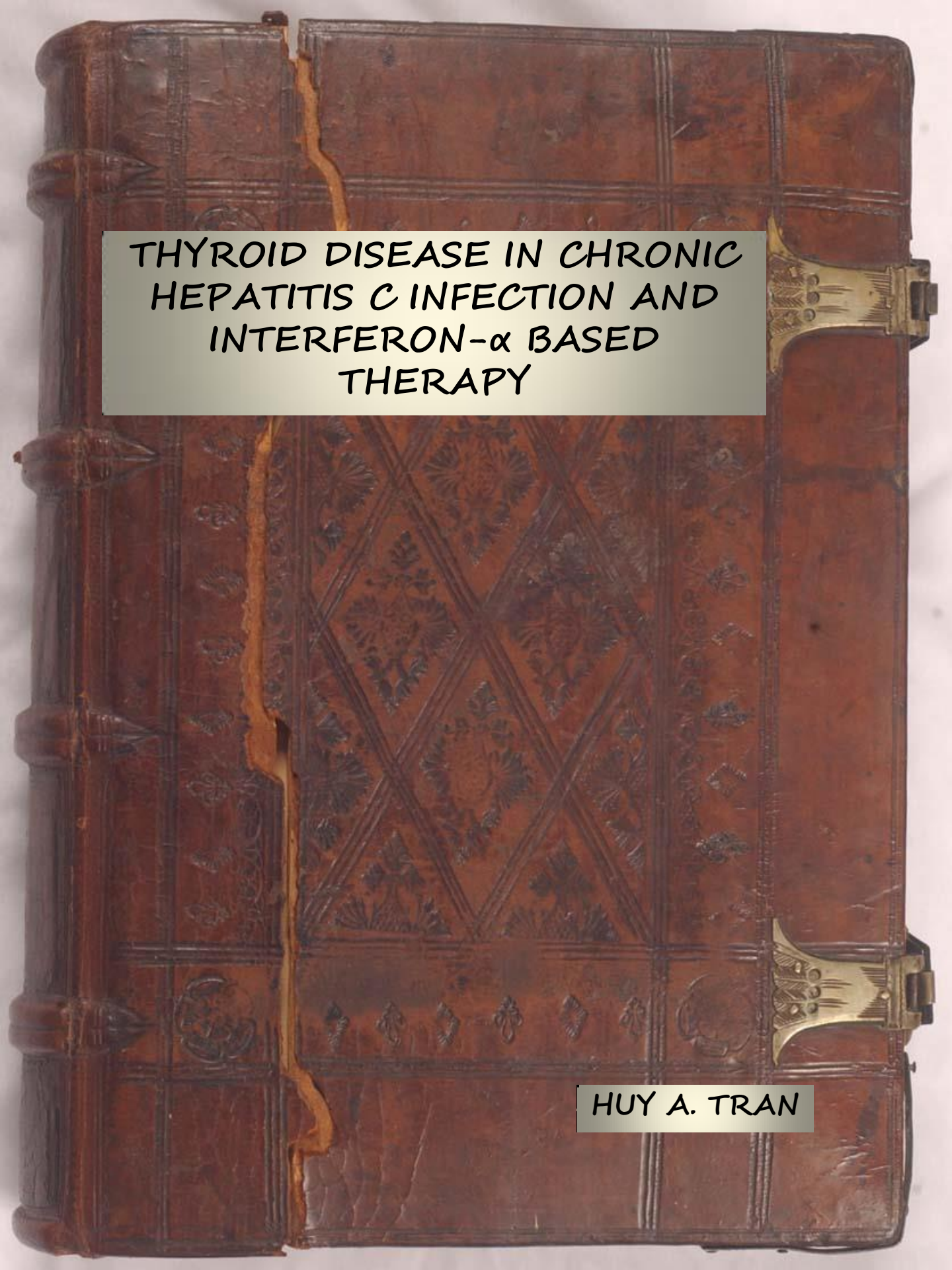
THYROID DISEASE IN CHRONIC HEPATITIS C INFECTION AND INTERFERON-α BASED THERAPY
HUY A. TRAN

THESIS SUBMISSION

THYROID DISEASE IN CHRONIC HEPATITIS C INFECTION AND INTERFERON-α BASED THERAPY

by

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DOCTORATE OF MEDICINE

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SIGNED: 13-November-2012 **HUY A. TRAN**

PREFACE

This study was performed over a 7 year period addressing the fascinating topic of thyroid diseases in association with chronic hepatitis C infection and its treatment. All the manuscripts were prepared in my own time whilst working in a full-time capacity as the Director of Chemical Pathology, Hunter Area Pathology Service, New South Wales.

I am very grateful and indebted to my family, my parents and brothers whose, whilst afar, moral advice, support and belief give me the absolute strength and motivation in the desperate hours of need. My father's wise and guiding words I will take to the grave: "You have two hands and feet. Others can, why can't you?" To Justin Tran whose sharp vision did the 'spotto' on the 'typos' within the first few glances and effectively enforced a redo of the manuscript. To Audrey Tran whose beautiful and natural personality made the experience all the richer. To Xuan Nguyen, my darling wife whose life-long support of my academic adventure is much more vast and valuable than anyone realizes.

I am also very grateful for the guidance and support of Professor Geoffrey M Kellerman and the late Professor Anthony S-Y Leong whose boundless and unparalleled academic achievements have inspired me to take on this mountainous project. I would not be able to achieve this project without the wonderful support of the Hepatitis C unit and

Gastroenterology Department at the John Hunter Hospital. I am very humbled and honored to have the support of Professor Aidan Foy, Drs. Robert Pickles and Brian Hughes, nursing sisters Elizabeth A Ianna, Tracey L Jones, Melissa Young and research scientist Nadine Leembruggen whose many joyful anecdotes have seeded the full bloom of many subsequent incisive scientific reports.

DEDICATION

THIS THESIS WAS DEDICATED TO THE MEMORY OF

PROFESSOR ANTHONY S-Y LEONG, MD, FRCPA, FRCPath, FCAP, 1945 – 2011.

DISTINGUISHED PROFESSOR OF PATHOLOGY AND MEDICAL DIRECTOR OF HUNTER AREA PATHOLOGY SERVICE, NEWCASTLE, NEW SOUTH WALES, 1999 – 2010.

A REVERED COLLEAGUE, FRIEND, MENTOR, NEIGHBOR AND FATHERLY FIGURE. MY SI PHU.

Obituaries

Anthony Siew-Yin Leong

MB BS, MD, FRCPA, FRCPath, FCAP, FHKCPath, FHKAM(Path)

ANTHONY LEONG was born in Singapore in 1945 and graduated from the University of Malaya in 1969. He pursued postgraduate training in America, where he completed his pathology residency at the University of Washington, Seattle, between 1971 and 1973. In 1976, he migrated to Adelaide to continue his work in lymphoma and tissue processing, especially tissue staining and immunohistochemistry. He excelled in research and, in 1980, received a doctorate in medicine from the University of Adelaide, where he was Clinical Professor of Pathology from 1981 until 1996.

From 1996, he dedicated most of his effort and time to the Asia–Pacific region, where he held a number of leading posts including Professor of Anatomical and Cellular Pathology at the Chinese University of Hong Kong and Honorary Professor of Pathology at the Post Graduate Medical Institute, Beijing. From 1999, Anthony was Professor of Anatomical Pathology at the University of Newcastle and Medical Director of the Hunter Area Pathology Service. He was a Fellow of the colleges of pathologists of Australasia, the United Kingdom and America, as well as an honorary Fellow of the Hong Kong and Thai colleges. He served as President of the International Academy of Pathology, Australasian Division in 1995–1996 and was the foundation President of the Asia–Pacific Society for Molecular Immunohistology in 2005–2006. He was also a founding member of the Society of Applied Immunohistochemistry and the International Society for Analytical and Molecular Morphology.

Anthony's most influential footprint was in the field of immunohistochemistry, where he left a great legacy of excellence in research and an unsurpassed love of pathology. He was a prolific author of over 370 original papers, reviews and book chapters, and more than 23 textbooks and monographs. He was a great teacher, a wise mentor and a wonderful leader, who was full of humour and interesting anecdotes. His favourite pastime was golf, and he was a proud member of the "four amigos" golf team at his local club. However, it is fair to say that his golfing never matched the dizzying heights of his academic record!

Anthony passed away in late June 2011 after a short battle with cancer. He is survived by his wife Wendy and two children Trishe and Joel, both pathologists.

Huy A Tran, Glenn E M Reeves, Frederick W Hetherington

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Summary: This chapter examines the evolution of interferon use and its subsequent graduation into the pharmacological management of many medical conditions. The initial treatment for hepatitis C involved monotherapy with interferon-α but as it soon became evident that combination therapy with ribavirin delivered a much superior outcome. As a result, it is now the current standard of practice in the treatment of chronic hepatitis C infection.

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Summary: As treatment for hepatitis C became readily available and further refined, an increased number of patients was anticipated to undergo treatment. As a result, adverse outcomes would also become more apparent, the commonest of which is thyroid disorders. It was essential that the prevalence of the condition be recognized, especially in Australia where few reports were available. The original research publication in this chapter addressed this concern and quantified the prevalence of thyroid disorders in an Australian cohort managed and treated in a specialized tertiary hospital unit.

Publications:

Tran HA. HEPATITIS C INFECTION, TREATMENT REGIMENS, AND THYROID ABNORMALITIES. *The Endocrinologist*, 2007; 17: 231-235.

Tran HA, Jones TL, Batey RG. THE SPECTRUM OF THYROID DYSFUNCTION IN AN AUSTRALIAN HEPATITIS C POPULATION TREATED WITH COMBINATION INTERFERON-α2β AND RIBAVIRIN. *BMC Endocr Disord*, 2005; 5: 8.

Tran HA, Reeves GEM, Lyons TJ, Attia JR. HISTOPATHOLOGICAL FINDINGS OF AUTOIMMUNITY IN THYROID, PITUITARY AND ADRENAL DISEASES IN CHRONIC HEPATITIS C POST-MORTEM CASES. Endocr Pract, 2010; 16: 566-569.

CHAPTER III. THE DEVELOPMENT OF THYROID DISEASES WITH THE CHANGE TO PEGYLATED INTERFERON

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Publication:

Tran HA, Attia JR, Jones TL, Batey RG. PEGYLATED INTERFERON-α2β IN COMBINATION WITH RIBAVIRIN DOES NOT AGGRAVATE THYROID DYSFUNCTION IN COMPARISON TO REGULAR INTERFERON-α2β IN A HEPATITIS C POPULATION: META-ANALYSIS. *J Hepatol Gastroenterol*, 2007; 22: 472-6.

CHAPTER IV. CHARACTERISATION OF THYROID DISEASE DURING TREATMENT WITH INTERFERON-α

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Summary: Although the development of thyroid disorders in association with interferon therapy became evident and widely reported, there still remained uncertainty and controversy regarding the patterns of thyroid disorders in this setting. This chapter critically examines the broad and fascinating spectrum of thyroid disorders during therapy. Where available, the natural history and outcome of a number of specific thyroid disorders was addressed in these original publications. Because of the unusual and rare occurrence of the disorder, many publications are case reports published for the purpose of stimulating and encouraging more reporting in the medical literature.

Publications:

Tran HA, Reeves GEM. CHARACTERISTICS OF GRAVES' DISEASE IN A COHORT OF CHRONIC HEPATITIS C PATIENTS TREATED WITH INTERFERON-α AND RIBAVIRIN. J Endocrinol Metab, 2011; 1: 14-20.

Tran HA, Reeves GE, Jones TL. THE NATURAL HISTORY OF INTERFERON-α2β-INDUCED THYROIDITIS AND ITS EXCLUSIVITY IN A COHORT OF PATIENTS WITH CHRONIC HEPATITIS C INFECTION. Q J Med, 2009; 102: 117-122.

Tran HA, Jones TL, Ianna EA, Reeves GE. THE NATURAL HISTORY OF INTERFERON-α INDUCED THYROIDITIS IN CHRONIC HEPATITIS C PATIENTS: A LONG TERM STUDY. Thyroid Res, 2011; 8: 4 (1): 2.

Tran HA. THE SWINGING THYROID IN HEPATITIS C INFECTION AND INTERFERON THERAPY. *Q J Med, 2010; 103: 187-191.*

CHAPTER V. THE METABOLIC EFFECTS OF THYROID DISEASE DURING INTERFERON TREATMENT

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Summary: In parallel with the recognition of thyroid disorders, a number offother metabolic and thyroid-associated conditions became evident. These cases were carefullyystudied and subsequentlyy published. Because of the rarity of such conditions, a review of other similar published cases was performed to compare and contrast the worldwwide clinical experience.

Publications:

Tran HA. HEPATITIS C INFECTION AND THYROTOXIC PERIODIC PARALYSIS – A NOVEL USE OF AN OLD DRUG. *Am J Med Sci*, 2008; 336: 515–518.

Tran HA, Reeves GEM. THE INFLUENCE OF HEPATITIS C INFECTION AND INTERFERON-α THERAPY ON THYROTROPIN BLOCKING AND STIMULATING ANTIBODIES IN GRAVES' OPHTHALMOPATHY: A CASE REPORT. Thyroid Res, 2009; Dec 2: 2(1): 12.

Tran HA, Song S, Lojewski R, Reeves GE. EXACERBATION OF HEPATITIS C INDUCED SUBCLINICAL HYPOADRENALISM BY INTERFERON α2β: A CASE REPORT. *Cases J, 2008; 1: 157.*

Tran HA, Crock PA, Reeves GEM. PITUITARY DISEASE IN CHRONIC HEPATITIS C INFECTION AND INTERFERON-A RELATED THERAPY: TWO CASE REPORTS. *J Endocrinol Metab, 2012; (In Press).*

CHAPTER VI. THYROID DISEASE FOLLOWING THE COMPLETION OF INTERFERON THERAPY

Summary: Once thyroid disorders occurring during combination treatment have been fully characterized, it remained to be determined whether they occur following treatment completion. These publications examined the need for short to medium term follow-up of thyroid status up to the time of the sustained virologic response review, 6 months after the completion of therapy. The question of whether a longer follow-up time is needed is unanswered, but is probably not warranted given the findings of these publications.

Publications:

Tran HA, Reeves GEM. THE SPECTRUM OF AUTOIMMUNE THYROID DISEASE IN THE SHORT TO MEDIUM TERM FOLLOWING INTERFERON-α THERAPY FOR CHRONIC HEPATITIS C. Int J Endocrinol, 2009; 2009: 241786.

Tran HA, Reeves GEM, Ianna EA, Leembruggen N. THYROID FUNCTION OUTCOMES AFTER PEGYLATED INTERFERON-A AND RIBAVIRIN THERAPY FOR CHRONIC HEPATITIS C. Endocr Pract, 2010; 16: 934-939.

CHAPTER VII. THE EFFECT OF THYROID DISEASE ON SUSTAINED VIROLOGIC RESPONSE

Summary: Whilst there are many factors to prognosticate outcome of hepatitis C treatment with interferon-α, it was observed in this meta-analysis that the development of thyroid disease is associated with a significant rate of sustained virologic response. Such speculation was heightened by two cases occurring in a natural experiment setting, followed by a nested case-control study. This observation is critical because, if confirmed, the finding offers a significant adjunct to the current standard of therapy.

Publications:

Tran HA, Reeves GEM, Gibson R, Attia JR. THE DEVELOPMENT OF THYROID DISEASES IN THE TREATMENT OF CHRONIC HEPATITIS C WITH INTERFERON- α MAY BE A GOOD PROGNOSTICATOR IN ACHIEVING A SUSTAINED VIROLOGICAL RESPONSE: A META-ANALYSIS. J Hepatol Gastroenterol, 2009; 24: 1163–1168.

Tran HA, Ianna EA, Jones TL, Reeves GEM. THE ADJUVANT ROLE OF THYROXINE IN THE TREATMENT OF CHRONIC HEPATITIS C INFECTION. Q J Med, 2012; 105: 683–687.

Tran HA, Jones TL, Gibson R, Reeves GEM. THYROID DISEASE IS A FAVORABLE PROGNOSTIC FACTOR IN ACHIEVING SUSTAINED VIROLOGIC RESPONSE IN CHRONIC HEPATITIS C UNDERGOING COMBINATION THERAPY: A NESTED CASE CONTROL STUDY. BMC Endocr Disord, 2011; 11: 10.

CHAPTER VIII. SUMMARY AND CLINICAL MANAGEMENT STRATEGIES

Final outcome: On the bases of the original data generated from this thesis and current literature, an evidence-based strategy for the surveillance and management of thyroid disease is formulated and proffered.

Publication:

Tran HA, Jones TL, Ianna EA, Foy A, Reeves GEM. THYROID DISEASE IN CHRONIC HEPATITIS C INFECTION TREATED WITH INTERFERON-α: MANAGEMENT STRATEGIES AND FUTURE PERSPECTIVE. Endocr Pract, September-2012; *In Press* (Appendix II).

SYNOPSIS

This thesis was conceived from a number of published letters in the mid 2000's, which subsequently developed and flourished into its completion (1, 2). The proposed questions and hypotheses led to the exploration of the topic of thyroid disease in the presence of chronic hepatitis C infection and combination interferon-α and ribavirin therapy.

Early in its evolution, hepatitis C infection was chronic and considered incurable. However, with the discovery and therapeutic development of interferon, the condition became readily treatable. The initial efficacy with interferon-α monotherapy was poor but, in combination with ribavirin, it soon became the gold standard for chronic hepatitis C infection.

As clinical experience with the combination therapy for hepatitis C grew, and together with the fact that this RNA virus is a highly immunogenic particle, extra-hepatic adverse events became increasingly evident. The most common of these is thyroid disease (3). This presented a golden opportunity to study this subject in great details.

Chapter 1 reviews the history of interferon and its therapeutic development, especially in the management of hepatitis C.

Chapter 2 assesses the prevalence of thyroid disease in an Australian population of 272 treated cases from a tertiary referred hospital hepatitis C unit (4). In a separate cohort (but with a similar geographic distribution to the aforementioned cohort), the histologic evidence of thyroid disease in 108 post-mortem hepatitis C cases is also presented. These subjects' major endocrine organs of pituitary, thyroid and adrenal tissues were reviewed to determine the possible underlying pathological process including autoimmunity. The histologic report is the first original study in the published English literature assessing and highlighting the magnitude of the problem in Australia (5).

Chapter 3 assesses the risk of developing thyroid diseases whilst receiving pegylated interferon therapy in comparison to regular interferon. Interferon was pegylated to improve compliance with a change from thrice to once weekly injection. It remained unknown if pegylation interferon, despite similar antiviral efficacy, differed from regular interferon in its effect on the thyroid. This analysis reassuringly found that the pegylated form conferred no additional risk (6).

Chapter 4 characterises the pattern of thyroid disease occurring during treatment. It became evident that the majority, if not all Australian cases, developed (bi-phasic) thyroiditis with complete recovery by the time of sustained virological response (SVR) assessment 6 months after the completion of therapy. This was a unique

finding in our cohort (7). A small percentage of patients developed Graves' like thyrotoxicosis *after* the end of therapy, some adjoining and following the thyroiditis, and thus the term 'tri-phasic' thyroiditis was employed. The natural history of Graves' like thyrotoxicosis was compared with the natural history of Graves' disease arising *de novo* (8,9). In addition, a 3-year long term follow-up study was carried out in these patients with thyroiditis and the outcome was reassuringly benign (10).

Chapter 5 reviews the extra-thyroid effects of interferon-α therapy. It is well documented that beside thyroid disease, other endocrine/metabolic effects may be observed. Some of these conditions such as subclinical hypoadrenalism, thyrotoxic periodic paralysis, associated Graves' ophthalmopathy and pituitary disease (11, 12, 13, 14) are reported in this chapter. A review of possible pituitary disease involvement was also performed.

Chapter 6 makes the critical observation that patients who developed thyroid disease during therapy for chronic hepatitis C also managed to achieve sustained virologic response (SVR) much more readily than their non-thyroiditis counterparts. This hypothesis was examined using pooled data for a meta-analysis (15). The result was negative although the published studies were quite heterogeneous. This observation was strengthened further in two natural experiments (16) and led to a nested case-control study that was performed to quantify the likelihood of SVR in this setting (17). The results are favorable,

particularly genotype 1 patients whose prognosis and response rate is much worse than other genotypes. Whether this will help to further refine the treatment of this condition remains to be elucidated.

Chapter 7 examines the spectrum of thyroid disorders (18) and outcomes in hepatitis C patients in the 6 months *after* the completion of treatment, at the time of SVR assessment. This is applicable to patients who did not develop thyroid disease during treatment and reassuringly, there were no excessive thyroid disease cases detected (19).

Chapter 8 summarises the current published literature on the topic and proposes an evidence-based strategy for the development of thyroid disease in this clinical setting.

In conclusion, the thesis reports the prevalence, development and natural history of thyroid disease during treatment with interferon-α based therapy for hepatitis C, the spectrum of thyroid disease seen during *and* after the period of treatment, and the possible underlying pathogenic mechanisms. These studies assist in the formulation of management strategies in this unique clinical setting. The influence of thyroid disease development on the final viral status remains encouraging but contentious and yet to be determined.

Publication:

Tran HA. THE UNCERTAIN NATURAL HISTORY OF THYROTOXIC PATIENTS TREATED WITH COMBINATION INTERFERON-α2β AND RIBAVIRIN. *Arch Intern Med*, 2005; 165: 1072.

5. Foster GD, Wyatt HR, Hill JO, et al. A randomized trial of a low-carbohydrate diet for obesity. *N Engl J Med*. 2003;348:2082-2090.
6. Yancy WS Jr, Guyton JR, Bakst RP, Westman EC. A randomized, controlled trial of a low-carbohydrate, ketogenic diet vs. a low-fat diet for obesity and hyperlipidemia. *Ann Intern Med*. 2004;140:769-777.
7. Meckling KA, O'Sullivan C, Saari D. Comparison of a low-fat diet to a low-carbohydrate diet on weight loss, body composition, and risk factors for diabetes and cardiovascular disease in free-living, overweight men and women. *J Clin Endocrinol Metab*. 2004;89:2717-2723.
8. Mensink RP, Katan MB. Effect of dietary fatty acids on serum lipids and lipoproteins: a meta-analysis of 27 trials. *Arterioscler Thromb*. 1992;12:911-919.
9. Mozaffarian D, Rimm EB, Herrington DM. Dietary fats, carbohydrate, and progression of coronary atherosclerosis in postmenopausal women. *Am J Clin Nutr*. 2004;80:1175-1184.

The Uncertain Natural History of Thyrotoxic Patients Treated With Combination Interferon Alfa-2β and Ribavirin

While reading with interest the recent publication regarding thyroid dysfunction and hepatitis C in men,[1] I noted a number of conundrums. First, there was no biochemical confirmation of thyroid function tests (TFTs) in the exclusion criteria other than simply excluding "patients with known thyroid disease." Second and similarly, there were no baseline TFTs in the recruited subjects. Third, the TFT testing protocol was an additional problem. Such frequency testing in this setting depends very much on the natural history, which can vary from weeks to months and is far from being completely understood.[2,3] Therefore, currently there is no definitive recommendation regarding TFTs during the treatment course.[4] Without such information, the concern is the probable misclassification of destructive autoimmune (bi-phasic) type "hyperthyroidism" into the "hypothyroidism" category. This point is best highlighted by the following clinical vignette.

A 40-year-old man with chronic hepatitis C (genotype 1) was treated with combination interferon alfa-2β identical to that in the study by Bini and Mehandru.[1] Six weeks into therapy, he complained of general lethargy and occasional myalgia. There was no history or family history of thyroid disease, and findings from examination showed peripheral stigmata of thyrotoxicosis. His serum thyrotropin level was suppressed to less than 0.03 mIU/L with free tetra-iodothyronine and free triiodothyronine levels of 2525 pg/dL (32.5 pmol/L) (reference range, 777-2020 pg/dL [10.0-26.0 pmol/L]) and 501 pg/dL (7.7 pmol/L) (reference range, 228-378 pg/dL [3.5-5.8 pmol/L]), respectively. His antithyroperoxidase antibody ratio was 1:6400 with undetectable thyroid-stimulating immunoglobulin and antithyroglobulin antibodies. His thyroid nuclear uptake scan was reduced at 7% (reference range, 12%-38%). A diagnosis of destructive autoimmune thyroiditis was made, and the patient was treated conservatively. His progress was reasonable but not ideal with persistent malaise and myalgia. Results from repeated TFTs at 6 weeks showed marked hypothyroidism with a thyrotropin level of 53.1 mIU/L and a free tetra-iodothyronine of 272 pg/dL (3.5 pmol/L). Thyroxine therapy was started, and the patient's symptoms improved. At 6 months after the completion of antiviral therapy, he still required thyroxine therapy at 100 µg/d.

According to the researchers' protocol, at 12 weeks into therapy, this patient would have been clearly misclassified. This and the absence of TFTs in the exclusion criteria and recruitment process, which would undoubtedly detect de novo hypothyroidism (either subclinical or overt), would falsely elevate the incidence of hypothyroidism in the final analysis. While the case presented may be an isolated one, further clinical studies into the natural history of this type of thyrotoxicosis are warranted so that appropriate testing frequency can be recommended.

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Correspondence: Dr Tran, Department of Clinical Chemistry, Hunter Area Pathology Service, John Hunter Hospital, Newcastle, New South Wales, Australia 2310 (huy.tran@hnehealth.nsw.gov.au).

1. Bini EJ, Mehandru S. Incidence of thyroid dysfunction during interferon alfa-2β and ribavirin therapy in men with chronic hepatitis C: a prospective cohort study. *Arch Intern Med*. 2004;164:2371-2376.
2. Carella C, Mazziotti G, Amato G, Braverman LE, Roti E. Clinical review 169: interferon-alpha-related thyroid disease: pathophysiological, epidemiological, and clinical aspects. *J Clin Endocrinol Metab*. 2004;89:3656-3661.
3. Prummel MF, Laurberg P. Interferon alpha and autoimmune thyroid disease. *Thyroid*. 2003;13:547-551.
4. Ward DL, Bing-You RG. Autoimmune thyroid dysfunction induced by interferon alpha treatment for chronic hepatitis C: screening and monitoring recommendations. *Endocr Pract*. 2001;7:52-58.

In reply

We appreciate Dr Tran's interest in our article[1] and welcome this opportunity to clarify the issues raised by him. Exclusion criteria for our study included a personal history of thyroid disease as well as current or prior treatment of thyroid disease. In addition, baseline thyrotropin (TSH) levels were obtained on all study subjects at week 0. Patients with abnormal TSH levels were excluded from the study.

We agree that there is a lack of consensus regarding how to screen or how often to screen for thyroid disease during interferon and ribavirin therapy. Some authors have suggested that screening for thyroid disease with a complete history and physical examination, TSH levels, and antithyroid peroxidase antibodies should be performed in all patients with hepatitis C virus (HCV) infection prior to therapy.[2] In addition, these authors recommended treatment of thyroid disease followed by TSH levels every 2 to 6 months during interferon and ribavirin therapy in patients with thyroid dysfunction or a clinical evaluation and TSH levels every 6 months in those without thyroid dysfunction at baseline.[2]

The case report by Dr Tran describes a patient with labile thyroid dysfunction during interferon and ribavirin combination therapy, cycling rapidly between hyperthyroidism and hypothyroidism. It is certainly possible that screenings for TSH levels performed every 12 weeks according to our protocol[1] or less frequently as recommended

by other investigators[2] would have missed transient hyperthyroidism. The case report by Dr Tran and our study highlights the need for additional studies to evaluate the optimal screening frequency for thyroid disease in HCV-infected patients treated with interferon and ribavirin therapy.

The development of recommendations to screen for thyroid disease in this population should consider risk stratification of patients. Several studies have described certain risk factors that predispose patients to develop thyroid dysfunction during HCV therapy, including female sex, personal or family history of thyroid disease, and preexisting antithyroid peroxidase antibodies.[1,3-6] In these "high-risk" individuals, screening for thyroid dysfunction should be more rigorous during therapy for HCV.

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1. Bini EJ, Mehandru S. Incidence of thyroid dysfunction during interferon alfa-2b and ribavirin therapy in men with chronic hepatitis C: a prospective cohort study. *Arch Intern Med*. 2004;164:2371-2376.
2. Ward DL, Bing-You RG. Autoimmune thyroid dysfunction induced by interferon-alfa treatment for chronic hepatitis C: screening and monitoring recommendations. *Endocr Pract*. 2001;7:52-58.
3. Koh LK, Greenspan FS, Yeo PP. Interferon-alpha induced thyroid dysfunction: three clinical presentations and a review of the literature. *Thyroid*. 1997;7:891-896.
4. Fernandez-Soto L, Gonzalez A, Escobar-Jimenez F, et al. Increased risk of autoimmune thyroid disease in hepatitis C vs hepatitis B before, during, and after discontinuing interferon therapy. *Arch Intern Med*. 1998;158:1445-1448.
5. Custro N, Montalto G, Scafidi V, et al. Prospective study on thyroid autoimmunity and dysfunction related to chronic hepatitis C and interferon therapy. *J Endocrinol Invest*. 1997;20:374-380.
6. Roti E, Minelli R, Giuberti T, et al. Multiple changes in thyroid function in patients with chronic active HCV hepatitis treated with recombinant interferon-alpha. *Am J Med*. 1996;101:482-487.

Publication:

Tran HA. THYROTOXICOSIS DURING PEGYLATED INTERFERON THERAPY IN A PATIENT WITH CHRONIC HEPATITIS C VIRUS. Endocr Pract, 2006; 12: 231-2.

Letters to the Editor

Thyrotoxicosis During Pegylated Interferon Therapy in a Patient With Chronic Hepatitis C Virus

To the Editor:

The recent report by Lin et al (1) in *Endocrine Practice* highlights the potential adverse problem with interferon-alfa-2b (IFN-α) therapy in patients with hepatitis C infection. The thyrotoxicosis in this case is specific to pegylated IFN-α (pIFN-α) only. Thyrotoxicosis associated with IFN-α is generally rare, and each case should be managed on its own merits, depending on the clinical status. To date, no published report has addressed thyroid diseases in relationship to this specific form of IFN as opposed to the standard IFN-α therapy, for which data are available but sparse. Therefore, it is difficult to make a generalized recommendation for thyroid surveillance during pIFN-α-based therapy.

Screening is clearly different from case finding, and the latter should apply only to high-risk groups, including patients with a genetic predisposition and other factors mentioned by the authors. In fact, of the two opposing thyroid conditions, primary hypothyroidism caused by IFN-α-based therapy is much more common by a factor of 2 to 3 times (2). Of note, the prevalence of primary hypothyroidism in patients with hepatitis C virus receiving IFN-α-based treatment is not higher than the prevalence in the general population. This fact also makes the case for regular testing in every patient (that is, screening) scientifically unsound and not an evidence-based strategy. Monetarily, however, the cost of surveying for thyroid disease in these patients would be relatively small in comparison with use of a full course of combination pIFN-α and ribavirin.

In the initial assessment of the patient's thyroid disease, 22 months before the start of IFN therapy, the indications for the triiodothyronine uptake and thyroid autoantibody studies are not clear and appear superfluous. In the presence of normal levels of thyrotropin and total thyroxine (or free thyroxine), no further investigation appears warranted, even in the presence of pathologically proven multinodular disease. Perhaps those investigations should be reserved in preparation for IFN-α-based therapy just before October 2002 for risk stratification because the patient clearly is in a high-risk group. In addition, the thyrotoxicosis on this occasion is more consistent with iodine-induced thyrotoxicosis on a background of multinodular goiter rather than thyroiditis, in light of the clinical details and the absence of thyroid autoantibodies from 22 months previously. Thyroiditis can certainly coexist with multinodular thyroid disease. In either situation, symptomatic treatment is all that is required, including a β-blocking agent, a corticosteroid, or both, if thyroiditis is the correct diagnosis. With the clear-cut history of multinodularity in mind, perhaps use of a contrast agent may have been averted or minimized or prophylactic therapy may have been instituted.

In the second admission, the presence of fever, persistent tenderness of the thyroid gland, and low blood pressure in a patient with known hypertension suggests the probability of a pyogenic thyroid abscess with disseminated sepsis. Although uncommon, suppurative thyroiditis, occurring more frequently in a multinodular goiter than in the setting of a single thyroid nodule (3), can easily mimic a thyrotoxic picture and must be rigorously excluded. This is a common scenario in patients who contract hepatitis C virus by intravenous drug use and are more likely than other patients to harbor unusual infections. This is important because corticosteroid treatment should then be used with extreme caution.

In the third admission, hypothyroidism due to overtreatment with propylthiouracil is best managed by cessation of treatment or dose reduction. There is very little scientific basis for "block-replace" therapy in this particular clinical scenario. Dual therapy with levothyroxine and propylthiouracil would only exacerbate the noncompliance in this patient, who had a proven track record for such a problem. The relapse in late April 2003 is also consistent with the natural history of iodine-induced thyrotoxicosis, which can be intractable and may require prolonged antithyroid medications (4). It is rather surprising that her radioiodine scan showed high uptake and homogeneous rather than heterogeneous activity, expected in a multinodular hemithyroid. Both forms of IFN-α-related *hyperthyroidism* are usually expected to remit soon after the withdrawal of IFN-α therapy (5,6). By this time, the patient has not received any IFN-α and ribavirin for ~6 months; thus, it is probable that her thyrotoxicosis bears no relationship to the antiviral medications whatsoever.

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REFERENCES

1. **Lin YQ, Wang X, Murthy MS, Agarwala S.** Life-threatening thyrotoxicosis induced by combination therapy with PEG-interferon and ribavirin in chronic hepatitis C. *Endocr Pract*. 2005;11:135-139.
2. **Prummel MF, Laurberg P.** Interferon-alpha and autoimmune thyroid disease. *Thyroid*. 2003;13:547-551.
3. **Farwell AP.** Infectious thyroiditis. In: Braverman LE, Utiger RD, eds. *Werner & Ingbar's The Thyroid: A Fundamental and Clinical Text*. 8th ed. Philadelphia, PA: Lippincott Williams & Wilkins, 2000: 1044-1050.

4. **Davies TF, Larsen PR.** Thyrotoxicosis. In: Larsen PR, Kronenberg HM, Melmed S, Polonsky KS, eds. *Williams Textbook of Endocrinology*. 10th ed. Philadelphia, PA: Saunders, Elsevier Science, 2003: 411.
5. **Carella C, Mazziotti G, Amato G, Braverman LE, Roti E.** Clinical review 169: interferon-alpha-related thyroid disease; pathophysiological, epidemiological, and clinical aspects. *J Clin Endocrinol Metab*. 2004;89:3656-3661.
6. **Pearce EN, Farwell AP, Braverman LE.** Thyroiditis [erratum in *N Engl J Med*. 2003;349:620]. *N Engl J Med*. 2003; 348:2646-2655.

In Response:

Thyroid diseases are frequent side effects of interferon (IFN)-α therapy for hepatitis C virus. Among the thyroid disorders associated with interferon therapy, thyrotoxicosis is infrequently observed; a recent report indicated that thyrotoxicosis occurs in about 3.7% of patients treated with IFN-α (1). The combination of ribavirin and interferon therapy does not modify the thyroid autoantibody pattern but is associated with a higher risk of hypothyroidism (2) and thyrotoxicosis (3). Because of the potentially severe adverse outcome of this combination therapy, as detailed in the reported case by Lin et al (4), screening and monitoring of such patients by measurement of thyrotropin (thyroid-stimulating hormone or TSH) and thyroid peroxidase antibody levels are a reasonable recommendation. If that patient had been monitored by an endocrinologist, the prophylactic therapy could have been instituted earlier, when the patient was found to have a suppressed TSH level, and perhaps the use of a contrast agent might have been avoided and the outcome may have been different.

IFN-induced thyrotoxicosis can be divided into two groups—Graves'-type thyrotoxicosis and thyroiditis-type thyrotoxicosis (1)--and Graves' hyperthyroidism may develop even after a transient phase of destructive thyrotoxicosis (3). At the first admission of the aforementioned patient (4), the thyrotoxicosis was consistent with destructive thyroiditis; iodine-induced thyrotoxicosis is very unlikely, inasmuch as no iodine was given before the admission. At the second admission, a pyogenic thyroid abscess with disseminated sepsis was completely ruled out by the clinical course because the patient was responding to corticosteroid, propylthiouracil, and β-adrenergic blocker therapy without intravenously administered antibiotics and surgical interventions. The fever is one of the signs of thyroid storm!

At the third admission, the diagnosis of Graves' disease was supported by a suppressed TSH level, elevated thyroxine level, and increased radioiodine uptake with homogeneous activity in the left lobe. The ideal management of IFN-induced Graves' disease is radioiodine treatment (5), but the patient refused this therapeutic option. I personally treat my patients with Graves' disease who refuse radioiodine therapy with propylthiouracil or methimazole for at least 12 months first, but I will add thyroid hormone if the TSH level increases, the so-called block-replace therapy, for the following reasons: (*1*) if the block treatment is discontinued too early, Graves' disease will relapse, especially in IFN-induced Graves' disease (4); (*2*) with use of dose reduction alone (without replacement therapy), maintenance of euthyroidism is extremely difficult; and (*3*) the possible immunosuppressive action of a thionamide could benefit Graves' disease directly, and some reports have shown that block-replace therapy could increase the remission rate (6).

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REFERENCES

1. **Wong V, Fu AX, George J, Cheung NW.** Thyrotoxicosis induced by alpha-interferon therapy in chronic viral hepatitis. *Clin Endocrinol (Oxf)*. 2002;56:793-798.
2. **Carella C, Mazziotti G, Morisco F, et al.** The addition of ribavirin to interferon-alpha therapy in patients with hepatitis C virus-related chronic hepatitis does not modify the thyroid autoantibody pattern but increases the risk of developing hypothyroidism. *Eur J Endocrinol*. 2002;146: 743-749.
3. **Mazziotti G, Sorvillo F, Stornaiuolo G, et al.** Temporal relationship between the appearance of thyroid autoantibodies and development of destructive thyroiditis in patients undergoing treatment with two different type-1 interferons for HCV-related chronic hepatitis: a prospective study. *J Endocrinol Invest*. 2002;25:624-630.
4. **Lin YQ, Wang X, Murthy MS, Agarwala S.** Life-threatening thyrotoxicosis induced by combination therapy with PEG-interferon and ribavirin in chronic hepatitis C. *Endocr Pract*. 2005;11:135-139.
5. **Carella C, Mazziotti G, Amato G, Braverman LE, Roti E.** Clinical review 169: interferon-alpha-related thyroid disease; pathophysiological, epidemiological, and clinical aspects. *J Clin Endocrinol Metab*. 2004;89:3656-3661.
6. **Hashizume K, Ichikawa K, Sakurai A, et al.** Administration of thyroxine in treated Graves' disease: effects on the level of antibodies to thyroid-stimulating hormone receptors and on the risk of recurrence of hyperthyroidism. *N Engl J Med.* 1991;324:947-953.

CHAPTER I

INTRODUCTION

A. GENERAL BACKGROUND ON INTERFERON THERAPY.

1. The history of interferon and its therapeutic development.

Interferon (IFN) was first discovered in 1945 and since then has been developed for use in many medical conditions. In short, there are 3 types of interferons: I, II and III. Type I consists of IFN-α, IFN-β and IFN-ω and type II IFN-γ. Interferon type III remains poorly defined and signals through a receptor complex consisting of IL10R2 (also known as CRF2-4) and IFNLR1 (CRF2-12). The latter is only a recent discovery (20) and its genetic make-up is highly relevant to the therapeutic response to therapy as discussed in chapter VII. Interferon types I and II are used widely in the treatment of many medical illnesses, especially immune-mediated conditions such as multiple sclerosis and cancers including haematological and renal malignancies (21, 22, 23). However, the commonest use of IFN-α, in combination with a nucleoside analogue, ribavirin, is in the treatment of chronic hepatitis C infection (24).

At the cellular level, IFNs work by binding directly to the specific IFN receptors. Each type of IFN has its own receptor and all appear to share a partial common post receptor pathway. They work via

the Janus Kinase (JAK)/Signal Transducer and Activation of Transcription (STAT) pathway to induce nuclear and protein production to exert its anti-viral effect. The IFN-α products bind to the IFN-stimulated response element (ISRE) while the IFN-γ the gamma activation site to induce the antiviral response. These physiological actions are summarized in *Figure 1*, (*adapted from 20*).

B. INTERFERON-α MONOTHERAPY IN THE TREATMENT OF HEPATITIS C.

In its earliest use, IFN-α alone was used for chronic non-A non-B hepatitis before hepatitis C was confirmed as the cause in the majority of cases. (25). Subsequent trials confirmed the effectiveness of IFN-α as monotherapy but many patients relapsed following its cessation (26, 27). In fact, the response then was very poor at ~30% necessitating a review and reevaluation of the treatment. It was then discovered that the addition of ribavirin (RBV), an old-fashioned antiviral agent, significantly improved the outcomes of chronic HCV cases. This was subsequently approved for chronic HCV treatment in combination with IFN-α in 1998 (28).

C. COMBINATION INTERFERON-α AND RIBAVIRIN THERAPY IN HEPATITIS C

(i) REGULAR INTERFERON-α

The combination of IFN-α and RBV became routine therapy which included thrice weekly injections of the IFN and daily oral RBV. The frequency of multiple injections, in addition to adverse effects, constituted a major barrier to the initiation of and compliance with

treatment. Interferon-α therapy further evolved in 1999 when it was pegylated (29). This involved the addition of an inert polyethylene glycol moiety to the regular IFN-α which then markedly increased its half-life and significantly improved treatment compliance because of reduced frequency of injections.

(ii) PEGYLATED INTERFERON-α

From 2000 onwards, combination pegylated IFN-α and RBV became standard therapy for HCV infection with sustained virologic response rates (defined as undetectable HCV Ribonucleic Acid activity in serum 6 months after the completion of therapy) ranging from 40-50% for patients with genotypes 1 and 4 and 70-80% for genotypes 2 and 3 (24). Since then, this has become the standard and optimal treatment for chronic hepatitis C infection, notwithstanding the recent introduction of protease inhibitor to combined IFN and RBV treatment (30).

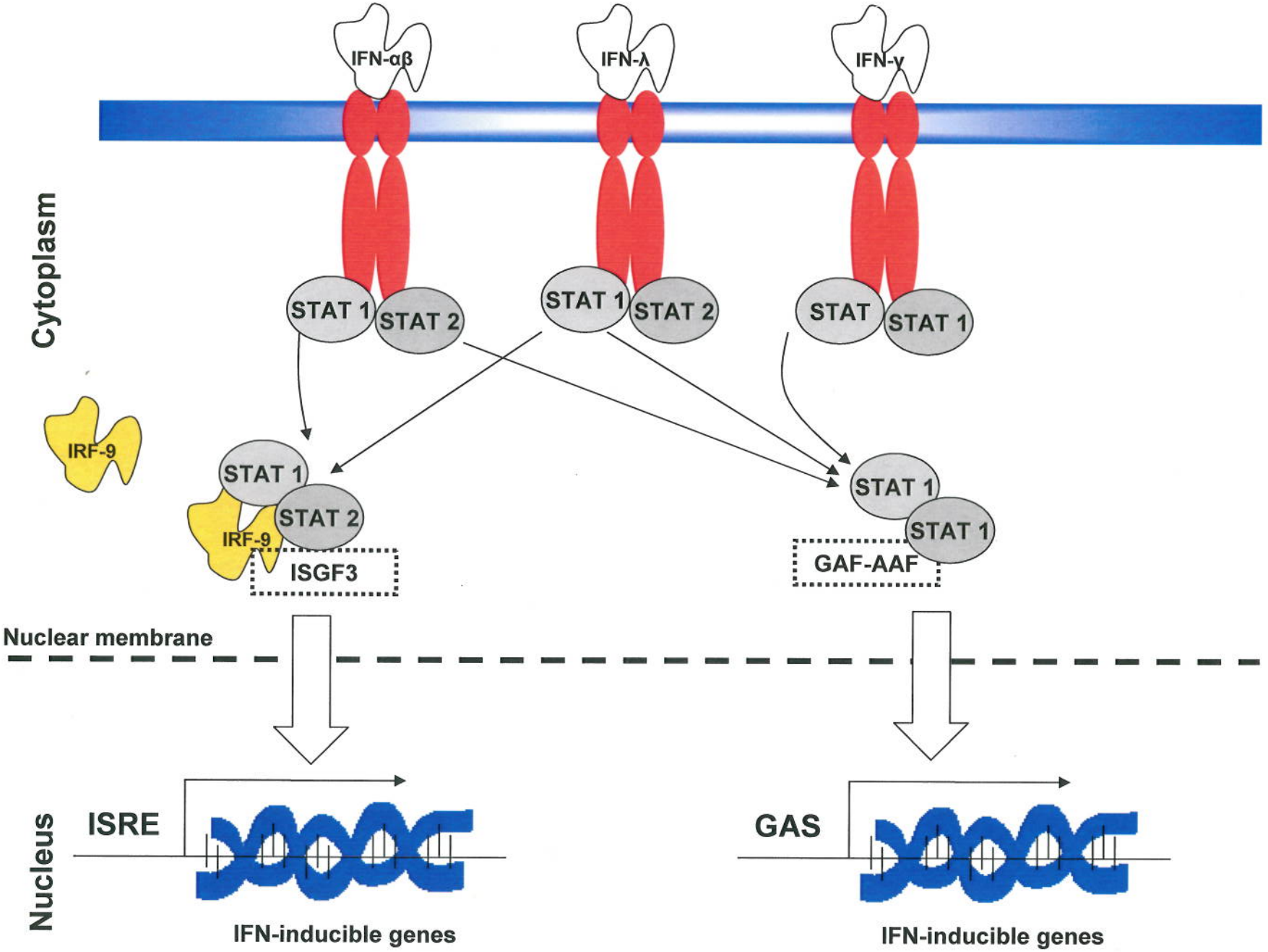

Figure 1. A schematic representation of the intracellular physiological action of the various interferon types

CHAPTER II. EPIDEMIOLOGY OF THYROID DISEASE, HEPATITIS C AND INTERFERON THERAPYY

A. INTERFERON-α AND THYROID DISEASE

As the use of IFN-α increased, it became evident that the therapy had a significant number of adverse effects, most of which are immune mediated. Thyroid disease became recognized as the most common such adverse effect in this setting. Indeed, the association between thyroid disease and IFN-α use was first observed in the treatment of breast cancer, well before the treatment of hepatitis C (31). Following the first use of IFN-α for chronic HCV infection, cases of thyroid related diseases were first reported in 1997 (32). Following this report, further publications supported this observation although the condition was not well characterized and under-appreciated.

Even though the incidence of hepatitis C infection has plateaued, if not declined early in the 21st century (33), it is anticipated that there will be an increase in the number of patients treated with IFN-α. It is additionally critical that related thyroid disease and its prognosis be better understood. Most reports of this condition were case series and hence the prevalence was unknown. A literature review of this condition is included (3).

B. PREVALENCE IN AUSTRALIA

(i) CLINICAL EVIDENCE

(ii) HISTOLOGIC/POST-MORTEM EVIDENCE

A retrospective study was performed to assess the epidemiological status and the prevalence of thyroid disease was found to be ~4% (4). Others reported similar results but these were also mostly retrospective and the thyroid surveillance frequency was inadequate to fully detect and clarify thyroid diseases. There were no clear cut criteria for the diagnosis of thyrotoxicosis (34). The diagnosis remains a clinical one. *Table 1* summaries the available published studies, the frequencies of thyroid function assessment and definitions of thyroid diseases. In many cases, the definitions of thyroid diseases are clearly unsatisfactory, the authors adopting parameters outside normal reference ranges to define hyper- and/or hypo-thyroidism (38). The definitions are even more ill-defined by some groups whilst some completely failed to mention the criteria for defining thyroid disease (44). It appears that many reports fail to appreciate the clinical entity of non-thyroidal illness (also known as sick euthyroid syndrome), in which the thyroid parameters can transiently mimic both hyper- and hypo-thyroidism (34). These thyroid parameters are expected to normalize in the fullness of time. Furthermore, the inappropriate frequency of testing, in which some series monitor the condition every 6 months, can easily fail to detect, define and diagnose the exact thyroid condition.

All these confounders make it very difficult to ascertain the true prevalence of true thyroid disease in this clinical setting and thus justify the introduction of a common management guideline. This proposal is presented in chapter VIII of this dissertation.

In addition to clinical studies, we were fortunate enough to investigate the prevalence of thyroid disease in a cohort of hepatitis C patients at post mortem. This was found to be at 13%, not inconsistent with recent reports (5).

The major difference of the 2010 post-mortem study and our 2005 study is that the earlier report detected the actual clinical entity whereas our subsequent post-mortem study detected histological evidence of thyroid disease without necessarily transforming or progressing to active thyroid disease. Of note no in-situ pituitary pathology was found. This issue will be further discussed in chapter V.

Authors	Publication year and study type	Number of patients	Number of thyroid patients (%)	TFT criteria	pIFN & RBV	Regular IFN & RBV	Type of thyroid disease	Frequency of monitoring
Andrade LJ et al (34)	2001 Pros	65	4 (4.6%)	Not stated	0	65	1 TTX and 2 Hypo	3 monthly
Costelloe et al (35)	2010 Pros	260	58 (2.6%)	TSH < 0.27 and TSH > 4.2	0	58	27 TTX and 31 Hypo	4 weekly
Dabrowska et al (36)	2010 Retro	114	12 (13.5%)	Not stated	0	89	12 TTX and 4 Hypo	3 monthly
Jamil et al (37)	2009 Retro	346	37 (8.8%)	TSH > 4 or fT4 < 10 and TSH < 4.0 or > 23	25	12	Not stated	3 monthly
Vezali et al (38)	2009 Retro	50	13 (21.3%)	TSH > 4.0 or < 0.3 irrespective of fT4/fT3 levels	50	0	Not stated	3 monthly
Gelu-Simeon et al (39)	2009 Retro	264	27 (10.0%)	TSH <0.3 or >4.0 on 2 successive tests	182	82	Not stated	2 monthly
Masood et al (40)	2008 Pros	100	18 (18.0%)	"Below or above normal range"	0	100 for 24 wks *irrespective of genotype*	Not stated	3 monthly
Kee et al (41)	2006 Pros	461	58 (12.6%)	TSH > 5.0, fT4 < 10.2 and TSH < 0.1, fT4 > 25.9	9	49	Not stated	3 monthly

Wirth et al (42)	2005 Pros	62	5 (10.3%)	TSH elevation, nil other	61	0	Elevated TSH	Not stated
Moncoucy et al (43)	2005 Retro	67	4 (7.0%)	Increase or decrease in TSH on 2 occasions (0.23-4.0)	Not stated	Not stated	Not stated	Every 2-3 months
Tran et al (4)	2005 Retro	272	18 (5.5%)	TSH < 0.1, fT4 > 26.0 or TSH > 4.0		272	15 Hypo and 3 Hyper	Monthly
Bini et al (44)	2004 Pros	225	15 (10.7%)	TSH > 5.5, fT4 < 10.3 or TSH<0.4, fT4>34.7		15	12 Hypo and 3 Hyper	Monthly
Kontorinis et al (45)	2003 Pros	81	8 (10.0%)	Not stated		8	6 Hypo and 2 Hyper	Not stated
Adinofi et al (46)	2003 Pros	114	1 (1.1%)	Not stated		1	Thyroiditis	Every 2-4 weeks
Carella et al (47)	2002 Pros	72	11 (15.3%)	TSH>3.5, fT4 < 9.0, no def for TTX		11	Not stated	Before and after 6 months of therapy
Kryczka et al (48)	2001 Pros	57	22 (38.6%)	Not defined			Not stated	

Table 1. Summary of publications on the prevalence of thyroid disease, their definition and frequency of thyroid testing in the setting of RBV and IFN-α. Abbreviations, TFT: Thyroid

Function Tests; pIFN: pegylated Interferon; RBV: Ribavirin; TSH: Thyrotropin; TTX: Thyrotoxicosis; Hypo: Hypothyroidism; Pros: Prospective; Retro: Retrospective.

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CME REVIEW ARTICLE #24

Chief Editor's Note: *This article is the 24th of 36 that will be published in 2007 for which a total of up to* 36 AMA PRA Category 1 Credits™ *can be earned. Instructions for how credits can be earned precede the CME Examination at the back of this issue.*

Hepatitis C Infection, Treatment Regimens, and Thyroid Function Abnormalities

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Abstract: Hepatitis C has become one of the major epidemics afflicting young people in both the developed and developing countries in the late 20th century. This peculiar infection has been associated with many extrahepatic manifestations, including renal glomerular and autoimmune diseases. The most common endocrine disorder, especially after treatment with interferon-α (IFN-α)-based therapy, is autoimmune thyroid disease. Although the pathophysiology of this condition is generally understood to be related to the immunomodulating properties of the hepatitis C virus particles, accentuated by exogenous IFN-α when treated, not every patient undergoing treatment encounters autoimmune thyroid disease, indicating there are other factors at play. This is particularly applicable to primary hypothyroidism. A surveillance program for thyroid diseases is proposed for patients undergoing IFN-α-based treatment.

Key Words: HCV, ribavirin, α-interferon, hypothyroidism, thyrotoxicosis

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Learning Objectives

- Outline the ways in which hepatitis C virus infection and its treatment may contribute to the development of autoimmune thyroid disease and consequent thyroid dysfunction.
- Identify relationships among human immunodeficiency virus infection, interferon therapy, and both thyrotoxicosis and hypothyroidism.
- Recall whether and how coinfection by hepatitis C virus and human immunodeficiency virus influences the risk of thyroid disease.

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Dr. Tran has disclosed that he has no significant relationships with or financial interests in any commercial company that pertains to this educational activity.

Lippincott Continuing Medical Education Institute, Inc. has identified and resolved all faculty conflicts of interest regarding this educational activity.

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Hepatitis C is one of the major epidemics afflicting young people worldwide. Fortunately, the incidence of hepatitis C virus (HCV) has either declined or plateaued in recent years.[1,2] The latter has occurred in Australia in the last 2–3 years due to better education and needle exchange programs.[3] In southeast Asia and Africa, the respective prevalences are 2.2% and 5.3%.[4] In 1999, the World Health Organization estimated that 170 million people were infected with HCV worldwide.[2] Although the prevalence of chronic HCV infection in the United States is 1.3%, it results in a substantial 3.2 million patients.[5]As a result of the high number of infected patients, it has been observed that HCV and related treatment regimens can result in an increased incidence of autoimmunity, including the generation of autoantibodies and some autoimmune diseases such as type I diabetes mellitus, systemic lupus erythromatosus, and crescentic glomerulonephritis.[6] The most common endocrine manifestation of HCV is autoimmune thyroid disease, leading to hyperthyroidism or more commonly primary hypothyroidism. Additionally, treatment regimens that include interferon (IFN)-α can increase the incidence of thyroid diseases, most commonly primary hypothyroidism. The available data regarding this issue, however, are incomplete and need to be interpreted with care. The current National Academy of Clinical Biochemistry guidelines make no recommendations regarding monitoring for thyroid disease in treating HCV.[7] As the number of HCV-infected patients are relatively young, this presages a marked increase in thyroid-related complications. Thus, it is vital that the association between HCV and thyroid disease be better understood so that appropriate management strategies can be implemented. The expected increase in the surveillance for thyroid disease will be reflected in the number of thyroid function tests and subsequent cost to the community health services. This report takes a detailed look at the available data regarding HCV, its treatments, and thyroid diseases.

