Clinical Significance of Serum Levels of Vascular Endothelial Growth Factor, Angiopoietin-1, and Angiopoietin-2 in Patients with Rheumatoid Arthritis

DAITARO KUROSAKA, KENICHIRO HIRAI, MAKIKO NISHIOKA, YUKIO MIYAMOTO, KEN YOSHIDA, KENTARO NODA, TARO UKICHI, MAIMI YANAGIMACHI, KAZUHIRO FURUYA, EIGO TAKAHASHI, ISAMU KINGETSU, KUNIHIKO FUKUDA, and AKIO YAMADA

ABSTRACT. ***Objective.*** To evaluate the clinical significance of serum levels of vascular endothelial growth factor (VEGF), angiopoietin-1 (Ang-1), and angiopoietin-2 (Ang-2) in patients with rheumatoid arthritis (RA).

Methods. The subjects were 70 patients with RA. Serum VEGF, Ang-1, and Ang-2 levels were determined by ELISA. As indices of disease activity, serum levels of C-reactive protein (CRP) and matrix metalloprotease (MMP)-3 were examined, and the 28-joint count Disease Activity Score (DAS28)-CRP was calculated. Power Doppler ultrasonography was performed in the bilateral wrists, elbows, shoulders, knees and ankles. The synovial blood flow signals were scored using a 3-grade scale (0–2), and the total of the scores in the 10 joints was regarded as the total signal score (TSS).

Results. Serum VEGF level showed significant correlations with serum CRP and MMP-3 levels, DAS28-CRP, and TSS. Serum Ang-1 level showed significant correlations with serum MMP-3 level and DAS28-CRP. Serum Ang-2 level showed significant correlations with serum CRP level and TSS.

Conclusion. The serum VEGF level is important as an index of the activity of RA based on angiogenesis and a prognostic factor regarding joint destruction. Serum Ang-1 level may be useful as an index of sustained arthritis based on the maintenance of newly formed vessels. Serum Ang-2 level may reflect a state of marked angiogenesis. (J Rheumatol First Release May 1 2010; doi:10.3899/jrheum.090941)

Key Indexing Terms:
VASCULAR ENDOTHELIAL GROWTH FACTOR
RHEUMATOID ARTHRITIS
CLINICAL PRACTICE
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Rheumatoid arthritis (RA) is a disease characterized by the chronic inflammation of joint synovial tissues. Inflammatory synovial tissue is called pannus. At such sites, many newly formed vessels are observed[1-3]. Angiogenic factors such as vascular endothelial growth factor (VEGF), angiopoietin-1 (Ang-1), and angiopoietin-2 (Ang-2) play important roles in the angiogenesis of pannus[3].

VEGF is particularly important[4]. It promotes angiogenesis by acting on vascular endothelial cells and promoting endothelial cell migration and lumen formation[5]. In patients with RA, VEGF is detected in joints and serum, and is considered to be closely related to the development of pathologic features of arthritis and especially angiogenesis[6-8].

Ang-1 and Ang-2 contribute to adhesion and detachment between vascular endothelial and wall cells[9]. Ang-1 is constantly secreted from wall cells when blood vessels are stable. Ang-1 is bound to Tie2 on endothelial cells, and this binding is related to the binding between vascular endothelial and wall cells. But the detachment of vascular endothelial and wall cells is necessary for the initiation of angiogenesis. At their detachment, Ang-2, an endogenous antagonist molecule highly homologous to Ang-1, is secreted by vascular endothelial cells and induces the inactivation of Tie2 by inhibiting the binding between Ang-1 and Tie2. As a result, the adhesion between vascular endothelial and wall cells is inhibited, and angiogenesis is initiated. In this state, Ang-2 is dominant over Ang-1. Once new vessels have been formed, their stabilization becomes important. Adhesion between vascular endothelial and wall cells becomes necessary again to stabilize the newly formed vessels. Therefore,

From the Division of Rheumatology and the Division of Radiology, Department of Internal Medicine, Jikei University School of Medicine, Tokyo, Japan.

D. Kurosaka, MD; K. Hirai, MD; K. Yoshida, MD; K. Noda, MD; T. Ukichi, MD; M. Yanagimachi, MD; K. Furuya, MD; E. Takahashi, MD, I. Kingetsu, MD; A. Yamada, MD, Division of Rheumatology, Department of Internal Medicine, Jikei University School of Medicine; M. Nishioka, MD; Y. Miyamoto, MD; K. Fukuda, MD, Division of Radiology, Department of Internal Medicine, Jikei University School of Medicine.

