Q J Med 2004; **97**:499–506
doi:10.1093/qjmed/hch088

Thyroid involvement in patients with overt HCV-related mixed cryoglobulinaemia

A. ANTONELLI[1], C. FERRI[2], P. FALLAHI[1], D. GIUGGIOLI[1], C. NESTI[1], G. LONGOMBARDO[1], P. FADDA[1], A. PAMPANA[3], M. MACCHERONI[4] and E. FERRANNINI[1]

From the [1]Department of Internal Medicine, University of Pisa; [2]Rheumatology Unit, University of Modena, and [3]Medical Division and [4]Endocrinological Laboratory, Azienda Ospedaliera Pisana, Pisa, Italy

Received 31 October 2002 and in revised form 21 January 2004

Summary

Background: Mixed cryoglobulinaemia (MC), a systemic vasculitis associated with hepatitis C virus (HCV) infection in >90% of cases, is frequently complicated by multiple organ involvement. The prevalence of thyroid disorders in MC has not yet been studied.
Aim: To investigate the prevalence and clinical features of thyroid involvement in patients with HCV-associated MC (HCV + MC).
Design: Case-control study.
Methods: HCV + MC patients ($n = 93$, 17 men and 76 women, mean ± SD age 63 ± 10 years, mean disease duration 14 ± 7 years) consecutively referred to the Rheumatology Unit were matched by sex and age (± 2 years) to (i) 93 patients with chronic C hepatitis (CH) without MC and (ii) 93 healthy (HCV-negative) controls from the local population. Measurements included prevalence of hypo- or hyperthyroidism, thyroid autoantibodies, thyroid nodules and thyroid cancer.
Results: By McNemar's χ^2 test, the following thyroid abnormalities were significantly more frequent in HCV + MC patients than in HCV-negative controls: serum anti-thyroperoxidase autoantibody (AbTPO) (28% vs. 9%, $p = 0.001$); serum AbTPO and/or anti-thyroglobulin autoantibody (31% vs. 12%, $p = 0.004$); subclinical hypothyroidism (11% vs. 2%, $p = 0.038$); thyroid autoimmunity (35% vs. 16%, $p = 0.006$). Serum AbTPO were also significantly more frequent in HCV + MC patients than in CH controls (28% vs. 14%, $p = 0.035$).
Discussion: The prevalence of thyroid disorders is increased in patients with HCV-related mixed cryoglobulinaemia. We suggest careful monitoring of thyroid function in these patients.

Introduction

Mixed cryoglobulinaemia (MC) is an immune-complex-mediated systemic vasculitis characterized by the classical clinical triad of purpura, arthralgias and weakness, along with several immune-mediated manifestations.[8,21,33] During the last decade, a large body of epidemiological, clinico-pathological and laboratory evidence has demonstrated the presence of hepatitis C virus (HCV) infection in >90% of MC patients.[1,2,15,16] In addition, chronic HCV infection may be responsible for a constellation of hepatic and extra-hepatic manifestations: hepatocellular carcinoma, MC, thyroid disorders, and B-cell lymphomas.[19,23] Among these autoimmune and neoplastic diseases, MC represents the most frequent HCV-related extra-hepatic complication.[19,23] The HCV

Address correspondence to Dr A. Antonelli, Department of Internal Medicine, University of Pisa, via Roma, 67, 56100, Pisa, Italy. e-mail: a.antonelli@med.unipi.it

lymphotropism[17,51] may trigger the B-lymphocyte expansion frequently observed in MC patients, with the resulting production of different autoantibodies, including rheumatoid factor and cryoprecipitable immune-complexes, leading to cryoglobulinaemic vasculitis with multiple organ damage.[1,19,21] Aminotransferase elevations and/or liver histological activity, renal involvement, peripheral neuropathy, and generalized vasculitis are the most frequent complications of MC.[1,8,19,21]

Thyroid involvement has been reported in HCV-infected subjects with variable frequency,[14,20,25–27,41,44] and we recently reported a high prevalence of papillary thyroid cancer in patients with hepatitis C.[4] We therefore investigated the prevalence and the characteristics of thyroid disorders in a large series of HCV-related MC patients with clinically evident MC syndrome, compared with age- and sex-matched controls from the general population, and with patients with chronic hepatitis type C but no MC.

