurs early in the course of synovitis [13, 14]. Angiogenic mediators such as vascular endothelial growth factor (VEGF), transforming growth factor (TGF)-β, interleukin (IL)-8, tumour necrosis factor (TNF)-α and IL-1β have all been demonstrated in RA synovium and implicated in the pathogenesis of joint destruction [15].

Previous studies have reported an association between the T pattern and the expression of angiogenic mediators in PsA [13, 14], but studies analysing the association between the different synovial vascular patterns reported in RA patients and the expression of angiogenic factors are lacking.

Stratification of RA patients according to their different vascular patterns could identify RA subsets with differing prognoses. This

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could have therapeutic implications, as angiogenic factors or cytokines involved in angiogenesis, such as TNF-α, have been shown to be potential therapeutic targets in inflammatory arthritis [16].

The aim of this study was to determine whether different subsets of RA patients classified by their synovial vascular pattern exhibit clinical or biological differences or a distinct expression of angiogenic markers in synovial tissue or serum.

Patients and methods

Patients

All consecutive patients meeting the ACR criteria for RA [17] who underwent a diagnostic or therapeutic arthroscopy in the Arthritis Unit of the Hospital Clinic of Barcelona between November 1999 and April 2001 were included. All 27 patients had active synovitis, with inflammatory knee or wrist synovial effusion. Parameters analysed at the time of the arthroscopy were: sex, age, disease duration, DMARDs, number of DMARDs taken, NSAIDs, oral steroids, rheumatoid factor (RF), erosive disease, ESR, CRP and IL-6 serum levels. The study was approved by the Ethics Committee of the Hospital Clinic of Barcelona.

Arthroscopy and biopsy handling

An arthroscopy was performed in all patients under local anaesthesia at the Rheumatological Arthroscopy Unit. All patients gave informed consent. A 2.7 mm diameter arthroscope (Storz, Tuttlingen, Germany) was used. The joint was systematically explored in all cases and images video-recorded using a computerized analysis system (Studio PCTV; Pinnacle Systems, Braunschweig, Germany). Vascular patterns were assigned according to the distribution of vascular morphology throughout the joint and classified in three patterns: straight (S), tortuous (T) and mixed (M) as described previously [9]. Eight synovial samples were obtained at each arthroscopy from the suprapatellar pouch and the medial gutter. Biopsy specimens were fixed in 4% formaldehyde and embedded in paraffin.

Immunohistochemical analysis

Paraffin sections were subjected to antigen retrieval in a pressure cooker when necessary. Slides were immunostained using an automated immunostainer (TechMate 500 Plus; Dako, Carpinteria, CA, USA). Serial sections were incubated with the following antibodies: anti-VEGF (polyclonal goat IgG; R&D Systems, Abingdon, UK), anti-VEGF receptor (VEGFR)-1 (flt-1) (polyclonal rabbit IgG; NeoMarkers, Fremont, CA, USA); anti-VEGFR-2 (KDR/flk-1) (polyclonal rabbit IgG; NeoMarkers); anti-TGF-β (polyclonal goat IgG; R&D Systems) and anti-IL-8 (polyclonal goat IgG; R&D Systems). Primary antibodies were detected by an avidin–biotin–peroxidase-based method (EnVision system, Dako). Peroxidase activity was developed with 0.05% hydrogen peroxide and aminoethylcarbazole (Sigma, St Louis, MO, USA). Slides were counterstained with haematoxylin. Preliminary studies in human tonsils, placenta and angiosarcoma tissue were used to optimize antibody concentrations. Negative controls included mouse or rabbit IgG, or normal goat serum, at the highest concentration of primary antibodies.

Digital image analysis

After immunohistochemical staining, all coded slides were subjected to digital image analysis (DIA) by an independent observer (B.G-T.) who was blinded to the clinical data. For all markers, three separate representative regions were chosen to evaluate each section. At least five high-power fields were measured from each region using the AnalySIS^{B} Image processing program (Olympus®) as described previously in detail [18, 19]. We calculated the integrated optical density as the product of staining area and intensity corrected for the area of each section stained.

