# PATIENTS WITH MIXED CRYOGLOBULINEMIA AND HCV INFECTION, IN PRESENCE OR ABSENCE OF AUTOIMMUNE THYROIDITIS, HAVE HIGH SERUM LEVELS OF (CXC MOTIF) LIGAND (CXCL)9 AND CXCL11 CHEMOKINES

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**No data are present in the literature regarding chemokine (CXC motif) ligand (CXCL)9 and CXCL11 circulating levels in cryoglobulinemia associated with hepatitis C (MC+HCV), in presence/absence of autoimmune thyroiditis (AT). Serum CXCL9 and CXCL11 have been measured in 38 MC+HCV patients without AT (MCo), 38 MC+HCV patients with AT (MC+AT), and in matched controls without (control 1) or with thyroiditis (control 2). Serum CXCL9 and CXCL11 were significantly higher: in control 2 than control 1 ($p<0.05$); in MCo than control 1 and control 2 ($p<0.001$, for both); in MC+AT than control 1 and control 2 ($p<0.0001$, for both), and than MCo ($p=0.01$, for both). Our study demonstrates markedly high serum levels of CXCL9 and CXCL11 in patients with MC+HCV compared to healthy controls; in MC+HCV patients increased CXCL9 and CXCL11 levels were significantly associated with the presence of AT. Moreover, a strong relation between circulating CXCL9 and CXCL11 in MC+HCV has been shown.**

Monokine induced by interferon (IFN)-γ, also known as chemokine (CXC motif) ligand (CXCL)9, IFN-inducible T cell α chemoattractant (CXCL11), and IFN-γ-induced protein 10/CXCL10, are members of a CXC chemokine subfamily that acts via the CXC chemokine receptor 3 (1, 2), expressed on activated T-helper (Th)1. CXCL9, CXCL10 and CXCL11 are "IFN-γ inducible chemokines", and they recruit activated Th1 lymphocytes to sites of inflammation.

Recent evidence has shown that CXC α-chemokine CXCL10 plays an important role in the active phases of mixed cryoglobulinemia (MC). In fact, circulating CXCL10 is high in particular in cryoglobulinemic patients with active vasculitis, suggesting a prevalence of the Th1 immune response

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in this phase (3, 4).

Furthermore, it has been shown CXCL10 is higher in MC with autoimmune thyroiditis (AT), with respect to those without (5, 6), accordingly with other studies in patients with autoimmune disorders without HCV infection (7, 8).

No study has evaluated CXCL11, together with CXCL9, circulating levels in patients with cryoglobulinemia associated with hepatitis C (MC+HCV), with or without AT. Furthermore, a few studies evaluated CXCL9 and CXCL11 in thyroid autoimmunity. In fact, it has been recently shown that CXCL9 and CXCL11 circulating levels are higher in patients with AT (9), and that the secretion of CXCL9 and CXCL11 in primary cultures of human thyrocytes can be stimulated by IFN-γ and tumor necrosis factor-α (10, 11). These data suggest that CXCL9 and CXCL11 chemokines are involved in the Th1 immune response that is pathogenetically associated with the initial phase of AT.

The aim of the present study therefore was to measure serum CXCL9 and CXCL11 levels in patients with MC+HCV, with or without AT, and to relate the findings to the clinical phenotype.