Methods

Patients

MC patients ($n = 111$) consecutively referred to the Rheumatology Unit of the University of Pisa were recruited into the study between 1999 and 2001. The diagnosis of MC was based on the presence of serum mixed (IgG-IgM) cryoglobulins and the classical clinical triad (purpura, weakness, arthralgias), and the exclusion of other well-known systemic disorders, such as immuno-rheumatic, neoplastic, and infectious diseases.[1,2,8,15,16,21] HCV infection was systematically evaluated in all patients, and nine were excluded because they were HCV-negative. Another nine patients were excluded from the study: four due to a previous treatment with external radiotherapy in the region of the neck or mediastinum; two because they were undergoing IFNα treatment at the time of the study; and three because it was not possible to obtain blood samples for thyroid hormone measurements. Each of the remaining 93 MC patients eligible for the study was matched, by sex and age, with a control from an HCV-negative group from the same geographic area (north-west Tuscany). This control group was extracted from a larger sample of 1640 subjects in a population-based survey of thyroid disorders. Another control group consisted of 93 sex- and age-matched patients with chronic type C hepatitis (CH) without CM, extracted from a larger cohort of 491 CH referrals. Extraction of either control group from the original populations was performed by finding the closest age match (± 2 years) to each case within either gender. When more than one age match was available per case, the choice was made at random. Thyroid status was investigated in all groups. The main demographic and clinico-serological features of the MC patients are reported in Table 1. Thirty-three had been previously treated with IFNα for a mean of 6.7 months (range 1–13), at a mean dosage of 10.2 MU/week (range 3–6); the time elapsed from the last course of IFNα treatment ranged from 6 to 84 months (mean 43). 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 IFNα.

At the time of study, 69 MC patients were taking low doses of corticosteroids, 13 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. In both patients and controls, a careful medical history was collected, with particular regard to family history of thyroid disease, residence in iodine-deficient areas, smoking habits, and drugs. Thyroid status was investigated by physical examination, thyroid ultrasonography, serum TSH, FT_3, and FT_4 measurements, and anti-thyroglobulin (AbTg), anti-thyroid peroxidase (AbTPO) and anti-TSH

Table 1 Clinical characteristics of 93 patients with HCV-related MC

Age (years)	63 ± 10
Men/women	17/76
Disease duration with MC (years)	14 ± 7
Purpura	88%
Weakness	98%
Arthralgias	93%
Arthritis	14%
Raynaud's phenomenon	46%
Sjögren's syndrome	52%
Peripheral neuropathy	78%
Renal involvement*	19%
Aminotransferases elevation and/or histological activity†	74%
Non-Hodgkin's lymphoma	4
Hepatocellular carcinoma	2
Cryocrit (%)	4.7 ± 9.1
CH50 (normal: 160-220 units)	114 ± 36
C3 (normal: 60-130 mg/dl)	82 ± 33
C4 (normal 20-55 mg/dl)	11 ± 9
Autoantibodies‡	25%

* Serum creatinine >1.5 mg/dl and/or proteinuria >0.5 g/24 h. † Increase of the liver enzyme (ALT) and/or histological alterations. ‡ Presence of ANA and/or AMA and/or ASMA and/or anti-ENA.

receptor autoantibodies (TRAb) determination. The presence of Raynaud's phenomenon, Sjögren's syndrome, skin ulcers, peripheral neuropathy, and renal and liver involvement in MC patients was evaluated as previously described.[15] Routine blood chemistry was carried out by standard methods.

The study protocol 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.[15] Antinuclear (ANA), anti-smooth muscle (ASMA), and anti-mitochondrial (AMA) autoantibodies were detected by current techniques.[15] 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 according to Bunn *et al.*[9]

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). A recombinant-based immunoblot assay (Chiron RIBA HCV, Second Generation) 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.[17,51] Amplification of HCV cDNA was performed using a 'nested' PCR, with primers located in the 5′ non-coding region.[51] The analysis of amplification products was performed by both ethidium bromide staining and hybridization with a radiolabelled oligonucleotide probe internal to the amplified sequence.

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

Neck ultrasonography and FNA were performed by the same operator, who was unaware of the results of thyroid hormone and autoantibody measurement, using a probe (Toshiba Tosbee) with a sectorial 7.5 MHz transducer, interposing a water bag.[5,35] The presence of hypoechoic echogenicity was arbitrarily rated at three levels (0 = normal echogenicity; 1 = slight hypoechoic; 2 = severely hypoechoic) in order to evaluate structural abnormalities of thyroid tissue associated with thyroid autoimmunity.[13,24,46] The presence of thyroid nodules was recorded, and palpable nodules or nodules with clinical or echographic pattern that suggested the opportunity of excluding malignancy were submitted to FNA.[6,40]

Thyroid laboratory evaluation

Thyroid function and thyroid autoantibodies were measured as previously described.[3,36] Circulating FT_3 (normal range 1.45–3.50 pg/ml) and FT_4 (normal range 0.70–1.90 ng/dl) were measured by commercial RIA kits (AMERLEX-MAB FT_3/FT_4 Kit). Serum TSH (normal range 0.3–4.0 mU/l) was determined by a ultrasensitive third-generation method (DPC). AbTPO (normal range 0–150 UI/ml) and AbTg (normal range 0–150 UI/ml) (ICN Pharmaceuticals) were evaluated by IRMA methods. TRAb (anti-thyroid stimulating hormone (TSH)-receptor autoantibodies) were measured in patients with hypo- or hyperthyroidism with the use of a radioreceptor assay (Radim) (normal range 0–16 UI/ml). When an abnormal result was obtained, a second measurement was done to confirm the data.