In addition, we studied the distribution of the immunostaining of every factor in different areas of synovial tissue (lining, endothelium, sublining, perivascular infiltrate).

ELISA of angiogenic factors in serum

Serum VEGF, TGF-β and IL-8 levels were determined at the time of arthroscopy, with a standard sandwich enzyme-linked immunoabsorbent assay (ELISA) (human quantikine, R&D Systems) using specific monoclonal and polyclonal antibodies, according to the manufacturer's protocols. For each analysis, 100 μl of serum was used. All the analyses were performed in duplicate. Sensitivity was 9 pg/ml for VEGF, 7 ng/ml for TGF-β and 10 pg/ml for IL-8.

Statistics

Differences between pairs of groups were analysed by the non-parametric Mann–Whitney U-test using SPSS version 11.0 software.

Results

Vascular patterns and clinical and demographic characteristics

Clinical and demographic characteristics are shown in Table 1. Twenty-seven consecutive RA patients were analysed. Disease duration (mean $\pm$ S.D.) was 60.3 $\pm$ 90 months, 28-joint disease activity score (DAS28) was 4.7 $\pm$ 1, 70.4% of patients were positive for RF, 40.7% had erosive disease, 19 out of 27 (70%) were being treated with DMARDs [15 with methotrexate (12.5–25 mg), two with methotrexate and infliximab, one with intramuscular gold and one with hydroxychloroquine] and had received 1.7 $\pm$ 2.1 DMARDs per patient. The DMARDs used were hydroxychloroquine, intramuscular gold, methotrexate, leflunomide, salazopyrine, cyclophosphamide, azathioprine and TNF-α antagonists.

Patients were classified in three subgroups according to their different vascular synovial patterns. Eleven out of 27 (41%) patients had the S pattern, nine (33%) the M pattern and seven (26%) the T pattern. RF was positive in 73 and 89% of patients in the S and M groups, respectively, in contrast to 43% in the T group, with a trend to significance ($P = 0.2$ and $P = 0.1$, respectively). Disease duration was significantly higher in the S (93.4 $\pm$ 110 months) than in the M (46.8 $\pm$ 81.5 months) and T (25.7 $\pm$ 48.5 months) groups ($p \leq 0.05$). Six out of seven patients with the T pattern were HLA-B27-negative. The only HLA-B27-positive patient was also RF-positive. There were no significant differences between the groups in DAS28 score or in the percentage of patients receiving DMARDs, NSAIDs or oral steroids at the time of arthroscopy. However, the total number of DMARDs received per patient showed a statistical trend to be higher in the S and M groups than in the T group ($P = 0.2$ and 0.15, respectively). Fifty-five per cent of RA patients in the S group had erosive disease compared with 44% in the M and 14% in the T group ($P = 0.04$ between the S and T groups and $P = 0.2$ between the M and T groups).

Inflammatory activity measured by CRP was significantly higher in the M group than in the T group ($P = 0.03$), and showed a strong trend to significance ($P = 0.07$) between the S and the T group. IL-6 serum values were significantly higher in the S and M groups than in the T group ($P < 0.02$) (Table 1).

