Observations on Cryoglobulin Testing: I. The Association of Cryoglobulins Containing Rheumatoid Factors with Manifestation of Cryoglobulinemic Vasculitis

FRANCESCO G. DE ROSA and VINCENT AGNELLO

ABSTRACT. ***Objective.*** To investigate the results of mixed cryoglobulin tests performed on patients with and without hepatitis C virus (HCV) infection, to determine whether type II cryoglobulins containing rheumatoid factor (Type II-RF) were associated with cryoglobulinemic vasculitis.

Methods. The cryoglobulin test protocol differed from the routine protocol. In addition to determination of both cryoglobulin concentration and immunoglobulin composition, the presence or absence of RF concentrated in the cryoglobulins was determined.

Results. A qualitative and quantitative association of Type II-RF cryoglobulins with cryoglobulinemic vasculitis was present among HCV-infected patients.

Conclusion. Detection and quantification of Type II-RF may enhance clinical monitoring of cryoglobulinemic vasculitis in HCV-infected patients. (First Release June 15 2009; J Rheumatol 2009; 36:1953–5; doi:10.3899/jrheum.081035)

Key Indexing Terms:
CRYOGLOBULINS
CRYOGLOBULINEMIC VASCULITIS
RHEUMATOID FACTOR
HEPATITIS C

Meltzer, Franklin, and colleagues first described the clinical syndrome of essential mixed cryoglobulinemia that is now called cryoglobulinemic vasculitis, and implicated IgG-IgM rheumatoid factor (RF) cryoglobulins in the pathogenesis of the vasculitis[1,2]. Eighty to ninety percent of what was essentially mixed cryoglobulinemia is now known to be secondary to hepatitis C virus (HCV) infection[3]. The current classification of cryoglobulinemia formulated by Brouet and colleagues defined 2 types of mixed cryoglobulinemia: Type II, consisting of polyclonal IgG and monoclonal IgM, and Type III, consisting of polyclonal IgG and polyclonal IgM[4]. In both types the IgM component was predominantly RF. Although these early studies delineated a role for RF in mixed cryoglobulins, almost all current clinical immunology laboratory protocols for assessing mixed cryoglobulins exclude cryoglobulin RF studies[5].

The main manifestations of cryoglobulinemic vasculitis are palpable purpura, peripheral neuropathy, and glomerulonephritis. To determine whether Type II cryoglobulins containing RF (Type II-RF) detected in the clinical laboratory were associated with the manifestations of cryoglobulinemic vasculitis, as previously demonstrated by clinical-pathologic studies, we examined the results of cryoglobulin tests that employed a protocol to assess RF in the cryoglobulins in addition to the routine cryoglobulin tests, that is, determination of cryoglobulin concentration, immunoglobulin isotype, and monoclonality.

MATERIALS AND METHODS

Tests on mixed cryoglobulins detected in 207 patients performed between January 1990 and March 2001 in the Clinical Immunology Laboratory, Lahey Clinic, were studied. Blood specimens were drawn into warm syringes, and clotted and centrifuged at 37°C. Cryoglobulins were isolated and quantitated using established protocols[5]. Typing of cryoglobulins was performed by Beckman Paragon Immunofixation Electrophoresis (Beckman Diagnostic Systems, La Brea, CA, USA). RF was quantified in serum and cryoglobulins using a Behring BNII nephelometer. Cryoglobulin RF was reported positive if the concentration was higher in the cryoglobulins than in the serum. Anti-HCV antibodies were detected using the Abbott assay (Abbott Laboratories, Chicago, IL, USA). Undetermined results were resolved by immunoblotting (Mayo Clinic, Rochester, MN, USA). Serum HCV-RNA was quantified using a reverse transcriptase-polymerase chain reaction (RT-PCR) assay as described[6]. Results were standardized to IU/ml. The serum C4 component of complement and alanine transaminase concentrations were determined in the clinical laboratory. Type I cryoglobulins were excluded from the study. Type II, Type IIa (oligoclonal), or Type III

From the Department of Laboratory Medicine, Lahey Clinic, Burlington; and Edith Nourse Rogers Memorial Veterans Affairs Hospital, Bedford, Massachusetts, USA.

Supported in part by a Department of Veterans Affairs Merit Review Grant. Dr. De Rosa was a fellow of the Robert E. Wise M.D. Research and Education Institute.

