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EXTENDED REPORT

Diagnostic strategy for the assessment of rheumatoid vasculitis

A E Voskuyl, J M W Hazes, A H Zwinderman, E M Paleolog, F J M van der Meer, M R Daha, F C Breedveld

Ann Rheum Dis 2003;**62**:407–413

Objective: To determine the clinical features associated with histologically proven rheumatoid vasculitis (HRV) and the additional diagnostic value of serological markers in an inception cohort of 81 patients with rheumatoid arthritis (RA) suspected of RV.

Methods: The presence and number of recently developed extra-articular manifestations (EAMs) and a weighted EAM score, as well as the levels of serological markers, were compared between 31 patients with RA with histologically proven vasculitis and 50 patients with RA in whom vasculitis could not be documented histologically. The following markers were evaluated: circulating immune complexes, complement components C3 and C4, class-specific rheumatoid factors (IgM RF, IgG RF, IgA RF), antineutrophil cytoplasmic antibodies, antinuclear antibodies, antiendothelial antibodies, circulating intercellular adhesion molecule-1 and -3, circulating vascular cell adhesion molecule and E-selectin, cellular fibronectin, von Willebrand factor antigen, and C reactive protein. The diagnostic value of these markers, in addition to the clinical features, was evaluated with logistic regression analysis.

Results: Peripheral neuropathy or purpura/petechiae, or both, were the most important clinical features to discriminate patients with RA with and without histologically proven RV. The presence of a high number of EAMs and a higher weighted EAM score in patients with RA suspected of vasculitis were also associated with an increased probability of histologically proven RV. After adjustment for EAMs, only the combination of an increased serum IgA RF level and a decreased serum C3 level appeared to make an additional contribution to the diagnosis histologically proven RV. Evidence of systemic vasculitis was found in a muscle biopsy of the rectus femoris in 9/14 (64%) patients with vasculitis with neuropathy and in 3/11 (27%) patients with purpura/petechiae and vasculitis of the skin.

Conclusions: In the diagnostic process of RV the presence of peripheral neuropathy and/or purpura/petechiae or a high weighted EAM score will increase the probability of histologically proven RV. Of the circulating factors previously suggested to be markers for RV only IgA RF and C3 further increase the probability of histologically proven RV and may be useful to guide diagnostic decisions.

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Vasculitis in rheumatoid arthritis (RA) has a heterogeneous clinical presentation. Neuropathy, rash, skin ulcers, gangrene, and abnormalities of visceral organs have been described in association with rheumatoid vasculitis (RV).^{1,2} In RV small and medium sized vessels can be affected, which is histologically characterised by the presence of an inflammatory infiltrate associated with destruction of the vessel wall.³ The diagnosis RV is associated with a considerable mortality^{1,4-6} and may have important implications for patient management.^{7,8} Therefore the diagnosis RV is generally pursued by histological examination of a biopsy specimen of affected organs or by muscle⁹ or rectum samples.¹⁰ The availability of non-invasive blood tests that mark the presence of vasculitis would be useful. Several serological markers have been suggested for their usefulness in the diagnosis and management of RV. These include circulating immune complexes,¹¹ complement components,¹¹ antiendothelial antibodies,¹² antineutrophil cytoplasmic antibodies,¹³ circulating adhesion molecules,¹⁴ and circulating cellular fibronectin.¹⁵ Furthermore, raised levels of serum rheumatoid factors (RFs) of different immunoglobulin classes¹⁶ and the presence of antinuclear antibodies¹⁷ were reported in a high proportion of patients with RV. In other forms of vasculitis von Willebrand factor (vWF) antigen was proposed as a possible disease marker.^{18,19}

At present little is known about the optimal diagnostic strategy to be pursued in patients with RA clinically suspected of RV. This study on a cohort of patients with RA suspected of RV aimed at answering the following questions: (a) Which

clinical symptoms or combinations of symptoms are associated with a high probability of histologically proven rheumatoid vasculitis (HRV)? (b) What is the additional diagnostic value of serological markers in patients with clinical suspicion of HRV?

PATIENTS AND METHODS

Patients

Consecutive patients (n=81) with RA and recent onset (≤ 6 months) of extra-articular manifestations (EAMs) who attended the Department of Rheumatology between June 1993 and August 1996 were enrolled in a diagnostic study on the assessment of vasculitis. The following EAMs were considered to be related to RV unless their presence could be explained otherwise: non-compressive peripheral neuropathy