Immunohistochemical evaluation (Table 2 and Fig. 1)

VEGF was localized predominantly in perivascular cells, sublining cells and, to a lesser extent, in the lining and endothelium. Using a

TABLE 1. Clinical, biological and serological factors of RA patients according to synovial vascular pattern

		Vascular pattern		
	All patients ($n=27$)	Straight ($n=11$)	Tortuous ($n=7$)	Mixed ($n=9$)
Female sex	16 (59.2%)	7 (63.6%)	4 (57.1%)	5 (55.6%)
Age (yr)	54 ± 15.7	52.2 ± 19.3	58.9 ± 7.6	52.5 ± 16.3
Disease				
Duration (months)	60.3 ± 90	93.4 ± 110.1[a]	25.7 ± 48.5[a]	46.8 ± 81.5[a]
DAS 28	4.66 ± 1.12	4.65 ± 0.93	4.24 ± 1.15	5 ± 1.31
Patients with DMARDs	19 (70%)	7 (63.6%)	4 (57.1%)	8 (88.8%)
Number of DMARDs received	1.7 ± 2.1	2.4 ± 2.7[b]	0.6 ± 0.5[b,c]	1.7 ± 1.7[c]
NSAIDs	24 (88.8%)	10 (90.9%)	6 (85.7%)	8 (88.8%)
Corticosteroids*	26 (96.3%)	10 (90.9%)	7 (100%)	9 (100%)
RF^+	19 (70.4%)	8 (72.7)[d]	3 (42.9)[d,e]	8 (88.9%)[e]
Erosive disease	11 (40.7%)	6 (54.5%)[f]	1 (14.3%)[f,g]	4 (44.4%)[g]
ESR (mm/h)	35.4 ± 30.8	37.4 ± 32	25.4 ± 18.4	40.5 ± 37.7
CRP (mg/dl)	2.3 ± 3.4	2.8 ± 4.6[h]	1 ± 1.3[h,i]	2.5 ± 2.7[i]
IL-6 (pg/ml)	92 ± 131.9	160.4 ± 178.9[j]	16.3 ± 14.4[j,k]	58.7 ± 47.6[k]

Values are mean ± S.E.M. or number (%). *Mean corticosteroid dose was equivalent to 5 mg prednisone.
[a] $P \leq 0.05$ between groups S and T or M; [b] $P=0.2$ between groups S and T; [c] $P=0.15$ between groups M and T; [d] $P=0.15$ between groups S and T; [e] $P=0.1$ between groups T and M; [f] $P=0.04$ between groups S and T; [g] $P=0.2$ between groups T and M; [h] $P=0.07$ between groups S and T; [i] $P=0.03$ between groups T and M; [j] $P=0.01$ between groups S and T; [k] $P=0.02$ between groups T and M.

TABLE 2. Serum levels and synovial tissue expression of angiogenic factors in RA patients according to synovial vascular pattern

	Vascular pattern		
	Straight ($n=11$)	Tortuous ($n=7$)	Mixed ($n=9$)
VEGF (S) pg/ml	284.8 ± 280.5[a]	190.8 ± 111.3	125.5 ± 75.4[a]
TGF-β (S) ng/ml	38.9 ± 15.3	38.3 ± 4.0	37.6 ± 8.0
IL-8 (S) pg/ml	13.2 ± 12.6	9.3 ± 6.3	20.4 ± 22.4
VEGF (ST) (IOD)	41 583 ± 12 115[b]	37 702 ± 14 941[c]	20 732 ± 13 266[b,c]
VEGFR-1 (ST) (IOD)	134 337 ± 164 234	116 415 ± 98 965	82 227 ± 60 485
VEGFR-2 (ST) (IOD)	166 618 ± 182 185	150 717 ± 79 643	149 883 ± 113 294
TGF-β (ST) (IOD)	61 698 ± 35 439[d]	128 895 ± 84 845[d,e]	103 664 ± 93 876[e]
IL-8 (ST) (IOD)	91 452 ± 71 776	59 985 ± 18 692[f]	127 145 ± 84 027[f]

All values are mean ± S.E.M. Angiogenic markers in synovial tissue (ST) are shown as integrated optical density (IOD).
S, serum.
[a] $P=0.1$ between groups S and M; [b] $P=0.008$ between groups S and M; [c] $P=0.08$ between groups T and M; [d] $P=0.04$ between groups S and T; [e] $P=0.10$ between groups T and M; [f] $P=0.1$ between groups M and T.

