CLINICAL STUDY

Serum CXCL10 levels and occurrence of thyroid dysfunction in patients treated with interferon- α therapy for hepatitis C virus-related hepatitis

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Abstract

Objective: Thyroid autoimmunity is a common side effect of interferon- α (IFN- α) treatment for chronic hepatitis C. There are currently no reliable parameters to predict the occurrence of thyroid dysfunctions in patients undergoing IFN- α therapy. CXC chemokine ligand 10 (CXCL10) is a chemokine known to play a role in both thyroid autoimmune disease and hepatitis C virus (HCV) hepatitis.

Design: The aim of this study was to evaluate serum CXCL10 levels in HCV patients treated with IFN- α in relation to the occurrence of thyroid dysfunctions. Serum CXCL10 levels were assayed in 25 HCV patients (proven to be negative for serum thyroid antibodies) before and during IFN- α therapy (2, 4 and 6 months) and in 50 healthy controls. HCV patients were retrospectively selected according to the occurrence of IFN- α -induced thyroid dysfunction and were assigned to two groups. Group I included 15 patients who did not develop thyroid antibody positivity or dysfunction; group II included ten patients who showed the appearance of serum thyroid antibodies, followed by clinically overt thyroid dysfunction.

Results: Patients with HCV, regardless of the development of thyroid dysfunctions, had significantly higher serum CXCL10 than controls (261.6 ± 123.4 vs 80.4 ± 33.6 pg/ml; $P < 0.00001$). Pretreatment mean serum CXCL10 levels were significantly higher in Group I versus Group II (308.6 ± 130.7 vs 191.1 ± 69.4 pg/ml; $P < 0.05$). Groups I and II showed different rates of favourable response to IFN- α treatment (33 and 90% respectively).

Conclusion: Our results suggest that measuring serum CXCL10 before IFN- α treatment may be helpful for identifying those patients with higher risk to develop thyroid dysfunction, and require a careful thyroid surveillance throughout the treatment.

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Introduction

Interferon- α (IFN- α) has become the standard therapy for chronic hepatitis C, inducing a biochemical (persistent normalisation of serum alanine-amino-transferase) and virological (sustained negative results for hepatitis C virus (HCV) RNA) response in about 15–25% of treated patients (1). It has been suggested that HCV might share partial sequences in a few amino acid segments with thyroid tissue antigens (2) and that virus-related factors, mainly HCV infection itself, might predispose to the development of thyroid autoimmune disease (3–5). In accordance with this hypothesis, some viral features (infection with mixed HCV genotypes and lower HCV RNA levels) have been reported to be associated with an increased risk for developing thyroid

disease (6). Thyroid autoimmunity has been widely reported, also as a side effect of interferon-treatment, with a frequency ranging from 2.5 to 45.3% (7–10), but its pathophysiological mechanisms remain still unclear.

Chemokines are a recently identified family of cytokines that induce the chemotaxis of different leukocyte subtypes (11). The major function of chemokines is the recruitment of leukocytes to inflammation sites, but they also play a role in tumoural growth, angiogenesis and organ sclerosis (12, 13). At present, more than 50 chemokines have been described, which have been classified into four major families (11, 14). Among chemokines of the CXC family, CXC chemokine ligand 10 (CXCL10) plays an important role in several human autoimmune and non-autoimmune diseases,

including chronic autoimmune Hashimoto's thyroiditis (HT), Graves' disease (GD) and HCV-related hepatitis (14–18). HCV infection induces an acute and chronic liver inflammation through an immune-mediated pathway that may lead to cirrhosis and liver failure (19). HCV-related hepatitis is characterised by a dramatic lymphocyte infiltration into the liver, mainly comprised of HCV non-specific cells. Several data indicated that IFN- γ secretion by intrahepatic lymphocytes may drive non-specific cell homing to the liver. This effect is mediated by CXCL10 production. Several clinical studies evaluated the changes of serum CXCL10 in patients with HCV hepatitis undergoing IFN- α therapy (18, 20), showing that a successful virological response to IFN- α therapy is characterised by a marked decrease in the intrahepatic, as well as in the serum, levels of CXCL10. Investigations concerning a possible relation between serum chemokines and treatment outcome in patients with HCV-hepatitis demonstrated that lower pretreatment levels of CXCL10 are significantly associated with a favourable therapeutic response to IFN- α . This finding indicated a role of CXCL10 measurement in patients with HCV-hepatitis for predicting a favourable response to the treatment with IFN- α (21).