Pathogenesis

The theory regarding the causality of HCV, thyroid disease, and its treatment focuses on immunodysregulation.[6,8,9] The exact mechanism remains uncertain; however, it is interesting to observe the common thyroid in the first 24 hours of IFN-α therapy. Thyrotropin (TSH) levels tend to decrease while free T4 and free T3 remain unchanged. Interleukin (IL)-6 levels increase, and tumor necrosis factor and IL-1 levels fall.[10] In chronic exposure, TSH levels remain fairly stable, dropping to a nadir at 4 months, followed by a return to pretreatment levels. All remain within the normal reference range.[11] It is hypothesized HCV particles will result in an induction of IFN-α and $-\beta$ production in the thyroid gland as part of the innate immune response.[12] IFN can also activate natural killer cells, maturation of dendritic cells, proliferation of dendritic cells, proliferation of memory T cells, and prevention of T-cell apoptosis.[13] These will induce a rise in the thyroid autoantibodies titer. These antibodies will, in turn, damage the thyroid gland, depending upon genetic predisposition. The addition of treatment regimens that include α-IFN may cause hypothyroidism, at least in part, by an abnormal expression and function of key proteins involved in iodine uptake and organification.[14] Beside the α form, β-IFN is reported to result in thyroid dysfunction, although not as frequently.[15] This is probably because treatments using β-IFN are often used in conditions without concomitant HCV infection such as multiple sclerosis. The pegylated form of IFN seems to have the same effects as standard IFN.[16] Much less information is available about thyroid function in subjects with HCV and human immunodeficiency virus (HIV). Preexisting thyroid nodularity, high thyroid autoantibody titer, and smoking seem to increase the effects of IFN. Iodine status does not seem to alter the effect of IFN on thyroid function.[17] Genetic and constitutional factors such as human leucocyte antigen-DR3, cytotoxic T-lymphocyte antigen-4, female gender, increased age, pregnancy, and family history all contribute to the final outcome.[18] The majority of patients treated with a combination IFN therapy, however, have no major thyroid dysfunction.[11,19] Figure 1 summarizes the proposed pathogenesis of this condition.

In studies documenting IFN-associated thyroid disease, some drawbacks include the lack of clarity in defining the condition. Some use the term *thyroid dysfunction* rather than indicating clearly hypo- or hyperthyroidism.[20–24] This makes the understanding of each form of thyroid disease more difficult.

The natural history of HCV alone and HCV treatment-associated autoimmune thyroid disease is irregular and unpredictable. Some studies report permanent or partial hypothyroidism[20,25,26] and some report complete recovery after the completion of therapy.[24,27] Some showed only biochemical TSH elevation, with subsequent normalization.[20] A similar scenario is applicable to the thyrotoxic states, although this is much less frequent.

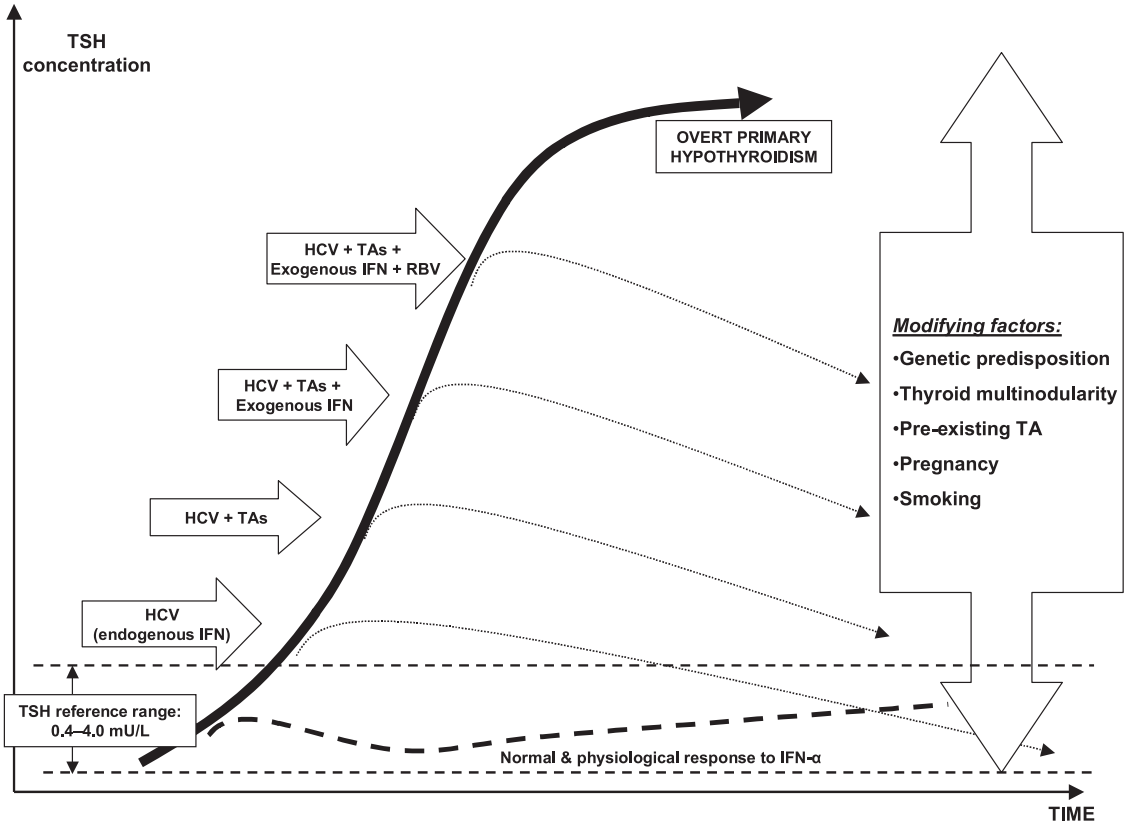

FIGURE 1. The proposed hypothesis regarding the evolution of PH in relation to HCV, treatment-related regimens, and associated modifying factors.

TABLE 1. The Prevalence of Thyrotoxicosis in the General Community, Hepatitis C Virus, and Hepatitis C Virus Treatment-Related Regimens

	General Community	HCV	HCV + sIFN	HCV + sIFN + RBV	HCV + pIFN + RBV	HCV/HIV + pIFN + RBV
Prevalence	0.5% USA (28) 2.0% Japan (28) 19% Sweden (28) 0.63% Scotland (55)	10.0% (31) 3.7% (With HBV coinfection) (29)	Not available; most reports include thyroid dysfunction	3.7% (29) 2.7% (30) 1.1% (11)	No data available	No data available

Numbers in parentheses indicate the actual references.
HCV indicates hepatitis C virus; HIV, human immunodeficiency virus; pIFN, pegylated interferon; RBV, ribavirin; sIFN, standard interferon.

Hepatitis C, IFN Therapy, and Thyrotoxicosis

Hyperthyroidism is not uncommon in the general community, and the prevalence varies between communities and countries, depending on iodine status. It ranges from 0.5% in the United States to 2% in Japan and 19% in Sweden.[28] The data on the prevalence of thyrotoxicosis in HCV and IFN-related treatment regimens are sparse. Wong et al[29] and Bini and Mehandru[30] report an incidence of 3.7% and 2.7% in 245 and 225 hepatitis C virus patients, respectively. Tran et al[16] found an incidence of ~1.1% in Australia. Antonelli et al[31] found an incidence of thyrotoxicosis of 10.0% but this is the same in the control group. Any thyrotoxicosis that occurs outside the time frame of IFN therapy should be assessed carefully for the usual and common causes. Hypothetically, it is possible that IFN therapy may lay the "fertile soil" for the subsequent development of autoimmune thyroid disease into the future.[32] In addition, the symptoms, due to IFN-α use can be confused with those of hyperthyroidism and thus complicate the diagnosis. There are essentially 2 types of thyrotoxicosis associated with hepatitis C virus and IFN-based treatments: Graves-like thyrotoxicosis and biphasic (destructive) thyroiditis. It is highly recommended that a radioisotope uptake scan be performed to differentiate between the 2 conditions. Also, thyroid testing and follow-up should be done at the correct time to avoid misdiagnosis as hypothyroidism.[24] Table 1 compares the prevalence of thyrotoxicosis

TABLE 2. The Prevalence of Primary Hypothyroidism and Thyroid Autoantibody in the General Community, Hepatitis C Virus, and Hepatitis C Virus Treatment-Related Regimens

	General Community	HCV	HCV + sIFN	HCV + sIFN + RBV	HCV + pIFN + RBV	HCV/HIV + pIFN + RBV
Primary Hypothyroidism						
Prevalence	United States: 4.6% (43) Italy: ~4.0% (31, 44) UK: 7.3% (45)	13% (31) 2.3% (23) 4.7% (27) 6.2% (46)	4.8% (24) 4.0% (23) 0.9% (35) 7.3% (47) 7.2% (48) 22.8% (34) 3.4% (20) 2.8% (27) 7.1% (26)	19% (9) 5.3% (30) 7.4% (19)	Similar to those using sIFN, although the exact percentages are not reported (37, 39)	No data available
Thyroid Autoimmunity						
Anti-TPO	13.0% (43) 10–15% (49) 11% (31) 14% (50)	21% (31) 4.8% (24) 12.3% (51) 5.1% (20) 10.3% (52) 6.2% (46)	10.0% (24) 10.9% (53)	4.4% (30)	No data available	No data available
Anti-Tg	11.5% (43) 3% (49) 10% (31) 14.6% (50)	17% (31) 1.7% (20) 2.1% (46)	20.0% (9)	21.4% (Combined anti-TPO and anti-Tg) (9), 4.8% (30)		
TSI	1–2% (49) 0.4% (54)					

Numbers in parentheses indicate the actual references.
HCV indicates hepatitis C virus; HIV, human immunodeficiency virus; pIFN, pegylated interferon; RBV, ribavirin; sIFN, standard interferon; Tg, thyroglobulin; TPO, myeloperoxidase; TSI, thyroid stimulating immunoglobulin.

in the general community and various HCV-related thyroid abnormalities.

Hepatitis C, IFN Therapy, and Hypothyroidism

Hypothyroidism is the most common end result of hepatitis C–related thyroid disease. It has been observed most commonly in the presence of IFN-α treatment. A combination IFN-α and ribavirin (RBV) therapy has become the gold standard treatment for patients with HCV. The data on primary hypothyroidism in this group of treatments are very limited (Table 2), and very few trials have found abnormalities in this setting. Carella et al[8] studied 72 HCV patients and found a significant increase in primary hypothyroidism in the group where RBV was added compared with α-IFN alone, suggesting a synergy between IFN-α and RBV. Kontorinis et al[19] documented an incidence of 7.4% in a cohort of 81 patients. Tran et al[11] found a primary hypothyroidism prevalence of 7.5% in 272 subjects. A recent meta-analysis found no overall increase in thyroid dysfunction with the addition of RBV to IFN-α.[35] Table 2 summarizes these studies.

Another treatment option for HCV is the introduction of pegylated interferon-α. This modified IFN-α involves the addition of a polyethylene glycol moiety to the rIFN molecule, which results in a longer half-life with increased therapeutic efficacy. Thus, medication can be given on a weekly basis rather than thrice weekly. Despite this difference, the pegylated IFN has a similar effect on thyroid functions,[36,37] with very few supportive published data.

Many patients are coinfected with HIV and HCV. Combination therapies[38–41] have not highlighted an increase in hypothyroidism or thyroid disease. However, it was noted that the adverse outcomes using pegylated interferon and RBV are very similar to those of rIFN and RBV.[38] It is reasonable to conclude that the incidence of primary hypothyroidism is too low to be included in the adverse outcomes or that the conditions were not designed to detect thyroid dysfunction. If the former is correct, it can be speculated that HIV might confer a protective effect on the development of thyroid disease. It is possible that there is inadequate immunostimulation to initiate an inflammatory process sufficient to induce thyroid dysfunction.

CONCLUSION

In patients with HCV and/or receiving IFN-based therapy, there appears to be a notable increase in thyroid dysfunction. Thyrotoxicosis is uncommon and should be diagnosed and treated on its own merit. Primary hypothyroidism should be monitored with monthly TSH measurement. There are very few data regarding thyroid outcomes in HCV/HIV coinfected patients treated with combination IFN-based therapy, as autoimmune disease is unusual in this cohort.

Recommendation for Thyroid Surveillance During Treatment With IFN-α Based Therapy

General

All patients should be assessed initially for a history or family history of thyroid disease, relevant medications, the presence of a goiter, baseline TSH, and anti-myeloperoxidase antibody titer.

Thyrotoxicosis

Case detection should be performed depending on clinical status and a high index of suspicion.

Each case should be managed similar to cases arising de novo, including TSI, anti-myeloperoxidase, and antithyroglobulin antibodies, radionuclide uptake studies.[8]

Immune-mediated thyrotoxicosis (Graves-like) should be clearly distinguished from destructive thyrotoxicosis. Monthly TSH level is recommended, especially in indeterminate cases, to detect the hypothyroid phase so that patients are not mistakenly misclassified as having primary hypothyroidism.[42]

Hypothyroidism

Those with preexisting hypothyroidism should be treated expectantly and anti–hepatitis C virus regimen continued forthwith.

Those in the high-risk groups as highlighted by the positive fining of any of the above should have monthly TSH until the end of therapy on the basis of the half-life of free tetra-iodothyronine. Similar to thyrotoxicosis,[42] this also assists in distinguishing between the hypothyroid phase of thyroiditis and primary hypothyroidism.[47]

In the absence of above highlighted risk factors, monthly TSH should be performed for the first 4 months when the TSH is expected to nadir and then 3 times monthly thereafter until the completion of therapy.[11]

Any symptoms or signs resembling primary hypothyroidism should be promptly followed up with TSH and free tetra-iodothyronine levels.[8,9]

REFERENCES

1. Alter MJ. Epidemiology of hepatitis C. *Hepatology*. 1997;26(3 suppl): 62S–65S.
2. World Health Organization website. Available at: http://www.who.int/csr/disease/hepatitis/whocdscsrlyo2003/en/index3. html. Accessed December 10, 2006.
3. Australia's notifiable diseases status, 2002. Annual report of the National Notifiable Diseases Surveillance System. Available at: http://www.cda.gov.au. Accessed December 10, 2006.
4. World Health Organization. Hepatitis C: global prevalence (update). *Wkly Epidemiol Rec*. 1999;74:425–427.
5. Armstrong GL, Wasley A, Simard EP, et al. The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. *Ann Intern Med*. 2006;144:705–714.
6. Wilson LE, Widman D, Dikman SH, et al. Autoimmune disease complicating antiviral therapy for hepatitis C virus infection. *Semin Arthritis Rheum*. 2002;32:163–173.
7. Demers LM, Spencer CA. Laboratory support for the diagnosis and monitoring of thyroid disease, 2003. Available at: www.nacb.org/lmpg/thyroid_lmpg_pub.htm. Accessed December 10, 2006.
8. Carella C, Mazziotti G, Amato G, et al. Interferon-α-related thyroid disease: pathophysiological, epidemiological, and clinical aspects. *J Clin Endocrinol Metab*. 2004;89:3656–3661.
9. Prummel MF, Laurberg P. Interferon-α and autoimmune thyroid disease. *Thyroid*. 2003;13:547–551.
10. Corssmit EPM, Heyligenberg R, Endert E, et al. Acute effects of interferon-alpha administration on thyroid hormone metabolism in health men. *J Clin Endocrinol Metab*. 1995;80:3140–3144.
11. Tran HA, Jones TL, Batey RG. The spectrum of thyroid disease treated with alpha-2-beta interferon and ribavirin in an Australian population. *BMC Endocr Disord*. 2005;5:8.

12. Lloyd AR, Jagger E, Post JJ, et al. Host and viral factors in the immunopathogenesis of primary hepatitis C virus infection. *Immunol Cell Biol.* Advance online publication, 28 November 2006.
13. Tilg H. New insights into the mechanisms of interferon alpha: an immunoregulatory and anti-inflammatory cytokine. *Gastroenterology*. 1997;112:1017–1021.
14. Caraccio N, Guannini R, Cuccato S, et al. Type I interferon modulate the expression of thyroid peroxidase, sodium/iodide symporter, and thyroglobulin genes in primary human thyrocyte cultures. *J Clin Endocrinol Metab*. 2005;90:1156–1162.
15. Durelli L, Ferrero B, Oggero A, et al. Thyroid function and autoimmunity during interferon beta-1b treatment: a multicenter prospective study. *J Clin Endocrinol Metab*. 2001;86:3525–3532.
16. Tran HA, Attia J, Jones TL, et al. Pegylated interferon-alpha-2-beta in combination with ribavirin does not aggravate thyroid dysfunction in comparison to regular interferon-alpha-2-beta in a hepatitis C population: meta-analysis. *J Hepatol Gastroenterol.* Advance online publication, 1 December 2006.
17. Minelli R, Braverman LE, Biuberti T, et al. Effects of excess iodine administration on thyroid function in euthyroid patients with a previous episode of thyroid dysfunction induced by interferon-alpha treatment. *Clin Endocrinol (Oxf)*. 1997;47:357–361.
18. Tomer Y, Davies TF. Searching for the autoimmune thyroid disease susceptibility genes: from gene mapping to gene function. *Endocrin Rev*. 2003;24:694–717.
19. Kontorinis N, Garas GM, Young J, et al. Outcome, tolerability and compliance of compassionate use interferon and ribavirin for hepatitis C infection in a shared care hospital clinic. *Intern Med J*. 2003;33:500–504.
20. Imagawa A, Itoh N, Hanafusa T, et al. Autoimmune endocrine disease induced by recombinant interferon-alpha therapy for chronic active type C hepatitis. *J Clin Endocrinol Metab*. 1995;80:922–926.
21. Ward LD, Bing-You GR. Autoimmune thyroid dysfunction induced by interferon-alfa treatment for chronic hepatitis C: screening and monitoring recommendations. *Endocr Pract*. 2001;7:52–58.
22. Chan WB, Chow CC, Cockram SC. Interferon alpha treatment and endocrine disease. *J R Soc Med*. 2003;96:484–485.
23. Hsieh M-C, Yu M-L, Chuang W-L, et al. Virologic factors related to interferon-α-induced thyroid dysfunction in patients with chronic hepatitis C. *Eur J Endocrinol* 2000;142:341–347.
24. Dalgard O, Bjoro K, Hellum K, et al. Thyroid dysfunction during treatment of chronic hepatitis C with interferon alpha: no association with either interferon dosage or efficacy of therapy. *J Intern Med*. 2002;251:400–406.
25. Fernandez-Soto L, Gonzalez A, Escobar-Jimenez F, et al. Increased risk of autoimmune thyroid disease in hepatitis C vs hepatitis B before, during, and after discontinuing interferon therapy. *Arch Intern Med*. 1998;158:1445–1448.
26. Carella C, Mazziotti G, Morisco F, et al. Long-term outcome of interferon-alpha-induced thyroid autoimmunity and prognostic influence of thyroid autoantibody pattern at the end of treatment. *J Clin Endocrinol Metab*. 2001;86:1925–1929.
27. Marazuela M, Garcia-Buey L, Gonzalez-Fernandez F, et al. Thyroid autoimmune disorders in patients with chronic hepatitis C before and during interferon-α therapy. *Clin Endocrinol*. 1996;44:635–642.
28. Wang C, Crapo LM. The epidemiology of thyroid disease and implications for screening. *Endocrinol Metab Clin North Am*. 1997;26:189–218.
29. Wong V, Fu Xi-Li A, George J, et al. Thyrotoxicosis induced by alpha-interferon therapy in chronic viral hepatitis. *Clin Endocrinol*. 2002;56:793–798.
30. Bini EJ, Mehandru S. Incidence of thyroid dysfunction during interferon-α-2β and ribavirin therapy in men with chronic hepatitis C. *Arch Intern Med*. 2004;164:2371–2376.
31. Antonelli A, Ferri C, Pampana A, et al. Thyroid disorders in chronic hepatitis C. *Am J Med*. 2004;117:10–13.
32. Lin YQ, Wang X, Murthy MS, et al. Life-threatening thyrotoxicosis induced by combination therapy with PEG-interferon and ribavirin in chronic hepatitis C. *Endocr Pract*. 2005;11:135–139.
33. Okanoue T, Sakamoto S, Itoh Y, et al. Side effects of high dose interferon therapy for chronic hepatitis C. *J Hepatol*. 1996;25:283–291.
34. Preziati D, La Rosa L, Covini G, et al. Autoimmunity and thyroid function in patients with chronic active hepatitis treated with recombinant interferon alpha-2a. *Eur J Endocrinol*. 1995;132:587–593.
35. Brok J, Gluud LL, Gluud C. Effects of adding ribavirin to interferon to treat chronic hepatitis C infection. *Arch Intern Med*. 2005;165:2206–2212.
36. Russe MW, Fried MW. Side effects of therapy for chronic hepatitis C. *Gastroenterology*. 2003;124:1711–1719.
37. Hadziyannis SJ, Sette H, Morgan HR, et al. Peginterferon-2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med*. 2004;140:346–355.
38. Myers RP, Benhamou Y, Bochet M, et al. Pegylated interferon alpha 2b and ribavirin in HIV/hepatitis C virus-co-infected non-responders and relapsers to IFN-based therapy. *AIDS*. 2004;18:75–79.
39. Fried MW, Shiffman ML, Reddy KR, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med*. 347;13:975–982.
40. Perez-Olmeda M, Nunez M, Romero M, et al. Pegylated IFN-2b plus ribavirin as therapy for chronic hepatitis C in HIV-infected patients. *AIDS*. 2003;17:1023–1028.
41. Moreno L, Quereda C, Moreno A, et al. Pegylated interferon 2b plus ribavirin for the treatment of chronic hepatitis C in HIV-infected patients. *AIDS*. 2004;18:67–73.
42. Tran HA. The uncertain natural history of thyrotoxic patient treated with combination interferon alfa-2β and ribavirin. *Arch Intern Med*. 2005;165:1072.
43. Hollowell JG, Staehling NW, Flanders WD, et al. Serum TSH, T4, and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). *J Clin Endocrinol Metab*. 2002;87:489–499.
44. Aghini-Lombardi F, Antonangeli L, Martino E, et al. The spectrum of thyroid disorders in an iodine-deficient community: the Pescopagano Survey. *J Clin Endocrinol Metab*. 1999;84:561–566.
45. Vanderpump MPJ, Tunbridge WMG, French JM, et al. The incidence of thyroid disorders in the community: a twenty-year follow-up of the Whickham Survey. *Clin Endocrinol*. 1995;43:55–68.
46. Ganne-Carrie N, Medini A, Coderc E, et al. Latent autoimmune thyroiditis in untreated patients with HCV chronic hepatitis: a case-control study. *J Autoimmun*. 2000;14:189–193.
47. Baudin E, Marcellin P, Pouteau M, et al. Reversibility or thyroid dysfunction induced by recombinant alpha interferon in chronic hepatitis C. *Clin Endocrinol*. 1993;39:657–661.
48. Marcellin P, Pouteau M, Benhamou J-P. Hepatitis C viral infection, alpha interferon therapy and thyroid dysfunction. *J Hepatol*. 1995;22:364–369.
49. Saravanan P, Dayan CM. Thyroid autoantibodies. *Endocrinol Metab Clin North Am*. 2001;30:315–337.
50. Salabe-Lotz H, Salabe GB. Population survey of thyroid autoimmunity in Italy: three year follow up. *Thyroidology*. 1990;2:107–112.
51. Huang MJ, Tsai SL, Huang BY, et al. Prevalence and significance of thyroid autoantibodies in patients with chronic hepatitis C virus infection: a prospective controlled study. *Clin Endocrinol*. 1999;50:503–509.
52. Rocco A, Gargano S, Provenzano A, et al. Incidence of autoimmune thyroiditis in interferon-alpha treated and untreated patients with chronic hepatitis C virus infection. *Neurol Endocrinol Lett*. 2001;22:39–44.
53. Piquer S, Hernandez C, Enriquez J, et al. Islet cell and thyroid antibody prevalence in patients with hepatitis C virus infection: effect of treatment with interferon. *J Lab Clin Med*. 2001;137:38–42.
54. Costagliola S, Morgenthaler NG, Hoermann R, et al. Second generation assay for thyrotropin receptor antibodies has superior diagnostic sensitivity for Graves' disease. *J Clin Endocrinol Metab*. 1999;84:90–97.
55. Flynn RWV, MacDonald TM, Morris AD, et al. The thyroid epidemiology, audit, and research study: thyroid dysfunction in the general population. *J Clin Endocrinol Metab*. 2004;89:3879–3884.

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The spectrum of thyroid dysfunction in an Australian hepatitis C population treated with combination Interferon-α2β and Ribavirin

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Abstract

Background: The study aims to assess the pattern of thyroid response to combination Interferon-α2β (IFN-α) and Ribavirin (RBV) anti-viral therapy in an Australian hepatitis C cohort. These include the prevalence of thyroid dysfunction (TD) including hyperthyroidism and hypothyroidism and their possible predictors, the common overall pattern of thyroid function tests whilst receiving therapy and TD outcomes, and the correlation with HCV status outcome.

Methods: A retrospective analysis of all medical records was performed to assess thyroid function in Hepatitis C Virus (HCV) patients who were treated at the Hunter Area hepatitis C treatment center between 1995 and March 2004. The centre is part of the John Hunter hospital, a major tertiary referral centre in New South Wales, Australia.

Results: There were 272 cases available for review. The prevalence of TD is 6.7 percent and is made up predominantly of females (80 percent). There were 3 (1.1 percent) cases of hyperthyroidism with 2 (67 percent) females. Thirteen out of fifteen (80 percent) cases of hypothyroidism were females with the overall prevalence of 5.5 percent. The majority of hypothyroid patients still required Thyroxine supplement at the end of follow up.

Conclusion: Ninety three percent of HCV treated patients have intact thyroid function at the end of treatment. The predominant TD is hypothyroidism. The predominant pattern of thyrotoxicosis (TTX) is that of thyroiditis although the number is small. Graves' like disease was not observed. People with pre-existing thyroid auto-antibodies should be closely monitored for thyroid dysfunction, particularly hypothyroidism.

Background

Hepatitis C infection is one of the major epidemics afflicting young adults with more than 150,000 known infected cases in Australia and a notification rate of ~16,000 cases per year in 2002 [1]. In the United States, HCV remains the most common chronic blood-born infection [2,3]. The effective management of the new cases is critically important because, without treatment, approximately 14,000 will develop chronic HCV infection, 6,500 will develop HCV-related cirrhosis, 175 liver failure and 50

with hepatocellular carcinoma. The treatment typically involves the combination of IFN-α and RBV therapy. This is an effective therapy with a 'cure' rate of up to 70% depending on genotype as judged by the negative HCV Ribonucleic Acid (RNA) polymerase chain reaction (PCR) detection [4]. However, no treatment is free from complication and the use of IFN-α is well documented to be associated with TD, the commonest autoimmune disorder associated with IFN-α therapy. This study looks at the combined effect of IFN-α and RBV in an exclusively Australian group of patients with hepatitis C to determine the pattern of thyroid behaviour with direct regards to *eu-*, *hyper-* and *hypo*-thyroidism. The prevalence of hypothyroidism prevalence and outcome of treatment, in terms of HCV RNA clearance, in direct relationship to thyroid condition are both determined.

Methods

Patients

The cases of 272 patients who received combination therapy between 1995 and end of March 2004 at the John Hunter hospital Hepatitis C service were reviewed. All other causes of chronic hepatitis were excluded. No patient had dual Hepatitis B and C. Baseline characteristics of all studied subjects are included in Table 1. Family history of thyroid disease is not available other than in patients who subsequently developed TD.

Table 1: Baseline characteristics of 272 patients who received combination IFN-α and RBV therapy for HCV

Demographics	
Mean age (years)	42 ± 8
Males	150 (55%)
Caucasians	204 (75%)
Asians	22 (8%)
Weight (kg)	79 ± 18
HCV Genotype	
1	136 (50%)
2	22 (8%)
3	103 (38%)
4	11 (4%)
Liver Function Tests (RR)	
Albumin (36–48 g/L)	41 ± 2
Serum Bilirubin (2–20 μmol/L)	15 ± 6
Alanine Aminotransferase (< 45 U/L)	133 ± 58
γ-Glutamyl Transpeptidase (1–30 U/L)	98 ± 46
Prothrombin time (11–18 seconds)	15 ± 3
Haematological Parameters (RR)	
Haemoglobin (115–165 g/L)	142 ± 16
White cell counts (4.0–11.0 × 10^6/mL)	7.1 ± 2.0
Platelets (150–400 × 10^9/mL)	168 ± 49

Laboratory assays

Serum autoantibodies to anti-thyroglobulin (anti-Tg) and anti-thyroperoxidase (anti-TPO) were measured by agglutination (Serodia-ATG and Serodia-AMC, Fujirebio, Inc., Tokyo, Japan). Titre of less than 1:400 was considered normal for both. Thyroid Stimulating Immunoglobulin (TSI) was measured using cell culture and radio-immunoassay. This is an in-house bioassay using Chinese Hamster Ovary (CHO) cells in culture to detect the presence of thyroid stimulating activity. The CHO cells are transfected with the TSH receptor genes and thus are responsive to TSI. Thyroid-stimulating activity is measured by evaluating the intracellular release of cyclic Adenosine Mono-Phosphate induced by the patient's serum immunoglobulin on the CHO cells. The results are reported as units/mL (U/mL). TSI should be absent in the normal population. A TSI level of <10 is considered negative, 10–50 as weakly, 50–100 as moderate and >100 U/mL as strongly positive.

Third generation serum thyrotropin (TSH), serum free tetra- and free tri-iodothyronine (fT4 and fT3) were determined by two-site sandwich immunoassay using an automated chemiluminescent system (Diagnostic Products Corporation, Immulite 2000). The reference range (RR) for TSH was 0.4–4.0 mU/L, fT4 10.0–26.0 and fT3 3.5–5.5 pmol/L. The coefficients of variations (CV) were 5.0 % and 5.1 % at TSH concentrations of 4.0 mU/L and 10.0 mU/L respectively. For fT4, the CV was 6.5% at 10.0 pmol/L and fT3 8.9% at 3.5 pmol/L.

Therapy

All patients were treated with combination IFN-α and RBV therapy. The duration of treatment depends on the HCV genotypes; genotypes 2 and 3 were treated for 24 weeks and types 1 and 4 for 48 weeks respectively. Treatment was continued to the end for the latter irrespective of the HCV RNA status at 24 weeks. The dosage for IFN-α was 3 MIU thrice a week with RBV dose ranging from 1000 to 1200 mg daily according to bodyweight.

Thyroid function assessments

All patients received routine thyroid function tests (TFT) at the start of treatment and at monthly intervals. If there was any concern, then the frequency was increased as clinically indicated. Most, particularly the thyrotoxic group, but not all patients were referred to the Endocrine service for review. Thyroxine supplement was prescribed for hypothyroidism when deemed clinically appropriate. All patients were followed up for a period of 12 months after the completion of anti-viral therapy. Some were followed additionally for a longer period in the Endocrine outpatient service. Thyroid autoantibodies were not routinely

Table 2: The pattern of TTX in patients receiving combination IFN-α and RBVNo thyroid nuclear or ultrasonic imaging was available for *all* thyrotoxic cases

Subjects	1	2	3
Gender	F	F	M
Age	26	35	36
HCV Genotype	1a	3a	1g
Regimen duration (weeks)	48	24	24
Adverse reactions	Myalgia, lethargy, emotional lability	None	Insomnia, rash
Baseline ALT	184	142	104
ALT at time of thyroid disease	33	28	17
Duration of therapy prior to thyroid disease (weeks)	16	8	32
Symptoms of thyroid disease	Anxiety and depression	Non-specific	Non-specific
Signs	No goitre	No goitre	No goitre
Peak fT4 (pmol/L)	56.7	57.0	27.0
Peak fT3 (pmol/L)	15.1	15.3	12.8
Thyroid autoantibody status	• Anti-Tg <1 • Anti-TPO 1:409,600 • TSI <1	• Anti-Tg <1 • Anti-TPO 1:409,600 • TSI <1	• Anti-Tg <1 • Anti-TPO <1:100 • TSI <1
Thyroid outcomes	Hypothyroidism on replacement therapy	Hypothyroidism on replacement therapy	Thyroid condition resolved with IFN-α dose reduction
Hepatic outcomes	PCR negative	PCR negative	PCR negative

performed until the patient developed hypo- or hyperthyroidism on clinical and/or biochemical grounds.

TTX was defined as having TSH of < 0.1 mU/L, fT4 levels > 26.0 and/or fT3 levels > 5.5 pmol/L respectively.

Hypothyroidism, including subclinical hypothyroidism, was defined as having TSH levels > 4.0 mIU/L. Thyroxine supplement was, however, given when deemed clinically appropriate regardless of the degree of TSH elevation.

Thyroid dysfunction

Thyroid dysfunction (TD) was defined as having *hypo*- or *hyper*-thyroidism, (clinically and/or biochemically based)

Statistics

Data are presented as percentage and mean ± Standard Error of Mean (SEM).

Results

Incidence of thyroid dysfunction in HCV treated group

The majority of patients (93%) had no TD at the end of treatment. Amongst the 272 patients, there were a total of 18 (6.7%) cases of TD: 3 *hyper*-thyroidism and 15 *hypo*-thyroidism.

There were 3 cases of pre-existing hypothyroidism detected at baseline and thus were excluded from the study. All cases were detected at the initial assessment for the treatment of hepatitis C and were shown to have autoimmune hypothyroidism requiring thyroxine supplement. This gave a pre-treatment hypothyroidism prevalence of 1.1% (3/275). Hypothyroidism was seen in 15 (5.5%) with 12 (80%) females after treatment. Thirteen (87%) patients required thyroxine therapy.

TTX was observed in 3 (1.1%) patients whose characteristics are listed in Table 2. Two (67%) were females and all required endocrinology attention. None had any thyroid imaging studies and thus the diagnosis was made on clinical grounds, auto-antibody detection and disease behaviour.

Thyroid response to combination Interferon-α2B and RBV therapy

1.1 Euthyroid patients

Patients who were treated with IFN-α and had normal TFTs were divided into 2 groups according to duration of treatment: at 24 (n = 117) and 48 (n = 136) weeks respectively. Both demonstrated a drop in TSH levels at about 4 months after entry into therapy. Interestingly, none of these patients lowered their TSH level below 0.4 U/L. This was followed by a return to pre-treatment levels and remained so in the follow-up period. Results are illustrated in Table 3 and Figure 1.

1.2 Hypothyroid patients

All hypothyroid patients had non-specific symptoms and relevant data are listed in table 4. Free T3 levels were not routinely measured in the presence of hypothyroidism.

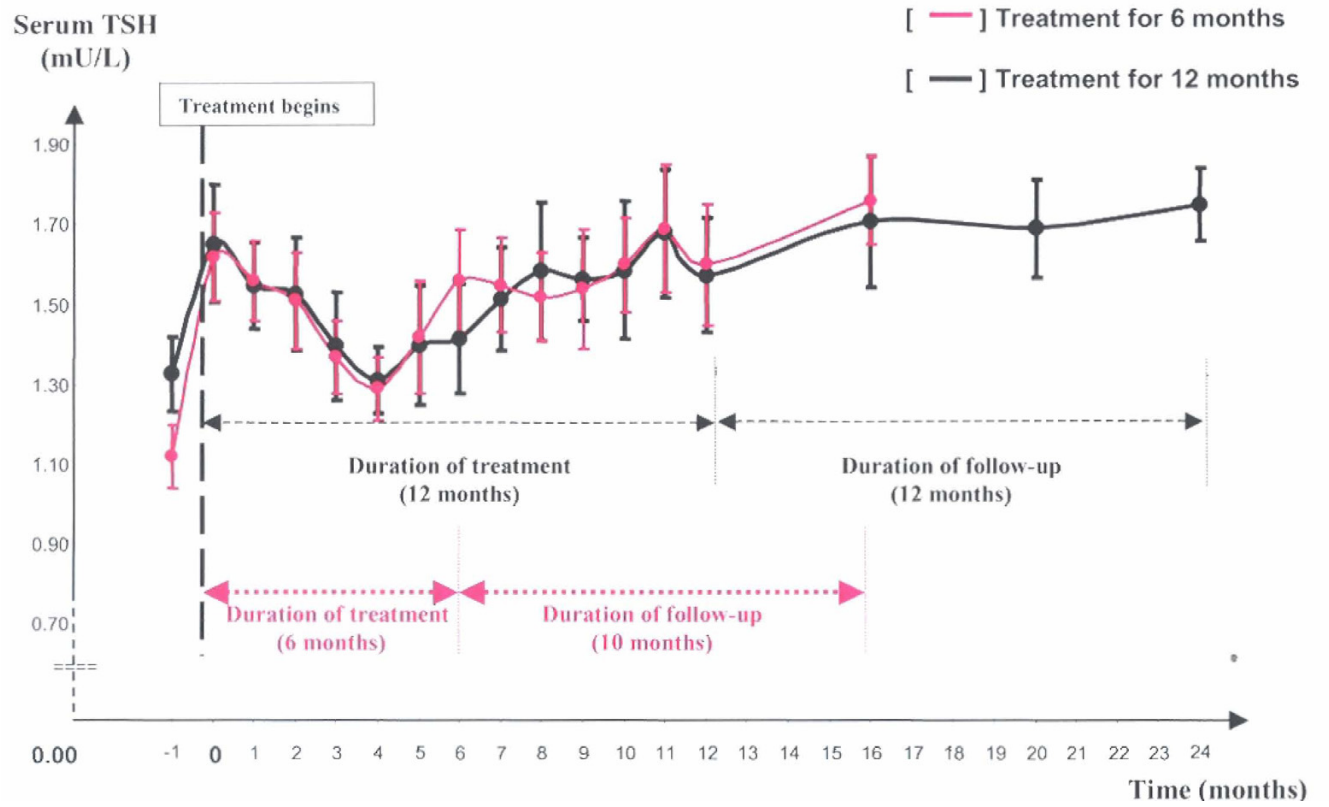

Figure 1
The normal patterns of TSH response in hepatitis C patients during and after receiving combination IFN-α and RBV therapy in the two treated groups at different durations.

Table 3: TSH data for the 6 and 12 month treated cohorts who did not develop TD

Time (months)	0	2	4	6	16
Mean TSH ± SEM (mU/L) for the *6 month treated cohort* n = 117	1.62 ± 0.110	1.51 ± 0.124	1.29 ± 0.088	1.56 ± 0.1312	1.76 ± 0.1116
Mean TSH ± SEM (mU/L) for the *12 month treated cohort n = 136*	1.65 ± 0.15	1.31 ± 0.08	1.58 ± 0.17	1.57 ± 0.14	1.71 ± 0.16

Anti-TPO antibodies were detected in 6 out of 15 hypothyroid patients (40%) with titres ranging from 1: 6,400 to 1: 409,600. The rest had titres <1:400. Baseline autoantibodies were not routinely requested at the initial assessment.

1.3 Hyperthyroid patients

All 3 thyrotoxic patients had suppressed TSH levels with varying levels of fT4 and fT3. The time to development of TTX was variable but most occurred whilst receiving treatment. Anti-TPO antibodies were elevated in patients 1 and 2 (2 out of 3 cases) and both behaved in a thyroiditis fashion. Hypothyroidism developed rapidly and Thyroxine supplement was subsequently required within 1 month. In patient 3, despite the negative antibody results, the TTX responded to a reduction in IFN-α dosage suggesting an IFN-α/autoimmune mediated mechanism. TSI was undetectable in all 3 patients.

All (100%) patients with thyroid dysfunction cleared their hepatitis C infection based on the aforementioned criteria

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Table 4: Data for hypothyroid patients receiving combination therapy for Hepatitis C infection

Number of subjects	15 *(9 treated for 48 weeks and 6 for 24 weeks)*
Gender	3 M : 12 F
Mean age	44 ± 2
Positive family history of thyroid disease (in first degree relatives)	8/18 (44%)
Duration of IFN-α before thyroid disease developed (months)	4.8 ± 1.2
Mean TSH levels (mU/L)	29.7 ± 8.8
Mean fT4 levels (pmol/L)	3.5 ± 0.9
The presence of TPO-autoantibodies	6/15 (40%)
Thyroxine requirement at the 12-month follow-up	13/15 (87%)
PCR negativity at the end of treatment	15/15 (100%)

against a background overall response rate of ~75% to the combination anti-viral therapy in our cohort.

Discussion

This is the first report to characterise thyroid responses to combination anti-viral treatment in an Australian cohort with hepatitis C infection. The prevalence of TD is ~7% of which 83% is hypothyroidism. The predominant gender is female at 80%. This is consistent with previous reports relevant to this condition [5-7]. The prevalence range is broad reflecting the differences in the definition of TD, some are biochemically based, others clinically based or both.

Overall, 93% (254/272 cases) of patients have normal thyroid outcome. In the majority of cases, the TSH levels remain within reference range throughout. Levels decrease slightly at 4 months but return to pre-existing level with the continuation of treatment irrespective of the duration of therapy. The range of TSH values is narrow ranging between 1.15 at the 4-month nadir and 2.03 at 16 months in 95% of cases whilst undergoing therapy. FT4 levels were not routinely measured in the presence of normal TSH levels which remain the single best assessment of thyroid function in the presence of intact hypothalamo-pituitary-thyroid axis [8], expected in this subgroup. Whilst it is tempting to attribute the decrease in TSH level during treatment to IFN-α and RBV, it is likely that non-thyroidal illness is the main underlying pathogenesis. Other co-morbidities associated with combination treatment (but particularly IFN-α) include fever, headache, depression and neuropsychiatric disturbance etc... all contribute to the changes in TFTs [9].

In TTX, the number is too small to arrive at any definitive conclusions. Only the destructive thyroiditis form is observed in this study. Goitre is absent in all cases. In patients 1 and 2, this type of hyperthyroidism rapidly converts to hypothyroidism in the presence of high auto-antibody titre and subsequently required permanent Thyroxine supplement. In patient 3, the TTX was mild and resolved with IFN dose reduction. This was presumed to be IFN-α/autoimmune mediated despite the absence of auto-antibodies. This pattern has been observed elsewhere [10-12]. Unfortunately, radioactive iodine uptake scans were not performed so that the diagnoses could be unequivocally confirmed. TTX resembling Graves' disease was not observed. In this scenario, TTX is uncommon and thus each case should be dealt with on its own merits. The index of clinical suspicion should remain high whilst on therapy. Management is best done in a specialist Endocrine clinic, with destructive thyroiditis being more common, often resulting in permanent hypothyroidism.

Hypothyroidism is clearly the commoner cause of TD in combination therapy. It has been suggested that the virus itself may play a role in the development of hypothyroidism in IFN-naïve patients as the prevalence is higher than the general population [13]. Also, if IFN-α alone is thought to cause hypothyroidism, the prevalence of hypothyroidism should be higher than in IFN-naïve patients and this is the case of this report with a 5 folds increase after treatment. However, this observation has not been consistent in the general literature, see Table 5[14-19]. To further add to the complexity of the situation, hypothyroidism is also more frequent in patients having combination therapy of IFN-α and RBV [20] (as opposed to IFN-α treated alone). Whilst HCV is well known to induce a higher prevalence of auto-antibody, this does not necessarily translate into hypothyroidism (either clinical or subclinical) [14]. It would have been additionally interesting to characterize, in parallel, the pattern of thyroid function in our HCV patients who did not receive HCV treatment for direct comparison. Unfortunately these data are not available.

The pathogenesis remains poorly understood but IFN-α is thought to be related to have a direct inhibitory effect on

Table 5: The prevalence of hypothyroidism in the IFN-naïve and IFN-treated HCV patients compared with the general populations. The prevalence of elevated TSH levels in the various population groups include: Australia ~2.0% [17], the United States of America ~7.3% [18], Spain ~2.0% [18], Japan ~1.3% [18], Great Britain ~7.3% [18] and Italy ~1.7% [19]

Studies N = number of subjects	Prevalence of hypothyroidism (before IFN)	Prevalence of hypothyroidism (after IFN)
Marcellin et al. [5] N = 74	0	7.2
Preziati et al. [15] N = 78	0	22.8
Imigawa et al. [14] N = 58	0	3.4
Marazuela et al. [7] N = 207	4.7	2.8
This work N = 272	**1.1%**	**7.5%**
Baudin et al. [16] N = 68	0	7.3
Antonelli et al. [13] N = 630	13%	N/A

thyrocytes preventing hormonogenesis and secretion. Another postulate is immunostimulation in the presence of hepatitis C infection. This is thought to include activation of lymphocytes and natural killer cells, increased production of Tumor Necrosis Factor, IFN-α, Interleukin and other cytokines and increased production of immunoglobulins [21]. All lead to the development of thyroid auto-antibodies with complete destruction and consequently permanent hypothyroidism in genetically susceptible individuals. Although this does occur in hepatitis B IFN-α treated patients, the prevalence of hypothyroidism is much lower. This suggests that the hepatitis C virus or its genome plays an integral part the development of thyroid dysfunction [10]. The virus itself has been postulated to induce thyroid auto-antibodies by ways of generating high *endogenous* IFN levels triggering off autoimmune thyroid disease in susceptible individuals, similar to Coxsackievirus. This virus and others have been shown to induce a higher level of endogenous IFN-α levels which have been associated with other auto-immune diseases such as type 1 diabetes [22]. When IFN-α is administered exogenously, another layer of complexity is added. It is possible but purely speculative that exogenous IFN-α synergises with the endogenous source, thus exaggerating the effect on the thyroid thus causing additional hypothyroidism.

Whatever the underlying pathophysiological process is, the major factors contributing to hypothyroidism includes the female gender with a relative risk ranging between 3–7 times [23] and the presence of anti-TPO antibodies (Ab). These factors feature prominently in our report although the prevalence of anti-TPO Ab is lower. The time to onset of biochemical hypothyroidism means at about 5 months, similar to other cohorts [6].

When IFN-α triggers destructive thyroid disease in genetically susceptible individuals, some may recover but the majority does not in our study. Thyroxine requirement therefore is expected to be long term as illustrated in 87% (13/15) of our cases. All 6 (100%) patients where anti-TPO antibodies are detected, are on thyroxine at the completion of follow up. The symptomatology of hypothyroidism is non-specific and often overlaps with IFN-α side-effects. The diagnosis often requires biochemical testing with some patients receiving thyroxine supplement at mildly elevated concentrations of TSH and/or low/normal fT4 levels. Where thyroxine was deemed necessary and thus given, all were continued with thyroxine supplement at 12-month follow-ups suggesting that thyroid damage is permanent although longer follow-up is preferred.

All patients with TD cleared their HCV infection up until end of follow-up. The number is too small to make any conclusion about the advantage of having thyroid dysfunction when it comes to eradicating the virus. Hypothetically, these patients may have developed an exaggerated response which helps to clear the virus and in the process induces thyroid dysfunction, an unfortunate but acceptable side-effect of the treatment modality. Whilst this is highly plausible, it has not been supported by other investigators [24].

Conclusion

IFN-α in combination with RBV therapy, by and large, does not cause thyroid dysfunction in the majority of HCV patients undergoing such treatment. Patients undergoing such therapy can expect a small decrease in TSH levels, which remained in the RR however, without going into frank hyper- or hypothyroidism. The incidence of TD in HCV patients receiving combination anti-viral therapy is generally small with hypothyroidism being the most common. All patients should be assessed thoroughly for relevant risk factors and monitoring for dysfunction at the appropriate time frame. Hyperthyroidism is uncommon

Table 6: Major summary points from the study

1. The majority of patients with HCV undergoing combination IFN-α and RBV therapy have normal thyroid function.
2. The commonest cause of thyroid dysfunction is hypothyroidism, a ratio of 4:1 compared with hyperthyroidism.
3. The average time from the start of anti-viral treatment to hypothyroidism is ~4–5 months, suggesting that this is the critical time to carry out thyroid testing.
4. Predisposing risk factors include female gender, family history of thyroid disease and existing thyroid auto-antibodies, especially anti-TPO antibodies. In this situation, initial TSH should be performed to exclude pre-existing hypothyroidism.
5. Hyperthyroidism is less common and each should be managed on its own merits in a specialised Endocrine service.

and thus is best managed in a specialised Endocrine clinic. The important points derived from this study are summarized in Table 6.

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

TLJ gathered, provided the data and participated in the discussion and drafting of the manuscript. RGB participated in the scientific discussion and drafting on the manuscript. HAT conceived the study, participated in its design, assisted with data collection, coordinated and helped to draft the manuscript. All authors read and approved the revised manuscript.

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References

1. Australia's notifiable diseases status: **Annual report of the National Notifiable Diseases Surveillance System.** 2002 [http://www.cda.gov.au]. accessed 29-September-2005.
2. CDC: **Recommendations for prevention and control of hepatitis C virus (HCV) infection and HCV-related disease.** *MMWR Recomm Rep* 1998, **47(RR-19):**1-40.
3. Alter MJ, Kruszon-Moran D, Nainan OV, McQuillan GM, Gao F, Moyer LA, Kaslow RA, Margolis HS: **The prevalence of hepatitis C virus infection in the United States, 1988 through 1994.** *N Engl J Med* 1999, **341:**556-562.
4. Sievert W: *An overview of antiviral therapy for chronic hepatitis C infection* Edited by: Crofts N, Dore G, Locarnini S. Hepatitis C: an Australian perspective. Melbourne: IP Communications; 2001:140-154.
5. Marcellin P, Pouteau M, Benhamou J-P: **Hepatitis C viral infection, alpha interferon therapy and thyroid dysfunction.** *J Hepatol* 1995, **22:**364-369.
6. Okanoue T, Sakamoto S, Itoh Y, Minami M, Yasui K, Sakamoto M, Nishioji K, Katagishi T, Nakagawa Y, Tada H, Sawa Y, Mizuno M, Kagawa K, Kashima K: **Side effects of high dose interferon therapy for chronic hepatitis C.** *J Hepatol* 1996, **25:**283-291.
7. Marazuela M, Garcia-Buey L, Gonzalez-Fernandez F, Garcia-Monzon D, Arranz A, Borque MJ, Moreno-Otero R: **Thyroid autoimmune disorders in patients with chronic hepatitis C before and during interferon-α therapy.** *Clin Endocrinol* 1996, **44:**635-642.
8. Larsen PR, Davies TF, Schlumberger M-J, Hay ID: *Chapter 10, Thyroid Physiology and Diagnostic Evaluation of Patients with Thyroid Disorders. Williams Textbook of Endocrinology* 10th edition. Edited by: Larsen PR, Kronenberg HM, Melmed S and Polonsky KS. Saunders, Elsevier Science Ltd; 2003:331-373.
9. Pegram M, Mitsuyasu RT: *Immune modulation: interferons and interleukins* Edited by: Rich RR, Fleisher TA. Clinical Immunology: Principles and Practice St. Louis: CV Mosby; 1996:2030-2036.
10. Fernandez-Soto L, Gonzalez A, Escobar-Jimenez F, Vazquez R, Ocete E, Olea N, Salmeron J: **Increased risk of autoimmune thyroid disease in hepatitis C vs hepatitis B before, during, and after discontinuing interferon therapy.** *Arch Intern Med* 1998, **158:**1445-1448.
11. Wong V, Fu Xi-Li A, George J, Cheung NW: **Thyrotoxicosis induced by alpha-interferon therapy in chronic viral hepatitis.** *Clin Endocrinol* 2002, **56:**793-798.
12. Minelli R, Valli MA, Di Secli C, Finardi L, Chiodera P, Bertoni R, Ferrari C, Barilli AL, Coiro V, Saccanti Jotti G, Delsignore R: **Is steroid therapy needed in the treatment of destructive thyrotoxicosis induced by alpha-interferon in chronic hepatitis C?** *Horm Res* 2005, **63:**194-199.
13. Antonelli A, Ferri C, Pampana A, Fallahi P, Nesti C, Pasquini M, Marchi S, Ferrannini E: **Thyroid disorders in chronic hepatitis C.** *Am J Med* 2004, **117:**10-13.
14. Imagawa A, Itoh N, Hanafusa , Oda Y, Waguri M, Miyagawa J-I, Kono N, Kuwajima M, Matsuzawa Y: **Autoimmune endocrine disease induced by recombinant interferon-alpha therapy for chronic active type C hepatitis.** *J Clin Endocrinol Metab* 1995, **80:**922-926.
15. Preziati D, La Rosa L, Covini G, Marcelli R, Rescalli S, Persani L, Ninno ED, Meroni PL, Colombo M, Beck-Peccoz P: **Autoimmunity and thyroid function in patients with chronic active hepatitis treated with recombinant interferon alpha-2a.** *Eur J Endocrinol* 1995, **132:**587-593.
16. Baudin E, Marcellin P, Pouteau M, Colas-Linhardt N, Le Floch JP, Lemonnier C, Benhamou JP, Bok B: **Reversibility of thyroid dysfunction induced by recombinant alpha interferon in chronic hepatitis C.** *Clin Endocrinol* 1993, **39:**657-661.
17. Topliss JD, Eastman CJ: **Diagnosis and management of hyperthyroidism and hypothyroidism.** *Med J Aust* 2004, **108:**186-193.
18. Wang C, Crapo LM: **The epidemiology of thyroid disease and implications for screening.** *Endocrinol Metab Clin North Am* 1997, **26:**189-218.
19. Salabe-Lotz H, Salabe GB: **Population of thyroid autoimmunity in Italy. Three year follow up.** *Thyroidology* 1999, **2:**107-112.
20. Carella C, Mazziotti G, Morisco F, Rotondi M, Cioffi M, Tuccillo C, Sorvillo F, Caporaso N, Amato G: **The addition of ribavirin to interferon-α therapy in patients with hepatitis C virus-related chronic hepatitis does not modify the thyroid autoantibody pattern but increases the risk of developing hypothyroidism.** *Eur J Endocrinol* 2002, **146:**743-749.
21. Carella C, Mazziotti G, Amato G, Braverman EL, Roti E: **Interferon-α-related thyroid disease: Pathophysiological, Epidemiological and Clinical Aspects.** *J Clin Endoc Metab* 2004, **89:**3656-3661.
22. Chehadeh W, Weill J, Vantyghem M-C, Alm G, Lefebvre J, Wattre P, Hober D: **Increased level of interferon-α in blood of patients with insulin-dependent diabetes mellitus: Relationship with Coxsackievirus B infection.** *J Infect Dis* 2000, **181:**1929-1939.
23. Prummel FM, Laurberg P: **Interferon-alpha and Autoimmune Thyroid Disease.** *Thyroid* 2003, **13:**547-551.
24. Dalgard O, Bjoro K, Hellum K, Myrvang B, Bjoro T, Haug E, Bell H: **Thyroid dysfunction during treatment of chronic hepatitis C with interferon alpha: no association with either interferon dosage or efficacy of therapy.** *J Intern Med* 2002, **251:**400-406.

