Clinical Biochemistry 144 (2026) 111145

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Cryoglobulinemia and Cryofibrinogenemia: Ten years of experience and diagnostic perspectives from a large laboratory-based cohort

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ARTICLE INFO

Keywords:
Cryoglobulins
Cryofibrinogen
Cryocrit
HCV
Systemic sclerosis

ABSTRACT

Objectives: Cryoproteins include cryoglobulins (CG), detectable in serum and plasma, and cryofibrinogen (CF), detectable only in plasma. Although they share clinical features, CG and CF differ in pathogenesis and diagnostic management. Cryofibrinogenemia remains poorly characterized and likely underdiagnosed.

Methods: We retrospectively analyzed CG tests performed between 2015 and 2024 and CF tests between 2020 and 2024 at the Laboratory of the Public Health Hospital of Modena, evaluating clinical and epidemiological characteristics.

Results: Among 10,874 patients tested for CG, 14% were positive: type III (50%), type II (45%), and type I (5%). Mean age at first detection differed significantly between males (60 years) and females (66 years; $p < 0.001$). Since 2020, male predominance was observed. Cryocrit levels were higher in HCV-positive patients ($p < 0.001$), with a marked reduction in HCV-related cases and cryocrit values over time. In patients achieving sustained virological response, cryocrit levels were comparable to HCV-negative patients. No temporal differences of cryocrit were observed in HCV-negative cases. In the 2020–2024 cohort ($n = 628$), CF was detected in 47%. Among 574 patients tested for both CG and CF, cryoprotein positivity was observed in 54%: isolated CF in 70%, combined CF/CG in 23%, and isolated CG in 7%. In systemic sclerosis patients ($n = 239$), isolated CF was significantly more frequent ($p = 0.022$; OR = 1.82).

Conclusions: Parallel CG and CF testing improves diagnostic accuracy in cryoproteinemias. CF determination should be systematically included in diagnostic protocols, as CG testing alone may be insufficient in complex clinical scenarios.

1. Introduction

Cryoproteins consist of a heterogeneous group of plasma proteins that precipitate at 4 °C and redissolve at 37 °C. They include two main entities: cryoglobulins (CG), detectable in both serum and plasma, and cryofibrinogen (CF), which is exclusively found in plasma [1]. CG are classified according to Brouet into three types [2]: type I, consisting of a single monoclonal immunoglobulin, and types II and III (mixed), composed of two immunoglobulins, one of which acts as an anti-immunoglobulin antibody. From a pathogenetic perspective, type I CG predominantly cause occlusive phenomena, whereas types II and III are responsible for immune complex–mediated disease [3].

CF, in contrast, forms an insoluble complex composed of fibrinogen, fibronectin, and other plasma proteins, often associated with inhibitors of fibrinolysis, thereby promoting thrombotic events [4].

Clinically, cryoglobulinemia and cryofibrinogenemia share the classic Meltzer triad (fatigue, arthralgia, and purpura) [5] and may present as essential forms or secondary to viral, autoimmune, and rheumatologic disorders, and—particularly in the case of CF—to neoplastic diseases [6]. Cryoglobulinemia is more frequently

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https://doi.org/10.1016/j.clinbiochem.2026.111145
Received 9 March 2026; Received in revised form 24 May 2026; Accepted 26 May 2026
Available online 27 May 2026

associated with vasculitis affecting the distal extremities or internal organs, such as kidneys and peripheral nervous system, whereas cryofibrinogenemia is typically linked to peripheral thrombosis, ulcers, and acral necrosis [7–9].

The differential diagnosis between these two conditions is not always straightforward on clinical grounds and requires laboratory support, including detection, quantification, and characterization of the cryoprecipitate (CG and/or CF), as well as meticulous management of the pre-analytical phase, which plays a major impact on analytical sensitivity [10–12].

Despite advances in the understanding of cryoglobulinemia, cryofibrinogenemia remains frequently underdiagnosed. The aim of this study is to describe the epidemiological and clinical data derived from ten years of CG testing and five years of CF testing performed at the Modena laboratory, highlighting their diagnostic and clinical implications.

2. Materials and methods

A retrospective study was conducted including all samples received at the Laboratory of the Civil Hospital of Baggiovara of AUSL of Modena with a request for CG testing between 2015 and 2024, and for CF testing between 2020 and 2024, following the introduction and validation of a dedicated analytical method.

2.1. Study population and data collection

Data were extracted from the Laboratory Information System (LIS) and organized in a dedicated database (Microsoft Excel 2019).

The collected variables included CG and CF test results (positive/negative), cryocrit (CCT) values, CG type, requesting clinical unit, and associated clinical diagnoses. Each test corresponds to a single physician request; therefore, the reported numbers reflect both the number of tests performed and the number of requests. Since testing for CF was introduced only in 2020, some patients had already been previously tested for CG. To avoid cumulative bias, all analyses were restricted to the first detection of CG positivity, as verified through comparison with historical laboratory databases.

As part of routine clinical practice and in accordance with good clinical practice guidelines, electronic medical records of patients testing positive for CG and/or CF were reviewed to explore potential clinical–laboratory correlations.

2.2. Statistical analysis

Continuous variables were expressed as median and 95% confidence interval (CI) in the case of non-normal distribution. Data normality was assessed using the D'Agostino–Pearson omnibus test. Comparisons between independent groups were performed using the Kruskal–Wallis and Mann–Whitney *U* tests, as appropriate. Associations between quantitative variables were evaluated using Pearson's correlation. Comparisons of quantitative parameters were performed using Student's *t*-test or the Wilcoxon test, according to data distribution. Categorical variables were analyzed using the chi-square test or Fisher's exact test, as appropriate. A p-value <0.05 was considered statistically significant.

Statistical analyses were performed using MedCalc® Statistical Software, version 23.1.3 (MedCalc Software Ltd., Ostend, Belgium).

2.3. Cryoprotein analysis methodology

Since CG and CF both belong to the group of cryoproteins, their laboratory evaluation should be performed concurrently in order to compare cryoprecipitate (CPT) formation in serum and plasma [4,13]. Accordingly, a standardized procedure for cryoprotein analysis was developed at our center, including pre-analytical, analytical, and post-analytical phases (Table 1).