F.G. De Rosa, MD, Department of Laboratory Medicine, Lahey Clinic (current address, Department of Infectious Diseases, University of Turin, Turin, Italy); V. Agnello, MD, Department of Laboratory Medicine, Lahey Clinic, Edith Nourse Rogers Memorial Veterans Affairs Hospital.

The views expressed in this article are those of the authors and do not necessarily reflect the position or policy of the Department of Veterans Affairs.

Address correspondence to Dr. V. Agnello, Lahey Clinic, 41 Mall Road, Burlington, MA 01805. E-mail: Vincent.Agnello@lahey.org

Accepted for publication March 6, 2009.

and the following cryoglobulin types containing RF designated as Type II-RF, Type IIa-RF and Type III-RF were studied.

Clinical findings were determined by examination of the medical records. Palpable purpura was diagnosed by a dermatologist and confirmed by the presence of leukocytoclastic vasculitis on skin biopsy. Glomerulonephritis was diagnosed by renal biopsy or clinically by the presence of at least one of the following criteria: persistent proteinuria > 500 mg/dl and hematuria, nephrotic syndrome, or acute or chronic renal failure in the absence of other causes. Peripheral neuropathy was diagnosed by electromyography and nerve conduction studies. Peripheral neuropathy secondary to other diseases was excluded. Cirrhosis was diagnosed with histiologic documentation or clinically by liver imaging consistent with cirrhosis or by evidence of decompensated liver function.

Statistical analysis. Univariate analyses were used to compare laboratory data. Differences in proportions were tested with the chi-square test or Fisher's exact test. An unpaired t test was used for continuous variables. All calculated p values are 2-tailed and $p < 0.05$ are noted.

RESULTS

The demographics and clinical characteristics of patients studied are shown in Table 1. The predominance of females among HCV-negative patients but not among HCV-positive patients may be due to the increased male prevalence among HCV-infected patients. The distribution of cryoglobulin types among patients with HCV infection differed in those patients with the most common signs of cryoglobulinemic vasculitis, palpable purpura, glomerulonephritis, and peripheral neuropathy, compared to patients without these signs (Table 2). For HCV-negative patients the distribution of cryoglobulin types differed only in patients with peripheral neuropathy. In both groups the differences appeared to be mainly due to the association of Type II-RF with signs of vasculitis.

Table 1. Comparison of demographics and clinical characteristics of patients positive or negative for HCV infection.

Characteristic	HCV-positive	HCV-negative
Total patients, no.	157	50
Mean age ± SD, yrs	50.6 ± 11.8	57.3 ± 15
Male/female	1.15 (84/73)	0.21 (9/41)
Mean ALT ± SD, IU/l	81.3 ± 70.3	23.9 ± 13.2
No. patients tested	143	38
Mean HCV RNA ($\times 10^6$ IU/ml)	2.99 ± 6.40	NA
No. patients tested	157	50
Mean Cryocrit ± SD, %	1.58 ± 1.74	1.89 ± 3.0
No. patients tested	157	50
Vasculitis signs, %	30.0	42.9
No. patients studied	155	49
Cirrhosis, %	33.3	5.0
No. patients studied	129	20

ALT: alanine transaminases; NA: not applicable.

The data were further analyzed by grouping patients with positive and negative HCV infections into symptomatic (vasculitis signs present) and asymptomatic (vasculitis sign absent) groups, and then comparing the cryoglobulin types detected by the 2 protocols for each group (Table 3). The association of Type II-RF cryoglobulins with signs of vasculitis was confirmed. There was a highly significant difference in the distribution of cryoglobulin types between the symptomatic and asymptomatic HCV-infected patients ($p < 0.002$). The difference appears to be due to a higher proportion of Type II-RF among symptomatic compared to asymptomatic patients, and a higher proportion of Type III among asymptomatic compared to symptomatic patients. The Type II-RF mean cryocrit concentration was significantly higher ($p < 0.002$) for symptomatic than for asymptomatic patients only in the HCV-infected patients. Also notable, 88% of Type II-RF cryoglobulins were associated with low C4 (data not shown).

DISCUSSION

The results of our study are notable because cryoglobulinemic vasculitis is uncommon and Type II-RF cryoglobulins were identified among mixed cryoglobulins detected in the clinical immunology laboratory rather than in populations

Table 2. Prevalence (%) of clinical manifestations associated with various cryoglobulin types in patients positive (+) and negative (–) for HCV infection.

