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Thyroid Autoantibodies and Thyroid Dysfunction During Treatment with Interferon-α for Chronic Hepatitis C

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Interferon-α $_{(2a \text{ or } 2b)}$ is increasingly used for treatment of chronic hepatitis C virus (HCV) infection. Recent reports suggested a correlation between increases in thyroid autoantibodies and the development of thyroid dysfunction during interferon-α therapy. In this study, we analyzed thyroid hormones and antithyroid antibodies at monthly intervals in 53 patients who received interferon α for chronic active hepatitis C infection. Of five patients with initially elevated levels of antithyroid peroxydase antibodies (anti-TPO), the antibodies increased further in two of them. Ten patients, who started interferon therapy with normal antibody levels, developed elevated anti-TPO antibodies for limited times during treatment. Levels of anti-TPO antibodies showed a marked fluctuation, and only three patients had increased anti-TPO antibodies persisting for longer than 3 mo. Antithyroglobulin antibodies appeared in four patients, all of whom were also positive for anti-TPO antibodies. No changes in TRAB levels were observed. All of these patients with elevated antithyroid antibodies remained in an euthyroid state. One patient with normal antithyroid antibodies developed thyroiditis with severe thyrotoxicosis after 9 wk of interferon therapy. These findings suggest that the induction of antithyroid antibodies during treatment with interferon-α does not indicate clinical relevant thyroid dysfunction. Routine measurement of antithyroid antibodies during interferon-α therapy does not seem to be mandatory.

Key Words: Thyroid autoantibodies; interferon; chronic hepatitis C.

Introduction

Treatment of chronic hepatitis C virus (HCV) infection with interferon-α is currently the only therapeutic option with potency to cure the disease *(1–3)* and, thus, to prevent the development of liver cirrhosis and hepatocellular carcinoma *(4)*. The widespread use of this therapy has provided a further insight into pathophysiological mechanisms in response to viral infection, but substantial side effects have been encountered *(1,5,6)*. Among these, thyroid dysfunction has been observed in 4–12% of patients *(7,8)* and leads to discontinuation of interferon therapy. Hypothyroidism, thyrotoxicosis, and a biphasic course have been described *(9,10)*. It is suspected that the immunomodulatory potency of interferon-α leads to an increase in formerly present levels of antithyroid antibodies or to a new expression of these antibodies (antithyroid peroxydase [anti-TPO] and antithyroglobulin [anti-TG]), for which an incidence of up to 40% has been reported in one study *(11)*. Antibodies against the TSH receptor (TRAB) were detected in only a few cases. It is unknown, however, if the development of these autoantibodies precedes the development of thyroid dysfunction. Since the increase of antithyroid antibodies during interferon therapy might unwarrantedly restrict this treatment, we investigated whether the presence of antithyroid antibodies or an increase of these autoantibodies during interferon-α treatment is correlated with the development of thyroid dysfunction.

Results

In all patients, thyroid hormone levels and TSH were normal before treatment, and no one had a history of thyroid disease. All patients included into this study had histologically confirmed chronic active hepatitis, and in seven patients (13%) a mild cirrhosis was found. No one had liver dysfunction. The duration of HCV infection before interferon therapy could be estimated in only 43% of all patients, with times ranging from 2 to 25 yr. Interferon therapy had to be discontinued after 2 mo in one patient because of severe depression, and in two patients because of fever and malaise after 2 and 4 mo, respectively. During interferon therapy, no patient complained of symptoms that could be unequivocally ascribed to hypothyreosis. Additionally, we did not observe enlargement of the thyroid gland or eye symptoms. In all patients, thyroid hormones and TSH remained in the normal range, except

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in one patient. A 23-yr-old female developed severe thyrotoxicosis 9 wk after starting interferon treatment. She complained of weight loss, weakness, and tachycardia, but no local thyroid or eye symptoms were reported or observed. Serum levels of thyroid hormones increased to a maximum of 240 ng/mL (T4), 5 ng/mL (T3), and 6 ng/mL free (T4) with a concomitant decrease of TSH levels below limit of detection. Antithyroid antibodies, especially TRAB levels, were normal before interferon therapy and remained so when thyrotoxicosis occurred. Radionuclide scanning with ^{99m}Tc revealed complete absence of ^{99m}Tc uptake, whereas ultrasonography showed normal size and echogenity of the thyroid gland. A fine-needle aspirate showed regressive and degenerative changes of thyrocytes with only minor infiltration of inflammatory cells.