Statistical analysis

Values are given as means ± SD. Mean group values were compared by using Student's *t* test or one-way analysis of variance (ANOVA) for normally distributed variables. Proportions were compared by the χ^2 test or Fisher's test. For comparison of proportions of MC patients, CH patients and controls, owing to the matched nature of the data, McNemar's χ^2 test was used and 'exact McNemar significance probability' (eM-p) reported if <0.05; comparisons were performed separately for (i) CM vs. HCV-negative controls, (ii) CM vs. CH patients and (iii) CH patients vs. HCV-negative controls. Univariate analyses were performed by standard methods.

Results

As a result of matching, age and sex distribution were quite similar in MC and CH patients and controls (Table 2). There were no significant differences among the three groups with regard to family history of thyroid disease, residence in iodine deficiency areas or smoking habits. The prevalence of subclinical hypothyroidism (i.e. TSH > 4.0 mcU/ml, FT_4 and FT_3 within normal range) was significantly higher in MC patients than in HCV-negative con-

Table 2 Data on thyroid involvement in MC and CH patients and controls

	MC patients	CH patients	Controls	p*
Age, mean ± SD (years)	63 ± 10	63.2 ± 6.4	63.8 ± 8.9	NS
Males/ females	17/76	17/76	17/76	NS
Iodine deficiency	81%	76.8%	90.0%	NS
Thyroid disease familiarity	7.8%	17.8%	13.0%	NS
Disease duration (years)	14 ± 7[b]	10 ± 6	–	–
Smoking	24%	19.6%	12.5%	NS
Hypothyroidism (TSH > 4mcU/ml)	10.8%[a]	7.5%	2.2%	
Antithyroglobulin antibody (AbTg)	9.5%	8.8%	5.5%	NS
Antithyroperoxidase antibody (AbTPO)	28.2%[a,b]	14.1%	8.8%	
AbTg and AbTPO	7.1%	3.3%	2.2%	NS
AbTg and/or AbTPO	30.6%[a]	18.7%	12.2%	
Thyroid autoimmunity	35.3%[a]	22.0%	15.8%	
Hyperthyroidism (TSH < 0.2mcU/ml)	9.7%	4.3%	4.3%	NS
Thyroid nodules	53.2%	53.7%	66.6%	NS
Thyroid papillary cancer	2.1%	0.0%	0.0%	NS

* ANOVA (for continuous variables) for all three groups of subjects; McNemar's χ^2 (for nominal variables). [a]MC patients vs controls, exact McNemar significance probability <0.05. [b]MC patients vs CH patients, exact McNemar significance probability <0.05.

trols (McNemars' $\chi^2 = 8.1$; eM-p = 0.038). The mean value of TSH in patients with subclinical hypothyroidism was 7.3 ± 2.0 mcU/ml in MC patients (range 5.1–17.7, 1.1 ± 0.7 in euthyroid MC patients), and it was 6.2 ± 2.0 mcU/ml in CH patients (range 4.3–9.6; 1.7 ± 1.0 in euthyroid CH patients). In contrast, no significant difference in the prevalence of subclinical hyperthyroidism (i.e. TSH <0.2 mcU/ml, FT_4 and FT_3 within normal range) was observed among the three groups. Clinically evident hypo- or hyperthyroidism were never observed. No MC patient had low FT_4, while three had low FT_3; these subjects had normal TSH, without any sign of central hypothyroidism, a finding compatible with sick euthyroid syndrome.[44] No CH patient or HCV-negative control had low FT_4 or FT_3 levels.

The percentage of patients with positive AbTPO was significantly higher in MC than in CH patients (McNemars' $\chi^2 = 5.1$; eM-p = 0.035) or HCV-negative controls (McNemars' $\chi^2 = 10.8$; eM-p = 0.001), while no significant difference was observed for AbTg positivity (Table 2). Moreover, in MC patients the presence of abnormally high AbTPO or AbTg titres was more frequent, compared to HCV-negative controls (McNemars' $\chi^2 = 8.7$; eM-p = 0.004). TRAb results were always negative in patients with altered TSH. The prevalence of thyroid nodules (independently of concomitant thyroid autoimmune disorder) was not significantly different in the three groups. A thyroid hypoechoic pattern, indicating inflammatory involvement of the thyroid tissue,[13,24,46] was more frequent in MC than in CH patients or controls (56% vs. 47% and 35%, respectively). FNA suggested the presence of a chronic thyroiditis in three MC and three CH patients. Thyroid autoimmunity was diagnosed in the absence of positive AbTg and AbTPO in four MC and three CH patients and in one control. Thyroid autoimmunity was significantly more frequent in MC than in HCV-negative controls (McNemars' $\chi^2 = 8.1$; eM-p = 0.006). On the whole, indices of thyroid autoimmunity were significantly more frequent in MC than in CH patients or controls. Moreover, these indices were significantly more frequent in MC patients with hypothyroidism than in the others (70% vs. 31%; p<0.02).

Finally, the possible association of thyroid disorders with clinical and serological parameters of MC was investigated (Table 3). Hypothyroidism was associated with proteinuria (but not with raised serum creatinine levels), higher cryocrit and with the presence of at least one autoantibody (ANA, AMA, ASMA, or anti-ENA). Moreover, thyroid autoimmunity was associated with longer MC duration and with the presence of active hepatitis.