Address correspondence to Dr. D. Kurosaka, Division of Rheumatology, Department of Internal Medicine, Jikei University School of Medicine, 3-25-8, Nishishinbashi, Minato-ku, Tokyo, Japan.
E-mail: d_kurosaka@jikei.ac.jp

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Ang-1 is secreted again by wall cells and binds with Tie2 on endothelial cells. In this state, Ang-1 is dominant over Ang-2[10-14].

Experiments using synovial tissues from patients with RA have confirmed the roles of Ang-1 and Ang-2 in angiogenesis[15-17]. Both have been observed to be important but to act in different periods. Ang-1 has been observed to be important for the maintenance of blood vessels, and Ang-2 to play a role at sites of marked angiogenesis.

While VEGF, Ang-1, and Ang-2 are connected to angiogenesis in synovial regions, the clinical significance of their levels in serum is unclear. The serum level of VEGF has been observed to serve as an index of the severity of joint destruction in RA, but there have been few studies concerning the levels of serum Ang-1 or Ang-2. One study said that the serum Ang-1 level was correlated with the erythrocyte sedimentation rate[8]. In addition, there has been no research on simultaneous measurement of serum levels of VEGF, Ang-1, and Ang-2, with evaluation of their relationships. In our study, therefore, we simultaneously measured the serum levels of these 3 factors in patients with RA and evaluated their relationships with indices of RA activity.

MATERIALS AND METHODS

The subjects were 70 patients (55 women and 15 men) with RA who fulfilled the diagnostic criteria of the American College of Rheumatology (1987)[18]. The mean age of the subjects was 56.2 ± 13.5 (range 24–85) years. The study was approved by the Ethical Committee of Jikei University School of Medicine in advance. Informed consent was obtained from all patients.

Measurement of serum levels of angiogenic factors. The serum samples were stored at –40˚C, and measurements were performed within 3 months after collection. Serum VEGF, Ang-1, and Ang-2 levels were determined using an ELISA kit (R&D Systems, Minneapolis, MN, USA). The mean values of double measurements were calculated.

Ultrasonography assessment. Power Doppler ultrasonography (PDUS) was performed in the dark. Ultrasound was emitted with a linear array transducer (10 MHz; GE Healthcare, Waukesha, WI, USA). 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. PDUS was performed in a total of 10 joints: the bilateral shoulders, elbows, wrists, knees, and ankles. The probe was applied to the posterior recess, biceps tendon sheath and subdeltoid bursa in the shoulder, anterior and posterior recesses in the elbow, dorsal carpal recesses, extensor tendon sheaths and flexor tendon sheaths in the wrist, suprapatellar, medial parapatellar and lateral parapatellar recesses in the knee, and anterior, medial and lateral tendon sheaths in the ankle. After PDUS, video clips recorded by 2 operators were analyzed. The clinical conditions and examination data of the analyzed patients were concealed from the analyzers. The blood flow signals at various sites of the synovial membrane were graded and scored as follows: grade 1: no flow (0 point); grade 2: mild or moderate flow (1 point); grade 3: intense flow (2 points). 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 analysis was performed using Graphpad Prism software (Version 4.0). The relationships of clinical data and findings on ultrasonography were analyzed using Spearman's rank correlation test. $P < 0.05$ was considered significant.

RESULTS

Patients' characteristics. Table 1 shows the patients' characteristics. The duration of the disease was 4.43 ± 4.81 years (mean ± SD). The duration was within 1 year after the onset in 14, 1-5 years in 29, 5-10 years in 17, and 10 years or longer in 9.

The dose of oral prednisolone was 0 mg in 29 patients, 5 mg or less in 11, 5–10 mg in 21, and 10 mg or more in 10. The dose of methotrexate was 0 mg in 33 patients, 0–4 mg in 7, 4–8 mg in 22, and 8 mg or more in 8. Biological preparations were used in 7 patients. The mean serum C-reactive protein (CRP) level was 2.166 ± 3.27 mg/dl, the mean serum matrix metalloprotease (MMP)-3 level was 1.77 ± 41 ng/ml, and mean 28-joint count Disease Activity Score (DAS28)-CRP was 3.324 ± 1.12. DAS28-CRP was 1–2 in 8 patients, 2–3 in 20, 3–4 in 26, 4–5 in 9, 5–6 in 6, and 6–7 in 1 patient. The mean serum VEGF level was 620.46 ± 567.3 pg/ml, mean serum Ang-1 level was 35,975.08 ± 11,200.65 pg/ml, and mean serum Ang-2 level was 3,377.614 ± 1,434.909 pg/ml.

