Synovial angiogenesis is considered to be an important early step in the pathogenesis of rheumatoid arthritis (RA) and in the perpetuation of disease [1, 2]. Furthermore, there is now much evidence to indicate that endothelial cell dysfunction is a feature of RA [3–5]. This review will discuss the role of vascular endothelial growth factor (VEGF), a marker of angiogenesis, in the pathophysiology of RA and consider evidence that serum VEGF concentrations correlate with disease activity and fall when synovitis is successfully suppressed by therapy. The potential of VEGF to predict disease outcome will also be discussed. Developments in ultrasonographic technologies that permit assessment of synovial vascularity and their potential application in the evaluation of RA disease activity, prediction of disease progression and monitoring of response to therapy will be reviewed.

Abnormal vasculature in the pathogenesis of RA

In health angiogenesis, or growth of new blood vessels from pre-existing vasculature, occurs during growth and the female reproductive cycle. It is also a feature of tissue repair following injury and contributes to the pathogenesis of a number of disease states. Examples include cancer, chronic gingivitis, diabetic retinopathy and RA. Angiogenesis arises when hypoxic, diseased or injured tissues secrete pro-angiogenic molecules and is regulated by a complex set of inducers and inhibitors. It occurs as a coordinated process comprising endothelial cell proliferation and migration followed by capillary tube formation, deposition of basement membrane and proliferation and migration of pericytes and smooth muscle cells. Anastomoses are created and flow of blood is established. Vascular reorganization follows in a process requiring the regression of redundant vessels by endothelial cell apoptosis [6]. To match function of the microvascular bed to local metabolic demand, the developing vessels begin to express vasoactive peptides and their receptors [1].

Histologically, RA synovitis is characterized by a mononuclear cell infiltrate and luxuriant vasculature [7]. Furthermore, the disease activity of a given joint is correlated with the synovial vascularization [8, 9]. Angiogenesis is evident on microscopic examination of synovial biopsies from the earliest stages of disease evolution [10] and is observed as a fine network of vessels over the rheumatoid synovium at arthroscopic inspection of RA joints. Angiogenesis is integral to the development of inflammatory pannus, and without it leucocyte ingress could not occur. Furthermore, formation of new blood vessels permits a supply of nutrients and oxygen to the augmented inflammatory cell mass and so contributes to the perpetuation of synovitis. Studies in experimental models of arthritis suggest that destruction of bone and cartilage may be more closely linked to angiogenesis than to pannus swelling [11, 12].

Patients with severe RA die prematurely, and cardiovascular disease is the major cause of excessive mortality. The earliest stages of atherosclerosis are characterized by endothelial dysfunction as demonstrated by abnormal blood flow responses to acetylcholine. There is now evidence that endothelial dysfunction is a feature of the early and established phases of RA [3–5].
Furthermore, this dysfunctional state is present independently of conventional cardiovascular risk factors for atherosclerotic disease [3], implicating other mechanisms associated with chronic inflammation [13].

VEGF in RA

A number of interdependent processes promote angiogenesis in the RA joint. These include shear stress on the endothelial wall as a result of increased blood flow as well as extravasated plasma proteins such as fibrinogen products. Similarly, inflammatory cells including macrophages, lymphocytes, mast cells, fibroblasts and their soluble products including the pro-inflammatory cytokines tumour necrosis factor alpha (TNFα), interleukin (IL)-1, and IL-8 promote angiogenesis. Many endothelial growth factors have been demonstrated in RA synovium [2, 14, 15] and tenosynovium [16]. Of these, VEGF is the most endothelial cell-specific growth factor characterized to date [17–19]. It also induces vascular permeability [20]. VEGF exists as several isoforms generated by alternative splicing of VEGF mRNA [21, 22]. There may be a link between VEGF gene polymorphisms and susceptibility to RA [23]. In RA synovium, fibroblast expression of VEGF is not only up-regulated by IL-1 and TNFα [24, 25] but also by the physical interaction of activated leucocytes and fibroblast-like synoviocytes [26] and engagement of the CD40α ligand [27]. VEGF induces endothelial decay-accelerating factor, which is cytoprotective against activated complement and may regulate endothelial proliferation and angiogenesis [28].

