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

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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 (FT₄) and free triiodothyroxine (FT₃) have been measured by RIA (commercial kit Ares-Serono, Italy); TSH detected by IRMA (Byk-Sangtec Diagnostic). The normal ranges for FT₃, FT₄ 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-

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**Table 1. Clinical and laboratory characteristics of HCV patients**

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

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

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

**Table 3. Viral genotypes of HCV patients**

<table>
<thead>
<tr>
<th>Total</th>
<th>1b</th>
<th>2a2c</th>
<th>3a</th>
<th>2a</th>
<th>1a</th>
<th>1a1b</th>
<th>4</th>
<th>N.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>179</td>
<td>79</td>
<td>37</td>
<td>13</td>
<td>10</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>23</td>
</tr>
<tr>
<td>%</td>
<td>44.1</td>
<td>20.7</td>
<td>7.3</td>
<td>5.6</td>
<td>3.3</td>
<td>3.3</td>
<td>2.8</td>
<td>12.9</td>
</tr>
</tbody>
</table>

**Table 4. Hormonal parameters in HCV patients**

<table>
<thead>
<tr>
<th>Basal</th>
<th>6 months</th>
<th>Total</th>
<th>A group</th>
<th>B group</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH</td>
<td>1.73±2.05</td>
<td>1.90±6.69</td>
<td>1.90±2.45</td>
<td>2.71±10.37</td>
</tr>
<tr>
<td>FT3</td>
<td>3.14±0.98</td>
<td>3.23±1.07</td>
<td>3.28±0.78</td>
<td>3.18±0.87</td>
</tr>
<tr>
<td>FT4</td>
<td>1.18±0.53</td>
<td>1.22±0.37</td>
<td>1.21±0.74</td>
<td>1.18±0.28</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Group A</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>TgAb</td>
<td>0/52</td>
<td>3/52 (5.7%)</td>
<td>0.255</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TPOAb</td>
<td>0/52</td>
<td>5/52 (9.7%)</td>
<td>0.069</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TgAb + TPOAb</td>
<td>0/52</td>
<td>3/52 (5.7%)</td>
<td>0.255</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>0/52</td>
<td>11/52 (21.1%)</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group B</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>TgAb</td>
<td>0/78</td>
<td>6/78 (7.7%)</td>
<td>0.039</td>
</tr>
<tr>
<td>TPOAb</td>
<td>0/78</td>
<td>1/78 (1.3%)</td>
<td>0.994</td>
</tr>
<tr>
<td>TgAb + TPOAb</td>
<td>0/78</td>
<td>1/78 (1.3%)</td>
<td>0.994</td>
</tr>
<tr>
<td>TOTAL</td>
<td>0/78</td>
<td>8/78 (10.3%)</td>
<td>0.011</td>
</tr>
</tbody>
</table>

**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 substitute 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
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