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ORIGINAL ARTICLE

Incidence of autoimmune thyroiditis in interferon-α treated and untreated patients with chronic hepatitis C virus infection

Antonio Rocco,[1] Serena Gargano,[1] Alessia Provenzano,[1] Maria Rosaria Nardone,[1] Giuseppe Maria De Sanctis,[2] Nadia Altavilla,[2] Lucia Valeria Chircu[2] & Francesca Grimaldi [2]

1. Dept. of Medical Patophysiology and
2. Dept. Of Tropical and Infectious Diseases, University of Rome "La Sapienza", Italy.

Correspondence to: Antonio Rocco,
Dipartimento di Fisiopatologia Medica, Università di Roma "La Sapienza", Policlinico Umberto I, Viale del Policlinico, I-00161 Rome, Italy.
TEL: +39 0649970708 FAX: +39 06490530
E-MAIL: arocco48@libero.it

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Abstract

OBJECTIVES: To clarify the relationship between interferon-α (IFN-α) therapy and autoimmune thyroiditis in chronic hepatitis C virus (HCV) infection, we investigated a selected number of patients without basal thyroid dysfunctions.
MATERIALS AND METHODS: 130 patients (average age: 20–70), with chronic HCV infection and without basal clinical and laboratory signs of autoimmune thyroiditis were divided into two groups: IFN-α treated (A) and untreated (B) patients. Group A received IFN-α (three million U.I./3 times a week) for six months; group B was followed for the same period. Thyroid peroxidase and thyroglobulin autoantibodies were measured by radioimmunoassay; thyroid function was measured by radioimmunoassay (free thyroxine and triiodothyronine) and immunoradiometric assay (thyroid stimulating hormone).
RESULTS: After a 6-month period, thyroid autoantibodies positivity was documented in 21.1% of group A and in 10.3% of group B patients, both statistically relevant ($p<0.001$ and $p< 0.011$, respectively). The comparison between the two groups was not statistically relevant ($p=0.142$).
CONCLUSIONS: Our study showed a prevalence of de novo thyroid autoimmunity in chronic HCV patients treated with IFN-α, confirming previous data in literature. The lack of a significant difference between treated and untreated patients strongly suggests that the anti-thyroid autoimmune response is linked to the HCV infection itself. Moreover, IFN-α therapy probably does not represent a risk factor in renewing the autoimmune processes of the thyroid gland. Thyroid function and autoantibodies must be systematically monitored in patients with HCV infection, especially in female and IFN-α treated population, not only to verify the possible thyroid abnormalities but also to rule out concomitant autoimmune diseases.

ABBREVIATIONS AND UNITS:

IFN-α:	Interferon-alpha
HCV:	Hepatitis c virus
PCR:	Polymerase chain reaction
TPO Ab:	Thyroid peroxidase autoantibodies
TG Ab:	Thyroglobulin autoantibodies
TSH-RAb:	Thyroid stimulating hormone receptor antibodies
TSH:	Thyroid stimulating hormone
FT3:	Free triiodothyronine
FT4:	Free thyroxine
ALT:	Alanine aminotransferase
AMA:	Anti-mitochondrial antibodies
ANA:	Anti nuclear antibodies
ASMA:	Anti-smooth muscle antibodies
α-FP:	Alpha fetoglobulin
HIV:	Human immunodeficiency virus
ELISA:	Enzyme-linked immunosorbent assay
IRMA:	Immunoradiometric assay
RIA:	Radioimmunoassay
I.V.:	Intravenous
S.C.:	Subcutaneous
I.U.:	International units
M/F:	Male/female
SD:	Standard deviation
n.s.:	Not significant
n.d.:	Not detectable

Introduction

Hepatitis C virus (HCV) infection has been associated to several autoimmune diseases.

A high prevalence of thyroid autoantibodies and permanent thyroid disorders have been repeatedly documented in HCV patients.

During the last two decades, interferon (IFN) showed positive results in host resistance to viral infection and it is the only treatment which proved to be effective on hepatitis C in controlled trials.