Hypoxia is often a feature of inflammation and is a potent inducer of VEGF. In vitro, hypoxic culture conditions greatly augment VEGF secretion from synovial fibroblasts following stimulation by IL-1 and transforming growth factor beta (TGFβ) [29]. Direct measurements confirm that the intra-articular environment is hypoxic in inflammatory arthritis [30]. Contributory factors include the high metabolic demands of inflamed synovial tissue and the rapid rate of synovial proliferation such that cells become more distant from the closest blood vessels, compound the hypoxic state [31]. Tissue hypoxia in the rheumatoid joint results in increased VEGF mRNA stability [32] and enhanced VEGF gene transcription through the binding of hypoxia-inducible transcription factors such as HIF-1 and HIF-2 that are over-expressed in the synovial lining and stromal cells of RA patients relative to synovial tissues from individuals without arthritis [33]. HIF-1 and HIF-2 are degraded within minutes of exposure to an oxygen tension >3–5% but are stabilized under conditions of hypoxia (<3% oxygen), then translocated to the nucleus, where they bind to hypoxia-responsive elements on hypoxia-inducible genes and up-regulate their expression [34]. In this way the hypoxic environment in the rheumatoid joint promotes transcriptional changes permissive for perpetuation of synovitis.

VEGF, disease activity and response to therapy

VEGF can be detected in serum, synovial tissue and fluids of patients with RA [24, 25, 35–39]. Human neutrophils secrete VEGF [40] and levels of neutrophil-associated VEGF in RA synovial fluids correlate well with free VEGF in joint effusions and with patient disease activity [41]. However, there is no correlation between VEGF concentrations measured in matched serum and synovial fluid samples from RA patients [42]. In early inflammatory arthritis, synovial fluid VEGF levels correlate with concentrations of matrix metalloproteinase (MMP)-9 in the fluid [43]. Several groups have reported that VEGF concentrations are elevated in the serum of RA patients compared with healthy controls and patients with osteoarthritis [36, 42, 44–46]. However, the tissue and cellular origins of the molecule are unclear. In vitro, human peripheral blood mononuclear cells release VEGF in response to cytokines present in RA joints, including TNFα [38]. Release of VEGF from platelets has also been reported [44]. Serum VEGF may therefore be derived from a number of sources including platelets, synovial fluid neutrophils, inflamed synovial tissue or others. Serum VEGF concentrations correlate with individual and composite measures of RA disease activity including acute phase markers and swollen and tender joint counts [42, 45, 46]. In a study of patients attending an early inflammatory arthritis and established RA clinic, we found that serum VEGF concentrations were higher in patients with early RA than in patients with longstanding treated RA [45]. This observation may represent a response to therapy, a view supported by other studies demonstrating reduction in serum VEGF concentrations after therapeutic intervention [36, 44, 45]. In our series, a total of 27 early RA patients responding to therapy with disease-modifying anti-rheumatic drugs (DMARDs) exhibited a significant reduction in serum VEGF levels, in contrast to patients unresponsive to DMARD treatment where there was no significant change in serum VEGF concentrations [45]. Treatment of RA patients with infliximab results in marked reduction, but not normalization, of serum VEGF concentrations [47]. The reduction correlates with changes in clinical and laboratory measures of disease activity.

