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Thyroid cancer in HCV-related mixed cryoglobulinemia patients

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ABSTRACT

Objective. *The prevalence of thyroid cancer in a series of unselected HCV-related mixed cryoglobulinemic patients was investigated in comparison with a control group.*

Methods. *Among 107 consecutive patients with mixed cryoglobulinemia (MC), 94 were eligible for the study. A control group was obtained from a sample of the general population (2,401 subjects), age > 50 years, who had undergone thyroid ultrasonography (582 subjects); 5 sex-matched controls were randomly assigned to each MC patient (470 individuals). The mean age was similar in the MC patients and controls (64.2 ± 10.0 vs. 63.4 ± 7.0).*

Results. *The prevalence of thyroid nodules was higher, although not significantly so, in control subjects than in MC patients (65.3 vs. 54.8%). Two patients with papillary thyroid cancer were found in the MC series, while no case was observed among controls (p = 0.001, chi-square P value; p = 0.02, Fisher's exact test). In both MC patients with papillary thyroid cancer lymphocytic infiltration was observed in the thyroid tissue.*

Conclusions. *The possible association between HCV-related MC and thyroid cancer indicates that a careful monitoring of the thyroid would be opportune during the clinical follow-up of HCV-associated MC patients, especially in those with signs of thyroid autoimmune disorders.*

Introduction

An oncogenetic role of hepatitis C virus (HCV) in the pathogenesis of different kinds of tumor has been suggested (1, 2). Furthermore, some studies have reported a high prevalence of thyroid papillary cancer in patients with HCV infection (3). Mixed cryoglobulinemia (MC) is a systemic vasculitis associated with hepatitis C virus (HCV) infection in over 90% of cases and frequently complicated by multiple organ involvement (autoimmune hepatitis, hepatocellular carcinoma, glomerulonephritis, polyarthritis, diabetes mellitus, thyroid disorders and B-cell lymphoma) (2,4-6). The HCV lymphotropism (7) may trigger the B-lymphocyte expansion frequently observed in MC patients; the consequent production of different autoantibodies, including rheumatoid factor and cryoprecipitable immune complexes, leads to cryoglobulinemic vasculitis with cutaneous and visceral organ damage (4-6). In some individuals the 'benign' mono-olygoclonal B-lymphocyte proliferation underlying the MC may evolve to a frank malignant lymphoma, usually after a long follow-up period (1, 2, 6).

Thyroid cancer is a relatively rare malignancy, but it is becoming more frequent in Italy. In 1983-1987, the standardized incidence rate per 100,000 inhabitants was 2.0 for males and 5.5 for females, while in 1988-1992 its incidence rate rose to 2.5 for males and 7.0 for females (8, 9). During the same time period an increasingly high prevalence of HCV infection was reported in the Italian general population (10).

The present study investigates the prevalence of thyroid cancer in a large series of unselected HCV-related MC patients in comparison to a control group from the general population.

Patients and methods

Patients

One hundred and sixteen consecutive MC patients referred to the Rheumatology Unit of the University of Pisa were prospectively studied over a 2-year period (1999-2001). The diagnosis of MC was based on the presence of serum mixed (IgG-IgM) cryoglobulins with rheumatoid factor activity and the classical clinical triad of purpura, weakness, arthralgias, and on the exclusion of other well-known systemic disorders (6, 7). In 40/116 a skin biopsy was carried out, and in all cases a leukocitoclastic vasculitis was found. HCV infection was systematically evaluated in all patients, and 9 were excluded because they were HCV-negative. Among the 107 patients with HCV-associated MC, 4 were excluded from the study because they had previously undergone treatment with external radiotherapy in the neck region or the mediastinum (3 for breast cancer and one for lymphoma) and 9 because it was not possible to perform thyroid

Table I. Main demographic and clinico-serological features of 94 HCV-related MC.

Age, mean ± SD years (range)	64.2 ± 10.0
No. males / no. females	20/74
Disease duration, mean ± SD years (range)	14.1 ± 7.3
Purpura	86.3%
Weakness	98.6%
Arthralgias	91.8%
Arthritis	15.3%
Raynaud's phenomenon	49.3%
Sjøgren's syndrome	52.1%
Peripheral neuropathy	80.3%
Renal involvement*	13.0%
Liver involvement §	58.9%
Non-Hodgkin's lymphoma	4
Hepatocarcinoma	1
Cryocrit, mean ± SD %	3.1 ± 4.9
CH50, mean ± SD units (normal 160-220)	113.6 ± 36.5
C3, mean ± SD mg/dl (normal 60-130)	83.6+33.6
C4, mean ± SD mg/dl (normal 20-55)	11.4 ± 8.7
Autoantibodies ◊	24.6%

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

ultrasonography.

A total of 94 patients (Table I) were eligible for the study. Among them 33 had been previously treated with alpha-IFN and 2 were in treatment with IFN at the moment of the study. At the time of the study, 70% of MC patients were taking low doses of corticosteroids. No patient had been treated with plasma exchange, cytotoxic and/or immunosuppressive treatment.