IFN is a cytokine acting through different mechanisms such as the induction of antiviral state into cells, the stimulation of natural killer activity and T cell-mediated immunity, the cytokine synthesis and the activation of macrophage [1–3]. IFN has also been reported to cause different thyroid disorders such as hypothyroidism, hyperthyroidism, destructive thyrotoxicosis and impaired intrathyroidal iodide organification [4, 5].

Thyroid antibodies and disorders have been reported in patients with chronic hepatitis, both before and after IFN-α treatment. The frequency and clinical characteristics of such thyroid dysfunctions during IFN-α therapy are not uniform, ranging from the presence of thyroid autoantibodies [6] to manifested hyper- or hypothyroidism [7–11]. The changes in thyroid assessment usually appear after three months of therapy, but they can occur as long as IFN-α is administered [12, 13]. It remains to be elucidated whether the thyroid disorders reported in IFN-α treated HCV patients are a direct consequence of the therapy, part of the natural history of the disease itself or depend on both [14, 15].

To better clarify the relationship between IFN-α therapy and thyroid disorders, in terms of prevalence of autoimmune response, patients with chronic HCV infection after IFN-α treatment were investigated. Therefore, a selected number of HCV patients without hormonal and clinical signs of autoimmune thyroiditis were enrolled in the study.

Material and methods

179 consecutive patients (67 men, 112 women) with chronic hepatitis C as a retrospective follow-up study were investigated. Patients were divided into two groups: the group of those treated with IFN-α and the group of untreated patients. The inclusion criteria were the following: age between 20 and 70 years and a persistent increase in serum alanine aminotransferase activity (ALT) for a minimum of 6 months prior to therapy. Exclusion criteria were represented by other causes of chronic liver disease and seropositivity for human immunodeficiency virus (HIV).

All patients were positive for hepatitis C viral RNA and negative for hepatitis B surface antigen. Serum anti-HCV antibodies were assayed using a third-generation enzyme-linked immunosorbent assay (ELISA-III; Ortho Diagnostic Systems, Raritan, N.J., USA). HCV RNA was carried out by polymerase chain reaction (PCR) analysis with type specific primers within the core region of HCV genome, as described by Okamoto et al. [16]. ALT was assessed by enzymatic assay (Beckman, Galway, Ireland; normal values < 60 UI/ml). The genotype results were named according to Simmonds classification [17].

The treated group consisted of 67 patients (36 men and 31 women), who were administered s.c. IFN α-2a (Roferon A, Hoffman LaRoche, Nutley, N.J.) or IFN α-2b (Intron A, Schering-Plough, Kenilworth, N.J.) or lymphoblastoid IFN α-n1 (Wellferon, Wellcome, Triangle Park, N.C.) at the dose of 3 million UI, three times a week, for 6 months. A liver biopsy documented inflammatory disease activity at histologic level. Since 15 patients showed altered thyroid function and/or positive titre of thyroid autoantibodies before starting IFN therapy, only 52 patients (30 men and 22 women, mean age 51.6 ± 12.6 years) were considered eligible for the study (group A).

During IFN-α administration, negligible side effects were present in a minority of patients. Informed consent was obtained from each patient.

The untreated group consisted of 112 patients (31 men, 81 women). They were not treated because of old age (> 65 years), low cytolysis (ALT) index, low viral load at PCR, microcythaemia or lack of compliance. A liver biopsy showed cirrhosis in 14.1% of patients. Since 34 patients showed altered thyroid function and/or positive titre of thyroid autoantibodies, only 78 patients (23 men and 55 women, mean age 56.9 ± 11.6 years) were enrolled in the study and considered as controls (group B).

Thyroglobulin (TgAb) and thyroid peroxidase (TPOAb) autoantibodies, as well as thyroid stimulating hormone receptor antibodies (TSH-RAb) were systematically assessed in all patients with HCV, both in basal condition and after 6 months. Serum TgAb and TPOAb has been performed by RIA (Bioline-Belgium; normal values <100 U/mL). Thyroid function was also measured in all patients. Free thyroxine (FT_4) and free triiodothyroxine (FT_3) have been measured by RIA (commercial kit Ares-Serono, Italy); TSH detected by IRMA (Byk-Sangtec Diagnostic). The normal ranges for FT_3, FT_4 and TSH were 2.5–6 pg/ml, 0.6–1.8 ng/dl and 0.1–3.5 μUI/ml, respectively.