Significant reductions in serum VEGF levels following response to treatment intervention in RA point to angiogenesis as an important pathogenic process in perpetuation of synovitis. This raises the possibility that an imbalance between inducers and inhibitors of angiogenesis contributes to persistence of joint inflammation. In support of this hypothesis, concentrations of endostatin, an angiogenesis inhibitor, are not elevated in serum and synovial fluid samples from patients in whom serum VEGF is elevated [44]. Interestingly, a preliminary report indicates that in RA serum endostatin levels rise after a single infliximab infusion [48]. Soluble Flt-1 (sFlt-1), another naturally occurring inhibitor of angiogenesis, is an alternatively spliced form of Flt-1, one of the tyrosine kinase receptors that mediates the action of VEGF [19, 49, 50]. We have reported sFlt-1 to be elevated in both early and long-standing RA groups compared with controls, and that elevated sFlt-1 levels correlate with VEGF concentrations in serum of the same RA patients [45]. Elevated levels of sFlt-1 in RA sera presumably represent an attempted homeostatic mechanism that is inadequate to inhibit VEGF activity, a finding analogous to that of raised levels of other pro-inflammatory cytokines and their naturally occurring inhibitors in this disease.

TNF blockade, synovial vascular markers and RA endothelial dysfunction

Diminished vascular permeability accompanying rapid suppression of VEGF levels is likely to be a factor contributing to the early reduction of joint swelling observed in RA patients after anti-TNFα therapy [47]. We have shown that TNF blockade is also accompanied by reduced synovial angiogenesis as assessed by immunohistological analysis of microvascular density and αVβ3 integrin expression in synovial biopsy tissue taken before and 4 weeks after a single 10 mg/kg infliximab infusion (Fig. 1) [51]. Similar findings are reported in the synovitis of psoriatic arthritis after infliximab therapy [52, 53]. At 8 weeks following three 5 mg/kg infliximab infusions Canete et al. [53] report significant reduction in synovial CD31+ immunopositive area fraction, αVβ3 integrin and VEGF expression but with an increase in expression of angiopoietin-2 suggesting that vascular
regression is a mechanism underlying the anti-angiogenic effect of TNFα blockade [53].

Is the endothelial dysfunction associated with RA reversible? Infliximab treatment reduces synovial endothelial activation in RA [54] and in psoriasis [52]. As measured by brachial ultrasound, it has recently been reported that infliximab has a beneficial, but transient, effect on endothelial-dependent vasodilatation [55, 56]. This benefit parallels improvement in disease activity and reduction in acute phase markers [55]. Endothelial-dependent (post-ischaemia), but not endothelial-independent (post-nitroglycerin), vasodilatation was significantly improved 2 and 7 days after infliximab infusion. However, by 4 weeks after infusion, endothelial function had returned to baseline levels [56]. Infliximab is also reported to improve endothelial dysfunction in antineutrophil cytoplasmic antibody (ANCA)-associated systemic vasculitis [57]. These studies implicate TNFα as a factor contributing to endothelial dysfunction in RA. Therefore restoration of endothelial homeostasis by TNFα blockade may potentially retard clinical progression of atherosclerosis and, in particular, reduce the risk of plaque rupture and acute coronary syndrome in these patients. Data being gathered in national registries for biologic therapies may help shed further light on this hypothesis.

### Ultrasonographic techniques for imaging synovial vascularity in RA

Recent studies addressing the use of conventional grey-scale (B-mode) ultrasonography (US) in the evaluation of RA synovial inflammation and joint damage indicate that clinical joint examination and conventional radiography are comparatively insensitive tools [58–61]. Conventional radiography offers only late signs of preceding disease activity and resulting cartilage and bone destruction. In comparison, images obtained using newer magnetic resonance and US technologies emphasize the inadequacy of radiography for soft tissue assessment in RA. The most important technical requirement for joint US is a high-quality imaging system. Resolution improves as the US frequency increases but at the expense of a dramatic decline in tissue penetration. Therefore the choice of ultrasound frequency for a particular purpose will represent a balance between resolution and penetration depth. Optimal ultrasound equipment for musculoskeletal work should be equipped with standard 7.5–10 MHz transducers for conventional examination. In order to depict fine details of superficial tissues including those associated with peripheral joints, higher-frequency transducers (13, 15, 20, 22, 30 MHz) are necessary. Twenty megahertz transducers have an axial resolution power of 0.038 mm but a limited image field of view, with poor beam penetration that does not permit evaluation of structures deeper than 1.5 cm below the skin surface. Recent developments in US technology include the availability of high-resolution broadband transducers (5–10 MHz, 8–16 MHz and 10–22 MHz).