The issue of IFN- α -related thyroid dysfunction has been widely investigated in patients with HCV hepatitis (22). Despite previous studies reporting that the risk for developing thyroid dysfunction during IFN- α therapy is closely correlated with mixed HCV genotype infection and lower HCV RNA levels, female gender, pretreatment positivity for thyroid antibodies (particularly TPO Ab) and a hypoechoic pattern of the thyroid gland at ultrasound, none of the above features has enough specificity and sensitivity to reliably predict the occurrence of thyroid dysfunction (22).

The aim of the present study was to evaluate pretreatment and on-treatment serum levels of CXCL10 in patients undergoing IFN- α treatment for HCV hepatitis, in relation to the development of thyroid autoimmune dysfunctions. IFN-related thyroid autoimmune diseases (IFN-AT) are commonly thought to reproduce the natural history of HT both in its short- and long-term outcome (23). As a consequence, IFN-AT has been proposed as an experimental model for human thyroid autoimmunity (24). For this reason, the evaluation of serum CXCL10 in HCV patients developing thyroid dysfunction may allow evaluating the chemokine changes from the onset of the autoimmune process throughout its variable clinical course, and hopefully provide further knowledge on CXCL10 behaviour in chronic autoimmune thyroiditis.

Patients and methods

The study group encompassed 25 patients (18 male and 7 female) with HCV-related chronic hepatitis and 50

sex- and age-matched healthy controls. All patients took a course of IFN- α –2a at doses ranging from 3 to 6 MU three times a week for at least 6 months. Patients were retrospectively selected from a larger population of HCV patients treated with recombinant IFN- α at the Department of Internal Medicine and Endocrinology of the University of Parma between 1992 and 1995. Selection criteria for patients were as follows: i) availability of frozen serum samples stored at -20°C ; ii) negative pre-IFN- α tests for thyroid antibodies; iii) availability of serum thyroid FT4, FT3, TSH, Tg Ab and TPO Ab levels before IFN- α therapy and every 2 months in the course of treatment and iv) detailed medical records, including both the outcome of HCV hepatitis and the thyroid disturbances. Patients were assigned to two groups according to the occurrence or not of thyroid autoimmune dysfunction during IFN- α treatment. In detail, Group I included 15 patients who neither develop thyroid dysfunction nor converted to thyroid antibody positivity throughout the study span. Group II included ten patients in whom the treatment with IFN- α led to the appearance of serum thyroid antibodies (Tg Ab and/or TPO Ab), followed by a clinically overt thyroid dysfunction. Mandatory inclusion criteria for patients assigned to Group II were that the thyroid dysfunction was persistent and required specific treatment. In detail, five patients (three male and two female) developed GD, while autoimmune thyroiditis was diagnosed in the remaining five patients. The diagnosis of GD was established from the clinical presentation and by thyroid hormones and thyroid autoantibodies measurements. Thyroid scintiscan was performed in all cases and was consistent with Graves' hyperthyroidism. All GD patients were given methimazole therapy, at doses ranging from 15 to 25 mg/day (and, when necessary, β -blockers to control cardiac rate). The diagnosis of autoimmune thyroiditis was established from the clinical presentation, thyroid hormones and thyroid autoantibodies measurements and/or thyroid ultrasonography (decreased and dyshomogeneous echogenicity). All patients were hypothyroid (TSH $> 3.5\ \mu\text{U/ml}$) and were given levothyroxine (L-T₄) at replacement doses.

The retrospective design of the present study, together with the fact that patients were specifically selected for the development of a clinically overt thyroid dysfunction requiring active treatment, does not allow drawing conclusions on the epidemiology of IFN- α -related thyroid dysfunction and/or to success rate of IFN- α treatment for liver disease. Pretreatment parameters, including viral and pharmacological information for patients belonging to different groups, are shown in Table 1. Serum CXCL10 levels were assayed in all patients before IFN- α and at 2, 4 and 6 months after the starting therapy. The study was performed according to Helsinki protocol and was approved by the local ethical committee.

Table 1 Pretreatment clinical characteristics of patients subdivided according to the development of thyroid dysfunction during the treatment with interferon- α (IFN- α) for hepatitis C virus-related hepatitis.

	Group I	Group II	<i>P</i> value
No. of patients	15	10	
Sex (M/F)	13/2	5/5	0.075
Age (years)	40 (29–63)	45 (25–62)	0.357
IFN- α dose (MU/week)	18 (9–18)	15 (6–18)	0.422
FT3 (pg/ml)	4.0 (2.7–5.6)	3.3 (2.9–5.4)	0.978
FT4 (pg/ml)	11.0 (7.3–17.0)	13.5 (8.0–16.8)	0.311
TSH (mU/l)	1.5 (0.9–2.3)	1.3 (1.0–1.6)	0.238
CXCL10 (pg/ml)	312.6 (78.6–456.5)	175.1 (83.9–282.4)	0.048

The parameters showing significant differences between the two groups are in bold characters.

