Concise report

Validation of the classification criteria for cryoglobulinaemic vasculitis

Luca Quartuccio¹, Miriam Isola², Laura Corazza¹, Manuel Ramos-Casals³, Soledad Retamozo³, Gaafar Mohamed Ragab⁴, Mostafa Naguib Zoheir⁴, Manal Abdel-Moneim El-Menyawi⁴, Mohamed Nabi Salem⁵, Domenico Sansonno⁶, Gianfranco Ferraccioli⁷, Elisa Gremese⁷, Athanasios Tzioufas⁸, Michael Voulgarelis⁹, Dimitris Vassilopoulos⁹, Salvatore Scarpato¹⁰, Nicolò Pipitone¹¹, Carlo Salvarani¹¹, Loic Guillemin¹², Benjamin Terrier¹², Patrice Cacoub¹³, Davide Filippini¹⁴, Francesco Saccardo¹⁵, Armando Gabrielli¹⁶, Paolo Fraticelli¹⁶, Marco Sebastiani¹⁷, Matija Tomsic¹⁸, Antonio Tavoni¹⁹, Cesare Mazzaro²⁰, Pietro Pioletti²¹, Norihiro Nishimoto²², Patrizia Scaini²³, Anna Linda Zignego²⁴, Clodoveo Ferrì²⁷, Giuseppe Monti²⁵, Maurizio Pietrogrande²⁵, Stefano Bombardieri²⁷, Massimo Galli²⁶ and Salvatore De Vita¹

Abstract

Objective. The aim of this study was to validate the classification criteria for cryoglobulinaemic vasculitis (CV).

Methods. Twenty-three centres were involved. New patients with CV (group A) and controls, i.e. subjects with serum cryoglobulins but lacking CV based on the gold standard of clinical judgment (group B) and subjects without cryoglobulins but with clinical features that can be observed in the course of CV (group C), were studied. Positivity of serum cryoglobulins was necessary for CV classification. Sensitivity and specificity of the criteria were calculated by comparing group A vs group B. The group A vs group C comparison was done to demonstrate the possible diagnostic utility of the criteria.

Results. The study included 268 patients in group A, 182 controls in group B and 193 controls in group C (small vessel vasculitis, 51.8%). The questionnaire (at least 2/3 positive answers) showed 89.0% sensitivity and 93.4% specificity; the clinical item (at least 3/4 clinical involvement) showed 75.7% sensitivity and 89.0% specificity and the laboratory item (at least 2/3 laboratory data) showed 80.2% sensitivity and
62.4% specificity. The sensitivity and specificity of the classification criteria (at least 2/3 positive items) were 89.9% and 93.5%, respectively. The comparison of group A with group C demonstrated the clinical utility of the criteria in differentiating CV from CV mimickers.

**Conclusion.** Classification criteria for CV were validated in a second, large, international study confirming good sensitivity and specificity in a complex systemic disease.

**Key words:** cryoglobulinaemia, hepatitis C, classification, vasculitis.

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**Introduction**

Cryoglobulinaemic syndrome or cryoglobulinaemic vasculitis (CV) is a systemic vasculitis associated with positive serum cryoglobulins, i.e. immune complexes composed of RF monoclonal or polyclonal against polyclonal IgG (type II or type III cryoglobulins, respectively) or monoclonal immunoglobulin without RF activity (type I), which reversibly precipitate at a temperature <37°C. CV is often linked to non-malignant B cell lymphoproliferation triggered by chronic infection by HCV [1-2]. Classification criteria developed with an accepted methodology were lacking, but represent a key step for clinical studies, research and epidemiological studies. Previous criteria were developed by taking into account only expert opinion and lacked any statistical support.

The first preliminary classification criteria for CV using an accepted methodology in a large, multicentre, international study have recently been published [7]. Notably, after a thorough discussion among experts, the presence of serum cryoglobulins was considered a *conditio sine qua non* for CV classification [7]. These criteria for CV showed sensitivity of 88.5% and specificity of 93.6%. The aim of this second study was to validate these preliminary classification criteria for CV in real cohorts of cases and controls, including some countries outside Europe.

**Methods**

A similar methodology as used in the first study was herein adopted [7]. A dedicated chart was developed, including the core set of items for classification already developed [7]. Briefly, three items were considered: (i) a validated questionnaire for CV [7]; (ii) data about the pattern of organ involvement (present and past), including constitutional symptoms, articular involvement, vascular involvement and neurological involvement and (iii) laboratory tests (positive RF, reduced C4 level, presence of serum monoclonal component). Consecutive, unselected new cases and controls based on the gold standard of diagnosis by an expert clinician, and not included in the previous study [7], were investigated and subdivided into the same three groups (A, B and C) as previously [7].

(i) Group A: patients with CV, HCV related or unrelated, essential or associated with other disorders with type I, II, III or non-typeable circulating cryoglobulins confirmed by at least two positive tests at an interval of ≥12 weeks [8].

(ii) Group B: subjects with serum cryoglobulins, but lacking CV based on the gold standard of diagnosis by an expert clinician.