The prevalence of Sjögren's syndrome in MC patients was 52%; it was almost invariably a sicca syndrome without serological and histological alterations. No CH patient had Sjögren's syndrome. No significant difference was observed in the prevalence of hypothyroidism, hyperthyroidism, AbTg or AbTPO in MC patients with or without Sjögren's

Table 3 Association between hypothyroidism or thyroid autoimmunity and clinico-serological findings in MC patients

	Hypothyroidism			Thyroid autoimmunity		
	Present (10)	Absent (83)	p*	Present (30)	Absent (63)	p*
Age (years) mean ± SD	61.9 ± 13.4	63.5 ± 9.8	NS	63.7 ± 8.3	63.0 ± 11.1	NS
Women	9	67	NS	25	45	NS
Males	1	16		5	10	
Disease duration (years) mean ± SD	15.7 ± 8.6	13.9 ± 7.3	NS	17.0 ± 8.4	12.4 ± 6.2	0.007*
Raynaud's phenomenon	66.7%	44.9%	NS	50.0%	47.2%	NS
Sjögren's syndrome	42.9%	52.6%	NS	44.0%	54.7%	NS
Peripheral neuropathy	40.0%	31.2%	NS	34.8%	28.8%	NS
Renal involvement (proteinuria g/24 h)	0.7 ± 0.5	0.2 ± 0.4	0.001*	0.1 ± 0.3	0.2 ± 0.4	NS
Aminotransferase elevation**	98.3 ± 91.3	177.4 ± 166.5	NS	196.7 ± 189.4	115.2 ± 64.8	0.044*
Cryocrit (%) mean ± SD	11.1 ± 13.1	4.0 ± 8.4	0.035*	3.5 ± 7.9	3.7 ± 5.5	NS
CH50 (IU, normal 160–220) mean ± SD	90.6 ± 25.5	115.0 ± 35.9	NS	107.4 ± 41.0	120.7 ± 32.6	NS
C3 (mg/dl, normal 60–130) mean ± SD	78.8 ± 43.0	81.8 ± 32.7	NS	76.8 ± 32.4	84.7 ± 34.3	NS
C4 (mg/dl, normal 20–55) mean ± SD	8.7 ± 3.6	11.5 ± 8.7	NS	11.6 ± 7.0	11.2 ± 9.4	NS
Autoantibodies***	71.4%	26.5%	0.025*	45.0%	30.0%	NS

* For the comparison between presence and absence of the trait. ** Increase of the liver enzyme ALT, expressed as % of upper limit of normal. *** Presence of ANA and/or AMA and /or ASMA and/or anti-ENA.

syndrome. Disease duration was significantly longer in MC than in CH patients ($p < 0.05$) (Table 2), but no correlation was found with the presence of thyroid disorders. A comparable prevalence of thyroid disorders was observed in MC type II (65%) and MC type III patients. The presence of monoclonal IgM component did not affect the occurrence of thyroid disorders.

HCV viraemia was detectable in 86% and 91% of MC and CH patients, respectively, with no apparent relation to the presence of thyroid disorders. Finally, in our MC series, the prevalence of HCV genotype 2 tended to be higher than in CH patients. However, no significant correlation was found between HCV genotype and thyroid disorders. FNA showed a papillary thyroid cancer in one MC patient, who was treated with total thyroidectomy. Histological examination confirmed the presence of a thyroid papillary cancer in the context of chronic thyroiditis. Another MC patient had been previously operated on for papillary thyroid cancer diagnosed 10 years after the disease onset. No case of thyroid papillary cancer was observed among CH or controls.

The subgroups of MC/IFN+ and MC/IFN– patients were comparable in terms of clinical phenotype and prevalence of thyroid disorders (Table 4). MC patients with or without corticosteroid treatment at the time of study or during the previous follow-up did not show significant difference in the prevalence of thyroid disorders.

No significant differences were observed between CH patients and HCV-negative controls.

Table 4 Data related to thyroid disorders in MC patients treated with interferon (IFN+) or not (IFN–)

	IFN– ($n = 60$)	IFN+ ($n = 33$)	p*
Age (years) mean ± SD	63.9 ± 10.7	62.2 ± 9.1	NS
Males/females (%)	20.0/80.0	15.2/84.8	NS
Iodine deficiency	83.0%	70.0%	NS
Thyroid disease familiarity	9.3%	4.8%	NS
Smoking habit	26.2%	19.0%	NS
Hypothyroidism (TSH > 4 mcU/ml)	13.3%	6.0%	NS
Antithyroglobulin antibody (AbTg)	13.2%	3.2%	NS
Antithyroperoxidase antibody (AbTPO)	30.2%	33.3%	NS
AbTg and AbTPO	11.3%	0.0%	NS
AbTg and/or AbTPO	32.1%	28.1%	NS
Thyroid autoimmunity	35.7%	34.5%	NS
Hyperthyroidism (TSH < 0.2 mcU/ml)	5.0%	18.1%	NS
Thyroid nodules	33.9%	44.8%	NS
Papillary thyroid cancer	1	1	NS

* ANOVA (for continuous variables); χ^2 and/or Fisher's exact test (for nominal variables).