Serum levels of angiogenic factors and indices of disease activity. Figure 1 shows the relationships of the serum VEGF level with the serum CRP and MMP-3 levels, DAS28-CRP, and TSS. The serum VEGF level was significantly correlated with the serum CRP level ($r = 0.5013$, $p < 0.0001$), serum MMP-3 level ($r = 0.3567$, $p = 0.0033$), DAS28-CRP ($r = 0.6527$, $p < 0.0001$), and TSS ($r = 0.4824$, $p < 0.0001$).

Figure 2 shows the relationships of the serum Ang-1 level with the serum CRP and MMP-3 levels, DAS28-CRP, and TSS. It was correlated with the serum MMP-3 level ($r = 0.3121$, $p = 0.0135$) and DAS28-CRP ($r = 0.2435$, $p = 0.0489$), but not with the serum CRP level ($r = 0.1923$, $p = 0.1218$) or TSS ($r = 0.1567$, $p = 0.2088$).

Figure 3 shows the relationships of the serum Ang-2 level with the serum CRP and MMP-3 levels, DAS28-CRP, and TSS. Serum Ang-2 was significantly correlated with the serum CRP level ($r = 0.3288$, $p = 0.0055$) and TSS ($r = 0.2790$, $p = 0.0193$), but not with the serum MMP-3 level ($r = 0.1035$, $p = 0.4081$) or DAS28-CRP ($r = 0.2147$, $p = 0.0743$).

Relationships among angiogenic factors. Figure 4 shows the relationships among the serum levels of angiogenic factors. A significant correlation ($r = 0.3005$, $p = 0.0142$) was noted between VEGF and Ang-1 but not between VEGF and Ang-2 ($r = 0.1429$, $p = 0.2378$) or between Ang-1 and Ang-2 ($r = 0.02165$, $p = 0.8630$).

DISCUSSION

We noted increases in the serum levels of angiogenic factors in patients with RA. These factors were correlated with at least 1 of the serum CRP level, serum MMP-3 level[19], DAS28-CRP[20], and TSS[21-23], which are considered to reflect the activity of RA. These factors have been observed

Table 1. Scores of blood signals and clinical data.