A control group was obtained from a sample of the general population (2,401 subjects) who had never undergone radiotherapy (6 subjects were excluded), age more than 50 years, and who had previously undergone thyroid ultrasonography (582 individuals). Among these 582 subjects, 5 controls (sex-matched) were randomly associated with each MC patient (470 subjects).

For both patients and controls a careful medical history was collected, in particular about risk factors for thyroid disorders: residence in iodine-deficient areas, smoking habits, drugs. In all cases thyroid disorders were investigated by means of physical examination and thyroid ultrasonography. The study had been approved by the local Ethical Commitee and informed consent was obtained from each patient included in the study.

Examinations

Immunological studies. Cryocrit levels, hemolytic complement activity (CH50) and C3-C4 fractions, antinuclear (ANA), -smooth muscle (ASMA), -mitochondrial (AMA), and -extractable nuclear antigen (ENA) autoantibodies were measured as previously described (6-7, 11).

Virological studies. Anti-HCV antibodies and HCV RNA (PCR) were determined on serum clotted and centrifuged at 37°C and stored at -70°C using current technologies as previously described (7).

Ultrasonography of the neck and fine-needle aspiration. Neck ultrasonography was performed by the same operator, who was unaware of the results of thyroid hormone and autoantibody measurements, in all patients using a probe (Toshiba Tosbee) with a sectorial 7.5 MHz transducer, interposing a water bag (12). The presence of hypoechoic and dyshomogeneous 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). The presence of thyroid nodules was recorded.

Fine neeedle aspiration (FNA) was recommended in all subjects with nodular goiter and was performed in subjects who gave their consents. FNA was performed in palpable nodules; nodules not palpable were examined by FNA only when clinical findings or the echographic pattern suggested the opportunity of excluding malignancy (14). FNA, was performed by the same operator, using a free-hand method (15) or by an ultrasonography-guided technique as already described (16).

Laboratory evaluations of the thyroid. Thyroid function and thyroid autoantibodies were measured in MC patients, as previously described (17, 18). 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 FT3/FT4 Kit; Amersham, UK). Serum TSH (normal range 0.3-4.0 mcU/ml) was determined by a ultrasensitive method (DPC, USA). AbTPO (normal range 0-150 UI/ml) and AbTg (normal range 0-150 UI/ml) (ICN Pharmaceuticals, USA) were evaluated by IRMA methods. When an abnormal result was

Table II. Prevalence of papillary thyroid cancer and thyroid nodules in MC patients and controls.

	MC (94)	Controls (470)	p*
Men	20 (21.3%)	100 (21.3%)	ns
Female	74 (78.7%)	370 (78.7%)	
Age (years ± SD)	64.2 ± 10.0	63.4 ± 7.0	ns
Thyroid nodules	54.80%	65.30%	ns
Thyroid cancer	2	0	0.001

*χ^2 P-value; ns: not significant.

obtained a second measurement was done to confirm the data.

Statistical analysis

Values are given as the mean ± SD; variables with a non-normal distribution are given as the median and interquartile range. 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 test. Univariate and multivariate analysis were performed by standard methods.

Results

The main clinico-epidemiological features of MC patients are reported in Table I. Mean age and sex distribution were closely similar in the MC patients and controls according to the matching procedure (Table II). No significant differences were observed between the two groups about smoking habits. Residence in iodine deficiency areas was more prevalent in the control group 92% than in MC patients 74% ($p < 0.05$).

The prevalence of preclinical hypothyroidism (TSH > 4 mcU/ml, FT4 and FT3 within normal range), preclinical hyperthyroidism (TSH < 0.3 mcU/ml, FT4 and FT3 within normal range) and the prevalence of positive AbTPO, AbTg and/or both thyroid autoantibodies are reported in Table III. Circulating positive AbTg and/or AbTPO, and/or a thyroid echografic pattern of diffuse hypoechogenicity indicative of an autoimmune thyroid disorder was observed in 32.5% of MC patients.

The prevalence of thyroid nodules was higher in control subjects than in MC patients (Table II). Fine needle aspiration (FNA) was performed in 13/94 (13.8%) of MC patients and in 66/470 (14%) of controls. Two patients with papillary thyroid cancer were detected in the MC series, while no case was observed among controls ($p = 0.001$, chi- square P value; $p = 0.02$, Fisher's exact test; Table II). In particular, FNA revealed in one MC patient, previously treated with alpha-IFN, a papillary thyroid cancer 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 for papillary thyroid cancer, diagnosed 10 years after the disease onset; also in this patient the revision of the histological specimen showed the presence of lymphocytic infiltration suggestive of the coexistence of a chronic thyroiditis. This last patient had been never treated with IFN.