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Original Article

HISTOPATHOLOGIC FINDINGS OF AUTOIMMUNITY IN THYROID, PITUITARY, AND ADRENAL DISEASES IN CHRONIC HEPATITIS C POSTMORTEM CASES

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ABSTRACT

Objective: To assess the histologic prevalence of immune-mediated thyroid, pituitary, and adrenal diseases in postmortem cases with hepatitis C.

Methods: We reviewed 108 consecutive cases of chronic hepatitis C in patients in whom a complete postmortem examination was performed. All microscopic and histologic slides of the thyroid, pituitary, and adrenal reports were reviewed and assessed for evidence of autoimmune diseases. These were compared with a control group of 100 postmortem cases without hepatitis C.

Results: In chronic hepatitis C infection, there is a heightened immune response resulting in many autoimmune diseases. The commonest endocrinopathy in association with this chronic infection is thyroid disease, a finding confirmed in our current study. Among the 108 postmortem cases of hepatitis C, there were 14 cases (13%) with evidence of thyroiditis. No cases of pituitary or adrenal disease were found. The mean age of the patients was 52 years (range, 29 to 68). This frequency compared with 7 cases of thyroid disease (7%) in the control group (no significant difference between the 2 groups).

Conclusion: On the basis of our postmortem data, thyroid disease was the only major endocrinopathy associated with hepatitis C infection, with a prevalence of 13%. This was comparable with other serologic and nonhistologic antemortem findings. There was no evidence of pituitary or adrenal involvement. **(Endocr Pract. 2010;16:566-569)**

Abbreviations:
CI = confidence interval; **FT_3** = free triiodothyronine; **FT_4** = free tetraiodothyronine (thyroxine)

INTRODUCTION

Endocrinopathies are the commonest extrahepatic manifestation of hepatitis C. Most epidemiologic studies involve the use of serology, antibody profile, and imaging techniques to define and categorize the endocrine condition, particularly thyroid disease (1-3). The definitive method for identifying the condition is to perform histologic assessment of tissue samples. Thus far, only one solitary non-English report in the literature has examined the "gold standard" of histologic involvement of the thyroid (4). For complete assessment of the involvement of the 3 major endocrine organs in patients with chronic hepatitis C, histologic slides were reviewed retrospectively for evidence of inflammation of the thyroid, pituitary, and adrenal glands.

METHODS

We reviewed 108 postmortem cases in which chronic hepatitis C had been documented. In addition, 100 postmortem cases without hepatitis C were also reviewed to serve as a control group. All cases were from the database of the Forensic Medicine Department of a major tertiary referral hospital, which performs approximately 1,000 postmortem forensic cases annually. Pediatric and pregnant cases were excluded from both the study group and the control group. No patients had received interferon-based therapy for their

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hepatitis C at the time of their death. The demographic data are summarized in Table 1.

All cases were documented to have hepatitis C, which had been confirmed serologically before death. The serologic findings were confirmed by immunochemiluminescence using the Architect assay (Abbott Laboratories, Abbott Park, Illinois). In addition, all liver histologic slides were reviewed and were found to be consistent with chronic viral hepatitis, although clearly not diagnostic. No immunohistochemical studies, including autoantibody staining, were performed on any of the histologic samples.

Histologic Definitions

1. *Autoimmune thyroiditis* was defined as the presence of interfollicular infiltration with lymphocytes and plasma cells. The presence of germinal centers, destruction of the follicles, and fibrosis may be added to the diagnosis, but these elements are not considered essential (5,6).
2. *Hypophysitis* was defined as the presence of predominantly lymphocytic infiltrate. Plasma cells and lymphoid follicles with germinal centers may be present but are not critical to the diagnosis (5,7).
3. *Adrenalitis* was defined as the presence of long-term cellular infiltrates, including lymphocytes, plasma cells, and histiocytes (5).

Statistical Analysis

Data are presented as mean ± standard error of the mean. The Fisher exact test was used for comparison of the prevalence of thyroid disease in the 2 groups.

RESULTS

Prevalence of Thyroid Disease

Among the 108 consecutive postmortem cases analyzed, the prevalence of thyroid disease was 13% (95% confidence interval [CI], 7% to 19%). There were no cases of pituitary or adrenal disease. The mean age was 52 years (range, 29 to 68). The main causes of death were trauma, suicide, or ischemic heart disease (Table 1). No cases of unexplained sudden death or death due to chronic liver failure were recorded.

In the control group, the prevalence of thyroid disease was 7% (95% CI, 2% to 12%); this finding was not significantly different from the prevalence in the hepatitis C group ($P = .13$). Similarly, no cases of adrenal or pituitary disease were noted.

Biochemical-Histologic Correlation

In 33 of the 108 hepatitis C cases, thyroid function tests, including thyrotropin, free tetraiodothyronine (thyroxine) (FT_4), and free triiodothyronine (FT_3) levels, were available. All thyroid variables were measured within 12

Table 1
Demographic Characteristics, Cause of Death, and Prevalence of Endocrinopathies on Postmortem Examination, Stratified by Study Group

Factor	Hepatitis C group (n = 108)	Control group (n = 100)
Mean age (range) (y)	52 (29-68)	49 (33-67)
Sex (M:F)	62:46	59:41
Cause of death (no.)		
Motor vehicle accident	68	50
Asphyxiation	20	26
Ischemic heart disease	12	9
Drug overdose	4	12
Subarachnoid hemorrhage	2	…
Miscellaneous	2	3
Thyroid disease, no. (%)	14 (13)	7 (7)
Pituitary disease, no. (%)	0 (0)	0 (0)
Adrenal disease, no. (%)	0 (0)	0 (0)

months before the postmortem examinations, and all results were normal. The mean thyrotropin, FT_4, and FT_3 levels with standard errors of the mean were 1.58 ± 0.13 mIU/L, 16.4 ± 2.1 pmol/L, and 4.4 ± 1.8 pmol/L, respectively. The reference ranges for these variables were as follows: thyrotropin, 0.4 to 4.0 mIU/L; FT_4, 10.1 to 25.4 pmol/L; and FT_3, 3.5 to 5.5 pmol/L.

DISCUSSION

This study is the first report in the English-language literature that documents the histologic findings (probably the criterion standard) in the assessment of endocrine organ inflammation in the pituitary, thyroid, and adrenal glands in the setting of hepatitis C. Our findings confirm that the thyroid is the predominant endocrine organ affected with autoimmune lymphocytic infiltration, with a prevalence of 13%. This frequency is consistent with previously reported values, ranging from 5.3% to 19% (8,9). Because of the relatively small number of subjects, it is not surprising that no significant difference was found in comparison with the control group.

The predominant histologic appearance involved clusters of lymphocytes. No distinct germinal centers or fibrosis was found. This scenario does not necessarily translate to active thyroid disease, inasmuch as there are no concomitant corresponding serologic thyroid function studies for validation. It is appreciated, however, that these findings represent various stages in the evolution of autoimmune thyroid disease before the clinical development and expression of active thyroid disease. It is probable that additional factors such as interferon therapy can activate these primed clusters of lymphocytes to clinical thyroid disease. Our postmortem thyroid disease prevalence is lower than the prevalences reported in some previous studies (10,11), likely attributable to the age difference in our relatively young cohort.

There is no histologic evidence of autoimmunity or inflammation in the adrenal and pituitary glands in this setting, although the confidence interval around this zero estimate extends to 3% to 4%. This is consistent with the fact that there are few case reports of hypophysitis and adrenal disease. The first case was described by Sakane et al (12) in 1995, in which the endocrinopathies developed 1 month after interferon therapy was discontinued. This case was shown to have pituitary antibodies against GH3 cells, a rat pituitary tumor cell line that secretes growth hormone and prolactin. In 2003, Concha et al (13) reported a second similar case. The reported panhypopituitarism was detected 1 year after the completion of interferon therapy, and there was no evidence of antipituitary antibodies. Chan and Cockram (14) described a case of panhypopituitarism but in the presence of hepatitis B infection. The patient developed amenorrhea while receiving interferon treatment and displayed permanent panhypopituitarism thereafter. In 2006, Ridruejo et al (15) reported a possible case of reversible or spontaneously recovered hypophysitis during combination interferon and ribavirin therapy. The diagnosis was based on the pituitary hormonal profile, a nonspecific pituitary magnetic resonance imaging finding, and the absence of thyroid and other autoimmune markers. Pituitary antibody tests were not performed. In light of the rare and unconvincing nature of these previous cases, it is not surprising that no evidence of lymphocytic infiltration of the pituitary was detected in our postmortem series. Together with the absence of adrenal disease, it was highly plausible that no cause of death was recorded as sudden or unexplained. Similarly, the absence of death attributable to chronic liver failure was a reflection of our relatively young cohort.

CLINICAL APPLICATION

Our study has several limitations. This is a snapshot of the autoimmune endocrinopathies in patients with hepatitis C and does not address the progression of the disease, especially the thyroid condition. Although the detection of lymphocytic infiltration in thyroid tissue is definitive of chronic thyroid inflammation, this finding may not indicate active disease. Graves disease also is associated with lymphocytic infiltration and thus could not be excluded fully in the absence of additional antemortem autoantibody results. Although Graves disease had been described previously in association with interferon therapy, our experience has been exclusively that of biphasic thyroiditis (16). The availability of biochemical thyroid function tests or an autoantibody profile (or both) would have been invaluable for assessment of the biochemical-histologic relationship.

Despite the aforementioned case reports on the development of adenohypophysitis, most investigators have observed the development of the condition in association with or sometime after the cessation of interferon therapy. These reports pointed toward an association without any definitive proof of causality. In addition, no published report has described the development of hypophysitis in association with hepatitis C alone, without interferon therapy. Similarly, there is no evidence of adrenalitis, consistent with the paucity of reports in the general literature. There is only one related report in the literature (17), which described reversible subclinical hypoadrenalism influenced by ribavirin and interferon-α.

These findings are reassuring in that, on occasions when treatment for hypothyroidism is required, our data suggest little need for assessment of the adrenal status. This consideration refers to the theoretical possibility that excessive thyroxine therapy or thyrotoxicosis can accelerate cortisol metabolism and result in transient hypocortisolemia or precipitate an actual addisonian crisis in patients with occult hypocortisolemia (18,19).

CONCLUSION

Although this investigation confirms the histologic evidence of lymphocytic infiltrative thyroid disease in patients with chronic hepatitis C infection, the prevalence does not exceed that in the general population. This finding suggests that additional contributing factors such as interferon are needed for complete evolution to active thyroid disease. We found no histologic evidence of pituitary or adrenal involvement, despite previous anecdotal case reports.

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DISCLOSURE

The authors have no multiplicity of interest to disclose.

AUTHOR CONTRIBUTIONS

Dr. Tran conceived the study, participated in its design, assisted with data collection and statistical analysis, and coordinated and drafted the manuscript. Dr. Lyons gathered the data and participated in the discussion and drafting of the manuscript. Drs. Attia and Reeves contributed to the statistical and analytical methods. All authors participated in the discussion and read and approved the final revised manuscript.

REFERENCES

1. **Tran HA, Jones TL, Batey RG.** The spectrum of thyroid dysfunction in an Australian hepatitis C population treated with combination interferon-alpha2beta and ribavirin. *BMC Endocr Disord*. 2005;5:8.
2. **Kabbaj N, Guedira MM, El Atmani H, et al.** Thyroid disorders during interferon alpha therapy in 625 patients with chronic hepatitis C: a prospective cohort study. *Ann Endocrinol (Paris)*. 2006;67:343-347.
3. **Rodriguez-Torres M, Rios-Bedoya CF, Ortiz-Lasanta G, Marxuach-Cuétara AM, Jiménez-Rivera J.** Thyroid dysfunction (TD) among chronic hepatitis C patients with mild and severe hepatic fibrosis. *Ann Hepatol*. 2008;7:72-77.
4. **Lun'kova LK, Mkhitarov VA, Makarova OV, Kuzantsova LV.** Morphofunctional characteristics of thyroid in drug addicts [in Russian]. *Ark Patol*. 2006;68:35-38.
5. **Lloyd RV, Douglas BR, Young WF Jr.** Endocrine diseases. In: King DW, ed. *Atlas of Nontumor Pathology*. Washington, DC: American Registry of Pathology and the Armed Forces Institute of Pathology, 2001: 1-257.
6. **Dayan CM, Daniels GH.** Chronic autoimmune thyroiditis. *N Engl J Med*. 1996;335:99-107.
7. **Caturegli P, Newschaffer C, Olivi A, Pomper MG, Burger PC, Rose NR.** Autoimmune hypophysitis. *Endocr Rev*. 2005;26:599-614.
8. **Bini EJ, Mehandru S.** Incidence of thyroid dysfunction during interferon-alfa-2b and ribavirin therapy in men with chronic hepatitis C: a prospective study. *Arch Intern Med*. 2004;164:2371-2376.
9. **Antonelli A, Ferri C, Pampana A, et al.** Thyroid disorders in chronic hepatitis C. *Am J Med*. 2004;117:10-13.
10. **Okayasu I, Hara Y, Nakamura K, Rose NR.** Racial and age-related differences in incidence and severity of focal autoimmune thyroiditis. *Am J Clin Pathol*. 1994;101:698-702.
11. **Williams ED, Doniach I.** Post-mortem incidence of focal thyroiditis. *J Pathol Bacteriol*. 1962;83:255-264.
12. **Sakane N, Yoshida T, Yoshioka K, Umekawa T, Kondo M, Shimatsu A.** Reversible hypopituitarism after interferon-α therapy. *Lancet*. 1995;345:1305.
13. **Concha LB, Carlson HE, Heimann A, Lake-Bakaar GV, Paal AF.** Interferon-induced hypopituitarism. *Am J Med*. 2003;114:161-163.
14. **Chan WB, Cockram CS.** Panhypopituitarism in association with interferon-alpha treatment. *Singapore Med J*. 2004;45:93-94.
15. **Ridruejo E, Christensen AF, Mando OG.** Central hypothyroidism and hypophysitis during treatment of chronic hepatitis C with pegylated interferon alpha and ribavirin. *Eur J Gastroenterol Hepatol*. 2006;18:693-694.
16. **Tran HA, Reeves GE, Jones TL.** The natural history of interferon-alpha2b-induced thyroiditis and its exclusivity in a cohort of patients with chronic hepatitis C infection. *QJM*. 2009;102:117-122.
17. **Tran HA, Song S, Lojewski RJ, Reeves GE.** Exacerbation of hepatitis C induced subclinical hypoadrenalism by interferon-alpha2beta: a case report. *Cases J*. 2008;18:157.
18. **Lewandowski KC, Macinkowska M, Skowroński-Jóźwiak E, Makarewicz J, Lewiński A.** New onset Graves' disease as a cause of an adrenal crisis in an individual with panhypopituitarism: brief report. *Thyroid Res*. 2008;1:7.
19. **Karl M, Onumah BM, Cole J, Golding J, Burman KD, Wartofsky L.** Hypocortisolemia in Graves hyperthyroidism. *Endocr Pract*. 2009;15:220-224.

CHAPTER III. THE DEVELOPMENT OF THYROID DISEASES WITH THE PARADIGM SHIFT TO PEGYLATED INTERFERON

(A) COMPARISON OF REGULAR VERSUS PEGYLATED INTERFERON IN THE DEVELOPMENT OF THYROID DISEASE

As treatment continued to evolve in order to simplify and improve compliance, interferon-α was pegylated to increase its half-life. This involved the addition of an inert polyethylene glycol moiety to interferon to increase its half-life and consequently improving treatment regimen. It also assisted in reducing the frequency of injections and hopefully improving the compliance rate of patients. Because the pegylation involves an inert moiety, it would not be expected to cause any extra adverse reaction. This original research meta-analysis confirmed that there was no difference between regular and pegylated interferon in the development of thyroid disorders.

Publication:

Tran HA, Attia JR, Jones TL, Batey RG. PEGYLATED INTERFERON-α2β IN COMBINATION WITH RIBAVIRIN DOES NOT AGGRAVATE THYROID DYSFUNCTION IN COMPARISON TO REGULAR INTERFERON-α2β IN A HEPATITIS C POPULATION: META-ANALYSIS. *J Hepatol Gastroenterol*, 2007; 22: 472-6.

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HEPATOLOGY

Pegylated interferon-α2β in combination with ribavirin does not aggravate thyroid dysfunction in comparison to regular interferon-α2β in a hepatitis C population: Meta-analysis

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Key words
hepatitis C, pegylated interferon, ribavirin, thyroid dysfunction.

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Abstract

Background: Interferon (IFN) has been well documented to cause thyroid dysfunction, especially in high risk patients and when combined with ribavirin (RBV). There is very sparse data to assess if pegylated IFN will further aggravate the thyroid disease risk in comparison to regular IFN. The purpose of this study was to assess the risk of developing thyroid disease with pegylated IFN (pIFN) *versus* regular IFN (rIFN) therapy (in combination with RBV). We also pooled our results with previous studies in a meta-analysis.
Methods: An observational study was made retrospectively of 24 patients who underwent a combination of rIFN and RBV therapy for hepatitis C virus (HCV) infection. As these patients failed to obtain an initial satisfactory response, they were retreated using pIFN and RBV. Monthly thyrotropin (TSH) levels were assessed while undergoing both treatment regimens. A meta-analysis was performed using available published data in PubMed.
Results: No difference in TSH levels was observed when comparing rIFN/RBV with pIFN/RBV. None of the patients developed hypo- or hyperthyroidism. TSH levels fluctuated during the treatment but did not extend outside the reference range. No further investigation was carried out in the absence of clinical and biochemical thyroid disease. The result of the meta-analysis failed to find any excess risk of thyroid dysfunction using pIFN above that of rIFN.
Conclusions: The pegylation of IFN, in combination with RBV, did not aggravate thyroid diseases in the hepatitis C population. This finding is reassuring and dictates that no deviation from current practice regarding thyroid surveillance is required whilst undergoing HCV treatment.

Introduction

Hepatitis C has become one of the major epidemics of the 21st century, afflicting mostly the young population across the world, including the USA and Australia.[1–4] It has become clear that the best and most effective therapy for the management of this chronic condition is the combination of interferon (IFN) and ribavirin (RBV).[5,6] The former used to be given subcutaneously thrice weekly until the development and introduction of pegylated IFN (pIFN). This modified IFN-á involves the addition of a polyethylene glycol (PEG) moiety to the regular IFN molecule which results in a longer half-life with increased therapeutic efficacy. The advantage of this medication is that it can be conveniently given on a weekly basis rather than thrice weekly.[7] Similar to regular IFN (rIFN), pIFN results in many autoimmune mediated endocrine conditions, the most common of which is thyroid disease.[8,9] Although there are many trials involving the use of pIFN and RBV, there are few data reported specifically regarding the outcome of thyroid function.[10–13] In order to see if there is a need to modify clinical surveillance for thyroid disease, this study aimed to assess if the risk of thyroid dysfunction is different with pIFN therapy in combination with RBV compared with a combination of rIFN and RBV using local data, as well as performing a meta-analysis from published data available on PubMed.

Methods

Patients

The Hunter Area Hepatitis C Treatment Center assesses and treats all cases of hepatitis C in northern New South Wales (NSW), Australia. It is part of the John Hunter Hospital, a major tertiary referral center in NSW. Up until the end of December 2005, the center had treated approximately 305 patients and the

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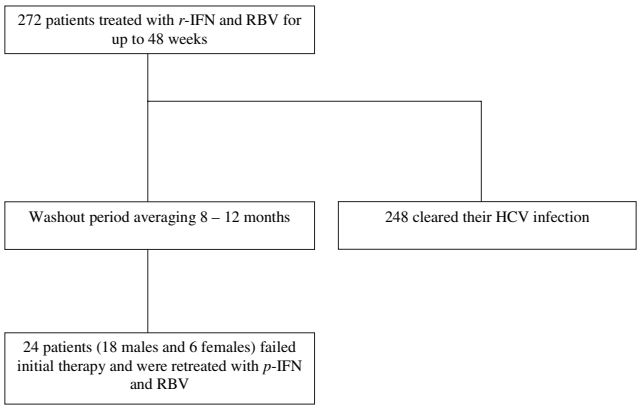

Figure 1 Study flow chart. Initially there were 272 patients treated with a combination of regular interferon (IFN) and ribavirin (RBV). Of these, 24 patients did not eliminate their hepatitis C virus (HCV) infection and thus were retreated with pegylated IFN (pIFN) and RBV.

Table 1 Baseline characteristics of 24 patients who received combination interferon (IFN)-α and ribavirin (RBV) therapy for hepatitis C virus (HCV)

Demographics	
Mean age (years)	45 ± 8
Males	18 (75%)
Caucasians	6 (25%)
Weight (kg)	74 ± 18
HCV genotype	
1	12 (50%)
2	2 (8%)
3	9 (38%)
4	1 (4%)
Liver function tests	
Albumin (36–48 g/L)	38 ± 2
Serum bilirubin (2–20 μmol/L)	14 ± 6
Alanine aminotransferase (<45 U/L)	89 ± 38
γ-Glutamyl transpeptidase (1–30 U/L)	55 ± 36
Prothrombin time (11–18 s)	14 ± 2
Hematological parameters	
Hemoglobin (115–165 g/L)	152 ± 14
White cell count (4.0–11.0 × 10^6/mL)	6.1 ± 1.8
Platelets (150–400 × 10^9/mL)	177 ± 38

Reference ranges are included in parentheses.

characteristics of 272 of these patients have been previously described.[14] Of the 272 patients, there were 24 who failed rIFN and RBV combination therapy and thus were retreated using pIFN and RBV therapy (Fig. 1). All other causes of chronic hepatitis were excluded, including hepatitis B and chronic alcoholic liver disease. Baseline characteristics of all studied patients are shown in Table 1. All 24 patients were assessed for thyroid disease clinically and biochemically prior to the beginning of therapy. No goiter was detected.

Therapy

For rIFN and RBV combination therapy, the duration of treatment depends on the HCV genotypes; genotypes 2 and 3 were treated for 24 weeks and types 1 and 4 for 48 weeks, respectively. For the latter group, treatment was continued for the full 48 weeks, irrespective of the HCV RNA status at 24 weeks. The dosage for regular IFN-α was 3 MIU thrice a week with the RBV dose ranging from 1000 to 1200 mg daily according to bodyweight.

Patients who did not respond to the above routine treatment or relapse were invited back for further treatment using a combination of pIFN and RBV. Non–responders were classified as those who remained polymerase chain reaction (PCR) positive for HCV RNA after 12 weeks of therapy. Relapsers were those who achieved viral clearance at the end of the aforementioned therapy, that is, PCR HCV RNA negative, but were found subsequently to be positive in the 24 month follow-up period. The dosage for pIFN was 180 μg once weekly and the RBV dose was identical to that above. Duration of treatments was also identical to that of rIFN and was genotype dependent. The average washout period between the two treatment courses was 10 months (range 8–12 months; Fig. 2).

Thyroid function assessments

All patients received routine assessments of thyrotropin (TSH) levels at the start of treatment and at monthly intervals. If there was any concern then the frequency was increased as clinically indicated. All patients were followed up for a period of 12 months after the completion of antiviral therapy.

Thyroid dysfunction

Thyroid dysfunction was defined as having hypo- or hyperthyroidism (clinically and/or biochemically based). Thyrotoxicosis was defined as having TSH of <0.1 mU/L, fT4 levels >26.0 and/or fT3 levels >5.5 pmol/L, respectively. Hypothyroidism, including subclinical hypothyroidism, was defined as having TSH levels >4.0 mIU/L. None of the patients were found to satisfy these criteria.

Thyroid autoantibodies were deemed unnecessary as the results, in the presence of normal thyroid values, would not deter patients' progression to therapy.

Laboratory assay characteristics

Third generation serum TSH, serum free tetra- and free triiodothyronine (fT4 and fT3) were determined by two-site sandwich immunoassay using an automated chemiluminescent system (Immulite 2000; Diagnostic Products, Los Angeles, CA, USA). The reference range (RR) for TSH was 0.4–4.0 mU/L, fT4 10.0–26.0 and fT3 3.5–5.5 pmol/L. The coefficients of variations (CV) were 5.0% and 5.1% at TSH concentrations of 4.0 mU/L and 10.0 mU/L, respectively. For fT4, the CV was 6.5% at 10.0 pmol/L while for fT3 it was 8.9% at 3.5 pmol/L.

Statistics and meta-analysis

Data from each study was summarized as a 2 × 2 table and these tables were pooled using the Mantel-Haenszel χ^2 method (fixed effects model) and the DerSimonian-Laird method (random effects), implemented using StatsDirect (vers. 2.2.8; StatsDirect,

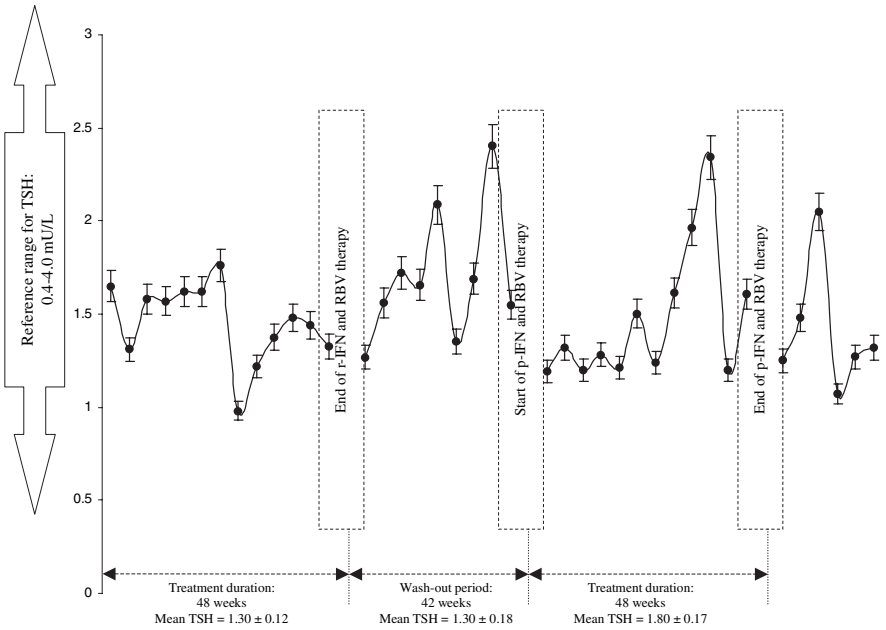

Figure 2 Mean thyrotropin (TSH) levels at each time point in the 24 patients treated with a combination of regular interferon (rIFN) followed by pegylated IFN (pIFN) in combination with ribavirin (RBV).

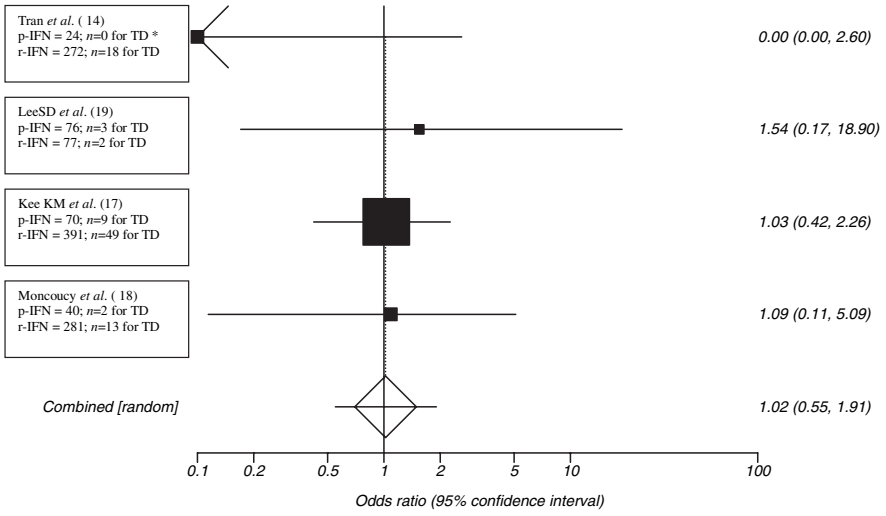

Figure 3 Meta-analysis of patients treated with regular interferon (rIFN) and pegylated IFN (pIFN) in combination with ribavirin (RBV). *Denotes current study. TD, thyroid dysfunction.

Cheshire, UK). Heterogeneity was checked using the Breslow-Day method and publication bias was checked using the Egger test.

Results

There were 18 males and six females undergoing both regimens of IFN in combination with RBV. None of the patients had any thyroid dysfunction whilst undergoing rIFN and RBV or during the follow-up period. Most of the patients were treated with the second course of pIFN within 12 months (mean 8 months) depending on the time of relapse. The selection process was judged according to HCV PCR status and was independent of thyroid status either in the preceding treatment regimen or during the follow-up period.

When undergoing combination pIFN therapy, similarly, there was no abnormality in TSH levels. In addition, there was no particular discernable pattern of thyroid dysfunction, as noted previously in patients undergoing rIFN therapy.[14] The graphical response of TSH to rIFN and then to pIFN in combination with RBV is illustrated in Figure 2, including the mean TSH ± SEM and associated intervals of management.

Our search on PubMed identified three other studies addressing this question, in addition to our data, yielding four studies for pooling[14–17] with a total of 1231 subjects. The pooled odds ratio was 0.94 (95% confidence interval [CI] 0.51–1.75) by the fixed effects model and 1.02 (95% CI 0.54–1.91) by the random effects model (Fig. 3). Both of these indicate no significant difference in thyroid dysfunction between the two regimens, and this estimate

was homogeneous ($P = 0.579$) with no publication bias (Egger's $P = 0.38$)

Discussion

There is a large body of evidence in the literature recognizing the immunostimulating effect of IFN, particularly on the thyroid, which remains the most common endocrine organ affected.[18,19] Although many studies have concentrated mostly on the effect of rIFN, there is few data on the thyroid effect of pIFN as this is a relatively new preparation. As a result, this study sought to clarify if there is any exacerbating effect on the thyroid above that which is associated with rIFN.

The results from this meta-analysis convincingly indicate that, based on all the evidence to date, there is no exacerbation of thyroid disease when the IFN is pegylated as compared to rIFN. This finding is not unexpected or surprising given the modification nature of pIFN. The pegylation of IFN-α-2β involves the addition of a 12 Kdalton polyethylene glycol molecule. Because of this relative benign molecular structure, this addition is not expected to have any effect on the thyroid organ. The majority of the immunomodulatory effect is expected to be due to IFN moiety itself. As pIFN is now widely accepted as the optimal therapy for chronic hepatitis C infection, it is reassuring that the incidence of thyroid dysfunction is not exacerbated. Thyroid dysfunction associated with IFN therapy is known to occur mainly in patients with an underlying predisposition, such as the presence of thyroid autoantibodies. These were not considered necessary in our cohort and their presence would not deny patients therapy. In addition, any thyroid conditions that developed during the treatment regimen would be assessed and treated as required on its own merit.

Although TSH levels fluctuate during treatments and the wash-out period, these range between ~0.90–2.5 mIU/L; fairly tight and well within the reference range. As a result, in the presence of likely intact hypothalamo-pituitary-thyroid axes, subclinical thyrotoxicosis, including thyroiditis with rebound hypothyroidism are unlikely. No thyroid abnormality was detected in the long term at the conclusion of therapy.

This report finding adds to what little information there is regarding thyroid dysfunction in relation to pIFN in the treatment of hepatitis C. Although many trials have been reported using pIFN and RBV for HCV alone or in combination with HIV infection, few included thyroid dysfunction in the reports. Most concentrated on hematological and psychological disturbance, which are the predominant adverse effects. Of note, both the British Society of Gastroenterology and the American Gastroenterological Association recognize the potential thyroid effect of IFN and recommend thyroid screening.[20,21] However, only the former specifies that a thyroid function test is recommended at *each* treatment visit.

The major drawback of all studies to date is the lack of randomization. Similar to a previous report,[16] our study included non-responders to rIFN for pIFN treatment. Although the total number of subjects in the meta-analysis is reasonable ($n = 1231$), the number on pIFN is a minority ($n = 210$), and hence limits the power of the study. The 10 month wash-out period is adequate to minimize the carry over effect, allowing for an adequate amount of time for the IFN to be completely eliminated. In addition, all treated patients had normal renal function and calculated glomerular filtration rate. Although the effect of RBV is long lasting, it was fully controlled for in both groups.

In conclusion, the use of pIFN in combination with RBV in the treatment of chronic HCV did not alter the incidence of thyroid dysfunction compared to rIFN and RBV. Thus there appears to be no need to alter current thyroid surveillance pattern in patients undergoing this new and effective derivative of IFN.

References

1 World Health Organization.*Hepatitis C Factsheet No. 164 (updated October 2000)* [Cited 18 June 2006]. Available from URL: http://www.who.International/mediacentre/factsheets
2 Australia's notifiable diseases status, 2002: annual report of the National Notifiable Diseases Surveillance System [Cited 18 June 2006]. Available from URL: http://www.cda.gov.au
3 Armstrong GL, Wasley N, Simard EP, McQuillan GM, Kuhnert WL, Alter MJ. The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. *Ann. Intern. Med.* 2006; **144**: 705–14.
4 Dienstag JL, Hepatitis C. A bitter harvest. *Ann. Intern. Med.* 2006; **144**: 770–1.
5 Patel K, Muir AJ, McHutchinson JG. Diagnosis and treatment of chronic hepatitis C infection. *BMJ* 2006; **332**: 1013–17.
6 Sievert W. An overview of antiviral therapy for chronic hepatitis C infection. In: Crofts N, Dore G, Locarnini S, eds. *Hepatitis C: an Australian Perspective.* Melbourne: IP Communications, 2001; 140–54.
7 Moreno-Otero R. Therapeutic modalities in hepatitis C: challenges and development. *J. Viral Hepat.* 2005; **12**: 10–19.
8 Antonelli A, Ferri C, Pampana A *et al.* Thyroid disorders in chronic hepatitis C. *Am. J. Med.* 2004; **117**: 10–13.
9 Larsen PR, Davies TF, Schlumberger M-J *et al.* Chapter 10, thyroid physiology and diagnostic evaluation of patients with thyroid disorders. In: Larsen PR, Kronenberg HM, Melmed S, Polonsky KS, eds. *Williams Textbook of Endocrinology*, 10th edn. Saunders: Elsevier Science, 2003; 331–73.
10 Fried MW, Shiffman ML, Reddy KR *et al.* Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N. Engl. J. Med.* 2002; **347**: 975–82.
11 Perez-Olmeda M, Nunez M, Romero M *et al.* Pegylated IFN-2b plus ribavirin as therapy for chronic hepatitis C in HIV-infected patients. *AIDS* 2003; **17**: 1023–8.
12 Moreno L, Quereda C, Moreno A *et al.* Pegylated interferon 2b plus ribavirin for the treatment of chronic hepatitis C in HIV-infected patients. *AIDS* 2004; **18**: 67–73.
13 Jacobson IM, Gonzalez SA, Ahmed F *et al.* A randomised trial of pegylated interferon alpha-2b plus ribavirin in the retreatment of chronic hepatitis C. *Am. J. Gastroenterol.* 2005; **100**: 2453–62.
14 Tran HA, Jones TL, Batey GR. The spectrum of thyroid dysfunction in an Australian hepatitis C population treated with combination interferon-alpha-2-beta and ribavirin. *BMC Endocr. Disord.* 2005; **5**: 8.
15 Kee K-M, Lee C-M, Wang J-H *et al.* Thyroid dysfunction in patients with chronic hepatitis C receiving a combined therapy of interferon and ribavirin: Incidence, associated factors and prognosis. *J. Gastroenterol. Hepatol.* 2006; **21**: 319–26.
16 Moncoucy X, Leymarie F, Delemer B *et al.* Risk factors and long-term course of thyroid dysfunction during anti-viral treatments in 221 patients with chronic hepatitis C. *Gastroenterol. Clin. Biol.* 2005; **29**: 339–45.
17 Lee SD, Yu ML, Chen PN *et al.* Comparison of a 6-month course peginterferon alpha-2-beta plus ribavirin and interferon alpha-2-beta

plus ribavirin in treating Chinese patients with chronic hepatitis C in Taiwan. *J. Viral Hepat.* 2005; **12**: 283–91.
18 Carella C, Mazziotti G, Amato G, Braverman EL, Roti E. Interferon-α-related thyroid disease: pathophysiological, epidemiological and clinical aspects. *J. Clin. Endoc. Metab.* 2004; **89**: 3656–61.
19 Prummel FM, Laurberg P. Interferon-alpha and autoimmune thyroid disease. *Thyroid* 2003; **13**: 547–51.
20 British Society of Gastroenterology. *Clinical Guidelines on the Management of Hepatitis C.* [Cited 18 June 2006]. Available from URL: http://www.bsg.org.uk/pdf_word_docs/clinguidehepc.pdf
21 Dienstag JL, McHutchison JG. American Gastroenterological Association medical position statement on the management of hepatitis C. *Gastroenterology* 2006; **130**: 225–30.

CHAPTER IV. CHARACTERISATION OF THYROID DISEASE DURING TREATMENT WITH INTERFERON-α

Although the development of thyroid disorders in association with interferon therapy became evident and widely reported, there was still uncertainty and controversy regarding the characteristics of thyroid disorders in this setting. This chapter critically examines the broad and fascinating spectrum of thyroid disorders during therapy. Where available, the natural history and outcome of a number of specific thyroid disorders was addressed in these original publications. Because of the unusual and rare occurrence of these disorders, many publications are case reports with the purpose of stimulating and encouraging more publications in the medical literature.

(A) HYPOTHYROIDISM

(i) PRIMARY HYPOTHYROIDISM

In our experience, patients undergoing treatment for chronic hepatitis C rarely develop primary hypothyroidism. It is likely that these patients have (bi-phasic) thyroiditis in which hypothyroidism is just one phase of the disease. However, the general literature often erroneously refers to frank hypothyroidism as an adverse effect of interferon therapy. This is suspected to be a sampling error, due to thyroid function tests being performed during the hypothyroid phase of disease. Please refer to section (C) of this chapter.

(ii) HYPOTHYROIDISM DUE TO THYROTROPIN RECEPTOR BLOCKING ANTIBODIES

Hypothyroidism due to thyrotropin (TSH) blocking antibody is exceedingly uncommon and thus is often underdiagnosed. It often occurs in the setting of immune disruption and chaos and in the presence of immunomodulators. We published a case report of this condition in the setting of chronic hepatitis C infection but it occurred in the immediate period following the cessation of IFN therapy (see chapter V). Although hypothyroidism by this mechanism is extremely rare, care must be taken not to confuse the diagnosis in this unique clinical setting in comparison with de novo primary hypothyroidism.

(B) GRAVES' LIKE THYROTOXICOSIS

(i) CHARACTERISTICS AND NATURAL HISTORY

Graves' like thyrotoxicosis occurring in this setting is uncommon and its natural history is poorly understood. We reported on a small series of patients and it appears that the thyrotoxicosis is heavily influenced by the use of IFN. Following its cessation, the disease abates slowly but surely and hence the duration can be shortened relative to that in de novo Graves' disease although the data are very sparse with very few publications.

Publication:

Tran HA, Reeves GEM. CHARACTERISTICS OF GRAVES' DISEASE IN A COHORT OF CHRONIC HEPATITIS C PATIENTS TREATED WITH INTERFERON-α AND RIBAVIRIN. J Endocrinol Metab, 2011; 1: 14-20.

Elmer Press

Original Article

J Endocrinol Metab • 2011;1(1):14-20

Characteristics of Graves' Disease in a Cohort of Chronic Hepatitis C Patients Treated With Interferon-α and Ribavirin

Huy A Tran[a, b], Glenn EM Reeves[a]

Abstract

Background: Thyrotoxicosis resembling Graves' disease (GD) in association with interferon-α therapy in the treatment of chronic hepatitis C is very uncommon, especially after treatment has ceased. Furthermore, it is unknown if this condition behaves differently from GD arising de novo.

Methods: A retrospective analysis was performed to detect and review all cases of GD occurring in our hepatitis C service unit over a five year period including long term outcomes.

Results: There were five cases of GD detected: 3 females and 2 males. These were: 1 case of co-existing GD and a toxic nodule developed during therapy, 2 cases developed GD after therapy and another 2 as part of the tri-phasic thyroiditis. All 5 patients were treated for 6 months with one exception and then followed up for another 12 months.

Conclusions: All patients responded satisfactorily to short term thionamides and remained in long term remission. Although the number is small, this report is a reminder that more cases can be expected as treatment for chronic HCV is likely to increase.

Keywords: Graves' disease; Chronic; Hepatitis C; Therapy; Interferon-α; Ribavirin; Thionamide

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Introduction

Spontaneous Graves' disease (GD) is not an uncommon condition in the general community with a prevalence of about 0.5 to 1.0% [1, 2]. Another form of GD has also been observed and described in association with interferon-α (IFN-α) therapy, either alone or as part of combination therapy for chronic hepatitis C infection [3]. The characteristics of this particular condition are poorly defined, primarily due to its low prevalence, compounded by a lack of awareness of its occurrence. Because of this deficiency, treatment is often ad hoc, occasionally with radioactive iodine which can render patients unnecessarily permanently hypothyroid. This report seeks to find out the prevalence of this condition, its response to conventional medical therapy and medium term follow-up as well as a review of the current literature.

Methods

Patients

The Hunter Area Hepatitis C treatment center assesses and treats referred cases of hepatitis C in Northern New South Wales, Australia. It is part of the John Hunter Hospital, a major tertiary referral center in the state. A total of 516 patients (282 males and 234 females) were treated between 1/1/2005 and 31/12/2009, a period of 60 months. All cases of GD were reviewed in patients with chronic hepatitis C who had undergone combination IFN-α and ribavirin (RBV) therapy. All were also reviewed and managed in consultation with a dedicated endocrinology team for the service.

Definition

Graves' disease was defined as clinical and biochemical thyrotoxicosis, elevated human thyrotropin receptor antibody (hTRAB) titres and/or increased radioisotope nuclear uptake scans. The parameters for thyrotoxicosis were thyrotropin (TSH) of < 0.1 mU/L, either free tetra-iodothyronine (fT4) levels > 20.6 and/or free tri-iodothyronine (fT3) levels > 6.0

pmol/L respectively.

Laboratory assay characteristics

Third generation TSH and serum fT4 levels were determined by two-site sandwich immunoassay using an automated chemiluminescent system (Diagnostic Products Corporation, Immulite 2000). The reference range (RR) for TSH was 0.4 - 4.0 mU/L and fT4 10.5 - 20.6 pmol/L. The coefficients of variation (CV) were 5.0% and 5.1% at TSH concentrations of 4.0 mU/L and 10.0 mU/L respectively. For fT4, the CV was 6.5% at 10.0 pmol/L.

Similarly, fT3 levels were performed using a two-site sandwich immunoassay using an automated chemiluminescent system (Beckman Coulter DXI). The RR was 3.5 - 6.0 pmol/L with 8.7% CV at 6.0 pmol/L.

Human TRAb assay was measured with the TRAK LUMI test (B.R.A.H.M.S.AG, Hennigsdorf/Berlin, Germany). An hTRAb level of < 1.0 IU/L is considered negative and > 2.0 as conclusively positive.

Serum autoantibodies to thyroglobulin and thyroperoxidase were measured by ELISA method (Aesku Diagnostics, Germany) with reference ranges being < 150 IU/mL and < 50 IU/mL respectively.

Thyroid nuclear uptake scans

These were performed using 99m-pertechnetate tracer with uptake studies taken at about 20 minutes post injection with a normal uptake ratio of 3 - 8 : 1.

Results

Five cases of GD were detected, either as the primary diagnosis or part of the tri-phasic thyroiditis as previously reported [4]. This constituted a prevalence of about 1%.

All patients consented to be part of this study. Because of the small number, each individual case is presented in detail below.

Clinical case notes

Case 1

A 53-year-old woman with chronic hepatitis C (genotype 3) presented with acute thyrotoxic symptoms. Her past medical history included intravenous drug use which led to the acquisition of hepatitis C about 20 years prior. She had been treated with combination IFN-α and RBV till the 16th week when she developed palpitation, anxiety, dyspnoea and recurrent diarrhoea. There was no prior or family of thyroid disease. She did not smoke nor drink and denied any eye or skin symptoms. Clinically she was unwell, anxious and lethargic with blood pressure of 130/60, pulse rate was regular at 108 beats per minute. Her weight was 58.3 kg, height of 1.65 m, body mass index (BMI) 21 kg/m^2. There was a diffuse non-tender goitre. There was no bruit or peripheral stigmata of thyrotoxicosis. Her TSH was < 0.03 IU/L, fT4 102.5, and fT3 of 39.7 pmol/L. Her hTRAb was elevated at 30.2 IU/L (< 2), anti-thyroperoxidase antibody (anti-TPO) 271 (< 50), anti-thyroglobulin antibody (anti-Tg) 10 (< 150). Her thyroid uptake scan showed an intense uptake in the right lobe, consistent with a hyperfunctioning nodule, and patchy uptake in the rest of the gland. The average overall uptake ratio was 6%. A diagnosis of interferon-induced GD was made with a co-existing toxic solitary nodule, not previously described in this setting.

Because of her anxiety and unstable psychological state, interferon therapy was terminated at this time, the 16th week of therapy. Carbimazole was started and the patient recovered slowly. She became hypothyroid on minimal carbimazole at 6 months and hence this was terminated with regular thyroid function surveillance. At a further 12 months after cessation, the patient remained euthyroid.

Case 2

A 47-year-old Caucasian man with chronic hepatitis C of genotype 1 presented with T3-toxicosis. He had been treated with combination IFN-α and RBV for 48 weeks. He tolerated treatment well until 6 weeks after completion when he developed general lethargy and mild intermittent palpitation. There were no other thyrotoxic symptoms, previous or family history thereof of thyroid disease. All his serial TSH levels during therapy had been normal.

Clinically, he appeared tired with weight of 67.2 kg and height of 1.75 m (BMI 22 kg/m^2). His cardiovascular examination was satisfactory with regular pulse rate of 88 beats per minute, blood pressure 130/80. There was no goitre or peripheral stigmata of thyrotoxicosis, including exophthalmopathy or dermatopathy. His TSH was < 0.03, with fT4 of 17.5 and fT3 of 8.2 pmol/L. His hTRAb was 16.6 IU/L, anti-Tg and anti-TPO were 188 and 26 respectively. His thyroid technetium uptake was elevated at 9%. He was assessed to have GD in association with IFN-α therapy. Carbimazole was initiated and remission was achieved after 8 weeks. Therapy was discontinued after 6 months at the patient's insistence. He was subsequently followed up at 12 months when he remained euthyroid.

Case 3

A 43-year-old woman with a history of breast cancer, chronic hepatitis C complicated by porphyria cutanae tarda presented with acute thyrotoxic symptoms. Her breast cancer was successfully treated 10 years prior and was deemed in remission with annual assessment. She carried the genotype 1 and thus

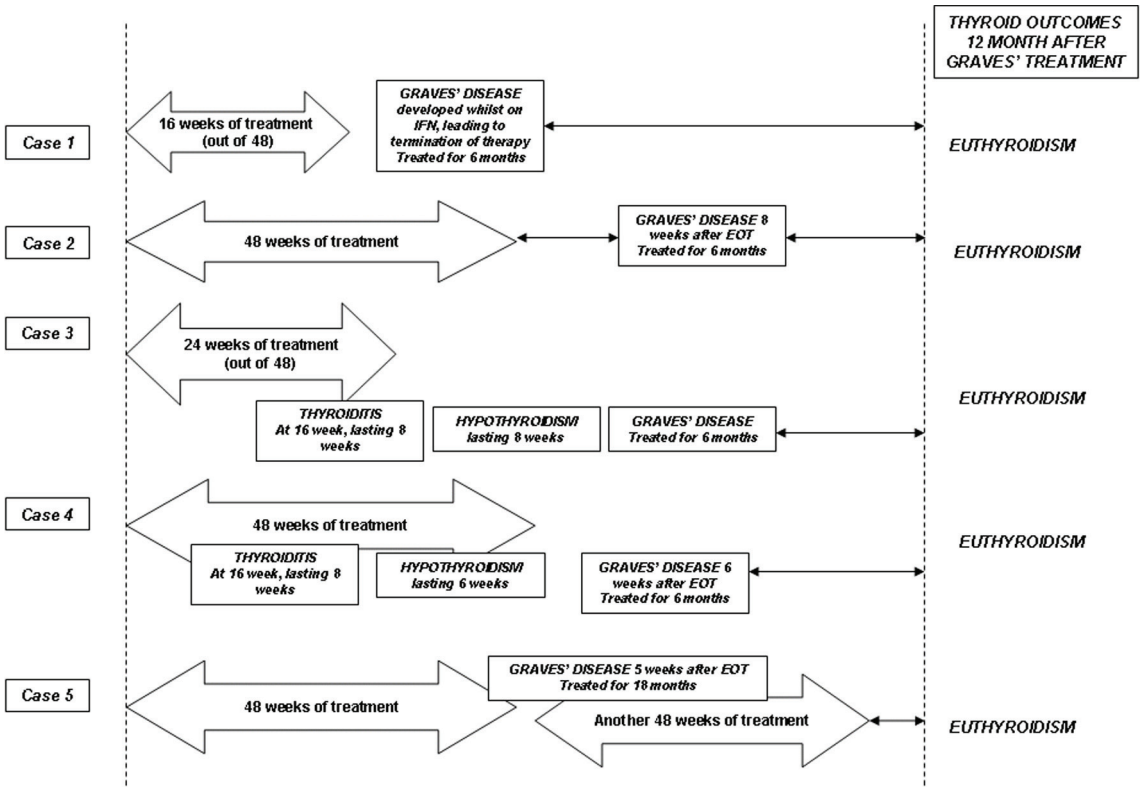

Figure 1. Schematic summaries of the cases and their final thyroid outcomes. The arrow bars indicate the duration of combination therapy with interferon-α and ribavirin. See text for detailed discussions.

was due to be treated for 42 weeks with RBV and IFN-α. She developed thyrotoxic symptoms at 16 weeks with diarrhoea and palpitation with weight loss. Clinical examination found her to be tired and lethargic with pulse of 98 regular and BP of 140/85. There was no goitre and no other evidence of GD. Her TSH was < 0.03, fT4 78.0 and fT3 6.9 pmol/L. Her anti-Tg and anti-TPO were elevated at 1597 and 1176 respectively. Her hTRAB was 3.3. Her thyroid scan was consistent with thyroiditis with a 20-minute uptake of 1%. She was treated with beta-blocker with good symptom relief. Hypothyroidism then developed with constipation and pedal edema. As a result, she was given a temporary thyroxine supplement. At week 24, she did not achieve early virological response and hence treatment was terminated. However, she began to feel again unwell at week 32 with tremors and general anxiety. She was found to be biochemically toxic with TSH < 0.03, fT4 of 28.9 and fT3 8.9 pmol/L. This time, her hTRAB was elevated at 5.4 with normalization of her anti-Tg and anti-TPO. Her repeat thyroid uptake scan showed an 8% uptake. This was confirmed to be GD, similar to case 4. The patient responded rapidly to carbimazole. She too was sensitive to this medication necessitating stopping at 6 months. After a further 12 month follow-up, she remained euthyroid.

Case 4

A 53-year-old Caucasian man presented with acute thyroiditis whilst undergoing combination RBV and IFN-α therapy for his hepatitis C infection. Because of his HCV genotype 1, treatment duration was to be 48 weeks. He had no medical history of note, smoked 5 - 10 cigarettes daily and has been tolerating the treatment regimen well. He developed IFN-induced thyroiditis at the 28th week of therapy and had recovered completely by the end of therapy. The patient completed treatment uneventfully at 48 weeks at which time his TSH, fT4 and fT3 levels had returned to normal. At 8 weeks post treatment, he complained of non-specific lethargy and a 2.5 kg weight loss. Repeat thyroid function tests showed T3-thyrotoxicosis with again, suppressed TSH, fT4 of 21.1 and fT3 8.0 pmol/L. His anti-Tg was 64, anti-TPO 228 and hTRAb was 19.3 U/mL. A pertechnetate uptake scan showed diffuse and increased uptake at 12%, consistent with GD. At 12 weeks post IFN therapy, his T3-toxicosis persisted with fT3 level of 8.7 pmol/L and propylthiouracil (PTU) was initiated. He was rendered euthyroid 6 weeks later. PTU was continued for another 6 months. His liver function tests remained stable throughout the duration. He self-terminated

Table 1. Characteristics and Long Term Follow-up of 5 Patients Who Developed Graves' Disease in Conjunction With Interferon-α and Ribavirin Therapy

Subject No.	Gender/Age	Ethnicity	Duration	Thyroid Scan	Final Diagnosis	Treatment	Long Term Outcome (12 months after end of treatment)
1	F/53	C	16	Toxic nodule & diffuse uptake 6%	Mixed Graves' disease and toxic solitary nodule	CBZ for 6 months	Euthyroidism
2	M/47	C	48	8% diffuse uptake	Graves' disease	CBZ for 6 months	Euthyroidism
3	Phase 1 (thyroiditis phase) F/43	C	24	Negligible uptake	Triphasic thyroiditis, Graves' disease	Symptomatic treatment	Euthyroidism
	Phase 2 (thyrotoxic phase)			5% diffuse uptake		CBZ for 6 months	
4	Phase 1 (thyroiditis phase) M/53	C	48	Negligible uptake	Triphasic thyroiditis, Graves' disease	Symptomatic treatment	Euthyroidism
	Phase 2 (thyrotoxic phase)			8% diffuse uptake		CBZ for 6 months	
5	F/49	C	48	9% diffuse uptake	Graves' disease	CBZ for 12 months	Euthyroidism

CBZ, Carbimazole

PTU after 6 months of therapy and remained euthyroid a further 12 months after.