Patient	Age, yrs	Sex	Disease Duration, yrs	Drugs			CRP, mg/dl	DAS28-CRP	MMP-3, ng/ml	VEGF, pg/ml	Ang-1, pg/ml	Ang-2, pg/ml	TSS
1	69	F	7	PSL 15 mg			1.44	4.52	268	279	16395	5205.5	5
2	51	F	0.2	—			6.87	5.29	177	1769	57755	6249.5	6
3	34	F	3	PSL 4 mg	MTX 8 mg		0.12	2.78	—	500	28800	4915.5	2
4	60	F	1	—			5.58	5.61	390	1725	54665	6019.5	4
5	55	F	22	PSL 6 mg			0.98	4.02	171	461	29730	2695.5	2
6	69	F	3	PSL 12.5 mg	MTX 12.5 mg	Infliximab	0.07	5.34	160	584	37535	2816	4
7	31	M	0.4	—			0.53	2.18	56.8	364	40545	2490	1
8	49	F	1	—			0.94	3.9	486	125	33725	2798.5	3
9	57	F	4	PSL 6 mg	MTX 8 mg	Infliximab	0.04>	1.92	101	432	50540	2225	0
10	85	M	1	—			11.49	6.54	361	1856	34935	4190	9
11	84	M	1	—			5.42	4.6	138	584	55545	6557.5	6
12	74	F	0.5	—			0.57	3.3	—	486	32845	5138.5	2
13	73	F	3	PSL 5 mg	MTX 4 mg		8.86	3.28	233	587	26195	5874	0
14	48	M	4		MTX 8 mg		0.04 >	1.78	143	346	36205	1445	0
15	52	F	2	—			0.11	2.77	54.3	350	42730	2225	2
16	64	F	4			Infliximab	0.04>	2.83	93.9	525	37255	2295.5	0
17	64	F	0.1	PSL 8 mg	MTX 8 mg		0.12	3.38	82.1	324	39815	2792.5	0
18	35	F	0.1	—			0.07	3.04	38	121	27605	3007	2
19	61	F	6	PSL 6 mg	MTX 10 mg		1.25	3.44	507	376	36590	6533	3
20	55	F	7			Etanercept	0.26	3.09	67.6	431	36040	2231	2
21	71	F	7	PSL 2 mg	MTX 8 mg		0.11	3.33	—	407	45275	809.5	1
22	49	M	1	PSL 4 mg			1.57	4.46	509	985	45335	1916	8
23	56	F	1	PSL 6 mg	MTX 8 mg		0.17	2.58	134	80	35045	2419.5	1
24	49	M	1			Infliximab	1.08	2.55	387	413	29075	3031	3
25	66	M	4	PSL 10 mg			0.86	3.3	533	1210	52275	2348.5	1
26	59	M	2	PSL 5 mg	MTX 8 mg		0.34	2.19	235	124	28205	1479	0
27	58	M	8	PSL 7 mg	MTX 12.5 mg		0.77	3	144	311	42055	1794.5	2
28	48	M	2	PSL 6 mg	MTX 10 mg		0.3	3.06	208	240	39035	2660	0
29	41	F	2		MTX 6 mg		0.04>	3.53	59.5	435	29130	2919	1
30	37	F	16	PSL 6 mg	MTX 8 mg		0.22	2.75	81.5	287	29945	3043.5	0
31	50	F	9	PSL 5 mg			0.43	2.82	284	225	31200	3193.5	4
32	68	F	5	PSL 5 mg	MTX 4 mg		0.82	2.19	268	609	26360	2894.5	1
33	42	F	0.5	PSL 8 mg	MTX 10 mg		3.9	2.99	176	491	35875	5076	1
34	54	F	5	PSL 12.5 mg	MTX 7.5 mg		1.64	3.67	35.8	740	28040	2635	4
35	73	F	17	—			0.18	2.03	49	379	40655	6754	1
36	75	M	3	PSL 10 mg	MTX 4 mg		0.78	4.54	284	485	77935	2580.5	9
37	58	F	15	PSL 4 mg			0.39	3.07	159	820	47695	3688	3
38	64	M	1	PSL 5 mg	MTX 8 mg		2.33	2.81	113	156	39695	3447	0
39	62	F	12		MTX 6 mg		0.08	1.87	45.1	105	29285	4365	0
40	62	F	5		MTX 6 mg		0.04>	1.78	—	239	31290	2830	0
41	64	F	3	PSL 6 mg	MTX 4 mg	Etanercept	2.24	3.35	384	612	73825	3120.5	4
42	39	F	5		MTX 6 mg		0.04>	1.78	45	60	31570	3878	0
43	39	F	5		MTX 8 mg		0.49	3.37	152	871	27155	1879.5	4
44	37	F	2	—			0.04>	2.85	38.9	694	22195	4830	3
45	54	F	2	PSL 10 mg			0.21	2.91	78	248	35790	4541	2
46	61	F	8	—	Bucillamine		0.34	3.03	48.3	418	23725	2851.5	0
47	58	F	0.2	—			4.91	5.85	267	380	25210	2994.5	4
48	47	F	5	PSL 3 mg	MTX 10 mg		0.04>	3.15	113	332	23955	2095	1
49	49	F	1.5	PSL 6 mg	MTX 8 mg		0.04>	1.78	103	224	30405	2453	1
50	32	F	11	PSL 4 mg	MTX 8 mg		0.09	3.35	116	434	30775	3665	3
51	58	F	6	PSL 12.5 mg	MTX 4 mg		0.18	3.31	113	903	35840	2400.5	1
52	69	F	0.5	—			7.88	5.61	77.9	1615	42540	4202	5
53	24	F	2	PSL 2 mg	MTX 8 mg		0.04>	1.78	46.8	345	31380	2609.5	0
54	26	F	4	PSL 1 mg	MTX 10 mg		0.04>	1.22	52	330	22285	5346.5	0
55	65	M	0.5	—			1.48	4.17	57.4	1013	30960	2201.5	1
56	72	F	1	PSL 4 mg	MTX 4 mg		0.35	2.2	114	284	34895	1534.5	2
57	62	M	3	PSL 10 mg			0.06	3.6	373	802	39215	2313	2
58	38	F	7	PSL 8 mg	MTX 10 mg		0.34	2.75	85.6	503	25810	1388	1

Table 1. Continued.

Patient	Age, yrs	Sex	Disease Duration, yrs	Drugs			CRP, mg/dl	DAS28-CRP	MMP-3, ng/ml	VEGF, pg/ml	Ang-1, pg/ml	Ang-2, pg/ml	TSS
59	74	F	0.2	—			12.67	5.5	545	2696	41570	6998	8
60	69	F	2	PSL 8 mg	MTX 8 mg	Infliximab	7.39	3.21	99.9	703	35460	3349.5	3
61	54	F	0.5	PSL 15 mg			0.92	4.36	219	2335	40750	4889.5	2
62	55	F	7	PSL 8 mg	MTX 10 mg		0.3	3.81	215	1162	38020	3212.5	4
63	56	F	7	PSL 2 mg	MTX 4 mg		0.31	3.01	58.2	61.3	20840	3120	2
64	57	F	2		MTX 6 mg		0.04>	2.62	31	336.1	24560	2499.5	3
65	43	F	0.5	PSL 1 mg	MTX 8 mg		0.04>	2.34	55.8	411.3	41330	3780	0
66	71	F	0.1	—			6.76	3.74	146	2602	25440	2650	7
67	73	M	0.5	—			3.13	4.79	279	734	—	3879.5	6
68	69	F	10		Bucillamine		12.12	4.81	278	660	—	4461	4
69	57	F	7	PSL 10 mg			0.18	2.82	134	137	—	3554	3
70	56	F	20	PSL 8 mg	MTX 8 mg		0.48	3.48	151	561	—	2148	0