DIA technique, we detected significant differences in the mean VEGF integrated optical density (IOD) between the S and the M groups ($P=0.008$), and a statistical trend between the T and M groups ($P=0.08$). Interestingly, VEGF expression in the lining was observed in 36% of patients with the S pattern, in 11% with the M pattern and in no patient with the T pattern. However, VEGF expression in endothelium was somewhat more frequent in the T (60%) than in the S (40%) and M (22%) groups.

TGF-β was observed in all synovial compartments, although expression was higher in the sublining and perivascular areas. TGF-β expression was significantly higher in the T than in the S group ($P=0.04$) and a statistical trend also was observed between the T and the M groups ($P=0.1$). All patients in the S and M groups displayed TGF-β in the perivascular infiltrate compared with 55% of patients in the T subgroup.

IL-8 expression was predominant in the sublining and perivascular infiltrate, but also was observed in the lining, endothelium and extracellular matrix in 30–60% of patients. IL-8 showed a statistical trend to higher expression in the M compared with the T group ($P=0.1$), while there were no marked differences in its distribution through the synovium.

VEGFR-1 (flt-1) displayed diffuse expression in the lining and sublining layers; VEGFR-2 (KDR/flk-1) was expressed predominantly in the endothelium and perivascular cells and diffusely in the sublining. Expression in endothelium was somewhat more frequent in samples with the T pattern. VEGFR-1 and VEGFR-2 expression did not differ significantly between groups.

Markers of angiogenesis in serum (Table 2)

VEGF serum level showed a trend to significance between the S and M groups ($P=0.1$). There were no significant differences in serum levels of TGF-β or IL-8 between the three vascular patterns. TGF-β and IL-8 serum levels were low, within the range of normality, and did not differ between groups.

Discussion

This study analysed synovial vascular patterns in a cohort of patients with non-selected active RA and their association with angiogenic mediators (VEGF, VEGFR-1, VEGFR-2, TGF-β and IL-8) in synovial tissue and serum. It is the first study to attempt to establish different RA subgroups based on different synovial vascular patterns.

The pioneer work by Reece *et al.* [7] reported that, of 18 patients with early RA, 89% had a synovial vascular pattern of straight, branching vessels (S) and 11% had tortuous, bushy vessels (T), whereas of 26 early SpA patients (14 with PsA and 12 with reactive arthritis), 73% displayed the T and 26% the S pattern. This suggested that distinctive vascular patterns assessed by arthroscopy could reflect different specific vascular factors in the pathogenesis of these arthritides. Other studies by the same group confirmed these results and, in addition, reported that there was a close relationship between angiopoietins, VEGF, MMP-9, TGF-β and vascular morphology in PsA [13, 14]. However, in a study assessing the diagnostic usefulness of the vascular pattern in 100 cases with early and late different inflammatory arthritides, we found that RA patients displayed three distinct synovial vascular patterns and, although the most characteristic was the S pattern,