Discussion

We investigated the prevalence of thyroid involvement in HCV-related MC patients, compared with age- and sex-matched groups of (a) patients with

HCV-related CH without MC and (b) subjects with neither MC nor HCV-positivity. We found autoimmune thyroid involvement, as judged from serum autoantibodies and/or echography and/or FNA, in significantly more MC patients than healthy controls. Subclinical hypothyroidism also was significantly more frequent in MC, while the prevalence of hyperthyroidism or thyroid nodules was comparable to that found in the general population. Interestingly, papillary thyroid cancer was found in two MC patients but in none of the controls. In CH patients, the prevalence of autoimmune thyroid involvement, evaluated by serum autoantibodies and subclinical hypothyroidism, was intermediate between that of MC patients and that of healthy controls.

MC is an immune-mediated disorder with multiple organ involvement, due to vessel deposition of immune-complexes, mainly cryoglobulins and complement, leading to vasculitis and consequent ischaemic lesions.[1,2,15,16,21] Other important pathogenic mechanisms responsible for some MC clinical manifestations, such as chronic hepatitis and possibly chronic thyroiditis, may involve cell-mediated and/or autoantibody-mediated tissue injury. HCV infection represents a chronic stimulus to the immune system, leading to polyclonal or oligo-clonal B-lymphocyte expansion, with organ- and non-organ-specific autoantibody production.[17,19,22,42] HCV also seems to have a direct pathogenic role in both autoimmune and/or neoplastic tissue alterations.[18,19] In this context, thyroid disorders can be included among the complications of HCV-associated MC.

A high prevalence of thyroid gland disorders has been reported in patients with chronic HCV infection.[14,20,25–27,41,44] In general, thyroid dysfunction in chronic C hepatitis may include all forms of thyroid alterations, i.e. hypothyroidism and hyperthyroidism, Hashimoto's disease and isolated increases in AbTPO.[14,20,25–27,41,44] The frequency of abnormally high levels of anti-thyroid antibodies in HCV-infected patients varies markedly in different series, ranging from 2% to 48%.[14,20,25–27,41,44] Differences in geographical distribution,[28] genetic variability in the populations studied,[43] and environmental co-factors such as iodine intake or other infectious agents,[32,37] could play an important role in the development of autoimmune thyroid disorders. In different studies, subclinical hypothyroidism was observed in 2%[30] to 9%[44] of patients with chronic HCV infection. The prevalence of various thyroid disorders and serum anti-thyroid autoantibodies is generally higher in chronic hepatitis type C than in hepatitis B or D[11,14,44] or control series.[20,27] Conversely, an increased prevalence of anti-HCV in thyroid autoimmune diseases has been reported by some authors[12,45,50] but has not been confirmed by others.[7,26,30,31,34] In summary, a careful analysis of previously published studies indicates that the thyroid abnormalities observed in chronic HCV infection are generally restricted to high titres of thyroid autoantibodies, most commonly found in female patients.[14] The results of our study in CH patients confirm a higher prevalence of autoimmune thyroid involvement in CH patients than in controls.

Recently, Cacoub reported a 40% prevalence of circulating cryoglobulins in patients with chronic HCV infection; these patients showed a significant association with low T_4, while no correlation was found between cryoglobulinaemia and abnormal TSH levels or serum thyroid autoantibodies.[10] The results of our study, using more tests and more sensitive methodology, demostrate a significantly higher prevalence of anti-thyroid autoantibodies and subclinical hypothyroidism in MC patients. Moreover, subjects with thyroid disorders exhibited more pronounced autoimmune phenomena (higher cryocrit levels, non-organ-specific autoantibodies) and severity of the cryoglobulinaemic syndrome (renal and liver involvement) along with a longer disease duration. The discrepancy between our study and previous reports could be entirely due to our inclusion only of patients with overt MC syndrome. We selected patients with the MC syndrome and not just HCV positivity—as the MC syndrome reflects a severe autoimmune disturbance, it is unsurprising that other autoimmune phenomena are associated with it. Whether thyroid disorders are present in a high proportion of HCV-positive patients with cryglobulinaemia but without clinically overt MC syndrome regardless of MC-related clinical manifestations, remains to be decided.

It is of interest that most of our MC patients were on corticosteroid treatment at the time of the study or before; nevertheless, they showed high levels of circulating AbTPO and/or AbTg. In no case did MC patients with thyroid dysfunction have high levels of TRAb; according to previous studies, this type of autoantibody is not involved in HCV-associated thyroid disorders.[49] HLA-B8-DR3, a well known 'autoimmune' haplotype, has been reported to confer susceptibility to HCV-related MC[29] in a similar fashion to DR3 for autoimmune thyroid disorders.[47] This genetic susceptibility may therefore explain the increased prevalence of autoimmune thyroid disorders in MC patients.

