Nail fold videocapillaroscopy in mixed cryoglobulinaemia

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Abstract

**Background.** Nail fold videocapillaroscopy (NVC) has been extensively used to examine morphological and functional changes of microcirculation in connective tissue diseases. The nutritional circulation that depends on tissue capillaries, can be expected to be significantly impacted in mixed cryoglobulinaemia (MC).

**Methods.** Using NVC, we evaluated 29 patients with MC (19 women), mean age 66 years (range 40–83). They included 28 hepatitis C virus (HCV) positive patients—14 genotype 1b, 10 genotype 2a 2c, two genotype 4, two with undetermined genotype. Of them, 18 had type II (IgMk-IgG) MC and 11 had type III. All patients were symptomatic, presenting with weakness (24 of 29 patients), arthralgia (24), purpura (16), peripheral neuropathy (20), Raynaud’s phenomenon (8), hypertension (19) and membranoproliferative glomerulonephritis (MPGN) (9). The nail fold capillaries of four fingers per hand were examined using a videomicroscope.

**Results.** Of the 29 patients, 27 had morphological abnormalities (including tortuosity and apical enlargement), 18 had capillaries with deeply altered orientations, 17 had shortened capillaries and 20 neoangiogenetic phenomena. These four types of capillary abnormalities were simultaneously present in 10, suggesting this combination to be a characteristic pattern in MC. Less common alterations included haemorrhages (10 cases), enlarged and giant capillaries (2) and avascular areas (2). The patients with MC-associated MPGN had a significantly greater number of capillary abnormalities (mean 4.5, range 4–6) than non-nephritic patients (mean 3.5, range 1–6, \( P = 0.01 \)). The number of capillary abnormalities was not related to the presence of Raynaud’s phenomenon.

**Conclusions.** Patients with MC show a variety of microcirculatory changes, often clustered in a characteristic pattern of abnormally oriented, short capillaries and neoangiogenetic phenomena. Capillary changes are more numerous in nephritic patients.

**Keywords:** cryoglobulinaemic glomerulonephritis; microcirculation; mixed cryoglobulinaemia; nail fold videocapillaroscopy

Introduction

Cryoglobulins are serum proteins mostly composed of immunoglobulins that precipitate at low temperatures and dissolve at higher temperatures. Type II cryoglobulins consist of monoclonal (usually IgM-k) and polyclonal (IgG) components, which sometimes are detected in patients with lymphoproliferative disorders infections, or autoimmune diseases [1]. Type III cryoglobulins are polyclonal immunoglobulins (IgG and IgM) also associated with infections and autoimmune disorders.

A number of clinical conditions, characterized by the presence of type II and III cryoglobulins without demonstrable underlying disease, were defined as ‘essential’ up to 1990, when a strong relation between the so-called ‘essential cryoglobulinaemia’ and hepatitis C virus (HCV) infection was established [2]. The cryoglobulinaemia syndrome is a chronic multiorgan disorder characterized by tissue deposition of cryoglobulins in the form of unique complexes consisting of IgM-k with rheumatoid activity linked to anti-HCV IgG antibody. The systemic signs of the syndrome include purpura, arthralgia, weakness and fever together with hepatic and neurological involvement.
Urinary abnormalities are present in 40–60% of patients. The most frequent histological picture is membranoproliferative glomerulonephritis with subendothelial deposits seen with light microscopy, IgG and IgM together with complement detected by immunofluorescopy and distinctive features visualized on electron microscopy [3]. Long-term, the clinical picture of the renal involvement varies during the course of the disease, with periods of temporary reactivation of nephritic syndrome, sometimes with rapidly advancing renal failure and long periods of partial remission [4].

Cryoglobulin entrapment in tissue capillaries triggers inflammatory reactions. As the cryoglobulinaemic vasculitis involves the small vessels of the skin and internal organs, a major impact on tissue capillaries, i.e. the nutritional microvasculature, can be expected in mixed cryoglobulinaemia (MC).

Nail fold videocapillaroscopy (NVC) has been extensively used to examine morphological and functional changes of microcirculation in connective tissue diseases that present with Raynaud’s phenomenon [5], especially systemic sclerosis [6].

In this study we examined a discrete cohort of patients with MC and a variety of multiorgan involvement. Attempts were made to rate capillary abnormalities in relation to selected characteristics of this systemic disorder.