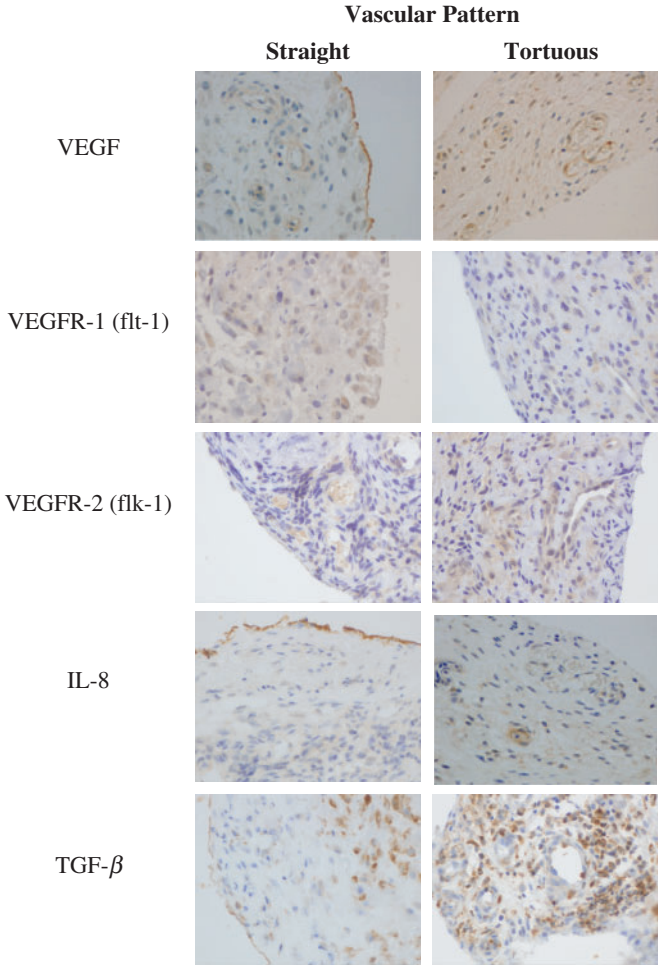

FIG. 1. Immunohistochemical detection of angiogenesis markers [VEGF, VEGFR-1 (flt-1), VEGFR-2 (KDR/flk-1), IL-8 and TGF-β] in representative sections obtained from two patients with rheumatoid arthritis (RA). For each marker, 'Straight' corresponds to a biopsy specimen obtained from the same patient with the S vascular pattern, and 'Tortuous' corresponds to a biopsy specimen obtained from another RA patient with the T pattern. Original magnification $\times$400.

there were also RA patients with the M or T patterns [9]. Other authors also observed heterogeneity in RA synovial morphology, with only 28% of patients displaying an S pattern [10]. These studies suggested the possibility of stratifying RA patients according to their vascular patterns in order to find out whether these patterns were associated with different clinical or pathogenic characteristics.

The present study shows that the S vascular pattern is the most frequent in our cohort of RA patients (41%), a percentage very similar to our previous results [9] but lower than the prevalence of 80–90% found in other studies [7, 8]. Taken together with our previous results [9], the present study supports the hypothesis of a higher prevalence of RF positivity in the S and M patterns compared with the T pattern. This suggests that vascular morphology could reflect pathophysiological changes characteristic of RA, which is clinically and pathogenically a heterogeneous disease. Unfortunately, these data cannot be compared with those of previous studies, as they did not include the prevalence of RF positivity according to vascular patterns.

The higher prevalence of erosive disease in the S and M groups compared with the T group could be related to shorter disease duration in patients with the T pattern, but there were no statistical differences in disease duration between the M and the T groups. Given that in a previous study we found no evidence of changes in the vascular pattern associated with disease duration [9], this increase in erosive disease may be due to the fact that the S and M patterns are associated with increased systemic inflammatory activity compared with the T pattern [20]. Although morphological characteristics of synovitis, including vascularity, have been suggested that could change with the intensity of local inflammation [10], all our patients had active joint inflammation at the time of arthroscopy. Furthermore, DAS28 and the percentage of patients on DMARDs were not different between the different RA vascular pattern groups at the time of arthroscopy. Therefore, the finding of a higher prevalence of erosive disease could also be interpreted to show that RA patients with the S pattern have a poorer outcome than RA patients with the T pattern. The finding that the S group show a statistical trend to higher number of DMARDs received compared with the T group supports this idea.

The prevalence of RF positivity, erosive disease, levels of markers of inflammatory activity (CRP and IL-6) and the number of DMARDs taken in the M and S groups were comparable, posing the question of whether these groups should be classified together, as they share markers of poor clinical outcome. In contrast, the T group had a lower prevalence of RF positivity, erosive disease, lower acute reactant phase levels and a trend to a smaller number of DMARDs received, probably identifying an RA group with a more favourable clinical outcome.