## MATERIALS AND METHODS

*Patients with MC+HCV*

Thirty-eight MC+HCV patients without autoimmune thyroiditis (MCo), and 38 patients with MC+HCV and autoimmune thyroiditis (MC+AT), consecutively referred to the Rheumatology Unit of the University of Modena and Reggio Emilia were recruited into the study (enrolment start date, January 1995). The diagnosis of MC was based on the presence of serum mixed (IgG-IgM) cryoglobulins and the classical clinical triad - purpura, weakness, arthralgias - and on the exclusion of other well-known systemic disorders, such as immuno-rheumatic, neoplastic, and infectious diseases (12, 13). HCV infection was systematically evaluated in all patients, and HCV negative were excluded. Only patients with MC+HCV, without liver cirrhosis (by histology, laboratory evidence of liver failure and/or ultrasound-proven portal hypertension) (14) and without hepatocellular carcinoma, in whom a thyroid screening [history, physical examination, thyroid-stimulating hormone (TSH), free triiodo-thyronine ($FT_3$), free-thyroxine ($FT_4$), anti-thyroglobulin (AbTg) and anti-thyroid peroxidase (AbTPO) antibodies measurements and ultrasonography] excluded (MCo), or revealed (MC+AT) the presence of associated thyroid autoimmune disorders, were included in these groups.

Fifty-one out of 76 (67%) MC+HCV patients underwent liver biopsy for diagnostic purposes; the mean activity index (grade) was 5.3±1.1, and the stage was 2.2±0.6; mean alanine aminotransferase (ALT) serum levels were 53±44 (reference values, 5-36 IU/L); high levels of Rheumatoid Factor were present in 98% of MC+HCV patients.

The main demographic and clinico-serological features of MCo patients are reported in Table I. Among them, 20 had been previously treated with IFN-α for an average of 7.2 months (range 1-14), at a mean dosage of 9 MU/week (range 3-9); the time elapsed from the last course of IFN-α treatment ranged from 5 to 92 months (mean 44). No statistically significant difference was observed in the main demographic and clinico-serological features of MC patients treated (MC/IFN+) or untreated (MC/IFN-) with IFN-α.

At the time of the study, 21 MCo patients were taking low doses of corticosteroids, 8 had previously been on corticosteroids and 9 had never been treated with corticosteroids. No MC patient had had plasma exchange treatment in the last year before the study. The presence of Raynaud's phenomenon, Sjøgren's syndrome, skin ulcers, peripheral neuropathy, and renal and liver involvement in MCo patients was evaluated as previously described (12). Routine blood chemistry was carried out by standard methods.

The main demographic and clinico-serological features of MC+AT patients are reported in Table I. Among them, 20 had been previously treated with IFN-α for an average of 5.9 months (range 1-12), at a mean dosage of 12 MU/week (range 3-10); the time elapsed from the last course of IFN-α treatment ranged from 3 to 65 months (mean 37). No statistically significant difference was observed in the main demographic and clinico-serological features of MC patients treated (MC/IFN+) or untreated (MC/IFN-) with IFN-α.

At the time of the study, 21 MC+AT patients were taking low doses of corticosteroids, 6 had previously been on corticosteroids and 11 had never been treated with corticosteroids. No MC patient had had plasma exchange treatment in the last year before the study. The presence of Raynaud's phenomenon, Sjøgren's syndrome, skin ulcers, peripheral neuropathy, and renal and liver involvement in MC+AT patients was evaluated as previously described (12). Routine blood chemistry was carried out by standard methods.

*Controls*

Two control groups were included, extracted from a random sample of the general population from the same geographic area (15).

The first control group (control 1) consisted of 38 subjects, matched by gender and age [that is a well known confounding factor (16)] with MCo patients, without HCV infection or other liver disorders, in whom a complete thyroid work-up (history, physical examination, TSH, $FT_3$, $FT_4$, AbTg, AbTPO, and ultrasonography) was available, and excluded the presence of thyroid or autoimmune disorders, or any kind of immunomodulant therapy.

The second control group (control 2) consisted of 38 subjects, matched by gender and age [that is a well known confounding factor (16)] with MC+AT patients, without HCV infection or other liver disorders, in whom a complete thyroid work-up (history, physical examination, TSH, $FT_3$, $FT_4$, AbTg, AbTPO, and ultrasonography) was available, and demonstrated the presence of thyroid autoimmune disorders, but excluded the presence of other autoimmune disorders and any kind of immunomodulant therapy.

In all patients and controls, a blood sample was collected in the morning, after overnight fasting, and serum was kept frozen until CXCL9 and CXCL11 measurement.