CRP: C-reactive protein; MMP-3: matrix metalloproteinase-3; VEGF: vascular endothelial growth factor; Ang-1: angiopoietin-1; Ang-2: angiopoietin-2; DAS: disease activity score; TSS: total signal score; PSL: prednisolone; MTX: methotrexate.

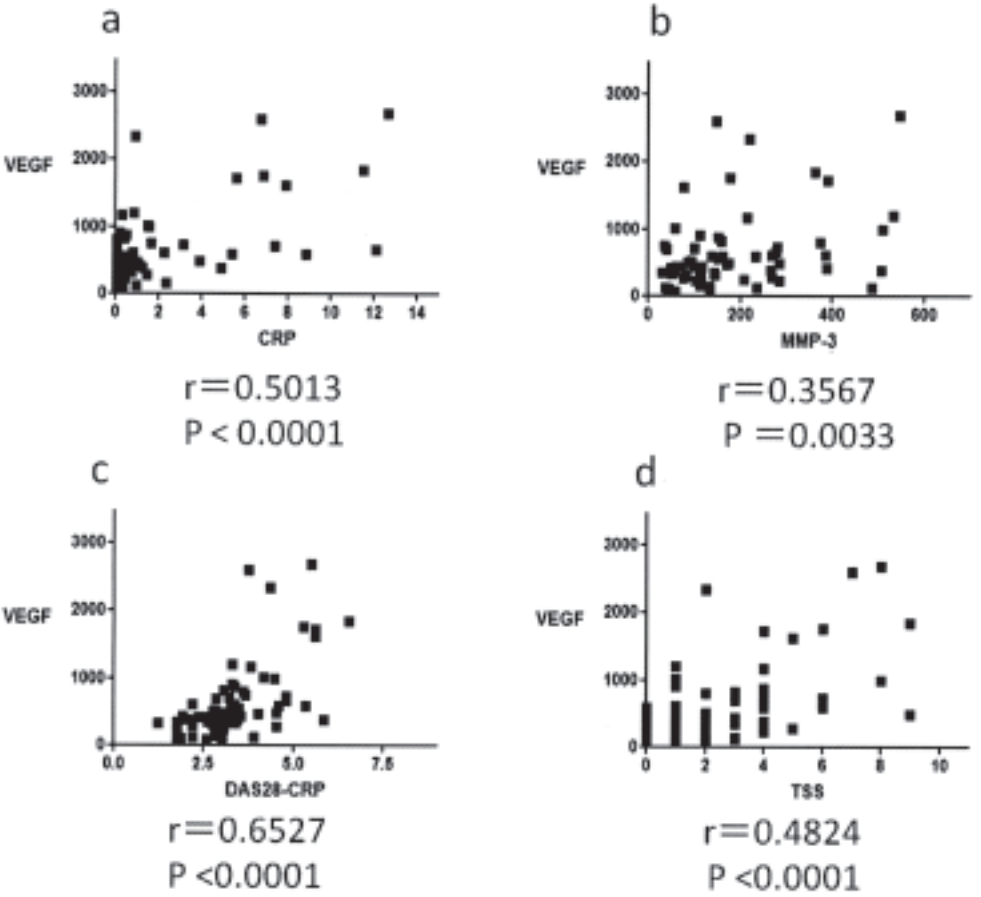

Figure 1. Relationships of the serum vascular endothelial growth factor (VEGF) level with indices of rheumatoid arthritis activity. (a) Serum VEGF and C-reactive protein (CRP) levels. (b) Serum VEGF and matrix metalloprotease (MMP)-3 levels. (c) Serum VEGF level and the 28-joint count Disease Activity Score (DAS28)-CRP. (d) Serum VEGF level and total signal score (TSS). Serum VEGF level was correlated with the serum CRP and MMP-3 levels, DAS28-CRP, and TSS.