Sera from HCV patients were screened for the presence of AMA, ASMA and ANA using indirect immunofluorescence as in the previously-described assays [18].

Thyroid sonography, cytology and radionuclide scintigraphy were performed if indicated.

Statistical analyses by two-tailed paired Student's t-test and chi-squared test for comparisons of proportions were carried out. P values < 0.05 were considered as statistically significant. All data are expressed as mean ± SD.

Results

The clinical and laboratory characteristics of the HCV patients are shown in table 1.

Percentages achieved for AMA, ANA, ASMA and for cryoglobulin positivity are shown in table 2.

The prevalence of viral genotype is reported in table III. No mixed genotypes (1b + 2b) were documented. 23 patients (12.9%) had no detectable viremia (<1000 U/ml). In our series of patients there was a 64.8 percentage of 1b and 2a2c genotypes (table 3).

The hormonal parameters of HVC patients are documented in table IV. TSH, FT3 and FT4 values did not show remarkable differences between group A and group B. TSH values of group A, even if consid-

Table 1. Clinical and laboratory characteristics of HCV patients

	total	IFN-α treated	untreated	P
n°	179	67	112	
Enrolled in the study	130	52	78	
Sex (M/F)	53/77	30/22	23/55	
M/F ratio	0.68	1.36	0.41	
Mean age (years)	54.8±12.2	51.6±12.6	56.9±11.6	n.s.
Mean ALT (UI/l)	71.0±61.2	91.5±54.1	57.4±62.3	< 0.002
αFP (>10 ng/ml)	14 (10.8%)	5 (9.6%)	9 (11.5%)	n.s
Cryoglobulin +	12 (9.2%)	5 (9.6%)	7 (9.0%)	n.s.

Table 2. Positivity of ANA, AMA and ASMA autoantibodies in HCV patients

	TOTAL (n= 130)	Group A (n= 52) before and after IFN		Group B (n= 78)	P
ANA	17 (13.1%)	3 (5.8%)	4 (7.7%)	10 (12.8%)	n.s.
AMA	1 (0.8%)	0	0	1 (1.3%)	n.s.
ASMA	3 (2.3%)	0	2 (3.8%)	1 (1.3%)	n.s.

Table 3. Viral genotypes of HCV patients

Total	1b	2a2c	3a	2a	1a	1a1b	4	N.D.
179	79	37	13	10	6	6	5	23
%	44.1	20.7	7.3	5.6	3.3	3.3	2.8	12.9

Tab. 4. Hormonal parameters in HCV patients

	total		A group		B group	
	Basal	6 months	basal	6 months	basal	6 months
TSH	1.73±2.05	1.90±6.69	1.90±2.45	2.71±10.37	1.62±1.74	1.37±1.72
FT3	3.14±0.98	3.23±1.07	3.28±0.78	3.18±0.87	3.07±1.05	3.27±1.18
FT4	1.18±0.53	1.22±0.37	1.21±0.74	1.18±0.28	1.17±0.34	1.25±0.42

Tab. 5. Prevalence of thyroid autoantibodies in HCV patients (A and B groups) in basal condition and after 6 months

	basal	6 months	P=
Group A			
TgAb	0/52	3/52 (5.7%)	0.255
TPOAb	0/52	5/52 (9.7%)	0.069
TgAb + TPOAb	0/52	3/52 (5.7%)	0.255
TOTAL	0/52	11/52 (21.1%)	0.001
Group B			
TgAb	0/78	6/78 (7.7%)	0.039
TPOAb	0/78	1/78 (1.3%)	0.994
TgAb + TPOAb	0/78	1/78 (1.3%)	0.994
TOTAL	0/78	8/78 (10.3%)	0.011
Group A vs. Group B			0.146

erably higher after 6 months of IFN-α therapy, were not statistically significant (p = 0.585), table 4.

Autoantibodies (only TgAb or only TPOAb or TgAb and TPOAb) positivity is shown in table V.