Materials and methods

Patients

We evaluated 29 consecutive patients with MC (10 men, 19 women, mean age 66.05 years, range 40–83) by NVC. They included 28 HCV positive patients—14 cases with genotype 1b, 10 genotype 2a/2c and two genotype 4 cases; genotype had not been determined in the remaining two cases. Of the cohort, 18 patients had type II (IgMk-IgG) MC, the remaining 11 had type III. No underlying disorders were identified, specifically collagenous tissue diseases. Prior to the discovery of HCV, all these cases would have been diagnosed as ‘essential’ MC patients. The patients were symptomatic, presenting with weakness (24 of 29 patients), arthralgia (24), purpura (16), peripheral neuropathy confirmed at the electro-physiological examination (20), Raynaud’s phenomenon (8) and hypertension (19). The nine patients with type II cryoglobulinaemia, who presented with major urinary abnormalities (proteinuria 2–6 g/day, haematuria 10–50 RBC/C14) in three cases associated with moderate renal failure [serum creatinine (1.7–2.5 mg/dl)]—had previously had renal biopsies examined by light microscopy, immunofluorescence staining and, in six out of nine cases, electron microscopy. They invariably had typical features of cryoglobulinaemic membranoproliferative glomerulonephritis [3]. One nephritic and three non-nephritic patients were taking ribavirin and 2-interferon when examined. No patient was taking immunosuppressants at the time of the study.

The ages of the nephritic patients (mean 64.3 ± 9.2 years, range 50–76) and non-nephritic patients (mean 65.2 ± 10.7 years, range 44–83) were similar. The mean disease durations of nephritic patients (7.6 ± 4.0 years, range 1–12) and non-nephritic ones (5.5 ± 4.3 years, range 2–14, P = 0.2) were comparable.

Nail fold videocapillaroscopy

After placing a drop of immersion oil on the nail fold to improve the resolution, the nail fold capillaries of four fingers of both hands were examined, using a videomicroscope (VideoCap DS; Medigroup, Milan, Italy). Each patient was indoors for at least half an hour before nail fold examination at room temperature. Locally traumatized fingers were not examined. Total examination times ranged between 34 and 41 min, including parameter rating and photography. A single inspector performed the NVC examinations, without advance knowledge of patients’ clinical conditions or characteristics. We evaluated deep and superficial venous plexuses, capillary tortuosity, capillary orientation and length, presence of enlarged and giant capillaries, avascular areas, haemorrhages, disorganization of the vascular array (i.e. striking modification of the normal architectural arrangement), and highly tortuous and arborized capillary loop clusters, as characteristic features of angiogenesis [7,8]. Only the capillaries in the distal row of the nail fold were analysed and scored. Haemorrhages were evaluated near the distal row.

A modified version of the cited semi-quantitative rating scales proposed for systemic sclerosis [8,9] was used in scoring microvascular changes in our patients. Although the detection of even a single loop with a homogeneous diameter >50 m was considered a marker of microangiopathy, any other abnormality was regarded as significant when present in at least one finger from each hand. Cumulative patient scores ranged from 0 (no changes) to 7 (presence of seven changes from among tortuous capillaries, altered orientation, shortened capillaries, giant capillaries, avascular areas, arborized capillary loop clusters and haemorrhages, regardless of the severity of a single type of alteration, as long as distributed on fingers).

Cryoglobulin detection

Venous blood was collected in a warm syringe and stored at 37°C until clotted. After centrifugation at 37°C, serum was collected and stored at 4°C for 7 days. After isolation and washing, the components of the cryoprecipitate were quantified using an immunonephelometric assay and characterized by immunofixation (Titan gel IFE kit; Helena Labs, Assago, Italy).

Laboratory tests

Anti-HCV antibodies were assessed using a second-generation enzyme-linked immunosorbent assay (Abbott Laboratories, North Chicago, IL). Reactive sera were confirmed using a second-generation recombinant immunoblot assay (RIBA HCV; Chiron, Emeryville, CA). A nested PCR was performed using primers that expand the highly conserved 5’ non-coding genomic region (Ambicor HCV monitor; Roche Diagnostic System, Branchburg, PA). HCV genotyping was performed by PCR, using primers specific for the HCV core region [11]. Rheumatoid factor, immunoglobulin and complement levels were measured by nephelometry.
Statistical analyses

Univariate analysis used the chi-square or Fisher’s exact tests for comparisons of qualitative values, including possible relations between NVC changes and clinical manifestations. The Student $t$-test and the Mann–Witney $U$-test for unpaired data were applied for quantitative evaluation.

Results

Figure 1 shows the main NVC findings in MC patients. Out of 29 patients, 27 had morphological abnormalities, including tortuosity and apical enlargement; 18 had capillaries with deeply altered orientations; 17 had shortened capillaries and 20 had neoangiogenetic phenomenon. In 10 patients (four with nephritis), these capillary abnormalities were present simultaneously, suggesting that their coexistence might be a characteristic capillaroscopic pattern in MC. Relatively common findings included evidence of deep venous plexuses (in 13 of 29 cases) and haemorrhages (10 cases). Less frequently, giant capillaries could be found (two cases). Table 1 summarizes the general results. It is of interest that patients with MC-associated glomerulonephritis had significantly higher numbers of capillary abnormalities (mean 4.5, range 4–6) than non-nephritic patients (mean 3.5, range 1–6, $P = 0.01$), even though only three of the nine patients with Raynaud’s phenomenon also had renal involvement ($P = \text{NS}$). Microcirculatory changes did not correlate with any of: patient age, other clinical manifestations (including hypertension), or serological indices (including cryocrit), cryoglobulin type, HCV genotype, viral load, haemoglobin, alanine aminotransferase, rheumatoid factor, IgM and C4...
levels. NCV changes were not significantly related to a single renal histological feature, including presence of intraluminal thrombi, endo- and extra-capillary proliferation, monocyte exudation, or interstitial leucocyte infiltration. While most nephritic patients had significant urinary abnormalities, especially proteinuria, NCV changes were not related to the amount of proteinuria. The patients with deep venous plexuses were not significantly older than those without (67.3 ± 8.8 years, range 52–83 vs 62.3 ± 10.8 years, range 40–73, P = 0.2), which suggests this finding is not merely an age-related phenomenon in MC.