All study subjects gave their informed consent to the study, which was approved by the local Ethics Committee.

*Immunological studies*

Cryocrit was measured as the percentage of packed cryoglobulins after cold centrifugation of the serum; cryoglobulin composition was determined by including the presence in cryoprecipitates of monoclonal or polyclonal IgM-rheumatoid factor, (i.e. MC type II or MC type III); haemolytic complement activity (CH50) and C3-C4 fractions were measured as previously described (12); anti-nuclear (ANA), anti-smooth muscle (ASMA), and anti-mitochondrial (AMA) autoantibodies were detected by current techniques (12). Sera with a titre > 1:40 were considered positive. Anti-extractable nuclear antigen (ENA) antibodies, including anti-Scl70, anti-Sm, -RNP, -SSA/SSB, -PCNA, -SL and -Jo1 specificities, were detected by counter-immunoelectrophoresis as previously reported (14).

*Virological studies*

Anti-HCV antibodies and HCV RNA were determined on serum clotted and centrifuged at 37°C and stored at -70°C. Antibodies against HCV (anti-HCV) were detected by an enzyme-linked immunoassay (Chiron ELISA HCV, Second Generation; Emeryville CA, USA). A recombinant-based immunoblot assay (Chiron RIBA HCV, Second Generation Assay; Emeryville CA, USA) was used to investigate the specificity of anti-HCV seropositivity. The presence of HCV RNA in the serum was investigated by a polymerase chain reaction (PCR) technique as previously described (17). Amplification of HCV cDNA was performed using a 'nested' PCR, with primers located in the 5' non-coding region (17). The analysis of amplification products was performed by both ethidium bromide staining and hybridisation with a radiolabelled oligonucleotide probe internal to the amplified sequence.

*Ultrasonography of the neck and fine-needle aspiration (FNA)*

Neck ultrasonography was performed by the same operator, who was unaware of the results of thyroid hormones, autoantibodies and CXCL10 measurements, using a probe (Esaote, Florence, Italy; AU5 with a sectorial 7.5 MHz transducer). Thyroid volume was calculated using the ellipsoid formula, as described (7). The presence of hypoechoic and dyshomogeneous echogenicity was arbitrarily rated at three levels (0=normal echogenicity; 1=slight hypoechoic and dyshomogeneous; 2=severely hypoechoic and dyshomogeneous) in order to evaluate structural abnormalities of thyroid tissue associated with thyroid autoimmunity (7). The presence of thyroid nodules was recorded, and nodules with a diameter >10 mm were submitted to ultrasonography-guided FNA, which was performed by the same operator, using a free-hand method as already described (7).

*Thyroid blood flow (TBF)*

TBF by color-flow doppler (CFD) was studied in all patients (7). The CFD pattern was defined as: normal (or type 0), TBF limited to peripheral thyroid arteries; type I, TBF mildly increased; type II, TBF clearly increased; or type III, TBF markedly increased (7). In AT patients TBF bore no relation to the thyroid status, and was type 0 in 58%, type I in 34%, type II in 8% of patients, while none had type III CFD pattern.

*Laboratory evaluation*

Thyroid function and thyroid autoantibodies were measured as previously described (7). Circulating $FT_3$ and $FT_4$ were measured by commercial radioimmunoassay kits (AMERLEX-MAB $FT_3$/$FT_4$ Kit; Amersham Biosciences, Little Chalfont, UK). Serum TSH (DiaSorin, Saluggia, Italy), AbTPO and AbTg (ICN Pharmaceuticals, Costa Mesa, CA, USA) were evaluated by immunoradiometric assay methods. For AbTg, AbTPO, positivity was set at >50, and >10 IU/mL, respectively.