Serum CXCL10 measurements

Serum CXCL10 levels were assayed by a quantitative sandwich immunoassay (25) using a commercially available kit (R & D Systems, Minneapolis, MN, USA), with a mean minimum detectable dose of 1.67 pg/ml and a maximum detectable dose of 500 pg/ml. The intra- and inter-assay coefficients of variation were 3 and 6.1% respectively. Samples were assayed in duplicate. Quality control pools at low, normal and high concentrations for all parameters were present in each assay respectively.

Statistical analysis

Statistical analysis was performed using SPSS software (SPSS, Inc., Evanston, IL, USA). Serum parameters were compared between groups by means of Student's *t*-test for unpaired data and Mann–Whitney *U* test owing to normal or non-parametric distribution. Correlation between two variables was ascertained by Pearson and Spearman's correlation tests as appropriate. Frequencies of favourable therapeutic response were compared among groups by χ^2 test and Fisher's exact correction. Successful response to the treatment was defined as persistent normalisation of serum aminotransferases. $P < 0.05$ was considered statistically significant. Results are expressed in the text as mean $\pm$ S.E.M. unless otherwise stated.

Results

Before treatment, the serum levels of CXCL10 were significantly higher in the entire cohort of patients with HCV-related hepatitis when compared with healthy subjects (261.6 ± 123.4 vs 80.4 ± 33.6 pg/ml; $P < 0.00001$). Group I patients had significantly higher pretreatment mean serum CXCL10 levels when compared with Group II patients (308.6 ± 130.7 vs

191.1 ± 69.4 pg/ml; $P < 0.05$). A difference was consistently observed at all time points during IFN- α treatment, with patients in Group I showing consistently higher serum CXCL10 levels when compared with Group II patients. The changes in serum levels of CXCL10 throughout IFN- α therapy are shown in Fig. 1.

In the whole group of patients receiving IFN- α , 14 out of 25 patients (56%) had a favourable response to the treatment. When patients from Groups I and II were analysed separately, the rate of favourable responses was different, with 5 out of 15 (33%) complete responders found in Group I as opposed to 9 out of 10 (90%) complete responders in Group II. As shown in Fig. 2, such a difference did not reach statistical significance, which was most likely due to the limited number of patients enrolled. Similarly, the pretreatment

Figure 1 Pretreatment and on-treatment serum levels of CXCL10 in HCV patients treated with IFN- α in relation to the occurrence of or less thyroid dysfunction in the course of therapy. Data are expressed as median plus 25th and 75th percentiles in boxes, and 5th and 95th percentiles as whiskers. *Indicates statistical significance (Mann–Whitney *U* test; $P < 0.05$).

Figure 2 Frequencies of favourable response to IFN- α therapy in the whole cohort of HCV patients and according to the occurrence of or less thyroid dysfunction in the course of treatment.

serum CXCL10 levels were lower in patients showing a favourable response to the treatment than patients who failed to achieve remission (305.7 ± 140.3 vs 226.9 ± 100.2 pg/ml respectively) but not in a statistically significant manner.

Discussion

The results of this study confirm that serum CXCL10 levels are significantly increased in patients with HCV chronic hepatitis, when compared with sex- and age-matched healthy volunteers. Furthermore, the development of overt thyroid dysfunction in the course of IFN- α therapy is associated with significantly lower serum levels of CXCL10, both before and during the treatment. This finding seems particularly relevant as it suggests that pretreatment serum levels of CXCL10, previously proven to have a predictive value for a favourable response to IFN- α , could also be useful to identify patients more prone to develop thyroid dysfunction during IFN- α treatment for HCV hepatitis. The clinical importance of such a finding stems from the fact that the difference in serum CXCL10 levels between patients who did or did not develop IFN- α -associated thyroid dysfunction was evident before starting IFN- α therapy. This would imply that serum CXCL10 measurement would allow identifying those patients in whom thyroid surveillance during IFN- α therapy should be more careful. Prompt diagnosis and early treatment of IFN- α -associated thyroid dysfunctions will be helpful to limit the necessity of stopping IFN- α therapy which, especially when a benefit for the liver disease is evident, may be disadvantageous (22).