Table 1
Procedure for cryoprotein determination. CG, cryoglobulins; CF, cryofibrinogen, CCT, cryocrit, IFE, immunofixation electrophoresis.

Phase	Procedure
Pre-analytical	• Sample collection: CG: 10 mL tube without anticoagulant/gel separator; CF: 10 mL K_2-EDTA tube. • Maintain tubes/syringes at 37 °C until separation. • Transport in 37 °C pre-heated container. • Centrifuge at 37 °C (2 500 g × 15 min); CG serum incubated at 37 °C for 2 h to complete coagulation. • Aliquot into two tubes; incubate at 4 °C for 7 days. • Test reversibility at 37 °C if precipitate present; measure CCT in Wintrobe tube. • Exclude hemolysed, lipemic, icteric samples. • Visual inspection: compare precipitate at 4 °C vs 37 °C in serum and plasma. • CCT quantification: centrifuge Wintrobe tube (500 *g* × 15 min); analytical sensitivity 1%.
Analytical	• Cryoprecipitate washing: ≥3 cycles with cold saline; centrifuge at 4 °C (1 500 g × 15 min). • Resolubilization at 37 °C: CG: saline + *N*-acetylcysteine; CF: saline. • Immunofixation electrophoresis (IFE) on Hydragel 4 IF, including CCT <1% samples. • CG panel: fixative, anti-IgG, IgA, IgM, κ, λ. • CF panel: fixative, anti-fibrinogen, anti-IgG, IgM, κ, λ. • Integrated evaluation of CCT and IFE patterns. • Confirm effective washing by absence of albumin band.
Post-analytical	• Interpretive outcomes: – CF only: precipitate in plasma at 4 °C; anti-fibrinogen band only. – CG only: precipitate in serum and plasma; immunoglobulin bands only. – CG + CF: precipitate in serum and plasma; both anti-fibrinogen and anti-Ig bands.

2.4. Pre-analytical phase

For CG determination, the method described by Sargur et al. was adopted [14], with sample collection in 10 mL tubes without anticoagulant and without gel separator (BD Vacutainer, BD Company, Plymouth, UK), in order to avoid potential interferences.

For CF analysis, a dedicated procedure was developed by integrating protocols previously reported in literature [15–20]. Blood samples were collected in 10 mL tubes containing K_2-EDTA (BD Vacutainer). Although other anticoagulants such as oxalate or citrate may be used for CF determination, heparin should be avoided, as it induces the formation of precipitable fractions unrelated to CF [21,22].

For both determinations, collection tubes and sampling devices were maintained at 37 °C to prevent in vitro cryoprotein precipitation. Samples were then rapidly transported to the laboratory in thermostated containers at 37 °C [23,24]. After at least one hour—required for complete coagulation of samples collected without anticoagulant—sera and plasma were centrifuged at 37 °C (2,500 g for 15 min) using a thermostated centrifuge (Rotina 380R Hettich, Kirchlengern, Germany). This procedure reduces platelet counts in plasma to <5000/μL, thereby minimizing interferences caused by platelet–fibrin complex formation, which may precipitate at low temperatures and adhere to tube walls, complicating subsequent CPT interpretation [25–27].

Following centrifugation, the liquid fraction was aliquoted into two tubes and incubated at 4 °C for seven days [15]. If CPT was detected, one aliquot was rewarmed to 37 °C to assess reversibility, while 1 mL of serum or plasma was transferred to a graduated Wintrobe tube (6.5 × 100 mm, Laboindustria, Padua, Italy) for CCT measurement. Hemolysed, lipemic, or markedly icteric samples were excluded from analysis.

2.5. Analytical phase

Cryoprotein analysis was performed through three sequential steps.

In the first step, CPT was assessed by comparative visual inspection of serum and plasma obtained from the same patient, incubated at 4 °C and subsequently rewarmed to 37 °C. The presence of CPT exclusively in

plasma at 4 °C was considered indicative of CF. The coexistence of CF and CG was hypothesized when precipitate was observed in both serum and plasma, with an equal or greater amount in plasma compared with serum.

The second step consisted of CCT quantification by centrifugation of the Wintrobe tube at 500 *g* for 15 min. When both cryoglobulins and cryofibrinogen are detected, the CCT measured in the plasma tube reflects the combined contribution of both cryoproteins. CCT was expressed as the percentage of CPT per 1 mL of serum or plasma, with an analytical sensitivity of 1%.

In the third step, CPT was subjected to washing, even when present in trace amounts (CCT <1%), using three consecutive cycles with 1 mL of cold saline solution at 4 °C, each followed by centrifugation at 1500 *g* for 15 min at 4 °C, in order to remove residual serum or plasma components. After the final wash, CG were solubilized at 37 °C with 50 μL of saline solution and 50 μL of *N*-acetylcysteine, to promote reduction of IgM disulfide bonds, whereas CF was dissolved at 37 °C using 100 μL of prewarmed PBS.

Subsequently, confirmatory immunofixation electrophoresis (IFE) was performed on Hydragel 4 IF (Sebia, Lissé, France). For CG analysis, in addition to the reference lane (with fixative), antisera against IgG, IgA, IgM, κ and λ (Sebia, Lissé, France) were used for immunoglobulin typing and classification according to Brouet [2]. For CF analysis, anti-fibrinogen antiserum (Dako-Agilent, USA) [16,28] and antisera against IgG, IgM, κ and λ were employed. Anti-IgA antiserum was not used, given its rare positivity in CG, while the reference lane was maintained to verify effective washing through the absence of the albumin band. Reactivity of anti-IgG, IgM, κ and λ antisera on plasma may provide indirect evidence of CG; however, it should be noted that the absence of Ca^{2+} in plasma, due to the chelating effect of anticoagulants, may in rare cases interfere with immunoglobulin precipitation [29].