Females did not show any significant difference in TPOAb, TgAb or both TPOAb + TgAb positivity in comparison to males (5/22 vs. 6/30 which means 22.7% vs. 20.0% in group A and 6/55 vs. 2/23, that is 10.9% vs. 8.7% in group B), table 5.

TSH-Rab were negative in both groups.

All the HCV patients treated with IFN-α had normal serum free T3, free T4 and TSH levels with the exception of 4 patients. They were consistent with one case of subclinical hypothyroidism, 2 cases of hypothyroidism and one case of subclinical hyperthyroidism. A single case of hypothyroidism is still in substitutive therapy.

Discussion

The study aimed at verifying the possible influence of IFN-α therapy in inducing clinical and laboratory signs of altered thyroid function, in terms of thyroid autoantibodies positivity.

All patients enrolled were of Caucasian and Italian origin, with a prevalence of female patients (77/130, 59.2%).

1b genotype was prevalent (44.1%). No HCV mixed (1b–2b) infections were documented, excluding HCV reinfection as potential pathogenetic mechanism of autoimmune thyroiditis, as reported in some Asian populations [19, 20]. The prevalence of thyroid dysfunctions in our HCV patients is given by the number of patients who were not enrolled in the final study (49/179, 27.3%). This high percentage, which is quite

different from the one found by other AA. [21, 22], could be related to the higher number of women.

130 HCV patients were enrolled and followed for a 6 month period. This follow-up period allowed us to verify the influence of the immunomodulatory compound in 52 patients and document the evolution of the thyroid function in 78 subjects never administered IFN-α therapy.

There was no evidence of clinical signs of systemic autoimmune disease in HCV patients positive for non-organ-specific autoantibodies in basal condition or during IFN-α therapy.

After 6 months of IFN-α administration, 21.1% of patients showed a statistically significant thyroid autoantibodies positivity, confirming that the compound is able to induce a *de novo* thyroid autoimmune process as also reported by other AA. [23]. Since our HCV patients were selected on the basis of the absence of thyroid autoantibodies positivity, the positive correlation between IFN-α therapy and the appearance of TGAb and TPOAb titres seems to substantiate the hypothesis that the autoimmune reactions found in our HCV patients are likely due to the immunomodulatory properties of IFN-α rather than to the exacerbation of pre-existing thyroid abnormalities.

After 6 months, 10.3% of patients who did not assume IFN-α therapy also showed a statistically significant autoimmune thyroid reaction. It is difficult to give a reliable explanation to this finding.

Since viral proteins of the hepatitis C virus may share amino acid sequence homology with those of thyroid antigens HCV patients could develop a predisposition to the autoimmune reaction through the mechanism of molecular mimicry [15, 24], even though the absence of cross-reaction between thyroid autoantibodies and anti-HCV has been reported by other authors [25]. According to this hypothesis, HCV infection could induce the autoimmune thyroid process by mimicking the structure of some components of the thyroid tissue [22]. Furthermore, it cannot be excluded that the autoimmune thyroid response could be related to the localization of hepatitis C virus into the thyroid, as suggested by Preziati et al. [22], but this remains to be confirmed. Another possible explanation could be given by the presence of a high number of females (70.5%), who show a higher natural incidence of autoimmune reactions if compared to the male population [26, 27].

The age of patients seems of negligible importance. In fact, in our case study the mean age of female and male patients is similar. Therefore, the observation that the prevalence of thyroid autoantibodies in ordinary general population usually increases with age [28–32] does not seem to be confirmed in our population. The lack of a statistically significant difference between autoantibodies positivity in treated and untreated patients adds further support to the hypothesis that IFN-α therapy does not represent a risk factor in inducing the autoimmune processes of the thyroid gland. However, the strict temporal correlation between IFN-α therapy and the development of thyroid autoantibodies seems to underline a close relationship between IFN-α and autoimmune processes.

It is still debated whether the thyroid dysfunction observed after IFN-α therapy is temporary or long-lasting, as some reports found the effects reversible [33, 34] while others found them long lasting [22, 35]. It has been reported that patients who developed permanent thyroid disorders showed high anti-thyroid autoantibodies before treatment [22, 36] although our data do not seem to be in line with this hypothesis. One out of 4 patients who developed hypothyroidism is still being administered substutive therapy. Indeed, thyroid function, at least autoantibodies titre and TSH, must be systematically monitored in patients with HCV infection, especially in the female population and in those who are administered IFN-α therapy, not only to verify the possible thyroid abnormalities but also to rule out concomitant autoimmune diseases.

