

# Baseline angiopoietin-2/angiopoietin-1 (Ang2/Ang1) ratio is correlated with the synovial vascularity measured 1 month later in rheumatoid arthritis

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## Background

Synovial angiogenesis is a component of rheumatoid pannus and considered to be an important early step in the pathogenesis of rheumatoid arthritis (RA).

## Objectives

The aim of this study was to evaluate the serum levels of angiopoietin-1 (Ang1), angiopoietin-2 (Ang2), as well as the significance of the Ang2/Ang1 ratio in RA in relation to the joints' blood flow signals and RA activity parameters. In addition, we studied their relevance in predicting the state of angiogenesis.

## Patients and methods

This study was carried out on 65 consecutive very early RA patients. Power Doppler ultrasonography was performed in a total of 10 joints. The blood flow signals of each joint were scored on a three-grade scaling system. The total of the scores of the 10 joints was defined as the total signal score (TSS). On the same day, serum variables including vascular endothelial growth factor (VEGF), Ang1 and Ang2 levels, and clinical disease activity were evaluated. All the parameters measured were reassessed after 1 month.

## Results

Serum VEGF, Ang2 levels, and Ang2/Ang1 ratio were significantly correlated with C-reactive protein, DAS28-CRP, and TSS at baseline and after 1 month. Moreover, significant correlations were observed between serum VEGF and Ang2 levels and Ang2/Ang1 ratio both at baseline and 1 month later. Interestingly, baseline serum VEGF level and Ang2/Ang1 ratio were significantly correlated with TSS after 1 month.

## Conclusion

Serum concentrations of VEGF and Ang2 as well as Ang2/Ang1 ratio were correlated with parameters of inflammation in early RA. Elevated serum VEGF and Ang2/Ang1 ratio (in favor of Ang2) could reflect a phase of vigorous angiogenesis.

## Keywords:

angiopoietin, power Doppler ultrasonography, rheumatoid arthritis, vascular endothelial growth factor

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## Introduction

The inflammatory process in rheumatoid arthritis (RA) is characterized by excessive, tumor-like proliferation of synoviocytes, which 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 upregulation of angiogenesis promoters and downregulation of inhibitors [2].

The angiogenic factor angiopoietin-1 (Ang1) is an agonist ligand of the endothelial receptor tyrosine kinase Tie-2. In adults, the Ang1/Tie-2 signaling system is essential for the maturation of vessels. Experiments in

transgenic mice have shown that vascular endothelial growth factor (VEGF) induces angiogenesis, whereas Ang1 induces vessels stabilization by maximizing the interactions between endothelial cells and their surrounding support cells and matrix [3].

Angiopoietin-2 (Ang2) is the natural antagonist of Ang1 [4]. By inhibiting Tie-2 signaling, Ang2 leads to a loosening of cell matrix and cell-cell interactions [5-7]. This antagonistic effect is considered to be a requirement for the sensitivity of endothelial cells to other angiogenic factors such as VEGF. In the absence of angiogenic growth or survival signals, Ang2 action results in destabilization and finally regression of vessels [5].

Because angiogenesis and formation of new blood vessels is a component of pannus in RA, we hypothesized that the balance between Ang1 and Ang2 is in favor of Ang2 (represented by Ang2/Ang1 ratio) in RA pannus.

The aim of this work is to study the serum levels of Ang1, Ang2, and the Ang2/Ang1 ratio in RA in relation to blood flow signals in a total of 10 joints and RA activity parameters. In addition, we aimed to study their relevance to predict the state of angiogenesis.

## **Patients and methods**

This study was carried out on 65 consecutive very early RA patients (59 women and six men) who were attending the outpatient clinic of Rheumatology and Rehabilitation, Mansoura University Hospital, Egypt. All patients fulfilled the American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) 2010 classification criteria for RA [8]. Their age ranged from 24 to 85 years. Disease duration was less than 12 months. All patients were naïve for disease-modifying antirheumatic drugs. All patients underwent a standardized interview for assessment of history, followed by a physical examination. The disease activity was assessed using the disease activity score DAS28-CRP tool [9]. In addition, 15 apparently healthy individuals were enrolled as controls. Written consent was obtained from every participant in this study after approval of this study from the local Ethical Committee.