2.6. Post-analytical phase

The post-analytical phase included integrated evaluation of IFE results and CCT percentages. Proper execution of CPT washing was confirmed by the absence of the albumin band. Based on the results, three main interpretative scenarios were identified:

1. Isolated CF positivity, characterized by the presence of a band exclusively corresponding to the anti-fibrinogen antiserum in plasma IFE;
2. Isolated CG positivity, with reactive bands to anti-immunoglobulin antisera in serum IFE and absence of reactivity with the anti-fibrinogen antiserum in plasma;
3. Coexistence of CF and CG, with bands detected for both the anti-fibrinogen antiserum and anti-immunoglobulin antisera in serum and plasma IFE.

2.7. Reporting

Laboratory reports indicated positivity or negativity for CF and CG, specifying, in positive samples, the percentage value of CCT (or < 1% when below the analytical sensitivity). In cases of CG positivity, the immunoglobulin class and type of cryoglobulinemia according to the Brouet classification [2] were also reported. When concomitant positivity for CF and CG was observed, the CCT value measured in plasma was explicitly reported as representing the combined contribution of both cryoproteins. The laboratory recommended concurrent testing for CG and CF to ensure appropriate clinical–laboratory interpretation of results.

3. Results

During the period 2015–2024, samples from 10,874 patients with a request for CG testing were received at the Provincial Laboratory of the Azienda Unità Sanitaria Locale of Modena. Between 2020 and 2024, 628 patients underwent CF testing, 574 patients had a concurrent request for both CG and CF, while in 54 patients, only CF testing was requested without CG assessment.

The clinical units submitting requests for CG and CF testing were, respectively: external/outpatient phlebotomy units (47% and 5%), Rheumatology (12% and 72%), Internal Medicine (12% and 7%), Nephrology/Dialysis (10% and 4%), Liver Transplant Surgery (8% and 6%), Gastroenterology (6% and 3%), Neurology (2% and 1%), Oncology (2% and 2%), and Hematology (1% and 0.4%) (Fig. 1). Requests originating from phlebotomy units were mainly generated by general practitioners, potentially following specialist recommendations; however, this cannot be reliably ascertained from the available data.

3.1. Cryoglobulins

Of the 10,847 patients tested for CG during the study period, 1521 (14%) were positive; among these, 1292 (85%) were typed. Type III CG was the most frequent (50%), followed by type II (45%) and type I (5%). The median of the CCT was 5.8% (95% CI 1.7–10.0) for type I, 1.9% (95% CI 1.5–2.3) for type II, and 0.8% (95% CI 0.7–0.8) for type III; for calculation purposes, CCT values <1% were conventionally set at 0.5%.

Age at first detection of CG showed a bimodal distribution with sex-related differences: the median age was 60 years in males (95% CI 59–61) and 66 years in females (95% CI 64–67), with a statistically significant difference ($p < 0.001$) that remained stable over time ($p = 0.769$) (Fig. 2). Sex distribution changed significantly over time, with a female predominance in 2015–2019 and a shift toward male predominance in 2020–2024 ($p < 0.001$), particularly for type II CG (Table 2).

Overall, 82% of patients were not retested. Among the 1954 patients (18%) who underwent repeat testing, the median retesting interval was 199 days (95% CI 179–229). Of the retested patients, 86% confirmed the initial result: 91% of initially negative subjects and 76% of initially positive subjects (Table 3). The probability of confirmation increased with higher CCT values: 68% for CCT <1%, 75% for CCT =1%, and 100% for CCT ≥2%.

Over the study period, a significant reduction in new CG cases was observed, both in absolute numbers and as a proportion of tested patients, decreasing from 248/1323 (19%) in 2015 to 112/979 (11%) in 2024 ($p < 0.001$). A transient decline in requests was noted in 2020–2021, coinciding with the COVID-19 pandemic. In parallel, the decade-long analysis showed a decrease in type II CG and a corresponding increase in type III CG.

3.2. Cryocrit and HCV infection

Among the 1521 patients with cryoglobulins, 1106 underwent virological screening: 428 (39%) were HCV-positive, 351 (32%) had other viral infections (HBV, HIV, CMV), and 294 (27%) were negative; 33 patients (3%) had achieved a sustained virological response (SVR). HCV-positive patients showed significantly higher CCT values compared with the other groups ($p < 0.001$), whereas in patients with SVR, CCT levels did not differ from those observed in HCV-negative subjects.

Over the observation period, a marked reduction in new CG cases among HCV-positive patients was observed, decreasing from 66 cases in 2015 to 17 cases in 2024 ($p < 0.001$), and this trend was accompanied by an overall reduction in CCT levels. Conversely, from 2020 onward, a slight increase in newly detected CG cases among HCV-negative patients was observed, predominantly associated with CCT <1%; however, this trend was also confirmed in patients with CCT >1% (Fig. 3).

Viral genotype analysis, available in 241 patients (1a, 19%; 1b, 36%; 2, 20%; 3, 21%; 4, 5%), did not reveal significant differences in CCT levels among genotypes ($p = 0.75$).

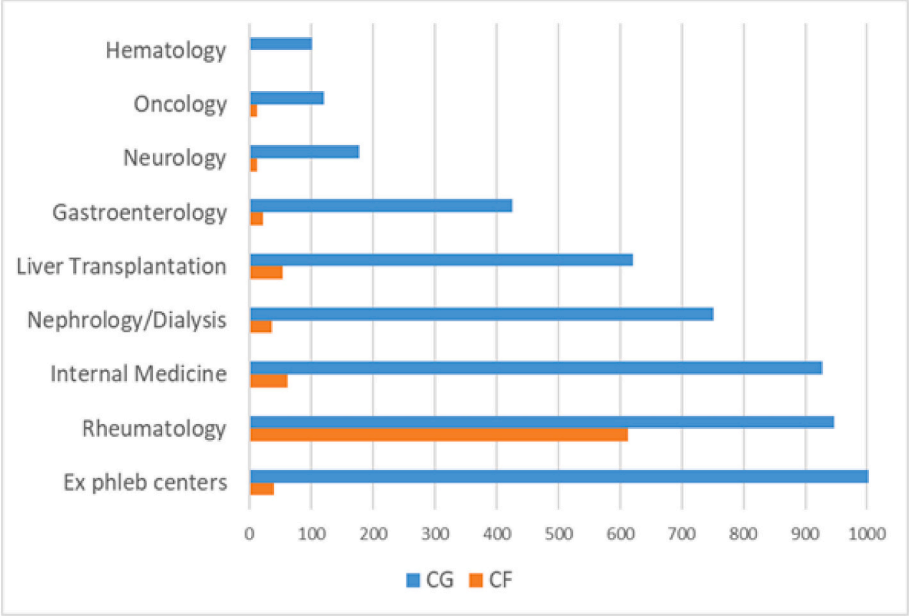

Fig. 1. Departments submitting requests for cryoglobulin (CG) and cryofibrinogen (CF) testing. Ex phleb center, external phlebotomy centers.