Case 5

A 48-year-old woman presented for ongoing management of her thyroid disease after her first course of interferon therapy. She had chronic hepatitis C of genotype 1 with cirrhosis. She underwent a first course of treatment with combination IFN-α and RBV for 48 weeks and did not achieve end of treatment response (ETR). Her thyroid surveillance throughout this had been entirely normal. Four weeks after completion the first course, she developed GD with hyperdynamic cardiovascular activity in the presence of a confirmed diffuse goitre. Her TSH was < 0.03, fT4 was 88.7 and fT3 of 11.3 pmol/L. Her anti-Tg was 80, anti-TPO was 234 and hTRAb 13.9 IU/L. The thyroid pertechnetate uptake scan showed diffuse uptake at 11%. Her thyroid condition came under control in the ensuing 12 weeks with carbimazole. Six weeks after completion of the first course, she commenced a second course of combination IFN therapy for an additional 48 weeks. Her thyroid condition came under control and remained normal during this period with a maintenance dose of carbimazole. Her antiviral therapy was otherwise uneventful. Carbimazole therapy was ceased after 18 months, coinciding with the time of sustained virologic response (SVR) assessment. When reviewed 24 months after Graves' diagnosis, she remained euthyroid without any medication. Figure 1 and Table 1 summarise the sequence of events in all cases.

Discussion

This is the first case series to document the prevalence, progress and natural history of GD in the setting of IFN therapy. Graves' disease per se is not an uncommon condition in the general population with a lifetime prevalence of 0.5 - 1.0%. However, in the setting of IFN-α and ribavirin treatment, our series estimates the prevalence to be at about 1 per 100 treatments, consistent with peer reports of between 0.8 and 1.3% [5, 6]. Other reports often mention patients with this condition but the diagnostic criteria for both GD and thyrotoxicosis are poorly defined or altogether lacking [7-9]. In addition, the diagnostic criteria for GD are poorly defined but may be characterized by a triad of clinical and biochemical thy-

Table 2. Thyroid Autoantibody Profiles Before and After Thionamide Therapy in the Five Cases

Subject No.	Anti-thyroglobulin		Anti-thyroperoxidase		hTRAb	
	Before	After	Before	After	Before	After
1	10	12	271	41	30.2	< 2
2	8	11	149	28	16.6	< 2
3	1597	89	1176	78	3.3	< 2
4	22	26	258	75	19.3	< 2
5	41	68	38	25	13.9	5.8

The 'After' antibody levels are measured after 6 months (at the end) of therapy.

rotoxicosis, positive hTRAb titres and/or increased nuclear uptake scans.

In this small series, the development of GD was quite unpredictable and could occur at any stage. Case 1 developed during therapy and in fact was so traumatic that treatment was terminated. This was the first case of co-existing GD and toxic nodular disease in the literature. Cases 2 & 3 both developed GD after the planned treatment for HCV infection. The latter developed GD eight weeks after treatment and it remains contentious whether IFN was directly responsible for its development. Cases 4 & 5 developed GD following tri-phasic thyroiditis which occurred in association with hepatitis C and interferon. The Graves' phase occurred in the post treatment period in both cases. This is consistent with other reports [10, 11]. Interestingly, the reverse is yet to be reported. A notable characteristic in this study is that the thyroid uptake is below that expected in more classic GD although the diagnosis is well supported by the hTRAb titres and the clinical course.

This condition is rare and is often missed due to lack of follow-up and inadequate frequent thyroid surveillance. Of the 5 cases, 3 occurred in conjunction with other co-existing symptoms such as that of nodular disease in case 1, and part of the tri-phasic thyroiditis in cases 4 and 5. Only 2 cases developed GD in isolation but after treatment completion. Symptoms are equally unreliable as they can be confused with those arising from interferon treatment. The occurrence of GD after treatment is unusual and may well be coincidental as it is expected the IFN effect to have waned by then. However, Savvas et al reported GD occurring 12 weeks after therapy [11]. Kee et al [12] reported 6 cases of hyperthyroidism that were consistent with GD. Four cases persisted requiring antithyroid drugs although the duration of treatment was not reported. One was given radioactive iodine I-131 after 54 months because of the persistence of symptoms. Other reports only involved IFN-α monotherapy. Doi et al [13] reported 9 cases of hyperthyroidism in which prolonged antithyroid was required, in one case up to 9 years. Wong et al [3] described 6 cases of GD occurring up to 9 months after therapy. Other manuscripts recognised this peculiar condition but did not study treatment outcomes and follow-ups [14-16].

Pathogenetically, GD occurring in this situation is IFN-driven although it must occur in genetically predisposed individuals [17]. Interferon-α induces high expression of IFN-inducible genes and MCH-II antigens and TSH receptors on thyroid gland, leading to the development of clinical GD [18]. Interferon is also thought to modulate the switching of the T-cell response to T_H2 and stimulate B cell proliferation which increases TSI production provoking the development of GD [19]. What remains undetermined is the prolonged influence of IFN after its termination to account for the development of GD post therapy. Conversely and logically, the condition should abate or regress spontaneously upon the withdrawal of this medication. However, once occurred, GD is unlikely to remit spontaneously and treatment is invariably necessary. What can be modified however is the duration of treatment, which can be reduced to 6 months. The remission of hTRAB titre is also a useful marker and may guide the termination of therapy.

It is also observed that the condition responded well to thionamide and was relatively easy to control, especially in the absence or removal of IFN. This is contrary to patients with spontaneous GD where conventional treatment is 12 - 18 months in total [20]. With the exception of case 5, where treatment with IFN was reintroduced and thionamide was required to continue, the other cases responded rapidly and necessitated withdrawal of anti-thyroid medication at 6 months due to hypothyroidism. Further regular thyroid surveillance did not reveal any relapse. This appears to be consistent with the withdrawal of the immunomodulating effect following IFN termination. Thionamides, especially Propylthiouracil, should be used cautiously in this population due to its potential adverse hepatic effects on an already compromised or-

gan. This series makes the observation that IFN-related GD responds well to thionamide therapy. The condition appears to parallel IFN therapy and abates with its termination. The removal of the immunostimulation effect is also evident by the normalization of hTRAb titres where the relevant cases all achieved and maintained remission (Table 2).

The major drawback from this report is clearly the small number of subjects, primarily due to the low prevalence of the condition. The second is the short duration of follow-up. Ideally, a longer follow-up period should apply although this is made more challenging by the itinerant tendencies of the subjects. These are observational cases and further reports with larger numbers are required to consolidate the understanding of the pathogenesis. It is important to that the medium to longer term natural history of this thyroid entity is understood to prevent unnecessary treatment such as radioactive iodine therapy which will render the patients permanently hypothyroid [12, 16].

In summary, and notwithstanding the small number of subjects, GD in this setting occurs in two forms: in isolation or adjoining thyroiditis. The condition appears to remit more rapidly than its spontaneous counterpart, especially with the cessation of IFN therapy as reflected by the resolution of hTRAb. Treatment for 6 months followed by 12 month follow-up appears satisfactory, especially with the resolution of hTRAb titre. Thionamide should be continued if IFN is extended. This study is important so that unnecessary radioactive iodine is not deployed earlier in the course as to render the patients permanently hypothyroid.

Major points of difference between IFN-induced and de novo Graves' disease

1) Interferon induced Graves' disease incidence approximates 1% in the setting of IFN treatment. 2) Interferon induced Graves' disease can occur at anytime; during, after or in conjunction with thyroiditides. 3) Human TRAb is invariably elevated and parallels IFN treatment duration. 4) Uptake studies can be normal, but not absent contrary to the thyroiditides. 5) Consideration should be given to 6 months of thionamide therapy as opposed to the standard 12 - 18 months in de novo GD therapy.

Competing Interests

There are no competing interests pertaining to any of the authors, either financial or non-financial.

Authors' Contributions

HAT conceived the study, participated in its design, assisted with data collection and coordinated and helped to draft the manuscript. GEMR participated in the design, discussion and drafting of the manuscript. Both authors read and approved the final report.

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References

1. Cooper DS. Hyperthyroidism. Lancet 2003;362(9382):459-468.
2. Weetman AP. Graves' disease. N Engl J Med 2000;343(17):1236-1248.
3. Wong V, Fu AX, George J, Cheung NW. Thyrotoxicosis induced by alpha-interferon therapy in chronic viral hepatitis. Clin Endocrinol (Oxf) 2002;56(6):793-798.
4. Tran HA. The swinging thyroid in hepatitis C infection and interferon therapy. QJM 2010;103(3):187-191.
5. Costelloe SJ, Wassef N, Schulz J, Vaghijiani T, Morris C, Whiting S, Thomas M, et al. Thyroid dysfunction in a UK hepatitis C population treated with interferon-alpha and ribavirin combination therapy. Clin Endocrinol (Oxf) 2010;73(2):249-256.
6. Gelu-Simeon M, Burlaud A, Young J, Pelletier G, Buffet C. Evolution and predictive factors of thyroid disorder due to interferon alpha in the treatment of hepatitis C. World J Gastroenterol 2009;15(3):328-333.
7. Masood N, Ghori R, Memon A, Memon S, Memon KI, Memon I, Jaffri M, et al. Frequency of thyroid disorders during interferon and ribavirin therapy in chronic hepatitis C infection. J Coll Physicians Surg Pak 2008;18(6):347-351.
8. Jamil KM, Leedman PJ, Kontorinis N, Tarquinio L, Nazareth S, McInerney M, Connelly C, et al. Interferon-induced thyroid dysfunction in chronic hepatitis C. J Gastroenterol Hepatol 2009;24(6):1017-1023.
9. Kryczka W, Brojer E, Kowalska A, Zarebska-Michaluk D. Thyroid gland dysfunctions during antiviral therapy of chronic hepatitis C. Med Sci Monit 2001;7 Suppl 1:221-225.
10. Bohbot NL, Young J, Orgiazzi J, Buffet C, Francois M, Bernard-Chabert B, Lukas-Croisier C, et al. Interferon-alpha-induced hyperthyroidism: a three-stage evolution from silent thyroiditis towards Graves' disease. Eur J Endocrinol 2006;154(3):367-372.
11. Savvas SP, Papakostas N, Giannaris M, Malaktari S, Koskinas J, Archimandritis AJ. Interferon alpha-induced hashimoto thyroiditis followed by transient graves disease in a patient with chronic HCV infection. South Med

J 2010;103(6):585-588.
12. Kee KM, Lee CM, Wang JH, Tung HD, Changchien CS, Lu SN, Wang PW. Thyroid dysfunction in patients with chronic hepatitis C receiving a combined therapy of interferon and ribavirin: incidence, associated factors and prognosis. J Gastroenterol Hepatol 2006;21(1 Pt 2):319-326.
13. Doi F, Kakizaki S, Takagi H, Murakami M, Sohara N, Otsuka T, Abe T, et al. Long-term outcome of interferon-alpha-induced autoimmune thyroid disorders in chronic hepatitis C. Liver Int 2005;25(2):242-246.
14. Lisker-Melman M, Di Bisceglie AM, Usala SJ, Weintraub B, Murray LM, Hoofnagle JH. Development of thyroid disease during therapy of chronic viral hepatitis with interferon alfa. Gastroenterology 1992;102(6):2155-2160.
15. Watanabe U, Hashimoto E, Hisamitsu T, Obata H, Hayashi N. The risk factor for development of thyroid disease during interferon-alpha therapy for chronic hepatitis C. Am J Gastroenterol 1994;89(3):399-403.
16. Ward DL, Bing-You RG. Autoimmune thyroid dysfunction induced by interferon-alpha treatment for chronic hepatitis C: screening and monitoring recommendations. Endocr Pract 2001;7(1):52-58.
17. Tomer Y. Hepatitis C and interferon induced thyroiditis. J Autoimmun 2010;34(3):J322-326.
18. Kuang M, Wang S, Wu M, Ning G, Yao Z, Li L. Expression of IFNalpha-inducible genes and modulation of HLA-DR and thyroid stimulating hormone receptors in Graves' disease. Mol Cell Endocrinol 2010;319(1-2):23-29.
19. Cargnel A, Gubertini G, Magnani C, Bosis S, Colombo F, Clerici M. Immune response in chronic hepatitis C-infected patients undergoing interferon treatment. Hepatology 1999;30(2):584-585.
20. Abraham P, Avenell A, McGeoch SC, Clark LF, Bevan JS. Antithyroid drug regimen for treating Graves' hyperthyroidism. Cochrane Database Syst Rev 2010;(1):CD003420.

(C) BIPHASIC THYROIDITIS

(i) SHORT AND LONG TERM NATURAL HISTORY

This section examines the most common thyroid condition seen in combination treatment, characterizing the natural history and long term outcomes. Although it can compound the potential adverse effect of IFN, the outcome of biphasic thyroiditis is relatively benign in both the short and longer terms. The condition should improve with close monitoring and none of the patients studied required long term thyroxine supplement for the hypothyroid phase.

Publications:

Tran HA, Reeves GE, Jones TL. THE NATURAL HISTORY OF INTERFERON-α2β-INDUCED THYROIDITIS AND ITS EXCLUSIVITY IN A COHORT OF PATIENTS WITH CHRONIC HEPATITIS C INFECTION. Q J Med, 2009; 102: 117–122.

Tran HA, Jones TL, Ianna EA, Reeves GE. THE NATURAL HISTORY OF INTERFERON-α INDUCED THYROIDITIS IN CHRONIC HEPATITIS C PATIENTS: A LONG TERM STUDY. Thyroid Res, 2011; 8: 4 (1): 2.

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The natural history of interferon-α2b-induced thyroiditis and its exclusivity in a cohort of patients with chronic hepatitis C infection

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Summary

Background: Interferon-α2b (IFN-α2b) is well known to cause both hyper- and hypo-thyroidism. In the former, the commonest aetiology is thyroiditis. As there is no previous data to fully characterize the entity of IFN-related thyroiditis, the aim of this study is to document in detail its evolution in a cohort of hepatitis C patients treated with pegylated IFN-α2b and Ribavirin (RBV).
Methods: A prospective observational study was conducted in patients who developed thyroid diseases whilst receiving combination of pegylated IFN-α2b and RBV for hepatitis C. The patients were followed with monthly thyrotropin (TSH). Where TSH was undetectable, free tetra- (fT4) and tri-iodothyronine (fT3) were added. Anti-thyroperoxidase (TPO), anti-thyroglobulin (Tg) and thyroid stimulating immunoglobulin (TSI) levels were also performed at diagnosis, during and at the end of IFN therapy. All patients were assessed and followed up closely with monthly TSH, fT4 and fT3 levels until the completion, after 6 and 12 months of treatment.
Results: There were seven females and four males over a 30-month period. All patients were found to have thyroiditis. On average, the time to the development of thyroid disease was 10 weeks and duration of disease 9 weeks. All patients eventually recovered normal biochemical thyroid function although two required short-term supplementation.
Conclusions: Thyroiditis was found exclusively in our patients. Both the hyper- and hypo-thyroid phase can be short lived, extreme and transient in nature which warrants strict monthly TSH monitoring. Careful follow-up of all patients is mandatory as complete recovery is expected.

Introduction

Treatment for hepatitis C infection often results in many endocrinological disturbances of which thyroid dysfunction is most prevalent.[1] Within the realm of thyroid-related diseases, thyroiditis is the commonest cause of interferon (IFN)-associated dysfunction. Others include Graves' like thyrotoxicosis and the tri-phasic thyroiditis in which the patient oscillated between increased and absent thyroid pertechnetate uptake on nuclear scan whilst being profoundly and clinically thyrotoxic.[2–4] Although the subject of much discussion, there are no previous reports that follow the clinical course of this peculiar clinical condition on a monthly basis. Previous studies measured thyroid parameters in an *ad hoc* basis. Kee *et al.*[7] and Dalgard *et al.*[8] measured thyroid function tests (TFTs) every 3 months; Moncoucy *et al.*[6] every 2 or 3 months and Hsieh *et al.* every 4 weeks for 24 weeks, followed by 8

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weeks for another 24 weeks.[9] Minelli *et al.*[10] evaluated thyroid functions at 1, 2, 3 and 6 months after diagnosis. Many studies were also performed in the setting of mono-IFN therapy[10] and/or combination regular IFN and Ribavirin (RBV),[7,9] not the pegylated form. To fully characterize, better understand and manage the condition, we prospectively studied 11 patients who developed thyrotoxicosis during the course of treatment. Their natural history and outcome were carefully recorded in the final analysis.

Methods

Patients

The Hunter Area Hepatitis C treatment centre assesses and treats all cases of hepatitis C in Northern New South Wales, Australia. It is part of the John Hunter Hospital, a major tertiary referral centre in the state. In total, 11 patients who developed thyroiditis whilst undergoing combination treatment for hepatitis C were included over a 24-month period. All were drug naive, i.e. all were undergoing therapy for the first time. All other causes of chronic hepatitis were excluded including hepatitis B and chronic alcoholic liver disease. Baseline characteristics of all studied subjects are summarized in Table 1. All patients were assessed for thyroid disease clinically and biochemically using TSH level prior to the beginning of therapy. No goiter was detected and baseline TSH levels were normal in all cases.

Therapy

All patients were treated with combination pegylated IFN-α2b and RBV therapy. The duration of treatment depended on the HCV genotypes; genotypes 2 and 3 were treated for 24 weeks and types 1 and 4 for 48 weeks, respectively. For the latter group, treatment was continued for the full 48 weeks irrespective of the HCV RNA status at 24 weeks. The dosage for pegylated IFN-α2b was 180 μg weekly with RBV dose ranging from 1000 to 1200 mg daily according to bodyweight.

Thyroid function assessments

All patients received routine TSH levels at the start of treatment and at monthly intervals. When the TSH level was undetectable, free tetra- (fT4) and triiodothyronine (fT3) levels were performed, followed by an endocrinological and clinical assessment from which further imaging investigations were determined. All had a thyroid ultrasound and thyroid pertechnetate uptake scan. Anti-thyroperoxidase (anti-TPO), anti-thyroglobulin (anti-Tg) antibodies and thyroid stimulating immunoglobulin (TSI) levels were performed at diagnosis, 4 weeks after thyroiditis and at the completion of IFN course. All patients were followed up at 4 weekly intervals until the end of therapy, in 6 months' time as part of the routine HCV treatment review and again in 12 months' time. Sustained virologic response (SVR) was defined as undetectable hepatitis C virus RNA at 24 weeks after the completion of treatment.[5]

Laboratory assay characteristics

Third generation serum TSH, serum fT4 and fT3 were determined by two-site sandwich immunoassay using an automated chemiluminescent system (Diagnostic Products Corporation, Immulite 2000, Los Angeles, CA, USA). The reference range (RR) for TSH was 0.4–4.0 mU/l, fT4 10.0–26.0 and fT3 3.5–5.5 pmol/l. The coefficients of variation (CV) were 5.0% and 5.1% at TSH concentrations of 4.0 mU/l and 10.0 mU/l, respectively. For fT4, the CV was 6.5% at 10.0 pmol/l and fT3 8.9% at 3.5 pmol/l.

Serum autoantibodies to thyroglobulin and TPO were measured by agglutination (Serodia-ATG and Serodia-AMC, Fujirebio, Inc., Tokyo, Japan). Titres of less than 1:400 were considered normal for both.

TSI was measured using cell culture and radioimmunoassay. This is an in-house bioassay using Chinese Hamster Ovary (CHO) cells in culture to detect the presence of thyroid-stimulating activity. The CHO cells are transfected with the TSH receptor genes and thus are responsive to TSI. Thyroid-stimulating activity is measured by evaluating the intracellular release of cAMP induced by the patient's serum immunoglobulin on the CHO cells. The results are reported as U/ml. TSI should be absent in the normal population. A TSI level of <10 is considered negative, 10–50 as weakly, 50–100 as moderately and >100 U/ml as strongly positive.

Definitions

Hyper- and Hypo-thyroidism

Thyrotoxicosis was defined as having TSH <0.1 mU/l, either fT4 level >26.0 and/or fT3 level >5.5 pmol/l, respectively. Hypothyroidism was defined as having TSH level >4.0 mIU/l, with normal or low (<10.0 pmol/l) fT4 levels.

Thyroiditis

Thyroiditis is defined as the triad of clinical and/or thyrotoxicosis, with a reduced/negligible thyroid pertechnetate uptake scan. All uptake scans were reviewed by a specialist nuclear

physician consultant. Thyroid autoantibodies may be present but are not considered essential to the diagnosis.

Statistical analysis

Data are presented as mean with 95% CI.

Results

There were 11 patients who developed the condition (seven females and four males) over the study period. Their characteristics are listed in Table 1. All thyroid ultrasound and pertechnetate uptake studies were performed within 14 days of diagnosis. In the former, the findings showed normal thyroid gland in seven patients (five females and two males). In the other four patients (two males and two females), there was multinodularity but without a size increase. All nuclear uptake results were absent or negligible. There appears to be no relationship between gender, genotype and the risk of developing thyroid disease. The duration to the development of disease, hyper- and hypo-thyroid phases are all quite variable. All cases returned to normality eventually. In some cases, the titres of all auto-antibodies increase with the development of thyroiditis but then recover. Two patients (cases 9 and 10, Table 2) developed significant hypothyroidism, one quite severe and prolonged, required thyroxine supplement but both were able to be weaned off therapy 12 months after the completion of HCV therapy. The range of fT4 and fT3 levels can be quite elevated. Equally, TSH levels can also be quite high, with undetectable fT4 level, representative of severe thyroid failure. All patients who developed thyroiditis achieved SVR and this was considered curative.[5] The evolutionary features of thyroiditis are listed individually in Table 2.

Discussion

Although there are many previous reports addressing thyroid dysfunction,[6–10,12] this is the first study to closely detail, on a monthly basis, the evolution of IFN-induced thyroiditis, to the authors' best knowledge. Thyroiditis is the most common thyroid-related disease in relation to IFN-based therapy. In general, there are four forms of thyroid dysfunction in this setting: non-thyroidal illness, thyroiditis (bi-phasic), hypothyroidism and Graves' like thyrotoxicosis.[4] The thyroiditis form, similar to other non-IFN mediated forms, demonstrates a bi-phasic nature, shifting from hyper- to hypo-thyroidism with subsequent full recovery, albeit at times prolonged. It is the single and exclusive form of thyroid disease observed in our cohort of 201 patients. Although the number of subject is modest, it offers a unique opportunity to comprehensively study this peculiar type of thyroid dysfunction. Graves' like thyrotoxicosis, although well described, was surprisingly and completely absent in our cohort. The reason for this discrepant observation is unknown, especially when compared with previous series described in Australia, Europe and North America.[4,8,11,12] It is possible that this is due to an ascertainment bias where pre-existing Graves' disease is detected due to the increased thyroid surveillance under IFN therapy. The contrasting findings on nuclear scintigraphy should rule out any potential misdiagnosis between the two conditions however. Similarly, tri-phasic

Table 1 Baseline characteristics, hepatitic outcomes and auto-antibody profiles in 11 thyroiditis patients

Subject No.	Gender	Age	Hepatitis C genotype	Duration of therapy (weeks)	SVR	Anti-thyroglobulin			Anti-TPO			TSI		
						Before	During	After	Before	During	After	Before	During	After
1	M	26	1a	48	Y	<1	16	<1	<1	<1	<1	<10	10	<10
2	M	51	2	24	Y	<1	16	<1	<1	<1	<1	<10	<10	<10
3	M	54	3	24	Y	<1	<1	<1	<1	<1	<1	<10	<10	<10
4	M	49	4	48	Y	<1	<1	<1	<1	<1	<1	<10	<10	<10
5	F	42	2	24	Y	<1	<1	<1	<1	256	1024	<10	<10	<10
6	F	34	3	24	Y	<1	<1	<1	<1	256	256	<10	<10	<10
7	F	49	1	48	Y	<1	<1	<1	<1	64	<1	<10	10	<10
8	F	49	1	48	Y	<1	16	<1	64	1024	16	<10	<10	19
9	F	50	2	24	Y	<1	<1	<1	<1	16	<1	<10	10	<10
10	F	37	1	48	Y	<1	<1	<1	<1	<1	<1	<10	14	<10
11	F	43	4	48	Y	<1	<1	<1	<1	<1	<1	<10	<10	<10

Mean age: 43.3 (95% CI, 25.7–60.9). All are Caucasians.

Table 2 Characteristics and evolutionary characteristics of 11 patients who developed thyroiditis and their thyroid status at the *completion* of IFN therapy

Subject No.	Time to onset of thyroiditis (weeks)	Duration of Toxic phase (weeks)	Duration of hypothyroid (weeks)	Hyperthyroid phase			Hypothyroid phase			Sonographic findings	Thyroid outcome
				TSH	Peak fT4	Peak fT3	TSH	FT4	FT3		
1	20	3	5	0	72.7	21.9	9.2	9.9	3.3	MN	N
2	31	7	4	0	25.1	5.9	34.6	8.2	3.5	N	HYPO
3	28	6	4	0	21.0	6.6	27.2	4.3	3.1	MN	N
4	21	5	5	0	33.8	7.8	14.6	8.6	3.4	MN	N
5	14	6	2	0	48.7	11.4	18.2	6.8	3.5	N	N
6	16	9	3	0	38.9	7	11.2	12.3	3.5	N	N
7	17	8	0	0	20.5	5.1	6.5	17.8	5.2	N	N
8	20	8	8	0	32.9	8.4	6.8	18.7	4.8	MN	N
9	12	4	4	0	23.5	5.8	115.8	3.7	2.1	N	HYPO
10	1	15	16	0	27.1	4.7	60.2	4.8	2.4	N	HYPO
11	8	6	4	0	25.5	8.2	34.8	8.3	3.3	N	N
Mean	18.2	6.8	5.1	0.0	33.9	6.9	18.2	8.2	3.4		
95% CI	2.5–33.9	0.3–13.3	0–13.1	N/A	23.9–45.5	5.0–11.6	7.7–51.5	5.9–12.7	2.8–4.1		

All three hypothyroid cases eventually recovered and did not require thyroxine supplement at 12 month follow-up. All cases had no/negligible uptake on Technetium scintigraphy. HYPO: hypothyroid; MN: multinodularity; N: normal.

thyroiditis was not seen although admittedly this pattern is rare, with a single report.[13]

The pathogenesis of thyroiditis in this particular setting is currently not fully understood and has been the subject of many recent reviews.[1,14] The opportunity to study the condition may also be made more difficult due to the condition's low frequency of <1%.[15] Concisely, there must be synergy between genetic predisposition, the presence of HCV virus and IFN therapy. The latter will result in an induction of IFN-α and -β production as part of the innate immune response.[16] IFN also causes the activation of natural killer cells, maturation of dendritic cells, proliferation of dendritic cells, proliferation of memory T cells and prevention of T-cell apoptosis.[17] These will induce a rise the thyroid auto-antibodies titre, which will in turn cause destructive changes in the thyroid gland in some of the cases. The presence of hepatitis C viral particle within the thyroid cells may additionally contribute further damage to the thyroid gland.[18] The addition of IFN-α then inflames an already vulnerable thyroid gland. Furthermore, IFN therapy has also been shown to have a direct toxic effect on thyroid cells, resulting in thyrocyte apoptosis, rupture of follicles and release of thyroid hormones.[16] These pathophysiologic events manifest themselves in the form of the bi-phasic thyroid response that is so classical of this type of thyroiditis, Figure 1.

The prevalence of thyroiditis is low in our cohort with 11 cases over a 2-year period and this is consistent with our previous report.[19] The time from

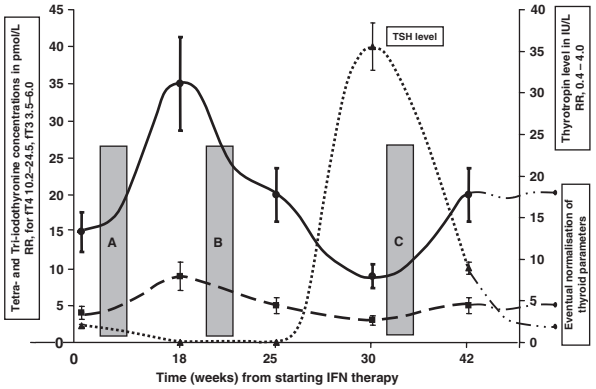

Figure 1. The evolving biochemical profile of thyroid hormones in IFN-induced thyroiditis. Each time point represents the *mean* value of each thyroid parameters with 95% CI bar. Testing at point [**A**] will falsely reassure with normal thyroid tests. Testing at point [**B**] will detect hyperthyroidism. Point [**C**] indicates hypothyroidism, which may be deemed permanent and required unnecessary life-long thyroxine therapy. fT4 levels (solid line); fT3 levels (dashed line), TSH concentrations (dotted line). The right *Y*-axis is the secondary axis for TSH.

exposure to the development of the disease, except for one case is long, up to 4 months, suggesting the need for prolonged IFN exposure to stimulate and modulate the immune system. Because of this unpredictability, it is important that thyroid surveillance is performed very early and frequently in the course of treatment to diagnose and manage the condition. The degrees of biochemical hyper- and hypo-thyroidism are quite extreme and can be

significantly incongruent with the clinical state. Symptomatology is also unreliable because of the side effects of IFN, which often override or mask thyroid-related symptoms. The urge to treat the biochemical values can be quite pressing and may need to be resisted as the natural history is relatively benign. Similarly, the hypothyroid phase can be prolonged and profound and thus should be closely followed up. The need to implement thyroxine replacement therapy should be judiciously considered in light of the clinical state as the potential to recover is high. Even if thyroxine is required, patients should be given a trial off therapy at the completion of the HCV treatment and observed for any potential recovery. This is particularly important as patients can be erroneously classified depending on the timing of the testing and the frequency. Patients who are found in the hypothyroid phase can be easily categorized as hypothyroidism, especially in the presence of positive thyroid autoantibodies. These patients are then subjected to life-long thyroid supplement without realizing the great potential of recovery and normalization of thyroid function.

This report also suggests that patients undergoing combination treatment for hepatitis C should have baseline and monthly TSH thereafter in order to fully assess thyroid status. The potential for misdiagnosis, depending on the timing of thyroid testing, is illustrated in Figure 1. The National Academy of Biochemistry is yet to recommend thyroid testing in this clinical scenario.[20] Three monthly TSH as suggested by Mandac *et al*.[21] may completely miss the diagnosis as thyroid functions have completely normalized in many cases. Of note, both the British Society of Gastroenterology and the American Gastroenterological Association recognize the potential thyroid effect of IFN and recommend thyroid screening.[22,23] However, only the former specifies that thyroid function test is recommended at each treatment visit, rather than monthly. The National Institute of Health consensus statement on hepatitis C management surprisingly makes no mention of the thyroid issue.[24] Alternately, testing in another phase can detect up hypothyroidism. In the presence of significantly slow recovery, this may be diagnosed as primary hypothyroidism leading to permanent therapy. It is also a concern that many reports in the literature may have indeed inadvertently misclassified these patients without ever recognizing the true nature of the diagnosis.[25] It is thus important that all cases of abnormal thyroid function in this setting undergo thyroid nuclear uptake scan to carefully define the diagnosis. Ultrasound studies may contribute and detect incidental nodularity in four of our cases but are not essential to the diagnosis. It may be debatable that due to the benign nature of the condition, thyroid surveillance is not important given the fact that recovery to normality is expected. Furthermore, the detection of thyroiditis does not necessarily warrant cessation of therapy. Many patients are willing to compromise thyroid function and risk hypothyroidism by completing their treatment regimen in order to achieve SVR and the potential cure. It is however important that the condition is recognized so that appropriate thyroid treatment is delivered. Furthermore, re-exposure can result in recurrence, albeit in isolated cases.[26] The rare possibility of IFN-mediated Graves' disease should also be fully excluded as management strategy is completely different. As IFN toxicity can easily mimic thyroid disease symptoms, it is equally critical to detect the two polarised phases of the thyroiditis so that temporary and alleviating measures can be implemented. It is important to identify thyroid involvement so that erroneous and premature termination of IFN therapy does not occur. Patients are often psychologically labile and finding an organic explanation for these symptoms can be additionally very reassuring. The hyperthyroid phase can be managed symptomatically with β-blockade. Glucocorticoid should be reserved as last line therapy, especially in the setting of chronic hepatitis. As the hypothyroid phase is temporary, there may be a strong argument to use tertroxin (fT3 supplement) which can be rapidly withdrawn at the completion of IFN with the expectation of complete thyroid recovery.

Conclusion

This study details clearly the natural history of IFN-induced thyroiditis. It also highlights the need for regular monthly thyroid testing to fully document and diagnose this prevalent and exclusive thyroid dysfunction in our cohort of hepatitis C patients. Short-term and rapid onset treatment should be considered to reduce the burden of psychological and physical symptoms contributed by thyroid diseases to patients undergoing IFN treatment.

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References

1. Tomer Y, Blackard JT, Akeno N. Interferon alpha treatment and thyroid dysfunction. *Endocrinol Metab Clin N Am* 2007; **36**:1051–66.
2. Carella C, Mazziotti G, Amato G, Braverman LE, Roti E. Interferon-α-related thyroid disease: pathophysiological, epidemiological, and clinical aspects. *J Clin Endocrinol Metab* 2004; **89**:3656–61.
3. Prummel MF, Laurberg P. Interferon-α and autoimmune thyroid disease. *Thyroid* 2003; **13**:547–51.
4. Wong V, Fu Xi-Li A, George J, Cheung NW. Thyrotoxicosis induced by alpha-interferon therapy in chronic viral hepatitis. *Clin Endocrinol* 2002; **56**:793–8.
5. Myers RP, Regimbeau C, Thevenot T, Leroy V, Mathurin P, Opolon P, *et al.* Interferon for interferon naive patients with chronic hepatitis C. *Cochrane Database System Rev* 2002; **2**:CD00370.
6. Moncoucy X, Leymarie F, Delemer B, Levy S, Bernard-Chabert B, Bouche O, *et al.* Risk factors and long-term course of thyroid dysfunction during antiviral treatments in 221 patients with chronic hepatitis C. *Gastroenterol Clin Biol* 2005; **29**:339–45.
7. Kee KM, Lee CM, Wang JH, Tung HD, Changchien CS, Lu SN, *et al.* Thyroid dysfunction in patients with chronic hepatitis C receiving a combined therapy of interferon and ribavirin: incidence, associated factors and prognosis. *J Gastroenterol Hepatol* 2006; **21**:319–26.
8. Dalgard O, Bjoro K, Hellum K, Byrvang B, Bjoro T, Haug E, *et al.* Thyroid dysfunction during treatment of chronic hepatitis C with interferon alpha: no association with either interferon dosage or efficacy of therapy. *J Intern Med* 2002; **251**:400–6.
9. Hsieh M-C, Yu M-L, Chuang W-L, Shin S-J, Dai C-Y, Chen S-C, *et al.* Virologic factors related to interferon-alpha-induced thyroid dysfunction in patients with chronic hepatitis C. *Eur J Endocrinol* 2000; **142**:431–7.
10. Minelli R, Valli MA, Di Secli D, Finardi L, Chiodera P, Bertoni R, *et al.* Is steroid therapy needed in the treatment of destructive thyrotoxicosis induced by α-interferon in chronic hepatitis C? *Horm Res* 2005; **63**:194–9.
11. Antonelli A, Ferri C, Pampana A, Fallahi P, Nesti C, Pasquini M, *et al.* Thyroid disorders in chronic hepatitis C. *Am J Med* 2004; **117**:10–3.
12. Minelli R, Coiro V, Valli MA, Finardi L, Di Secli C, Bertoni R, *et al.* Graves' disease in interferon-alpha-treated and untreated patients with chronic hepatitis C virus infection. *J Investig Med* 2005; **53**:26–30.
13. Bohbot NL, Young J, Orgiazzi J, Buffet C, Francois M, Bernard-Chabert B, *et al.* Interferon-alpha-induced hyperthyroidism: a three-stage evolution from silent thyroiditis towards Graves' disease. *Eur J Endocrinol* 2006; **154**:367–72.
14. Tran HA. Hepatitis C infection, treatment regimens, and thyroid function abnormalities. *Endocrinologist* 2007; **17**:231–5.
15. Hoofnagle JH, Seeff LB. Peginterferon and Ribavirin for chronic hepatitis C. *N Engl J Med* 2006; **352**:2444–51.
16. Lloyd AR, Jagger E, Post JJ, Crooks LA, Rawlinson WD, Hahn YS, *et al.* Host and viral factors in the immunopathogenesis of primary hepatitis C virus infection. *Immunol Cell Biol* 2007; **85**:24–32.
17. Tilg H. New insights into the mechanisms of interferon alpha: an immunoregulatory and anti-inflammatory cytokine. *Gastroenterology* 1997; **112**:1017–21.
18. Gowans EJ. Distribution of markers of hepatitis C virus infection throughout the body. *Semin Liver Dis* 2000; **20**:85–102.
19. Tran HA, Jones TL, Batey RG. The spectrum of thyroid disease treated with alpha-2-beta interferon and ribavirin in an Australian population. *BMC Endocr Disord* 2005; **5**:8.
20. Demers LM, Spencer CA. Laboratory support for the diagnosis and monitoring of thyroid disease, 2003. [www.nacb.org/lmpg/thyroid_lmpg_pub.htm], Accessed 27 July 2008.
21. Mandac JC, Chaudhry S, Sherman KE, Tomer Y. The clinical and physiological spectrum of interferon-alpha induced thyroiditis: toward a new classification. *Hepatology* 2006; **43**:661–72.
22. British Society of Gastroenterology. Clinical Guidelines on the management of hepatitis C. [www.bsg.org.uk/pdf_word_docs/clinguidehepc.pdf] Accessed 28 July 2008.
23. Dienstag JL, McHutchison JG. American Gastroenterological Association medical position statement on the management of hepatitis C. *Gastroenterology* 2006; **130**:225–30.
24. Management of hepatitis C: 2002. *NIH Consens State Sci Statements* 2002; 19:1–46.
25. Tran HA. The uncertain natural history of thyrotoxic patients treated with combination interferon alfa-2β and Ribavirin. *Arch Intern Med* 2005; **165**:1072.
26. Panara R, Cruz M, Lyra L, T Cruz. Subacute thyroiditis during treatment with combination therapy (interferon plus ribavirin) for hepatitis C virus. *J Viral Hepat* 2000; **7**:393–5.

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THYROID RESEARCH

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The natural history of interferon-α induced thyroiditis in chronic hepatitis c patients: a long term study

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Abstract

Background: Autoimmune thyroid disease is a common complication of patients with chronic hepatitis C undergoing combination pegylated interferon-α and ribavirin treatment. A small proportion develops interferon-induced thyroiditis of which the long term natural history is unknown and how it compares with de novo thyroiditis. The aim of the study is to determine the natural history of thyroid disease including antibody profile in this particular setting 36 months from the completion of therapy.

Methods: A cohort of 18 hepatitis C patients (mean age 45 ± 8 years (standard deviation)) who developed exclusively thyroiditis in this setting was followed every 12 months after the completion of therapy for 36 months. Investigations included thyrotropin, free tetra-iodothyronine, free tri-iodothyronine levels and thyroid autoantibodies.

Results: None of the patients developed any long term thyroid disease. Two patients had a prolonged hypothyroid phase of the thyroiditis early after the completion of treatment but recovered fully. The remaining 16 patients remained euthyroid. Similarly, thyroid autoantibodies all declined and returned to reference range.

Conclusions: The long term natural history in this small series of interferon induced thyroiditis was benign. If a larger series confirms a similar outcome then there is no long term residual effect on thyroid function and follow-up testing would not be warranted.

Background

Hepatitis C remains one of the major causes of chronic liver infection and cirrhosis worldwide. The most effective and established treatment available for this condition is the combination ribavirin and pegylated interferon-α (IFN-α). A major and common adverse effect of this treatment is the development of thyroid disease during therapy. A large spectrum of autoimmune thyroid disease (TD) has been described to occur ranging from Graves' disease to thyroiditis to frank primary hypothyroidism [1-3]. As the number of patients undertaking treatment is expected to rise, a proportion of these patients will progress to develop thyroid disease. It is therefore important to understand and fully clarify the natural progression of the disease. This forms a critical part of the long term management and counselling for these patients. It has been reported that ~50% of these patients recover from this complication [4,5]. Furthermore, other reports are retrospective and follow patients in an ad hoc fashion with very variable and unstandardised duration. In addition, some of the studied groups included the use of IFN as monotherapy and regular IFN (rather than pegylated) in combination with ribavirin [6]. In some cases, patients remain permanently thyroxine dependent although no withdrawal trials were attempted. The long term natural history of this condition beyond this time frame remains largely unknown and it is uncertain whether it is different from other forms of thyroiditides arising de novo.

The aim of this report is to follow prospectively the long term natural history of TD 36 months after the completion of treatment in a cohort of patients who

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developed thyroid disease during therapy with pegylated interferon and also to compare the outcome with the natural history of the thyroiditides arising de novo.

Methods

Patients

The patients are all recruited from a Hepatitis C service centre in a major tertiary referral hospital. A total of 18 patients were available for study. These were retrieved from a pool of 358 patients over a 5 year period, giving an annual incidence of ~5.0%. All were followed-up over a 36-month period. All were medication naive, i.e. all were undergoing therapy for the first time. All other causes of chronic hepatitis were excluded including hepatitis B and chronic alcoholic liver disease. Baseline characteristics of all studied subjects are summarised in Table 1.

Thyroid function assessments

All patients were assessed by an endocrinology team and confirmed to have thyroiditis whilst receiving interferon therapy as previously reported [7]. At the end of interferon treatment, all had baseline thyroid function studies and thyroid autoantibodies, at 12, 24 and 36 months post therapy. Follow-ups at 24 and 36 months were performed by mail correspondence and telephone interviews. Patients were recalled and reviewed formally if clinically indicated.

Laboratory assay characteristics

Third generation serum TSH, serum fT4 and fT3 were determined by two-site sandwich immunoassay using an automated chemiluminescent system (Diagnostic Products Corporation, Immulite 2000). The reference range (RR) for TSH was 0.4-4.0 mU/L, fT4 10.0-26.0 and fT3 3.5-5.5 pmol/L. The coefficients of variation (CV) were 5.0% and 5.1% at TSH concentrations of 4.0 mU/L and 10.0 mU/L respectively. For fT4, the CV was 6.5% at 10.0 pmol/L and fT3 8.9% at 3.5 pmol/L.

Serum autoantibodies to thyroglobulin and thyroperoxidase were measured by agglutination (Serodia-ATG and Serodia-AMC, Fujirebio, Inc., Tokyo, Japan). Titres of less than 1:40 were considered normal for both.

Thyroid Stimulating Immunoglobulin was measured using cell culture and radio-immunoassay. This is an in-house bioassay using Chinese Hamster Ovary (CHO) cells in culture to detect the presence of thyroid stimulating activity. The CHO cells are transfected with the TSH receptor genes and thus are responsive to TSI. Thyroid-stimulating activity is measured by evaluating the intracellular release of cAMP induced by the patient's serum immunoglobulin on the CHO cells. The results are reported as units/mL (U/mL). TSI should be absent in the normal population. A TSI level of <10 is considered negative, 10-50 as weakly, 50-100 as moderately and >100 U/mL as strongly positive.

Table 1 Baseline characteristics, hepatitic outcomes and thyrotropin outcome profiles in all 18 thyroiditis patients

Subject No.	*Gender*	*Age*	*Hepatitis C Genotype*	*Duration of Therapy (weeks)*	*SVR*	*End of Rx TSH*	*TSH at 12 months*	*TSH at 24 months*	*TSH at 36 months*
1	M	26	1a	48	Y	2.1	1.8	2.8	3.4
2	M	51	2	24	Y	1.7	3.2	3.1	2.7
3	M	54	3	24	Y	3.8	2.3	2.2	3.2
4	M	49	4	48	Y	1.1	1.4	2.1	2.3
5*	F	42	2	24	Y	1.6	1.8	2.1	1.9
6*	F	34	3	24	Y	2.4	2.7	2.1	2.0
7	F	49	4	48	Y	3.1	2.9	2.7	2.2
8*	F	49	1	48	Y	0.03	1.5	1.2	1.3
9	F	50	2	24	Y	2.2	2.1	2.4	2.5
10*	F	37	4	48	Y	6.5	4.5	5.2	2.3
11	F	43	4	48	Y	1.8	1.9	2.3	2.1
12	F	42	3	24	Y	2.1	2.3	1.7	2.5
13*	F	43	1	48	N	10.5	2.3	1.8	2.4
14*	F	51	3	24	Y	8.8	4.0	3.8	2.3
15	M	57	1	48	N	2.2	2.7	1.9	1.2
16	F	38	3	24	Y	1.9	2.2	2.3	3.7
17	M	57	1	48	Y	4.5	2.5	3.6	2.8
18	F	37	3	24	Y	3.4	3.3	2.5	2.8

*; Cases 5 and 6 required short-term thyroxine supplement, case 8 progressed to develop post-interferon Graves' like thyrotoxicosis, cases 10 and 14 had subclinical hypothyroidism and case 13 was in the hypothyroid phase at the end of treatment. SVR; Sustained virologic response, Rx; Treatment, TSH; Thyrotropin.

Duration of follow-ups

Duration of follow-ups started from the completion of interferon treatment, be it 24 or 48 weeks.

Definition of thyroiditis

Thyroiditis is defined as the triad of clinical and/or biochemical thyrotoxicosis followed by a hypothyroid phase, with an initial reduced/negligible thyroid pertechnetate uptake scan. All uptake scans were reviewed by a specialist nuclear physician consultant. Thyroid autoantibodies may be present but are not considered essential to the diagnosis.

Results

There were 18 cases of thyroiditis available for the study. All recovered from their TD at the end of the study. Cases 5 and 6 required short-term thyroxine supplement but subsequently were successfully withdrawn from thyroxine. Case 8 progressed to developed temporary Graves-like thyrotoxicosis demonstrating the uncommon tri-phasic thyroiditis that had previously been reported [8,9]. In addition, cases 10 and 14 had subclinical hypothyroidism which resolved with time and did not require thyroxine at any stage, Table 2. Similarly, case 13 was in the hypothyroid phase of the thyroiditis at the end of treatment, was treated expectantly and resolved without thyroxine. At 36 months, all cases had normal thyrotropin levels and all thyroid autoantibodies were undetectable. No patient required thyroxine at the end of the study.

With the exception of case 8, thyroid autoantibody titres declined towards normal at the end of treatment and remained unaltered throughout the duration of follow-up.

Discussion

This is the first report to prospectively investigate the natural history of patients who develop thyroiditis during treatment with pegylated interferon-α and ribavirin for chronic hepatitis C infection. It is reassuring to ascertain that this form of TD is benign and that there is no long term recurrence or hypothyroidism. This benign prognosis is encouraging for both patients and clinicians. There appears to be little need to monitor for associated thyroid condition beyond 36 months after completion of treatment.

This benign outcome also parallels the thyroid autoantibodies which similarly resolved. With the exceptions of one patient (case 8), all thyroid antibodies normalise. In this affected case, autoantibodies persisted at the end of treatment suggesting ongoing immunomodulation but the consequent mild Graves' disease resolved after 12 months.

The involution of thyroid disease after interferon exposure is as expected and is probably due to two reasons. Firstly, it is because of the pharmacokinetics of the

Table 2 Auto-antibody profiles in 18 thyroiditis patients

Subject No.	*End of Rx anti-TPO*	*Anti-TPO at 12 months*	*Anti-TPO at 24 months*	*Anti-TPO at 36 months*	*End of Rx anti-Tg*	*Anti-Tg at 12 months*	*Anti-Tg at 24 months*	*Anti-Tg at 36 months*	*End of Rx TSI*	*TSI at 12 months*	*TSI at 24 months*	*TSI at 36 months*
1	<1	<1	<1	<1	<1	<1	<1	<1	<10	<10	<10	<10
2	<1	<1	<1	<1	<1	<1	<1	<1	<10	<10	<10	<10
3	<1	<1	<1	<1	<1	<1	<1	<1	<10	<10	<10	<10
4	<1	<1	<1	<1	<1	<1	<1	<1	<10	<10	<10	<10
5*	1024	<1	<1	<1	<1	<1	<1	<1	<10	<10	<10	<10
6*	256	<1	<1	<1	<1	<1	<1	<1	<10	<10	<10	<10
7	<1	<1	<1	<1	<1	<1	<1	<1	<10	<10	<10	<10
8*	16	<1	<1	<1	<1	<1	<1	<1	19	10	<10	<10
9	<1	<1	<1	<1	<1	<1	<1	<1	<10	<10	<10	<10
10*	<1	<1	<1	<1	<1	<1	<1	<1	<10	<10	<10	<10
11	<1	<1	<1	<1	<1	<1	<1	<1	<10	<10	<10	<10
12	<1	<1	<1	<1	<1	<1	<1	<1	<10	<10	<10	<10
13*	<1	<1	<1	<1	<1	<1	<1	<1	<10	<10	<10	<10
14*	16	<1	<1	<1	<1	<1	<1	<1	<10	<10	<10	<10
15	<1	<1	<1	<1	<1	<1	<1	<1	<10	<10	<10	<10
16	<1	<1	<1	<1	<1	<1	<1	<1	<10	<10	<10	<10
17	<1	<1	<1	<1	<1	<1	<1	<1	<10	<10	<10	<10
18	<1	<1	<1	<1	<1	<1	<1	<1	<10	<10	<10	<10

*; see Table 1 legend. Anti-TPO; anti-thyroperoxidase antibody, Anti-TgI; anti-thyroglobulin antibody, TSI; Thyrotropin stimulating immunoglobulins.

pegylated IFN which is expected to clear within 4 to 6 weeks. As a result, most of the TDs that occur post-therapy tend to do so in this time frame [10]. Secondly, once beyond the 6 month period where sustained virologic response (SVR) had been attained, the immunomodulating effect of the hepatitis C viral particle has been removed and hence its influence on thyroid tissues is no longer pertinent [11].

However our findings are inconsistent with other retrospective reports. Carella et al [12] studied a group of 114 chronic hepatitis C patients after an average of 6.2 years of follow-up and found some persistence of thyroid autoantibodies after 6.2 years of follow-up but without clinical findings of TDs. However, these patients were treated with IFN-α alone for 12 months. However the treatment was monotherapy with regular IFN. Vezali et al [13] described 2 cases which developed thyroiditis within 1 month of treatment completion and another 3 cases at 6, 6.5 and 26 months respectively. No further details were forthcoming. The frequency of thyroid testings was not mentioned during this post-treatment period. Jamil et al [6] looked at 45 cases of thyroid disease over a 12 year period but did not comment on the long-term outcomes of thyroid diseased patients nor the frequency of thyroid monitoring. Generally speaking in other retrospective series, the thyroid follow-ups are often ad hoc as a result and the outcome is not uniform and inconsistent [14,6]. The thyroid surveillance frequency is also inadequate to assess for the thyroid condition.

Most series report hypothyroidism as the commonest thyroid disorder in this setting but our experience has been that of thyroiditis almost exclusively [7]. In this setting, the prevalence of permanent hypothyroidism has been reported at ~50% [13] but where attempts or trials of thyroxine withdrawal were done, none of our patients required long term supplements. This suggests that the thyroid has completely recovered from the interferon-elicited injury and that hypothyroidism occurring in this setting deserved a trial of withdrawal before being committed to life-long therapy and all its potential complications.

The presence of high thyroid autoantibody titres also heavily influences the involution of thyroid diseases. Where they persisted or evolved, clinical diseases appear to follow as evident in cases 5, 6 and 8. However, because of the sporadic nature of post-treatment thyroid diseases, it remains uncertain whether routine thyroid autoantibodies should be performed routinely in this setting.

The major limitation of this study is the small number of subjects due the low prevalence of the condition. The second concern is the testing frequency of 12 month intervals during which it is conceivable that thyroid function may have fluctuated during this time. Regardless, it appears that thyroid disease sustained during interferon therapy for chronic hepatitis C infection is self-limiting and without any long last consequences. All cases recovered, compared with de novo thyroiditis where the progression to hypothyroidism can be as high as 20% in both adults and children [15,16]. Although there is no data beyond 3 years, it can safely be assumed that any development of thyroid conditions beyond this time should be deemed unrelated and treated each on its own merit. Perhaps monitoring for thyroid disease could be safely ceased at 6 months follow-up, coinciding with the SVR review.

Conclusion

Despite the small number, the long term outcome of interferon induced thyroiditis appears benign. There is no long term risk of hypothyroidism. Larger studies will be needed to confirm this outcome. If this is the case, perhaps there is little need to follow these patients longer than 6 months after the completion of interferon therapy. This is to cover for any immediate thyroid conditions that may occur during this time frame. Follow-ups beyond 3 years appear unwarranted although this time frame is completely arbitrary.

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Authors' contributions

HAT conceived the study, participated in its design, assisted with data collection and statistical analysis, and coordinated and helped to draft the manuscript. GEMR contributed to the statistical and meta-analytical methods, and participated in the discussion and drafting of the manuscript. TLJ, EAI gathered, provided the data, and participated in the discussion and drafting of the manuscript. All authors read and approved the final revised manuscript.

Competing interests

The authors declare that they have no competing interests.

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References

1. Tomer Y, Blackard JT, Akeno N: **Interferon alpha treatment and thyroid dysfunction.** *Endocrinol Metab Clin N Am* 2007, **36**:1051-66.
2. Carella C, Mazziotti G, Amato G, Braverman LE, Roti E: **Interferon-α-related thyroid disease: Pathophysiological, epidemiological, and clinical aspects.** *J Clin Endocrinol Metab* 2004, **89**:3656-61.

3. Prummel MF, Laurberg P: **Interferon-α and autoimmune thyroid disease.** *Thyroid* 2003, **13**:547-51.
4. Antonelli A, Ferri C, Fallahi P: **Thyroid dysfunction in patients with hepatitis C on IFN-α therapy.** *Nat Rev Gastroenterol Hepatol* 2009, **6**:633-5.
5. Wong V W-S, Cheng A Y-S, Chan H L-Y: **Interferon-induced thyroid dysfunction - not always transient.** *J Gastroenterol Hepatol* 2009, **24**:933-940.
6. Jamil KM, Leedman PJ, Kontorinis N, Taruinio L, Nazareth S, McInerney M, Connelly C, Flexman J, Burke V, Metcalf C, Cheng W: **Interferon-induced thyroid dysfunction in chronic hepatitis C.** *J Gastroenterol Hepatol* 2009, **24**:1017-23.
7. Tran HA, Reeves GEM, Jones TL: **The natural history of interferon-alpha2b-induced thyroiditis and its exclusivity in a cohorte of patients with chronic hepatitis C infection.** *QJM* 2009, **102**:117-22.
8. Bohbot NL, Young J, Orgiazzi J, Buffet C, Francois M, Bernard-Chabert B, Lukas-Croisier C, Delemer B: **Interferon-alpha-induced hyperthyroidism: a three-stage evolution from silent thyroiditis towards Graves' disease.** *Eur J Endocrinol* 2006, **154**:367-72.
9. Tran HA: **The swinging thyroid in hepatitis C infection and interferon therapy.** *QJM* 2009, **183**:187-191.
10. Tran HA, Reeves GE: **The spectrum of autoimmune thyroid disease in the short to medium term following interferon-alpha therapy for chronic hepatitis C.** *Int J Endocrinol* 2009, **2009**:241786.
11. Akeno N, Blackard JT, Tomer Y: **HCV E2 protein binds directly to thyroid cells and induces IL-8 production: a new mechanism for HCV induced thyroid autoimmunity.** *J Autoimmun* 2008, **31**:339-344.
12. Carella C, Mazziotti G, Morisco F, Manganella G, Rotondi M, Tuccillo C, Sorvillo F, Caporaso N, Amato G: **Long-term outcome of interferon-α-induced thyroid autoimmunity and prognostic influence of thyroid autoantibody pattern at the end of treatment.** *J Clin Endocrinol Metab* 2001, **86**:1925-1929.
13. Vezali E, Elefsiniotis I, Mihas C, Konstantinou E, Saroglou G: **Thyroid dysfunction in patients with chronic hepatitis C: Virus- or therapy-related?** *J Gastroenterol Hepatol* 2009, **24**:1024-1029.
14. Masood N, Shori R, Memon A, Memon S, Memon KI, Memon I, Jaffri M, Baloch GH: **Frequency of thyroid disorders during interferon and ribavirin therapy in chronic hepatitis C infection.** *J Coll Physicians Surg Pak* 2008, **18**:347-351.
15. Demirbilek H, Kandemir N, Gonc EN, Ozon A, Alikasifoglu A: **Assessment of thyroid function during the long course of Hashimoto's thyroiditis in children and adolescents.** *Clin Endocrinol* 2009, **71**:451-454.
16. Bindra A, Braunstein GD: **Thyroiditis.** *Am Fam Physician* 2006, **73**:1769-1776.