Discussion

A high prevalence of papillary thyroid cancer was observed in HCV associated MC patients (2.1%), while no case was observed in control subjects. This last result is in agreement with the low prevalence of thyroid cancer recently reported in a population-based study in southern Italy (14). The thyroid status of 1,411 subjects, representing virtually the entire resident population of an iodine-deficient village, was assessed by ultrasound and FNA; thyroid cancer was found in only 1 case. It is worth noting that in our MC patients and especially in our control series the prevalence of thyroid nodules is particularly high (Table II), probably because these subjects lived in the past decades in iodine deficient areas; in fact, iodine deficiency is largely diffuse in Italy (18). Compared to the higher prevalence of thyroid nodules in the control subjects the prevalence of thyroid cancer in MC patients was particularly significant. The present observation agrees with our first report on thyroid cancer complicating type C chronic hepatitis (3,19). Furthermore Montella *et al.*,have been recently confirmed the result in a case-control study conducted in Southern Italy (10). In 186 patients operated for papillary thyroid cancer the presence of HCV infection was significantly higher than that observed in 186 subjects operated for benign disorders; particularly in female (OR = 4.0; 95% CI 1,1-8.8), or in subjects over 50 (OR = 3.1; 95% CI 1.1-8.2). More recently Montella *et al*. confirmed the association between thyroid cancer and HCV infection in a new case-control study (20).

Of interest, in our HCV-associated MC patients a high prevalence of thyroid gland disorders has been recorded (Table III). The prevalence of anti-thyroid autoantibodies in our patient series (29.5%) is higher than that reported in a population-based study in Italy (14) in subjects with age 56-75 (that was in the range between 15% and 20%). In general, the presence of thyroid disorders in chronic hepatitis type C has been widely reported; all forms of thyroid alterations, i.e. hypo- and hyperthyroidism, Hashimoto's disease and isolated increase of AbTPO (21-23) have been observed, similarly to that found in our MC patients. Thyroid autoimmunity and chronic thyroiditis have been regarded as preneoplastic conditions. Okayasu *et al*. (24) studied the prevalence and severity of thyroiditis associated with adenomatous goiter, follicular adenoma, or papillary carcinoma by examination of surgically resected materials from Japanese (626 patients), and white and African Americans (330 and 90 patients, respectively). The prevalence of lymphocytic infiltrates, which are indicative of autoimmune thyroiditis, was significantly higher in patients with papillary carcinoma than in patients with adenomatous goiter or follicular adenoma among Japanese females (63.0%) and males (50.0%), white females (76.0%), and African American females (46.2%). Lymphocyte infiltration into the follicular adenoma or papillary carcinoma correlated with the severity of concomitant thyroiditis. The possibility of autoimmune thyroiditis as a predisposing factor for papillary thyroid carcinoma has been suggested. It is of interest that in our MC series both patients with thyroid

Table III. Thyroid disorders in MC patients.

	MC (94)
Antithyroglobulin antibody (AbTg)	7.8%
Antithyroperoxidase antibody (AbTPO)	28.2%
AbTg and AbTPO	6.5%
AbTg and/or AbTPO	29.5%
Thyroid autoimmunity	32.5.%
Hypothyroidism (TSH > 4 mcU/ml)	6.3%
Hyperthyroidism (TSH<0.2 mcU/ml)	6.3%

papillary cancer had lymphocytic infiltration of the thyroid. This finding together with the high prevalence of thyroid autoantibodies in our MC patients suggest that thyroid autoimmunity may be a predisposing condition for papillary cancer. No relationship between the presence of thyroid cancer and the treatment with interferon is suggested by our study.

Cryoglobulinemic vasculitis is the most frequent extrahepatic complication of HCV infection (25) characterized by large amount of cryo- and non-cryoimmunecomplexes and autoantibodies, responsible of a wide spectrum of autoimmune manifestations (Table I). A clonal expansion of IgMk-bearing B-cells (26), lymphocyte and plasmocytoid cell infiltrates in the bone marrow and liver (2), with frequent Bcl-2 protooncogene translocation (27), have been definitely demonstrated in HCV-associated MC (1, 2). Thus, MC may represent a benign lymphoproliferative disorder (6); an indolent B-cell lymphoma is the underlying disorder in a significant number of MC patients (2, 6). This clinically indolent lymphoproliferation can switch over to frank malignant lymphoma (2, 6). A large body of clinicoserological and pathological observations indicate that there is a continuum between chronic HCV infection, MC and other autoimmune-lymphoproliferative or neoplastic disorders (1, 2, 6).

An oncogenetic role for HCV has been definitely demonstrated for hepatocellular carcinoma complicating chronic HCV infection with or without the intermediate cirrhosis (1, 2); while several clinico-epidemiological studies suggested a possible role of this virus also in B-cell non-Hodgkin lymphomas (1, 2). Since HCV is an RNA virus that cannot be integrated in the host genome, it can exert its oncogenetic potential through indirect mechanisms with the possible contribution of genetic and enviromental factors. It may be supposable that the same oncogenetic potential can be also involved in the thyroid cancer complicating HCV-infected individuals with or without MC syndrome. Although the possible association between MC and thyroid cancer needs to be confirmed by further clinico-epidemiolgical studies, our preliminary observation indicate that a careful thyroid monitoring is opportune during the clinical follow-up of HCV-associated MC patients, above all in the presence of signs of thyroid autoimmunity.

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