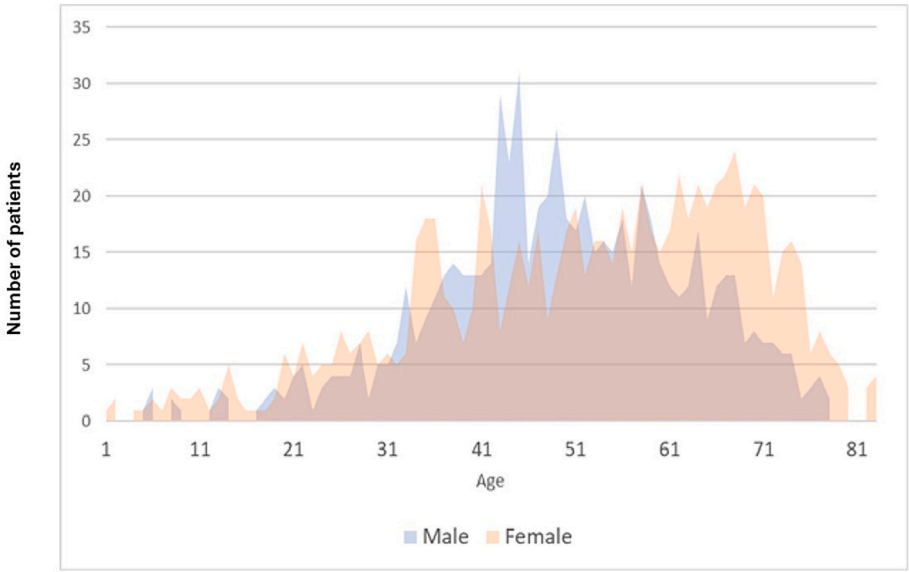

Fig. 2. Patients tested for cryoglobulins from 2015 to 2024 at first detection: age and sex distribution.

Table 2
Demographic characteristics of patients tested for cryoglobulins at first detection (2015–2024).

Characteristic	Males	Females	Total	*p*-value
Patients, n (%)	5056 (46.4)	5818 (54.6)	10,874 (100)	<0.001
CG-positive, 2015–2024, n (%)	693 (45.5)	828 (54.5)	1521 (14.0)	<0.001
CG-positive, 2015–2019, n (%)	388 (41.0)	548 (59.0)	936 (8.0)	<0.001
CG-positive, 2020–2024, n (%)	305 (52.0)	280 (48.0)	585 (6.0)	<0.001
Age, median (95% CI), years	60 (59–61)	66 (64–67)	63 (62–64)	<0.001

Table 3
Patients tested for cryoglobulins from 2015 to 2024: follow-up testing.

Characteristic	Positive	Negative	Total	*p*-value
Patients retested, n (%)	634 (32)	1320 (68)	1954 (18)	<0.001
Retesting interval, median days (95% CI)	147 (114–199)	232 (194–260)	199 (179–229)	0.002
Confirmed results, n (%)	486 (76)	1204 (91)	1690 (86)	<0.001
Not confirmed, n (%)	148 (24)	116 (9)	264 (14)	<0.001

3.3. *Cryofibrinogen*

During the 2020–2024 period, 628 patients underwent CF testing, with an overall positivity rate of 47%. Among CF-positive subjects, 31% were male; the median age was 62 years (95% CI 60–63), with no significant differences between sexes ($p = 0.71$). Among the 574 patients

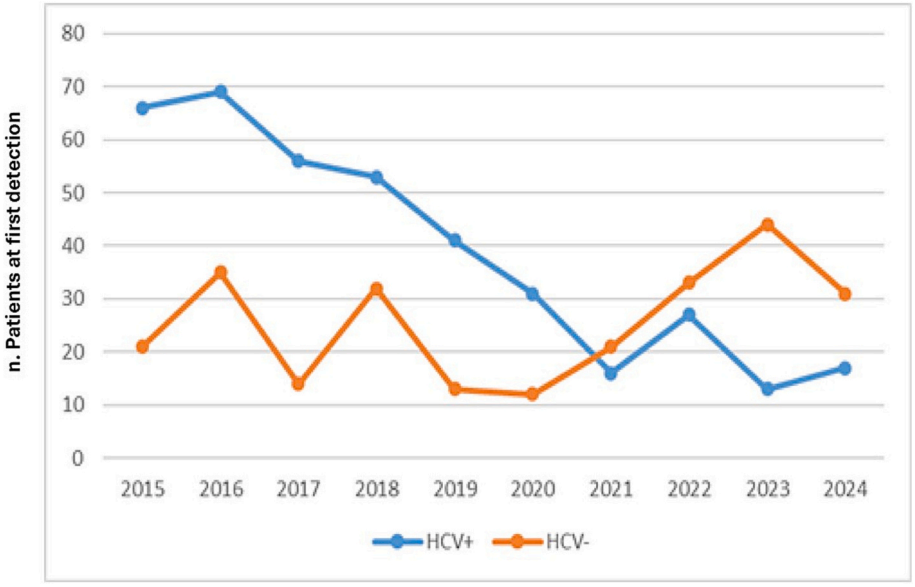

Fig. 3. Temporal trends in newly identified cryoglobulin-positive patients at first detection, comparing HCV-positive and HCV-negative cases.

who were tested concurrently for CG and CF, 46% were negative for both cryoproteins, whereas 54% showed positivity for one or both.