There are no previous studies analysing vascular patterns in RA and their association with the expression of angiogenic factors. Fraser *et al.* [13] and Fearon *et al.* [14] compared the T pattern in early PsA with the S pattern in early RA and found that the T pattern was associated with higher levels of VEGF, MMP-9, TGF-β and angiopoietin-2 in synovial tissue/fluid compared with the S pattern. Although we found no significant differences in serum levels of VEGF, TGF-β and IL-8 between the RA groups studied, we observed a statistical trend to higher VEGF in the S compared with the M group. However, different serum levels of IL-8 were associated with distinct histological variants of rheumatoid synovitis in a recent study [21]. TGF-β levels were similar to those reported in RA by other authors [22]. Our negative findings may be due to the small sample size and/or to the fact that angiogenic factors are produced in the synovium and serum levels are usually very low, making it difficult to find significant differences between RA groups.

We observed differential expression of some angiogenic factors in synovial tissue. With respect to VEGF, the S group was characterized by higher levels in the synovium compared with the M group, consistent with serum findings, probably reflecting some pathophysiological differences between these more clinically homogeneous groups. TGF-β showed higher levels in the T group compared with the S and M groups. Although TGF-β is a strong inducer of VEGF production, its precise role in arthritis remains unclear, but it is thought that the balance between its pro-inflammatory and anti-inflammatory effects in joint inflammation is crucial to RA outcome [23]. However, it has recently been shown that TGF-β1 exerts anti-inflammatory effects on antibody-induced arthritis [24]. Given the better clinical outcomes associated with the T group compared with the S and M groups, it is tempting to speculate that high TGF-β expression could be protective in RA patients in the T group.

VEGFR-1 and VEGF-R2 were observed in the same synovial sites as VEGF, without differences between groups, emphasizing that angiogenesis was active in our patients, as the VEGF/VEGFR2 system is responsible for most of the growth signals for vascular endothelial cells [25]. IL-8 expression only showed a statistical trend to higher expression in the M compared with the T group, probably reflecting the higher level of inflammatory activity in the M group.

Some mild differences were observed in the synovial location of some factors between the different RA groups. The higher frequency of VEGF expression in the lining of the S group compared with the T group could lead to higher macrophage activity with increased production of pro-inflammatory cytokines and metalloproteinases by signalling of VEGFR-1 (flt-1) [26]. Furthermore, suppression of joint destruction in animal models of RA was shown by blocking VEGFR-1, suggesting that high levels of VEGF expression in lining cells could be a factor promoting joint destruction [27].

VEGF is a critical mediator of blood vessel formation during development and in pathological conditions. After secretion, VEGF becomes bound to the extracellular matrix. A recent study demonstrated that a subset of metalloproteinases can cleave VEGF, and that soluble VEGF and matrix-bound VEGF provide different angiogenic outcomes. Matrix-bound VEGF induces extensive growth and branching of vessels, while cleaved VEGF promotes dilatation of existing vessels [28]. In addition to other factors, a higher frequency of VEGF expression (both matrix-bound and soluble VEGF) in the vessels of the T pattern group might participate in the generation of the dense, dilated, bushy morphology characteristic of this pattern by signalling of VEGFR-2 (KDR/flk). However, it will be necessary to be able to discriminate matrix-bound from cleaved VEGF isoforms to achieve greater insight into this process. Although the global expression of TGF-β was higher in the T than in the S pattern, almost 50% of patients with the T pattern did not express TGF-β in the perivascular infiltrate. The pathophysiological relevance of these findings is unclear. However, in order to determine the real role of differential synovial location of angiogenic mediators, microdissection studies would be necessary. Furthermore, angiogenesis and vessel patterning are very complex phenomena and they implicate multiple mediators acting simultaneously or sequentially, such as angiopoietins, neuropilins, semaphorins, ephrins and ephs receptors, making it extraordinarily difficult to determine its clinical implications [29].