(iii) Group C: subjects without serum cryoglobulins (by at least two repeated tests during a follow-up of at least 1 year), but with clinical or laboratory features that can be observed in the course of CV. This group was included to try to answer the question: if a patient has some features that pose a differential diagnosis of CV, even if negative for serum cryoglobulins by initial testing, when should CV be suspected? Based on the results of the previous study [7], the experts recommended the inclusion of this second group of controls to further support the clinical utility of the criteria, even if this was not required for validation, and so that patients with systemic vasculitis represented at least half of the subjects recruited.

A sample size of at least 140 patients for group A and 140 controls for group B was estimated in order to obtain sensitivity and specificity of at least 90% (s.d. 5) based on the results of the first study [7]. Sensitivity and specificity were calculated by comparing group A vs group B. Validation of the criteria was based on comparison of these two groups, since the positivity of serum cryoglobulins was a *conditio sine qua non* for classification [7]. Each centre was asked to recruit 12 patients in each group (A, B and C). The study was approved by the Independent Ethical Committee of the co-ordinating centre and then by the local ethics committees of each centre involved. Signed informed consent was obtained from all the patients enrolled in the study.

**Results**

**Patients**

Six hundred and forty-three patients were enrolled in 23 centres in Italy, France, Spain, Greece, Slovenia, Egypt and Japan. Organizational or local issues did not allow the participation of the invited American experts. Group A comprised 268 patients, group B comprised 182 controls with serum cryoglobulins and group C comprised 193 controls without serum cryoglobulins but with clinical features of CV. The composition and demographic characteristics of groups A, B and C are shown in Table 1.
Twenty patients showed type I cryoglobulinaemia, 13 in group A and 7 in group B. In group C, 108/193 (55.9%) patients suffered from systemic vasculitides, of whom 100/108 (92.6%) had vasculitides involving the small vessels. Missing data were <10%.

Comparison of group A vs group B for validation of the classification criteria

Groups A and B were compared for criteria development and validation, since the positivity of serum cryoglobulins is a conditio sine qua non for the classification of CV [7]. The sensitivity and specificity of the questionnaire item (at least 2/3 positive answers) were 89.0% (95% CI 85.1, 92.9) and 56.5% (95% CI 59.1, 73.1), respectively; for the clinical item (at least 3/4 clinical involvements), 75.7% (95% CI 70.5, 80.8) and 42.0% (95% CI 35.0, 49.0), respectively; and for the laboratory item (positivity of at least 2/3 laboratory data), 80.2% (95% CI 75.3, 85.2) and 92.1% (95% CI 88.1, 96.1), respectively. The sensitivity and specificity of the single questions, clinical manifestations and laboratory tests included in each item are shown in Table 2. The final classification criteria (at least 2/3 positive items) showed a sensitivity of 89.9% (95% CI 86.1, 93.6), similar to the previous study (88.5% sensitivity and 93.6% specificity) [7]. Since the issue of whether a patient has CV even if cryoglobulins are negative or not determined is of critical importance for clinical purposes (although not for classification), further investigation was done. One of the items, i.e. the laboratory item, continued to show high specificity in the present study. Therefore the possibility that this item should be positive to suggest CV, despite the lack of cryoglobulins, was further explored. With the assumption of positivity of the laboratory item (questionnaire plus laboratory item, clinical plus laboratory or positivity of all three items), the CV classification criteria showed sensitivity of 77.8% (95% CI 72.6, 83.0) and specificity of 92.6% (95% CI 88.8, 96.5) in the comparison of group A and group C.

Discussion

The present study validates preliminary classification criteria for CV (see supplementary Table S2, available at Rheumatology Online). Overall, in this setting the sensitivity and specificity of the single items were as follows: for the questionnaire (at least 2/3 positive answers), 89.6% (95% CI 85.9, 93.2) and 56.5% (95% CI 59.1, 73.1), respectively; for the clinical item (at least 3/4 clinical involvements), 75.7% (95% CI 70.5, 80.8) and 42.0% (95% CI 35.0, 49.0), respectively; and for the laboratory item (positivity of at least 2/3 laboratory data), 80.2% (95% CI 75.3, 85.2) and 92.1% (95% CI 88.1, 96.1), respectively. The sensitivity and specificity of the single questions, clinical manifestations and laboratory tests included in each item are shown in Supplementary Table S1, available at Rheumatology Online.

The comparison of group A with group C was done not to validate the classification criteria, but to further support the clinical relevance of the criteria for patients suspected to suffer from CV even with negative serum cryoglobulins by initial testing. These patients deserve particular attention [7]. In this setting, the sensitivity and specificity of the single items were as follows: for the questionnaire (at least 2/3 positive answers), 89.6% (95% CI 85.9, 93.2) and 56.5% (95% CI 59.1, 73.1), respectively; for the clinical item (at least 3/4 clinical involvements), 75.7% (95% CI 70.5, 80.8) and 42.0% (95% CI 35.0, 49.0), respectively; and for the laboratory item (positivity of at least 2/3 laboratory data), 80.2% (95% CI 75.3, 85.2) and 92.1% (95% CI 88.1, 96.1), respectively. The sensitivity and specificity of the single questions, clinical manifestations and laboratory tests included in each item are shown in Supplementary Table S1, available at Rheumatology Online.