In patients with cryoproteinemia, isolated CF represented the most frequent finding (70%), followed by combined CF and CG positivity (23%), while isolated CG was observed in only 7% of cases. When considering exclusively CF-positive patients, CF was detected as an isolated finding in 75% of cases, whereas CG were isolated in 22%.

Among the 432 patients with a defined clinical diagnosis who were tested for both cryoproteins, 246 (57%) were positive for at least one cryoprotein. Of these, 239 (55%) were affected by systemic sclerosis (SSc). In patients with SSc, isolated CF was detected in 114 cases (48%), compared with 66 (34%) in patients with other diagnoses, demonstrating a significant association between isolated CF and SSc ($p = 0.022$; OR = 1.82) (Table 4).

4. Discussion

The ten-year analysis confirms that type III cryoglobulinemia represents the most frequently detected form in the study population. However, the literature is not uniform: while type I cryoglobulinemia is consistently reported as the least common, the relative prevalence of type II and type III forms varies across studies. Some authors report a higher frequency of type II cryoglobulinemia [14,30,31], whereas others describe a predominance of type III [2,29,32]. In line with previous reports, the highest CCT values were observed in type I cryoglobulinemia, followed by type II and, lastly, type III [33–35].

The median age at first detection of cryoglobulinemia remained stable throughout the observation period but differed significantly between sexes: males showed a lower median age than females, resulting in a bimodal age distribution. This difference may reflect underlying etiological factors, particularly HCV infection, which tends to be diagnosed at a later age in women [7,36]. From an epidemiological perspective, a female predominance was observed during the 2015–2019 period, followed by a shift from 2020 onward toward a higher prevalence among males. This change may be attributable to emerging factors, including indirect effects of the COVID-19 pandemic or an increased referral of patients from the liver transplant center, particularly individuals with HCV-related hepatocellular carcinoma, a condition more frequently observed in males.

A further relevant finding of this study concerns the limited repetition of CG testing. The majority of patients were not re-evaluated after the initial assessment; among those who underwent follow-up testing, repeat analysis was more frequent in initially negative subjects and confirmed the initial result in most cases. In patients with discordant results, lack of confirmation more commonly involved those who were initially positive. Moreover, in positive cases, the probability of result confirmation clearly increased with higher CCT values, reaching 100% in cases with CCT >2%. Low CCT values (<1%) are associated with lower reproducibility, underscoring the need for confirmatory testing.

The importance of confirming CG testing after an initial detection is essential, regardless of the result. False-negative results may be attributed to pre-analytical and analytical criticalities, particularly difficulties in maintaining an appropriate warm chain during the pre-analytical phase and a cold chain during the analytical phase [37,38]. Conversely, false-positive results may reflect transient conditions, such as acute infections, which can induce a temporary production of cold-precipitable immunoglobulins that subsequently disappear following resolution of the infectious event [7,32,39,40]. In this context, repeat testing after at least 12 weeks is essential to distinguish transient cryoglobulinemia from persistent positivity [31,41].

The role of HCV infection in the pathogenesis of cryoglobulinemia remains central [42,43]. HCV-positive patients exhibit higher CCT values compared with HCV-negative subjects or those infected with other viruses. The introduction of direct-acting antiviral agents has significantly modified the clinical scenario, reducing the severity of residual manifestations [44]. In patients achieving a SVR, even in the absence of complete remission of cryoglobulinemia, CCT levels tend to decrease to values comparable to those observed in HCV-negative patients or in individuals infected with other viruses [45–47].

Accordingly, HCV-associated cryoglobulinemia has shown a marked decline over the last decade, currently accounting for approximately one

Table 4
Comparison of cryoglobulins (CG) and cryofibrinogen (CF) in patients with systemic sclerosis (SSc) and in patients with other non–systemic sclerosis conditions (non-SSc).

Diagnosis	CF+/CG-	CF+/ CG+	CF-/ CG+	CF-/ CG-	Total
SSc	114	20	4	101	239
Non-SSc	66	33	9	85	193
Total	180	53	13	186	432
Odds ratio (95% CI)	1.82 (1.09–3.03)				
p-value	0.022				

quarter of the initially observed cases. In parallel, a slight increase in HCV-negative cases has been observed, particularly evident from 2021 onward. In this population, most cases are characterized by low-grade CCT values (<1%); however, even among newly detected cases with CCT >1%, the prevalence of HCV-negative patients exceeds that of HCV-positive patients, suggesting a growing contribution of non–HCV-related disorders [48]. With regard to viral subtypes, genotype 1b remains the most prevalent, in agreement with Italian epidemiological data [49–52].

In the present study, the association between CF and CG is consistent with the existing literature, although it shows considerable heterogeneity related to patient selection criteria. In laboratory-based studies, isolated CF is more frequently detected, as reported in investigations conducted on unselected populations [29,53]. In contrast, studies involving selected clinical cohorts, particularly patients with cryoglobulinemic vasculitis and HCV-positive status, demonstrate a higher prevalence of combined CF–CG positivity, often associated with more severe clinical manifestations [4,54]. Comparison across available studies [4,29,53,54] suggests that isolated or combined detection of CF and CG reflects distinct pathophysiological patterns rather than quantitative variations of a single disease process. Requests for CF and CG testing are influenced by heterogeneous diagnostic suspicions and clinical presentations, underscoring the need for an integrated interpretation of laboratory findings within the appropriate clinical context.

As summarized in Box 1, in the population analyzed in this study, CF emerged as the most frequently detected cryoprotein. Although CF testing is commonly requested together with CG determination, CF was more often identified as an isolated finding rather than in association with CG, in line with previous observations [10,29]. This result, however, may be partially influenced by selection bias, as the study population was predominantly derived from a specialized Rheumatology unit serving as a regional referral center for SSc. Consequently, CF testing was mainly requested in patients with rheumatologic diseases, particularly those affected by SSc.

Overall, a high prevalence of cryoprotein positivity was observed, with 57% of patients testing positive for at least one cryoprotein. Notably, more than half of these patients were diagnosed with SSc, indicating a non-random distribution of cryoprotein positivity across clinical diagnoses. The most relevant finding was the significantly higher frequency of isolated CF detected in patients with SSc compared with those affected by other conditions (48% vs 34%). This association was statistically significant ($p = 0.022$; OR = 1.82), confirming that isolated CF positivity occurs more frequently in SSc and supporting previous reports suggesting a potential role of CF as a biomarker in this disease [10,55,56].