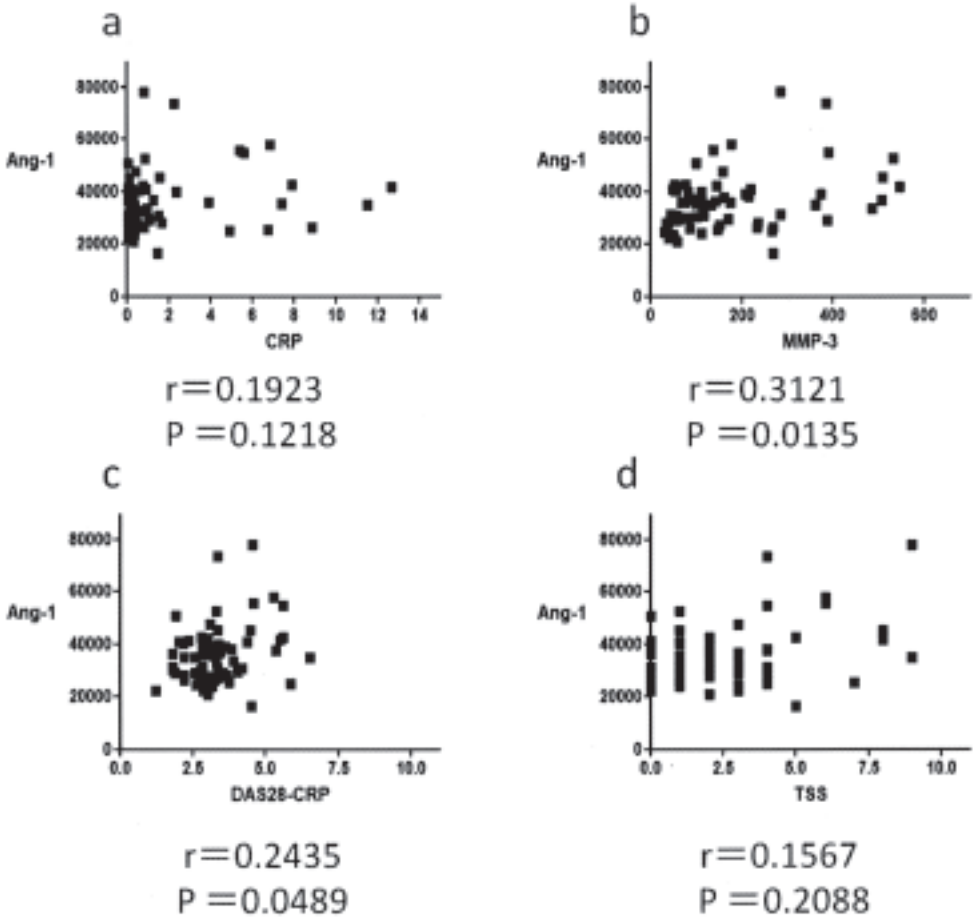

Figure 2. Relationships of the serum angiopoietin-1 (Ang-1) level with indices of RA activity. (a) Serum Ang-1 and C-reactive protein (CRP) levels. (b) Serum Ang-1 and matrix metalloprotease (MMP)-3 levels. (c) Serum Ang-1 level and the 28-joint count Disease Activity Score (DAS28)-CRP. (d) Serum Ang-1 level and total signal score (TSS). Serum Ang-1 level was correlated with the serum MMP-3 level and DAS28-CRP. It was not correlated with the serum CRP level or TSS.

to increase in inflamed areas of the synovial membrane in patients with RA. Therefore, part of the increases in the angiogenic factors observed in this study is considered to have been due to their release from inflamed synovial membrane, although not all of their sources could be determined.

The serum VEGF level was correlated with the serum CRP and MMP-3 levels, DAS28-CRP, and TSS. The serum VEGF level has been observed to be correlated with the serum CRP level[7] and DAS28[8], but the relationship between the serum VEGF and MMP-3 levels has not been observed. The serum MMP-3 level is useful as a predictive factor for joint destruction[24]. The serum VEGF level has been observed by radiographic analysis to be an index of joint destruction[8]. Thus, as both the serum VEGF and MMP-3 levels have been observed to reflect joint destruction, their correlation may be explained from this perspective.

In addition to our 2009 study[25], Strunk, *et al*[26] observed the relationship between the serum VEGF level and synovial blood flow signals. We observed that the serum VEGF level was correlated with the synovial blood flow signal level in the wrist, but Strunk, *et al* did not observe such a correlation. In these 2 studies, evaluation was made of the wrists alone. While 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 was considered necessary to examine the relationship between serum VEGF level and synovial blood flow signals. So we examined multiple joints. The number of patients was also markedly increased. 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, i.e., angiogenesis[27]. Thus, the correlation between the serum level of VEGF, which is a major angiogenic factor, and TSS appears reasonable.