Abbreviations: AEA, antiendothelial antibodies; ANA, antinuclear antibodies; ANCA, antineutrophil cytoplasmic antibodies; cFN, cellular fibronectin; CI, confidence interval; CICs, circulating immune complexes; DMARDs, disease modifying antirheumatic drugs; DSSN, distal symmetric sensory or sensorimotor neuropathy; EAM, extra-articular manifestation; ELISA, enzyme linked immunosorbent assay; ESR, erythrocyte sedimentation rate; HRV, histologically proven rheumatoid vasculitis; ICAM, intercellular adhesion molecule; LF, lactoferrin; MPO, myeloperoxidase; PR3, proteinase 3; RA, rheumatoid arthritis; RF, rheumatoid factor; ROC, receiver operating characteristic; RV, rheumatoid vasculitis; VCAM, vascular cell adhesion molecule; vWF, von Willebrand factor

(mononeuritis multiplex, distal symmetric sensory or sensorimotor neuropathy (DSSN)), deep and superficial skin ulcers, gangrene, necrotising glomerulonephritis, fibrosing alveolitis, ischaemic colitis, skin rash, pulp lesions, nailfold lesions, pericarditis, pleuritis, (epi-)scleritis, scleromalacia perforans, weight loss ($\geq 10\%$ in six months), fever, and multiple rheumatoid nodules. All patients with EAMs were extensively investigated for RV following a predefined protocol. This included histological examination of a full thickness (skin/muscle/fascia) biopsy specimen of the rectus femoris muscle in all patients and a biopsy sample of the tibial muscle in those patients who were willing to participate in a substudy on the value of such a biopsy procedure. The former muscle biopsy was obtained by an open surgical procedure, whereas the latter muscle biopsy was obtained with a forceps.²⁰ The size of the sample obtained was $\pm 1 \times 1 \times 1$ cm for the rectus femoris muscle and $\pm 0.5 \times 0.5 \times 0.5$ cm for the tibial muscle. If no vasculitis was seen in either muscle biopsy, a sural nerve biopsy was done in patients with peripheral neuropathy, confirmed by electromyographic abnormalities of the sural nerve. Furthermore, a skin biopsy was performed in all patients with skin rash—that is, petechiae or purpura or rash, but not of skin ulcers. Vasculitis was defined as the observation of fibrinoid necrosis in a vessel wall within a muscle or sural nerve biopsy, or of leucocytoclasia in a skin biopsy. No immunofluorescent analysis on tissue was done. A large number of sections ($n=45$) from each muscle biopsy were examined to avoid, as far as possible, false negative test results due to discontinuous distribution of the lesions.²¹ Patients with EAMs were classified as HRV or RA+EAM according to, respectively, the presence or absence of histological evidence of vasculitis in any tissue biopsy. Thus, 31/81 patients with RA suspected of vasculitis were classified as HRV and 50 patients as RA+EAM.

Controls for the laboratory parameters included 44 consecutive patients with RA without clinical suspicion of vasculitis (RA-). These control patients attended the clinic between August 1995 and August 1996 and were matched for age, sex, disease duration, and clinic referral status of the patients with HRV and RA+EAM. The RA- control patients showed no sign of the above mentioned EAMs within six months before blood sampling or after at least six months of follow up. No histological diagnostic procedure was performed in these patients.

All patients fulfilled the criteria of the American College of Rheumatology for RA.²²

Blood samples

Blood samples were obtained at the time of enrolment in the study and before biopsies were performed in patients with HRV and RA+EAM. For the assessment of the vWF antigen venous blood was collected in 1/10 volume of 0.11 M trisodium citrate and centrifuged at 2500 *g* for 20 minutes. Serum and plasma samples were obtained simultaneously and were stored at, respectively, -20° and -80°C until use.

Laboratory parameters

In serum samples, levels of circulating immune complexes (CICs) were measured by a C1q binding assay.²³ Levels of complement components C3 and C4 were measured as previously described.²⁴ The normal ranges are: CICs 0 $\mu\text{gEq/ml}$ of aggregated IgG, C3 0.7–1.0 g/l, C4 0.2–0.3 g/l. Enzyme linked immunosorbent assays (ELISAs) were used to determine levels of antiendothelial antibodies (AEA),²⁵ circulating intercellular adhesion molecule (ICAM)-1 and -3, circulating vascular cell adhesion molecule (VCAM), and E-selectin,²⁶ as well as cellular fibronectin (cFN).¹⁵ Normal ranges are: AEA 0 U/ml, ICAM-1 115–306 ng/ml, ICAM-3 15–37 ng/ml, VCAM 395–714 ng/ml, E-selectin 29–63 ng/ml, cFN $< 208\%$ of pooled normal plasma. Antineutrophil cytoplasmic antibodies (ANCA) were determined as described¹³ and included indirect immunofluorescence, ELISA for the levels of antibodies against protei-

Table 1 Demographic and disease characteristics of 81 patients with recent onset of extra-articular manifestations and a suspicion of rheumatoid vasculitis