From a laboratory perspective, CF detection may reflect underlying endothelial dysfunction and alterations in coagulation pathways, which are well-recognized features of SSc. In this context, CF may represent a laboratory marker associated with the vascular component of the disease rather than a nonspecific epiphenomenon.

Overall, these results suggest a possible role for CF as a biomarker associated with SSc, opening interesting perspectives from both diagnostic and prognostic standpoints. Further studies will be required to clarify the clinical significance of CF positivity, its potential correlation with specific disease phenotypes, disease activity, or severity, and to evaluate whether it may contribute to improved stratification of patients with SSc.

Another critical issue concerns the underdiagnosis of cryofibrinogenemia. CF determination is currently not included in the Italian National Essential Levels of Care (LEA), nor is there an established laboratory and clinical culture supporting its systematic use in routine diagnostic practice. In patients with suggestive clinical manifestations, but in the absence of detectable CG or in the presence of disproportionately low CCT values relative to clinical severity, CF testing may provide important interpretative insights [4,36]. Indeed, the clinical features associated with cryofibrinogenemia largely overlap with those observed in cryoglobulinemia and may even be more severe; therefore, CF positivity may explain the lack of correlation between CCT and clinical presentation observed in some patients [4,11,57].

Box 1
Schematic overview of the study population.

Study Population	Testing Pathways
• CG tested patients $n = 10,874$ (2015–2024) • CF tested patients $n = 628$ (2020–2024)	• Concurrent CG + CF $n = 574$ • CF only $n = 54$
Main Findings	**Conclusion**
• CG positivity: 14% • CF positivity: 47% • Isolated CF most frequent finding	• Integrated CG + CF testing improves diagnostic accuracy • Isolated CF associated with systemic sclerosis (SSc) • Supports inclusion of CF in diagnostic protocols

This large laboratory-based study supports the systematic integration of CF testing into routine diagnostic algorithms for cryoproteinemias. Parallel CG and CF assessment improves diagnostic accuracy, particularly in rheumatologic diseases such as SSc, and may explain clinical–laboratory discrepancies in patients with low CCT values.

In light of these findings, the need emerges for an integrated diagnostic approach that systematically includes CF assessment in appropriate clinical settings. The development of shared recommendations and a consensus document, supported by scientific societies, could represent a crucial step toward standardizing CF testing and improving the diagnostic work-up of cryopathies.

CRediT authorship contribution statement

Patrizia Natali: Conceptualization. **Daria Debbia:** Methodology. **Cecilia Napodano:** Methodology. **Umberto Basile:** Supervision, Methodology. **Francesca D'Ambrosio:** Data curation. **Valerio Basile:** Data curation. **Mariapaola Marino:** Writing – review & editing, Supervision. **Maria Teresa Mascia:** Writing – original draft. **Gilda Sandri:** Writing – review & editing, Conceptualization.

Informed consent statement

All participants provided written informed consent.

Institutional review board statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee (Comitato Etico Area Vasta Emilia Nord, protocol number 275/16).

Funding

This research and its publication have been funded from Università Cattolica del Sacro Cuore Fondazione Policlinico Universitario "A. Gemelli" I.R.C.C.S. as a part of its programs on promotion and dissemination of scientific research (Linea D1 to M.M.).

DATA AVAILABILITY STATEMENT

The data presented in this study are available upon reasonable request to the corresponding author.

References

[1] C.T. Nebe, G. Stamminger, H. Baum, M. Ossendorf, G. Wittmann, Relevance and diagnostics of cryoproteins, J. Lab. Med. 38 (2015), https://doi.org/10.1515/labmed-2015-0048.

[2] J.C. Brouet, J.P. Clauvel, F. Danon, M. Klein, M. Seligmann, Biological and clinical significance of cryoglobulins, Am. J. Med. 57 (1974) 775–788.

[3] M.N. Kolopp-Sarda, P. Miossec, Cryoglobulins: an update on detection, mechanisms and clinical contribution, Autoimmun. Rev. 17 (2018) 457–464.

[4] M. Michaud, G. Moulis, B. Puissant, L. Balardy, A. Huart, F. Gaches, et al., Cryofibrinogenemia: a marker of severity of Cryoglobulinemic Vasculitis, Am. J. Med. 128 (2015) 916–921.

[5] M. Meltzer, E.C. Franklin, K. Elias, R.T. McCluskey, N. Cooper, Cryoglobulinemia-a clinical and laboratory study. II. Cryoglobulins with rheumatoid factor activity, Am. J. Med. 40 (1966) 837–856.

[6] C. Belizna, L. Loufrani, J.F. Subra, M. Godin, P. Jolly, O. Vitecocq, et al., A 5-year prospective follow-up study in essential cryofibrinogenemia patients, Autoimmun. Rev. 10 (2011) 559–562, https://doi.org/10.1016/j.autrev.2011.04.009.

[7] C. Ferri, Mixed cryoglobulinemia, Orphanet J. Rare Dis. 3 (2008) 1–17.

[8] M. Trendelenburg, J.A. Schifferli, Cryoglobulins are not essential, Ann. Rheum. Dis. 57 (1998) 3–5.

[9] C.C. Belizna, F. Tron, P. Joly, M. Godin, M. Hamidou, H. Lévesque, Outcome of essential cryofibrinogenaemia in a series of 61 patients, Rheumatol 47 (2008) 205–207.

[10] P. Natali, D. Debbia, M.R. Cucinelli, T. Trenti, G. Amati, A. Spinella, et al., Analysis of cryoproteins with a focus on cryofibrinogen: a study on 103 patients, Clin. Chem. Lab. Med. 60 (2022) 1796–1803.

[11] N. Belfeki, S. Abroug, A. Strazzulla, S. Diamantis, Rare cause of bilateral foot gangrene: coexisting essential cryofibrogenaemia and cryoglobulinaemic vasculitis, BMJ Case Rep. 12 (2019) 2018–2020.