	Protocol I											
Cryocrit Type	II		II-RF		IIa		IIa-RF		III		III-RF	
HCV status	HCV+	HCV–	HCV+	HCV–	HCV+	HCV–	HCV+	HCV–	HCV+	HCV–	HCV+	HCV–
No. of patients	28	9	20	8	2	0	1	0	97	31	7	1
Palpable purpura*												
n	7	2	9	2	0	0	0	0	3	1	0	0
%	25	22	45	22	0	0	0	0	3	3	0	0
Glomerulonephritis†												
n	6	3	4	2	0	0	0	0	2	9	1	0
%	21	33	20	25	0	0	0	0	2	29	14	0
Peripheral neuropathy**††												
n	4	1	6	2	0	0	0	0	5	2	0	1
%	14	11	30	25	0	0	0	0	5	6	0	100

* $p < 0.01$ for difference in distribution of cryoglobulin types in HCV-positive patients with and without palpable purpura. ** $p < 0.01$for difference in distribution of cryoglobulin types in HCV-positive patients with and without glomerulonephritis. † $p = 0.03$ for difference in distribution of cryoglobulin types in HCV-positive patients with and without peripheral neuropathy. †† $p = 0.04$ for difference in distribution of cryoglobulin types in HCV-negative patients with and without peripheral neuropathy.

Table 3. Association of Type II-RF cryoglobulins with cryoglobulinemic vasculitis in patients positive (total 155) and negative (total 49) for HCV infection.

	HCV-positive		HCV-negative	
	Symptomatic	Asymptomatic	Symptomatic	Asymptomatic
No. of patients (%)	47 (30.3)	108 (69.7)	25 (51)	24 (49)
Male/female ratio	0.96 (23/24)	1.25 (60/48)	0.92 (12/13)	0.47 (8/17)
Type II (% of column)*†	17 (36.2)	11 (10.2)	6 (24)	3 (12.5)
Mean cryocrit	1.6 ± 0.89	1.2 ± 0.65	1.3 ± 0.57	1.2 ± 0.51
Type II-RF (% of column)	19 (40.4)	1 (0.9)	6 (24)	2 (8.3)
Mean cryocrit**††	6.2 ± 6.68	1.5 ± 0.57	4 ± 4.2	3.6 ± 3
Type IIa (% of column)	0	2 (1.8)	0	0
Mean cryocrit	0	1.5 ± 0.7	0	0
Type IIa-RF (% of column)	0	1 (0.9)	0	0
Mean cryocrit	0	1	0	0
Type III (% of column)	10 (21.3)	87 (80.6)	12 (48)	19 (79.2)
Mean cryocrit	1 ± 0.21	1.1 ± 0.39	1 ± 0	1 ± 0
Type III-RF (% of column)	1 (2.1)	6 (5.6)	1 (4)	0
Mean cryocrit	2.5 ± 0.7	1	1	

* $p < 0.002$ for difference in distribution of cryoglobulin types between symptomatic and asymptomatic HCV-infected patients, Fisher exact test. ** No difference in distribution of cryoglobulin types between symptomatic and asymptomatic uninfected patients, Fisher exact test. † $p = 0.08$ for difference in mean cryocrit between symptomatic and asymptomatic HCV-infected patients, t test. †† No difference in mean cryocrit between symptomatic and asymptomatic uninfected patients, t test.

selected for signs of vasculitis, as in previous studies. The quantification of Type II-RF differentiated patients who were symptomatic from those who were asymptomatic for major signs of vasculitis, whereas previous studies demonstrated increased cryoglobulin concentrations in patients with Type II cryoglobulinemia with no correlation to disease activity[7]. The clinical associations of Type II-RF cryoglobulins described in this study are consistent with the well established association of Type II cryoglobulins containing monoclonal IgMκ RF with cryoglobulinemic vasculitis[2,7-10] and the pathologic role for Type II-RF cryoglobulins demonstrated in the palpable purpura and membranoproliferative glomerulonephritis lesions of patients with cryoglobulinemic vasculitis[8,11]. Both the qualitative and quantitative associations of Type II-RF cryoglobulins with cryoglobulinemic vasculitis appeared to occur mainly in HCV-infected patients rather than in those without infection.

Detection and quantification Type II-RF cryoglobulins may enhance the monitoring of HCV-infected patients with cryoglobulinemic vasculitis.

ACKNOWLEDGMENT

We thank Dr. Cindy L. Christiansen for performing the statistical studies, Carol Spencer for editing the manuscript, and the staff of the Clinical Immunology Laboratory and especially Gale Kennedy for their assistance in obtaining data for this study.

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