	HRV (n=31)	RA+EAM (n=50)	RA- (n=44)
Age, years (range)	70 (49–85)	66 (38–85)	65 (38–84)
Female (No (%))	18 (58)	29 (58)	26 (59)
RA disease duration, years (range)	14 (1–52)	13 (0–44)	10 (0–33)
Patients with (%)			
Nodules (previous onset)	74	60	18
RF	100	96	96
Joint erosions	97	98	91
Arthritis activity (range)			
Swollen joint count	8 (0–16)	7 (0–15)	ND
Painful joint count	8 (0–24)	8 (0–35)	ND
Treatment (%)			
Corticosteroids	32	28	NA
Methotrexate	10	18	NA
Others	49	42	NA
None	16	8	NA
Vasculitis observed in (n)			
Muscle	17/31	0/50	ND
Skin	9/11	0/3	ND
Muscle and skin	3/3		ND
Sural nerve	2/2	0/6	ND

HRV, histologically proven rheumatoid vasculitis and recent onset of extra-articular manifestations; RA+EAM, rheumatoid arthritis with recent onset of extra-articular manifestations, but without histological evidence of vasculitis; RA-, rheumatoid arthritis, without recent onset of extra-articular manifestations or a suspicion of vasculitis; RF, increased levels of serum rheumatoid factor, measured at any time during the disease course of RA.

nase 3 (PR3), myeloperoxidase (MPO), and lactoferrin (LF). Normal values are: anti-PR3 < 8.0 U/ml, anti-MPO < 31.6 U/ml, anti-LF < 50.1 U/ml. Levels of class-specific rheumatoid factors were measured by an ELISA.²⁷ Normal values are: IgM RF < 5 U/ml, IgG RF < 200 U/ml, IgA RF < 4 U/ml. Determinations of antinuclear antibodies (ANA) were performed by indirect immunofluorescence on cultured Hep-2 cells. The presence or absence of cryoglobulins was determined. In plasma samples, levels of vWF antigen were determined by ELISA.²⁸ The normal range is 0.36–2.15 IU/ml.

Statistical analysis

Clinical features

Differences in demographic and disease characteristics, as well as the presence and number of EAMs between patients with HRV and RA+EAM were tested by χ^2 test or Mann-Whitney U test, where appropriate.

The association of EAMs with HRV was determined for each EAM separately and for combinations of EAMs, using logistic regression analysis and expressed as odds ratios with 95% confidence intervals. Subsequently, all EAMs were entered in a logistic regression model to evaluate which of the entry variables were independently associated with HRV.

Furthermore, the association between HRV and the number of EAMs, as well as a weighted EAM score, was determined. The term weighted EAM score represents the clinical suspicion for RV which depends on the type of EAM seen in a patient. Based on the consensus judgment of clinicians and information from published reports, each EAM was given a score ranging from 1 to 4, a score of 4 representing the highest suspicion of RV. The weighted EAM score was calculated by adding up the scores of each EAM seen in one patient. The following score was given to the EAMs: peripheral neuropathy, deep skin ulcers, gangrene, necrotising glomerulonephritis, fibrosing alveolitis, and ischaemic colitis were each given a score of 4; skin rash a score of 3; nailfold lesions, scleromalacia perforans, (epi-)scleritis, and superficial skin ulcers a score

Table 2 Extra-articular manifestations (EAMs) of recent onset in 81 patients suspected of rheumatoid vasculitis and associations between EAMs and histologically proven rheumatoid vasculitis. Results are shown as No (%)

	HRV (n=31)	RA+EAM (n=50)	Odds ratio* (95% confidence interval)
Peripheral neuropathy	14 (45)†	6 (12)	6.04 (1.9 to 18.9)
Mononeuritis multiplex	3 (10)	–	∞
DSSN	14 (45)	6 (12)	6.04 (1.9 to 18.9)
Skin	25 (81)	29 (58)	3.02 (1.0 to 8.8)
Ulcers	7 (23)	11 (22)	1.03 (0.3 to 3.1)
Deep skin ulcer	3 (10)	2 (4)	2.56 (0.4 to 16.8)
Superficial skin ulcer	4 (13)	9 (18)	0.68 (0.2 to 2.5)
Gangrene	1 (3)	1 (2)	1.63 (0.1 to 28.5)
Purpura/petechiae	14 (45)	3 (6)	12.94 (3.2 to 52.5)
Nailfold lesions	14 (45)	21 (42)	1.14 (0.5 to 2.9)
Systemic features (weight loss+ fever)	14 (45)	24 (48)	1.65 (0.2 to 12.9)
Weight loss	13 (42)	19 (38)	1.17 (0.5 to 3.0)
Fever	3 (10)	7 (14)	0.66 (0.2 to 2.8)
Eye involvement	5 (16)	4 (8)	2.21 (0.5 to 9.2)
Scleromalacia perforans	1 (3)	1 (2)	1.63 (0.19 to 28.5)
(Epi-)scleritis	4 (13)	3 (6)	2.32 (0.5 to 11.5)
Pericarditis	0	1 (2)	0 (-)
Pleuritis	2 (6)	6 (12)	0.51 (0.1 to 2.3)
Fibrosing alveolitis	1 (3)	1 (2)	1.63 (0.1 to 28.5)
Multiple rheumatoid nodules (recent onset)	2 (6)	4 (8)	0.79 (0.1 to 4.8)

HRV, histologically proven rheumatoid vasculitis and recent onset of extra-articular manifestations; RA+EAM, rheumatoid arthritis with recent onset of extra-articular manifestations, but without histological evidence of vasculitis; DSSN, distal symmetric sensory or sensorimotor neuropathy.