IFNα therapy is associated with the development of thyroid autoimmunity and/or thyroid dysfunction in 5%–12% of patients with chronic HCV infection, usually expressing pre-existing subclinical

abnormalities.[14,25,44,46] These thyroid abnormalities resolve in >50% of patients 6 months after the discontinuation of IFNα treatment.[14,44] In our IFNα-treated patients with MC, the prevalence of thyroid autoantibodies and subclinical hypothyroidism was similar to that observed in untreated MC patients, whereas subclinical hyperthyroidism was definitely, although not significantly, more frequent (18% vs. 5%). This finding can be explained by the fact that discontinuation of IFNα treatment for >6 months was a selection criterion in our study. It does nevertheless suggest that the thyroid problems triggered by IFNα in MC are generally mild and reversible.

Interestingly, thyroid papillary cancer was observed in two MC patients, but in none of the controls. This observation resonates with our initial report on thyroid cancer complicating chronic C hepatitis,[4] as well as with the results of two recent case-control studies in Southern Italy.[38,39] It is therefore possible that the oncogenetic potential of HCV, responsible for liver cancer and B-cell lymphoma, may also be relevant to thyroid cancer in HCV-positive patients with or without MC. However, the association between MC and thyroid cancer needs to be confirmed by more extensive epidemiological studies.

In conclusion, the high prevalence of different thyroid disorders in HCV-associated MC mandates careful thyroid monitoring in these patients.

References

1. Abel G, Zhang QX, Agnello V. Hepatitis C virus infection in type II mixed cryoglobulinemia (review). *Arthritis Rheum* 1993; **36**:1341–9.
2. Agnello V, Chung RT, Kaplan LM. A role for hepatitis C virus infection in type II cryoglobulinemia. *N Engl J Med* 1992; **327**:1490–5.
3. Antonelli A, Fallahi P, Nesti C, Pupilli C, Marchetti P, Takasawa S, Okamoto H, Ferrannini E. Anti-CD38 autoimmunity in patients with chronic autoimmune thyroiditis or Graves' disease. *Clin Exp Immunol* 2001; **126**:426–31.
4. Antonelli A, Ferri C, Fallahi P. Thyroid cancer in patients with hepatitis C infection [Letter]. *JAMA* 1999; **281**:1588.
5. Antonelli A, Miccoli P, Derzhitski VE, Panasiuk G, Solovieva N, Baschieri L. Epidemiologic and clinical evaluation of thyroid cancer in children coming from the Gomel region (Belarus). *World J Surg* 1996; **20**:867–61.
6. Antonelli A, Miccoli P, Ferdeghini M, Di Coscio G, Alberti B, Iacconi P, Baldi V, Fallahi P, Baschieri L. Role of neck ultrasonography in follow-up of patients operated on for differentiated thyroid cancer after surgery. *Thyroid* 1995; **5**:25–9.
7. Baudin E, Marcellin P, Pouteau M, Colas-Linhart N, Le Floch JP, Lemmonier C, Benhamou JP, Bok B. Reversibility of thyroid disfunction induced by recombinant interferon alpha in chronic hepatitis C. *Clin Endocrinol (Oxf)* 1993; **39**:657–61.
8. Brouet JC, Clouvel JP, Danon F, Klein M, Seligmann M. Biologic and clinical significance of cryoglobulins. *Am J Med* 1974; **57**:775–88.
9. Bunn CC, Gharavi AE, Hughes GRV. Antibodies to extractable nuclear antigens in 173 patients with DNA binding positive SLE: an association between antibodies to ribonucleoprotein and Sm antigen observed by counterimmunoelectrophoresis. *J Clin Lab Immunol* 1982; **8**:13–17.
10. Cacoub P, Poynard T, Ghillani P, Charlotte F, Olivi M, Piette JC, Opolon P. Extrahepatic manifestations of chronic hepatitis C. *Arthritis Rheum* 1999; **42**:2204–12.
11. Deutsch M, Dourakis S, Manesis EK, Gioustozi A, Hess G, Horsch A, Hadziyannis S. Thyroid abnormalities in chronic viral hepatitis and their relationship to interferon alfa therapy. *Hepatology* 1997; **26**:206–10.
12. Duclos-Vallee JC, Johanet C, Trinchet JC, Deny P, Laurent MF, Duron F, Valensi P, Weil B, Homberg JC, Pateron D, *et al.* High prevalence of serum antibodies to hepatitis C virus in patients with Hashimoto's thyroiditis. *Br Med J* 1994; **309**:846–7.
13. Espinasse P. L'echographie thyroidienne dans les thyroidites lymphocytaires chroniques autoimmunes. *J Radiol* 1983; **64**:537–44.
14. Fernandez-Soto L, Gonzales A, Escobar-Jimenez F, Vazquez R, Ocete E, Olea N, Salmeron J. Increased risk of autoimmune thyroid disease in hepatitis C vs hepatitis B before, during and after discontinuing interferon therapy. *Arch Intern Med* 1998; **158**:1445–8.
15. Ferri C, Greco F, Longombardo G, Palla P, Moretti A, Marzo E, Fosella PV, Pasero G, Bombardieri S. Antibodies to hepatitis C virus in patients with mixed cryoglobulinemia. *Arthr Rheum* 1991; **34**:1606–10.
16. Ferri C, La Civita L, Longombardo G, Greco F, Bombardieri S. Hepatitis C virus and mixed cryoglobulinemia (review). *Eur J Clin Invest* 1993; **23**:399–405.
17. Ferri C, Monti M, La Civita L, Longombardo G, Greco F, Pasero G, Gentilini P, Bombardieri S, Zignego AL. Infection of peripheral blood mononuclear cells by hepatitis C virus in mixed cryoglobulinemia. *Blood* 1993; **82**:3701–4.
18. Ferri C, Pileri S, Zignego AL. Hepatitis C Virus, B-cell Disorders, and Non-Hodgkin's Lymphoma. In: Goedert JJ, eds. *Infectious Causes of Cancer. Targets for Intervention. National Cancer Institute (NIH).* Totowa NJ, The Humana Press Inc., 2000:349–68.
19. Ferri C, Zignego AL. Relation between infection and autoimmunity in mixed cryoglobulinemia. *Curr Opin Rheumatol* 2000; **12**:53–60.
20. Ganne-Carrie N, Medini A, Coderc E, Seror O, Christidis C, Grimbert S, Trinchet JC, Beaugrand M. Latent autoimmune thyroiditis in untreated patients with HCV chronic hepatitis: a case-control study. *J Autoimmun* 2000; **14**:189–93.
21. Gorevic PD, Kassab HJ, Levo Y, Kohn R, Meltzer M, Prose P, Franklin EC. Mixed Cryoglobulinaemia. Clinical aspects and long-term follow-up of 40 patients. *Am J Med* 1980; **69**:287–308.
22. Gorevic PD, Frangione B. Mixed cryoglobulinemia cross-reactive idiotypes: implication for relationship of MC to rheumatic and lymphoproliferative diseases. *Semin Hematol.* 1991; **28**:79–94.