## **Collection and preparation of samples**

After a 12 h fast, venous blood samples were collected from every participant by sterile venipuncture on the morning of the same day of assessment of history and clinical examination. The separated serum was kept frozen at  $-20^{\circ}\text{C}$  until the time of analyses, which were carried out at the latest after 3 months. Samples were used for estimation of serum C-reactive protein (CRP) and the angiogenic factors.

## **Evaluation of angiogenic factors and calculation of Ang2/Ang1 ratio**

Serum VEGF and Ang1 and Ang2 levels were determined using an ELISA kit (R&D Systems, Minneapolis, Nebraska, USA). The mean values of double measurements were calculated. Concentrations are reported as pg/ml. After obtaining Ang1 and Ang2, the Ang2/Ang1 ratio was calculated using the statistical program for each patient.

## **CRP assay**

Quantitative assay of the CRP kit was supplied by Turbox CRP (Orion Diagnostica, Espoo, Finland). The Turbox assay for CRP is a liquid-phase immunoprecipitation assay with nephelometric detection. Concentrations are reported as mg/dl.

## **Assessment using PDUS**

Ultrasound was emitted using a linear array transducer (7.5–10 MHz, Esaote-GPX-color Doppler apparatus, Genoa, Italy). The pulse repetition frequency was set at the lowest level in the tolerated range to achieve the maximum sensitivity: 500–750 Hz. Low wall filters were used. The dynamic range was set at 20–40 dB. Power Doppler ultrasonography (PDUS) was performed in a total of 10 joints: the bilateral elbows, wrists, metacarpophalangeal, knees, and ankles. The probe was applied to the anterior and posterior recesses in the elbow, dorsal carpal recesses, extensor tendon sheaths and flexor tendon sheaths in the wrist, anterior and posterior recesses in the wrist, suprapatellar, medial parapatellar and lateral parapatellar recesses in the knee, and anterior, medial, and lateral tendon sheaths in the ankle. The degree of vascularity at various sites of the synovial membrane was quantified by counting color pixels of region of interest using a special computer software, followed by calculation of the color fraction (defined as the ratio of color pixels number to the total number of pixels in the region of interest [10]). The color fraction was scored as follows: score 0, no PDUS signals; score 1, color fraction less than 25%; score 2, color fraction from 25 to 50%; and score 3, color fraction greater than 50% [11]. The score at the site with the strongest finding in each joint was adopted as the score of the joint and the total of the scores of the 10 joints was defined as the total signal score (TSS).

## **Statistical analyses**

All statistical analyses were carried out using SPSS for windows version 17.0 (SPSS, Chicago, Illinois, USA). Continuous data were expressed as mean $\pm$ SD and median. The correlations between the angiogenic markers (VEGF, Ang1, Ang2, and Ang2/Ang1 ratio) and the parameters of inflammation (CRP and DAS28-CRP) were assessed by Spearman's  $r$  coefficient. Correlations among serum angiogenic markers as well as the Ang2/Ang1 ratio were also assessed using Spearman's  $r$  coefficient.  $P$ -values of less than 0.05 were considered to be statistically significant.

## **Results**

Patient characteristics at baseline and after 1 month are summarized in Table 1. This study included 65 patients (59 women and six men); their mean age was  $56.1 \pm 13.7$  years (age range from 24 to 85 years). The disease duration was  $6.8 \pm 1.2$  months (ranging from 4 to 10 months). Serum VEGF concentrations were significantly higher in patients with early RA than in the controls.

### Serum levels of angiogenic factors and Ang2/Ang1 ratio with indices of disease activity

Table 2 shows the relationship of the CRP and DAS28-CRP with the angiogenic factors and the Ang2/Ang1 ratio at baseline and after 1 month. Serum VEGF level was correlated significantly with CRP at baseline ( $r = 0.345, P = 0.005$ ) and after 1 month ( $r = 0.261, P = 0.036$ ) and correlated significantly with DAS28-CRP at baseline ( $r = 0.347, P = 0.005$ ) and after 1 month ( $r = 0.371, P = 0.002$ ). The Serum Ang2 level was correlated significantly with CRP at baseline ( $r = 0.377, P = 0.002$ ) and after 1 month ( $r = 0.399, P = 0.001$ ) and correlated significantly with DAS28-CRP at baseline ( $r = 0.369, P = 0.002$ ) and after 1 month ( $r = 0.371, P = 0.002$ ). The Ang2/Ang1 ratio was correlated significantly with CRP at baseline ( $r = 0.254, P = 0.041$ ) and after 1 month ( $r = 0.280, P = 0.024$ ) and correlated significantly with DAS28-CRP at baseline ( $r = 0.307, P = 0.013$ ) and after 1 month ( $r = 0.275, P = 0.027$ ). Serum Ang1 level did not correlate with the indices of disease activity.