### Discussion

The predictive value of nail fold capillaroscopy in the diagnosis and monitoring of some connective tissue diseases has been definitely established [12]. NVC analysis has proven to be particularly useful in evaluating the progression of sclerodermic microangiopathy [9]. Indeed, different patterns were found to be related with different disease durations in this connective tissue disease. Moreover, cerebral hypoperfusion assessed by 133-xenon fluximetry along with single-photon emission computed tomography analysis has been recently reported to be related to major NVC patterns seen in scleroderma [13]. The relationship between changes induced by the same disease in two microcirculation systems, i.e. brain and skin capillaries, suggests an unexpected opportunity for monitoring the severity of disease by a simple and non-invasive method.

MC is a multiorgan disorder characterized by the malfunction of the reticulendothelial system with deposition of immune complexes (mostly consisting of a monoclonal IgM directed to an IgG that usually reacts with HCV) at the level of tissue microcirculation [14]. These cryoprecipitable immune complexes have the unique property of accumulating inside the monocytes of affected organs [3] producing a thesaurismosis-like phenomenon [15]. This accumulation is possibly due to abnormalities of the enzymatic assessment of HCV-infected monocytes, which impair intraphagosome degradation of phagocytosed cryoglobulins.

In this study, patients with MC were found to have a variety of microcirculatory changes. Certain vascular abnormalities may be detected in several connective tissue diseases. For instance, the enlargement of the entire capillary loop and loss of blood vessels are observed in patients with dermatomyositis and, even more often, with scleroderma [12]. However, while individually non-specific, the abnormalities detected in MC could be clustered in a pattern of tortuous and short capillaries with abnormal orientations and with neoangiogenetic shapes, which is consistent with a high degree of deterioration of nutritional circulation.

The microcirculatory changes occurring in MC might be related to ischaemic events due either to rheological disturbances (as a consequence of hyper-viscosity) or to subclinical perivascular leucocyte infiltration of small- or medium-sized tributary vessels [16]. The angiogenetic phenomena could represent an attempt to compensate for the defective nutritional circulation. This study failed to show significant correlations between NVC abnormalities and patients’ age or disease duration, as has been found for other clinical and biochemical parameters in HCV-infected patients [17]. Somewhat analogous to encephalopathic manifestations in scleroderma, NVC abnormalities in MC have been found to be particularly numerous in the presence of renal involvement, a known factor of worsening prognosis [4]. Additional and differently designed studies are needed to verify if the extent of microangiopathy may be regarded as a predictor of the development of cryoglobulinaemic nephritis. Thus, the apparent association between renal involvement and a greater score of NVC abnormalities must be regarded cautiously. Moreover, attempts to correlate observed NVC changes with histological features were limited by the relatively low number of affected patients. The specificity of the relation to the membranoproliferative pattern of glomerulonephritis could not be verified by examining patients with other types of glomerular involvement, such as membranous or mesangial glomerulonephritis, which can occasionally be detected in these patients [18]. Finally, more obvious parameters of renal involvement, like proteinuria, clearly reflect microcirculatory abnormalities.

Despite these limitations, which will call for a more extensive analysis, definite abnormalities have been detected by NVC in patients with MC, with some prevalence in those presenting with glomerulonephritis.

#### Acknowledgements

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#### Conflict of interest statement

None to declare.

#### References


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**Table 1. NVC features in patients with MC**

<table>
<thead>
<tr>
<th>Morphological abnormalities</th>
<th>Patients (n = 29)</th>
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<tr>
<td>Morphological abnormalities include tortuosity and apical enlargements. Deep venous plexuses are not uncommon in aged healthy subjects, but have been found to be unexpectedly frequent in MC patients (see in text).</td>
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<tr>
<td>Altered orientation</td>
<td>18 (62%)</td>
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<td>Shortened capillaries</td>
<td>17 (59%)</td>
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<tr>
<td>Neoangiogenesis</td>
<td>20 (69%)</td>
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<tr>
<td>Evidence of deep venous plexus</td>
<td>13 (45%)</td>
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<tr>
<td>Haemorrhages</td>
<td>10 (34%)</td>
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<tr>
<td>Giant capillaries</td>
<td>2 (7%)</td>
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