*Association of EAM with RV; †number of patients (%);

of 2; weight loss, fever, pericarditis, pleuritis, and multiple rheumatoid nodules a score of 1. The weighted EAM score could reach a maximum score of 40. The weighted EAM score was constructed by the authors before the study design.

The sensitivity, specificity, accuracy, and predictive values of the EAMs, number of EAMs, and the weighted EAM score were calculated. Accuracy is defined as the percentage patients correctly classified as either HRV or RA+EAM. To determine the diagnostic value of non-dichotomous variables the optimal cut off point was estimated by receiver operating characteristic (ROC) curves.

Laboratory parameters

Differences in serum or plasma levels of laboratory parameters of patients with HRV, RA+EAM and RA– controls were tested by χ^2 test or Mann-Whitney U test, where appropriate. In addition, laboratory parameters were dichotomised. Thresholds were determined such that 95% of the RA control group was normal. The laboratory parameters which were found to differ between HRV and RA+EAM at a p value <0.10 were selected for further evaluation.

The strength of association between HRV and selected laboratory parameters was determined univariately by logistic regression analysis, expressed as odds ratios with 95% confidence interval. Subsequently, to evaluate the additional diagnostic value of laboratory parameters above that of clinical symptoms, the strength of association between HRV and each selected laboratory parameter was adjusted for the weighted EAM score. Finally, to evaluate which of the selected laboratory parameters were independently associated with HRV, a stepwise logistic regression analysis was done on all selected laboratory parameters adjusted for the weighted EAM score.

RESULTS

Clinical features

In total, 31/81 (38%) patients were found to have vasculitis in any of the biopsy samples taken. In the 81 patients suspected

of RV, vasculitis was found on histological examination of the rectus femoris muscle (17/81 (21%)), tibial muscle (10/77 (13%)), skin (12/17 (71%)), and sural nerve (2/8 (25%)). Both rectus femoris and tibial muscle were sampled in 27/31 patients with HRV. Of those 27 patients with HRV, vasculitis was found in both muscle biopsies in seven patients with HRV (26%), only in the rectus femoris muscle in six patients with HRV (22%), only in the tibial muscle in three patients with HRV (11%), and in neither muscle biopsies in 11 patients with HRV (41%).

Demographic and disease characteristics of HRV, RA+EAM, and RA– control patient groups did not differ significantly, except for a longer median disease duration and a higher proportion of nodules in patients with HRV than in RA– control patients (table 1).

Table 2 presents the EAMs occurring in the preceding six months that motivated the diagnostic procedure for RV. The most common were the presence of weight loss, nailfold lesions, purpura/petechiae, peripheral neuropathy (in particular, DSSN), and skin ulcers. The EAMs most strongly associated with RV were petechiae/purpura and DSSN (table 2). Of 14 patients with HRV with peripheral neuropathy, two had peripheral neuropathy only and 12 patients had additional features: gangrene (n=1), deep skin ulcer (n=2), purpura/petechiae (n=3), scleromalacia perforans (n=1), weight loss (n=7), fever (n=2), nailfold lesions (n=7), superficial skin ulcer (n=1), pleuritis (n=1), and multiple rheumatoid nodules (n=2). Of the 14 patients with HRV with peripheral neuropathy, nine (64%) had vasculitis in a rectus femoris muscle biopsy, an additional two only in the tibial muscle biopsy, and an additional two only in a sural nerve biopsy. Of the 11 patients with HRV with purpura/petechiae but no neuropathy, six had purpura/petechiae only, and five had purpura/petechiae with additional features: deep skin ulcer (n=1), weight loss (n=1), fever (n=1), nailfold (n=3), episcleritis (n=1). Of the 11 patients with HRV with purpura/petechiae but no peripheral neuropathy, 10 had vasculitis in a skin biopsy, and three (27%) had vasculitis in a rectus femoris

Table 3 Diagnostic characteristics of extra-articular manifestations (EAMs) for histologically proven rheumatoid vasculitis in 81 patients suspected of rheumatoid vasculitis

	Sensitivity	Specificity	Accuracy	PPV	NPV
EAM					
Peripheral neuropathy	45	88	72	70	72
Purpura/petechiae	45	94	75	82	73
Peripheral neuropathy or purpura/petechiae	81	84	83	76	88
Number of EAM					
≥1	100	0	38	38	–
≥2	77	46	58	47	77
≥3	42	84	68	62	70
≥4	23	100	70	100	68
Weighted EAM score*					
≥3	100	50	56	55	100
≥5	58	80	72	64	76
≥6	45	90	73	74	73
≥9	19	100	69	100	67

Accuracy, percentage patients correctly classified as either HRV or RA+EAM; PPV, positive predictive value; NPV, negative predictive value.