High frequency (grey-scale) US measurements of joint space are robust and allow reproducible delineation of synovial thickening in the small joints of the hands in patients with active RA. However, analysis of such images does not necessarily demonstrate clear relationships with clinical assessments of disease activity [62], an observation likely to reflect the fact that high-frequency US identifies synovial thickening without necessarily differentiating actively inflamed tissue from fibrous tissue, blood clot, fibrin, complex effusion or tissue debris. Using Doppler methods, in which a signal is generated by moving blood cells, flow information can be obtained easily and non-invasively [58, 63, 64]. Therefore, by assessing vascularized synovium, additional use of Doppler techniques might be predicted to better reflect the presence of active synovitis. Large vessel blood flow is at high velocity and is readily detected by conventional colour Doppler sonography that encodes the mean Doppler frequency shift. However, blood flow at the microvascular level, which is most relevant to rheumatoid synovitis, is at a lower velocity and less readily detectable by this method. In contrast, power Doppler (PD) sonography encodes the amplitude of the power spectral density of the Doppler signal and is a sensitive tool for demonstrating the presence of blood flow in small vessels. The PD signal is actually a measure of the density of moving reflectors at a particular level, and thus of fractional vascular volume [65, 68]. It is not a measure of angiogenesis as such; PD (in common with most other ultrasound methods) is insensitive to flow in submillimetre vessels, and is thus only an indirect surrogate for measurement of capillary flow. However, several studies have confirmed that PD is capable of detecting synovial hyperaemia in the inflamed RA joint [58, 62, 67, 68]. Furthermore, recent studies confirm the value of combined high-frequency US and PD as a tool for detection of peripheral enthesitis in patients with spondyloarthropathy [69].

Numerical quantification of synovial vascularization detected by PD permits investigation of this imaging technology to assess disease activity and response to therapeutic intervention. For simplicity, many researchers have favoured semiquantitative assessments [63, 70]. However, absolute quantification of the number of colour pixels in a region of interest can be achieved using digital image analysis software packages [58, 62, 67, 71]. Good correlations are reported between the scores derived using global semiquantitative methods and digital image analysis [64]. Quantitative PD assessment of vascularized synovium in metacarpophalangeal joints of patients with RA is reported to correlate with erythrocyte sedimentation rate (ESR) [62]. Interestingly, no clear relationships are observed between serum VEGF concentrations and semiquantitative scores for intra-articular synovial vascularity, as determined by PD, at clinically involved wrists [72]. However, this is perhaps not surprising in view of the uncertainty regarding the source of VEGF measured in the serum and the heterogeneity in vascular signal observed in any given RA patient between various peripheral joints.