The issue of predicting thyroid dysfunction occurring in patients with HCV hepatitis during IFN- α treatment has been previously addressed and several virus-related, therapeutic regimen-related and patient-related parameters have been identified (6, 10, 22, 24). Among the virus-related variables, it has been demonstrated that lower serum HCV RNA levels before IFN- α therapy are significantly associated with a higher frequency of thyroid dysfunction in the course of treatment (6). This evidence may be regarded as indirect confirmation of our results, in that lower pretreatment serum levels of CXCL10 are likely to result from a lower intra-hepatic INF- γ production and lymphocyte infiltration sustained by a low viral load (18, 25). Treatment-related variables, influencing the occurrence of thyroid dysfunction, are mainly caused by the use of different IFN preparations and the dose received. Patients with cancer treated with lymphoblastoid, IFN- α -1, had a higher frequency of early thyroid dysfunction than those treated with recombinant IFN- α . The presence of trace amounts of IFN- γ in natural IFN- α could explain the different rates of thyroid autoimmunity (26). Because all our patients received recombinant IFN- α and similar doses were used, we can reasonably assume that the

occurrence of thyroid dysfunction was unrelated to different therapeutic modalities. The most studied and widely reported factor predisposing to thyroid dysfunction in the course of IFN- α therapy is pretreatment subclinical thyroid autoimmunity, as assessed by positive tests for serum Tg Ab and TPO Ab and/or an hypoechoic pattern of the thyroid at ultrasound (10, 22). In accordance with the design of the present study, negative tests for thyroid antibodies at baseline constituted a mandatory inclusion criterion, therefore the predictive efficacy of measuring serum CXCL10 and/or TPO Ab before treatment cannot be compared. Nevertheless, the assessment of serum CXCL10 levels may prove useful at least for those patients showing normal serum thyroid function and undetectable thyroid antibodies at baseline.

In our series, patients developing thyroid dysfunction were also more likely to display a favourable response to IFN- α treatment, showing nearly a three times higher rate of success. This last aspect deserves a comment. Indeed, as shown in a recent article, lower pretreatment CXCL10 levels in patients with HCV hepatitis are related to a more favourable response to IFN- α . Furthermore, Gogas *et al.* (27) have recently reported that the appearance of autoantibodies or clinical manifestations of autoimmunity, during the treatment with interferon- α , is associated with a statistically significant improvement in the relapse-free survival and in the overall survival of the patients with melanoma (27). Taken together, the results of these studies would be in line with our findings indicating that patients with lower pretreatment serum CXCL10 levels develop autoimmune thyroid dysfunction more frequently and also show a higher rate of successful therapeutic response. The above findings would suggest that the measurements of CXCL10 in the serum of HCV patients before IFN- α therapy might predict both the development of an autoimmune thyroid dysfunction and an amelioration of the liver disease.

An additional aim of the present study was to evaluate the serum changes in CXCL10 from the onset of the thyroid autoimmune process throughout its clinical course, in order to better define the natural history of circulating chemokines in AITD. IFN- α -induced thyroid dysfunction was thought to represent a good experimental model in that, at variance with naturally occurring thyroiditis, a precise estimation of the timing of thyroiditis development would be possible. Unfortunately, the extremely high serum levels of CXCL10 in HCV patients did not allow drawing conclusions in this regard. A more reliable model to investigate this aspect could be to evaluate CXCL10 in autoimmune thyroiditis occurring after IFN- β therapy in patients with multiple sclerosis (28). Indeed, the latter patients show pretreatment serum CXCL10 levels similar to healthy subjects (29).

A limitation of the present study is that patients developing thyroid autoimmunity with preserved

thyroid function were not taken into account. Indeed, it is reasonable to hypothesise that the changes in serum CXCL10 might be more evident in patients developing overt thyroid dysfunction, who also show a microenvironment more enriched in T helper type 1 (Th1) molecules (17). Nevertheless, given the aim of the study, which was to identify patients who require a particularly strict thyroid function surveillance during IFN- α therapy, it was decided to include only those patients developing overt thyroid dysfunction.

It has been recently reported that HCV patients developing thyroid dysfunction during IFN- α treatment displayed circulating markers of a Th1 immune reaction, as assessed by IFN- γ expression by peripheral blood lymphocytes (30). Therefore, the results of our study, indicating no significant change of a Th1-related chemokine in a correspondent group of patients, would, at least partially, disagree with this previous observation (30). A possible explanation for such a discrepancy, besides the different experimental procedures, could be that, at variance with the study of Mazziotti *et al.* (30), our patients did not receive ribavirin, which is known to be a potent Th1 inducer.

In conclusion, our retrospective study suggests that measuring serum CXCL10 before IFN- α treatment may be a helpful tool for identifying the patients who will develop overt thyroid dysfunction, and therefore will require a careful thyroid surveillance throughout the treatment. Future longitudinal prospective studies enrolling large numbers of patients will be required to confirm these data.

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