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Table 2

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<th>Specificity</th>
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<td>93.4</td>
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<tr>
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<td>84.7</td>
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<td>Serum monoclonal component</td>
<td>69.4</td>
<td>63.7, 75.1</td>
<td>54.0</td>
<td>46.6, 61.4</td>
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</table>

Rheumatology Online) [7], with very similar results observed. These criteria have been developed and validated in large and independent series of real patients and controls and are now available for epidemiological, clinical and research studies. An accepted methodology and adequate statistical methods were used for the first time, whereas the previous criteria were developed by taking into account only expert opinion [9–12]. Experts from different countries and from different branches of medicine [7, 13] were involved, supporting the robustness of the results. In patients with serum cryoglobulins, positivity of at least two of three items of the questionnaire is required for the classification of CV.

The questionnaire, validated in the first study [7], is herein shown to have good sensitivity and specificity. This simple questionnaire may be useful for the initial evaluation of the patient, especially by physicians without great experience in the field. Also, a validated questionnaire is helpful for epidemiological studies of CV, which have been scarce and difficult to compare. Overall, the good sensitivity and specificity of the questionnaire in a patient with serum cryoglobulins support the notion that when skin purpura is associated with HCV infection, CV is very likely. In addition, the positivity of the validated questions for purpura included in the questionnaire may disclose CV in HCV-uninfected subjects, e.g. in the course of SS [14].

In any case, no single item (questionnaire, clinical or laboratory) included in the criteria is absolutely needed for classification [7]. This represents a major advantage in several situations: e.g. for patients with difficulty completing a questionnaire, with clinical features less common in CV or where laboratory data are uncertain for any reason [7]. Although the classification criteria have not been developed and should not be employed for diagnostic purposes, their use in clinical practice may occur, in particular, for disease recognition in the early stages [15, 16]. As previously mentioned, according to the experts [7], positive serum cryoglobulinaemia is a conditio sine qua non for the classification of CV; therefore, patients in whom CV is suspected on clinical grounds, but where cryoglobulins are negative, cannot be classified as CV. However, there may be patients with true CV, where serum cryoglobulins may initially be negative for various reasons [7]. Investigation of the possible usefulness of the criteria in these patients was recommended by experts [7]. To this end (and therefore not for classification purposes) a separate group C of controls was included, as previously [7]. Patients in group C lacked serum cryoglobulins, although they suffered from other systemic vasculitides or other systemic diseases where signs and symptoms may support the differential diagnosis of CV. When comparing group A with group C, the classification criteria for CV maintained good sensitivity but showed a lower specificity than previously [7]. This might be explained by the composition of group C, since more patients with systemic vasculitides were included in the present study (55.9% vs 40.7%, P < 0.001), with particular regard to small vessel vasculitides (92.6% vs 57.2%, P < 0.0001). On the other hand, the laboratory item maintained high specificity for CV in this study, and for this reason the positivity of the laboratory item as a necessary factor for suspecting CV in clinical practice, despite the negativity of cryoglobulins, was further investigated. Based on this assumption, a much higher specificity (92.6%) of the criteria, with acceptable sensitivity (77.8%), was noticed in the comparison of group A vs group C. Furthermore, the laboratory item appears as a surrogate marker for the presence of cryoglobulins and is useful to help distinguish CV from other vasculitides in the absence of serum cryoglobulins. Overall, these results improve previously published data about an unmet need underscored by experts [7] and the key role of laboratory investigations to discriminate CV from other vasculitides is reinforced. Besides the well-known importance of optimal and repeated testing of serum cryoglobulins [7], reliable assays for the determination of RF, serum monoclonal component and C4 are of major clinical value (see supplementary Table S2, available at Rheumatology Online). Finally, the number of
patients with type I cryoglobulinaemia studied was increased in the present study with respect to the previous one [7]. The evidence reported here that CV may be correctly classified in this subset of patients is noteworthy, given its low prevalence.

In conclusion, classification criteria for CV have been validated in a new, large cohort of cases and controls from both European and non-European countries. Both in the initial study [7] and in the present one, the criteria show good sensitivity and specificity for this systemic and polymorphic disease. Besides classification, laboratory tests are crucial to support the possible diagnosis of CV, when the determination of serum cryoglobulins is negative by initial testing.

**Rheumatology key messages**

- Classification criteria for cryoglobulinaemic vasculitis have been validated in patients and controls.
- Classification criteria for cryoglobulinaemic vasculitis have high sensitivity and specificity.
- The laboratory item is required to suspect cryoglobulinaemic vasculitis if serum cryoglobulins are negative by initial testing.

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**Supplementary data**

Supplementary data are available at Rheumatology Online.

**References**