Several recent publications have highlighted the potential value of high-frequency US and PD for the assessment of response to a therapeutic intervention. Reduction in PD signal in RA joints has been reported in small, uncontrolled studies.
following intravenous or intra-articular steroid [63, 73–76] and anti-TNF therapies [77–79]. Our group have undertaken the first study to successfully employ ultrasonographic measures of synovial thickness and vascularity to assess outcome in the context of a placebo-controlled, double-blind, randomized study [71]. In this study, we investigated the capability of high-frequency ultrasound measures of synovial thickness and quantitative PD measurement of synovial vascularity to discriminate between patients with early RA receiving either infliximab or placebo infusions added to pre-existing, stable methotrexate (MTX) therapy. Twenty-four patients with early RA (<3yr duration) on stable doses of MTX were randomized to receive infusions of infliximab (5 mg/kg) or placebo at weeks 0, 2, 6, and thereafter every 8 weeks to week 46. At baseline and at 18 weeks, all the metacarpophalangeal joints were scanned over the dorsal surface by high-frequency ultrasound and PD using a 15L8 Sequoia transducer (Acuson, USA) with constant B-mode settings. For each joint, synovial thickening was assigned a score ranging from 0 to 5. A total synovial thickness score was calculated as the sum of the individual joint scores. Similarly, the number of colour Doppler pixels in images demonstrating maximal synovial vascularity was determined and the total colour Doppler area calculated as the sum of the individual joint scores. At baseline and week 54, radiographs of the hands and feet were taken and evaluated in chronological sequence using the van der Heijde modification of Sharp’s method (van der Heijde–Sharp score). The Mann–Whitney U-test was used to assess differences in progression in the three imaging modalities between the two treatment groups. Median reduction in synovial thickness as assessed by high-resolution US was 50% in the infliximab group as compared with an increase of 1.2% in the placebo group (P = 0.014). Similarly, median colour Doppler area diminished by 98.4% in the infliximab plus MTX group as compared with a reduction of only 30.7% in the placebo plus MTX group, a statistically significant difference (P = 0.017). In this study, the ultrasonographic measures were better able to discriminate between patients receiving infliximab and those receiving placebo infusions than were changes in disease activity score (DAS28) at the 18-week time point. The delay or reversal of inflammatory and joint destructive mechanisms in patients with early RA was apparent following 18 weeks of treatment with infliximab plus MTX and was reflected in radiographic changes at 54 weeks.

Intravenous microbubble echo-contrast agents may raise the intensity of weak PD signals to a detectable threshold, thus enhancing the sensitivity of PD imaging in inflammatory arthritis [62, 80]. Applications of echo-contrast agents warranting further investigation in the characterization of inflammatory joint tissue include assessment of time–intensity curves that provide quantitative estimation of synovial perfusion [76, 81]. In the case of RA knee joint synovial tissue, significant relationships are reported between the area underlying time–intensity curves and DAS [81] as well as C-reactive protein [76]. However, there are several relative disadvantages including an increase in cost, time and invasiveness. Other potential disadvantages are that gain in sensitivity may be accompanied by loss of specificity, as observed in a recent study comparing contrast-enhanced and unenhanced PD with villous vascular marking on arthroscopy as a reference [82]. Similarly, there may be no correlation between synovitis as assessed clinically and contrast-enhanced PD signal [83]. As sensitive imaging technologies such as PD become more widely employed in clinical practice, it is important to delineate the pathophysiologial correlates of imaging abnormalities. In patients with osteoarthritis and RA, the PD signal intensity correlates well with histological assessment of synovial membrane microvascular density in tissue taken at arthroplasty from the previously imaged site [67, 84]. There is a very close relationship between the presence or absence of vascular flow signal on PD imaging and the rate of early synovial enhancement on dynamic gadolinium-enhanced magnetic resonance imaging (MRI) of RA metacarpophalangeal joints [69]. Similarly, findings on imaging of metacarpophalangeal joints in RA patients using the microbubble PD contrast agent Levovist are closely related to those with dynamic gadolinium-enhanced MRI; the rate of early synovial enhancement on MRI is significantly higher in those joints observed to have a contrast-enhanced PD signal than in those without such a signal [83]. Collectively, these findings further validate the hypothesis that a synovial vascular signal on PD is associated with inflammatory processes.

The main advantages of US as compared with other imaging techniques include absence of radiation, good visualization of tendons and joint space, low running costs, multiplanar imaging capability and easy comparison with the contralateral side. US can be performed at the bedside and is readily acceptable to patients. However, the image acquisition procedure for high-frequency US and PD has yet to be standardized and the quality of the examination is highly dependent upon the use of optimal equipment and the skill of the operator. For example, movement of the transducer or patient may result in a phenomenon termed ‘flash’ artefact that artificially enhances the Doppler effect. This may compromise quantitation and interpretation of vascular signal but can be minimized by appropriate adjustment of machine settings such as increasing the pulse repetition frequency, adjusting persistence and reducing gain. Other measures include the use of splints to maintain the particular region being examined in a position of rest. Temperature fluctuations may alter the PD signal, and therefore scanning should ideally be performed in a room where constant temperatures are maintained all year round with a delay of at least 10 min if the patient arrives from outside. Vessel occlusion with concomitant reduction in PD signal may result if excessive pressure from the transducer is exerted; a stand-off gel pad may reduce this tendency. Furthermore, there are potential problems with reproducibility based on intra- and inter-observer variability and the use of different machines. These issues are now beginning to be addressed and encouraging data are emerging. In a recent study of ultrasonographic assessment of finger and toe joints in RA, using a semiquantitative scoring system for evaluation of synovial thickening, bone erosions and PD signal, an experienced radiologist and a rheumatologist with limited ultrasound training achieved high inter-observer agreement rates [61].