*Weighted EAM score; for explanation see "Patients and methods".

muscle biopsy. The remaining six patients with HRV, who had no peripheral neuropathy or purpura/petechiae, presented a combination of any of the following features: weight loss (n=5), nailfold lesions (n=4), superficial skin ulcer (n=3), pleuritis (n=1), or episcleritis (n=2). In these latter six patients, vasculitis was seen in a rectus femoris muscle biopsy.

The number of EAMs (mean 2.5, range 1–7) in the patients with HRV was significantly higher ($p < 0.01$) than that in the patients with RA+EAM (mean 1.7, range 1–3). The weighted EAM score was also significantly ($p < 0.0001$) higher in patients with HRV (mean 5.9, range 3–15) than that in patients with RA+EAM (mean 3.0, range 1–8). Table 3 presents the diagnostic value of each EAM of interest in patients suspected of RV (that is, patients with HRV and RA+EAM). As shown, the presence of skin rash (purpura/petechiae) or neuropathy increases the probability of the pres-

ence of HRV from 38% among the total study group to 82% among those patients with skin rash or neuropathy. The highest accuracy was found for a combination of peripheral neuropathy and purpura/petechiae. The accuracy of the number of EAMs and the weighted EAM score was found to be the highest at a optimal cut off point of ≥ 4 EAMs and at a weighted EAM score ≥ 6 , respectively. The association between HRV and the EAMs, expressed as odds ratios, at the optimal cut off points was 3.79 (95% confidence interval (CI) 1.3 to 10.7) for the number of EAMs (≤ 2 v ≥ 3) and 7.41 (95% CI 2.3 to 24.1) for the weighted EAM score (≤ 5 v ≥ 6).

To evaluate which of the EAMs were independently associated with HRV, a stepwise logistic regression analysis was done. Peripheral neuropathy and purpura/petechiae were found to be the only EAMs that best explained the presence or absence of HRV; the odds ratios for peripheral neuropathy and

Table 4 Laboratory variables measured in patients with rheumatoid vasculitis (HRV) and in patients with rheumatoid arthritis with EAMs, but no histological evidence of vasculitis (RA+EAM). Results are shown as No (%)

	HRV (n=31)	RA+EAM (n=50)	Odds ratio (95 CI)	p Value
CICs >52 $\mu\text{gEq/ml}$	16 (55)	11 (22)	4.3 (1.6 to 11.5)	0.006
C3 <0.7 g/l	19 (66)	9 (18)	8.4 (2.9 to 24.2)	<0.0001
C4 <0.2 g/l	10 (35)	12 (25)	1.6 (0.6 to 4.4)	0.44
vWF >3.75 IU/ml	8 (35)	1 (2)	22.4 (2.6 to 194)	0.001
AEA >33 U/ml	7 (26)	13 (26)	1.0 (0.3 to 2.9)	0.99
ANCA				
Cytoplasmatic	0	1 (2)	–	0.99
Perinuclear	13 (48)	15 (30)	2.1 (0.8 to 5.5)	0.21
Non-specific	0	0	–	–
Absent	14 (52)	34 (68)	1.0 (–)	0.21
Anti-PR3 ≥ 0.8 U/ml	1 (4)	2 (4)	0.9 (0.1 to 11.7)	0.99
Anti-MPO >30 U/ml	3 (11)	4 (8)	1.4 (0.3 to 7.0)	0.69
Antilactoferrin >25 U/ml	2 (7)	3 (6)	1.3 (0.2 to 8.0)	0.99
ICAM-1 >720 ng/ml	0 (0)	1 (2)	0 (–)	0.99
ICAM-3 >90 ng/ml	15 (54)	35 (71)	0.5 (0.2 to 1.2)	0.14
VCAM >1200 ng/ml	8 (29)	8 (16)	2.1 (0.7 to 6.3)	0.25
E-selectin >115 ng/ml	5 (18)	17 (35)	0.4 (0.1 to 1.3)	0.19
Cellular fibronectin $>400\%$	18 (62)	13 (26)	4.7 (1.7 to 12.4)	0.002
IgM RF >265 U/ml	11 (37)	9 (18)	2.6 (0.9 to 7.4)	0.10
IgG RF >550 U/ml	13 (43)	7 (14)	4.7 (1.6 to 13.8)	0.007
IgA RF >14 U/ml	19 (63)	10 (20)	6.9 (2.5 to 19.1)	<0.0001
ANA positive	13 (42)	20 (40)	1.1 (0.5 to 2.9)	0.82
ESR >59 mm/1st h	26 (84)	27 (54)	4.4 (1.5 to 13.4)	0.008
CRP >104.5 mg/l	2 (7)	10 (20)	0.3 (0.1 to 1.5)	0.19

Where appropriate, thresholds were determined such that 95% of the "RA–" control group was normal, except for C3, IgA RF, and ESR. For these parameters thresholds were determined by ROC analysis of the "HRV" versus "RA+EAM" groups.