*Serum CXCL9 and CXCL11 enzyme-linked immunosorbent assay*

Serum CXCL9 and CXCL11 levels were assayed by a quantitative sandwich immunoassay (R&D Systems, Minneapolis, MN, USA). Sensitivity ranged from 1.3-12.7 pg/mL with a mean minimum detectable dose of 3.4

pg/mL for CXCL9; the intra- and inter-assay coefficients of variation were 4.1% and 6.4%. The CXCL11 sensitivity ranged from 0.4-3.7 pg/mL with a mean minimum detectable dose of 11.8 pg/mL; the intra- and inter-assay coefficients of variation were 4.2% and 6.7%.

*Data analysis*

Values are given as mean±standard deviation (SD) for normally distributed variables, otherwise as median and [interquartile range]. Mean group values were compared by using one-way analysis of variance (ANOVA) for normally distributed variables (age and body mass index), otherwise by the Mann-Whitney *U* or Kruskal-Wallis test. Proportions were compared by the $\chi^2$ test. *Post-hoc* comparisons on normally distributed variables were carried out using the Bonferroni-Dunn test. Univariate and multivariate analysis were performed by simple or multiple linear regression analysis. Statistical power (ex post analysis) (stat-power) was calculated.

## RESULTS

MCo and MC+AT patients were not significantly different in relation to the clinical phenotype of cryoglobulinemia (Table I). The demographic and clinical thyroid features of patients and controls are reported in Table II. As expected, MC+AT patients, and controls with AT (control 2) showed significantly higher thyroid autoantibodies levels, as well as hypoechogenicity and hypervascularity of the thyroid gland, and subclinical hypothyroidism in comparison to control 1 and MCo.

Serum CXCL9 levels were significantly (Table II) higher in control 2 thyroiditis, than in control 1 ($p<0.01$). MCo have serum CXCL9 levels significantly ($p<0.0001$) (stat-power=1) higher than control 1 without thyroiditis (Fig. 1) and controls 2 with thyroiditis. MC+AT have serum CXCL9 levels significantly higher ($p<0.0001$) (stat-power=1) than control 1 and 2 (Fig. 1), or MCo ($p=0.01$) (stat-power=0.8). Serum CXCL9 levels were not associated with any of the clinical features of cryoglobulinemia in patients MCo and MC+AT (data not shown).

Serum CXCL11 levels were significantly (Table II) higher in control 2 thyroiditis, than in control 1 ($p=0.02$). MCo have serum CXCL11 levels significantly higher than control 1 and control 2 ($p<0.001$, for both) (stat-power=1) (Fig. 2). MC+AT have serum CXCL11 levels significantly higher than control 1 and 2 ($p<0.0001$, for both) (stat-power=1) (Fig. 2), and than MCo ($p=0.01$) (stat-power=0.8). Serum CXCL11 levels were not associated with any of the clinical fetaures of cryoglobulinemia in patients MCo and MC+AT (data not shown).

In order to better define the role of increased serum chemokines in AT, CXCL9 and CXCL11 were studied in relation to clinical features of AT (age; gender; thyroid volume <6 mL; thyroid hypoechoic pattern, or hypervascularity; AbTg or AbTPO positivity; subclinical hypothyroidism) in MC+AT patients and control 2, without any significant result.

By defining a high CXCL9 level as a value of at least 2 SD above the mean value of the control group (>125 pg/mL), 4% of control 1, 29% of control 2, 87% of MCo and 98% of MC+AT, had high CXCL9 ($p<0.0001$; $\chi^2$ test).

By defining a high CXCL11 level as a value of at least 2 SD above the mean value of the control group (>110 pg/mL), 4% of control 1, 28% of control 2, 88% of MCo and 98% of MC+AT, had high CXCL11 ($p<0.0001$; $\chi^2$ test).

No significant association was observed in relation to the presence or absence of active or previous treatments, or in relation to the duration of MC, and CXCL9 or CXCL11 circulating levels.

In a simple regression analysis CXCL9 and CXCL11 serum levels were significantly related to each other (r=0.547, $p<0.0001$) in MC+HCV patients.