[12] P. Natali, D. Debbia, F. Turra, M. Savoia, D. Farci Santarcangeli, G. Cigliana, U. Basile, et al., Survey on cryoproteins testing among Italian laboratories: from pre-analytical phase to reporting, Biochim. Clin. 46 (2023) 1–12.

[13] D. Saadoun, I. Elalamy, P. Ghillani-Dalbin, D. Sene, A. Delluc, P. Cacoub, Cryofibrinogenemia: new insights into clinical and pathogenic features, Am. J. Med. 122 (2009) 1128–1135, https://doi.org/10.1016/j.amjmed.2009.03.040.

[14] R. Sargur, P. White, W. Egner, Review article Cryoglobulin evaluation: best practice? Ann. Clin. Biochem. 47 (2010) 8–17.

[15] M. Michaud, J. Pourrat, Cryofibrinogenemia, J. Clin. Rheumatol. 19 (2013) 142–148.

[16] H. Blain, P. Cacoub, L. Musset, N. Costedoat-Chalumeau, C. Silberstein, O. Chosidow, et al., Cryofibrinogenaemia: a study of 49 patients, Clin. Exp. Immunol. 120 (2000) 253–260.

[17] N. Stathakis, D. Karamanolis, G. Koukolis, E. Tsianos, Characterization of Cryofibrinogen isolated from patients' plasma, Haemostasis 10 (1981) 195–202.

[18] M.S. Soyfoo, A. Goubella, E. Cogan, J.C. Wautrecht, A. Ocmant, P. Stordeur, Clinical significance of cryofibrinogenemia: possible pathophysiological link with Raynaud's phenomenon, J. Rheumatol. 39 (2012) 119–124.

[19] D. Saadoun, L. Musset, P. Cacoub, Les cryofibrinogénémies, Rev. Med. Interne 32 (2011) 287–291.

[20] J. Damoiseaux, The diagnosis and classification of the cryoglobulinemic syndrome, Autoimmun. Rev. 13 (2014) 359–362.

[21] R. Smith, R. Von Korff, A heparin-precipitate fraction of human plasma. I. Isolation and characterization of the fraction, J. Clin. Invest. 36 (1956) 596–604.

[22] R. Smith, A heparin-precipitable fraction of human plasma. II. occurrence and significance of the fraction in normal individuals and in various disease states, J. Clin. Invest. 36 (1957) 605–616.

[23] U. Basile, E. Torti, M.T. Dell'Abate, L. Colacicco, F. Gulli, C. Zuppi, et al., Pre-analytical phase in cryoglobulin (CRG) detection: an alternative method for sample transport, Clin. Chem. Lab. Med. 54 (2016) e123–e126.

[24] M.H. Nahm, W.W. Chatham, W.H. Benjamin, Device for carrying blood samples at 37 °C for cryoglobulin test, Clin. Vaccine Immunol. 19 (2012) 1555–1556.

[25] V.J. Kogler, J.A. Miles, T. Özpolat, S.L. Bailey, D.A. Byrne, M. Bawcom-Randall, et al., Platelet dysfunction reversal with cold-stored vs room temperature–stored platelet transfusions, Blood 143 (2024) 2073–2088.

[26] H.E. Kattlove, B. Alexander, F. White, The effect of cold on platelets. II. Platelet function after short-term storage at cold temperatures, Blood 40 (1972) 688–696.

[27] P.V. Drossos, S.P. Fortis, A.T. Anastasiadi, E.G. Pavlou, A.G. Tsantes, G.A. Spyratos, et al., Cold vs. room temperature: a comparative analysis of platelet functionality in cold storage, Biomedicines 13 (2025) 1–15.

[28] L. Musset, M. Diemert, F. Taibi, L. Thi Huong Du, P. Cacoub, J. Leger, et al., Characterization of cryoglobulins by immunoblotting, Clin. Chem. 38 (1992) 798–802.

[29] M.N. Sarda-Kolopp, P. Miossec, Cryofibrinogen-characteristics and association with Cryoglobulin: a retrospective study out of a series of 1,712 samples over 7 years, Thromb. Haemost. 123 (2023) 669–678, https://doi.org/10.1055/a-2075-8204. 30.

[30] G. Monti, M. Galli, F. Invernizzi, P. Pioltelli, F. Saccardo, A. Monteverde, et al., Cryoglobulinaemias: a multi-Centre study of the early clinical and laboratory manifestations of primary and secondary disease. GISC. Italian Group for the Study of Cryoglobulinaemias, QJM (1995) 115–126.

[31] L. Quartuccio, M. Isola, L. Corazza, M. Ramos-Casals, S. Retamozo, G.M. Ragab, et al., Validation of the classification criteria for cryoglobulinaemic vasculitis, Rheumatology 53 (2014) 2209–2213.

[32] C. Napodano, F. Gulli, G.L. Rapaccini, M. Marino, U. Basile, Cryoglobulins: identification, classification, and novel biomarkers of mysterious proteins, Adv. Clin. Chem. 104 (2021) 299–340, https://doi.org/10.1016/bs.acc.2020.09.006.

[33] J. Khwaja, S.J. Salter, S. D'Sa, IgM-associated cryoglobulinaemia, Hemato 4 (2023) 240–249, https://doi.org/10.3390/hemato4030019.

[34] E. Muchtar, H. Magen, M.A. Gertz, How I treat cryoglobulinemia, Blood 129 (2017) 289–298.

[35] K.E. Nelveg-Kristensen, A. Paterson, L.C. Willcocks, Cryoglobulinaemic vasculitis diagnostic and treatment recommendations, Rheumatol (United Kingdom) 60 (Suppl 3):III39–42 (2021).

[36] F. Dammacco, G. Lauletta, S. Russi, P. Leone, M. Tucci, C. Manno, et al., Clinical practice: hepatitis C virus infection, cryoglobulinemia and cryoglobulinemic vasculitis, Clin. Exp. Med. 19 (2019) 1–21.

[37] M.N. Kolopp-Sarda, A. Nombel, P. Miossec, Cryoglobulins today: detection and immunologic characteristics of 1,675 positive samples from 13,439 patients obtained over six years, Arthritis Rheum. 71 (2019) 1904–1912.