REFERENCES

1 Baron S, Tyring SK, Fleischmann Jr WR, Coppenhaver DH, Nielsel DW, Klimpel GR, et al. The interferons. Mechanisms of action and clinical applications. J Amer Med Assoc 1991; **266**:1375–83.
2 Yoshie O, Mellman IS, Broeze RJ, Garcia-Blanco M, Lengyel P Interferon action: effects of mouse α and β-interferons on rosette formation, phagocytosis, and surface-antigen expression of cells of macrophage-type line RAW309Cr 1. Cell Immunol 1992; **73**:128–40.
3 Itoh Y, Okanoue T, Enjyo F , Morimoto M, Takeuchi T, Kagawa K, et al. Elevated interleukin-6 and γ-globulin during interferon therapy for hepatitis B. Amer J Gastroenterol 1992; **87**:1485–7.
4 Marcellin P, Pouteau M and Benhamou JP. Hepatitis C virus infection, α-interferon therapy and thyroid dysfunction. J Hepatol 1995; **22**:364–9.
5 Roti E, Minelli R, Giuberti T, Marchelli S, Schianchi C, Gardini E, et al. Multiple changes in thyroid function in patients with chronic active HCV hepatitis treated with recombinant interferon-α. Am J Med 1996; **172**:482–7.
6 Fonesca V, Thomas M & Duseiko G. Thyrotropin receptor antibodies following treatment with recombinant α-interferon in patients with hepatitis. Acta Endocrinol 1991; **125**:491–3.
7 Burman P, Totterman TH, Oberg K, Karlsson FA. Thyroid