This study had several limitations. Firstly, the small number of patients included, typical of this type of study, makes it difficult to find significant differences between groups and, therefore, to draw definite conclusions. However, we believe that the study may provide useful pathophysiological insights into the disease. Secondly, the design of the study was cross-sectional; a prospective study would permit comparison of patients with distinct vascular patterns and the same disease duration, allowing clarification of the relationship between vascular pattern and clinical and radiographic outcome. Thirdly, the classification of the M vascular pattern is subjective and a consensus between researchers in this area is necessary [9].

In conclusion, this study confirms that there are three different synovial vascular patterns in RA and suggests for the first time that patients with the S pattern have a poorer outcome than patients with the T pattern. Patients with the M pattern seem clinically and biologically very similar to patients with the S pattern. Furthermore, differential synovial expression and location of VEGF and TGF-β could have pathophysiological relevance. However, larger, prospective multicentre studies would be necessary to confirm and extend these findings.

Rheumatology	Key messages
	• This preliminary study suggests that RA patients with the straight and mixed synovial vascular patterns could have poor clinical outcomes compared with RA patients with the tortuous pattern.

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Reference

1. Tak PP. Analyzing synovial tissue samples. What can we learn about early rheumatoid arthritis, the heterogeneity of the disease, and the effects of treatment? J Rheumatol 2005;32:25–6.
2. van der Pouw Kraan TC, van Gaalen FA, Karpeskovitz PV *et al.* Rheumatoid arthritis is a heterogeneous disease: evidence for differences in the activation of the STAT-1 pathway between rheumatoid tissues. Arthritis Rheum 2003;48:2132–45.
3. Kraan MC, Haringman JJ, Post WJ, Verseendaal J, Breedveld FC, Tak PP. Immunohistological analysis of synovial tissue for differential diagnosis in early arthritis. Rheumatology 1999;38:1074–80.
4. Salvador G, Sanmarti R, Garcia-Peiró A, Rodríguez-Cros JR, Muñoz-Gómez J, Cañete JD. p53 Expression in rheumatoid and psoriatic arthritis synovial tissue and association with joint damage. Ann Rheum Dis 2005;64:183–7.
5. Kruithof E, Baeten D, De Rycke RL *et al.* Synovial histopathology of psoriatic arthritis, both oligo- and polyarticular, resembles spondyloarthropathy more than it does rheumatoid arthritis. Arthritis Res Ther 2005;7:R569–80.
6. Cañete JD, Martinez SE, Farrés J *et al.* Differential Th1/Th2 cytokine patterns in chronic arthritis: interferon γ is highly expressed in synovium of rheumatoid arthritis compared with seronegative spondyloarthropathies. Ann Rheum Dis 2000;59:263–8.
7. Reece RJ, Cañete JD, Parsons WJ, Emery P, Veale DJ. Distinct vascular patterns of early synovitis in psoriatic, reactive, and rheumatoid arthritis. Arthritis Rheum 1999;42:1481–4.
8. Fiocco U, Cozzi L, Chieco-Bianchi F *et al.* Vascular changes in psoriatic knee joint synovitis. J Rheumatol 2001;28:2480–6.
9. Cañete JD, Rodriguez JR, Salvador G, Gomez-Centeno A, Muñoz-Gomez J, Sanmarti R. Diagnostic usefulness of synovial vascular morphology in chronic arthritis. A systematic survey of 100 cases. Semin Arthritis Rheum 2003;32:378–87.
10. Baeten D, Demetter P, Cuvelier C *et al.* Comparative study of the synovial histology in rheumatoid arthritis, spondyloarthropathy, and osteoarthritis: influence of disease duration and activity. Ann Rheum Dis 2000;59:945–53.
11. Paleolog EM. Angiogenesis in rheumatoid arthritis. Arthritis Res 2002;4(Suppl. 3):S81–S90.
12. Taylor PC, Sivakumar B. Hypoxia and angiogenesis in rheumatoid arthritis. Curr Opin Rheumatol 2005;17:293–8.
13. Fraser A, Fearon U, Reece R, Emery P, Veale D. Matrix metalloproteinase 9, apoptosis, and vascular morphology in early arthritis. Arthritis Rheum 2001;44:2024–8.
14. Fearon U, Griosios K, Fraser A *et al.* Angiopoietins, growth factors, and vascular morphology in early arthritis. J Rheumatol 2003; 30:260–8.
15. Tak PP, Brenishan B. The pathogenesis and prevention of joint damage in rheumatoid arthritis. Arthritis Rheum 2000;43: 2619–33.
16. Cañete JD, Pablos JL, Sanmartí R *et al.* Antiangiogenic effects of anti-tumour necrosis factor α therapy with infliximab in psoriatic arthritis. Arthritis Rheum 2004;50:1636–41.
17. Arnett FC, Edworthy SM, Bloch DA *et al.* The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.