**Table 1 Patients' characteristics at baseline and after 1 month**

	Range	Mean $\pm$ SD	Median
Age (years)	24–85	56.1 $\pm$ 13.7	
RA duration (months)	4–10	6.8 $\pm$ 1.2	
CRP (mg/dl)			
Baseline	0.04–12.67	1.9 $\pm$ 3.2	
After 1 month	0.5–6.84	1.4 $\pm$ 1.6	
DAS28-CRP			
Baseline	1.22–6.54	3.3 $\pm$ 1.1	
After 1 month	1.16–6.21	3.2 $\pm$ 1.1	
VEGF (pg/ml)			
Baseline	60–2696	590.6 $\pm$ 543.1	432
After 1 month	66–2307	517 $\pm$ 461.7	382
Ang1 (pg/ml)			
Baseline	8145–58451	26110.8 $\pm$ 9842.9	24634
After 1 month	8959.5–64296	28721.8 $\pm$ 10827.2	27097.50
Ang2 (pg/ml)			
Baseline	971.5–8397.5	3976 $\pm$ 1648.1	3503
After 1 month	826–7138	3394.9 $\pm$ 1419.3	2977.50
Ang2/Ang1 ratio			
Baseline	0.03–0.57	0.18 $\pm$ 0.11	0.15
After 1 month	0.02–0.62	0.14 $\pm$ 0.1	0.12
TSS			
Baseline	3–22	9 $\pm$ 4.8	
After 1 month	3–23	9 $\pm$ 4.7	

Ang1, angiopoietin-1; Ang2, angiopoietin-2; CRP, C-reactive protein; RA, rheumatoid arthritis; TSS, total signal score; VEGF, vascular endothelial growth factor.

**Table 2 Correlation of angiogenesis markers with inflammatory parameters at baseline and after 1 month**

	Correlation at baseline				Correlation after 1 month			
	CRP		DAS28-CRP		CRP		DAS28-CRP	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
VEGF	0.345	0.005	0.347	0.005	0.261	0.036	0.371	0.002
Ang1	0.066	>0.05	0.042	>0.05	0.04	>0.05	0.049	>0.05
Ang2	0.377	0.002	0.369	0.002	0.399	0.001	0.371	0.002
Ang2/Ang1 ratio	0.254	0.041	0.307	0.013	0.280	0.024	0.275	0.027

Ang1, angiopoietin-1; Ang2, angiopoietin-2; CRP, C-reactive protein; VEGF, vascular endothelial growth factor.

### Serum levels of angiogenic factors and Ang2/Ang1 ratio with TSS

Serum VEGF level was correlated significantly with TSS at baseline ( $r = 0.564, P < 0.001$ ) and after 1 month ( $r = 0.350, P = 0.004$ ). Serum Ang2 level was correlated significantly with TSS at baseline ( $r = 0.423, P < 0.001$ ) and after 1 month ( $r = 0.352, P = 0.004$ ). The Ang2/Ang1 ratio was correlated significantly with TSS at baseline ( $r = 0.355, P = 0.004$ ) and after 1 month ( $r = 0.360, P = 0.003$ ; Table 3).

### Relationship among angiogenic factors

Table 4 shows the relationship among angiogenic factors at baseline and after 1 month. A significant correlation was observed between serum VEGF level and Ang2 ( $r = 0.518, P < 0.001$ ) and Ang2/Ang1 ratio ( $r = 0.398, P < 0.001$ ) at baseline. After 1 month, a significant correlation was also observed between serum VEGF level and Ang2 ( $r = 0.348, P = 0.004$ ) and Ang2/Ang1 ratio ( $r = 0.283, P = 0.022$ ). Serum Ang1 level was significantly inversely correlated with the Ang2/Ang1 ratio at baseline ( $r = -0.579, P < 0.001$ ) and after 1 month ( $r = -0.569, P < 0.001$ ). Serum Ang2 level was significantly correlated with the Ang2/Ang1 ratio at baseline ( $r = 0.628, P < 0.001$ ) and after 1 month ( $r = 0.647, P < 0.001$ ).