autoimmunity in patients on long term therapy with leukocytes-derived interferon. J Clin Endocrinol Metab 1986; **63**:1086–90.
8 Schultz M, Muller R, Von zur Muheln A and Brabant G. Induction of hyperthyroidism by interferon-α2b. Lancet 1989; **1**:1452–6
9 Lesker-Melman M, Di Bisceglie AM, Usala SJ, Weintraub B, Murray LM and Hoffnagle JH. Development of thyroid disease during therapy for chronic viral hepatitis with interferon-α. Gastroenterol 1992; **102**:2155–60.
10 Koh LK, Greenspan FS, Yeo PP. Interferon-α induced thyroid dysfunction: three clinical presentations and a review of the literature. Thyroid 1997; **7**:891–6.
11 Reddy KR, Krawitt EL, Jacobsen I et al. Thyroid dysfunction in chronic hepatitis C and following interferon-α2a treatment. Hepatology, 1997; **26**:368A
12 Deutsch M, Dourakis S, Manesis EK, Gioustozi A, Hcss G, Horsch A, et al. Thyroid abnormalities in chronic viral hepatitis and their relationship to interferon-α therapy. Hepatology 1997; **26**:206–10.
13 Metcalfe RA, Ball G, Kudesia G and Weetman AP. Failure to find an association between hepatitis C virus and thyroid autoimmunity. Thyroid 1997; **7**:421–4.
14 Marazuela M. Lymphocyte traffic and homing in autoimmune thyroid disorders. Eur J Endocrinol 1999; **29**:1890–1900.
15 Huang MJ, Tsai SL, Huang BY, Sheen IS, Yeh CT and 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.
16 Okamoto HY, Sugiyama Y, Okada S. Typing hepatitis C virus by polymerase chain reaction with type-specific primers: applications to clinical surveys and tracing infection source. J Gener Virol 1992; **73**:673.
17 Simmonds P. Variability of hepatitis C virus. Hepatology 1995; **21**:570–83.
18 Meroni PL, De Bartolo G, Barcellini W, Riboldi PS, Basile R, Zanussi C. Anti-ribosomal ribonucleoprotein autoantibodies in systemic lupus erythematosus. J Clin Immunol 1984; **1**; 145–54.
19 Dusheiko G, Schmilowitz-Weiss H, Brown D, Mc Omish F, Yap PL, Sherlock S, et al. Hepatitis C virus genotypes: an investigation of type-specific differences in geographic origin and disease. Hepatology 1994; **19**:13–8.
20 Kao JH, Chen PJ, Lai MY, Yang PM, Sheu JC, Wang TH et al. Genotypes of hepatitis C virus in Taiwan and the progression of liver disease. J Clin Gastroenterol 1995; **21**:233–7.
21 Tran A, Quaranta JF, Benzaken S, Thiers V, Chau HT, Hastier P, et al. High prevalence of thyroid autoantibodies in a prospective series of patients with chronic hepatitis C before interferon therapy. Hepatology 1993; **18**:253–7.
22 Preziati D, Rosa LL, Covini G, Marcelli R, Rescalli S, Persani L, et al. Autoimmunity thyroid function in patients with chronic active hepatitis treated with recombinant interferon α-2a. Eur J Endocrinol 1995; **132**:587–93.
23 Marazuela M, Garcia-Buey L, Gonzalez-Fernandez B, Garcia-Monzon C, Arranz A, Borque MJ, et al. Thyroid autoimmune disorders in patients with chronic hepatitis C before and during interferon-α therapy. Clin Endocrinol 1996; **44**:635–42.
24 Wucherpfennig KW and Strominger JL. Molecular mimicry in T cell-mediated autoimmunity: viral peptides activated human T cell clones specific for myelin basic protein. Cell 1995; **80**:695–705.
25 Quaranta IF, Tran A, Regnier D, Letestu R, Beusnel C, Fuzibet JG, et al. High prevalence of autoantibodies to hepatitis-C virus (HCV) in patients with anti-thyroid autoantibodies. J Hepatol 1993; **18**:136–8.
26 Hawkins BR, Cheah PS, Dawkins RL, Whittigham S, Burger H, Patel Y, et al. Diagnostic significance of thyroid microsomal antibodies in randomly selected population. Lancet 1980; **2**:1057–9.
27 Ansar Ahmed S, Penhale WJ, Talal N. Sex hormones, immune responses, and autoimmune disease: mechanism of sex hormone action. Am J Pathol 1985; **121**:531–51.
28 De Groot LJ, Reed Larsen P, Refetoff S, Stanbury JB. The thyroid gland disease. In: The thyroid and its disease. New York: Wiley & Sons; 1984. p. 125.
29 Mariotti S and Pinchera A. Role of the immune system in the control of thyroid function. In: Greer MA, editor. Thyroid gland. New York: Raven Press; 1990. p. 147.
30 Davies TF. Human autoimmune thyroid disease. Newer views on commonbreakdown in T cell tolerance. Israel J Med Sci 1994; **30**:2–11.
31 Weetman AP. Thyroid autoimmune disease. In: Braverman LE & Utiger RD, editors. The thyroid: a fundamental and clinical text. Philadelphia: Werner & Ingbar's, Lippincott-Raven; 1996. p. 1295.
32 Tunbridge WMG, Evered DC, Hall R, Appleton D, Brewis M, Clark F, et al. The spectrum of thyroid disease in a community: the Whickham survey. Clin Endocrinol 1997; **47**:481–93.
33 Imagawa A, Itoh N, Hanafusa T, Oda Y, Waguri M, Miyagawa J, et al. Autoimmune endocrine disease induced by recombinant interferon α therapy for chronic active type C hepatitis. J Clin Endocrinol Metab 1995; **80**:922–26.
34 Baudin E, Marcellin P, Pouteau M, Colas-Linhart N, Le Floch JP, Lemonnier C, et al. Reversibility of thyroid dysfunction induced by recombinant α interferon in chronic hepatitis C Clin Endocrinol 1993; **39**:657–61.
35 Marcellin P. Treatment of chronic hepatitis C with alpha-interferon. Eur J Med 1992; **1**:194–5.
36 Ronnblom LE, Alm GV, Oberg KE. Autoimmunity after α-interferon therapy for malignant carcinoid tumor. Ann Int Med 1991; **115**:178–83.