*p Value of Fisher's exact test.

Table 5 Association between the diagnosis histologically proven rheumatoid vasculitis and selected laboratory variables, presented as univariate crude odds ratios and adjusted odds ratios, adjusted for the weighed EAM score

	Crude		Adjusted for weighed EAM score	
	Odds ratio (95% CI)	p Value	Odds ratio (95% CI)	p Value
CIC >52 µgEq/ml	4.3 (1.6 to 11.5)	0.006	3.7 (1.2 to 11.8)	0.024
C3 <0.7 g/l	8.4 (2.9 to 24.2)	<0.0001	7.3 (2.2 to 24.2)	0.001
vWF >3.75 IU/ml	22.4 (2.6 to 194)	0.001	11.9 (1.2 to 116)	0.033
Cellular fibronectin >400%	4.7 (1.7 to 12.4)	0.002	2.8 (0.9 to 8.3)	0.11
IgM RF >265 U/ml	2.6 (0.9 to 7.4)	0.11	1.3 (0.4 to 4.7)	0.66
IgG RF >550 U/ml	4.7 (1.6 to 13.8)	0.007	3.6 (1.1 to 12.4)	0.038
IgA RF >14 U/ml	6.9 (2.5 to 19.1)	<0.0001	5.5 (1.7 to 17.9)	0.004
ESR >59 mm/1st h	4.4 (1.5 to 13.4)	0.008	2.1 (0.6 to 7.2)	0.23

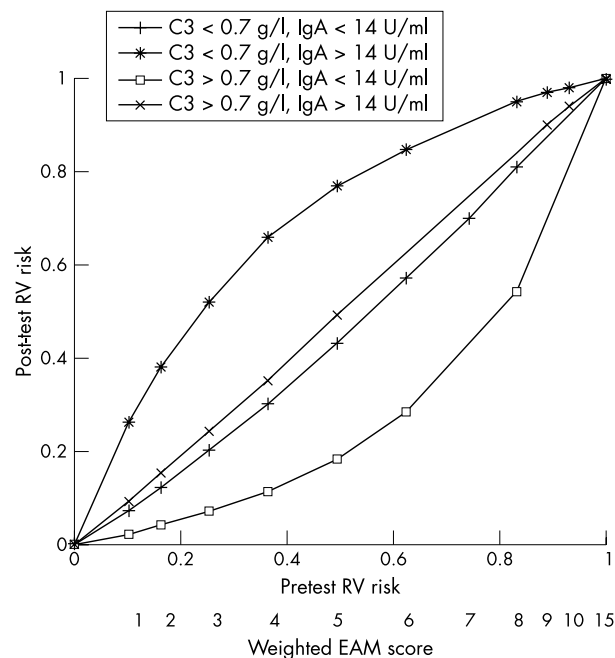


Figure 1 Relation between the pretest probability of HRV (on the x axis), as calculated with the weighted EAM score, and the post-test probability of HRV (on the y axis) after measuring IgA RF and C3 levels. Increased IgA RF levels were defined as >14 U/ml. Normal IgA RF levels were defined as ≤14 U/ml. C3 levels were decreased if <0.7 g/l and normal if ≥0.7 g/l.

purpura/petechiae were 10.24 (95% CI 2.8 to 37.5) and 20.86 (95% CI 4.6 to 95.4), respectively. When all other possible combinations of EAM were examined, no significant interaction was found. The strengths of association of combinations of EAMs with HRV were similar to the strength of association of the separate EAMs with HRV as found by univariate analysis.