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(D) TRIPHASIC THYROIDITIS

This is a very uncommon condition and rarely reported in the literature. Our publication is only the second report and is the first outside Europe. Although rare, it questions the need for ongoing thyroid, albeit limited, surveillance after the completion of therapy, (see chapter VIII).

Publication:

Tran HA. THE SWINGING THYROID IN HEPATITIS C INFECTION AND INTERFERON THERAPY. *Q J Med, 2010; 103: 187-191*.

Q J Med 2010; **103**:187–191
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Case report

QJM

The swinging thyroid in hepatitis C infection and interferon therapy

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Introduction

Thyroid diseases are the commonest endocrine manifestations in chronic hepatitis C infection. These are further exacerbated with the use of interferon-α related therapy. Beside the non-specific non-thyroidal illness, the spectrum of thyroid diseases range from frank hypothyroidism to overt thyroiditis. The latter is a specific condition in which there is a particular pattern of an initial thyrotoxicosis, followed by hypothyroidism with subsequent normalization, commonly known as biphasic thyroiditis. Presented is a case of tri-phasic thyroid response in which the interferon-induced bi-phasic thyroiditis was followed closely with Graves' like thyrotoxicosis post therapy. This case offers a unique opportunity to understand the immunopathogenesis by analysis of the thyroid autoantibody pattern response. This is a recently described entity and the management needs to be specific for each particular phase of the condition.

Clinical notes

A 53-year-old Caucasian man presented with acute thyroiditis whilst undergoing combination Ribavirin and Interferon-α2b therapy for his hepatitis C infection. Because of his HCV genotype 1, treatment duration was to be 48 weeks. He had no medical history of note, smoked 5–10 cigarettes daily and has been tolerating the treatment regimen well. At the 28th week of therapy, he complained of palpitations, unintentional weight loss of 4 kg over 4 weeks and intermittent diarrhoea. There was no family or prior history of thyroid disease. He did not take any other prescribed or over-the-counter medications. Clinical examination showed a thin man, weight 56 kg, height 1.62 m, (body mass index of ~22 kg/m^2). He was in sinus tachycardia at 101 beats per minute, blood pressure of 120/80 with no postural hypotension. There were peripheral stigmata of thyrotoxicosis but no signs of Graves' ophthalmopathy or dermopathy. No goitre was present. His monthly TSH levels which had been normal were now undetectable [reference range (RR), 0.4–4.0 mU/l]; free tetra-iodothyronine (fT4) was 28.7 (RR, 10.1–24.5 pmol/l), free tri-iodothyronine (fT3) was 6.9 (RR, 3.3–5.8 pmol/l). His anti-thyroglobulin antibody and thyrotropin stimulating immunoglobulin (TSI) titres were normal at 1 : 64 (RR, <1 : 400) and <10 (RR, <10 U/ml), respectively. His anti-thyroperoxidase titre was elevated at 1 : 640 (RR, 1 : 400). His thyroid ultrasound revealed normal size without any nodular disease and a pertechnetate thyroid uptake was undetectable at <1% (RR, 3–8%). A diagnosis of thyroiditis was made and Propanolol was initiated. No specific medications were required, specifically no corticosteroid. The patient remained generally well and was keen to complete therapy. Interferon therapy was thus continued with monthly thyroid function tests. At 36 week, he became hypothyroid with a TSH level of 27.2 mU/l, fT4 of 4.3 and fT3 of 3.9 pmol/l. As there were no hypothyroid symptoms,

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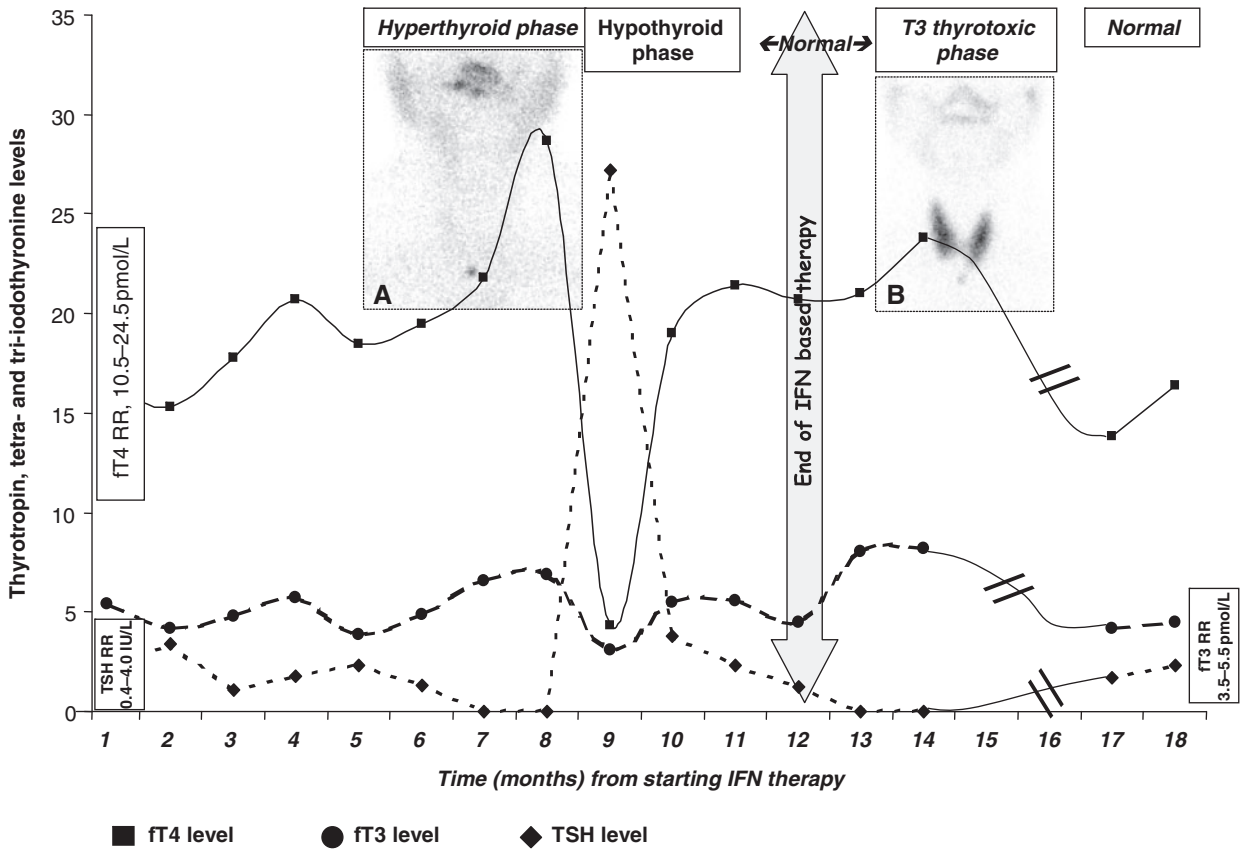

Figure 1. The swinging pattern of thyroid function in the patient with hepatitis C infection during and after IFN-based therapy. Insets A and B show the thyroid uptake scan appearances at the two respective thyrotoxic incidents.

no thyroxine replacement therapy was given. The patient completed his IFN-base treatment uneventfully at 48 weeks at which time his TSH, fT4 and fT3 levels had returned to normal. At 8 weeks post treatment, he complained of non-specific lethargy and a 2.5 kg weight loss. Repeat thyroid function tests showed T3-thyrotoxicosis with again, suppressed TSH, fT4 of 21.1 and fT3 8.0 pmol/l. His anti-thyroglobulin antibody titre was 1:64, anti-thyroperoxidase 1:128 and TSI was elevated at 19 U/ml. A repeat pertechnetate uptake scan showed diffuse and increased uptake at 12%, consistent with Graves' disease (GD). At 12 weeks post IFN therapy, his T3-toxicosis persisted with fT3 level of 8.7 pmol/l. A diagnosis of GD was made and propylthiouracil (PTU) treatment was initiated. He was rendered euthyroid 6 weeks later at which time his fT4 and fT3 levels had normalized. PTU was continued for another 18 months. His HCV RNA viral load was absent at 24 weeks post IFN therapy confirming a sustained virological response. He remained euthyroid 3 months after cessation of PTU. Figure 1 demonstrates the swinging pattern of thyroid function tests and pertechnetate uptake scans.

Hepatitis C was first identified in 1989,[1] and since then millions of people around the world have been diagnosed with this condition. It is estimated that ~3% of the world population, 180-million people are infected with the virus.[2] In Australia there estimated to be ~13 000–20 000 cases per year although the number is decreasing annually with improved control.[3] In the United States, the prevalence is 1.3% resulting in a substantial 3.2-million cases.[4] As the number of treated cases is expected to escalate, it is important that side effects of treatment are recognized and managed appropriately. Hepatitis C infection, together with IFN-based treatment, is associated with a number of extra-hepatic endocrinopathies, the commonest of which is thyroid disease. This is not well appreciated because it is an uncommon adverse reaction and thus is not often reported in clinical trails, limiting its understanding.[5] As treatment is increasing, the number of thyroid-related complications can only be expected to increase.

The spectrum of thyroid disease in this setting is broad and ranges from overt hypothyroidism to frank hyperthyroidism. Commonly, most patients developed a non-thyroidal illness like pattern

with low/undetectable TSH and normal/low free thyroid hormone levels biochemically and with subsequent recovery.[6] Approximately 3–5% develops (bi-phasic) thyroiditis, 5–8% hypothyroidism and 1% Graves' like thyrotoxicosis.[7] Each entity often follows its own course with characteristic natural history and therapeutic response.

This report, the second worldwide, illustrates a recently described specific thyroid disease in patients with hepatitis C undergoing IFN-base medication.[8] The patient developed tri-phasic thyroid dysfunction with two peaks of thyrotoxicosis, bisected by hypothyroidism. This is opposed to the classical bi-phasic condition that is common in thyroiditis. This swinging pattern of thyroid disease from one extreme to the other is a relatively novel finding. This clinical observation offers an opportunity to add to what little is known about this entity.

The overall pathogenesis of thyroiditis remains unknown. Drug naive hepatitis C infection is known to modulate the immune system, particularly the cytotoxic immune response in CD_4 T cells, producing high concentrations of interferon-γ (IFN-γ) and interleukin-2 (IL-2). In the presence of therapeutic IFN, first, the cytotoxic CD_4 T cell response is further exaggerated, particularly the T_H1 immunity which induces apoptosis by aberrant and enhancement of major histocompatability complex class I antigen surface expression on thyrocytes. This subsequently evolves into the destruction of thyroid cells and associated follicles.[9] This is further accentuated by the increased IFN-γ level. Furthermore, there is a reduction in T-cell apoptosis which further fuels the immuno-modulation induced by the hepatitis C infection. Second, there is the T_H2 response which guides the autoantibody response, particularly of anti-thyroperoxidase and anti-thyroglobulin.[10,11] Together with the T_H1 immune response, these result in the hyperthyroid phase of thyroiditis in which there is rupture of the thyroid follicles leading to the release of stored thyroid hormones into the circulation. This first phase is further characterized by the absent/negligible pertechnetate uptake scan and thyroid auto-antibodies positivity. Following this destructive phase, the thyroid is then depleted of all its hormone reserve, resulting in the second hypothyroid phase. With time, the thyroid recovers back to normality. In *de novo* thyroiditis, ~5% remain permanently hypothyroid, but in IFN-related cases, the ultimate outcome is still uncertain but most resolve and remain quiescent. With the development of the high uptake thyrotoxic third phase, there must be further modulation of the immune system. This phase behaves similarly to GD in the presence of an elevated TSI titre. It is probable that the initial exposure to IFN swings the immune system in favour of the T_H2 system, activating the B cells to generate TSI, resulting in the clinical Graves' like thyrotoxicosis. This occurs after the cessation of IFN therapy, suggesting that the entire pathogenic process must have been triggered off whilst undergoing treatment but has taken sometime to develop.[12] In addition, there must be an underlying genetic predisposition so that in the suitable biological milieu, the condition is allowed to proceed inexorably to clinical thyroid disease.[13] Figure 2 summarises the proposed pathogenesis of this relatively novel entity.

The persistence of T3-toxicosis is another peculiarity in this case. Although T3 toxicosis does occur in *de novo* GD,[14] this, and its persistence without therapy, has not been previously described in IFN-related toxicosis. The diagnosis is further confirmed by the increased uptake scan and positive TSI. This plausibly indicates that TSI in HCV infection preferentially stimulates iodothyronine deiodinase type 1 (D1) activity, the bulk of which resides primarily in the thyroid and liver, resulting in T3-toxicosis alone. As D1 enzyme is potently inhibited by PTU rather carbimazole,[15] the former was selected and subsequently proven to be very effective.

The management strategies for each segment of this interesting condition are quite different and thus it is important that the diagnosis is correctly made. In the initial thyrotoxic phase of thyroiditis, thionamides should not be used as they contradict the underlying pathogenesis. If the thyrotoxic phase is too severe, symptomatic control should be managed with beta-blockade. In severe and symptomatic hypothyroidism, short-term thyroxine supplement should be considered as this phase too is short lived. Corticosteroid should not be used primarily due to its ineffectiveness[16] but also due to the theoretical risk of precipitating hepatic necrosis.[17] The final thyrotoxic phase should be treated as GD *de novo*. This entails prolonged use of thionamides, ranging between 12–18 months (Table 1).

The surveillance for thyroid disease during and after IFN therapy remains inconsistent. The recommendations are either varying or absent according to various governing bodies. Both the British Society of Gastroenterology recommends thyroid function test at *each* treatment visit, rather than monthly.[18] The American Gastroenterological Association does not offer any recommendation.[19] Both the National Institute of Health consensus statement on hepatitis C management and the National Academy of Clinical Biochemistry surprisingly makes no mention of the thyroid issue in relation to IFN therapy.[20,21] With this report in mind, it is plausible and physiological that thyroid screening, using

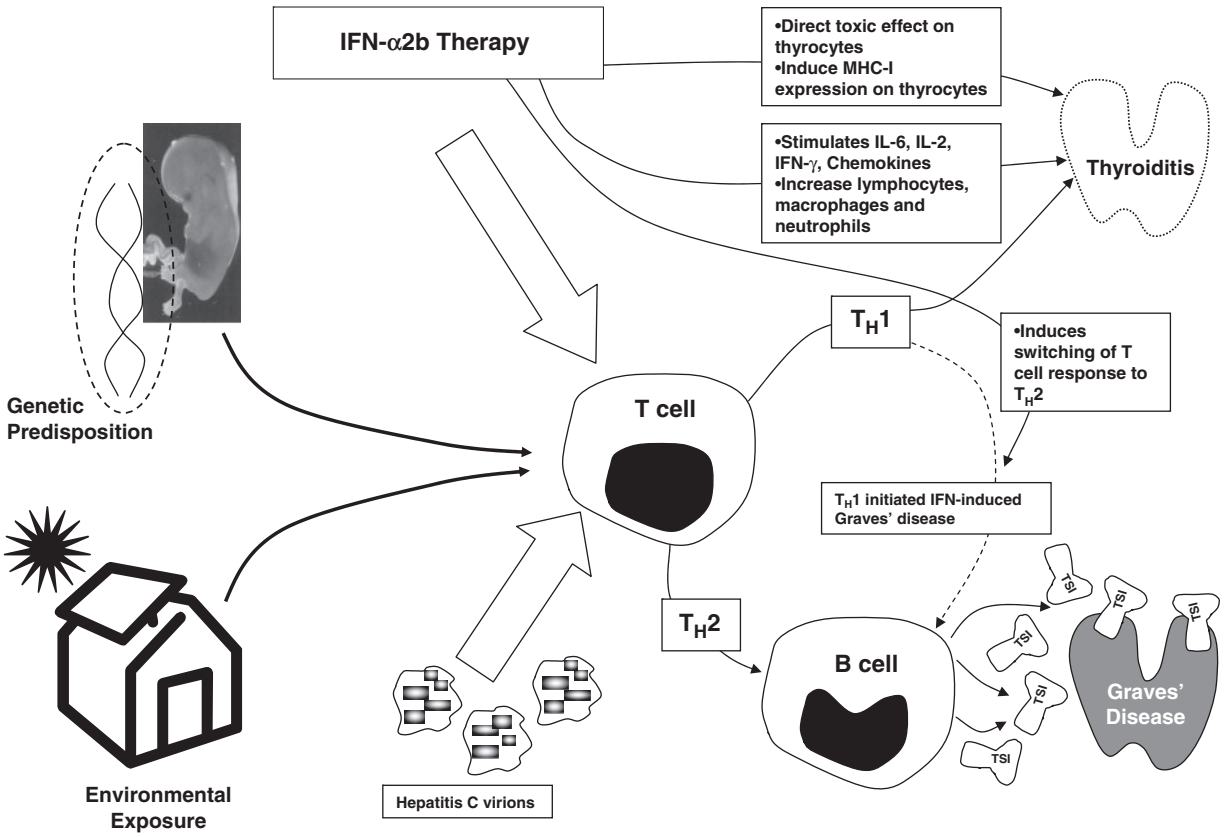

Figure 2. The proposed pathogenesis of 'tri-phasic' thyroiditis under the influence of IFN-α2b in the presence of chronic hepatitis C infection.

Table 1 Contrasting features of thyroiditis and GD in patients with hepatitis C infection treated with IFN-based therapy

	Thyroiditis	Graves' disease
Characteristics	Biphasic resposnse	Persistent thyroxicosis
Pathogenesis	Rupture/destructive of thyroid gland	Immunostimulation with TSI
Thyroid pertechnetate uptake scan	Absent/negligible	Increased uptake
Natural history	Hyperthyroid phase followed by hypothyrodism with subsequent normalization	Persistent hyperthyroidism requiring treatment
Treatment	Observation and β-blocker if required Corticosteroid is relatively contr indicated	Long-term thionamide therapy

TSH, is performed monthly for the duration and 6 months after the completion of therapy. Beyond this time frame, individual cases should be assessed according to symptomatology.

This case highlights the newly described condition of tri-phasic thyroid disease,[8] in response to IFN-based therapy, offering some crucial understanding of the condition. Each phase of this multiphasic thyroid disease needs to be managed appropriately with the background appreciation of the pathogenesis of the condition.

Acknowledgements

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Conflict of interest: None declared.

References

1. Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* 1989; **244**:359–62.
2. WHO website: http://www.who.int/topics/hepatitis/en/ (last accessed 7 April 2008).
3. Hepatitis C virus projections working group. *Estimates and projections of the hepatitis C virus epidemic in Australia 2006*. National Centre in HIV Epidemiology and Clinical Research, the University of New South Wales, October 2006.
4. Batey RG. Hepatitis C: where are we at and where are we going? *Med J Aust* 2002; **176**:361–2.
5. Manns MP, Wedemeyer H, Comberg M. Treating viral hepatitis C: efficacy, side effects and complications. *Gut* 2006; **55**:1350–9.
6. Tran HA, Jones TL, Batey RG. The spectrum of thyroid disease treated with alpha-2-beta interferon and ribavirin in an Australian population. *BMC Endocr Disord* 2005; **5**:8.
7. Wong V, Fu Xi-Li A, George J, Cheung NW. Thyrotoxicosis induced by alpha-interferon therapy in chronic viral hepatitis. *Clin Endocrinol* 2002; **56**:793–8.
8. Bohbot NL, Young J, Orgiazzi J, Buffet C, Francois M, Bernard-Chabert B, *et al.* Interferon-alpha-induced hyperthyroidism: a three-stage evolution from silent thyroiditis towards Graves' disease. *Eur J Endocrinol* 2006; **154**: 367–72.
9. Lloyd AR, Jagger E, Post JJ, Crooks L-A, Rawlinson WD, Hahn YS, *et al.* Host and viral factors in the immunopathogenesis of primary hepatitis C virus infection. *Immunol Cell Biol* 2007; **85**:24–32.
10. Carella C, Mazziotti G, Amato G, Braverman LE, Roti E. Interferon-α-related thyroid disease: Pathophysiological, epidemiological, and clinical aspects. *J Clin Endocrinol Metab* 2004; **89**:3656–61.
11. Prummel MF, Laurberg P. Interferon-α and autoimmune thyroid disease. *Thyroid* 2003; **13**:547–51.
12. Tran HA. Hepatitis C infection, treatment regimens, and thyroid function abnormalities. *The Endocrinologist* 2007; **17**:231–5.
13. Tomer Y, Davies TF. Searching for the autoimmune thyroid disease susceptibility genes. From gene mapping to gene function. *Endocr Rev* 2003; **24**:694–717.
14. Weetman AP. Graves' disease. *N Engl J Med* 2000; **343**:1236–48.
15. Kuiper GGJM, Kester MHA, Peeters RP, Visser TJ. Biochemical mechanisms of thyroid hormone deiodination. *Thyroid* 2005; **15**:787–98.
16. Minelli R, Valli MA, Di Secli C, Finardi L, Chiodera P, Bertoni R, *et al.* Is steroid therapy needed in the treatment of destructive thyrotoxicosis induced by α-interferon in chronic hepatitis C? *Horm Res* 2005; **63**:194–9.
17. Tomer Y, Blackard JT, Akeno N. Interferon alpha treatment and thyroid dysfunction. *Endocrinol Metab Clin N Am* 2007; **36**:1051–66.
18. British Society of Gastroentrology. *Clinical Guidelines on the Management of Hepatitis C*. http://www.bsg.org.uk/pdf_word_docs/clinguidehepc.pdf, accessed 18 October 2009.
19. Dienstag JL, McHutchison JG. American Gastroenterological Association medical position statement on the management of hepatitis C. *Gastroenterology* 2006; **130**:225–30.
20. Management of Hepatitis C. *NIH Consens State Sci Statements* 2002; **19(3)**:1–46.
21. NACB. *Laboratory Support for the Diagnosis and Monitoring of Thyroid Disease*. http://www.nacb.org, accessed 18 October 2009.

CHAPTER V. THE METABOLIC AND EXTRA-THYROID EFFECTS ARISING FROM INTERFERON-α TREATMENT

In parallel with the recognition of thyroid disorders, a number of other metabolic and thyroid-associated conditions became evident. These cases were carefully studied and subsequently published. Because of the rarity of such conditions, similar published cases were performed to compare and contrast the worldwide clinical experience.

(A) THYROTOXIC PERIODIC PARALYSIS

Publication:

Tran HA, Reeves GE. HEPATITIS C INFECTION AND THYROTOXIC PERIODIC PARALYSIS – A NOVEL USE OF AN OLD DRUG. *Am J Med Sci*, 2008; 336: 515–518.

Hepatitis C Infection and Thyrotoxic Periodic Paralysis—A Novel Use of an Old Drug

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ABSTRACT: Hypokalaemic thyrotoxic periodic paralysis is an enigmatic and uncommon condition which occurs exclusively in males of Asian descent. The underlying causes of thyrotoxicosis may be any of the well-recognized etiologies including a toxic multinodular goiter, Graves' disease or iodine excess. Beside thyrotoxicosis, a number of other hormonal factors have been hypothesized to contribute to hypokalaemic thyrotoxic periodic paralysis, particularly postprandial hyperinsulinaemia and testosterone. We hereby present a case of a 48-year-old hepatitis C positive gender-assigned man in whom all of these factors are proposed to interact, lending further support to these hypotheses. The patient presented with interferon-induced thyroiditis causing acute generalized weakness whilst undergoing combination interferon-α-2β and ribavirin therapy. As part of his hepatitis C infection, marked insulin resistance with hyperinsulinaemia was also present, exacerbating the paresis. Initial treatment with beta-blocker failed to normalize his serum potassium concentration, requiring the novel use of spironolactone, despite euthyroidism. This continued to be required until his testosterone supplement dissipated. **KEY INDEXING TERMS:** Hepatitis C infection; Hypokalemia; Thyrotoxic periodic paralysis; Spironolactone. **[Am J Med Sci 2008;336(6):515–518.]**

Clinical Course

A 48-year-old Filipino gender-assigned man presented with acute onset of quadraparesis after breakfast one morning. He had been generally well until the event. There were no respiratory symptoms or sphincter disturbances. System review showed recent weight loss of about 6 kg over 3 months with general lethargy and tiredness. No cardiovascular or gastrointestinal symptoms were forthcoming. His medical history included hepatitis C infection, chronic alcoholism (ceased 5 years prior), ex-smoker of 10 years, and gender reassignment since 19 years of age from female to male. His hepatitis C was of genotype 2, contracted from intravenous drug use in his early 20s. He was in the 18th week of his combination pegylated interferon-α-2β and ribavirin treatment for his hepatitis C infection. His major reactions to this therapy included emotional lability, listlessness, and mild anemia which required no intervention. Other medications include testosterone implants at 800 mg every 6 months, last given 4 weeks prior. The patient denied any use of alternative or over-the-counter medicines. There was no family or prior personal history of thyroid disease. Clinical examination showed a well-masculinized patient, weight of 65 kg, height of 1.64 m (body mass index of ~24 kg/m^2). Blood pressure was 140/70 mm Hg with no postural drop and resting regular pulse rate of 100 beats per minute. Peripheral stigmata of thyrotoxicosis were present. His Achilles tendon reflexes were absent, despite the Jendrassik manoeuvre. No goiter or ophthalmopathy was detected. Respiratory examination was unremarkable. The bedside peak expiratory flow rate was ~350 L/min. Electrocardiogram showed sinus rhythm with a rate of 104 beats per minute.

Laboratory test results are shown in Tables 1 and 2. Note that his serum potassium level was 1.8 mmol/L. A 3-hour oral glucose tolerance test with insulin levels was performed 7 days after presentation. The thyroid pertechnetate uptake was absent at 0% uptake at 6 hours, consistent with the diagnosis of biphasic interferon-induced thyroiditis.

The patient was treated with 240 mg of oral propanolol (at 3 mg/kg) followed by 80 mg thrice daily. At 1 hour, his hypokalemia remained unchanged at 1.9 mmol/L and only slightly improved to 2.3 mmol/L at 4 hours. At 8 hours, it remained static at 2.4 mmol/L. The patient had a slight improvement in symptoms although he felt weak and could only walk with 1 person assisting. His tendon reflexes remained absent, and his peak expiratory flow rate had decreased to 300 L/min. At 24 hours, his serum potassium level remained low at 2.5 mmol/L with no further improvement of symptoms. His propanolol

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Table 1. Summary of the Patient's Laboratory Test Results

	Results	Reference Intervals
Random glucose level	5.7	5.5–7.8 mmol/L
pH	7.39	7.35–7.45
Po_2	109	>75 mmHg
Pco_2	32	35–45 mmHg
Bicarbonate	38	35–45 mmol/L
Base excess	−1	±2
Anion gap	14	7–15 mmol/L
Sodium	142	136–146 mmol/L
Potassium	2.1	3.5–5.5 mmol/L
Urea	9.1	3.0–7.0 mmol/L
Creatinine	90	40–90 μmol/L
Bilirubin	18	6–10 μmol/L
Alanine aminotransferase	55	<50 IU/L
Alkaline phosphatase	136	<110 IU/L
Gamma glutamyl transpeptidase	25	<60 IU/L
Albumin	38	35–45 g/L
Hemoglobin	158	115–165 g/L
Neutrophil count	11.7	$4.0–7.0 \times 10^6$ per mL
Platelet	289	$150–450 \times 10^6$ per mL
Thyrotropin	<0.03	0.4–4.0 U/L
Free tetra-iodothyronine	72.8	10.5–24.5 pmol/L
Free tri-iodothyronine	21.94	3.3–6.2 pmol/L
Antithyroglobulin titre	<1	$<4 \times 10^2$
Antimicrosomal titre	64	$<4 \times 10^2$
Serum testosterone	15.4	<2.1 nmol/L; for adult females 10.5–21.5 nmol/L; for adult males
Serum sex hormone binding globulin	12	10–50 μg/L

Note that testosterone reference ranges for both adult males and females have been included.

was increased to 120 mg thrice daily and as a result his heart rate decreased to 54 beats per minute. Given that his gender reassignment history with testosterone supplementation may have played in exacerbating his hypokalemia, spironolactone was empirically given at 400 mg daily. At 48 hours his potassium level was 3.2 mmol/L, and at 60 hours 3.8 mmol/L. The patient felt that his leg strength had improved and was able to mobilize short distances unaided. At 72 hours his potassium level was 4.0 mmol/L. No potassium supplement was given at any stage and spironolactone was continued unchanged.

His thyroiditis was treated expectantly according to the natural progression of the disease. Four weeks later, his potassium level remained stable at 4.3 mmol/L despite ongoing spironolactone use. Thyroid function tests showed a free T4 level of 24.5 and a free T3 level of 5.8 pmol/L, with suppressed thyroid stimulating hormone. He continued to require spironolactone despite the attainment of euthyroidism, as his potassium level dropped to 3.0 and 3.2 mmol/L on 2 occasions when this medication was temporarily withheld. His potassium level became progressively elevated to 5.8 and 6.2 mmol/L when his testosterone levels began to wear off, necessitating a reduction and subsequent removal of the spironolactone. Figure 1 summarizes the relationship between his serum potassium concentration, free T4 level and testosterone level. It is noteworthy that the potassium level increase was only possible after the testosterone effect had worn off. His interferon therapy was discontinued at the development of thyroid disease. When last reviewed, he remained euthyroid and was not on any medication.

Table 2. The Patient's 3-hour Oral Glucose Tolerance Test Which Demonstrated Impaired Fasting Glycemia, Insulin Resistance (the Homeostasis Model of Assessment Index is 8.8, Which is Moderately High)

Relative Times	Serum Glucose (mmol/L)	Serum Insulin (mIU/L)	Potassium (mmol/L)
0	5.8	34	3.8
60	10.9	108	3.5
120	7.8	119	3.3
180	6.0	112	3.2

The test was aborted at 180 minutes due to progressive decline in potassium level and the risk of redeveloping generalized weakness.

Discussion

Hypokalaemic thyrotoxic periodic paralysis (HTPP) is an uncommon condition that is spreading beyond its traditional geographic Asian areas due to increased migration and ease of transport.[1] Adding this to the prevalence of hepatitis C, especially in developing countries, the development of this particular case scenario is not unexpected. HTPP in the setting of hepatitis C infection has been described previously but in association with Graves' disease.[2,3] Thyroid disease is the most common endocrine-related problem associated with hepatitis C and interferon-γ treatment. As part of this spectrum, Graves' like thyrotoxicosis has been described, but thyroiditis of a biphasic nature is more common.[4] In the absence of any other discernable causes, and through unknown mechanisms, the thyrotoxicosis leads to hypokalemia with subsequent paralysis in genetically predisposed patients.[5] Whilst many thyrotoxic conditions have been previously described to be associated with HTPP, this is the first case report to describe it in associated with interferon-γ induced thyroiditis in patient with hepatitis C infection. The course of thyroiditis, in this situation, unfortunately cannot be modified by any therapy. Prednisolone has little place in the treatment of thyroiditis[6] and may indeed exacerbate the hypokalemia, further aggravating the clinical state.[7] Potassium supplements are effective in alleviating the hypokalemic symptoms but rebound hyperkalemia is often a problem once the patient is rendered euthyroid.[8] Beta-blockers such as labetolol or atenolol have been employed effectively in the acute hypokalemic phase

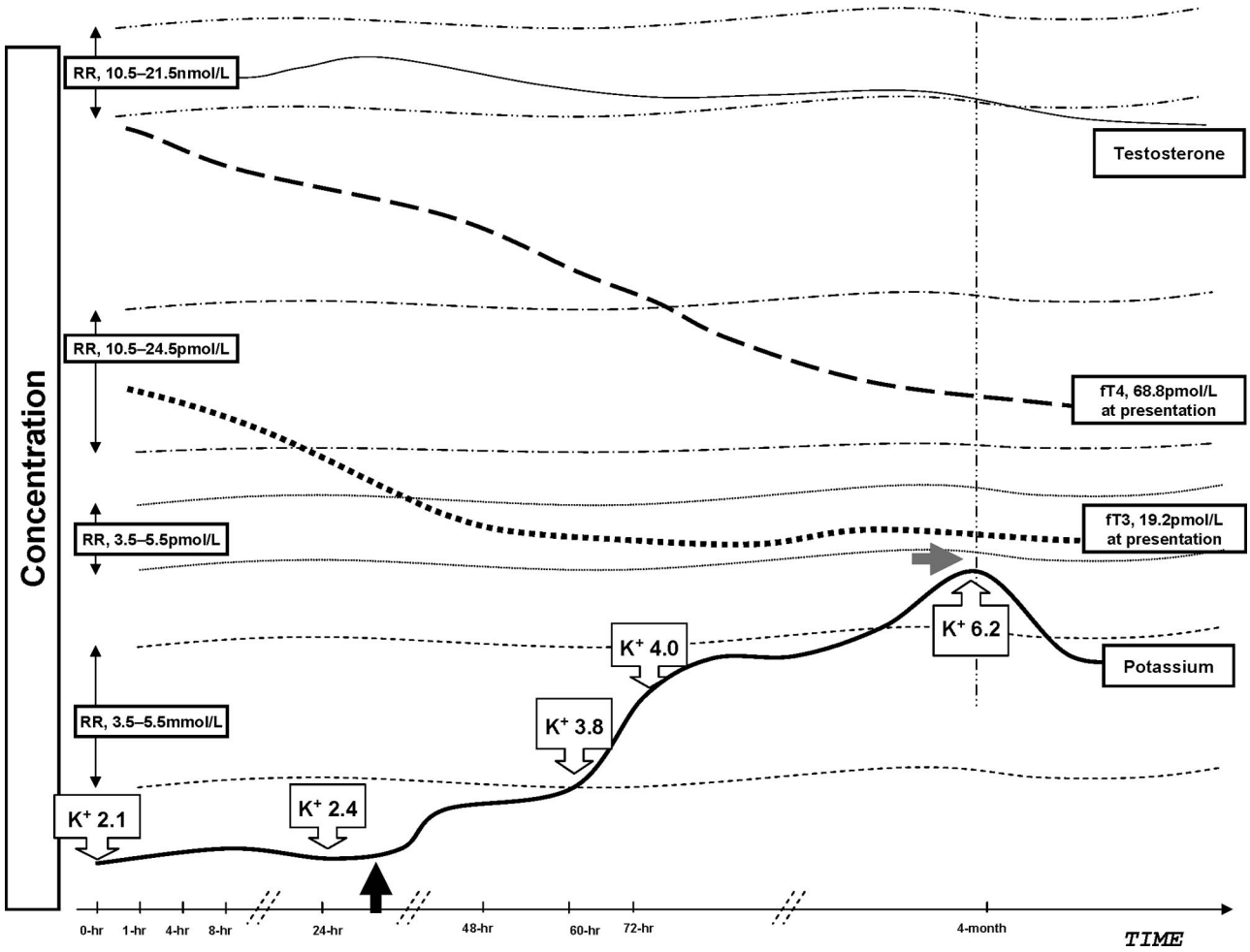

Figure 1. The temporal relationship between potassium concentrations and the critical hormones involved in the pathogenesis of hypokalaemic thyrotoxic periodic paralysis (HTPP). The black arrow indicates the introduction of spironolactone and the grey hyperkalaemia, approximately at the time of testosterone wearing off. Note: Both the axes are not to scale.

both to improve serum potassium concentration and overcome the adrenergic activity of the thyrotoxicosis without the rebound hyperkalemia.[9] Unfortunately, the patient did not tolerate high dose beta-blockade very well due to bradycardia and the rise in potassium level was only modest. In addition, beta-blockade may itself induce quadriparesis, further aggravating the situation.[10]

Hyperinsulinaemia is another factor that is thought to contribute to hypokalemia in this setting.[11] In patients with HTPP, there is a demonstrable increase in the expression of Na^+/K^+-ATPase on platelets and an increase in responsiveness to insulin. Insulin also activates and increases translocation of Na^+/K^+-ATPase pumps to skeletal muscle plasma membranes.[12,13] This is the likely explanation of the postprandial exacerbation of the condition, where there is an excessive and disproportionate increase of insulin to the carbohydrate load as demonstrated in the glucose tolerance test (Table 1). In addition, hepatitis C infection confers an insulin resistance state, further increasing the endogenous insulin concentration.[14,15] This would plausibly aggravate the hypokalemia with subsequent paralysis.

Testosterone is the 3rd factor thought to be contributing to the hypokalemia. Certainly, it has been shown to increase Na^+/K^+-ATPase activity in animal model.[16,17] Animal studies indicate that, at least in some tissues, Na^+/K^+-ATPase activity is stimulated by androgens[18,19] and inhibited by estrogen[20] or progesterone.[21,22] Androgen also synergises with thyroxine and accelerates the binding of beta-adrenergic receptor in animal models. In human skeletal muscle, exposure to androgen up-regulates many of the anabolic hormones such as insulin-like growth factor 1 and beta-catenin binding transcription factor, TCF-4.[23] Many of these genes are located on chromosome 1 where the gene for Na^+/K^+-ATPase pumps is also located. It is, thus, very plausible that the Na^+/K^+-ATPase protein increases in expression when exposed to androgen, hence contributing to the pathogenesis. This is highly consistent with the con-

sistent clinical observation that HTPP seems to occur in predominantly postpubertal males where androgen is clearly a determining factor. Although HTPP has been rarely described in genetic females, the exposure to male level of androgen may overcome the inhibitory effects of estrogen and progesterone mentioned above and precipitates the underlying elusive pathogenesis. Indeed, androgen continues to exert its influence on Na^+/K^+-ATPase activity even when thyrotoxicosis has been controlled. This was reflected by the continuing requirement for potassium supplementation. In addition to the stimulatory effects of testosterone, inhibition of Na^+/K^+-ATPase with the antiandrogen canrenoate, the active metabolite of spironolactone, has been demonstrated, but only in animal model.[24] On this basis and also to avert the risk of rebound hyperkalemia with potassium supplementation as testosterone effects wane, spinorolactone was employed. This was selected for its androgen receptor blocking effect and relative short half life of 10 to 12 hours (compared with cyproterone acetate or bicalutamide), allowing for easy manoeuvrability in situations of rebound hyperkalemia. The extra-advantage of using spironolactone is its antagonism of aldosterone, which has a clear stimulating effect on Na^+/K^+-ATPase activity and expression, especially in the collecting ducts.[25] In this case, spironolactone was effective in restoring and maintaining potassium level and blunting the androgenic effect of testosterone on the Na^+/K^+-ATPase pumps.

This report reinforces the currently understood pathophysiology of HTPP where the pivotal and regulating agent is Na^+/K^+-ATPase function and activity. Beside the normal contributing including hyperinsulinaemia (in the presence of hepatitis C infection) and hyperthyroidism (in biphasic thyroiditis), testosterone has been shown to clearly influence Na^+/K^+-ATPase activity. This unique clinical scenario further clarifies the pathogenesis of HTPP by applying a novel use of a commonly known medication in spironolactone. Although very effective in this case, it should be used as an adjunct to antithyroid medications and exclusively in postpubertal males.

References

1. **Tran HA, Kay SE, Kende M, et al.** Thyrotoxic hypokalaemic periodic paralysis in Australasian men. Intern Med J 2003;33:91–4.
2. **Cesur M, Gursoy A, Avcioglu U, et al.** Thyrotoxic hypokalemic periodic paralysis as the first manifestation of Interferon-alpha-induced Graves disease. J Clin Gastroenterol 2006;40:864–5.
3. **Kodali VR, Jeffcote B, Clague RB.** Sudden weakness in a young Chinese man. Post grad Med J 1997;73:829–930.
4. **Tran HA.** Hepatitis C infection: treatment regimen and thyroid function abnormalities. Endocrinologist 2007;17: 231–5.
5. **Kung AWC.** Thyrotoxic periodic paralysis: a diagnostic challenge. J Clin Endocrinol Metab 2006;91:2490–95.
6. **Minelli R, Valli MA, Secli CD, et al.** Is steroid therapy needed in the treatment of destructive thyrotoxicosis induced by alpha-Interferon in chronic hepatitis C? Horm Res 2005; 63:194–9.
7. **Liu Z, Braverman LE, Malabanan A.** Thyrotoxic periodic paralysis in a Hispanic man after the administration of prednisone. Endocr Pract 2006;12:427–31.
8. **Manoukian MA, Foote JA, Crapo LM.** Clinical and metabolic features of thyrotoxic periodic paralysis in 24 episodes. Arch Intern Med 1999;159:601–6.
9. **Lin S-H, Lin Y-F.** Propanolol rapidly reverses paralysis, hypokalaemia, and hypophosphataemia in thyrotoxic periodic paralysis. Am J Kidney Dis 2001;37:620–3.
10. **Izzedine H, Launay-vacher V, Hulot JS, et al.** Beta-blocker-induced quadriparesis. Ann Int Med 2004;141:62.
11. **Chan A, Shinde R, Chow CC, et al.** Hyperinsulinaemia and Na+-K+ATPase activity in thyrotoxic periodic paralysis. Clin Endocrinol (Oxf) 1994;41:213–6.
12. **Al-Khalili L, Yu M, Chibalin AV.** Na+/K+ATPase trafficking in skeletal muscle: insulin stimulates translocation of both alpha1- and alpha2-subunit isoforms. FEBS Lett 2003; 536:198–202.
13. **Al-Khalili L, Kotova O, Tschida H, et al.** ERK1/2 mediates insulin stimulation of Na, K-ATPase by phosphorylation of the alpha-subunit in human skeletal muscle cells. J Biol Chem 2004;279:25211–8.
14. **Lecube A, Genesca J, Hernandez C, et al.** Proinflammatory cytokines, insulin resistance, and insulin secretion in chronic hepatitis C patients. Diabetes Care 2006;29:1096–101.
15. **Narita R, Abe S, Kihara Y, et al.** Insulin resistance and insulin secretion in chronic hepatitis C virus infection. J Hepatol 2004;41:132–8.
16. **Azzarolo AM, Mircheff AK, Kaswan RL, et al.** Androgen support of lacrimal gland function. Endocrine 1997;6:39–45.
17. **Farnsworth WE.** Androgen regulation of prostatic membrane ATPase. Biol Reprod 1970;3:218–22.
18. **Fraser CL, Sarnacki P.** Na+-K+ATPase pump function in rat brain synaptosomes in different in males and females. Am J Physiol 1989;257:E284–9.
19. **Guerra M, Rodriguez del Castillo A, Battaner E, et al.** Androgen stimulate preoptic area Na+-K+ATPase activity in male rates. Neurosci Lett 1987;78:97–100.
20. **Davis RA, Kern F, Showalter R, et al.** Alterations of hepatic Na+-K+ATPase and bile flow by estrogen: effects on liver surface membrane lipid structure and function. Proc Natl Acad Sci U S A 1978;75:4130–4.
21. **Lijnen PD, Groeseneken L, Lommelen L, et al.** In vitro inhibition of renal sodium-potassium ATPase activity by progesterone. Methods Find Exp Clin Pharmacol 1985;7:347–9.
22. **Temma K, Ng YC, Brody TM, et al.** Effects of progesterone derivatives on sodium pump activity and force of myocardial contraction in isolated guinea pig heart. Res Commun Chem Pathol 1983;41:51–63.
23. **Montano M, Flanagan JN, Jiang L, et al.** Transcriptional profiling of testosterone-regulated genes in the skeletal muscle of human immunodeficiency virus-infected men experiencing weight loss. J Clin Endocrinol Metab 2007;92:2793–802.
24. **Miner PB Jr, Sneller M, Crawford SS.** Spironolactone- and canrenone-induced changes in hepatic Na/K-ATPase activity, surface membrane cholesterol and phospholipids, and fluorescence polarization in the rat. Hepatology 1983; 3:481–8.
25. **Feraille E, Doucet A.** Sodium-potassium-adenosinetriphosphatase-dependent sodium transport in the kidney: hormonal control. Physiol Rev 2001;81:345–418.

(B) GRAVES' OPHTHALMOPATHY

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Case report

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The influence of hepatitis C infection and interferon-α therapy on thyrotropin blocking and stimulating autoantibodies in Graves' ophthalmopathy: a case report

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Abstract

Background: Hepatitis C virus is a highly immunogenic pathogen often inducing autoimmune activation changes and this can often be further exacerbated by Interferon therapy. As HCV is lymphocytotropic, it can modulate T cell and B cell antibody responses, affecting many endocrine organs, most commonly the thyroid.

Case presentation: We hereby describe a case of fluctuating and wavering thyrotropin autoantibodies of both stimulating and blocking nature in the setting of Graves's ophthalmopathy, hepatitis C infection and interferon-α, causing hypo- and subsequently hyper-thyroidism. The autoantibody profile was clearly modified during interferon therapy and settled into a new equilibrium at the completion of treatment.

Conclusion: The case highlights the possible existence of a dual thyroid autoantibody population associated with hepatitis C, and its modulation by interferon therapy, which further compounds the difficulties in the assessment thyroid disease in this setting.

Background

Hepatitis C virus is a highly immunogenic pathogen often inducing autoimmune activation changes and this can often be further exacerbated by interferon therapy. As HCV is lymphocytotropic, it can modulate T cell and B cell antibody responses, affecting many endocrine organs, most commonly the thyroid. As a result of this modulating effect, the thyroid autoantibody profile can be severely affected, especially in the setting of Graves' disease superimposed with hepatitis C infection and interferon-based treatment. The following case report illustrates this phenomenon with fluctuating thyrotropin autoantibodies of both stimulating and blocking nature during interferon therapy. It is the existence of these changing antibodies that compounds the difficulties of assessing thyroid disease in this setting.

Case Presentation

A 44 year-old Caucasian man with chronic hepatitis C infection and known, long-standing primary hypothyroidism presented with recent onset Graves' ophthalmopathy (GO). There was no other medical problem and the patient had otherwise been well. His hypothyroidism was diagnosed 10 years prior, approximately at the same time

as his hepatitis C. At that time, the thyrotropin (TSH) was found to be 34 IU/L, free tetra-iodothyronine (fT4) 10.2 and free tri-iodothyronine (fT3) 3.2 pmol/L. He was started on thyroxine therapy with subsequent satisfactory control. No information on autoantibodies or imaging was available from this presentation.

Clinical examination showed a tattooed man in euthyroidism. His ophthalmopathy was graded as moderate-to-severe including >3 mm lid retraction and marked congestion with a clinical activity score (CAS) of 5/7 [1]. Visual acuities were 6/6 bilaterally. No goitre, dermatopathy or acanthosis nigricans was detected. His liver span was normal at 11 cm and there was no evidence of chronic liver disease, ascites or portal hypertension. His thyroid ultrasound scan showed 2 small nodules but was otherwise normal in volume. His thyroid pertechnetate uptake scan was reduced at 1% (reference range (RR), 3-8%) whilst on 150 μg of thyroxine daily, at which time his TSH was 1.98 (RR, 0.4-4.0 mU/L) and fT4 21.5 (RR, 10.2-24.5 pmol/L). His antithyroperoxidase and antithyroglobulin antibodies were undetectable. The thyrotropin receptor antibody (TRAb) was 4.0 IU/L (reference interval, < 1.0 IU/L). Other routine laboratory tests were normal including aspartate and alanine aminotransferase activity. His baseline viral load was 6.08 log IU/mL. His ocular magnetic resonance imaging (MRI) supported the diagnosis of GO (Figure 1).

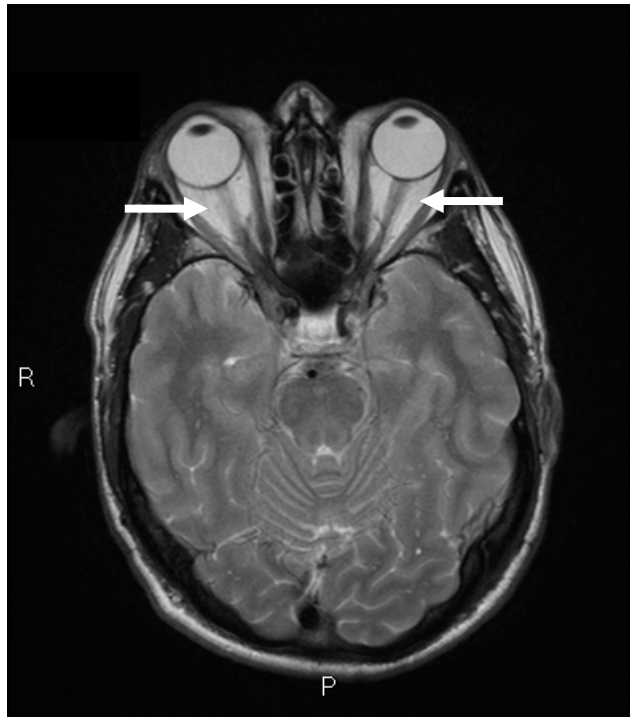

Figure 1
Magnetic Resonance Imaging of the orbits, showing congestion of the retro-orbital space and enlargement of the extraocular muscles *(arrows)*, consistent with the diagnosis of Graves' Ophthalmopathy.

Due to the diametrically opposite clinical findings of hypothyroidism and GO, further bioassays were performed to assess antibodies affecting the function of the TSH receptors. The thyrotropin stimulating antibody (TSAb) was 192 (RR, <180%) and thyrotropin blocking antibody (TBAb) 124 (RR, <40%), suggesting that the mechanism for his hypothyroidism was immune mediated with TSH blocking activity.

Progress

Due to the presence of hypothyroidism and the potential additional effect of interferon on thyroid tissue, hepatitis C treatment was carefully started. Because of his hepatitis C genotype 2, liver biopsy was deemed unnecessary and thus was not performed [2]. Treatment then included combination interferon-α and ribavirin for 24 weeks. The ophthalmopathy did not worsen and was managed conservatively with liquid film eye drops and protective glasses. The CAS remained unchanged. Both his eye and thyroid status was monitored and closely reviewed every month. On the 8th week, his TSH declined, necessitating a reduction in thyroxine dosage. At 16 week, he was found to be biochemically thyrotoxic with suppressed TSH, fT4 of 28.9 and fT3 of 6.9 pmol/L at which time his thyroxine was ceased. The TSH stimulating activity increased whilst blocking activity declined. His TSAb rose further whilst blocking activity declined. The evolution of the antibody profile is summarised in Figure 2. The thyroid uptake scan evolved to show a diffuse and increased in uptake at 12%. The potential and aggravating effects of elevated TSAb titres on the ophthalmopathy were duly considered but observation was continued (*see below*).

As the patient was asymptomatic, his thyroid condition was also observed closely. His serial fT4 and fT3 levels remained high but unaltered throughout the course of therapy (Figure 2). Four weeks after the completion of therapy, his TSH was 0.05 mIU/L, fT4 21.5 and fT3 4.9 pmol/L. At 12 weeks, his level had become progressively hypothyroid with TSH of 5.9 mIU/L and fT4 17.9 pmol/L. At the subsequent 6, 12, 24 and 36 months follow-ups, his TSH levels ranged between 5.3 and 6.4 mIU/L. His viral load was undetectable at the 6-month follow-up consistent with sustained virologic response.

Methods

TRAb assay was measured with the TRAK LUMI test (B.R.A.H.M.S.AG, Hennigsdorf/Berlin, Germany). A TRAb level of <1.0 IU/L is considered negative and > 1.5 as conclusively positive. TSAb and TBAb bioassays were meas-

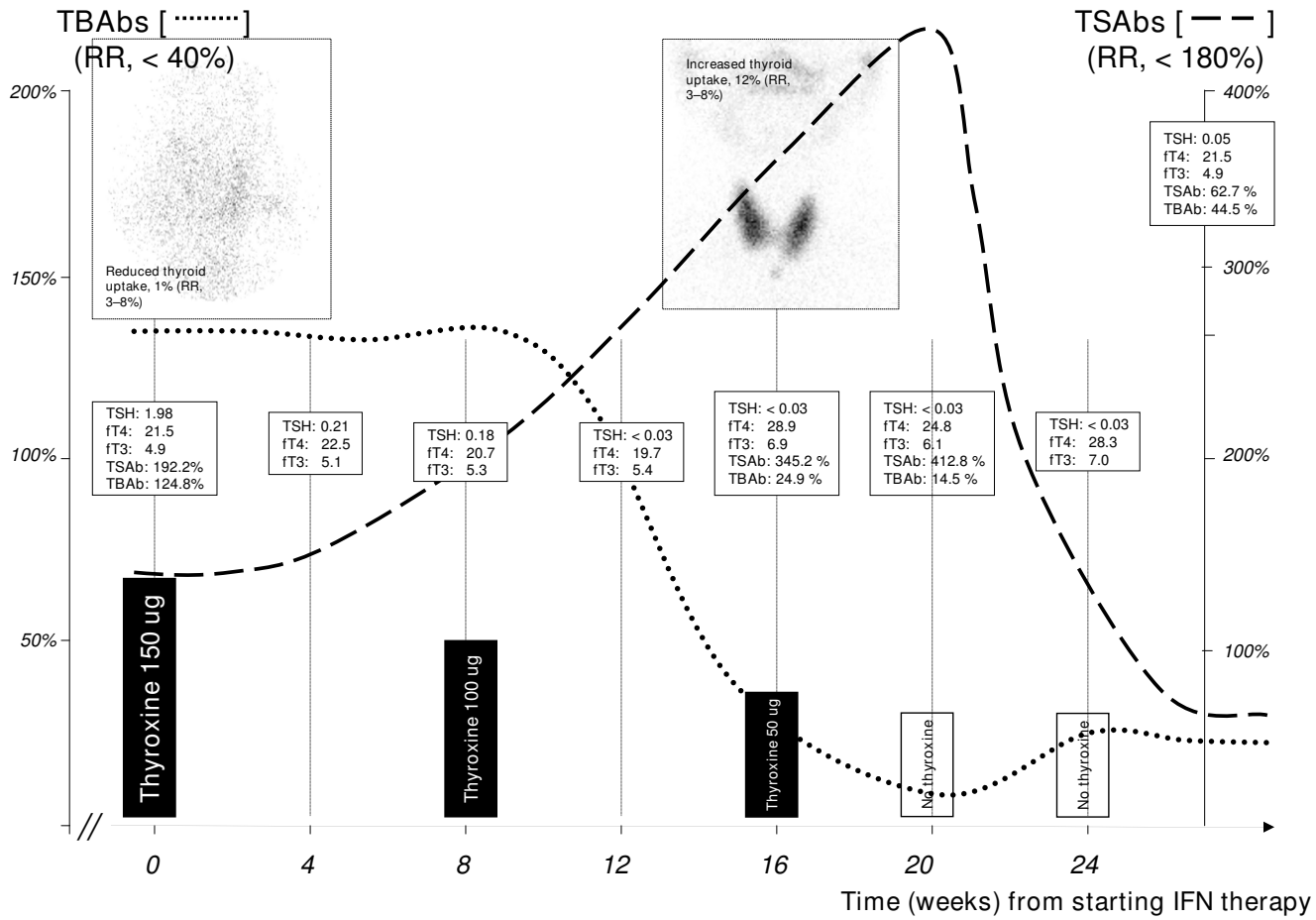

Figure 2
The immunomodulating effect of Interferon therapy on the TS and TB subsets of autoantibodies and its effects on thyroxine dosage and nuclear uptake patterns. *Note*: the graphs are only approximate representations, not to exact scale.

ured as previously described [3,4]. Generally, the detection was carried out in low salt conditioning using JPO9 Chinese hampster ovary cells transfected with the human TSH-R. Cyclic AMP was measured in a commercial RIA (Amersham, Aylesbury, UK). Thyroid stimulation index (SI) was calculated as: SI (percent) = 100 × (cAMP patient/cAMP euthyroid control). For TBAb detection, bovine TSH (1 mU/mL; Sigma, St. Louis, MO) was added with either euthyroid control or test serum. The inhibition index (InI) was calculated as: InI (percent) = 100 × [1 - (counts per minute patient/counts per minute euthyroid control)].