23. Gumber S, Chopra S. Hepatitis C: a multifaceted disease. Review of extrahepatic manifestations (review). *Ann Intern Med*. 1995; **123**:615–20.

24. Gutenkust R, Hafermann W, Mansky T, Scriba PC. Ultrasonography related to clinical and laboratory findings in lymphocytic thyroiditis. *Acta Endocrinologica (Copenh)*. 1989; **121**:129–35.

25. Hsieh MC, Yu ML, Chuang WL, Shin SJ, Dai CY, Chen SC, Lin ZY, Hsieh MY, Liu JF, Wang LY, Chang WY. Virologic factors related to interferon-induced thyroid dysfunction in patients with chronic hepatitis C. *Eur J Endocrinol* 2000; **142**:431–7.

26. Huang MJ, Wu SS, Liaw YF. Thyroid abnormalities in patients with chronic viral hepatitis. *Hepatology* 1994; **20**:1651–2.

27. Huang MJ, Tsai SL, Huang BY, Sheen IS, Yeh CT, Liaw YF. Prevalence and significance of thyroid autoantibodies in patients with chronic hepatitis C virus infection: a prospective controlled study. *Clin Endocrinol* 1999; **50**:503–9.

28. Lenzi M, Johnson PJ, McFarlane IG, Ballardini G, Smith HM, McFarlane BM, Bridger C, Vergani D, Bianchi FB, Williams R. Antibodies to hepatitis C virus in autoimmune liver disease: evidence for geographical heterogeneity. *Lancet* 1991; **338**:277–80.

29. Lenzi M, Frisoni M, Mantovani V, Ricci P, Muratori L, Francesconi R, Cuccia M, Ferri S, Bianchi FB. Haplotype HLA-B8-DR3 confers susceptibility to hepatitis C virus-related mixed cryoglobulinemia. *Blood* 1998; **91**:2062–6.

30. Loviselli A, Oppo A, Velluzzi F, Atzeni F, Mastinu GL, Farci P, Orgiana G, Balestrieri A, Cocco PL, Mariotti S. Independent expression of serological markers of thyroid autoimmunity and hepatitis virus C infection in the general population: results of a community-based study in north-western Sardinia. *J Endocrinol Invest* 1999; **22**:660–5.

31. Marazuela M, Garcia-Buey L, Gonzalez-Fernandez B, Garcia-Monzon C, Arranz A, Borque MJ, Moreno-Otero R. Thyroid autoimmune disorders in patients with chronic hepatitis C before and during interferon-α therapy. *Clin Endocrinol* 1996; **44**:635–42.

32. McFarlane IC, Path MR. Autoimmunity and hepatotropic viruses. *Semin Liver Dis* 1991; **11**:223–33.

33. Meltzer M, Franklin EC, Elias K, McCluskey RT, Cooper N. Cryoglobulinemia. A clinical and laboratory study. II. Cryoglobulins with rheumatoid factor activity. *Am J Med* 1966; **40**:837–56.