Immediately after detection of thyrotoxicosis, therapy with carbimazol was initiated, but treatment with interferon was continued because HCV-RNA was eliminated 4 wk before the onset of thyrotoxicosis. Under this regimen, levels of thyroid hormones could not be normalized. Finally, interferon had to be withdrawn after a total of 11 wk of interferon therapy, and a treatment with carbimazol and prednisolone was begun, under which the thyroid hormone levels returned to normal.

Before interferon therapy, anti-TPO antibodies were within the normal range in 48 patients (90.6%). In five patients (9.4%), four males and one female, elevated levels of anti-TPO autoantibodies were present before interferon therapy. The initial antibody levels ranged from 109 to 314 U/mL, which is slightly to moderately elevated above the normal limit (<100 U/mL). In two of these patients, the antibody levels increased further and persisted for longer than 3 mo (Fig. 1), with a more pronounced elevation during the later course of therapy. Antibody levels in one patient normalized under interferon therapy. In two patients of this group, interferon therapy had to be stopped after 2 and 4 mo, respectively, because of side effects other than thyroid dysfunction (fever, depression).

As shown in Fig. 2, 10 patients (9 males, 1 female) developed de novo anti-TPO antibodies during interferon therapy. The increase in antibody levels was moderate in 9 of 10 patients. Only one patient had a more pronounced increase during the later course of interferon therapy. The antibody levels showed a marked fluctuation, with only three patients in this group having elevated anti-TPO antibodies for longer than 3 mo. Neither in this group nor in the group of patients with initially elevated anti-TPO antibodies could a correlation be found between antibody levels and histological findings or liver function.

Anti-TG autoantibodies were undetectable in all patients before interferon therapy. Four patients (three males, one female) developed increased levels of anti-TG antibodies (Fig. 3), and all of these patients consecutively had increased levels of anti-TPO autoantibodies. In two patients, the anti-TG antibodies persisted for longer than 3 mo. Both

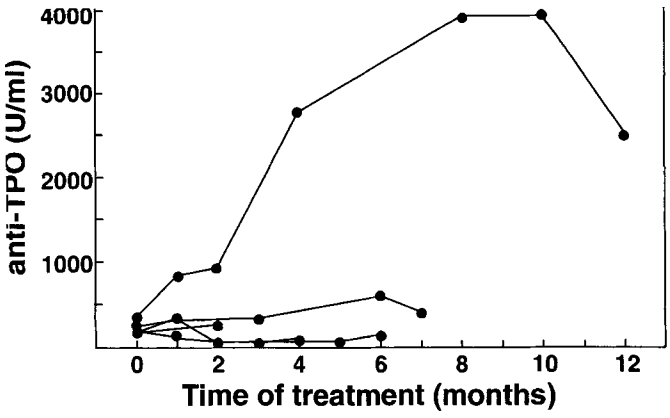

Fig. 1. Time-course of anti-TPO antibodies in five patients with elevated anti-TPO antibodies before interferon therapy. Normal values of anti-TPO antibodies are below 100 U/mL.

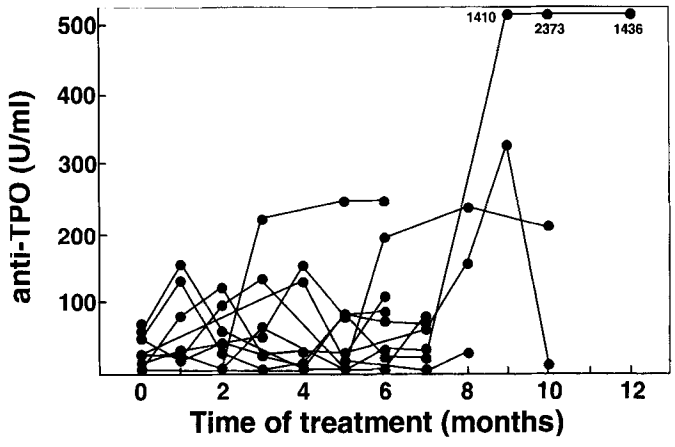

Fig. 2. Time-course of anti-TPO antibodies in 10 patients with normal anti-TPO antibodies before interferon therapy and elevated antibody during therapy. Normal values of anti-TPO antibodies are below 100 U/mL.