Laboratory parameters

Serum or plasma levels of CICs, vWF, ICAM-3, VCAM, E-selectin, cFN, IgM RF, IgG RF, IgA RF, and erythrocyte sedimentation rate (ESR) were significantly higher in patients with HRV than in RA– control patients. Serum levels of C3 and C4 were significantly lower in patients with HRV than in the control RA– patients. Between HRV and the control RA– patients, no significant differences were found in the median serum levels of AEA, anti-PR3, anti-MPO, anti-LF, ICAM-1, CRP and in the presence of ANA or ANCA. When compared with patients with RA+EAM, the serum or plasma levels of CICs, vWF, cFN, IgM RF, and IgA RF were significantly higher

in patients with HRV. The serum levels of C3 were significantly lower in patients with HRV than in patients with RA+EAM. Between HRV and RA+EAM patients, no significant differences were found in serum levels of C4, AEA, anti-PR3, anti-MPO, anti-LF, ICAM-1, ICAM-3, E-selectin, IgG RF, ESR, CRP and in the presence of ANA or the ANCA pattern on immunofluorescence. Cryoglobulins were not found in any of the patients studied. Table 4 shows the laboratory parameters as measured in patients with HRV and RA+EAM, in which the laboratory parameters were dichotomised. Thresholds were determined such that 95% of the RA control group was normal, except for C3, IgA RF and ESR. For these parameters thresholds were determined by ROC analysis of the HRV versus RA+EAM groups. Significant differences between HRV and RA+EAM were found for CICs, C3, vWF, cFN, IgG RF, IgA RF, and ESR.

Diagnostic value of laboratory parameters in addition to the clinical features

Table 5 shows the associations between HRV and selected laboratory parameters, unadjusted and adjusted for the weighted EAM score. Statistically significant associations with HRV were found for increased serum CIC levels, decreased serum C3 levels, increased plasma vWF levels, increased serum cFN levels, increased serum levels of IgG RF, IgA RF, and ESR. After adjustment for the weighted EAM score, CIC, C3, vWF, IgA RF, and IgG RF were still significantly associated with HRV (table 5). Entering all selected laboratory variables and the weighted EAM score simultaneously, only IgA RF and C3 were still significantly associated with HRV, with a trend for vWF (data not shown). Given a weighted EAM score, the additional diagnostic value of measuring both IgA RF and C3 levels is higher than the additional value of measuring IgA RF levels only. For instance, if only IgA RF were measured and found to be increased (>14 IU/ml = median value of patients with HRV and RA+EAM), at a pretest risk of 50% the post-test probability for HRV was 66%. If both an increased IgA RF level and a decreased C3 level (<0.7 g/l = median value of patients with HRV and RA+EAM) were found, the post-test probability for HRV was 78%. Figure 1 illustrates the relation between the pretest probability of HRV (on the x axis), as calculated with the weighted EAM score, and the post-test probability of HRV (on the y axis) after measuring IgA RF and C3 levels.

DISCUSSION

This study determines the diagnostic value of EAM and serological markers for histologically proven RV (HRV) in an inception cohort of patients with RA clinically suspected of vasculitis. The presence of peripheral neuropathy or skin rash (purpura/ petechiae) increases the probability of HRV from 38% to 82%. The probability of HRV also increased considerably with an increasing number of EAM present or with an

increasing weighted EAM score. Of the serological markers tested, only IgA RF and C3 appeared to make an additional contribution to the probability of HRV.

In contrast with previous studies, this study used histology as a "gold standard" for RV. The results show that the various EAMs differ in their predictive value for the presence of HRV. The strong association of peripheral neuropathy and/or skin rash with HRV is in line with the findings of previous studies.^{1-4,29} Mononeuritis multiplex had always been found in association with vasculitis. In contrast with previous observations,^{1,29} no relevant association was found between HRV and nailfold lesions in combination with serositis or (epi-)scleritis.

The question may be raised whether patients have subclinical vasculitis and the gold standard used in this study is adequate. Firstly, in our opinion histological vasculitis is probably always associated with clinical signs of vasculitis as all our patients with HRV had EAMs of recent onset, and histological evidence of vasculitis could not be found in any patient with RA without extra-articular manifestations.⁹ To minimise the possibility of misclassification an extensive histological procedure was performed, with at least one muscle biopsy (the majority had two muscle biopsies) and if indicated with skin or sural nerve biopsy. Furthermore, none of the patients with RA+EAM developed progressive signs of vasculitis by at least one year after the diagnostic procedure. We therefore think that the gold standard used in this study was adequate.

The predictive value of the weighted EAM score for the presence of HRV was no better than the presence of neuropathy and/or skin rash. Nevertheless, this score, which was based on clinical experience and designed before the study, seemed to be remarkably accurate. The possible usefulness of such a score is suggested by the fact that not all patients with HRV present peripheral neuropathy and/or skin rash, though further validation of such a score seems appropriate.

The additional diagnostic value of serological markers of HRV was disappointing. All investigated serological tests have been previously reported as markers for RV. In this study, changed levels of CICs, C3, C4, von Willebrand factor antigen, ICAM-3, VCAM, E-selectin, cFN, IgM RF, IgG RF, IgA RF, and ESR were significantly different between patients with HRV and RA controls. However, when diagnostically used in patients with RA suspected of RV, only the changes in levels of CIC, C3, vWF antigen, cFN, IgG RF, IgA RF, and ESR were still significantly associated with HRV. Discrepancies between this study and previous studies may be explained by differences in the definition of the RV and RA control groups. The absence of antiendothelial antibodies and antilactoferrin antibodies contrasts with the results of previous studies and remains unexplained.^{12,13} The differences in CICs, vWF antigen, cFN, IgM RF, and IgG RF between patients with RA, with and without HRV, are of interest for an understanding of the pathogenesis of RV, but have no additional value for the diagnostic process of RV.