## DISCUSSION

Our study first demonstrates high serum levels of CXCL9 and CXCL11 chemokine in patients with MC, in particular in the presence of AT. Moreover, to the best of our knowledge, we have first shown strong association between CXCL9 and CXCL11 circulating levels in these patients, strongly supporting the role of a Th1 immune response in the pathogenesis of MC+HCV.

Our results agree with those of other studies in patients with HCV infection without cryoglobulinemia.

Increased circulating levels of CXCL9 (18-22) and CXCL11 (18, 20, 23), were observed in some studies, while other studies were no able to show any significant difference with controls (24). Bieche et

**Table I.** *Clinical characteristics of 38 patients with HCV-related MC without autoimmune thyroiditis (MCo) and 38 with autoimmune thyroiditis (MC+AT).*

| | MCo n=38 | MC+AT n=38 |
|---|---|---|
| Age (years) | 61±13 | 64±11 |
| Men/Women | 9/29 | 7/31 |
| Disease duration with MC (years) | 11±11 | 12±10 |
| Purpura | 91% | 95% |
| Weakness | 94% | 88% |
| Arthralgias | 89% | 87% |
| Arthritis | 14% | 12% |
| Raynaud's phenomenon | 46% | 49% |
| Sjøgren's syndrome | 43% | 44% |
| Peripheral neuropathy | 62% | 55% |
| Renal involvement* | 15% | 12% |
| Cryocrit (%) | 4.1±10.3 | 4.7±9.1 |
| CH50 (normal: 160-220 units) | 116±38 | 113±37 |
| C3 (normal: 60-130 mg/dL) | 86±38 | 81±36 |
| C4 (normal:20-55 mg/dL) | 15±16 | 13±15 |
| Autoantibodies † | 25% | 33% |

** Serum creatinine >1.5 mg/dL and/or proteinuria >0.5 gr/24h. † Presence of ANA and/or AMA and /or ASMA and/or anti-ENA No significant difference was observed regarding the the above-mentioned characteristics in the 2 groups.*

al. studied the expression of 240 genes in patients with HCV chronic infection (25). They showed mRNA level up-regulation of IFN-γ-inducible genes (CXCL9, CXCL10, CXCL11). These results suggest that CXCL9 and CXCL11, potent chemo attractants for activated T cells, are produced by hepatocytes in the HCV-infected liver and play an important role in the pathogenesis of MC.

In our MC+HCV patients CXCL9 and CXCL11 serum levels were significantly higher than in controls. The possible contribution of chronic hepatitis C to high CXCL9 and CXCL11 serum levels in MC+HCV patients cannot be excluded. Furthermore, the increase of CXCL9 and CXCL11 is in agreement with recent evidences, that have shown that CXCL10 plays an important role in

**Table II.** *Thyroid status and circulating levels of CXCL9 and CXCL11, in control subjects (control 1), control with autoimmune thyroiditis (control 2), patients with HCV-related MC without (MCo) or with autoimmune thyroiditis (MC+AT).*

| | controls 1 | controls 2 thyroiditis | MCo | MC+AT | *p* |
|---|---|---|---|---|---|
| n | 38 | 38 | 38 | 38 | |
| Age (years) | 63±11 | 62±13 | 61±13 | 64±11 | ns |
| Gender (M/F) | 8/30 | 7/31 | 9/29 | 7/31 | ns |
| Thyroid volume (mL) | 9±10 | 13±12 | 10±11 | 11±14 | ns |
| Hypoechoic (%) | 0 | 71° | 0 | 83° | 0.0001 |
| Hypervascular (%) | 0 | 43° | 0 | 48° | 0.0001 |
| Serum TSH (μU/mL) | 1.2±0.8 | 1.6±1.7 | 1.1±1.1 | 2.8±3.4* | 0.001 |
| AbTPO (IU/mL) | 10±10 | 267±361° | 11±9 | 348±325° | 0.0001 |
| AbTg (IU/mL) | 11± 12 | 326±224° | 9±11 | 252±273° | 0.0007 |
| Anti-thyrotropin-receptor antibody (TRAb) (IU/mL) | 0 | 0 | 0 | 0 | ns |
| AbTPO positivity (%) | 0 | 81 | 0 | 88 | 0.0001 |
| AbTg positivity (%) | 0 | 78 | 0 | 81 | 0.0001 |
| Subclinical hypothyroidim (%) | 0 | 5° | 0 | 39° | 0.002 |
| CXCL9 (pg/mL) | 69±28 | 151±91§ | 403±149^ | 507±232* | <0.0001 |
| CXCL11 (pg/mL) | 60±25 | 139±84§ | 256±197^ | 348±279* | <0.0001 |

