Concise Report

A new approach to studying angiogenesis in rheumatoid arthritis by means of power Doppler ultrasonography and measurement of serum vascular endothelial growth factor


Objective. To evaluate angiogenesis as an essential component of pannus formation and cartilage destruction in rheumatoid arthritis (RA) using power Doppler ultrasonography (PDUS) and serum vascular endothelial growth factor (VEGF) measurement.

Methods. Twenty-one RA patients with a painful and swollen wrist and 12 healthy controls were examined with ultrasound. By means of standard scans, vascularity near and inside the joint capsule was visualized with PDUS. Two trained investigators performed sonography. Representative video clips were stored and read by two independent investigators, under blinded conditions, with regard to the microvascular Doppler flow being either inside or outside the joint capsule and with respect to a qualitative estimate of the intensity of blood flow, according to a grading from 1 to 3. Serum levels of VEGF were measured with a standard quantitative sandwich ELISA.

Results. The power Doppler mode identified increased synovial microvascular blood flow inside the joint capsule in 17 of 21 RA patients (81%) vs one of the healthy controls. We found large variation in serum VEGF levels in RA patients and in healthy controls. The degree of synovial vascularity determined by PDUS showed no correlation with the immediate serum VEGF level in the same patient.

Conclusion. The high correlation between intra-articular microvascular power Doppler flow and clinical synovitis in RA patients (P < 0.0001) indicates that PDUS may be helpful in studying the role of synovial blood vessels in rheumatoid inflammation.

Key words: Angiogenesis, Vascular endothelial growth factor, Rheumatoid arthritis, Ultrasound, Power Doppler sonography.

The inflammatory process in rheumatoid arthritis (RA) is characterized by excessive, tumour-like proliferation of synoviocytes that is associated with an increase in the vasculature needed to support metabolic requirements. Neovascularization is a complex process in which new blood vessels develop from the existing microvascular bed; it involves endothelial cell division, selective degradation of vascular basement membranes and the surrounding extracellular matrix, and endothelial cell migration [1]. This process is driven by a combination of up-regulation of angiogenesis promoters and down-regulation of inhibitors [2]. Vascular endothelial growth factor (VEGF) is the most endothelial cell-specific angiogenic factor characterized to date [3]. In RA, regardless of its duration, the localization of VEGF polypeptide could be demonstrated in macrophages and fibroblasts surrounding microvessels, vascular smooth muscle cells and synovial lining cells. Furthermore, serum VEGF concentrations are higher in patients with RA than in patients with osteoarthritis and normal controls; however, the source of VEGF in the serum is unclear. Although the mechanisms by which VEGF expression and activity are regulated in RA are poorly understood, it very probably plays a crucial role in the neovascularization that occurs during pannus formation in rheumatoid synovitis [4–6].

Technological improvements, such as high-resolution ultrasound and the power Doppler mode, make possible the assessment of synovial pannus and vascular tissues along with the detection of low-velocity blood flow at the microvascular level [7]. There are several studies on the visualization of vascularized synovium in joints and tendon sheaths with power Doppler ultrasonography (PDUS) in patients with RA that emphasize the correlation between the qualitative estimates of blood flow obtained by PDUS and synovial blood vessel density in a histological tissue section [8–10].

Since VEGF is the most important and best known soluble angiogenesis promoter in RA, we measured serum VEGF levels in patients with RA to compare it with intra-articular microvascular power Doppler flow of a clinical symptomatic joint.

Patients and methods

Because arthritis of the wrist is common in RA, we selected it as a criterion for ultrasound investigation. Twenty-one patients with active RA according to the ACR criteria with a painful and swollen wrist were included in the study. The group consisted...
of 16 women and five men ranging from 38 to 79 yr of age. Ten patients received no treatment, other than non-steroidal anti-inflammatory drugs at the time of examination (non-treated patients), whereas the others had already been treated with glucocorticoids and/or disease-modifying anti-rheumatic drugs over a long period (treated patients). Wrist arthritis was clinically evaluated by joint tenderness and soft tissue swelling. Twelve healthy controls (six women and six men), ranging from 17 to 58 yr of age, were also investigated.