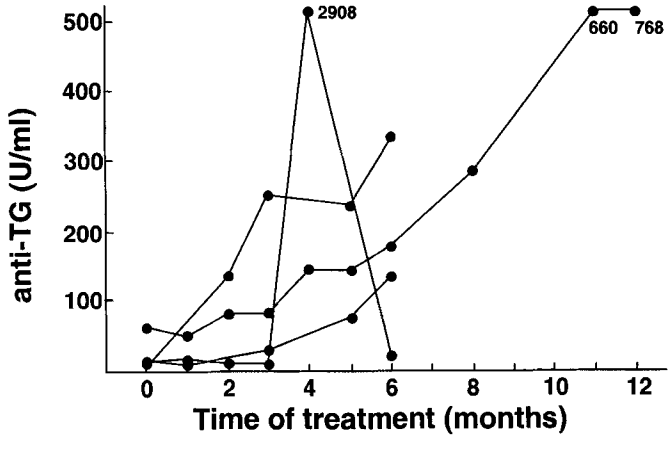

Fig. 3. Course of anti-TG antibodies in four patients with initially normal anti-TG antibodies and elevated antibody levels under interferon therapy. Normal values of anti-TG antibodies are below 100 U/mL.

patients had simultaneously elevated levels of anti-TPO antibodies for the same length of time. Overall, in 5 of 53 patients (9.4%) elevated thyroid autoantibodies were detectable for at least 3 mo, whereas the increases in all other patients were only transient. As mentioned above, the patients remained in an euthyroid state despite changes in thyroid autoantibodies. Levels of TRAB remained in the normal range in all 53 patients during interferon therapy (data not shown).

Discussion

The induction of thyroid autoantibodies during interferon-α therapy is a well-known phenomenon. De novo appearance or increasing levels of pre-existing antithyroid antibodies have been reported in up to 40% of patients treated for chronic hepatitis C *(7,11)*. Furthermore, a wide range of elevated autoantibodies other than thyroid autoantibodies have been reported, including antismooth muscle cell, antiparietal cell, or antinuclear antibodies *(5)*. The expression of autoantibodies under interferon-α is thought to be mediated by enhanced expression of MHC class I antigens on various target tissues *(12)*. This alone or in addition to inappropriate expression of MHC class II antigens *(13)* results in presentation of normal cell-surface antigens to activated lymphocytes. Thyroid dysfunction then might be caused by activation of cytotoxic lymphocytes or by stimulatory or blocking TSH-receptor autoantibodies.

In our study, 9.4% of the patients had elevated antibody levels before starting interferon therapy. In 18.8% of the patients, elevated thyroid autoantibody levels were detectable at some time during interferon therapy, giving a total of 28.2% with abnormal thyroid autoantibodies. The antibody levels did not correlate with liver function or histological findings. Since we were unable to determine the time of infection in most of our patients, we could not analyze this parameter in thyroid autoantibody expression. The only parameter associated with persistently elevated antibody levels was the duration of interferon treatment.

In the present study, it is important to note that only five of our patients (9.4%) had elevated levels of autoantibodies for longer than 3 mo. All other patients with elevated thyroid autoantibodies showed a marked fluctuation in antibody levels, being within normal limits for most of the time of interferon therapy. Taking into account the transient nature of the increased antibody levels in most of the observed cases, we judged only those patients as antibody-positive who had a persistent elevated antibody level for longer than 3 mo. This resulted in the low rate of 9.4% antibody-positive patients, which is less than the rates reported by others, ranging from 12.5 *(14)* to 42.3% *(11)*. The low incidence of antithyroid antibodies compared to other studies may be attributed to different laboratory methods and/or the shorter observation intervals in our study. It is well documented that the older assays for antithyroid antibodies, especially hemagglutination tests for antimicrosomal antibodies, had a high rate of false positive results compared to recent anti-TPO test systems *(15)*. Furthermore, most studies tested serum for antithyroid antibodies at only four time-points during interferon therapy. Thus, a transient elevation of antithyroid antibodies may have been overestimated. Because of closer monitoring of our patients at 4 wk intervals, we found the increase in antithyroid antibodies to be transient in 66% of antibody-positive patients (10 of 15 patients). This might explain the low rate of 9.4% patients with persistent thyroid autoantibodies in our study.

Clinical overt thyroid dysfunction was reported in patients with interferon α therapy ranging from 4 *(8)* to 12% *(16)*, with hypothyroidism occurring at a slightly higher frequency than thyrotoxicosis. The lowest frequency of thyroid dysfunction was found in studies in which thyroid function was assessed exclusively on clinical grounds.