*Anti-thyroperoxidase antibody: AbTPO; Anti-thyroglobulin antibody: AbTg; Thyroid-stimulating hormone: TSH; Anti-thyrotropin-receptor antibody:TRAb * p <0.05 or less vs controls 1 or vs autoimmune thyroiditis control 2, or vs MCo ° p<0.05 or less vs controls 1 and vs MCo § p<0.05 or less vs controls 1 ^ p <0.05 or less vs controls 1 or vs autoimmune thyroiditis control 2*

cryoglobulinemia. In fact, circulating CXCL10 is high in particular in cryoglobulinaemic patients with active vasculitis, suggesting a prevalence of the Th1 immune response in this phase (3, 4).

In our study we did not find any relation among serum CXCL9 or CXCL11 levels and the duration of MC+HCV, probably because the disease is characterized by a relapsing clinical course whose

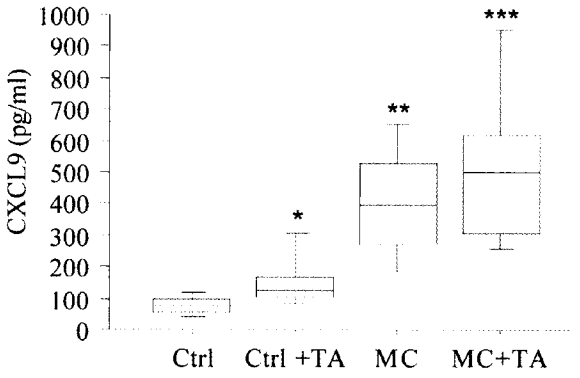


**Fig. 1.** *Serum CXCL9 levels in patients with MC+HCV with or without AT and controls. Serum CXCL9 levels were significantly (*) higher in control 2 with thyroiditis (Ctrl+AT), than in control 1 (Ctrl) ($p<0.01$). MC+HCV patients (MC) have serum CXCL9 levels significantly (**) ($p<0.0001$) (stat-power=1) higher than control 1 without thyroiditis (Ctrl) and controls 2 with thyroiditis (Ctrl+TA). MC+HCV patients with AT (MC+TA) have serum CXCL9 levels significantly (***) higher ($p<0.0001$) (stat-power=1) than control 1 (Ctrl) and 2 (Ctrl+TA), or MC ($p=0.01$) (ANOVA) (stat-power=0.8). The box indicates the lower and upper quartiles and the central line is the median value; the horizontal lines at the end of the vertical lines are the 2.5% and 97.5% values.*