### Relationship between baseline levels of angiogenic factors and TSS after 1 month

Figure 1 shows the relationship between baseline serum VEGF level and TSS after 1 month, whereas Figure 2 shows the relationship between baseline Ang2/Ang1 ratio and TSS after 1 month. Interestingly, baseline serum VEGF level was correlated significantly with TSS after 1 month. Also, baseline Ang2/Ang1 ratio was correlated significantly with TSS after 1 month.

### Discussion

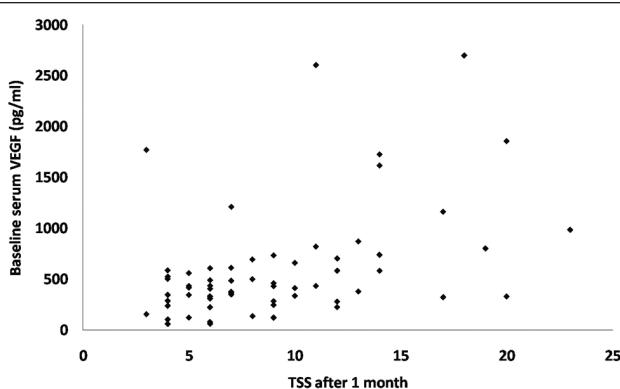
The main findings of this study are as follows: (a) interestingly, only serum VEGF levels and Ang2/Ang1 ratio measured at baseline were correlated with TSS measured 1 month later and (b) serum VEGF,

levels, serum Ang2 levels, and Ang2/Ang1 ratio are correlated with CRP and DAS28-CRP and thus seem to reflect the RA activity.

The serum VEGF level and serum Ang2 level were correlated with the serum CRP levels, DAS28-CRP, and TSS. Conflicting data on the association of angiogenic factors with parameters of inflammation in RA have been reported by previous studies. In addition to our study, the serum VEGF level has been observed to be correlated with the serum CRP level [12], parameters of inflammation, and DAS28-CRP [13]. Kurosaka *et al.* [14] found that serum Ang2 levels are correlated with serum CRP but not with DAS28-CRP; they also reported a relationship between serum VEGF and Ang2 levels, but not Ang1, with synovial blood flow signals. However, Strunk *et al.* [15] did not observe a correlation between serum VEGF level and

synovial blood flow signals in the wrist. In the study of Strunk and colleagues, only the wrists were evaluated. Although the serum VEGF level is a systemic finding, the synovial blood flow signals of the wrist are local findings. Therefore, evaluation of various joints of the body would be more relevant to examination of the relationship between serum angiogenesis factors and synovial blood flow signals. Hence, we examined multiple joints. Moreover, Strunk and colleagues examined only 21 RA patients, whereas in the current study, 65 RA patients were examined. As a result, a correlation between the serum VEGF level and TSS could be confirmed. The increase in synovial blood flow signals has been observed histologically to be caused by an increase in the number of blood vessels in the synovial tissue, that is, angiogenesis [16]. Thus, the correlation between the serum level of VEGF, which is a major angiogenic factor, and TSS appears reasonable.

**Figure 1**



Baseline serum vascular endothelial growth factor (VEGF) level is correlated significantly with the total signal score (TSS) measured 1 month later ( $r = 0.452$ ,  $P < 0.001$ ).

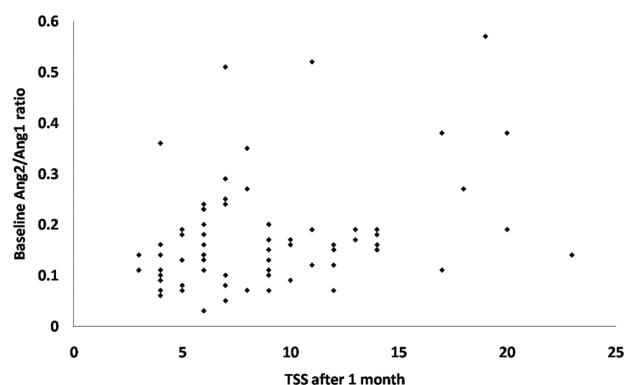
**Table 3 Correlation between angiogenesis markers and TSS**

	Correlation at baseline		Correlation after 1 month	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
VEGF	0.564	<0.001	0.350	0.004
Ang1	-0.092	>0.05	-0.123	>0.05
Ang2	0.423	<0.001	0.352	0.004
Ang2/Ang1 ratio	0.355	0.004	0.360	0.003

Ang1, angiopoietin-1; Ang2, angiopoietin-2; TSS, total signal score; VEGF, vascular endothelial growth factor.