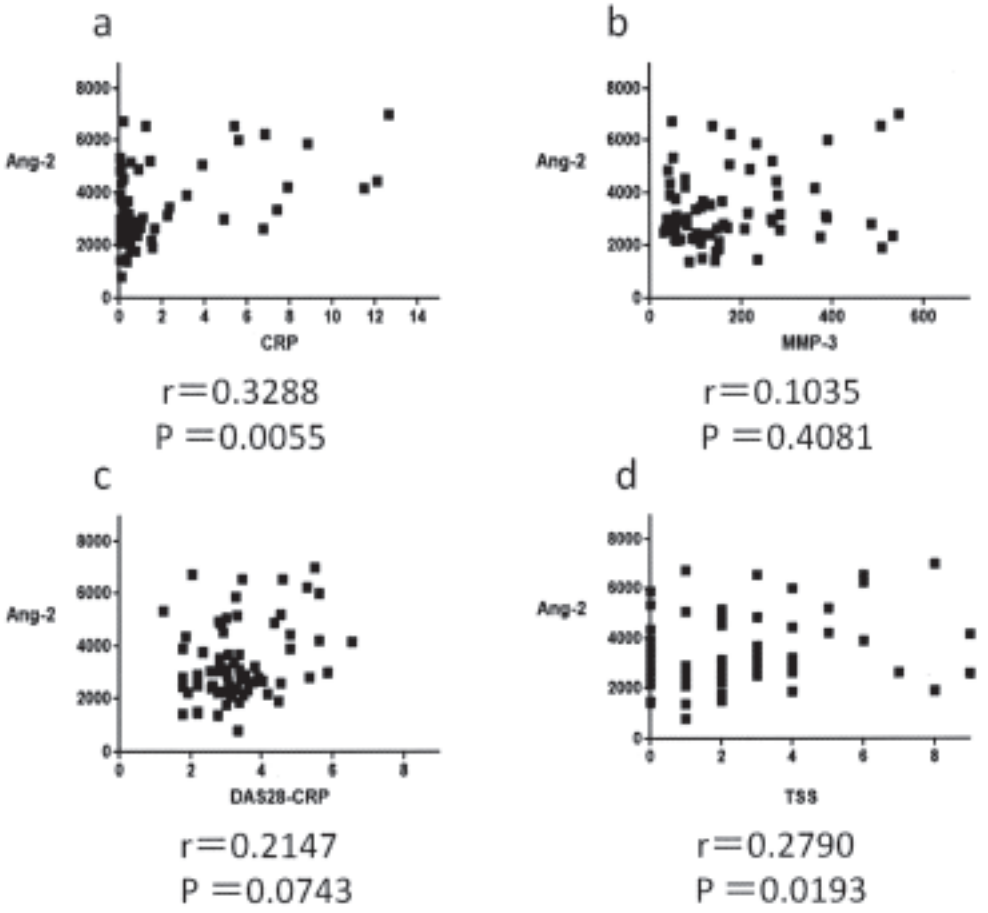

Figure 3. Relationships of serum angiopoietin-2 (Ang-2) level with indices of RA activity. (a) Serum Ang-2 and C-reactive protein (CRP) levels. (b) Serum Ang-2 and matrix metalloprotease (MMP)-3 levels. (c) Serum Ang-2 level and the 28-joint count Disease Activity Score (DAS28)-CRP. (d) Serum Ang-2 level and total signal score (TSS). Serum Ang-2 level was correlated with the serum CRP level and TSS. It was not correlated with the serum MMP-3 level or DAS28-CRP.

The serum VEGF level was correlated with all 4 measures of RA activity examined in our study. Therefore, the serum VEGF level is considered useful as an index of RA activity and a predictive factor for joint destruction based on angiogenesis.

The correlations of the serum Ang-1 and Ang-2 levels with indices of RA activity were weaker than the correlations of the serum VEGF level, and their clinical significance was difficult to determine. A close examination of the data revealed a comparison between the serum Ang-1 and Ang-2 levels that was of interest. The serum Ang-1 level was correlated with the serum MMP-3 level but not with the serum CRP level. The serum Ang-2 level, on the other hand, was correlated with the serum CRP level but not with the serum MMP-3 level. While the serum MMP-3 level reflects disease activity, it is less useful than the serum CRP level as a disease activity marker. But the serum MMP-3 level correlates closely with the outcome of patients with RA, particularly the degree of radiological joint destruction. Thus, the serum MMP-3 level is an index of sustained arthritis and is useful as a predictive factor for the radiological outcome of RA. In contrast, serum CRP level is considered to be most useful as an index of disease activity on examination. The serum level of Ang-1, which is important for the maintenance of newly formed vessels, is related to indices of sustained arthritis, and the serum level of Ang-2, which is important in marked angiogenesis, is related to indices of disease activity on examination.