18. Holten J, Smeets TJM, Blankert P, Tak PP. Expression of interferon β (IFNβ) in synovial tissue from rheumatoid arthritis patients compared to osteoarthritis and reactive arthritis patients. Ann Rheum Dis 2005;64:1780–2.
19. Haringmann JJ, Vinkenoog M, Gerlag DM, Smeets TJM, Zwinderman AH, Tak PP. Reliability of computerized image analysis for the evaluation of serial synovial biopsies in randomised controlled trials in rheumatoid arthritis. Arthritis Res Ther 2005; 7:R862–7.
20. Wolfe F, Sharp JT. Radiographic outcome of recent-onset rheumatoid arthritis: a 19-year study of radiographic progression. Arthritis Rheum 1998;41:1571–82.
21. Klimiuk PA, Sierakowski S, Latosiewicz R *et al*. Histological patterns of synovitis and serum chemokines in patients with rheumatoid arthritis. J Rheumatol 2005;32:1666–71.
22. Eriksson P, Andersson C, Ekerfelt C, Ernerudh J, Skogh T. Relationship between serum levels of IL-18 and IgG1 in patients with primary Sjögren's syndrome, rheumatoid arthritis and healthy controls. Clin Exp Immunol 2004;137:617–20.
23. O'Shea JJ, Ma A, Lipsky P. Cytokines and autoimmunity. Nat Rev Immunol 2002;2:37–45.
24. Kim HY, Kim HJ, Min HS *et al*. NKT cells promote antibody-induced joint inflammation by suppressing transforming growth factor β1 production. J Exp Med 2005;201:41–7.
25. Korpelaine EI, Karkkainen M, Gunji Y, Vikkula M, Alitalo K. Endothelial receptor tyrosine kinases activate the STAT signaling pathway: mutant Tie-2 causing venous malformations signals a distinct STAT activation response. Oncogene 1999;18:1–8.
26. Mezquita J, Mezquita B, Pau M, Mezquita C. Down-regulation of Flt-1 gene expression by the proteasome inhibitor MG262. J Cell Biochem 2003;89:1138–47.
27. De Bandt M, Ben Mahdi M, Ollivier V *et al*. Blockade of vascular endothelial growth factor receptor I (VEGF-RI), but not VEGF-RII, suppresses joint destruction in the K/BxN model of rheumatoid arthritis. J Immunol 2003;171:4853–9.
28. Lee S, Jilani SM, Nikolova GV, Carpizo D, Iruela-Arispe ML. Processing of VEGF-A by matrix metalloproteinases regulates bioavailability and vascular patterning in tumors. J Cell Biol 2005; 169:681–91.
29. Klagsbrun M, Eichmann A. A role for axon guidance receptors and ligands in blood vessel development and tumor angiogenesis. Cytokine Growth Factor Rev 2005;16:535–48.