Ultrasonography was carried out with a 7.5 MHz linear array transducer (Image Point; Hewlett Packard, Bad Homburg, Germany). With the use of standard scans according to the ‘Guidelines for musculoskeletal ultrasound in rheumatology’ [11], joint effusion and the vascularity near and/or inside the joint capsule were visualized. Power Doppler settings were standardized with a pulse repetition frequency of between 700 and 1000 Hz, and the gain was set as suggested by Rubin et al. [12]. Two examiners (JS and EH) performed sonography alternately. Stored images (video clips) were read by two independent investigators (KS and PK), who were blinded to the patients’ clinical and laboratory findings. They localized the Doppler signals as being either inside or outside the joint capsule and estimated the intensity of Doppler flow according to a grading system modified after that of Newman et al.: 1 = no flow; 2 = mild or moderate flow; 3 = intense flow [7].

Since this grading classifies microvascular Doppler flow in relation to the surrounding tissue perfusion, we adapted grade 1 from ‘normal or minimal perfusion’ to ‘no flow’ because normally we could not find any blood flow inside the joint capsule with the ultrasound machine (Image Point) that was used, and we combined mild and moderate flow as grade 2. In order to exclude the interpretation of artefacts, in every patient that demonstrated intra-articular Doppler signals, pulse-wave Doppler was used to obtain a Doppler flow spectrum of a small artery.

Blood samples for serum VEGF measurement were taken during the morning of the same day or a maximum of 1 day after ultrasound investigation was performed. Each sample was centrifuged 2 h later and then stored at –22°C until further analysis, which was performed at the latest after 4 months. Serum VEGF was measured using a standard quantitative sandwich ELISA (Human VEGF; Bio Source International, Camarillo, CA, USA). The intra- and inter-assay coefficients of variation of this test are 4.9 and 6.5% respectively. Double measurements were performed in 10 out of 21 RA patients, and high agreement was found between the two values. The system uses microplates with wells precoated with specific polyclonal human VEGF antibodies.

For each analysis, 50 µl of serum was used. At the end of the test, optical densities were determined with a microplate reader (Tecan Deutschland, Crailsheim, Germany). Concentrations are reported as pg/ml.

Because blood sample taking and ultrasound investigation was done during the routine clinical diagnostic procedure, ethical approval was not required. We obtained informed consent from every patient involved in the study.

**Results**

PDUS identified microvascular blood flow inside a hypoechoic space with a width ranging from 1.5 to 4.6 mm in 17 RA patients, in comparison to one of the healthy controls ($P < 0.0001$; $\chi^2$ test). In every patient with Doppler signals it was possible to demonstrate a spectral flow curve of a small intra-articular artery to prove blood flow. Interobserver agreement for localization of Doppler signals inside the joint capsule was $\kappa = 0.87$; for qualitative estimation of blood flow it was $\kappa = 0.69$.

The serum VEGF levels in RA patients (median = 795 pg/ml) were slightly elevated compared with healthy controls (median = 569 pg/ml) (Mann–Whitney test, $P = 0.04$). We found a broad range of serum VEGF levels in both groups: from 211 to 1974 pg/ml in the RA group and from 70 to 1090 pg/ml in the healthy controls.

Qualitative estimation of intra-articular blood flow according to the modified Newman grading system in RA patients showed no correlation with the immediate serum VEGF level of the same patient (Fig. 1). It is interesting to note that in two RA patients without evidence of intra-articular Doppler flow (grade 1) and in two patients with intense flow (grade 3), higher serum VEGF levels were measured than in patients with mild and moderate (grade 2) intra-articular Doppler flow (Fig. 2).