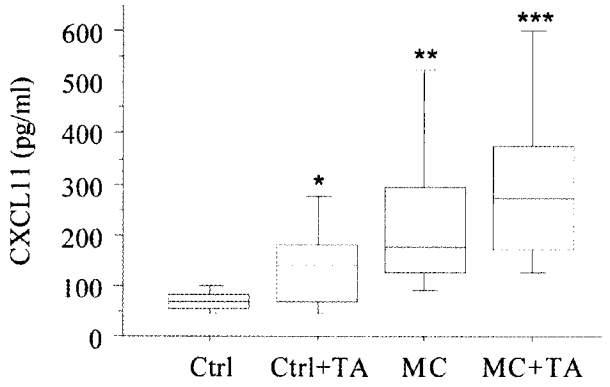


**Fig.2.** *Serum CXCL11 levels in patients with MC+HCV with or without AT and controls. Serum CXCL11 levels were significantly (*) higher in control 2 with thyroiditis (Ctrl+TA), than in control 1 (Ctrl) ($p=0.02$). MC+HCV patients (MC) have serum CXCL11 levels significantly (**) higher than control 1 (Ctrl) and control 2 (Ctrl+TA) ($p<0.001$, for both) (stat-power=1). MC+HCV patients with AT (MC+TA) have serum CXCL11 levels significantly (***) higher than control 1 (Ctrl) and 2 (Ctrl+TA) ($p<0.0001$, for both) (stat-power=1), and than MC ($p=0.01$) (ANOVA) (stat-power=0.8). The box indicates the lower and upper quartiles and the central line is the median value; the horizontal lines at the end of the vertical lines are the 2.5% and 97.5% values.*

most common expression are vasculitic symptoms (26). Clinico-pathological alterations of MC+HCV may recognize at least two synergical pathogenetic mechanisms triggered by HCV. Firstly, B-cell proliferation leads to immune-complexes production (cryoglobulins) (26), that are responsible for immune-complex-mediated vasculitis (13, 26), while high serum CXCL9 and CXCL11 levels secondary to both HCV-related vascular and hepatic cell injury may amplify the inflammatory process through Th1-mediated immune response.

Change of serum chemokine levels in the course of other autoimmune disorders has been demonstrated. Recent experimental evidence has demonstrated that CXC chemokines and particularly CXCL10 play an important physiopathological role in the initial phases of autoimmune thyroid disorders (7, 27). Furthermore, we have recently shown that increased serum CXCL10 levels in patients with Graves' disease (GD) are associated mainly with the active phase of GD, not related to hyperthyroidism itself, but mainly to autoimmune response (8).

Interestingly, this study first shows serum CXCL9 and CXCL11 were higher in MC+AT patients with respect to MCo, such as in AT patients with respect to controls without AT, in agreement with the above-mentioned studies. Furthermore, it has been recently shown that CXCL9 and CXCL11 circulating levels in AT patients were associated with high serum TSH concentrations, suggesting that raised CXCL9 and CXCL11 chemokines are not only associated with the autoimmune process itself, but may be a marker of a thyroiditic process eventually leading to the destruction of thyroid follicular cells (9, 28).

We were not able to find any relationship among CXCL9 or CXCL11 levels and hypothyroidism in the present study, probably because of the limited number of patients studied. Longitudinal studies evaluating serum CXCL9 and CXCL11 levels in large MC+HCV patient series will be necessary to evaluate whether serum CXCL9 or CXCL11 measurement could represent an easily detectable prognostic marker for clinical management of MC+HCV patients, with or without thyroiditis.

Recently, it was shown that a low baseline CXCL10 level was significantly associated with low baseline viral load, rapid viral response, and sustained viral response in HCV+ patients treated with IFN-α

(29-31).

In another study, levels of CXCL10 and CXCL9 decreased following successful antiviral therapy in HCV+ patients, while CXCL11 did not decline (18).

Since IFN-α is a well known effective therapy for MC+HCV (26), pretreatment evaluation of serum of CXCL9 or CXCL11 levels might suggest the virological response to IFN also in MC+HCV patients.

Interestingly, to the best of our knowledge, this is the first demonstration of a strong relation between circulating CXCL9 and CXCL11, in a human pathology, such as MC+HCV in presence or absence of AT. This finding strongly supports the role of a Th1 immune response in the pathogenesis of MC+HCV.

In conclusion, our study demonstrates markedly high serum levels of CXCL9 and CXCL11 in patients with MC+HCV compared to healthy controls; in MC+HCV patients increased CXCL9 and CXCL11 levels were significantly associated with the presence of AT. Moreover, a strong relation between circulating CXCL9 and CXCL11, in MC+HCV has been shown. Future studies in larger cohort of patient will be needed to evaluate the relevance of serum CXCL9 and CXCL11 in the therapeutic approach to these patients.

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