While DAS28-CRP was correlated with the serum Ang-1 level but not the serum Ang-2 level, the p or r value showed no marked difference, and the correlations of DAS28-CRP with the serum Ang-1 and Ang-2 levels were weaker than those of the VEGF level. Serum Ang-1 and Ang-2 levels are considered to be less sensitive indices of RA activity than the serum VEGF level.

The relationship with the TSS also differed between the serum Ang-1 and Ang-2 levels. The TSS correlated with the level of Ang-2 but not with the Ang-1 level. Synovial blood flow signals have been observed to be related to angiogenesis. It remains unclear in which period it is related more

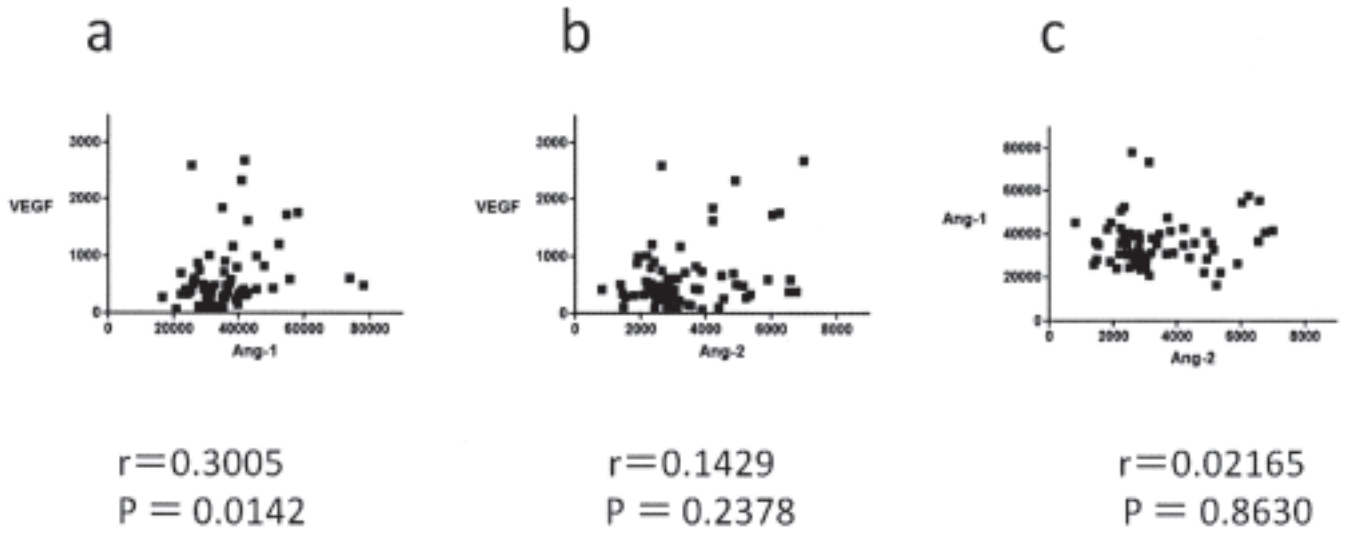

Figure 4. Relationships among angiogenic factors. (a) Serum vascular endothelial growth factor (VEGF) and angiopoietin-1 (Ang-1) levels. (b) Serum VEGF and angiopoietin-2 (Ang-2) levels. (c) Serum Ang-1 and Ang-2 levels. A correlation was observed between the serum VEGF and Ang-1 levels but not between the serum VEGF and Ang-2 levels or between the serum Ang-1 and Ang-2 levels.

closely to angiogenesis. Since the TSS correlated with Ang-2 but not with Ang-1, synovial blood flow signals are considered to represent angiogenesis particularly in a phase of the disease showing vigorous angiogenesis. Synovial blood flow signals have been seen to fluctuate dynamically, responding to various treatments, particularly treatment with tumor necrosis factor-α inhibitors[28-30]. The data from our study may explain this.

Among the angiogenic factors we examined, a correlation was observed between the serum levels of VEGF and of Ang-1 but not in the other combinations. The absence of a correlation between the serum Ang-1 and Ang-2 levels is understandable based on other data. The correlation of VEGF level with the level of Ang-1 rather than the level of Ang-2 may indicate the usefulness of the serum VEGF level as an index of sustained arthritis.

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