Can angiogenic markers predict disease outcome?

Our group have reported that serum VEGF levels at presentation with early RA correlate highly significantly with development of radiographic damage over the subsequent year [45] as assessed in radiographs of the hands and feet by the van der Heijde modification of Sharp’s method [85]. We have also observed that RA patients with persistent disease activity despite conventional therapy have relatively high serum VEGF concentrations at first presentation. If confirmed in larger series, these observations would support the case for early introduction of a more aggressive therapeutic regime in patients with highly elevated serum VEGF levels early in their disease course.

We have also recently reported a remarkable and previously undescribed finding in a study using PD to assess response to therapy in a randomized double-blind study in early RA in which either infliximab or placebo infusions were added to MTX treatment. Quantitative PD measures of synovial vascularity at baseline were strikingly correlated with the magnitude of radiological joint damage over the following year. There was a highly significant, strongly positive correlation between the baseline summed colour Doppler area in pixels for all 10 metacarpophalangeal joints imaged and the progression in total modified van
der Heijde–Sharp score over 54 weeks (Spearman correlation 0.78; \( P = 0.005 \)) (Fig. 2). Treatment with infliximab plus MTX not only abolished the positive relationship between baseline vascular signal and progression of joint damage, but also resulted in a weakly negative but non-significant correlation. This negative correlation following infliximab plus MTX therapy suggests that patients with high baseline disease activity, as assessed by PD, may derive the greatest benefit from anti-TNF treatment with respect to arrest of structural damage to joints [71].

Collectively, these observations warrant testing the hypothesis that serum measurement of angiogenic markers and PD imaging can be used to identify early RA patients at highest risk of accelerated joint damage who therefore merit intervention with biologic agents targeting TNFα.

**Conclusions**

Evaluation of disease activity and structural damage to joints in RA is important in both routine clinical management and clinical trials. Recent years have witnessed the emergence of a new paradigm in the treatment strategy for RA involving early and aggressive suppression of synovitis by means of pharmacological intervention with drugs proven to modify the rate of progression of structural damage to joints. In particular, it has emerged that combination therapy with once weekly oral methotrexate and an anti-TNF agent can even prevent the progression of structural damage, previously thought to be an unavoidable disease characteristic [86, 87]. Preservation of joint integrity is closely associated with maintenance of functional capability. The economic implications of long-term biologic therapy are such that rationing is necessary in all health-care systems. It is therefore highly desirable to identify those patients most in need of these drugs early in their disease course. It is also necessary to ascertain whether patients treated with biologic therapies are responding satisfactorily not only with respect to reduction in symptoms and signs of disease but also with respect to retardation of structural damage to joints. However, as compared with ultrasonographic technology, clinical joint examination and plain radiography are relatively insensitive tools for assessment of synovitis and erosive disease [88]. Power Doppler is a sensitive, non-invasive method for visualizing RA synovial vascularization that is emerging as a clinically important tool for the assessment of disease activity and holds promise as a novel means of evaluating the response of patients to therapy. Now that arrest of structural damage to joints is becoming a realistic goal in the management of RA, serum vascular markers and power Doppler imaging may also have the potential to predict those patients most at risk of accelerated joint destruction and therefore to inform treatment decisions with respect to early introduction of anti-TNF therapies.

The author has no direct or indirect financial interest which might affect the conduct or reporting of the work described.

**References**