Third generation serum thyrotropin (TSH), serum free tetra- and free tri-iodothyronine (fT4 and fT3) were determined by two-site sandwich immunoassay using an automated chemiluminescent system (Diagnostic Products Corporation, Immulite 2000). The reference range (RR) for TSH was 0.4-4.0 mU/L, fT4 10.0-26.0 and fT3 3.5-5.5 pmol/L. The coefficients of variations (CV) were 5.0% and 5.1% at TSH concentrations of 4.0 mU/L and 10.0 mU/L respectively. For fT4, the CV was 6.5% at 10.0 pmol/L and fT3 8.9% at 3.5 pmol/L.

Serum autoantibodies to thyroglobulin and thyroperoxidase were measured by agglutination (Serodia-ATG and Serodia-AMC, Fujirebio, Inc., Tokyo, Japan). Titres of less than 1:400 were considered normal for both.

Discussion

Although the co-existence of dual thyroid TSAb and TBAb has been well documented [5], its pattern in the presence of hepatitis C and its modulation by interferon treatment has rarely been reported previously [6]. The present case highlights the population of thyroid autoantibodies whose evolution is significantly influenced in the pres-

ence of hepatitis C and interferon. It was highly likely that the initial diagnosis of hypothyroidism was the result of TSH blocking activity resulting in the biochemical expression of hypothyroidism. In the absence of further information at the time of diagnosis, it was hard to confirm this hypothesis. However, in favour of this diagnosis was the absent/negligible nuclear uptake in the thyroid scan, the presence of a normal-size thyroid on ultrasound and the absence of any thyroid auto-antibodies. Although excessive thyroxine can affect thyroid uptake scan appearance, in the presence of a normal (non-suppressed) TSH level, it is very likely that the uptake scan reflects the activity of TBAbs.

The development of GO is a fascinating feature that must closely involve the presence of TRAb. Although the pathogenesis of GO remains undetermined, TSAb is one of the major contributors in inducing the inflammatory process in the orbital fat and ocular muscles resulting in swelling and congestion of the orbit [7]. In addition, recent case reports suggested an association between hepatitis C infection and GO [8,9]. Hypothetically, prior to the development of GO, there must have existed an equilibrium between these two sub-classes of thyroid antibodies, albeit unbalanced in favour of TBAb, just sufficient to elicit the hypothyroidism but with enough stimulating activity to participate in the development of GO. In the presence of interferon as an immuno-modulator, the equilibrium then shifted in favour of TSAb and hence the progressive thyrotoxic biochemical profile with marked nuclear uptake. It was conceivable but unlikely that this shift was spontaneous and coincidental because his thyroid status was stable in the intervals following interferon therapy. This would have been further bolstered if his thyroid parameters were available in the period preceding hepatitis C treatment. These changes appeared permanent as the hypothyroidism moderated compared to before treatment. Despite the mildly elevated TSH level, no thyroxine was required 3 years after the completion of interferon therapy. This is underlined by the newly equilibrated antibody profile at the end of treatment, with both TS- and TB-Ab subclass activities being normal. These were not performed further in the convalescing period. Interestingly, the relatively higher TS-Ab activity did not further compound the ophthalmopathy.

The management of GO in this case was both challenging and difficult, involving plentiful of discussion with the patient and his spouse. As mentioned, the addition of interferon may aggravate the GO which in turn may be further exacerbated by the evolving TSAb, potentially precipitating an ophthalmic crisis and loss of vision [9]. On the other hand, independent treatment with immunosuppressants such as glucocorticoid, calcineurin inhibiting agents or methotrexate can potentially lead to fulminant hepatic necrosis and failure. After much deliberation and risk estimation, the GO was monitored closely with visual acuities and color charts weekly, tapering to monthly as the condition remained progressively stable. Although the natural history of this condition is unknown in this setting and to be safe, orbital irradiation and decompressive surgery were also consulted and made readily available. Fortunately, his ophthalmic condition did not deteriorate.

The underlying pathogenesis of this swinging antibody profile is unknown. Although the prevalence of hypothyroidism in the setting of hepatitis C and interferon is not uncommon, this rare type of hypothyroidism often goes unsuspected unless there are other indicators such as ophthalmopathy. The antibodies switching to the TSH receptors must be modulated, evolving from blocking to predominantly stimulating. It is both a relief and fascination that GO failed to progress as TSAb has been suggested to initiate and stimulate orbital adipogenesis [10]. Recent studies were able to further investigate the inhibitory and stimulatory nature of these antibodies. In fact monoclonal antibodies with both stimulating and block activities were recently developed. Plausibly the relevant B cells are selected to alter the variable regions of the immunoglobulins to specifically affect the critical areas for TSH receptors stimulation and blockade, particularly region M22 and 5C9 of the TSH autoantibodies [11,12]. It must be noted however that the switching between hyper- and hypothyroid phases of Graves' disease can occur independent of hepatitis C infection and interferon therapy [13]

In HCV infection, there is also an increased secretion of IFN-γ and chemokine ligand 10 (CXCL10) generally as well as by thyrocytes [14]. The high CXCL10 level is higher in patients who develop thyroid disease, especially hypothyroidism in the setting of hepatitis C. It is possible that the endo-, exo-genous interferon and CXCL 10 combine to modify the immune response. The elevated CXCL10 level has also been implicated in GO recently [15]. These factors may add to the aforementioned mechanisms to result in the complex immuno-chemical cascade representative of this case. This is purely speculative however and this observed immune phenomenon remains poorly understood. It adds to the spectrum of thyroid diseases in the presence of hepatitis C and in particular its modulation by interferon therapy [16].

Conclusion

This case highlights another fascinating and complex interaction between the thyroid and interferon therapy in hepatitis C infection. Although highly unusual, hypothyroidism potentially due to TBAb should be thoroughly considered in this setting.

Consent

Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Both authors contribute equally and substantially to, and approved the final version of the manuscript.

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References

1. Bartalena L, Baldeschi L, Dickinson AJ, Eckstein A, Kendall-Taylor P, Marcocci C, Mourits MP, Perros P, Boboridis K, Boschi A, Currò N, Daumerie C, Kahaly CJ, Krassas G, Lane CM, Lazarus JH, Marinò M, Nardi M, Neoh C, Orgiazzi J, Pearce S, Pinchera A, Pitz S, Salvi M, Sivelli P, Stahl M, von Arx G, Wiersinga WM: **Consensus statement of the European Group on Graves' Orbitopathy (EUGOGO) on management of GO.** *Eur J Endocrinol* 2008, **158:**273-285.
2. Dienstag JL, McHutchinson JG: **American gastroenterological association medical position statement on the management of hepatitis C.** *Gastroenterol* 2006, **130:**225-230.
3. Morgenthaler NG, Pampel I, Aust G, Seissler J, Scherbaum WA: **Application of bioassay with CHO cells for the routine detection of stimulating and blocking autoantibodies to the TSH-receptor.** *Horm Metab Res* 1998, **30:**162-168.
4. Ajjan RA, Weetman AP: **Techniques to quantify TSH receptor antibodies.** *Nature Clin Pract Endocrinol Metab* 2008, **4:**461-468.
5. Rapoport B, Chazenbalk GD, Jaume JC, McLachlan SM: **The thyrotropin (TSH) receptor: interaction with TSH and autoantibodies.** *Endocr Rev* 1998, **19:**673-716.
6. Chen F-Q, Okamura K, Sato K, Kuroda T, Mizokami T, Fujikawa M, Tsuji H, Okamura S, Fujishima M: **Reversible primary hypothyroidism with blocking or stimulating type TSH binding inhibitor immunoglobulin following recombinant interferon-alpha therapy in patients with pre-existing thyroid disorders.** *Clin Endocrinol* 1996, **45:**207-214.
7. Khoo TK, Bahn RS: **Pathogenesis of Graves' ophthalmopathy: The role of autoantibodies.** *Thyroid* 2007, **17:**1013-1018.
8. Villanueva RB, Brau N: **Graves' ophthalmopathy associated with interferon-alpha treatment for hepatitis C.** *Thyroid* 2002, **12:**737-738.
9. DeMartelaere SL, Green KM, Shore JW: **Exacerbation of Graves' ophthalmopathy with interferon-alpha therapy.** *Ophthal Plast Reconstrr Surg* 2007, **23:**319-321.
10. Goh SY, Ho SC, Seah LL, Fong KS, Khoo DHC: **Thyroid autoantibody profiles in ophthalmic dominant and thyroid dominant Graves' disease differ and suggest ophthalmopathy is a multiantigenic disease.** *Clin Endocrinol* 2004, **60:**600-607.
11. Sanders J, Jeffreys J, Depraetere H, Evans M, Richards T, Kiddie A, Brereton K, Premawardhana LD, Chiradze DY, Nunez Miguel R, Blundell TL, Furmaniak J, Rees Smith B: **Chracteristics of a human monocloncal autoantibody to the thyrotropin receptor: sequence structure and function.** *Thyroid* 2004, **14:**560-570.
12. Sanders J, Evans M, Betterle C, Sanders P, Bhardwaja A, Young S, Roberts E, Wilmot J, Richards T, Kiddie A, Small K, Platt H, Summerhayes S, Harries R, Reeve M, Coco G, Zanchetta G, Chen S, Furmaniak J, Rees Smith B: **A human monoclonal autoantibody to the thyrotropin receptor with thyroid-stimulating blocking activity.** *Thyroid* 2008, **18:**735-746.
13. Kim WB, Chung HK, Park YJ, Park DJ, Tahara K, Kohn LD, Cho BY: **The prevalence and clinical significance of blocking thyrotropin receptor antibodies in untreated hyperthyroid Graves' disease.** *Thyroid* 2000, **10:**579-586.
14. Antonelli A, Rotondi M, Fallahi P, Romagnani P, Ferrari SM, Buonamano A, Ferrannini E, Serio M: **High levels of circulating CX chemokine ligand 10 are associated with chronic autoimmune thyroiditis and hypothyroidism.** *J Clin Endocrinol Metab* 2004, **89:**5496-5499.
15. Antonelli A, Rotondi M, Ferrar SM, Fallahi P, Romagnani P, Francheschini S, Serio M, Ferrannini E: **Interferon-gamma-inducible alpha-chemokine CXCL10 involvement in Graves' ophthalmopathy: modulation by peroxisome proliferator-activated receptor-gamma agonists.** *J Clin Endocrinol Metab* 2006, **91:**614-620.
16. Tran HA: **Hepatitis C infection, treatment regimens, and thyroid function abnormalities.** *The Endocrinologist* 2007, **17:**231-235.

(C) ADRENAL DISEASE

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Exacerbation of hepatitis C induced subclinical hypoadrenalism by Interferon-alpha2beta: A case report

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Abstract

Adrenal disease is an uncommon manifestation of hepatitis C infection and its related treatment regimen. This is a case of subclinical hypoadrenalism, probably induced by hepatitis C infection and further exacerbated by interferon-α2β and Ribavirin therapy. The adrenal deterioration during the treatment course was observed closely with 24-hour salivary profiles and 250 μg adrenocorticotropin stimulation tests using parallel serum and salivary cortisol concentrations. A number of possible pathogenic mechanisms are discussed, and the controversy over its management is emphasized.

Case presentation

A 55 year old post menopausal Caucasian female presented with vitiligo on her face, arms and legs. Her past medical history included only mild asthma requiring only intermittent bronchodilators without glucocorticoids. There was no other significant personal or family medical history. Clinical examination showed a well woman, weight of 69.1 kg, height 1.67 metre (body mass index ~25). Her blood pressure was 120/75 lying and 120/70 sitting with a regular pulse of 78 beats per minute. There were 3 vitiligo patches each measuring approximately 3 × 5 cm on her forehead, anterior abdomen and left cubital fossa. No goitre or liver enlargement was detected. Biochemical investigations are as follow: sodium 130 mmol/L (reference range (RR), 136–146), potassium 5.4 mmol/L (RR, 3.5–5.5), chloride 99 mmol/L (RR, 98–108), bicarbonate 21 mmol/L (RR, 24–30), urea 9.1 mmol/L (3.0–7.0), creatinine 90 umol/L (RR, 40–90), bilirubin 18 μmol/L (6–10), alanine aminotransferase 105 IU/L (RR, < 50), aspartate aminotransferase 56 IU/L (RR, < 45), alkaline phosphatase 136 IU/L (RR, < 110), γ-glutamyl transferase 40 IU/L (< 60), albumin 33 g/L (RR, 35–45), plasma aldosterone 172 pmol/L (RR, 80–1040), plasma renin activity > 35.7 ng/mL/hr (RR, 1.2–2.8). In essence, they showed mild hyperkalaemic metabolic acidosis and hepatocellular dysfunction. Because the latter persisted, chronic hepatitis C was confirmed with positive serology of genotype 1. The liver biopsy showed changes consistent with chronic persistent hepatitis, the inflammatory and fibrotic changes were both graded 1 according to the scoring method [1]. Together with additional biochemical and immunological studies, other causes of persistent abnormal liver function tests were excluded. In view of her vitiligo, hepatitis C and biochemical disturbance, the Adrenocorticotropin (ACTH) stimulation test (AST), also known as the Cosyntropin or Short Synacthen test, was performed which revealed the presence of subclinical adrenal insufficiency (AI), additional file 1. Further inves-

tigations for the causes of AI revealed positive adrenal cell antibody (ACA) serology. Computerised tomographic scan showed small adrenal glands bilaterally with normal anatomy and appearance.

The patient underwent routine combination treatment of Interferon-α2β (IFN) and Ribavirin (RBV) for 48 weeks for her genotype 1 HCV infection. The patient's subclinical adrenal disease was also followed closely using salivary as well as serum cortisol levels to assess the hypothalamo-pituitary-adrenal axis (HPA) every 12 weeks, starting at baseline, during treatment, 6 and 12 month follow-ups. The results suggested a progressive decline of her adrenal function during the treatment phase. Glucocorticoid replacement therapy was seriously considered but with apprehension due to possible exacerbation of the hepatitis. The risks were discussed in detail with the patient and her family and it was decided to continue with anti-viral therapy but without glucocorticoid supplement. The patient and her husband were counselled comprehensively regarding the emergency management of Addisonian crisis, provided with a carrying note and required to wear an alert bracelet. Other immediate family members were also involved in her management plan, with her consent. Her management plan was also forwarded to the local hospital Emergency Department and Hepatitis C Service. Fortunately, there was no crisis other than the common side effects of the treatment regimen. Her pattern of steroid profile and ACA returned to its pre-existing state and remained unchanged at 6 and 12 month follow-up after the cessation of therapy. The patient has remained well since but her subclinical AI persists.

Discussion

Hepatitis C is well-documented to be associated with many auto-immune endocrinopathies, especially thyroid but also including the hypothalamus, pituitary, renal organs [2,3]. This is the first case to explore the challenge of subclinical AI and the influence of interferon treatment upon it. In the presence of positive ACA, it was very probable that AI is the result of HCV infection and its immunomodulating effect on adrenal tissue, as most alternative causes are not apparent.

It was interesting that patient was found to have subclinical AI in which the diagnosis was confirmed by the failure to stimulate an adequate cortisol rise after 60 minutes of Cosyntropin stimulation even though the baseline levels were satisfactory throughout. In healthy subjects, there must be an absolute increase of 300 nmol/L or more at the 60 minute sample [4]. Another definition of non-responders has been suggested to be less than a 20% increase compared with baseline value [5], directly relevant to this patient. Additionally, the elevated ACTH and renin levels and reduced aldosterone/renin ratio conclusively indicated adrenal cortical failure. Various stages of progressive adrenal failure have been described but this case clearly does not fit into any of the prescribed categories [6]. According to this proposal, our patient belongs in the '*Symptomatic Under Stress*' category in which she satisfied all criteria except that her baseline cortisol was normal rather less than 83 nmol/L.

During IFN and RBV therapy, there was a steady and gradual decline in baseline salivary and serum cortisol levels prior to ASTs. Serum ACTH levels increased slightly throughout the treatment course and returned to pre-treatment level after. The failure of cortisol levels to rise at 60 minutes is consistent in both saliva and serum throughout the 48 weeks of treatment. There was also a significant decrease in cortisol levels during therapy (additional file 1). At 12 months following the end of IFN therapy, the baseline cortisol levels returned to their pre-treatment levels.

The longitudinal 24-hour salivary cortisol profile throughout the course of treatment is rewarding and revealing. The normal physiological diurnal pattern has been lost with the deletion of the second late-afternoon cortisol peak. Although the first morning peak was retained, it was significantly attenuated to a mean of 6.2 nmol/L followed by a gradual decline to an undetectable nadir at midnight. The effect and influence of IFN on the 24-hour salivary profile also paralleled that observed in serum. There was a distinct difference in salivary cortisol concentrations during therapy in comparison to levels before and after. Following the completion of therapy, adrenal function recovered and returned to pre-treatment level, i.e. subclinical hypoadrenalism where the second afternoon cortisol peak was lost (additional file 1, Figure 1). In this case, salivary cortisol levels appear to be an additionally useful tool in the assessment of HPA axis. In ASTs, it compares satisfactorily with serum cortisol in detecting AI. Although AI is best assessed by serum cortisol dynamics, this report strongly suggests that salivary concentration dynamics are a reliable and dependable tool in the diagnosis of AI. It is additionally convenient for patients, especially in the ambulatory setting. This assay is not widely and routinely available however. The loss of diurnal variation in salivary concentration also remains to be validated but is also indicative of AI. Our testing frequency may not have detected the second diurnal peak which may occur anywhere between 15:00 and 18:00 hours. Frequent samplings may be more revealing but at the risk of non-compliance. The absolute morning salivary cortisol concentrations of less than 3.8 nmol/L is a good indicator of (impending) adrenal failure [7,8]. This level declined steadily following IFN therapy indicating a continuing decrease of adrenal reserve where it is

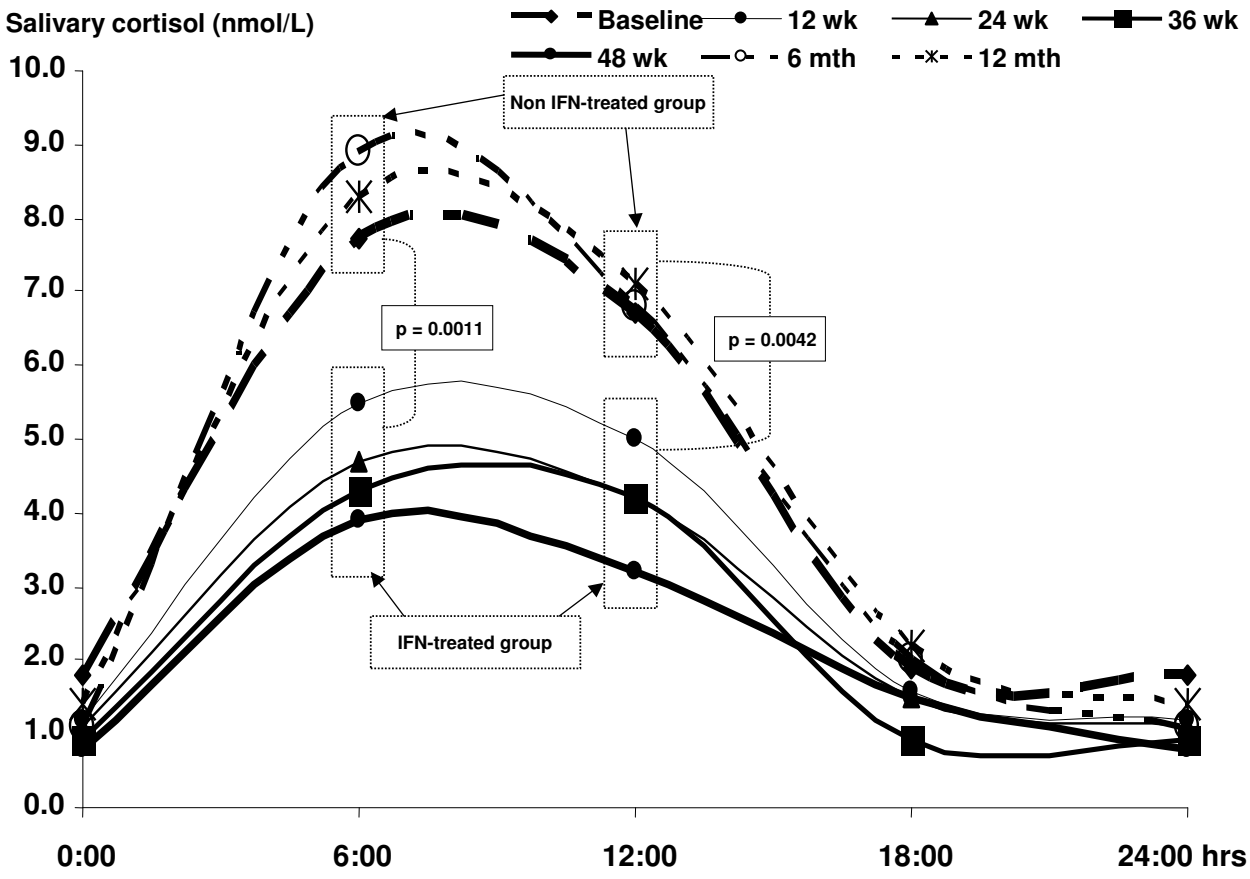

Figure 1
Twenty-four hour salivary cortisol profiles *before, during and after* the patient underwent combination Interferon-$\alpha 2\beta$ and Ribavirin therapy. There is a significant difference between the IFN-treated vs. non-IFN treated groups.

maximally stimulated by ACTH as indicated by the ACTH failure to rise further with progressive hypoadrenalism.

The ACA titres peaked at the 48th week. Thereafter, it returned to pre-treatment titre, paralleling the cortisol profiles.

The pathogenesis of HCV associated adrenal disease remains unknown and the molecular basis by which this occurs is undelineated. Normal CT imaging excluded anatomical causes such as haemorrhage, disseminated carcinoma or rarely antiphospholipid syndrome [9]. In autoimmune adrenal disease, it is thought that there must be an antigen presented on the adrenal cortical cells to stimulate the immune response. This response includes both T and B cells although the major determinant is in the former. The immune system is often stimulated by HCV infection, further amplified by IFN therapy. This is reflected by the positive but low titre of ACA at baseline, peaking at the nadir of adrenal function. This observation is consistent with a previous report [10] although our case additionally details the effect of the rise in ACA titre on cortisol metabolism. It is possible that IFN directly inhibits the synthetic function of adrenal cells and the swinging cortisol dynamics before and during treatment represents a balance between the inhibiting effect of IFN and the trophic effect of ACTH. Alternatively, the IFN may act by stimulating the T cells to destroy more adrenal cortical cells. ACTH was almost at its peak level pre-treatment and consequently failed to rise adequately to counter the adrenalytic effect of IFN. Other case report suggested an increase in cortisol consumption in AI proper [11]. Furthermore, the natural history of adrenalitis is unknown including its reversibility, the influence of IFN therapy

and response to glucocorticoid supplement. Reversible autoimmune adrenalitis treated with high dose glucocorticoid associated with Graves' disease was previously described but this was distinctly different from our case [12]. The ACA titres parallel the dynamic of cortisol levels, peaking at the nadir of adrenal function although the absolute titre is modest. Nevertheless, this observation lends strong evidence to the immunopathogenesis of this condition.

Although the entity of subclinical AI has been well described [13], its management remains controversial. In *de novo* autoimmune situation, its progress depends on a number of factors such as ACA titres, the degree of adrenal gland failure, age and gender of the patient [14,15]. In the presence of hepatitis C infection, the progress is unknown although it can be surmised that the risk is higher due to the presence of ACA antibodies and subsequent interferon therapy. It is debatable as to the true 'subclinical' state of the condition given the presence of vitiligo. The patient however, had no other adrenal related symptoms although admittedly in the presence of HCV and IFN therapy, the clinical symptoms of adrenal failure can be very hard to elicit.

It was both tempting to treat this patient with replacement hydrocortisone given the biochemical results and the difficulties in separating adrenal failure symptoms and treatment related side effects. The latter made it very difficult to determine the active component of the symptomatology due to adrenal insufficiency. Treatment with supraphysiological corticosteroid has been reported to reverse the condition in a similar clinical scenario but in the absence of HCV infection [12]. Physiological replacement, which was non-immunosuppresive, was not required as the patient has adequate baseline cortisol, but emergency supraphysiological supplement was made available in the contingency plan.

Conclusion

This case explores the immunopathogenic effect of hepatitis C virus and IFN therapy on the adrenal cortex. It is made more interesting by the presence of incidental and subclinical AI. Whilst clinically silent, the clinical scenario allows for the exploration of the use of salivary cortisol levels which appears to be a useful tool in detecting AI, either in a AST or a 24-hour profile.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

HAT provided the clinical case, consented the patient, conceived the study, participated in its design, assisted with data collection and statistical analysis, and coordinated and helped to draft the manuscript. SS and RJL analysed, provided the data, and participated in the discussion and drafting of the manuscript. GEMR contributed to the statistical and statistical analysis, participated in the discussion and drafting of the manuscript. All authors read and approved the final revised manuscript.

Consent

Written informed consent was obtained from the patient for publication of this case report and biochemical data. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Additional material

Additional file 1

Table 1. Salivary and plasma cortisol, ACTH and ACA levels at various time points of IFN-based therapy
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[http://www.biomedcentral.com/content/supplementary/1757-1626-1-157-S1.doc]

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References

1. Batts KP, Ludwig J: **Chronic hepatitis.** *Am J Surg Pathol* 1995, **19:**1409-17.
2. Tran HA: **Hepatitis C infection, treatment regimens, and thyroid function abnormalities.** *The Endocrinologist* 2007, **17:**231-235.
3. Tebben PJ, Atkinson JLD, Scheithauer BW, Erickson D: **Granulomatous adenohypophysitis after interferon and ribavirin therapy.** *Endocr Pract* 2007, **13:**169-175.
4. Dorin RI, Qualls CR, Crapo LM: **Diagnosis of adrenal insufficiency.** *Ann Intern Med* 2003, **139:**194-204.
5. Boscaro M, Betterle C, Sonino N, Volpato M: **Early adrenal hypofunction in patients with organ-specific autoantibodies and no clinical adrenal insufficiency.** *J Clin Endocrinol Metab* 1994, **79:**452-455.
6. Eisenbarth GS, Gottlieb PA: **Autoimmune polyendocrine syndromes.** *N Eng J Med* 2004, **350:**2068-2079.
7. Patel RS, Shaw SR, McIntyre HE, McGarry GW, Wallace MA: **Morning salivary cortisol versus Synacthen test as a test of adrenal suppression.** *Ann Clin Biochem* 2004, **41:**408-410.
8. Restituto P, Galofre JC, Gil MJ, Mugueta C, Santos S, Monreal JI, Varo N: **Advantage of salivary cortisol measurements in the diagnosis of Glucocorticoid Related Disorders.** *Clinical Biochemistry* 2008.
9. Balderramo DC, Garcia O, Colmenero J, Espinosa G, Forns X, Gines P: **Antiphospholipid syndrome during pegylated interferon alpha-2a therapy for chronic hepatitis C.** *Dig Liver Dis* in press. 2008 Jan 31
10. Wesche B, Jaeckel E, Trautwein C, Wedemeyer H, Falorni A, Frank H, Muhlen A von zur, Manns M-P, Brabant G: **Induction of autoantibodies to the adrenal cortex and pancreatic islet cells by interferon alpha therapy for chronic hepatitis C.** *Gut* 2001, **48:**378-383.
11. Oshimoto K, Shimiz H, Sato N, Mori M: **A case of Addison's disease which became worse during interferon therapy: insulin secretion under hyposmolarity.** *Nippon Naibunpi Gakkai Zasshi* 1994, **70:**511-516. *[Abstract only, Article in Japanese]*

12. De Bellis A, Falorni A, Laureti S, Perrino S, Coronella C, Forini F, Bizzarro E, Bizzarro A, Abbate G, Bellastella A: **Time course of 21-hydroxylase antibodies and long-term remission of subclinical autoimmune adrenalitis after corticosteroid therapy: Case Report.** *J Clin Endocrinol Metab* 2001, **86:**675-678.
13. Tran HA: **Incidental adrenal insufficiency.** *JAMA* 2006, **295:**1124.
14. Betterle C, Dal Pra C, Mantero F, Zanchetta R: **Autoimmune adrenal insufficiency and autoimmune polyendocrine syndromes: Autoantibodies, autoantigens, and their applicability in diagnosis and disease prediction.** *Endoc Rev* 2002, **23:**327-364.
15. Coco G, Dal Pra C, Presotto F, Albergoni MP, Canova C, Pedini B, Zanchetta R, Chen S, Furmaniak J, Smith BR, Mantero F, Betterle C: **Estimated risk for developing autoiommune Addison's disease in patients with adrenal cortex autoantibodies.** *J Clin Endocrinol Metab* 2006, **91:**1637-1645.

(D) PITUITARY DISEASE

Publication:

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Case Report

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Pituitary Disease in Chronic Hepatitis C Infection and Interferon-alpha Related Therapy: Two Case Reports

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Abstract

Pituitary dysfunction in chronic hepatitis C infection treated with interferon-α is a rare condition with 4 case reports world wide. We hereby report two cases of pituitary dysfunction in HCV patients, with and without interferon-α therapy. Case 1: A 34-year-old man co-infected with HIV and HCV presented with a 3 month history of lethargy, listlessness and a general lack of energy. Past medical histories include inactive neurosyphillis, chronic schizophrenia and seizure. His HCV is genotype 1 without cirrhosis and he completed a 48-week course of combination IFN-α and RBV for 48 weeks uneventfully 3 months prior. Examination and investigation found him to isolated ACTH deficiency. His condition improved markedly with corticosteroid replacement therapy. Case 2: A 45 year-old and treatment naive man with chronic HCV infection presented with a 20 kg weight loss, lack of energy and the occasional dizziness. Examination and investigation found him to have panhypopituitarism. Replacement therapy was initiated including hydrocortisone, testosterone and hydrocortisone. He made a slow but steady recovery and regained about 15 kg of weight but unfortunately was lost to follow up. It concluded that hepatitis C infection on its own or in conjunction with interferon-α based therapy can result in pituitary failure. The condition is readily treatable and hence should be considered in the appropriate clinical setting.

Keywords: Pituitary; Hypophysitis; Hepatitis C; Interferon-alpha

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Introduction

Pituitary pathology associated with interferon-α (IFN-α) therapy is an uncommon condition which so far has been poorly described and reported. Most are anecdotal with few unconvincing case reports. The mechanism as a result then is poorly understood but perhaps and similar to IFN-α related thyroid disease, immune-modulation is the major underlying pathogenesis. We hereby describe two cases of hepatitis C and IFN-α related disease where pituitary failure developed.

Case Report

Case 1

A 34-year-old man co-infected with Human Immunodeficiency Virus (HIV) and Hepatitis C Virus (HCV) presented with a 3 month history of lethargy, listlessness and a general lack of energy. Past medical histories include treated and inactive neurosyphilis, chronic schizophrenia and seizure. His HCV is genotype 1 without cirrhosis and he completed a 48-week course of combination IFN-α and RBV for 48 weeks uneventfully 3 months prior.

Clinically he was unwell with BP of 110/70 sitting and 100/60 standing and PR of 89 beats per minute (bpm). General examination was unremarkable and there was no pigmentation. A baseline serum cortisol was 36nmol/L at 07:05 hrs with Adrenocorticotropic (ACTH) level of 3.3 pmol/L (Reference Range (RR), < 10). His Thyrotropin (TSH) level was 0.96 mIU/L (RR, 0.4 - 4.0), free tetra-iodothyronine (fT4) of 19.1 pmol/L (RR, 10.8 - 21.0), Luteinising Hormone (LH) 13.8 IU/L (RR, 5.5 - 11.5), Follicular Stimulating Hormone (FSH) 7.7 IU/L (RR, 2.1 - 8.0), Testosterone 13.9 nmol/L (RR, 8.0 - 25.9), Growth Hormone (GH) < 0.2mIU/L, Insulin-like Growth Factor 1 (IgF-1) 0.73 U/mL (RR, 0.5 - 2.0), Prolactin 402 mIU/L (RR, < 410). A 250 µg Synacthen stimulation test showed a rise from baseline of 70 to 304 nmol/L at 60 minutes. His electrolytes were normal with Na of 137 and K 4.1 mmol/L. His pituitary Magnetic Resonance Imaging was normal. Pituitary antibodies were

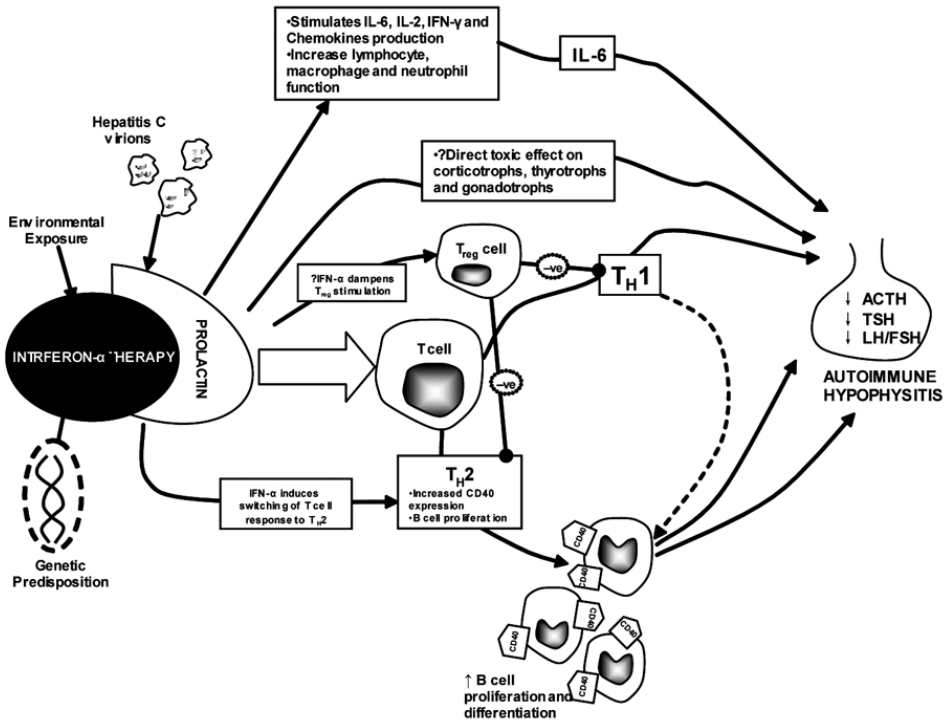

Figure 1. The proposed hypothesis for the development of autoimmune hypophysitis in HCV infection and IFN-based therapy. IL-6: Interleukin-6; IFN: Interferon; MHC-II: major histocompatability complex-II; T_H: T helper.

not available.

The patient was started on Hydrocortisone with marked improvement. Mineralocorticoid replacement therapy was not indicated as this is likely to be secondary adrenal insufficiency. The patient was to be followed up for an assessment of possible pituitary recovery.

Case 2

A 45-year-old man presented with cachexia, unintentional 25 kg weight loss over 6 months, recurrent nausea and vomiting on a background of chronic hepatitis C infection which he had acquired 20 years before from intravenous drug use. His past medical history included type 2 diabetes which recently became labile. He also developed recurrent hypoglycaemia without any major changes in his routine dietary and oral intake. There was no change or non-compliance with his oral hypoglycaemic regimen. Clinically he was unwell, cachectic with weight of 56.2 kg and height of 1.65 m, body mass index of about 20 kg/m^2. His BP was 120/70 sitting and 100/60 standing with pulse rate of 88 bpm. He appeared hypo-androgenic with sparse body hair distribution and an absence of pubic and axillary hair. His testes were 6 and 8 mL in size bilaterally. Further investigations are as follow: TSH 1.58 mU/L, fT4 9.1 pmol/L, fT3 4.7 pmol/L, ACTH < 1.1 pmol/L, Cortisol 326 nmol/L, LH 0.6 IU/L, FSH 0.3 IU/L, Testosterone < 0.7 nmol/L, Prolactin 252 mIU/L. A short-synacthen test revealed a rise from 326 to 430 nmol/L over 60 minutes consistent a sub-optimal response. On the basis of these results, no dynamic stimulation test was warranted.

The patient was given triple replacement therapy including thyroxine, cortisone acetate and testosterone isocaproate (Sustanon) injections. He made a rapid recovery and great symptomatic improvement. His hypoglycaemic crises resolved. Indeed, he became hyperglycaemic. He gained about 5 kg and was referred for treatment consideration with IFN-α therapy. However, he did not return for review and was subsequently lost to follow up.

Discussion

These two cases highlight the immuno-modulating effects of the hepatitis C viral particles and IFN-α therapy individually, especially in regards to pituitary pathology. Due to its rarity, the condition is poorly understood [1]. Theoretically however, it is thought that prolactin plays a major part in the pathogenesis of the condition [2]. The presence of HCV particles and IFN-α, both of which are potent immuno-modulators, further inflame the condition. The three combine to act through a similar pathway which was previously proposed for IFN-α related thyroid disease [3]. Prolactin is thought to activate the JAK/STAT pathways which lead to the activation of Interferon Regulatory Factor 1 (IRF1). Prolactin also

Table 1. Summary of Our Cases and Available Published Reports, Please Note Case 3 Involved Hepatitis B Infection

Authors and Year of publications	Gender:Age	Pituitary Antibody status	MRI findings	Treatment modality	Panhypopituitarism and therapy	Reversibility
1. Sakane et al, 1995	F:44	YES: GH3 cell	Normal	IFN-α monotherapy for 3 months	Y: Hydrocortisone and Thyroxine	Yes, after 11 months
2. Concha et al, 2003	M:39	NO:Normal Human Pituitary Tissues	Normal	IFN-α and RBV for 1 year	Y: Testosterone and Growth hormone	No
3. Chan et al, 2004	F:30 (HBV infection)	Not done	Anterior pituitary cyst	IFN-α monotherapy for 3 years	Y: Hydrocortisone, Oestrogen and Thyroxine	No
4. Ridruejo et al, 2006	F:54	Not done	Not done	IFN-α and RBV for 48 weeks	Y: No therapy	Yes, transient
5. Our case 1	M:34	Not available	Normal	IFN-α and RBV for 48 weeks	Y: Hydrocortisone	Unknown. Lost to follow up
6. Our case 2	M:45	Not available	Normal	Untreated	Y: Hydrocortisone, Testosterone and Thyroxine	No

activates T_H1 and T_H2 cytokine activities which lead to the development of autoimmunity. In addition, the T helpers are further in turn regulated by T regulator cells (T_{reg}). The latter function is dampened in the presence of IFN-α therapy, amplifying the PRL response, leading to the clinical expression of anterior pituitary deficiency [3]. The PRL hypothesis is probably more relevant in post-partum nursing mothers where hyperprolactinaemia predominates. However, only 50% of the discussed cases are females and none was breast-feeding. Genetic predisposition must play a part, as is the vascular supply. The anterior pituitary has an extensive vascular supply, exposing the pituitary cells to the HCV particles, IFN-α and associated antibodies, Figure 1. It remains unknown if the condition is reversible, especially once the virus has been terminated or cured with IFN-α therapy. The extermination of the HCV particles also reduces the stimulating effect helping the reversibility of the condition.

Previous published cases in the literature were sparse. The first case was described by Sakane et al [4] in 1995 in which the endocrinopathies developed 2 months (out of six) after stopping IFN therapy. This case was shown to have pituitary antibodies against GH3 cells, a rat pituitary tumor cell line that secretes growth hormone and prolactin. Fortunately, the condition was reversible. In 2003, Concha et al [5] reported a second similar case. The proposed panhypopituitarism was detected 1 year after the completion of therapy although there was no evidence of antipituitary antibodies. Chan et al [6] described a case of panhypopituitarism but in the presence of hepatitis B infection. The patient developed amenorrhoea whilst on treatment and displayed permanent panhypopituitarism thereafter. Ridruejo et al [7] in 2006 reported a possible case of reversible or spontaneously recovered hypophysitis whilst on combination IFN and RBV therapy. The diagnosis was clinically based in all cases using the temporal relationship with treatment, pituitary hormonal profile, pituitary magnetic resonance imagings, all of which are normal or non-contributory, and the absence of thyroid and other autoimmune markers. Except for case 3, all demonstrated the typical sequence of deficiencies in autoimmune hypophysitis where ACTH is the first to be affected, followed by TSH and then LH/FSH [8]. Antipituitary antibodies are also not available in most case as these remain poorly defined and the test is not routinely available in practice [8]. Contrary to de novo cases, none developed headache and/or visual disturbance. These few published cases are summarized in Table 1.

In addition, INF-α can unmask previously undetected pituitary Sheehan's syndrome or syndrome of inappropriate antidiuresis [9-11] which can be fatal if unrecognized.

The prevalence of pituitary dysfunction in relation to hepatitis C infection and IFN-α therapy is poorly known and appears very rare. Our previous report in postmortem cases found no evidence of pituitary involvement in untreated HCV cases [12]. This is not surprising given the rarity of clinical panhypopituitarism in relation to HCV infection and suggests that the condition occurs in an ad hoc fashion, presumably in genetically susceptible individuals. Surveillance therefore is not recommended. However, the diagnosis of pituitary dysfunction, either partial or complete should be considered in HCV patients with the appropriate clinical symptomatology. Similarly and not evidence based, patients should be followed up to assess for reversibility of the condition.

Conclusion

Hepatitis C infection and IFN-α associated pituitary dysfunction is rare but should be considered in the appropriate clinical setting. The condition is readily treatable and is potentially reversible.

Disclosure Statement

All authors have no conflict of interests relevant to this work.

References

1. Caturegli P, Newschaffer C, Olivi A, Pomper MG, Burger PC, Rose NR. Autoimmune hypophysitis. Endocr Rev. 2005;26(5):599-614.
2. De Bellis A, Ruocco G, Battaglia M, Conte M, Coronella C, Tirelli G, Bellastella A, et al. Immunological and clinical aspects of lymphocytic hypophysitis. Clin Sci (Lond). 2008;114(6):413-421.
3. Tran HA, Reeves GE. The Spectrum of Autoimmune Thyroid Disease in the Short to Medium Term Following Interferon-alpha Therapy for Chronic Hepatitis C. Int J Endocrinol. 2009;2009:241786.
4. Sakane N, Yoshida T, Yoshioka K, Umekawa T, Kondo M, Shimatsu A. Reversible hypopituitarism after interferonalfa therapy. Lancet. 1995;345(8960):1305.
5. Concha LB, Carlson HE, Heimann A, Lake-Bakaar GV, Paal AF. Interferon-induced hypopituitarism. Am J Med. 2003;114(2):161-163.
6. Chan WB, Cockram CS. Panhypopituitarism in association with interferon-alpha treatment. Singapore Med J. 2004;45(2):93-94.
7. Ridruejo E, Christensen AF, Mando OG. Central hypothyroidism and hypophysitis during treatment of chronic hepatitis C with pegylated interferon alpha and ribavirin. Eur J Gastroenterol Hepatol. 2006;18(6):693-694.
8. Howlett TA, Levy MJ, Robertson IJ. How reliably can autoimmune hypophysitis be diagnosed without pituitary biopsy. Clin Endocrinol (Oxf). 2010;73(1):18-21.
9. Kanda K, Kayahara T, Seno H, Yamashita Y, Chiba T.

Conversion from latent to symptomatic Sheehan's syndrome by pegylated interferon therapy for chronic hepatitis C. Intern Med. 2008;47(10):939-941.
10. Mabe K, Shinzawa H, Yamatani K, Takeda T, Ishibashi M, Yamada N, Misawa H, et al. Case report: interferon induced coma in Sheehan's syndrome. J Gastroenterol Hepatol. 1997;12(7):551-553.
11. Tanaka M, Kamoi K, Takahashi T. Interferon-alpha is a predisposing risk factor for carbamazepine-induced hyponatremia: A case of syndrome of inappropriate antidiuresis caused by interferon-alpha therapy. Int J Gen Med. 2008;1:21-25.
12. Tran HA, Reeves GE, Lyons TJ, Attia JR. Histopathologic findings of autoimmunity in thyroid, pituitary, and adrenal diseases in chronic hepatitis C postmortem cases. Endocr Pract. 2010;16(4):566-569.

Other endocrine diseases have also been reported including skin disease (50), gastric disease (51) and diabetes (52, 53) to be associated with interferon-α therapy but causality remains difficult to delineate.

CHAPTER VI. THYROID DISEASES FOLLOWING THE COMPLETION OF INTERFERON THERAPY

Once thyroid disorders occurring in this setting has been fully characterized, it remains to be determined if they are independent of or causally follow the treatment period. Pattern of thyroid disease, frequency of outcomes and the rationale (or lack) for the need to follow these patients with regards to thyroid disorders are to be determined at the time of SVR review, that is 6 months after the completion of treatment. One of our manuscripts looks at the prevalence and spectrum of actual thyroid diseases occurring during this immediate 6 month period. We also assessed thyroid outcomes in the general HCV population treated with combination therapy in a similar duration.

Publication:

Tran HA, Reeves GEM. THE SPECTRUM OF AUTOIMMUNE THYROID DISEASE IN THE SHORT TO MEDIUM TERM FOLLOWING INTERFERON-α THERAPY FOR CHRONIC HEPATITIS C. Int J Endocrinol, 2009; 2009: 241786. Epub 2009 Aug 31.

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Case Report

The Spectrum of Autoimmune Thyroid Disease in the Short to Medium Term Following Interferon-α Therapy for Chronic Hepatitis C

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Autoimmune thyroid diseases are common manifestations of hepatitis C infection, exacerbated by interferon-based treatment. However, the occurrence and pattern of thyroid disease in the short/medium term following the completion of IFN-based therapy is relatively unknown and there are very few previous reports regarding the specific spectrum of autoimmune thyroid disease that may follow such therapy. We hereby report 3 cases which demonstrate the range of thyroid diseases that may occur following interferon therapy. The hypothesis advanced is that in the pathogenesis of these conditions there must be both triggering and sustaining mechanisms as thyroid diseases occur well outside the immediate effect window of pegylated interferon. This paper suggests the need to continue thyroid surveillance in IFN-treated HCV patients following the completion of therapy, perhaps for the first 6 months.

1. Clinical Case Notes

1.1. Case 1. A 53-year-old man underwent combination Ribavirin (RBV) and Interferon-$\alpha 2\beta$ (IFN-$\alpha 2\beta$) for a total of 48 weeks because of his HCV genotype 1 and had achieved sustained virological response (SVR). During this time, he developed IFN-induced thyroiditis at 18 weeks with the classical biphasic thyrotoxic phase followed by hypothyroidism. The condition completely resolved by the 38th week, including normal thyrotropin (TSH), free tetra-iodothyronine (fT4), and free tri-iodothyronine (fT3). Thyroid hormone levels were followed monthly throughout the duration of treatment. There was no family or prior history of thyroid disease and antiviral therapy was continued despite his thyroid condition. He represented with general lethargy and weight loss of 3 kg two months *after* the completion of treatment for his chronic hepatitis C (HCV) infection. Clinical examination at this time showed sinus tachycardia at 101 beats per minute (bpm), blood pressure (BP) of 120/80 with no postural hypotension. There were peripheral stigmata of thyrotoxicosis but no signs of Graves' ophthalmopathy or dermopathy. No goitre was present. His TSH was undetectable (reference range (RR), 0.4–4.0 IU/L), fT4 19.8 (RR, 10.5–24.5 pmol/L) and fT3 8.9 (RR, 3.5–5.5 pmol/L). His thyroid pertechnetate uptake study was diffusely uniform and increased to 9% (RR, 3–8%), consistent with Graves' disease (GD). His TSH Stimulating Immunoglobulin (TSI) returned positive at 30 IU/L (RR, <10), his human TSH receptor antibody (hTRAB) was 15.8 IU/L (RR, <2). His antithyroglobulin (Tg), (RR, < 1 : 400), and antithyroperoxidase (TPO), (RR, < 1 : 400), levels were undetectable. A diagnosis of GD was made but the patient was reluctant to take medication as he was well with excellent exercise tolerance and thus treatment was withheld. Six weeks later, his fT3 level was 8.2 pmol/L and carbimazole was started. Three months later, he was clinically well and his fT3 level has normalised. Treatment was continued for 12 months during which his thyroid function tests were normal. His follow-up TSI and hTRAB antibodies had become undetectable at the end of treatment.

1.2. Case 2. A 56-year-old woman presented with T3-toxicosis 6 weeks following the completion of combination RBV and IFN-$\alpha 2\beta$ for her HCV infection. She had undergone antiviral therapy over the previous 48 weeks for her HCV genotype 4 without any thyroid complications and had achieved SVR. There was no previous personal or family history of thyroid disease. As part of treatment protocols, her monthly thyroid function tests for the duration of treatment had been entirely normal. Four weeks after the completion of therapy, she began to notice mild dyspnoea on exertion, intermittent palpitation and heat intolerance. There were no other symptoms of thyrotoxicosis. Clinically, she appeared well with a regular pulse of 92 bpm, BP of 130/80. No goitre was detected nor were there any signs of thyrotoxicosis. Her TSH was undetectable, fT4 was 24.1 and fT3 8.9 pmol/L. Her thyroid uptake scan was reduced at 2% (RR, 3–8). The thyroid ultrasound was also normal in size and appearance; there was no evidence of nodularity but mild increase in vascularity. Her TSI, hTRAB, anti-Tg, and anti-TPO antibodies were not detectable. One week later, her T3-toxicosis persisted at 8.4 pmol/L. A diagnosis of IFN-induced thyroiditis was made and low dose propanolol was prescribed given her symptoms. She was followed closely with monthly TSH, fT4, and fT3 levels. Eight weeks later, she had entered into the hypothyroid phase with TSH of 54.6 IU/L, fT4 8.8, and fT3 2.3 pmol/L. As the patient remained free of any hypothyroid symptoms and given the expected recovery in thyroiditides, thyroxine therapy was withheld. At 16 weeks, her thyroid function had returned to normal. Propanolol was ceased and when last reviewed, the patient was in excellent health with ongoing normal thyroid function tests.

1.3. Case 3. A 45-year-old woman presented for a routine review 8 weeks following her failed therapy with IFN-$\alpha 2\beta$ for her chronic HCV. She had been generally well with no significant previous medical history although there was a strong family history of thyroid disease in her family, with both her mother and grandmother experiencing thyroid diseases of undetermined nature, culminating in both requiring thyroxine supplement. Treatment for her HCV infection (genotype 1) was to be 48 weeks of RBV and IFN-$\alpha 2\beta$. However, after 24 weeks, there was no reduction in viral load and treatment was terminated. Her monthly thyroid function tests had been normal till then. System review on this occasion did not reveal any symptoms to suggest thyroid disease. Clinical examination at the review visit showed no signs of hypothyroidism. Her vital signs were satisfactory, with normal tendon reflexes and no goitre. Her routine TSH was found to be 48.0 IU/L with undetectable fT4 level. The follow-up thyroid ultrasound revealed the presence of a small atrophic gland with total volume of 6 mls (RR, 6–10) [1]. Her anti-Tg and anti-TPO titres were 1 : 256 and 1 : 512, respectively. The TSI and hTRAB were 11 IU/L and 11.7 IU/L respectively. Her thyroid uptake study was low at 3%. A diagnosis of autoimmune hypothyroidism was made and consequently, thyroxine was started. She was stabilised on 100 μg of thyroxine daily. At six month review, her anti-Tg, anti-TPO, TSI and hTRAB antibodies were undetectable. In retrospect, the combination of high TSI and hTRAB titres with hypothyroidism and a low pertechnetate uptake study was consistent with the presence of thyrotropin blocking antibodies (TB-Ab). Based on this probable underlying mechanism for the hypothyroidism, thyroxine was ceased. The patient remained well and independent of thyroxine supplement in the following 12 months. Interferon-induced thyroiditis (in the hypothyroid phase) was unlikely given that there was no hyperthyroid phase. Functional TB-Ab assay would have been definitive but unfortunately was not available.

The clinical profiles of the three cases are represented schematically in Figure 1.

2. Discussion

The cases highlight the peculiar and fascinating spectrum of thyroid disease in the ensuing months following the completion of IFN-based therapy for chronic HCV infection. Our series demonstrates a wide spectrum of autoimmune thyroid diseases, ranging from GD to thyroiditis to profound subclinical hypothyroidism following IFN treatment. In case 1, the pattern includes GD following thyroiditis that occurred during the treatment phase. This unusual occurrence, referred to as "tri-phasic", has been reported only once previously [2]. The predominance of T3 activity was also peculiar, without progressing to florid GD. In case 2, T3 thyroiditis occurred; this has not been described previously nor has it been described in this particular clinical setting. The clinical and biochemical behaviour is not very different from those arising de novo, expressing the classical biphasic response despite the negative antibody findings. Case 3 illustrates a complex mechanism of developing hypothyroidism in, presumably, a pre-existing abnormal thyroid gland. Despite normal TSH levels throughout the treatment duration, the development and resolution of hTRAB suggested the presence of TSH blocking antibody (TB-Ab) to account for the hypothyroidism, although this was never conclusively proven. The latter resolved, possibly following TBAB disappearance after the IFN effects had waned. In all 3 cases, no baseline antibody profiles were performed as they did not alter clinical management and thyroid status was monitored monthly during therapy.