**Discussion**

The current approach to the management of RA recommends early and aggressive treatment to prevent structural joint damage that leads to pain, loss of function and disability. For the detection of early inflammatory changes in active arthritis in RA patients, sensitive and specific diagnostic methods that are simple to perform are urgently needed [13]. The initiation of synovial inflammation is characterized by periarticular vasodilatation followed by synovial proliferation, which is accompanied by

![Patient 12](image1.png)

**Patient 12**

Grading: 1 = no intra-articular blood flow

VEGF: 1700 pg/ml

![Patient 6](image2.png)

**Patient 6**

Grading: 3 = intense intra-articular flow

VEGF: 1183 pg/ml

**Fig. 1.** Dorsal longitudinal radial scans of the wrists of two RA patients, demonstrating different intensity of power Doppler flow. Patient 12 shows no intra-articular microvascular Doppler flow even though a high serum VEGF level was measured. In patient 6, intense blood flow and a high serum VEGF level was found.
angiogenesis resulting in intra-articular new blood vessel formation. Technological improvements, such as high-resolution ultrasound and power Doppler mode, make it possible to discriminate between peri- and intra-articular blood flow and to demonstrate synovial proliferation [14]. Walther et al. could demonstrate a positive correlation between qualitative estimation of blood flow by power Doppler sonography and blood vessel density in a histological tissue section [10]. Using dynamic magnetic resonance imaging (MRI) as a reference, Szkudlarek et al. showed PDUS to be a reliable method for assessing inflammatory activity in arthritic joints of RA patients [15].

In swollen and painful wrists of RA patients we measured a hypoechoic joint space of 1.5 to 4.6 mm. The carpal is characterized by an irregular surface because it consists of many different bones. For this reason the measured hypoechoic area could not be conclusively assumed to be pathological [16]. However, detection of power Doppler flow inside the hypoechoic space allows differentiation between synovial fluid and hypervascularized synovitis. In our study, we found a statistically significant correlation between increased intra-articular microvascular power Doppler flow and clinical signs of wrist synovitis \( (P < 0.0001) \). Since inflammation of the synovial membrane along with vasodilatation and blood vessel proliferation are the earliest recognizable pathological changes in RA, visual representation of this process is a new and challenging method of detecting early disease activity. In comparison with MRI, ultrasound is a widely available, easy-to-use, inexpensive method with no risk for the patient [17], which makes the use of PDUS greatly advantageous in the assessment of disease activity in RA.

VEGF, originally described as a tumour cell-derived vascular permeability factor, has been implicated as an angiogenic mediator in RA [18, 19]. Several investigators have described local VEGF expression in the joints of RA patients, where it is synthesized and released by different cell types, such as subsynovial macrophages, fibroblasts surrounding microvessels, vascular smooth muscle cells and synovial lining cells [20–22]. Other groups found correlations between serum VEGF levels and laboratory [23] and clinical [24] disease activity variables or the development of radiographic damage [25, 26]. Pinheiro et al. could find no correlation with laboratory and clinical disease activity scores in long-standing disease [27]. Lee et al. found no correlation between VEGF levels in serum and levels in synovial fluid from the same RA patient [28], indicating uncertainty about whether angiogenesis in RA is a local or systemically driven process [29, 30]. In our study we compared local intra-articular synovial hyperaemia of one symptomatic joint (wrist) with the immediate serum VEGF level of the same patient as a parameter of systemic disease or angiogenic activity. Serum VEGF levels show a great range in RA patients and healthy controls, indicating an individual variability that depends on different regulatory mechanisms and possibly factors such as sex, age, disease duration, arthritic joint count, present treatment and the large variations in disease activity in general. Moreover, blood centrifugation leads to efflux of VEGF from thrombocytes, which may have contributed to the wide range of VEGF concentrations measured.

Since previous studies using ultrasonography or MRI have shown only weak correlations between biomarkers of disease activity (primarily CRP and ESR) and local signs of disease activity in the individual joint [31–33], it is not surprising that we did not find a significant correlation between serum VEGF levels and the degree of synovial vascularity determined by means of PDUS (Fig. 2). Qualitative grading of power Doppler signals allows only inaccurate estimation of synovial blood flow, so that further longitudinal studies with a greater number of patients and the use of improved ultrasound techniques, such as three-dimensional Doppler sonography [34], will possibly be more effective in investigating the connection between systemic angiogenic activity and local intra-articular inflammatory vascular alterations, including angiogenesis, in RA.

The authors have declared no conflicts of interest.

References