Ang1 and Ang2 are ligands of the tyrosine kinase receptor Tie-2 that contribute toward blood vessel formation during angiogenesis [17]. Stabilization of the vascular wall is regulated by Ang1 binding to the Tie-2 receptor. In contrast, Ang2 Tie-2 binding induces vessel destabilization, which leads to angiogenic sprouting [6,18]. The balance between these factors and the level of other angiogenic factors, particularly VEGF, determines whether new blood vessels form or blood vessels become stable [19].

**Figure 2**



Baseline angiopoietin-2 (Ang2)/angiopoietin-1 (Ang1) is correlated significantly with the total signal score (TSS) measured 1 month later ( $r = 0.328$ ,  $P = 0.008$ ).

**Table 4 Correlation between angiogenesis markers at baseline**

	Correlation at baseline						Correlation after 1 month					
	Ang1		Ang2		Ang2/Ang1		Ang1		Ang2		Ang2/Ang1	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
VEGF	0.029	>0.05	0.518	<0.001	0.398	0.001	0.029	>0.05	0.348	0.004	0.283	0.022
Ang1			0.042	>0.05	-0.579	<0.001			-0.025	>0.05	0.569	<0.001
Ang2					0.628	<0.001					0.647	<0.001

Ang1, angiopoietin-1; Ang2, angiopoietin-2; VEGF, vascular endothelial growth factor.

As the TSS in the current study is correlated with serum VEGF and Ang2 levels but not with serum Ang1 levels, the synovial flow signals are considered to represent a phase of vigorous angiogenesis. Interestingly, our finding of a correlation between baseline serum VEGF level and baseline Ang2/Ang1 ratio with the TSS measured 1 month later seems to confirm this concept.

Among the angiogenic factors we examined, a correlation was observed between the serum levels of VEGF and Ang2 but not between serum levels of VEGF or Ang2 with Ang1. The absence of a correlation between the serum levels of VEGF and Ang1 levels is understandable on the basis of these data. The correlation of VEGF level with the level of Ang2 rather than the level of Ang1 may indicate the usefulness of both markers as an index of active arthritis.

Thus, as Ang1 stabilizes the blood vessels, whereas Ang2 induces angiogenesis, it is reasonable that the elevated Ang2/Ang1 ratio (in favor of Ang2) reflects the phase of vigorous angiogenesis. The correlation of the baseline VEGF and Ang2/Ang1 ratio with TSS measured 1 month later observed in the current study may be explained from this prospective. To our knowledge, this is the first study in which the ratio of Ang2 to Ang1 is determined in relation to synovial blood flow.

## Conclusion

Serum concentrations of VEGF and Ang2 as well as Ang2/Ang1 ratio were correlated with parameters of inflammation in early RA. Our results show that the elevated serum VEGF and Ang2/Ang1 ratio (in favor of Ang2) reflect a phase of vigorous angiogenesis.

## Acknowledgements

### Conflicts of interest

None declared.