There are only two previous studies documenting the long-term outcome of thyroid diseases but with IFN-α monotherapy, rather than combination therapy. Carella et al. [3] followed 114 patients for 6.2 years on average and did not find any overt thyroid dysfunction, only subclinical hypothyroidism in 12 cases at the end of therapy and 7 cases at the end of followup. Doi et al. [4] followed 17 patients to an average of 71 months in which there were 9 hyper- and 8 hypothyroid cases. No overt thyroiditis was observed, contrary to our current series. Tong et al. [5] studied the efficacy of *consensus*-IFN versus IFN-α (both in combination with RBV). This study included a follow-up period of 24 weeks posttreatment to coincide with the SVR review. Although both hypothyroidism and hyperthyroidism were included, no specific mention was made of their timing or characteristics.

The pathogenesis of this condition is in essence unknown but is probably distinct from the thyroid diseases arising

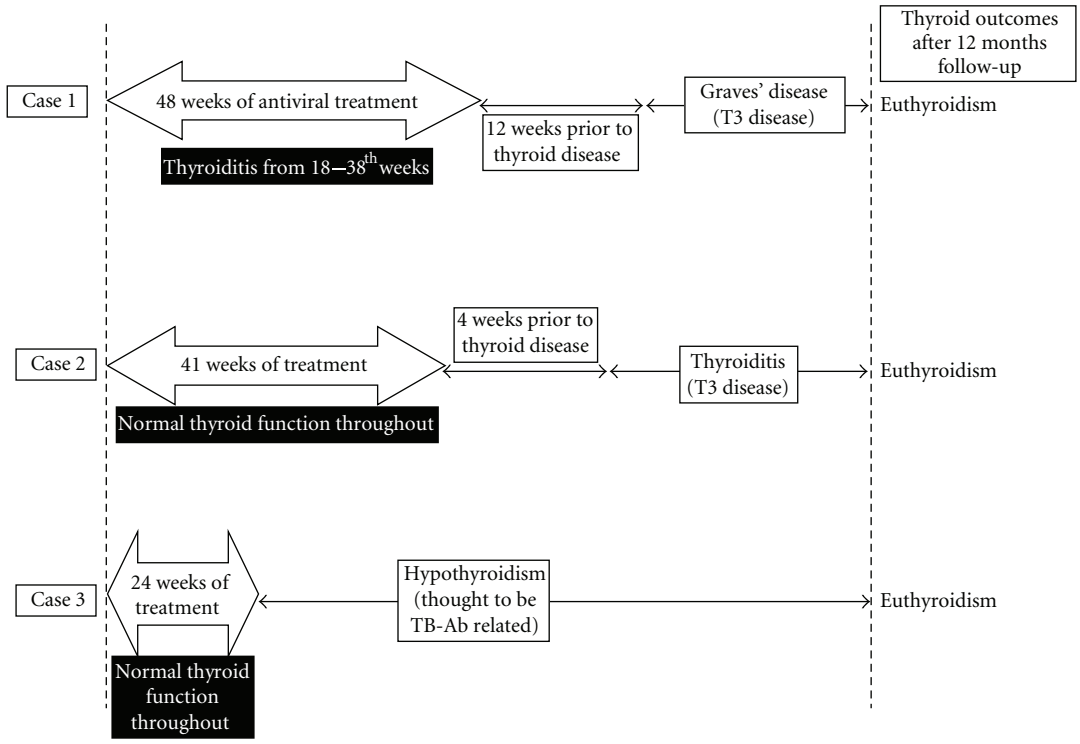

Figure 1: Schematic summaries of the cases and their final thyroid outcomes. The arrow bars indicate the duration of combination therapy with interferon-α and ribavirin. See text for detailed discussions.

during the course of IFN therapy [6, 7]. Clearly, a common denominator is recent exposure to IFN therapy for chronic HCV infection but it also clear that the process must have been perpetuated by additional factors. This persistence of the immune reaction up to 12 months after therapy had been observed previously in 3 patients [8]. All of these individuals achieved sustained virological response (SVR) whereas only 1 of our 3 cases did not. The exogenous IFN-α is believed to stimulate lymphocyte, macrophage and neutrophil function as well as increasing cytokine and chemokine concentrations, especially, interleukin-6 (IL-6) [8]. IFN-α induces MHC-II expression and probably CD40 expression on thyrocytes [9]. The latter results in an increased T-cell activation of the CD40 signalling pathway within the thyroid gland. This leads to an overexpression of intrathyroidal IL-6, synergising with pre-existing and circulating IL-6, in turn induces the development of thyroiditis. The high IL-6 is also thought to block TSH-mediated iodine uptake leading to the absent uptake scan [10]. Beside MHC-II, IFN also induces MHC-I expression on thyrocytes by way of IL-2 and chemokines [11], adding to the inflammatory response and the thyroiditis. IFN is also known to have a direct toxic effect on thyrocytes [10]. Furthermore, HCV particles have been found inside the thyrocytes [12] which could trigger and sustain an intracellular T-cell response and inflammation but this is unlikely assuming that there are no remaining intrathyroidal HCV particles in the presence of SVR. All these mechanisms are further amplified, exacerbated and maintained by the exogenous IFN-α therapy [13].

Of recent interest is the function and place of regulatory T-cells (T_{reg}) in HCV infection and IFN-associated thyroiditis. In HCV, the frequency of T_{reg} is high which directly suppresses T-cell overall function allowing the infection to persist [14]. In animal models, T_{reg} depletion induced lymphocytic infiltration of the thyroid leading to transient and/or permanent hypothyroidism [15]. Treatment with interferon-β in patients with multiple sclerosis results in an increased T_{reg} inhibitory capacity leading to the favourable outcome [16]. However, it is not known if this is the case for IFN-α. Furthermore, for the T-cell immunity to elicit the various immune thyroid disease, T_{reg} response should be dampened rather than enhanced to removs its inhibitory capacity and allow the T-cell helpers (T_H) to initiate the autoimmunity process.

The discussed immune mechanism above is thought to be under the auspices of the T_H1 response. In GD, IFN is thought to induce or modulate switching of the T-cell response to T_H2. This, in turn, stimulates B cell proliferation and differentiation under the influence of IL-6 and increased CD40 overexpression, resulting in an increase in TSI, simulating GD [9]. However, Nagayama et al. [17] suggested additionally that T_H1 in itself might be a potential pathogenic mechanism in GD. Similarly, the apparent hypothyroidism is thought to be elicited by a similar mechanism but involving the alternative TB-Ab. There is no apparent reason to explain why the induced antibodies should be preferentially be stimulating, inhibitory, neutral or even a mixture. The pathogenesis is highly IFN dependent and resolves once

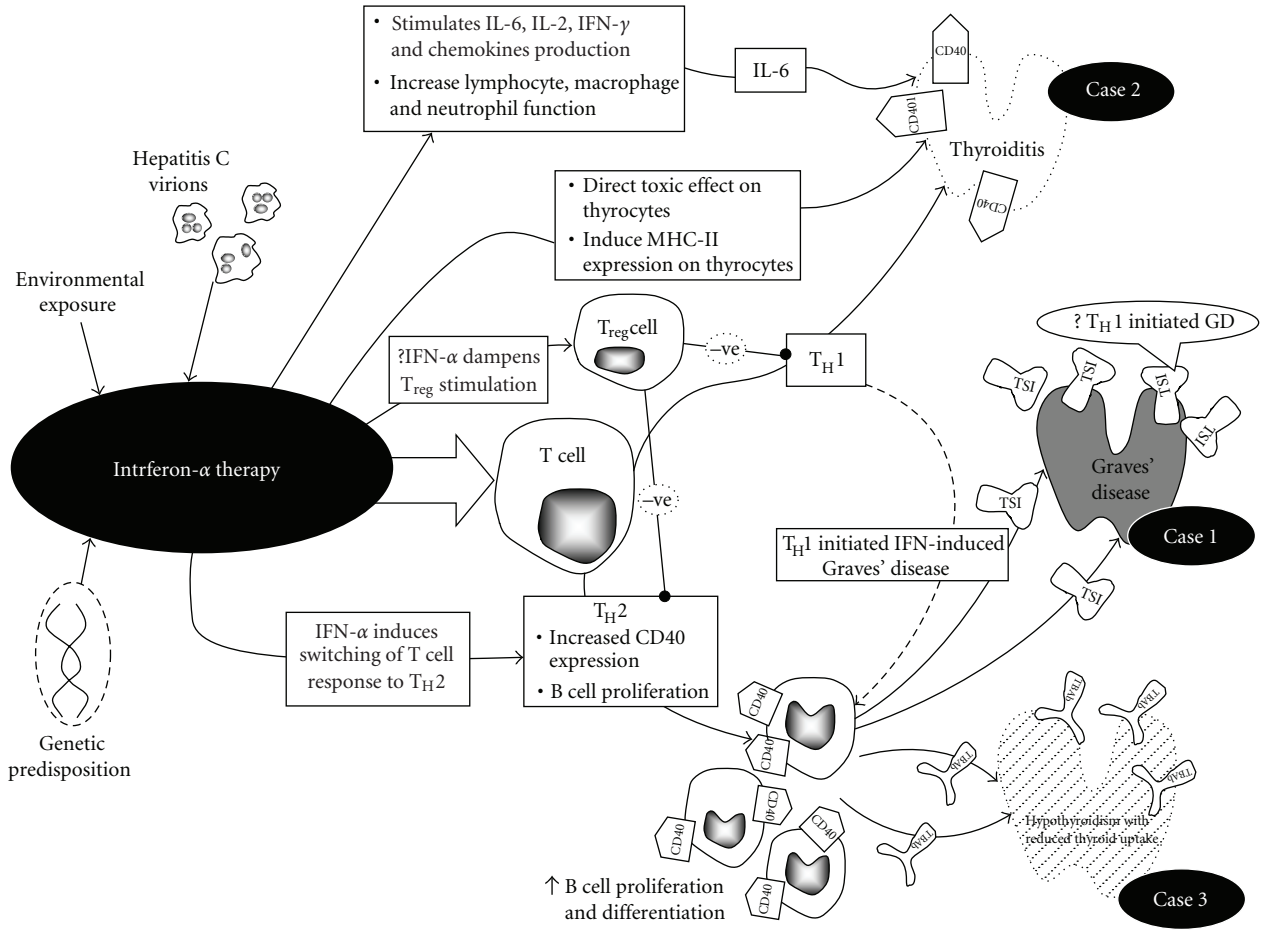

Figure 2: The proposed hypotheses for the development of the full spectrum of thyroid diseases after the completion of combination IFN-based therapy. IL-6: Interleukin-6; IFN: Interferon; MHC-II: major histocompatability complex-II; GD: Graves' disease; T_H: T helper.

the prolonged IFN effect has waned. This represents a potentially reversible pathway for hypothyroidism other than the hypothyroid phase of thyroiditis previously described [18]. The complex pathogenesis of this condition as currently understood has been summarised in Figure 2.

It is well documented that hepatitis C infection is associated with an increase in endocrinopathies, especially autoimmune thyroid diseases, prior to and during therapy [7, 19], with a prevalence of 7–19% [20] depending on the studied population. Previous reports suggested that IFN therapy and female gender contribute to disease risk [20, 21]. Our previous published data suggested that thyroid surveillance is carried out monthly during treatment [7], although the frequency of surveillance remains contentious and the National Academy of Biochemistry is yet to recommend thyroid testing in this clinical scenario [22]. Three monthly TSH as suggested by Mandac et al. [19] may miss the entire diagnosis as thyroid functions have completely normalized in many cases. Both the British Society of Gastroenterology and the American Gastroenterological Association recognise the potential thyroid effect of IFN and recommend thyroid screening [23, 24]. However, only the former specifies that thyroid function testing is recommended at *each* treatment visit, rather than monthly. The National Institute of Health consensus statement on hepatitis C management surprisingly did not address this issue [25].

To add to the complexity of the issue, it remains unknown if thyroid diseases are increased post-IFN therapy and if female gender is a risk factor, especially once this highly immunostimulatory virus has been eradicated. This is the first case series to explore the wide but probably incomplete spectrum of autoimmune thyroid dysfunction immediately following the completion of IFN-based treatment. This case series is small and the development of thyroid disease may arguably be incidental. However, the observation strongly suggests a persisting and disrupted immune system. These cases highlight the need for ongoing suspicion of thyroid disease and probable thyroid surveillance strategy, at least during the immediate 6–12 weeks following therapy completion and perhaps extending it to coincide with the time of SVR review at 6 months posttherapy followup.

References

[1] X. Barrère, P. Valeix, P. Preziosi, et al., "Determinants of thyroid volume in healthy French adults participating in the SU.VI.MAX cohort," *Clinical Endocrinology*, vol. 52, no. 3, pp. 273–278, 2000.

[2] N. L. Bohbot, J. Young, J. Orgiazzi, et al., "Interferon-α-induced hyperthyroidism: a three-stage evolution from silent thyroiditis towards Graves' disease," *European Journal of Endocrinology*, vol. 154, no. 3, pp. 367–372, 2006.

[3] C. Carella, G. Mazziotti, F. Morisco, et al., "Long-term outcome of interferon-α-induced thyroid autoimmunity and prognostic influence of thyroid autoantibody pattern at the end of treatment," *The Journal of Clinical Endocrinology & Metabolism*, vol. 86, no. 5, pp. 1925–1929, 2001.

[4] F. Doi, S. Kakizaki, H. Takagi, et al., "Long-term outcome in interferon-α-induced autoimmune thyroid disorders in chronic hepatitis C," *Liver International*, vol. 25, no. 2, pp. 242–246, 2005.

[5] M. J. Tong, K. R. Reddy, W. M. Lee, et al., "Treatment of chronic hepatitis C with consensus interferon: a multicenter, randomized, controlled trial," *Hepatology*, vol. 26, no. 3, pp. 747–754, 1997.

[6] V. Wong, A. X.-L. Fu, J. George, and N. W. Cheung, "Thyrotoxicosis induced by alpha-interferon therapy in chronic viral hepatitis," *Clinical Endocrinology*, vol. 56, no. 6, pp. 793–798, 2002.

[7] H. A. Tran, G. E. M. Reeves, and T. l. Jones, "The natural history of interferon-α2b-induced thyroiditis and its exclusivity in a cohort of patients with chronic hepatitis C infection," *QJM*, vol. 102, no. 2, pp. 117–122, 2009.

[8] A. Cargnel, G. Gubertini, C. Magnani, S. Bosis, F. Colombo, and M. Clerici, "Immune response in chronic hepatitis C-infected patients undergoing interferon treatment," *Hepatology*, vol. 30, no. 2, pp. 584–585, 1999.

[9] E. C. Ridgway, Y. Tomer, and S. M. McLachlan, "Update in thyroidology," *The Journal of Clinical Endocrinology & Metabolism*, vol. 92, no. 10, pp. 3755–3761, 2007.

[10] E. P. M. Corssmit, J. de Metz, H. P. Sauerwein, and J. A. Romijn, "Biologic responses to IFN-α administration in humans," *Journal of Interferon and Cytokine Research*, vol. 20, no. 12, pp. 1039–1047, 2000.

[11] A. R. Lloyd, E. Jagger, J. J. Post, et al., "Host and viral factors in the immunopathogenesis of primary hepatitis C virus infection," *Immunology and Cell Biology*, vol. 85, no. 1, pp. 24–32, 2007.

[12] E. J. Gowans, "Distribution of markers of hepatitis C virus infection throughout the body," *Seminars in Liver Disease*, vol. 20, no. 1, pp. 85–102, 2000.

[13] E. Roti, R. Minelli, T. Giuberti, et al., "Multiple changes in thyroid function in patients with chronic active HCV hepatitis treated with recombinant interferon-alpha," *American Journal of Medicine*, vol. 101, no. 5, pp. 482–487, 1996.

[14] T. Boettler, H. C. Spangenberg, C. Neumann-Haefelin, et al., "T cells with a $CD4^+CD25^+$ regulatory phenotype suppress in vitro proliferation of virus-specific $CD8^+$ T cells during chronic hepatitis C virus infection," *Journal of Virology*, vol. 79, no. 12, pp. 7860–7867, 2005.

[15] S. M. McLachlan, Y. Nagayama, P. N. Pichurin, et al., "The link between Graves' disease and Hashimoto's thyroiditis: a role for regulatory T cells," *Endocrinology*, vol. 148, no. 12, pp. 5724–5733, 2007.

[16] C. de Andrés, C. Aristimuño, M. Bartolomé, et al., "Clinical response to interferon-β-1a may be linked to low baseline circulating BDCA1 myeloid dendritic cells. Differential role of circulating dendritic cells and $CD4^+$ regulatory T-cells in relapsing-remitting multiple sclerosis: a 1-year longitudinal study," *Journal of Neuroimmunology*, vol. 212, no. 1-2, pp. 112–120, 2009.

[17] Y. Nagayama, H. Mizuguchi, T. Hayakawa, M. Niwa, S. M. McLachlan, and B. Rapoport, "Prevention of autoantibody-mediated Graves'-like hyperthyroidism in mice with IL-4, a Th2 cytokine," *The Journal of Immunology*, vol. 170, no. 7, pp. 3522–3527, 2003.

[18] Y. Tomer, J. T. Blackard, and N. Akeno, "Interferon alpha treatment and thyroid dysfunction," *Endocrinology and Metabolism Clinics of North America*, vol. 36, no. 4, pp. 1051–1066, 2007.

[19] J. C. Mandac, S. Chaudhry, K. E. Sherman, and Y. Tomer, "The clinical and physiological spectrum of interferon-alpha induced thyroiditis: toward a new classification," *Hepatology*, vol. 43, no. 4, pp. 661–672, 2006.

[20] H. A. Tran, "Hepatitis C infection, treatment regimens, and thyroid function abnormalities," *Endocrinologist*, vol. 17, no. 4, pp. 231–235, 2007.

[21] M. Gelu-Simeon, A. Burlaud, J. Young, G. Pelletier, and C. Buffet, "Evolution and predictive factors of thyroid disorder due to interferon alpha in the treatment of hepatitis C," *World Journal of Gastroenterology*, vol. 15, no. 3, pp. 328–333, 2009.

[22] L. M. Demers and C. A. Spencer, "Laboratory support for the diagnosis and monitoring of thyroid disease," June 2009, http://www.aacc.org/members/nacb/pages/default.aspx.

[23] British Society of Gastroenterology, "Clinical Guidelines on the management of hepatitis C," June 2009, http://www.bsg.org.uk/pdf_word_docs/clinguidehepc.pdf.

[24] J. L. Dienstag and J. G. McHutchison, "American gastroenterological association medical position statement on the management of hepatitis C," *Gastroenterology*, vol. 130, no. 1, pp. 225–230, 2006.

[25] "NIH consensus statement on management of hepatitis C: 2002," *NIH Consensus and State-of-the-Science Statements*, vol. 19, no. 3, pp. 1–46, 2002.

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Original Article

THYROID FUNCTION OUTCOMES AFTER PEGYLATED INTERFERON-α AND RIBAVIRIN THERAPY FOR CHRONIC HEPATITIS C

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ABSTRACT

Objective: To assess the frequency of new thyroid disease, in patients who did not develop thyroid disease during treatment with interferon-α in combination with ribavirin for hepatitis C, during the 6-month period after the end of therapy.

Methods: A prospective study was performed in 190 patients who underwent a combination of interferon-α and ribavirin therapy for hepatitis C infection during the 36-month period between 2006 and 2008. Thyroid function tests were performed at the completion of treatment and at 4, 12, and 24 weeks of follow-up.

Results: During the 6 months after the completion of interferon-α and ribavirin therapy in the 190 study patients with hepatitis C infection, there were 2 cases of thyroid disease. One patient had the typical biphasic thyroiditis, and the other had primary hypothyroidism. Thus, the prevalence of thyroid disease in this setting was 2 of 190 patients (1.0%).

Conclusion: The majority (99%) of patients had normal thyroid outcomes at 6-month follow-up. Only 1 patient had symptoms. This finding is reassuring and eliminates the need for ongoing thyroid surveillance during this time and probably longer. In the absence of symptoms, only a single thyroid-stimulating hormone measurement at 6-month review is recommended. **(Endocr Pract. 2010;16:934-939)**

Abbreviations:
FT_3 = free triiodothyronine; **FT_4** = free thyroxine; **HCV** = hepatitis C virus; **hTRAb** = human thyroid receptor antibodies; **SVR** = sustained virologic response; **Tg** = thyroglobulin; **TPO** = thyroperoxidase; **TSH** = thyroid-stimulating hormone; **TSI** = thyroid-stimulating immunoglobulins

BACKGROUND

Hepatitis C has become one of the major epidemics of the 20th century, afflicting mostly the young populations across the world, including the United States and Australia (1-4). It has become clear that the best and most effective therapy for the management of this chronic condition is the combination of pegylated interferon and ribavirin (5,6). Although thyroid disease associated with this treatment has been well documented, it remains unknown whether the immunomodulating effects are long lasting and may cause thyroid disease beyond the duration of treatment. In this study, further information was sought to clarify this matter and to assess any clinical need for ongoing monitoring during the 6-month period leading to the time of a sustained virologic response (SVR).

PATIENTS AND METHODS

Study Subjects

The Hunter Area Hepatitis C Treatment Centre assesses and treats all cases of hepatitis C in northern New South Wales, Australia. It is part of the John Hunter Hospital, a major tertiary referral center in the state. A total of 216 patients (118 male and 98 female patients) were treated between 2006 and 2008, a period of 36 months. Those patients in whom thyroid disease developed before or during

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therapy were excluded from the final analysis. All patients with other causes of chronic hepatitis were excluded, including hepatitis B and chronic alcoholic liver disease. Baseline characteristics of all studied subjects are presented in Table 1.

Fifteen patients developed thyroiditis and were excluded from the study because they underwent separate follow-up. In addition, 9 patients were lost to follow-up. Two patients had their treatment terminated early because of response failure and severe anemia. Thus, 190 patients completed the assigned treatment regimens. All patients consented to be part of this study.

Therapy

All study patients were treated with a combination of pegylated interferon and ribavirin; the duration of treatment depended on the hepatitis C virus (HCV) genotypes, with genotypes 2 and 3 treated for 24 weeks and genotypes 1 and 4 for 48 weeks. The continuation of treatment was further adjudicated on the basis of viral load results for genotypes 1 and 4. For genotypes 2 and 3, treatment was completed irrespective of the qualitative viral load at 4 weeks. The dosage for pegylated interferon-α ranged from 80 to 120 μg weekly, and the ribavirin dosage ranged from 1,000 to 1,200 mg daily—both based on body weight.

Thyroid Function Assessments

All patients had routine thyroid-stimulating hormone (TSH) measurements at monthly intervals throughout the course of therapy. Measurements of free thyroxine (FT_4) and free triiodothyronine (FT_3) were sequentially performed when TSH levels were abnormal. In addition, all 190 study patients were assessed for thyroid disease clinically and biochemically at the completion of the treatment and at 4, 12, and 24 weeks of follow-up. The last review coincided with the assessment of SVR.

Table 1
Baseline Characteristics of 190 Patients Assessed With Regard to Thyroid Outcome During the 6-Month Interval After Interferon Therapy[a]

Factor	Result
Demographics	
Mean age (y)	49 ± 7
Male patients	112 (59%)
White patients	133 (70%)
Weight (kg)	77 ± 18
Hepatitis C virus genotype	
1	96 (51)
2	17 (9)
3	68 (36)
4	7 (4)
Liver function tests[b]	
Albumin (36-48 g/L)	37 ± 3
Serum bilirubin (2-20 μmol/L)	15 ± 6
Alanine aminotransferase (<45 U/L)	79 ± 34
γ-Glutamyltransferase (<30 U/L)	58 ± 35
Prothrombin time (11-18 seconds)	15 ± 2
Hematologic variables[b]	
Hemoglobin (115-165 g/L)	154 ± 13
White blood cell count (4.0-11.0 × 10^9/L)	5.8 ± 1.5
Platelet count (150-400 × 10^9/L)	187 ± 34

[a] Results are expressed as means ± standard error or absolute number (%).
[b] Reference ranges are shown in parentheses.

Thyroid Dysfunction

Thyroid dysfunction was defined as having hypothyroidism or hyperthyroidism (clinically and biochemically based). Thyrotoxicosis was defined as having TSH levels of <0.1 mIU/L, FT_4 levels >26.0 pmol/L, FT_3 levels >6.0 pmol/L, or any combination of these findings.

Hypothyroidism, including subclinical hypothyroidism, was defined as having TSH levels >4.0 mIU/L. No patients fulfilled this criterion. Measurement of thyroid autoantibodies was deemed unnecessary because the results, in the presence of normal thyroid values, would not influence the patients' progression to therapy.

Laboratory Assay Characteristics

Third-generation TSH and serum FT_4 levels were determined by 2-site sandwich immunoassay with use of an automated chemiluminescent system (Diagnostic Products Corporation, Immulite 2000). The reference range for TSH was 0.4 to 4.0 mIU/L and for FT_4 was 10.0 to 26.0 pmol/L. The coefficients of variation were 5.0% and 5.1% at TSH concentrations of 4.0 mIU/L and 10.0 mIU/L, respectively. For FT_4, the coefficient of variation was 6.5% at 10.0 pmol/L.

Similarly, FT_3 levels were determined by 2-site sandwich immunoassay with use of an automated chemiluminescent system (Beckman Coulter DXI). The reference range was 3.5 to 6.0 pmol/L, with an 8.7% coefficient of variation at 6.0 pmol/L.

Thyroid Nuclear Uptake Scans

Thyroid nuclear uptake scans were performed with use of 99m pertechnetate tracer. The uptake studies were done at approximately 20 minutes after injections.

Statistical Analysis

Thyrotropin values were reviewed graphically and assessed for normality with use of normal distribution plots. Because the results were not normally distributed, logarithmic transformation was applied to data, with normalization of the distribution demonstrated for these transformed values. Mean and standard deviation for the log-transformed values were used to calculate a point estimate for geometric mean TSH with 95% confidence intervals. The results are expressed as means with 95% confidence intervals. Differences between these summary statistics were analyzed with use of the Student *t* test and the Mann-Whitney or Wilcoxon test. The Fisher exact test was used when the minimal expected value with χ^2 testing was less than 5. All statistical analyses were performed by using Stata version 10 software (Stata Corporation, College Station, Texas).

RESULTS

There were 2 patients with thyroid disease during the immediate 6-month follow-up period after treatment of chronic hepatitis C. This constituted a prevalence of approximately 1.0%. The spectrum of autoimmune thyroid disease included thyroiditis (with predominant triiodothyronine activity) and hypothyroidism (likely to be attributable to thyrotropin blocking autoantibody activity). Detailed cases of the 2 patients are presented in the subsequent material. Otherwise, there were no abnormal TSH levels detected at 4-, 12-, and 24-week follow-up assessments. Thyrotropin levels are listed in Table 2. There was no statistically significant difference between any of the follow-up time points.

Because all the TSH levels were normal, no free thyroid measurements were performed. The following 2 new cases of thyroid disease were detected after the end of interferon-based treatment.

Case 1

A 56-year-old woman presented with triiodothyronine-toxicosis 6 weeks after the completion of combination therapy with ribavirin and pegylated interferon-α for HCV infection. She had undergone antiviral therapy during the previous 48 weeks for HCV genotype 1 infection without any thyroid complications and had achieved SVR.

Table 2
Mean Thyroid-Stimulating Hormone Levels With 95% Confidence Intervals at the End of Treatment and at Weeks 4, 12, and 24 After Interferon-Based Therapy for Both 24-Week and 48-Week Treatment Groups[a]

	Time (weeks)			
Factor	0 (end of therapy)	4	12	24
Thyroid-stimulating hormone (mIU/L)				
Mean	1.15	1.10	1.15	1.25
95% confidence intervals	0.37 to 3.58	0.31 to 3.93	0.35 to 3.78	0.36 to 3.97

[a] See "Therapy" section for further details.

She had no previous personal or family history of thyroid disease. As part of treatment protocols, her monthly thyroid function tests for the duration of treatment had shown entirely normal results. Four weeks after the completion of therapy, she began to notice mild dyspnea on exertion, intermittent palpitations, and heat intolerance. There were no other symptoms of thyrotoxicosis. Clinically, she appeared well with a regular pulse of 92 beats//min and a blood pressure of 130/80 mm Hg. No goiter was detected, nor were there any signs of thyrotoxicosis. Her TSH level was undetectable, FT_4 was 24.1 pmol/L, and FT_3 was 8.9 pmol/L. A thyroid pertechnetate uptake scan demonstrated reduced uptake at 2% (reference range, 3% to 8%). Ultrasonography of the thyroid showed that it was normal in size and appearance, except for a mild increase in vascularity. Her thyroid-stimulating immunoglobulins (TSI), human thyroid receptor antibodies (hTRAb), and antithyroglobulin (anti-Tg) and antithyroperoxidase (anti-TPO) antibodies were not detectable. One week later, her thyrotoxicosis persisted (FT_3, 8.4 pmol/L). A diagnosis of interferon-induced thyroiditis (with predominant triiodothyronine activity) was made, and low-dose propranolol was prescribed for symptom relief.

The patient was followed closely with monthly TSH, FT_4, and FT_3 determinations. Eight weeks later, she had entered into the hypothyroid phase, with a TSH level of 54.6 mIU/L, FT_4 of 8.8 pmol/L, and FT_3 of 2.3 pmol/L. Because the patient remained free of any hypothyroid symptoms and in light of the expected recovery from thyroiditides, thyroxine therapy was withheld and propranolol therapy was discontinued. At 16 weeks after the completion of therapy, her thyroid function had returned to normal. At the time of the last consultation, the patient was in excellent health and had normal results of thyroid function tests.

Case 2

A 45-year-old woman presented for a routine examination 8 weeks after her failed combination therapy for chronic HCV. She had generally been well with no remarkable previous medical history, although there was a strong history of thyroid disease in her family, with both her mother and her grandmother experiencing thyroid diseases of undetermined nature, culminating in both requiring thyroxine supplementation. Treatment for her HCV infection (genotype 1) was to be 48 weeks of ribavirin and pegylated interferon-α. After 24 weeks, however, there was no reduction in viral load, and treatment was terminated. Results of her monthly thyroid function tests had been normal until then, and system review on this occasion did not reveal any symptoms suggestive of thyroid disease. Clinical examination at this consultation showed no signs of hypothyroidism. Her vital signs were satisfactory, with normal tendon reflexes and no goiter. A routine TSH level was found to be 48.0 mIU/L in conjunction with an undetectable FT_4 level. The follow-up thyroid ultrasound study revealed the presence of a small gland with a total volume of 6 mL (reference range, 6 to 10). Her anti-Tg and anti-TPO titers were 1:256 and 1:512, respectively. The TSI and hTRAb were 11 IU/L and 11.7 IU/L, respectively. Her thyroid uptake study was reduced at 3% (reference range, 3% to 8%). A diagnosis of autoimmune hypothyroidism was made, and levothyroxine therapy was started. The patient's condition was stabilized with use of 100 μg of levothyroxine daily.

At 6-month review, the anti-Tg, anti-TPO, and TSI antibodies as well as hTRAb were undetectable. In retrospect, the combination of high TSI and hTRAb titers with hypothyroidism and a low result on a thyroid pertechnetate uptake study was consistent with the presence of thyrotropin blocking antibodies. On the basis of this probable underlying mechanism for the hypothyroidism, levothyroxine therapy was discontinued. The patient remained well and independent of thyroxine supplementation for the subsequent 12 months. Interferon-induced thyroiditis (in the hypothyroid phase) was unlikely because there was no hyperthyroid phase. A functional thyrotropin blocking antibody assay would have been definitive but, unfortunately, was not available.

DISCUSSION

A large body of literature has recognized the immunostimulating effect of interferon—particularly on the thyroid, which remains the commonest endocrine organ affected (7-9). Because of the long-lasting effects of pegylated interferon in which the half-life is prolonged to between 15 and 44 hours (10), there are lingering concerns regarding possible residual immunologic effects beyond the duration of treatment. This is our second report, to complement our previous one (11), focusing specifically on the cohort of patients who did not develop thyroid disease during therapy. This can be compared with several previous studies that documented the long-term outcome of thyroid disease but with use of regular (unpegylated) interferon-α monotherapy, rather than combination ribavirin and pegylated interferon.

Carella et al (12), who conducted follow-up on 114 patients for a mean of 6.2 years, did not find any overt thyroid dysfunction, apart from subclinical hypothyroidism in 12 cases at the end of therapy and 7 cases at the end of follow-up, when TSH levels ranged between 5.9 and 8.1 mIU/L. Doi et al (13) found 6 cases of thyroid disorders after the completion of interferon therapy in patients with chronic hepatitis C. No overt thyroiditis was observed, contrary to this current series. In a study by Morisco et al (14) of 136 patients with chronic hepatitis C, 2 cases of hypothyroidism were found at the end of follow-up. Both patients had normal thyroid function throughout therapy. Tong et al (15) studied the efficacy of another unpegylated form, consensus interferon, versus interferon-α (both in combination

with ribavirin). This study included a follow-up period of 24 weeks after treatment, coinciding with the SVR review. Although both hypothyroidism and hyperthyroidism were found, no specific mention was made of their timing or characteristics. Our previous analysis (11) arrived at a similar conclusion, in which thyroid disease was uncommon in this setting. Among 61 patients, Vezali et al (16) detected 2 cases of thyroid disease at 1 month and a further 3 cases at 6, 6.5, and 26 months after treatment completion. Details of the 2 relevant cases were not apparent, but the definition of thyroid disease in this report appeared unconventional. On the basis of this definition, many of the cases of thyroid dysfunction may simply represent nonthyroidal illness as a direct effect of HCV infection or treatment.

In other forms of autoimmunity after interferon therapy, Fabbri et al (17) analyzed the prevalence of autoimmune gastritis during the 12 months after therapy and similarly found a regression of the condition. Betterle et al (18) studied pancreatic and gastric autoantibodies in 70 patients for at least 4 years after interferon therapy. The former tended to remain stable; however, the gastric autoantibodies increased titers, or antibody-negative patients underwent seroconversion. Both these cohorts were treated with regular interferon monotherapy only. Our study was limited to 6 months after treatment and addressed the hard end point of elevation of the TSH level without thyroid autoantibodies. Thus far, it is the only report to study the aftereffect of combination ribavirin and pegylated interferon therapy on thyroid tissue during this time interval.

The pathophysiologic features of this condition are consistent with the waning influence of interferon therapy, with no long-lasting effect on thyroid tissue. The individual mechanisms in each case remain to be determined, but certainly there is an individual or genetic predisposition to the development of such disease. Exposure to iodine was considered a factor, but it does not appear to influence thyroid outcome in patients treated with interferon-α monotherapy (19). In itself, HCV has an immunostimulatory effect on the thyroid, and perhaps this influence has been substantially diminished, especially in those patients who have achieved an early virologic response (at 12 weeks of therapy) or end-of-treatment virologic response.

Although thyroid disease developed in only 1.0% of patients in our study, these 2 patients demonstrated quite different forms of autoimmune thyroid disease. Nevertheless, too few cases are involved to arrive at any definitive pattern of thyroid disease in this clinical setting. Silent thyroiditis of both biphasic and triphasic nature was considered but deemed unlikely because of the presence of normal thyroid surveillance profiles before the development of thyroid disease. It is, however, reassuring that the prevalence is low, a finding that potentially eliminates the need for routine and repetitive thyroid function tests after therapy. In light of the relatively low cost of TSH testing, it is worthwhile considering a screening TSH measurement at each visit (1, 3, and 6 months after treatment) in the absence of symptoms. On analysis of our data, the number needed to test is 190 for every case of *asymptomatic* thyroid disease. At approximately 30 A$ per TSH test, the total would approximate 6,000 A$ for each case of thyroid disease detected, in comparison with 24,000 A$ for a 48-week course of interferon therapy, not including ribavirin. An alternative strategy would be to monitor for symptoms and allow for case detection. This is not applicable to our case 2, however. The latter appears a more logical option because our patients are relatively young and cognizant of early symptoms. In addition, follow-up visits are more challenging because they tend to be more mobile and itinerant. Although it is already common practice that most hepatologists do not assess TSH levels in this setting, these data provide reassuring and supportive evidence.

As previously mentioned, it is important to note that these recommendations apply strictly to the thyroid-naïve population who had been treated with combination interferon and ribavirin. For those patients in whom thyroid disease (predominantly thyroiditis) develops during treatment, the long-term natural history remains far from being determined, and recent recommendations regarding the long-term monitoring of thyroid outcomes in this setting appear premature and lacking in details (20).

CONCLUSION

There is a very small effect (if any) of interferon-based therapy on thyroid function after its completion. Despite the negative results of the current study, it is reassuring that patients who do not develop thyroid disease during therapy and who remain asymptomatic with a normal ensuing TSH level do not need to undergo further follow-up (from the aspect of thyroid function) after their SVR review.

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DISCLOSURE

The authors have no multiplicity of interest to disclose.

AUTHORS' CONTRIBUTIONS

Professor Huy A. Tran conceived the study, participated in its design, assisted with data collection, and coordinated the drafting of the manuscript. Professor Glenn E. M. Reeves, Elizabeth A. Ianna, and Nadine Leembruggen collected the data and participated in the study design. All authors participated in the discussion and drafting of the manuscript. All authors read and approved the final revised manuscript.

REFERENCES

1. **World Health Organization.** Hepatitis C. Factsheet No. 164, revised October 2000. https://apps.who.int/inf-fs/en/fact164.html. Accessed for verification August 15, 2010.
2. **Yohannes K, Roche P, Blumer C, et al.** Australia's notifiable diseases status, 2002: annual report of the National Notifiable Diseases Surveillance System. *Commun Dis Intell*. 2004;28:6-68.
3. **Armstrong GL, Wasley N, Simard EP, McQuillan GM, Kuhnert WL, Alter MJ.** The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. *Ann Intern Med*. 2006;144:705-714.
4. **Dienstag JL.** Hepatitis C: a bitter harvest. *Ann Intern Med*. 2006;144:770-771.
5. **Patel K, Muir AJ, McHutchison JG.** Diagnosis and treatment of chronic hepatitis C infection. *BMJ*. 2006;332:1013-1017.
6. **Moreno-Otero R.** Therapeutic modalities in hepatitis C: challenges and development. *J Viral Hepat*. 2005;12:10-19.
7. **Carella C, Mazziotti G, Amato G, Braverman LE, Roti E.** Clinical review 169: interferon-alpha-related thyroid disease; pathophysiological, epidemiological, and clinical aspects. *J Clin Endocrinol Metab*. 2004;89:3656-3661.
8. **Tran HA.** Hepatitis C infection, treatment regimens, and thyroid function abnormalities. *Endocrinologist*. 2007;17:231-235.
9. **Prummel MF, Laurberg P.** Interferon-alpha and autoimmune thyroid disease. *Thyroid*. 2003;13:547-551.
10. **François C, Castelain S, Duverlie G, Capron D, Nguyen-Khac E.** Optimizing the treatment of chronic viral hepatitis C. *Expert Rev Gastroenterol Hepatol*. 2009;3:607-613.
11. **Tran HA, Jones TL, Batey RG.** The spectrum of thyroid dysfunction in an Australian hepatitis C population treated with combination interferon-alpha2beta and ribavirin. *BMC Endocr Disord*. 2005;5:8.
12. **Carella C, Mazziotti G, Morisco F, et al.** Long-term outcome of interferon-alpha-induced thyroid autoimmunity and prognostic influence of thyroid autoantibody pattern at the end of treatment. *J Clin Endocrinol Metab*. 2001;86:1925-1929.
13. **Doi F, Kakizaki S, Takagi H, et al.** Long-term outcome of interferon-alpha-induced autoimmune thyroid disorders in chronic hepatitis C. *Liver Int*. 2005;25:242-246.
14. **Morisco F, Mazziotti G, Rotondi M, et al.** Interferon-related thyroid autoimmunity and long-term clinical outcome of chronic hepatitis C. *Dig Liver Dis*. 2001;33:247-253.
15. **Tong MJ, Reddy KR, Lee WM, et al (Consensus Interferon Study Group).** Treatment of chronic hepatitis C with consensus interferon: a multicenter, randomized, controlled trial. *Hepatology*. 1997;26:747-754.
16. **Vezali E, Elefsiniotis I, Mihas C, Konstantinou E, Saroglou G.** Thyroid dysfunction in patients with chronic hepatitis C: virus- or therapy-related? *J Gastroenterol Hepatol*. 2009;24:1024-1029.
17. **Fabbri C, Jaboli MF, Giovanelli S, et al.** Gastric autoimmune disorders in patients with chronic hepatitis C before, during and after interferon-alpha therapy. *World J Gastroenterol*. 2003;9:1487-1490.
18. **Betterle C, Fabris P, Zanchetta R, et al.** Autoimmunity against pancreatic islets and other tissues before and after interferon-alpha therapy in patients with hepatitis C virus chronic infection. *Diabetes Care*. 2000;23:1177-1181.
19. **Minelli R, Braverman LE, Valli MA, et al.** Recombinant interferon alpha (rIFN-alpha) does not potentiate the effect of iodine excess on the development of thyroid abnormalities in patients with HCV chronic active hepatitis. *Clin Endocrinol (Oxf)*. 1999;50:95-100.
20. **Wong VW, Cheng AY, Chan HL.** Interferon-induced thyroid dysfunction—not always transient. *J Gastroenterol Hepatol*. 2009;24:938-940.

CHAPTER VII. THE EFFECT OF THYROID DISEASE ON SUSTAINED VIROLOGIC RESPONSE OUTCOMES

As thyroid disorders in this setting are better understood, it was speculatively observed in a meta-analysis that the development of thyroid diseases might be associated with an increased rate of SVR. This is despite of many other favorable treatment response prognostic factors. The speculation heightened with two reported cases occurring in a natural experimental setting, followed by a nested case-control study. This observation is critical because if confirmed, thyroxine supplement offers a significant adjunctive and relatively safe addition to the current standard of therapy.

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HEPATOLOGY

Development of thyroid diseases in the treatment of chronic hepatitis C with α-interferon may be a good prognosticator in achieving a sustained virological response: A meta-analysis

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Abstract

Background and Aim: Thyroid dysfunction is the most common endocrinopathy associated with hepatitis C and its interferon-based treatment. When undergoing treatment, interferon and ribavirin synergize to potently stimulate the immune system in order to eradicate the virus. One of the innocent bystanders in this accentuated response is the thyroid. The present study investigated whether thyroid dysfunction while undergoing combination treatment for hepatitis C is a favorable prognostic maker for a sustained virological response.

Methods: We carried out a prospective clinical audit in 201 patients treated with combination ribavirin and α-interferon and determined the prevalence of sustained virological response in patients in association with thyroid disease. A meta-analysis was also carried out pooling 741 patients from four previous studies on this topic.

Results: There was positive and significant association between thyroid disease and viral clearance. This was not supported by the meta-analysis, however, and some plausible explanations are proffered for this inconsistency.

Conclusion: Despite lacking supportive evidence from the meta-analysis, it is important that this information is confirmed (or refuted) in future studies.

Introduction

Hepatitis C is one of the major chronic hepatic infections in the world and carries with it a significant rate of cirrhosis and hepatoma, particularly in developing countries. In Australia[1,2] and the USA,[3,4] the total number of cases is not insignificant. Unfortunately, the incidence, and associated sequelae, has been predicted to increase in the coming decades.[5] Consequently, a large and escalating number of patients will be expected to undergo treatment for hepatitis C. Of those receiving combination treatment with α-interferon (IFN-α) and ribavirin (RBV), approximately 5–10% will develop thyroid-related complications. While there are a number of factors in the prediction of a favourable hepatic outcome such as genotype, ethnicity and early viral load reduction,[6] there are few published reports that assess the development of thyroid disease (TD) in relation to sustained virological response (SVR). The presence of TD may signify a supercharged and heightened immune response which, in turn, increases the chance of eradicating the virus. Thus, the TD can be looked upon as a potential prognostic marker of SVR. The aim of the present report is to investigate this hypothesis in a cohort of treated hepatitis C patients at our center and to review other recent studies on this question.

Methods

Patients

A prospective clinical audit was carried out involving 201 patients who received combination therapy over a 36-month period between 2005 and 2007 in a major tertiary referral hospital in New South Wales (NSW), Australia. All patients were treated according to clinical needs with a predetermined IFN-based treatment regimen and thyroid surveillance protocol.[7] No ethical approval was required. All other causes of chronic hepatitis were excluded. No patient had dual hepatitis B and C. All were monitored carefully for protocol adherence. All patients were reviewed at monthly intervals, including those who developed TD (as defined below) with thyrotropin (TSH) levels, full blood counts, renal and liver function tests. No routine thyroid autoantibodies were carried out as previously reported.[7] Where TSH levels were above the reference range or undetectable, either at baseline or at any time

during the study, the patients were reviewed by the supporting endocrinologist.

Laboratory assays

Third generation serum thyrotropin (TSH), serum free tetra- and tri-iodothyronine (fT4 and fT3) were determined by two-site sandwich immunoassay using an automated chemiluminescent system (Immulite 2000; Diagnostic Products Corporation, Los Angeles, CA, USA). The reference range (RR) for TSH was 0.4–4.0 mU/L, fT4 10.0–26.0 and fT3 3.5–5.5 pmol/L. The coefficients of variations (CV) were 5.0% and 5.1% at TSH concentrations of 4.0 mU/L and 10.0 mU/L, respectively. For fT4, the CV was 6.5% at 10.0 pmol/L and for fT3 the CV was 8.9% at 3.5 pmol/L.

Quantitative measurement of serum hepatitis C virus (HCV)-RNA was detected using the Cobas AmpliPrep/Amplicor™ HCV test, version 2.0 (Roche Diagnostics, Basel, Switzerland). The lowest limit of detection of this assay is 20 IU per mL. HCV genotype testing was assayed using a second-generation reverse hybridization, line probe assay (Inno-LiPA HCV II; Innogenetics, Zwijndrecht, Belgium).

Therapy

All patients were treated with combination pegylated IFN-α and RBV therapy. The duration of treatment depends on the HCV genotypes; genotypes 2 and 3 were treated for 24 weeks and types 1 and 4 for 48 weeks. Treatment was further adjudicated according to viral load results for genotypes 1 and 4 (see below). For genotypes 2 and 3, treatment was continued to the end irrespective of the qualitative viral load at 4 weeks. Where TD developed, treatment was continued in 10 (out of 11) cases. This was done after consultation with the patients who were informed and prepared to continue, fully aware of the potential TD. In a solitary case, treatment was ceased at 14 weeks (out of 24) for genotype 3. The dosage for pegylated IFN-α ranged between 80 and 120 μg weekly and the RBV dose ranged from 1000 to 1200 mg daily; both according to bodyweight.

Thyroid disease assessment

All patients underwent routine TSH level assessments at the start of treatment and at monthly intervals. When the TSH level was undetectable, free T4- and T3 levels were carried out, followed by an endocrinological and clinical assessment from which further imaging investigations were determined. All patients had thyroid ultrasounds and thyroid pertechnetate uptake scans. Anti-thyroperoxidase (anti-TPO), anti-thyroglobulin (anti-Tg) antibodies and thyroid stimulating immunoglobulin (TSI) levels were carried out at diagnosis of thyroid disease, 4 weeks after thyroiditis and at the completion of the IFN course. All were followed up at 4-weekly intervals until the end of therapy, in 6 months time as part of the routine HCV treatment review and again in 12 months time.

Thyroid disease was clinically and/or biochemically defined as hypo- or hyperthyroidism. The biochemical criteria for thyrotoxicosis were TSH of < 0.1 mU/L, fT4 levels > 26.0 and/or fT3 levels > 5.5 pmol/L. For hypothyroidism, including subclinical hypothyroidism, TSH levels had to be > 4.0 mIU/L.

Virological response surveillance protocol

The following viral load testing protocols were used during the study.

1. Quantitative HCV-RNA polymerase chain reaction (PCR) assays were carried out on all patients irrespective of genotype.
2. For genotypes 1 and 4, qualitative viral load assays were done at weeks 4, 24, 48 and 24 weeks after the completion of therapy. An additional quantitative assay was done at week 12. Treatment was terminated if there was ≤ 2 $\log_{10}$ reduction of viral load compared with baseline at the 12th week of therapy or if the qualitative viral load was positive at the 24th week.
3. For genotypes 2 and 3, qualitative viral load assay was done at weeks 4, 24 (at the completion of therapy) and at week 24 follow up.

Early virological response (EVR) was defined as ≥ 2 $\log_{10}$ reduction in viral load at 12 weeks of therapy compared with baseline.

SVR was defined as serum HCV-RNA being undetectable by PCR at 24 weeks following the completion of therapy.

Meta-analysis and statistical methods

Because of the scarcity of reported thyroid diseases in relation to SVR in the literature, we carried out a literature search over a 10-year period from 1998 to the present time using PubMed. Keywords entered in the search consisted of 'hepatitis C, thyroid disease, thyroid dysfunction, interferon treatment, ribavirin and sustained virological response'. The inclusion criteria were:

1 Case–control or cohort design of the studies.
2 Availability of data on thyroid and non-thyroid disease subgroups.
3 Final SVR status.
4 Treatment must include either IFN alone or in combination with RBV.

Data are presented as percentage and mean ± standard error of mean (SEM). Fisher's exact test was used to compare sustained viral response in those with and without TD. Meta-analysis of previous studies was carried out using StatsDirect (StatsDirect, Cheshire, UK).

Results

The baseline characteristics of the cohort are presented in Table 1.

Thyroid disease

No thyroid abnormality was detected at baseline. Eleven patients (seven females and four males) developed TD during the course of treatment. All developed biphasic thyroiditis and characteristics have been previously documented.[8] In summary, mean time to the onset of thyroiditis after the initiation of treatment was 18 weeks (95% confidence interval [CI]: 2.5–33.9), thyrotoxic phase 6.8 weeks (95% CI: 0.3–13.3), hypothyroid phase 5.1 weeks (95% CI: 0.0–13.1). All made a complete and sustained thyroid recovery

Table 1 Characteristics of the 201 patients including those with and without thyroid disease

	All patients $n = 201$	Thyroid dysfunction $n = 11$	No thyroid dysfunction $n = 190$	*P* value
Female gender (no. [%])	97 (48%)	7 (63%)	90 (47%)	0.35
Mean age (years)	49 ± 8	48 ± 9	50 ± 8	0.42
Weight (kg)	74 ± 18	76 ± 21	74 ± 18	0.36
Body mass index (kg/m^2)	27 ± 7	26 ± 9	28 ± 8	0.42
Asian origin (no. [%])	36 (18%)	0	36 (19%)	0.53
Caucasians (no. [%])	151 (75%)	11 (100%)	140 (74%)	0.80
Genotypes (no. [%])				< 0.001
1	100 (50%)	4 (36%)	95 (50%)	
2	16 (8%)	3 (27%)	13 (7%)	
3	76 (38%)	2 (18%)	74 (39%)	
4	9 (4%)	2 (18%)	8 (4%)	
Viral load				
At baseline (log IU/mL)	5.97 ± 0.74	5.92 ± 0.80	5.98 ± 0.79	
Positive EVR at 12 weeks (this is only applicable to genotypes 1 & 4)†	75/109 (69%)	6/6 (100%)	70/103 (68%)	0.59
Positive ETR (at 24 weeks for genotypes 2 & 3 and 48 weeks for genotypes 1&4)‡	161/201 (80%)	11/11 (100%)	143/190 (75%)	0.75
SVR (24 weeks post-therapy)	110/201 (54%)	11/11 (100%)	99/190 (53%)	0.99
Albumin (36–48 g/L)	36 ± 2	36 ± 3	35 ± 8	0.68
Serum bilirubin (2–20 μmol/L)	15 ± 5	14 ± 7	16 ± 4	0.13
Alanine aminotransferase (< 45 U/L)	79 ± 22	65 ± 18	72 ± 20	0.26
γ-Glutamyl transpeptidase (1–30 U/L)	53 ± 14	62 ± 11	54 ± 15	0.08
Prothrombin time (11–18 s)	15 ± 2	14 ± 3	15 ± 3	0.28
Hemoglobin (115–165 g/L)	154 ± 12	154 ± 13	153 ± 10	0.75
White cell counts (4.0–11.0 × 10^6/mL)	5.8 ± 1.9	6.9 ± 2.5	5.9 ± 2.2	0.15
Platelets (150–400 × 10^9/mL)	166 ± 37	181 ± 29	168 ± 38	0.27

†Defined as ≥ 2 $\log_{10}$ viral load reduction compared with baseline by quantitation.

‡No viral RNA is detected at the completion of treatment.

Due to the small number in the TD group, there is inadequate statistical power to arrive at any definitive conclusions.

ETR, end of treatment response; EVR, early virological response; SVR, sustained virological response.

after completion of treatment. Fisher's exact test shows that this difference in viral response between the thyroid groups is markedly significant ($P = 0.0001$).

Early virological response

All six patients (100%) with genotypes 1 and 4 achieved EVR as defined. This is compared with 68% in the non-TD group.

Sustained virological response

Out of a total of 201 patients, 91 eradicated their hepatitis C virus. Of the 11 patients who developed TD, 11 out of 11 (100%) achieved SVR as defined. All six patients with genotypes 1 and 4 achieved EVR as defined by a more than 2 $\log_{10}$ reduction in viral load at 12 weeks of therapy. The characteristics of patients with and without TD are summarized in Table 1.

Meta-analyses

Out of 21 publications, four studies were identified as suitable (excluding this report), yielding a total of 741 subjects.[9–12] The pooled odds ratio was 1.24 (95% confidence interval [CI] = 0.77 to 2.01) by the fixed effects model, and 1.22 (95% CI = 0.68 to 2.19) by the random effects model (Fig. 1). Both of these indicate no significant difference in viral response between thyroid dysfunction groups, and this estimate was homogeneous (Breslow-Day, $P = 0.27$, I^2 [inconsistency] = 20%) with no publication bias (Egger's $P = 0.52$).