When thyroid function tests were performed systematically, a higher frequency of thyroid dysfunction was found. In most patients with thyroid dysfunction, antithyroid antibodies were elevated, but only few reports presented data on individual follow-up of these patients. In our study with clinical and biochemical evaluation of thyroid function at monthly intervals, no patient with elevated antibodies developed thyroid dysfunction. The one patient who developed thyrotoxicosis had no elevation in antibody levels. The incidence of thyroid dysfunction in our patients is in the lower range compared to other reports. Theoretically, there could be genetic or nutritious factors, in addition to parameters related to HCV infection, having an influence on the susceptibility for thyroid dysfunction, but we are not aware of such circumstances in our patients, which could make them different to other study populations. Another possible cause for the low incidence of thyroid dysfunction, although apparently not of relevance in most other studies, is that the development of thyroid dysfunction in patients with thyroid autoantibodies might occur after a longer latent period than the follow-up time in the present study. Lisker-Melmann et al. *(8)* reported a similar low incidence of thyroid dysfunction (4%), and in two of their patients, thyroid dysfunction occured 2 mo after the end of treatment. Thus far, none of our patients developed thyroid dysfunction after the end of therapy.

The hyperthyroidism observed in one of our patients appears to be the result of interferon-induced thyroiditis, since no definite findings for Hashimoto's or Graves' disease were observed. In addition to the negative TRAB assay, which measures TSH-binding inhibitory immunoglobulins at high specificity *(17)*, we found absence of ^{99m}Tc uptake in this hyperthyroid patient. Anti-TSH receptor antibodies (TRAB) have rarely been studied in patients under interferon therapy, and we are aware of only

one report *(8)* describing a patient with hyperthyroidism similar to our patient, without any detectable antithyroid autoantibodies. TRAB antibodies could be divided in stimulatory and blocking TSH-receptor antibodies. The TRAB assay used in this study does not discriminate between stimulatory or blocking TSH-receptor antibodies, but it detects the vast majority of either antibody. For future studies, in addition to the TRAB assay, measurement of anti-thyroid antibodies with stimulatory or blocking action on the TSH receptor seems to be of interest, especially to clarify the nature of hyperthyroidism under interferon therapy.

In conclusion, our data show a low rate of persistently elevated antithyroid antibodies (anti-TPO, anti-TG) in patients treated with interferon-α for chronic hepatitis C. At least in this population, the appearance of antithyroid antibodies is mostly transient and not predictive of the development of thyroid dysfunction. For practical purposes, we would suggest monitoring thyroid function only by measurement of TSH, T3, and fT4. Regular determinations of antithyroid antibodies seem to be of no value during interferon-α therapy for chronic hepatitis C.

Materials and Methods

Patients and Treatment

Fifty-three patients with chronic hepatitis C (37 men and 16 women) undergoing therapy with interferon-α_{2a} (Roferon®, Hoffman LaRoche) or interferon-α_{2b} (Intron A®, Schering) were investigated. Diagnosis of chronic active hepatitis C was established by documented long-term elevation of serum alanine aminotransferase levels, positive serological testing for HCV antibodies (using a second-generation enzyme linked immunosorbent assay), and HCV-RNA by polymerase chain reaction. Histological examination of liver biopsies confirmed chronic active HCV hepatitis. Other causes for chronic hepatitis were excluded. Interferon-α was administered subcutanously at initial doses of 6 Mega units (MU) three times weekly. Doses were reduced to 3 MU after 3 mo, when serological response was documented. Interferon doses were increased to 9 MU three times per week, and therapy was prolonged to a total duration of 12 mo when viral breakthrough occurred. Interferon therapy was terminated when therapy did not eliminate the virus after 3 mo or failed to reduce elevated liver enzymes, when patients reported severe and persisting side effects, or after 6 mo of therapy, when remission was achieved. Blood was obtained before and at 4 wk interval during the treatment and analyzed for viral parameters, liver enzymes, TSH, thyroid hormones, and thyroid autoantibodies. In addition, the patients were assessed clinically for signs and symptoms of thyroid dysfunction.

T3, fT4, and TSH were measured using an enzyme immunoassay (Abbot, Germany), and T4 was determined by a fluorescence-photometric immunoassay (Abbot, Germany). Anti-Tg and anti-TPO were measured with an immunoluminometric and a competitive luminescence immunoassay, respectively (Brahms, Germany). TSH receptor autoantibodies (TRAB) were determined with a radioimmunoassay (Brahms, Germany). Normal values for anti-TPO, anti-TG, and TRAB were <100 U/mL, <100 U/mL, and < 10 U/L, respectively.

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