Of the circulating factors previously suggested to be markers of RV, only increased IgA RF and decreased C3 levels increase the probability of HRV in addition to the readily available clinical information. Measurement of IgA RF and C3 was found to be of diagnostic value in all subgroups of patients with RA suspected of RV—for example, patients with recent onset of EAMs but no skin rash or neuropathy (data not shown). The present results confirm previously reported observations of increased serum IgA RF in patients with RV compared with patients with RA without vasculitis.¹⁶ The role of circulating IgA RF and C3 in the pathogenesis of RV is suggested by the high prevalence of IgA immune complex deposits in combination with C3 deposits in the affected skin of patients with HRV and the absence of such deposits in the skin of patients with RA without vasculitis.³⁰

Histological confirmation of RV should be directed to an affected organ (skin or nerve), but the observation of vasculitis in medium sized arteries in muscle or rectum is also important. Previous observations suggest that patients with vasculitis in the rectum or muscle have a worse outcome than those with vasculitis limited to the skin only.^{10,31} There are two reasons why we prefer a muscle biopsy. Firstly, a muscle biopsy is easy to perform and less invasive than a rectum biopsy and, secondly, the diagnostic yield is similar to a sural nerve biopsy in patients with RV with peripheral neuropathy.⁵ Of the patients who were biopsied in two muscles, vasculitis was seen in 48% of the rectus femoris muscle biopsy specimens and in 37% of the tibial muscle biopsy specimens. The tibial muscle biopsy obtained by forceps is easy to perform in a regular outpatient clinic²⁰ and less invasive than an open surgical biopsy of the rectus femoris muscle. Although the investigation of the diagnostic yield of the various invasive procedures was not the primary aim of this study, the results suggests that a tibial muscle biopsy obtained by a forceps is the best first step for the documentation of vasculitis, particularly in patients without skin rash. If no vasculitis is seen in a tibial muscle biopsy than a biopsy of the rectus femoris muscle can be done.

One may argue whether in clinical practice RV can be diagnosed only on the basis of clinical signs or whether histological confirmation is necessary. The clinical signs of RV are heterogeneous and mostly non-specific, in particular when these appear as an isolated feature, excepted for mononeuritis multiplex which is diagnostic of RV. The differential diagnosis of several features of RV is extensive, as has been reported for skin ulcers³² and polyneuropathy,³³ but also for other features—for example, skin rash, weight loss, and fever. To obtain a proper and prompt diagnosis and to have additional support to initiate aggressive immunosuppressive treatment, histological confirmation of the diagnosis RV is advocated. It is important to start aggressive immunosuppressive treatment quickly because the survival of patients with RV with severe organ lesions (neuropathy) who are treated promptly with immunosuppressive drugs—that is, high dose corticosteroids in combination with cyclophosphamide or azathioprine, seems better than survival in those patients who are not treated in this way.^{34,35} Likewise, patients with RV with other severe organ lesions—for example, deep skin ulcers, might be considered as having a poor prognosis and aggressive immunosuppressive treatment could be started. However, patients with RV with non-severe superficial skin lesions—for example, purpura/petechiae or superficial skin ulcers, do not have a poor prognosis and a change of disease modifying antirheumatic drug (DMARD) treatment may be sufficient for these patients, instead of treating them with aggressive immunosuppressive drugs.³¹ Three of our patients with RV had a rash as the only clinical manifestation in combination with vasculitis in the skin biopsy and, interestingly, also in a muscle biopsy. This observation suggests that some patients with skin rash apparently may have systemic vasculitis. It has been found that patients with systemic vasculitis in a biopsy have a worse prognosis,¹ which suggests that aggressive treatment should be considered in patients with RV with rash and systemic vasculitis in a muscle biopsy. For patients with RA with EAMs but without HRV—that is, RA+EAM, vasculitis only limited to a small part of an organ cannot be ruled out, such as in nailfold lesions, episcleritis, pericarditis, pleuritis. The prognosis of patients with RA with such lesions is generally good³⁶ and adequate treatment with DMARDS or low dose corticosteroids is probably sufficient.

In conclusion, the results of this study confirm that the development of EAMs, particularly the presence of peripheral neuropathy and skin rash, in RA increases the probability of HRV. The study further demonstrates that serum IgA RF and C3 levels in patients with RA are the only markers for HRV that add to the diagnostic information obtained by careful physical examination.

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