34. Metcalfe RA, Ball G, Kudesia G, Weetman AP. Failure to find an association between hepatitis C virus and thyroid autoimmunity. *Thyroid* 1997; **7**:421–4.

35. Miccoli P, Antonelli A, Iacconi P, Alberti B, Gambuzza C, Baschieri L. A prospective, randomized, double blind study about T4 suppressive therapy effectiveness in the prevention of nodular thyroid recurrence after surgery: evaluation of results at the third year of follow up. *Surgery* 1993; **114**:1097–102.

36. Miccoli P, Antonelli A, Spinelli C, Ferdeghini M, Fallahi P, Baschieri L. Completion total thyroidectomy in children with thyroid cancer secondary to the Chernobyl accident. *Arch Surg* 1998; **133**:89–93.

37. Minelli R, Braverman LE, Giuberti T, Schianchi C, Gardini E, Salvi M, Fiaccadori F, Ugolotti G, Roti E. Effects of excess iodine administration on thyroid function in euthyroid patients with a previous episode of thyroid dysfunction induced by interferon-alpha treatment. *Clin Endocrinol*. 1997; **47**:357–61.

38. Montella M, Crispo A, Pezzullo L, Izzo F, Fabbrocini G, Ronga D, Tamburini M. Is hepatitis C virus infection associated with thyroid cancer? A case-control study [letter]. *Int J Cancer* 2000; **87**:611–12.

39. Montella M, Crispo A, de Bellis G, Izzo F, Frigeri F, Ronga D, Spada O, Mettivier V, Tamburini M, Cuomo O. HCV and cancer: a case-control study in a high endemic area. *Liver* 2001; **21**:335–41.

40. Pacini F, Antonelli A, Lari R, Gasperini L, Baschieri L, Pinchera A. Unsuspected parathyroid cysts diagnosed by thyroglobulin and parathyroid hormone measurement in fluid aspirates. *Ann Intern Med* 1985; **102**:793–4.

41. Pateron D, Hartmann DJ, Duclos-Vallee JC, Jouanolle H, Beaugrand M. Latent autoimmune thyroid disease in patients with chronic HCV hepatitis. *J Hepatol* 1992; **16**:244–5.

42. Perl A, Gorevic PD, Ryan DH, Condemi JJ, Ruszkowski RJ, Abraham GN. Clonal B cell expansion in patients with essential mixed cryoglobulinemia. *Clin Exp Immunol* 1989; **76**:54–60.

43. Prentice LM, Phillips DI, Sarsero D, Beever K, McLachlan SM, Smith BR. Geographical distribution of subclinical autoimmune thyroid disease in Britain: a study using highly sensitive direct assay for autoantibodies to thyroglobulin and thyroid peroxidase. *Acta Endocrinol* 1990; **123**:493–8.

44. Preziati D, La Rosa L, Covini G, Marcelli R, Rescalli S, Persani L, Del Ninno E, Meroni PL, Colombo M, Beck-Peccoz P. Autoimmunity and thyroid function in patients with chronic active hepatitis treated with recombinant interferon alpha-2a. *Eur J Endocrinol* 1995; **132**:587–93.

45. Quaranta JF, Tran A, Regnier D, Letestu R, Beusnel C, Fuzibet JG, Thiers V, Rampal P. High prevalence of antibodies to hepatitis C virus (HCV) in patients with anti-thyroid autoantibodies. *J Hepatol* 1993; **18**:136–8.

46. Roti E, Minelli R, Giuberti T, Marchelli S, Schianchi C, Gardini E, Salvi M, Fiaccadori F, Ugolotti G, Neri TM, Braverman LE. Multiple changes in thyroid function in patients with chronic active HCV hepatitis treated with recombinant interferon-alpha. *Am J Med*. 1996; **172**:482–7.

47. Tandon N, Zhang L, Weetman AP. HLA associations with Hashimoto's thyroiditis. *Clin Endocrinol*. 1991; **34**:383–6.

48. Vitti P. Grey scale thyroid ultrasonography in the evaluation of patients with Graves' disease. *Eur J Endocrinol*. 2000; **142**:22–4.

49. Wada M, Miyoshi T, Fujimoto M, Kamimoto H, Kinugasa A, Sawada K, Shimoyama T. Serum levels of thyroid-stimulating hormone receptor antibodies during IFN-alpha2a treatment of chronic hepatitis C. *J Interferon Cytokine Res* 1999; **19**:85–8.

50. Wagner B, Vierhapper H, Hofmann H. Prevalence of hepatitis C virus infection in Hashimoto's thyroiditis. *Br Med J* 1996; **312**:640–1.

51. Zignego AL, Deny P, Feray O, Ponzetto A, Gentilini P, Tiollais P, Brechot C. Amplification of hepatitis delta virus RNA sequences by polymerase chain reation: a tool for viral detection and cloning. *Mol Cell Probes* 1990; **4**:45–51.