[38] P. Natali, D. Debbia, T. Trenti, G. Galassi, J. Chester, G. Sandri, et al., Frequency and results of Cryoglobulin retesting in 4,963 patients: comment on the article by Kolopp-Sarda et al, Arthritis Rheum. 72 (2020) 1955–1956.

[39] M. Galli, L. Oreni, F. Saccardo, L. Castelnovo, D. Filippini, P. Marson, et al., HCV-unrelated cryoglobulinaemic vasculitis: the results of a prospective observational study by the Italian Group for the Study of Cryoglobulinaemias (GISC), Clin. Exp. Rheumatol. 35 (2017) 67–76.

[40] U. Basile, C. Napodano, M. Marino, F. Gulli, S. Colantuono, M. Casato, et al., Cryoglobulins: putative effectors of adaptive immune response, Clin. Exp. Rheumatol. 39 (Suppl. 129) (2021) 171–179, https://doi.org/10.55563/clinexprheumatol/keip4u.

[41] S. De Vita, F. Soldano, M. Isola, G. Monti, A. Gabrielli, A. Tzioufas, et al., Preliminary classification criteria for the cryoglobulinaemic vasculitis, Ann. Rheum. Dis. 70 (2011) 1183–1190.

[42] U. Basile, F. Gulli, L. Gragnani, K. Pocino, C. Napodano, L. Miele, et al., Different biochemical patterns in type II and type III mixed cryoglobulinemia in HCV positive patients, Dig. Liver Dis. 50 (9) (2018) 938–943, https://doi.org/10.1016/j.dld.2018.03.028.

[43] C. Napodano, G. Ciasca, P. Chiusolo, K. Pocino, L. Gragnani, A. Stefanile, et al., Serological and molecular characterization of hepatitis C virus-related cryoglobulinemic vasculitis in patients without cryoprecipitate, Int. J. Mol. Sci. 24 (14) (2023) 11602, https://doi.org/10.3390/ijms241411602.

[44] A.L. Zignego, S. Marri, L. Gragnani, Impact of direct acting antivirals on hepatitis C virus-related cryoglobulinemic syndrome, Minerva Gastroenterol. 67 (2021) 218–226.

[45] L. Gragnani, M. Visentini, E. Fognani, T. Urraro, A. De Santis, L. Petraccia, et al., Prospective study of guideline-tailored therapy with direct-acting antivirals for hepatitis C virus-associated mixed cryoglobulinemia, Hepatology 64 (2016) 1473–1482.

[46] M. Bonacci, S. Lens, Z. Mariño, M.C. Londoño, S. Rodriguez-Tajes, J.M. Sánchez-Tapias, et al., Long-term outcomes of patients with HCV-associated cryoglobulinemic vasculitis after virologic cure, Gastroenterol 155 (2018) 311–315.

[47] G. Lauletta, S. Russi, F. Pavone, A. Vacca, F. Dammacco, Direct-acting antiviral agents in the therapy of hepatitis C virus-related mixed cryoglobulinaemia: a single-Centre experience, Arthritis Res. Ther. 19 (2017) 1–10.

[48] K. Pocino, A. Stefanile, P. Natali, C. Napodano, V. Basile, G. Ciasca, et al., Cryoproteins in non-HCV-related autoimmune disorders: a serious cold-induced problem, Diagnostics (Basel) 15 (2025) 1933, https://doi.org/10.3390/diagnostics15151933.

[49] A. Petruzziello, S. Marigliano, G. Loquercio, A. Cozzolino, C. Cacciapuoti, Global epidemiology of hepatitis C virus infection: an up-date of the distribution and circulation of hepatitis C virus genotypes, World J. Gastroenterol. 22 (2016) 7824–7840.

[50] N. Landini, C. Chiericati, M. Boni, L. Simone, M. Trombini, C. Zanforlin, et al., Italian screening protocol and genotypes characterization for HCV elimination (2022−2023) in Ferrara's province: a real-world study, Sci. Rep. 15 (2025) 1–13.

[51] M. Ciccozzi, A. Lo Presti, A.R. Ciccaglione, G. Zehender, M. Ciotti, Phylogeny and phylodinamic of hepatitis C in Italy, BMC Infect. Dis. 12 (Suppl. 2) (2012) S5, https://doi.org/10.1186/1471-2334-12-S2-S5.

[52] N. Marascio, M.C. Liberto, G.S. Barreca, E. Zicca, A. Quirino, A.G. Lamberti, et al., Update on epidemiology of HCV in Italy: focus on the Calabria region, BMC Infect. Dis. 14 (Suppl. 5) (2014) S2, https://doi.org/10.1186/1471-2334-14-S5-S2.

[53] T. Troiano, A. Marinaccio, P. Patierno, A. Di Geronimo, L. Demarinis, F. Di Serio, Laboratory practices for the detection and characterization of Cryoglobulins and Cryofibrinogen, J. Clin. Lab. Anal. 39 (2025) e70074, https://doi.org/10.1002/jcla.70074.

[54] A. Delluc, D. Saadoun, P. Ghillani-Dalbin, D. Sene, J.C. Piette, P. Cacoub, Cryofibrinogen in patients with hepatitis C virus infection, Am. J. Med. 121 (2008) 624–631.

[55] G. Amati, A. Spinella, N. Patrizia, D. Daria, O. Martina, S. Ottavio, et al., Secondary cryofibrinogenemia is related to more severe microangiopathic involvement in systemic sclerosis: results from a retrospective observational study, Clin. Rheumatol. 44 (2025) 1173–1185, https://doi.org/10.1007/s10067-025-07324-z.

[56] S. De Almeida Chaves, B. Puissant, T. Porel, E. Bories, D. Adoue, L. Alric, et al., Clinical impact and prognosis of cryoglobulinemia and cryofibrinogenemia in systemic sclerosis, Autoimmun. Rev. 21 (2022) 103133, https://doi.org/10.1016/j.autrev.2022.103133.

[57] S. Moiseev, R. Luqmani, P. Novikov, T. Shevtsova, Cryofibrinogenaemia-a neglected disease, Rheumatology 56 (2017) 1445–1451.