## References

- 1 Schrieber L, Jackson JC. In: Klippel HJ, Dieppe PA, editors. *Angiogenesis in rheumatoid arthritis*. Rheumatology 2<sup>nd</sup> ed. London, UK: Mosby International; 1988. 12.1–12.4.
- 2 Koch AE, Volin MV, Woods JM, Kunkel SL, Connors MA, Harlow LA, et al. Regulation of angiogenesis by the C-X-C chemokines, interleukin-8 and epithelial neutrophil activating peptide 78 in the rheumatoid joint. *Arthritis Rheum* 2001; **44**:31–40.
- 3 Thurston G, Suri C, Smith K, McClain J, Sato TN, Yancopoulos GD, et al. Leakage-resistant blood vessels in mice transgenically overexpressing angiopoietin-1. *Science* 1999; **286**:2511–2514.
- 4 Maisonneuve PC, Suri C, Jones PF, Bartunkova S, Wiegand SJ, Radziejewski C, et al. Angiopoietin-2, a natural antagonist for Tie2 that disrupts *in vivo* angiogenesis. *Science* 1997; **277**:55–60.
- 5 Yancopoulos GD, Davis S, Gale NW, Rudge JS, Wiegand SJ, Holash J. Vascular-specific growth factors and blood vessel formation. *Nature* 2000; **407**:242–248.
- 6 Scharpfenecker M, Fiedler U, Reiss Y, Augustin HG. The Tie-2 ligand angiopoietin-2 destabilizes quiescent endothelium through an internal autocrine loop mechanism. *J Cell Sci* 2005; **118**:771–780.
- 7 Rovizze F, Tsikatos S, Kotanidou A, Bucci M, Brancaleone V, Cirino G, et al. Angiopoietin-2 causes inflammation *in vivo* by promoting vascular leakage. *J Pharmacol Exp Ther* 2005; **314**:738–744.
- 8 Funovits J, Aletaha D, Bykerk V, Combe B, Dougados M, Emery P, et al. The 2010 American College of Rheumatology/European League against rheumatism classification criteria for rheumatoid arthritis: methodological report phase I. *Ann Rheum Dis* 2010; **69**:1589–1595.
- 9 Fransen J, Welsing P, Keijzer RD, Riel PV. Disease activity scores using C-reactive protein: CRP may replace ESR in the assessment of RA disease activity. *Ann Rheum Dis* 2004; **62**:151.
- 10 Ellegaard K, Christensen R, Torp Pedersen S, Terslev L, Holm CC, Kønig MJ, et al. Ultrasound Doppler measurements predict success of treatment with anti-TNF- $\alpha$  drug in patients with rheumatoid arthritis: a prospective cohort study. *Rheumatology* 2011; **50**:506–512.
- 11 Seymour MW, Kelly S, Beals CR, Malice MP, Bolognese JA, Dardzinski BJ, et al. Ultrasound of metacarpophalangeal joints is a sensitive and reliable endpoint for drug therapies in rheumatoid arthritis: results of a randomized, two-center placebo-controlled study. *Arthritis Res Ther* 2012; **14**:R198.
- 12 Ballara S, Taylor PC, Reusch P, Marmé D, Feldmann M, Maini RN, et al. Raised serum vascular endothelial growth factor levels are associated with destructive change in inflammatory arthritis. *Arthritis Rheum* 2001; **44**:2055–2064.
- 13 Clavel G, Bessis N, Lemeiter D, Fardellone P, Mejjad O, Ménard JF, et al. Angiogenesis markers (VEGF, soluble receptor of VEGF and angiopoietin-1) in very early arthritis and their association with inflammation and joint destruction. *Clin Immunol* 2007; **124**:158–164.
- 14 Kurosaka D, Hirai K, Nishioka M, Miyamoto Y, Yoshida K, Noda K, et al. Clinical significance of serum levels of vascular endothelial growth factor, angiopoietin-1 and angiopoietin-2 in patients with rheumatoid arthritis. *J Rheumatol* 2010; **37**:1121–1128.
- 15 Strunk J, Heinemann E, Neeck G, Schmidt KL, Lange U. A new approach to studying angiogenesis in rheumatoid arthritis by means of power Doppler ultrasonography and measurement of serum vascular endothelial growth factor. *Rheumatology* 2004; **43**:1480–1483.
- 16 Walther M, Harms H, Krenn V, Radke S, Faehndrich TP, Gohlke F. Correlation of power Doppler sonography with vascularity of the synovial tissue of the knee joint in patients with osteoarthritis and rheumatoid arthritis. *Arthritis Rheum* 2001; **44**:331–338.
- 17 Szekanec Z, Koch AE. Mechanisms of disease: angiogenesis in inflammatory diseases. *Nat Clin Pract Rheumatol* 2007; **3**:635–643.
- 18 Davis S, Aldrich TH, Jones PF, Acheson A, Compton DL, Jain V, et al. Isolation of angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning. *Cell* 1996; **87**:1161–1169.
- 19 Shahara S, Volin MV, Connors MA, Haines GK, Koch AE. Differential expression of the angiogenic Tie receptor family in arthritic and normal synovial tissue. *Arthritis Res* 2002; **4**:201–208.