Discussion

The prevalence of hepatitis C is now controlled in the Western world[3,4] but continues to increase in developing countries due to lack of needle exchange programs and medical infrastructure. The current established and accepted treatment for chronic hepatitis C infection is the combination of pegylated IFN-α2b or –α2a and RBV. This treatment regimen delivers a cure rate of approximately 50% of cases.[13] The favorable factors in response to treatment include genotypes (genotypes 2 and 3 have a better response), compliance, duration of treatment and early viral load reduction, particularly for genotype 1.[6] Additional positive prognosticators consist of body mass index, coexisting liver disease and ethnicity (Asians and Caucasians have a higher SVR than African Americans).[14] However, the relationship between TD and SVR has not been addressed previously. Hypothetically, when treatment is delivered, the immune system is stimulated sufficiently in an attempt to eradicate the virus and, in the process,

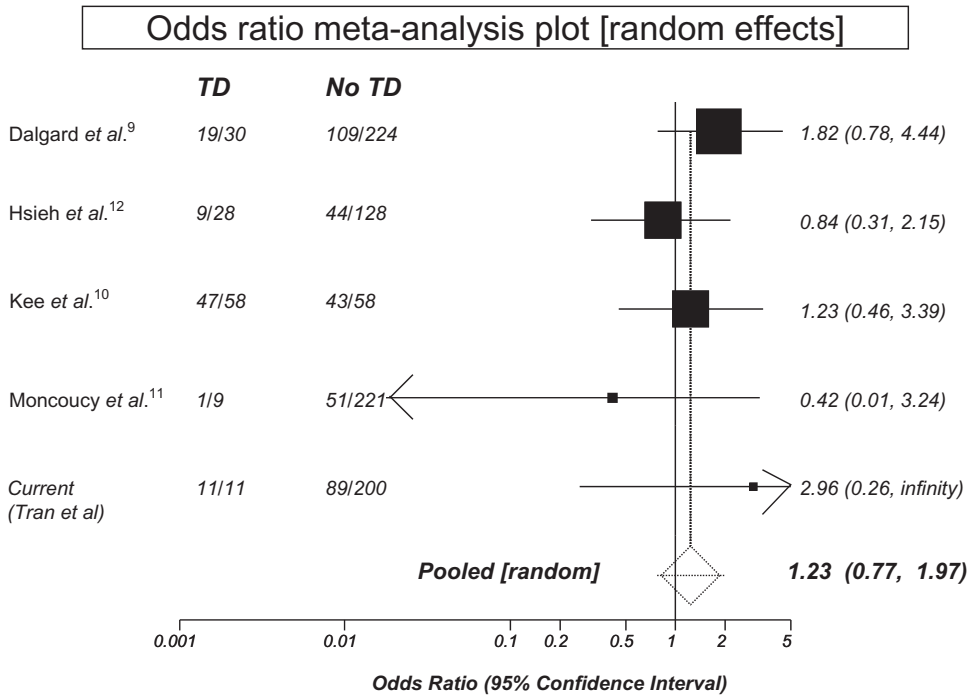

Figure 1 Meta-analysis from five reported studies, including the present report, showing a null effect. TD, thyroid disease.

Table 2 Summary of the major characteristics of the four reports in the meta-analysis

Demographics	Dalgard *et al.*[9]	Hsieh *et al.*[12]	Kee *et al.*[10]	Moncoucy *et al.*[11]	Current report
No. patients	254	150	116	221	201
Gender (F)	101 (40%)	69 (46%)	50 (43%)	77 (35%)	97 (48%)
Mean age	39	46 ± 12	50 ± 12	42 ± 13	49 ± 8
Ethnicity	19 (7%) Asians	NS	NS	NS	50 (25%) Caucasians
Genotype distribution	Type 1: 52%	Type 1: 36%	Type 1: 46%	Incomplete	Type 1&4: 54%
	Type 2&3: 49%	Type 2a, 2b and mixed: 64%	Non-type 1: 54%		Type 2&3: 46%
Treatment regimen	IFN & RBV	IFN alone	IFN & RBV	IFN & RBV	IFN & RBV
Overall SVR	128 (55%)	53 (35%)	92 (80%)	51 (24%)	90 (45%)

The genotype distribution in the report by Moncoucy *et al.*[11] was incomplete and the majority of the reports did not state genotype 4 percentage. The inconsistently high overall SVR observed by Kee *et al.*[10] is poorly understood despite the similar genotype distribution.
IFN, interferon; NS, not stated; RBV, ribavirin.

may also be enough to trigger TD as an unintended consequence. Studies have shown that in patients who developed TD, immune marker levels such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) and chemokines are relatively higher and thus, in the process, are potentially more effective at achieving SVR.[15,16] Furthermore, the presence of thyroid hormones *in vitro* also potentiates the antiviral action of IFN-γ in cultured human cells.[17] This hypothesis is clearly confirmed in our cohort, albeit in an isolated study in which all 11 IFN-related thyroid cases achieved SVR. This observation was further explored with the review of the literature, as the prevalence of TD in IFN-treated patients is low. Four studies (other than the present study), totalling 741 subjects (Table 2), were found to be suitable for further investigating this question.

The pooled data indicate a lack of association between TD and SVR in chronic hepatitis C infection and do not support our observation. The reasons for this discrepancy are not clear but possibilities include the following. First, previous patients were treated with regular IFN-α (in combination with RBV) rather than the pegylated form. However, despite the evidence to date that SVR is superior with the pegylated form,[13] the pegylation of IFN does not aggravate thyroid function in comparison with regular IFN.[18] Second, there may be differences in the epidemiology of the population in terms of age. At a population level, thyroid dysfunction is uncommon before 50 years of age, paralleling the prevalence of autoimmune thyroid disease and, hence, accounting for the low prevalence of thyroid diseases in the younger age group.[19] This plausibly accounts for the infrequent abnormal thyroid function

tests at baseline prior to therapy initiation. However, females are well documented to develop TD,[20] although there is no gender difference between the studies (Table 2). Third, the frequency with which thyroid indices were checked in previous studies may have been insufficient and thus may have been completely overlooked in the development of TD. These patients would then be misclassified as having no TD which would bias towards the null hypothesis and obscure this association. With the exception of our study in which thyroid testing is based on the half-life of T4, other studies measured thyroid function tests in an ad hoc basis. Dalgard *et al*.[9] and Kee *et al*.[10] measured TFT every 3 months, Moncoucy *et al*.[11] every 2 or 3 months and Hsieh *et al*. every 4 weeks for 24 weeks, followed by 8 weeks for another 24 weeks.[12] Furthermore, the development of TD may lead to premature and perhaps unwarranted termination of treatment.[11,12] There is no formal recommendation or randomized data in such a clinical scenario and the decision to continue with treatment depends primarily on the treating physicians. In our cohort, all patients were informed of the potential risks versus benefits and all but one decided to complete treatment. This therefore may account for the higher SVR. Fourth, the usefulness of TD as a marker of adequate immunostimulation may be modified by the iodine status according to the local geography of the studied cohorts. In an iodine adequate state, there is a higher prevalence of thyroid autoimmunity in comparison with iodine-deficient areas.[21] Iodine increases or modulates autoimmune reactions in animal models with genetic susceptibility but the evidence is less clear in humans.[22] There are no data on iodine status specific to our cohort. However, the data suggest a generally mild/borderline iodine deficiency in the larger surrounding NSW area.[23]

There are some caveats to this study. First is the lack of complete histopathological data on liver biopsies. The latter became optional towards the second half of the study, especially for genotypes 2 and 3. Second, the meta-analysis includes small studies and is not individual/patient-based. While much more revealing, an individual patient data meta-analysis (IPDMA) is a much more labor-intensive process and is beyond the scope and resources of this project. In addition, the response to collaborative requests for IPDMA is invariably poor. IPDMA is also particularly relevant where the pooled effect size is heterogeneous and this is not the case in the present report; indeed, the pooled effect size is clinically and statistically homogeneous, negating the need for an IPDMA. Third, as this is a clinical audit, we had group and not individual variables, making a multivariate analysis impossible. While audits lack control over variables, these are arguably more representative of the heterogeneous population in clinical practice, extending and improving on the validity of the study.

Notwithstanding the aforementioned shortcomings, our study finds supportive evidence that the development of TD in HCV patients while undergoing combination with RBV and IFN-α treatment is a potentially favorable prognostic factor for achieving SVR. The meta-analysis does not support our findings, but points to the ad hoc thyroid surveillance as a possible explanation. This report merely seeks to generate and highlight this hypothesis and this valuable piece of information clearly requires further research and validation in other cohorts. If confirmed, both patients and clinicians may be heartened to persevere with treatment when this complication arises, with the expectation that SVR is likely to be achieved in the end.

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References

1 World Health Organization. *Hepatitis C. Factsheet No. 164 (updated October 2000)*. Cited 21 May 2009. Available from URL: http://www.who.int/mediacentre/factsheets/fs164/en/print.html
2 Yohannes K, Roche P, Blumer C, Spencer J, Milton A, Bunn C, Gidding H, Kirk M, Della-Porta T. *Australia's Notifiable Diseases Status, 2002: Annual report of the National Notifiable Diseases Surveillance System*. Cited 21 May 2009. Available from URL: http://www.health.gov.au/internet/main/publishing.nsf/Content/cda-pubs-2004-cdi2801-pdf-cnt.htm/$FILE/cdi2801b.pdf
3 Armstrong GL, Wasley N, Simard EP, McQuillan GM, Kuhnert WL, Alter MJ. The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. *Ann. Intern. Med.* 2006; **144**: 705–14.
4 Dienstag JL. Hepatitis C: a bitter harvest. *Ann. Intern. Med.* 2006; **144**: 770–1.
5 Shephard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. *Lancet Infect. Dis.* 2005; **5**: 558–67.
6 Patel K, Muir AJ, McHutchinson JG. Diagnosis and treatment of chronic hepatitis C infection. *BMJ* 2006; **332**: 1013–17.
7 Tran HA, Jones TL, Batey RG. The spectrum of thyroid disease treated with interferon-α2b and ribavirin in an Australian population. *BMC Endocr. Disord.* 2005; **5**: 8.
8 Tran HA, Reeves GEM, Jones TL. The natural history of interferon-α2b induced thyroiditis and its exclusivity in a cohort of patients with chronic hepatitis C infection. *QJM* 2008 Nov 25, Epub ahead of print.
9 Dalgard O, Bjoro K, Hellum K *et al.* Thyroid dysfunction during treatment of chronic hepatitis C with interferon alpha: no association with either interferon dosage or efficacy of therapy. *J. Intern. Med.* 2002; **251**: 400–6.
10 Kee K-M, Lee C-M, Wang J-H *et al.* Thyroid dysfunction in patients with chronic hepatitis C receiving a combined therapy of interferon and ribavirin: Incidence, associated factors and prognosis. *J. Gastroenterol. Hepatol.* 2006; **21**: 319–26.
11 Moncoucy X, Leymarie F, Delemer B *et al.* Risk factors and long-term course of thyroid dysfunction during anti-viral treatments in 221 patients with chronic hepatitis C. *Gastroenterol. Clin. Biol.* 2005; **29**: 339–45.
12 Hsieh M-C, Yu M-L, Chuang W-L *et al.* Virologic factors related to interferon-alpha-induced thyroid dysfunction in patients with chronic hepatitis C. *Eur. J. Endocrinol.* 2000; **142**: 431–7.
13 Manns MP, Wedemeyer H, Cornberg C. Treating viral hepatitis C: efficacy, side effects and complications. *Gut* 2006; **55**: 1350–9.
14 Yan KK, Guirgis M, Ding T *et al.* Treatment responses in Asians and Caucasians with chronic hepatitis C infection. *World J. Gastroenterol.* 2008; **14**: 3416–20.
15 Cargnel A, Gubertini G, Magnani C, Bosis S, Colombo F, Clerici M. Immune response in chronic hepatitis C-infected patients undergoing interferon treatment. *Hepatology* 1999; **30**: 584–5.
16 Rotondi M, Minelli R, Magri F *et al.* Serum CXCL10 levels and occurrence of thyroid dysfunction in patients treated with interferon-alpha therapy for hepatitis C virus-related hepatitis. *Eur. J. Endocrinol.* 2007; **156**: 409–14.
17 Lin H-Y, Thacore HR, Davis PJ, David FB. Thyroid hormone potentiates the antiviral action of interferon-gamma in cultured human cells. *J. Clin. Endocrinol. Metab.* 1994; **79**: 62–5.
18 Tran HA, Attia JR, Jones TL, Batey RG. Pegylated interferon-α2b in

combination with ribavirin does not aggravate thyroid dysfunction in comparison to regular interferon-α2b in a hepatitis C population: Meta-analysis. *J. Gastroenterol. Hepatol.* 2007; **22**: 472–6.
19 Hollowell JG, Staehling NW, Flanders WD *et al.* T_4, and thyroid antibodies in the United States population (1988 to 1994): national health and nutrition examination survey (NHANES III). *J. Clin. Endocrinol. Metab.* 2002; **87**: 489–99.
20 Tran HA. Hepatitis C infection, treatment regimens, and thyroid function abnormalities. *Endocrinologist* 2007; **17**: 231–5.
21 Papanastasiou L, Vatalas I-A, Koutras DA, Mastorakos G. Thyroid autoimmunity in the current iodine environment. *Thyroid* 2007; **17**: 729–39.
22 Ruwhof C, Drexhage HA. Iodine and thyroid autoimmune disease in animal models. *Thyroid* 2001; **11**: 427–36.
23 Li M, Eastman CJ, Waite KV *et al.* Australian children iodine deficient? Results of the Australian National Iodine Nutrition Study. *Med. J. Aust.* 2006; **184**: 165–9.

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Case report

QJM

The adjuvant role of thyroxine in the treatment of chronic hepatitis C infection

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Introduction

Thyroid disease (TD) is the commonest extra-hepatic complication in patients with chronic hepatitis C undergoing combination interferon-α and ribavirin treatment. About 5–10% develops TD of various aetiologies during the course of treatment.[1,2] It had been suggested that patients who developed thyroid diseases tend to achieve sustained virological response more readily[3] although a meta-analysis did not support this contention.[4] Furthermore, the underlying pathogenetic mechanisms are poorly understood. The following 2 cases are presented to support this observation. In both cases, initial therapies were unsuccessful with normal thyroid function, whereas re-treatment with interferon-α (and ribavirin) in the presence of overt TD resulted in successful sustained viral clearance. Although anecdotal, these cases lend further evidence to the potential synergistic effect of thyroid hormone and interferon therapy. Further research is required to support this hypothesis.

Case presentation

Case 1

A 48-year-old woman presented for ongoing management of her TD after her first course of interferon therapy. She had chronic hepatitis C of genotype 1 with cirrhosis. She underwent a first course of treatment with combination interferon-α (IFN-α) and ribavirin (RBV) for 48 weeks and did not achieve sustained virological response (SVR) at the end of treatment despite good compliance. She had regular monthly thyroid function tests throughout and they were entirely normal. Four weeks after completion, she developed Graves' disease (GD) with hyperdynamic cardiovascular activity in the presence of a confirmed diffuse goitre. Her thyrotropin (TSH) was undetectable [reference range (RR), 0.4–4.0 mU/l]; free tetra-iodothyronine (fT4) was 28.7 (RR, 10.1–24.5 pmol/l), free tri-iodothyronine (fT3) was 6.9 (RR, 3.3–5.8 pmol/l). Her anti-thyroglobulin (anti-Tg) antibody was normal at 1:64 (RR, < 1 : 400), anti-thyroperoxidase (anti-TPO) titre 1 : 640 (RR, 1: 400), and thyrotropin stimulating immunoglobulin (TSI) titre 14 (RR, < 10 U/ml). The thyroid pertechnetate uptake scan showed diffuse uptake at 11%, (RR: 3–8%). The endocrinology team confirmed the diagnosis and began treatment with carbimazole. Her thyroid condition came under control within the ensuing 12 weeks. Six weeks after completion of the first course of combination interferon therapy, she commenced a second course for an additional 48 weeks. Her thyroid status remained normal during this period with a maintenance dose of carbimazole. Her antiviral therapy was otherwise uneventful. Carbimazole therapy was ceased after 18 months, coinciding with the time of SVR. Her viral load was found to be undetectable, confirming SVR. When

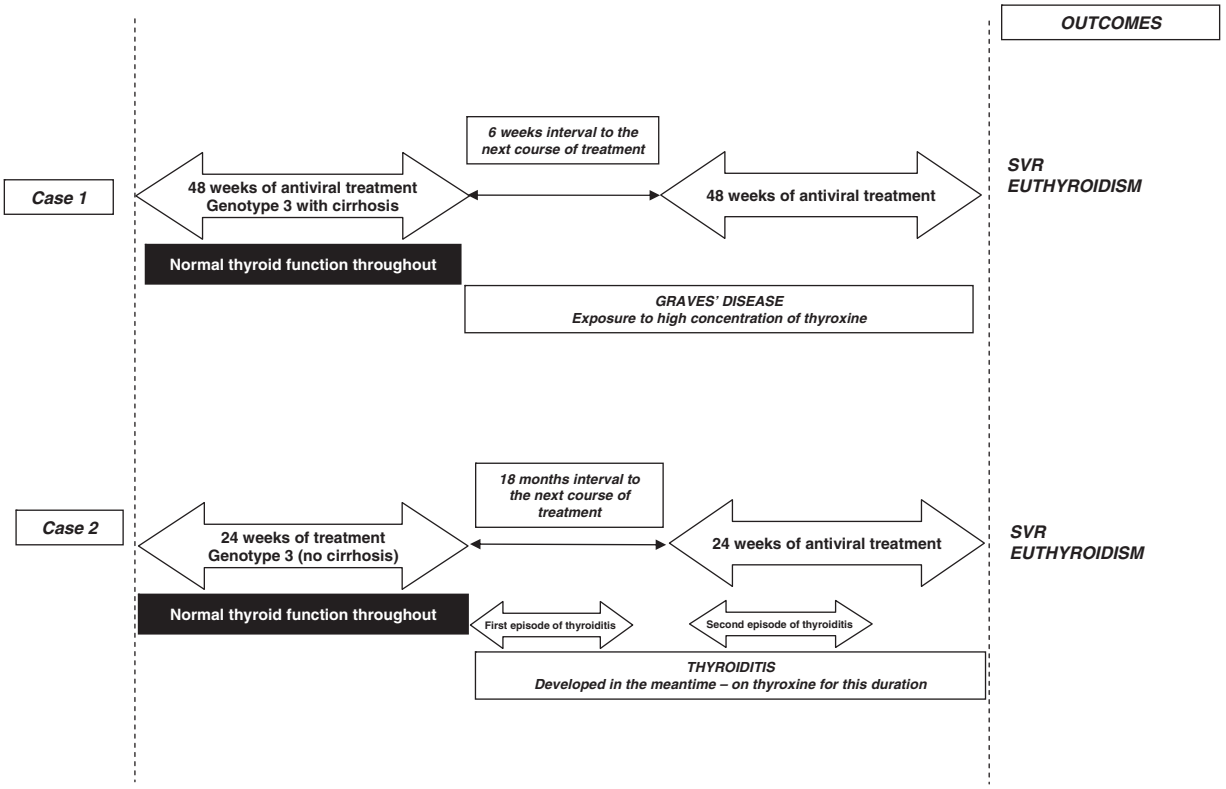

Figure 1. Schematic summaries of the cases and their final thyroid outcomes. The arrow bars indicate the duration of combination therapy with interferon-α and ribavirin. See text for detailed discussions. SVR: Sustained Virologic Response.

reviewed 24 months after Graves' diagnosis, she remained euthyroid without any medication. Figure 1 summarizes her sequence of events diagrammatically.

Case 2

A 45-year-old female, the biological younger sister of Case 1, presented with acute thyroiditis following the completion of a course of IFN-α and RBV therapy. She had chronic hepatitis C of genotype 3 and had been treated with the combination therapy for the previous 24 weeks uneventfully. She too had regular monthly thyroid function tests during treatment without any abnormalities. She then developed diarrhoea and palpitations 2 weeks after the completion of therapy. Further clinical assessment found her to be thyrotoxic in the absence of a goitre. Her thyroid pertechnetate uptake scan was negligible at 1%. Her anti-Tg antibodies were elevated at 1 : 1280. Her anti-TPO antibody and TSI were not elevated. A diagnosis of interferon-induced thyroiditis was made. This was further supported by the natural progression of the disease when she developed hypothyroidism and required thyroxine supplement. Unfortunately, SVR was not achieved after the first course of thyroid treatment. Eighteen months further on, she underwent a second antiviral course with interferon-α and ribavirin. Thyroxine was continued throughout. However, she developed a second episode of thyroiditis 12 weeks into the second course of treatment with anti-Tg titre rising to 1 : 5120. Her TSH became suppressed with fT4 of 22.5 and fT3 6.5 pmol/l although on a stable dose of thyroxine. Her thyroid uptake scan was again at 2% but uninterpretable in the presence of thyroxine. Thyroxine was ceased and her condition improved following symptomatic treatment. However, 8 weeks later, she again became hypothyroid necessitating the resumption of her thyroxine. Her antiviral therapy continued independent of her thyroid event. She remained well through the remaining treatment and completed the interferon therapy uneventfully. SVR was achieved at 24-week follow-up. Her thyroxine was ceased at the same time. Her follow-up TSH levels at 8 and 24 weeks were normal. Please refer to Figure 1 for her sequence of events.

Laboratory assay characteristics

Third generation TSH and serum fT4 levels were determined by two-site sandwich immunoassay using an automated chemiluminescent system

(Diagnostic Products Corporation, Immulite 2000). The RR for TSH was 0.4–4.0 mU/l and fT4 10.5–20.6 pmol/l. The coefficients of variation (CV) were 5.0% and 5.1% at TSH concentrations of 4.0 mU/l and 10.0 mU/l, respectively. For fT4, the CV was 6.5% at 10.0 pmol/l.

Similarly, fT3 levels were performed using a two-site sandwich immunoassay using an automated chemiluminescent system (Beckman Coulter DXI). The RR was 3.5–6.0 pmol/l with 8.7% CV at 6.0 pmol/l.

Thyroid Stimulating Immunoglobulin was measured using cell culture and radio-immunoassay. This is an in-house bioassay using Chinese Hamster Ovary (CHO) cells in culture to detect the presence of thyroid stimulating activity. The CHO cells are transfected with the TSH receptor genes and thus are responsive to TSI. Thyroid-stimulating activity is measured by evaluating the intracellular release of cAMP induced by the patient's serum immunoglobulin on the CHO cells. The results are reported as units/ml (U/ml). TSI should be absent in the normal population. A TSI level of <10 is considered negative, 10–50 as weakly, 50–100 as moderately and >100 U/ml as strongly positive.

Serum autoantibodies to thyroglobulin and thyroperoxidase were measured by agglutination (Serodia-ATG and Serodia-AMC, Fujirebio, Inc., Tokyo, Japan). Normal titres were less than 1:400 for both.

Thyroid nuclear uptake scans

These were performed using 99 m-pertechnetate tracer with uptake studies taken at ~20 minutes post injection with a normal uptake ratio of 3–8:1.

Discussion

TD is the commonest extra-hepatic manifestation of interferon-based treatment in patients with chronic hepatitis C.[1,2] While still preliminary, it has been our observation that patients who developed TD during treatment achieved a much higher rate of cure or SVR.[3] This was not supported by our meta-analysis however,[4] which was additionally compounded by inherent reporting differences in treatment, the ad hoc method of surveying for TD while having treatment and that some patients were having regular IFN rather than pegylated IFN. These two case reports, although anecdotal, mimic the natural experiment in which neither patient achieved SVR from hepatitis C treatment in the absence of TD. Conversely, both achieved the desired outcome in the presence of TD. It is also fortuitous that the two cases carry both the favourable and unfavourable genotypes in types 3 and 1, respectively. Further more, Case 1 carried the additional poor prognostic factor of cirrhosis and yet managed to achieve SVR. The second case is inherently more responsive because genotype 3 patients often have a much better response rate of ~70–80%, independent of thyroid disease.[5] Despite this favourable factor, the patient did not clear the virus in the absence of TD following the first course of treatment but did so in the presence of TD.

These two natural experimental cases highlight the potential influence of thyroid involvement in achieving SVR. While the thyroid gland is clearly implicated, it remains undetermined whether it is the actual underlying inflammatory autoimmunity of TD that is the culprit or the exposure to supraphysiological concentrations of thyroid hormones (TH). Pathogenetically and pertinent to Case 1, GD is a highly immune-mediated condition which has been reported to be precipitated by interferon therapy.[6] It is currently unclear whether the behaviour of this condition is any different from de novo GDs which may influence the final hepatitic viral outcome. Thyroiditis such as that seen in the second case also shares a similar immunological mechanism, with the rising anti-Tg levels with each episode, lending strong support to this observation. While demonstration of autoantibodies is not proof of autoimmunity, the timing of these events soon after administration of an immunomodulators in a primed medium strongly implicates a causal link rather than an epiphenomenon. However recent report suggested a direct thyroid-toxic effect of IFN on thyrocytes,[7] and alluded to in Figure 2. Because the two cases are biologically related, it is possible that in genetically predisposed individuals, IFN therapy is able to induce an unusually amplified response, successful in clearing the virus but damaging the thyroid in the process. Recent publication indicated that genetic variations in the IL-28B gene enhanced the natural spontaneous clearance of the virus.[8] It is not known if this was the case in both our cases as haplotype studies were not available. Hepatitis C infection by itself will result in an induction of IFN–α and –β production as part of the innate immune response.[5] IFN also causes the activation of natural killer cells, maturation and proliferation of dendritic cells, proliferation of memory T cells, and prevention of T-cell apoptosis.[9] These factors will induce a rise in thyroid auto-antibody titres, which will in turn cause the immune-related changes demonstrated in both cases. The addition of exogenous IFN–α then further inflames an already vulnerable thyroid gland. Studies have shown that in patients who developed TD, immune marker levels such as Interleukin-6 (IL-6), Tumor Necrosis

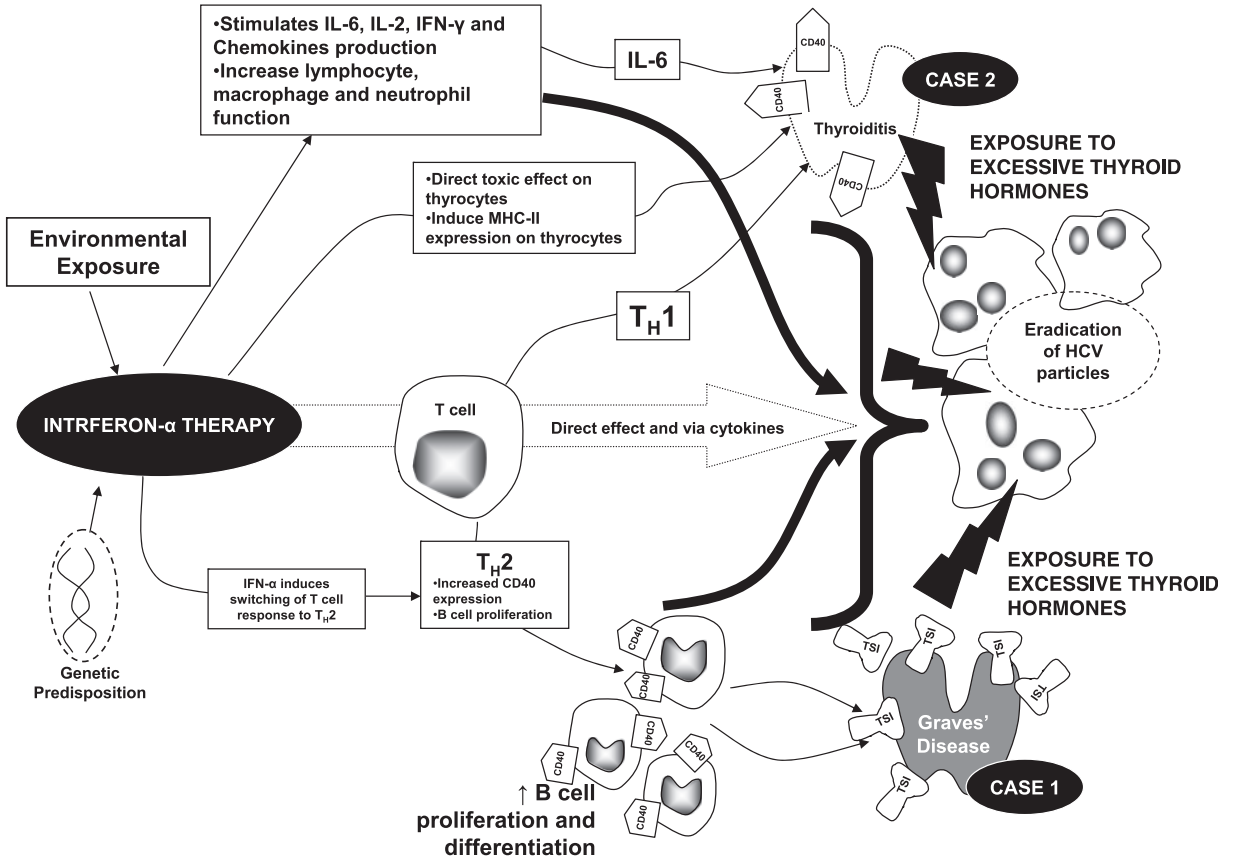

Figure 2. The proposed hypotheses for the eradication of HCV with TD and IFN-based therapy. IL-6, Interleukin-6; IFN, Interferon; MHC-II, major histocompatability complex-II; T_H, T helper.

Factor-alpha (TNF-α) and Chemokine Ligand 10 are higher and these factors may in involved in eliciting SVR.[10,11]

The second potential influencing factor is the exposure to TH. Normal physiological TH levels are unlikely to be effective as all patients would otherwise have normal circulating concentrations. The first case was exposed to high doses of TH early in the second course of therapy while her GD was coming under control. The second developed thyroiditis both before and during the second course of treatment, again exposed to the same milieu. In both cases, the presence of TD in the intervening periods may have indeed primed the tissue for a favourable response. However, the presence of TH *in vitro* also potentiates the antiviral action of interferon in cultured human cells.[12] In the presence of IFN, TH can also enhance the immune activation pathways such as HLA-DR antigen expression.[13] It is therefore plausible that the favourable outcome is related to the exposure to supraphysiological concentrations of TH.

A third consideration is whether repeated treatment with interferon will confer an improved chance of SVR. Patients receiving retreatment course have ∼16% of achieving SVR, usually in the absence of TD.[14] Retreatment in non-responders is often less effective than relapsers, especially with genotype 1.[15] This is pertinent to Case 1 who, at least on the grounds of prognostic markers, is least favoured to achieve SVR. In addition, re-exposure to interferon does not appear to increase the risk of thyroid disease.[16] Figure 2 summarizes the hypothetical mechanism of achieving SVR in these 2 cases.

Our report has a number of drawbacks. Firstly, we did not have the opportunity to study the single nucleotide polymorphisms (SNPs) near the interleukin (IL) 28B gene locus in both cases. These SNPs have been shown to play an important part in the spontaneous recovery, response to treatment and attaining SVR from HCV infection.[17–20] It would be fascinating to know of the cases harbour the favourable SNPs although the clinical utility of this novel finding remains to be refined.[21] Secondly and clearly, these are anecdotal cases and may be considered coincidental. Nevertheless, they suggest a potential existing mechanism in accentuating the SVR rate in the presence of thyroid disease. It is critical that further clinical studies are performed

to see if this observation can be reproduced before any definitive conclusions can be extracted. Further, demonstration that thyroxine (both in supraphysiological and physiological concentrations) can enhance the IFN-α antiviral effects *in vitro* would also considerably strengthen this hypothesis.

Conclusion

These two biologically-related cases illustrate the genetic tendency to develop autoimmune-mediated TD when undergoing IFN-α based treatment for chronic hepatitis C. The presence of the TD, either by way of the immune mediation or exposure to high concentrations of TH (or both), represents a favourable prognostic factor in achieving SVR for hepatitis C. Further investigation is required to confirm this potentially enhanced prognostic outcome, especially for the resistant genotype 1 patients. This is very important because patients who have sustained viral eradication can have regression of cirrhosis and a reduced risk for cirrhosis-related complications.[22]

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References

1. Tomer Y, Blackard JT, Akeno N. Interferon alpha treatment and thyroid dysfunction. *Endocrinol Metab Clin N Am* 2007; **36**:1051–66.
2. Tran HA. Hepatitis C infection, treatment regimens, and thyroid function abnormalities. *The Endocrinologist* 2007; **17**:231–5.
3. Tran HA, Jones TL, Batey RG. The spectrum of thyroid dysfunction in an Australian hepatitis C population treated with combination Interferon-α2β and Ribavirin. *BMC Endocr Disor* 2005; **5**:8.
4. Tran HA, Reeves GEM, Gibson R, Attia JR. Development of thyroid diseases in the treatment of chronic hepatitis C with α-interferon may be a good prognosticator in achieving a sustained virologic response: A meta-analysis. *J Gastroenterol Hepatol* 2009; **24**:1163–8.
5. Zeuzem S, Berg T, Moeller B, Hinrichsen H, Mauss S, Wedemeyer H, *et al.* Expert opinion on the treatment of patients with chronic hepatitis C. *J Viral Hepat* 2009; **16**:75–90.
6. Wong V, Fu Xi-Li A, George J, Cheung NW. Thyrotoxicosis induced by alpha-interferon therapy in chronic viral hepatitis. *Clin Endocrinol* 2002; **56**:793–8.
7. Tomer Y. Hepatitis C and interferon induced thyroiditis. *J Autoimmunity* 2010; **34**:J322–6.
8. Thomas DL, Thio CL, Martin MP, Qi y, Ge D, O'Huigin C, *et al.* Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature* 2009; **461**:798–801.
9. Tilg H. New insights into the mechanisms of interferon alpha: an immunoregulatory and anti-inflammatory cytokine. *Gastroenterology* 1997; **112**:1017–21.
10. Lloyd AR, Jagger E, Post JJ, Crooks LA, Rawlinson WD, Hahn YS, *et al.* Host and viral factors in the immunopathogenesis of primary hepatitis C virus infection. *Immunol Cell Biol* 2007; **85**:24–32.
11. Antonelli A, Rotondi M, Fallahi P, Romagnani P, Ferrari SM, Buonamano A, *et al.* High levels of circulating CX chemokine ligand 10 are associated with chronic autoimmune thyroiditis and hypothyroidism. *J Clin Endocrinol Metab* 2004; **89**:5496–9.
12. Lin H-Y, Thacore Hr, David FB, Davis PJ. Thyroid hormone analogues potentiate the antiviral action of interferon-gamma. *J Cell Physiol* 1996; **167**:269–76.
13. Lin H-Y, Thacore Hr, David FB, Davis PJ. Potentiation by thyroxine of interferon-gamma-induced HLA-DR expression in protein kinas A- and C-dependent. *J Interferon Cytokine Res* 1996; **16**:17–24.
14. Camma C, Cabibbo G, Bronte F, Enea M, Licata A, Attanasio M, *et al.* Retreatment with pegylated interferon plus ribavirin of chronic hepatitis C non-responders to interferon plus ribavirin: a meta-analysis. *J Hepatol* 2009; **51**:675–81.
15. Dieterich DT, Rizzetto M, Manns MP. Management of chronic hepatitis C patients who have relapsed or not responded to pegylated interferon alfa plus ribavirin. *J Viral Hepat* 2009; **16**:833–43.
16. Moncoucy X, Leymarie F, Delemer B, Levy S, Bernard-Chabert B, Bouche O, *et al.* Risk factors and long-term course of thyroid dysfunction during antiviral treatments in 221 patients with chronic hepatitis C. *Gastroenterol Clin Biol* 2005; **29**:339–45.
17. Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, *et al.* Genome-wide association of IL28B with response to pegylated interferon-α and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009; **41**:1105–9.
18. Ge D, Fellay J, Thomas AJ, Simon JS, Shianna KV, Urban TJ, *et al.* Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009; **461**:399–401.
19. Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, *et al.* Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature* 2009; **461**:798–801.
20. Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, *et al.* IL28B is associated with response to chronic hepatitis C interferon-α and ribavirin therapy. *Nat Genet* 2009; **41**:1100–4.
21. Ahlenstiel G, Booth DR, George J. IL28B in hepatitis C virus infection: translating pharmacogenomics into clinical practice. *J Gastroenterol* 2010; **45**:903–10.
22. Mallet V, Gilgenkrantz H, Serpaggi J, Verkarre V, Vallet-Pichard A, Fontaine H, *et al.* Effective treatment of hepatitis C often results in regression of cirrhosis and a reduced risk for cirrhosis-related complications. *Ann Intern Med* 2008; **149**:399–403.

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RESEARCH ARTICLE Open Access

Thyroid disease is a favorable prognostic factor in achieving sustained virologic response in chronic hepatitis C undergoing combination therapy: A nested case control study

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Abstract

Background: Interferon-α in combination with ribavirin is the current gold standard for treatment of chronic hepatitis C. It is unknown if the development of autoimmune thyroid disease (TD) during treatment confers an improved chance of achieving sustained virologic response. The aim of this study is to assess the chance of achieving sustained virologic response (SVR) in patients who developed TD during treatment when compared with those who did not.

Methods: We performed a tertiary hospital-based retrospective nested case-control analysis of 19 patients treated for hepatitis C who developed thyroid disease, and 76 controls (matched for age, weight, gender, cirrhosis and aminotransferase levels) who did not develop TD during treatment. Multivariate logistic-regression models were used to compare cases and controls.

Results: The development of TD was associated with a high likelihood of achieving SVR (odds ratio, 6.0; 95% confidence interval, 1.5 to 24.6) for the pooled group containing all genotypes. The likelihood of achieving SVR was increased in individuals with genotype 1 HCV infection who developed TD (odds ratio, 5.2; 95% confidence interval, 1.2 to 22.3), and all genotype 3 patients who developed TD achieved SVR.

Conclusions: Development of TD during treatment for hepatitis C infection is associated with a significantly increased chance of achieving SVR. The pathophysiogical mechanisms for this observation remain to be determined.

Trial Registration: *The Australian New Zealand Clinical Trials Registry (ANZCTR)*: ACTRB12610000830099

Background

Hepatitis C is one of the major global causes of chronic hepatic infections, particularly in third world countries, and is associated with a significant rate of cirrhosis and hepatoma. In Australia [1,2] and the United States of America [3,4], the burden of disease is significant. Although the incidence has come under control, the associated sequelae unfortunately have been predicted to increase in the coming decades [5]. Consequently, a large and growing number of patients will be expected to undergo treatment for hepatitis C. Of those receiving combination treatment with interferon (IFN)-α and ribavirin (RBV), approximately 5-10% will develop thyroid-related complications [6]. Whilst there are a number of factors in the prediction of favourable hepatic outcome such as genotype, ethnicity, and early viral load reduction [7,8], there are few published reports that assess the development of thyroid disease (TD) in relation to sustained virological response (SVR). Our previous meta-analysis did not find any difference, although this may be due to inherent differences in the published reports [9]. The aim of this study is to investigate the hypothesis that the development of TD in patients

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treated for HCV is associated with a significantly increased likelihood of attaining SVR.

Methods

Patients and control participants

We enrolled 19 patients who developed TD while receiving combination therapy in a major tertiary referral hospital, New South Wales (NSW), Australia. These patients had been assessed by the endocrinology team. All were found to have thyroiditis as previously described [10], except one who developed primary hypothyroidism.

Patients were treated with predetermined IFN-based treatment regimen and thyroid surveillance protocol. Alternative causes of chronic hepatitis were excluded. No patient had dual Hepatitis B and C. All were monitored carefully for protocol adherence and had normal thyroid functions prior to entering treatment. Monthly reviews were performed, including those who developed TD (as defined below) with thyrotropin (TSH) levels, full blood counts, renal and liver function tests as previously described [10]. Thyroid autoantibodies, whilst predictive, were not performed as it did not affect management in the event of TD development.

Because of the scarcity of reported thyroid diseases in relation to SVR in the literature, we performed a nested case control study using a 1:4 ratio of cases to controls. Patients were matched for age, gender, viral load, body weight, alanine and aspartate aminotransferase levels, cirrhotic state and individualised IFN-α2 treatments (either α2a or α2b). The cirrhotic status was determined clinically including ultrasonography and/or computerised tomography.

Therapy

All patients were treated with combination pegylated IFN-α and RBV therapy. Pegylated IFN- α2a (fixed dose 180 ug) or pegylated IFN-α2b (weight based dose, 1.5 ug/kg of body weight) was injected subcutaneously weekly. Oral RBV was administered twice daily base on the patient's weight (1.0 gm, <75 kg; 1.2 gm, >75 kg) for patients with genotype 1 and the fixed dose (0.8 gm) for patients with non-1 genotype. The duration of treatment depends on the HCV genotypes; genotypes 2 and 3 were treated for 24 weeks and types 1 and 4 for 48 weeks respectively. Treatment was further adjudicated according to viral load results for genotypes 1 and 4 (see below). For genotypes 2 and 3, treatment was continued to the end irrespective of the qualitative viral load at 4 weeks.

Thyroid disease definition

Thyroid disease was defined as having hypo- or hyperthyroidism, (clinically and biochemically based). Thyrotoxicosis was defined as having TSH of <0.1 mU/L, fT4 levels >26.0 and/or fT3 levels >6.0 pmol/L respectively.

Hypothyroidism, including subclinical hypothyroidism, was defined as having TSH levels >4.0 mIU/L.

Thyroiditis is defined as the triad of clinical and/or biochemical thyrotoxicosis the current clinical setting, with a reduced/negligible thyroid pertechnetate uptake scan. All uptake scans were reviewed by a specialist nuclear physician consultant. Thyroid autoantibodies may be present but are not considered essential to the diagnosis.

Virological Response Surveillance Protocol

The following viral load testing protocols were used during the study:

1. Baseline quantitative HCV RNA PCR assays were performed on *all* patients irrespective of genotype.
2. For genotypes 1 and 4, *qualitative* viral load assays were done at weeks 4, 24, 48 and 24 weeks after the completion of therapy. Additional q*uantitative* assay was done at week 12. Treatment was terminated if there was ≤ 2 $\log_{10}$ reduction of viral load compared with baseline at the 12^{th} week of therapy or if the qualitative viral load was positive at the 24^{th} week.
3. For genotypes 2 & 3, *qualitative* viral load assay was done at week 4, 24 (at the completion of therapy) and at week 24 follow-up.

SVR was defined as serum HCV-RNA being undetectable by polymerase chain reaction (PCR) at 24 weeks *following* the completion of therapy.

Statistical Analyses

The study was designed to detect an odds ratio of 2 or more for associations between SVR and thyroid disease development, with 80% power. A 1:4 ratio of cases to controls was used, with significance tests performed at a two-tailed alpha level of 0.05. Odds ratios were adjusted for age, weight, gender and aminotransferase levels. Unconditional multivariate logistic-regression analysis was used to estimated odds ratios and 95% confidence intervals (CIs). Statistical significance was determined using the likelihood-ratio test. Demographic data are presented as percentage and means with standard deviations (SDs). Results were also analysed using a 2 × 2 contingency table with χ^2 analysis for comparison of percentages between groups. The Fisher's exact analysis and continuity correction were applied when appropriate. All analyses were performed using Stata 10 software (Stata).

Results

All subject cases had thyroiditis as previously defined except for one case of primary hypothyroidism and all patients were well matched for all stated criteria, Table 1.

Sustained virologic response was observed in 84.2% of patients who developed TD compared with 53.9% in those who did not. The difference between proportions was significant at 0.30 (95% Confidence Interval (CI), 0.10 - 0.50, $p < 0.05$).

Overall, univariate logistic regression analysis revealed that cases which developed TD, had a 6.0 times greater likelihood of achieving SVR compared with controls (95% confidence interval (CI), 1.5 to 24.6). The likelihood of achieving SVR was even greater for genotype 1 cases with TD versus those without TD (OR, 5.2; 95% CI, 1.2 to 22.3), and all genotype 3 patients who developed TD also achieved SVR, Table 2.

Multivariate logistic regression analysis showed that presence of TD and genotype was the only two variables significantly associated with SVR. Modelling with these two independent variables revealed that individuals who developed TD had a 6 folds greater likelihood of achieving SVR compared with controls (95% confidence interval (CI), 1.5 to 24.6).

Discussion

The prevalence of hepatitis C is now controlled in the Western world [5] but continues to increase in third world and developing countries due to lack of needle exchange program and medical infrastructure. In Australia, the numbers have stabilized and more recently declined [2]. The current established and accepted treatment for chronic hepatitis C infection is the combination of pegylated IFN-α and RBV. This treatment regimen delivers a cure rate of approximately 50% of cases [11]. The favourable factors in response to treatment include genotypes (2 and 3 genotypes have better response), compliance, duration of treatment and early

Table 1 Characteristics of 95 patients and separate case/control subjects

	All Patients	Cases TD	Controls No TD	P-values 95% CI
	N = 95	N = 19	N = 76	
Demographic factors				
Female gender - number (%)	55 (57.9%)	11 (57.9%)	44 (57.9%)	NSS
Mean age (years) ± SD	47 ± 8	47 ± 8	48 ± 7	NSS
Weight (kg) ± SD	69.7 ± 10.1	70.6 ± 8.5	69.8 ± 10.7	NSS
Hepatic factors				
Cirrhosis - number (%)	5 (5.3%)	1 (5.3%)	4 (5.3%)	NSS
ALT (<45 U/L) ± SD	54 ± 24	56 ± 26	53 ± 25	NSS
AST (1-30 U/L) ± SD	39 ± 11	41 ± 12	38 ± 12	NSS
Virologic factors				
Genotypes - number (%)				
• 1	55 (58.0%)	11 (58.0%)	44 (58.0%)	NSS
• 3	40 (42.0%)	8 (42.0%)	32 (42.0%)	NSS
Viral Load				
- At baseline (log IU/mL) ± SD	6.44 ± 0.52	6.22 ± 0.56	6.64 ± 0.53	NSS
Treatment regimens - number (%)				
IFN-α2a	55 (57.9%)	11 (57.9%)	44 (57.9%)	NSS
together with				
Ribavirin				
1.0 gm (patients ≤ 75 kg)	20 (36.4%)	4 (36.4%)	16 (36.4%)	
1.2 gm (patients >75 kg)	15 (27.3%)	3 (27.3%)	12 (27.3%)	
0.8 gm	20 (36.4%)	4 (36.4%)	16 (36.4%)	
IFN-α2b	40 (42.1%)	8 (42.1%)	32 (42.1%)	NSS
together with				
Ribavirin				
1.0 gm (patients ≤ 75 kg)	10 (25.0%)	2 (25.0%)	8 (25.0%)	
1.2 gm (patients >75 kg)	10 (25.0%)	2 (25.0%)	8 (25.0%)	
0.8 gm	20 (50.0%)	4 (50.0%)	16 (50.0%)	

Results are expressed as Means ± Standard Deviations (SDs). CI, Confidence Intervals; IFN, Interferon; NSS, Non Statistically Significant; ALT, Alanine Aminotransferase, AST; Aspartate Aminotransferase.

Table 2 Sustained Virologic Responses (SVR), Odds Ratios (OR) and 95% Confidence Intervals (CI) analyses for *all* patients, genotype 1, 3 and regimen-specific subgroups

GENOTYPE 1 (N = 55)								
Treatment regimens	Pegylated IFN-α2a and RBV 1000 mg		Pegylated IFN-α2a and RBV 1200 mg		Pegylated IFN-α2b and RBV 1000 mg		Pegylated IFN-α2b and RBV 1200 mg	
	Control 16	TD 4	Control 12	TD 3	Control 8	TD 2	Control 8	TD 2
EVR*	Not applicable (see text)							
ETR - number (%)	9 (56.3%)	3 (75.0%)	9 (75.0%)	3 (100.0%)	4 (50.0%)	2 (100.0%)	5 (62.5%)	2 (100.0%)
SVR - number (%)	8 (50.0%)	3 (75.0%)	7 (58.3%)	2 (66.6%)	4 (50.0%)	2 (100.0%)	5 (62.5%)	2 (100.0%)
GENOTYPE 3 (N = 40)								
Treatment regimens	Pegylated IFN-α2a and RBV 800 mg				Pegylated IFN-α2b and RBV 800 mg			
	Control N = 16		TD N = 4		Control N = 16		TD N = 4	
EVR*	Not applicable (see text)							
ETR - number (%)	**16 (100.0%)**		**4 (100.0%)**		**16 (100.0%)**		**4 (100.0%)**	
SVR - number (%)	16 (100.0%)		4 (100.0%)		16 (100.0%)		4 (100.0%)	
SVR for genotype 1: Odds Ratio, 95% CI	5.2, 1.2 to 22.3							
SVR for genotype 3	Control (n = 32) 100.0%				TD (n = 8) 100.0%			
	All 40 cases achieved SVR making the combination of Genotype 3 and TD a perfect predictor of SVR in this group.							
Treatment related SVR IFN-α2a vs IFNα2b	Non Statistically Significant							
OVERALL SVR: ODDS RATIO, 95% CI	**6.0, 1.5 to 24.6**							

viral load reduction [8]. Additional positive prognosticators consist of lower body mass index, lack of co-existing liver disease and ethnicity (Asians and Caucasians have a higher SVR than African Americans) [7]. The relationship between TD and SVR had been scarcely addressed and in our solitary meta-analysis, the outcome was negative [9].

Hypothetically, when treatment is delivered, the immune system is stimulated sufficiently in an attempt to eradicate the virus and in the process may also be intense enough to trigger TD, as an unintended consequence. Because the presence of TD may signify a supercharged and heightened immune response which in turn increases the chance of eradicating the virus. Studies have shown that in patients who developed TD, immune marker levels such as Interleukin-6 (IL-6), Tumor Necrosis Factor-alpha (TNF-α) and chemokines are relatively higher and thus in the process may potentially be more effective at achieving SVR [12]. Furthermore, the presence of thyroid hormones *in vitro* also potentiates the antiviral action of IFN in cultured human cells [13]. This hypothesis was observed in our previous report in which all 11 IFN-related thyroid cases achieved SVR [10]. However, a subsequent meta-analysis failed to find any relationship although this might be due to inherent differences within the various reports [9]. This additional nested case control study in which the cases were matched in a ratio of 1 case to 4 controls finds further supportive evidence that the development of TD in HCV patients whilst undergoing combination with RBV and IFN-α treatment is a favorable prognostic factor for achieving SVR. It is reassuring that other factors are matched including age, gender, weight, viral load, cirrhosis and transaminase levels; some of which can influence final SVR status. It is also interesting that there was one primary hypothyroid case which was clearly not the hypothyroid phase of the thyroiditis. In this case, the patient responded well to routine thyroxine supplement.

The viral kinetic responses bear little relationship to the development of TD. It is not possible to assess the relationship between the early viral response (EVR), its associated viral load and the timing of TD development. This is because TD tends to develop approximately at the 18th week of treatment [10]. Furthermore, the critical end-point of this report is the assessment of SVR, irrespective of other landmark viral load studies during treatment. However, end of treatment responses (ETR) appear to confirm previous observation that it is often a reliable predictor of SVR. Most patients who achieved ETR also completed SVR except for the two genotype 1 patients who relapsed as defined by failure to achieve SVR. There was a 6-fold increase in the chance of achieving SVR with TD development for the pooled

group containing all genotypes (odds ratio, 6.0; 95% confidence interval, 1.5 to 24.6). The likelihood of achieving SVR was increased in individuals with genotype 1 HCV infection who developed TD (odds ratio, 5.2; 95% confidence interval, 1.2 to 22.3). All genotype 3 patients who developed TD achieved SVR. Although our control cohort has a SVR rate of 100%, the overall reported and accepted rate approximates 80%, perhaps suggesting an additional synergistic effect in this genotype subgroup. Because of this overall high response rate and in order to quantify this amplified increment, there would need to be a much larger cohort. This is unlikely to be achievable given the low incidence of true TD occurring in this setting.

Whilst the thyroid gland is clearly implicated in this study, it remains undetermined whether thyroiditis is a mediator or the result of processes leading to SVR. While the development of a more effective immune response to HCV may unmask underlying thyroid autoimmune tendencies, it is also possible that acute exposure to supraphysiological concentrations of thyroid hormones (THs) may confer favourable immunomodulating activities leading to SVR. It is not yet known if the behaviour of the thyroid conditions in this setting is any different from those arising de novo and hence provides differing influences on the final hepatitic viral status.

HCV infection results in the induction of IFN-α and -β production as part of the innate immune response [14], with subsequent activation of natural killer cells, maturation and proliferation of dendritic cells, proliferation of memory T cells, and prevention of T-cell apoptosis [15]. These effects are important in the mediation of a heightened tendency toward organ-specific autoimmunity (including TD) in patients with HCV. The addition of exogenous IFN-α potentiates the risk of inflammatory autoimmunity in an already vulnerable and primed thyroid gland.

The exposure to the two different forms of IFN-α may be a potential confounder in eliciting SVR. These two IFNs differ significantly in their pegylation characteristics which may translate into their pharmacokinetics and biological activity. Recent reports indicated that there was a slightly higher SVR rate with pegylated IFN-α2a compared with α2b [16]. However, recent practice guidelines did not distinguish between the two in their recommendations [17-19]. There appears to be insufficient data to guide preference of one over the other. Similarly, our findings do not indicate any difference in SVR with regards to the two types of IFN-α, but again this is due to the small numbers.

The second potential influencing factor is the exposure to THs. Normal levels are unlikely to be effective as all other euthyroid and treated patients would otherwise have normal circulating levels. However, the presence of THs *in vitro* also potentiates the antiviral action of IFN in cultured human cells (13). In the presence of IFN, THs can enhance the immunomodulation such as HLA-DR antigen expression (15). It is therefore possible but highly speculative that the favorable outcome seen in these patients is related to the exposure to supraphysiological concentrations of THs. However, it is equally possible that THs are not involved but is a mere para-phenomenon in a vigorous and exaggerated response to IFN therapy.

Finally, the recent discovery of the association between single nucleotide polymorphisms (SNPs) near the interleukin (IL) 28B gene locus showed that this plays an important part in the spontaneous recovery, response to treatment and attaining SVR from HCV infection [20-23]. This SNPs may reflect differential levels of the immunomodulating cytokine IFN-λ-3, but the possibility that individuals at higher risk of TD development harbor favorable SNPs remains to be explored [24].

One of the shortcomings of this report is the small sample size, due primarily to the low prevalence of the condition. This was a major determinant of the choice of study design, with nested case-control studies offering important data on relatively uncommon end-points with little potential for additional bias. Nevertheless, collaboration with prominent centers would potentially allow prospective designs to be pursued, hence enhancing the power of the study; such studies would require standardization of thyroid surveying and definitions of thyroid disease. Secondly, there may be different genetic or regional differences as other reports, albeit retrospective, failed to find such a consistent pattern of SVR amongst TD cases [25-27]. The sampling of both cases and controls from the same patient cohort reduced the chance of selection bias.

Clinical implications

The finding from this study tentatively raises the question of whether thyroxine might be useful as an adjuvant addition to the treatment armamentarium. It is critical that this finding is confirmed (or refuted) by larger and independent studies because recent data suggested that in HCV cases with SVR, cirrhosis and its associated morbidities can be reversible [28].

Conclusions

This nested case control study strongly indicates that TD is associated with a higher chance of achieving SVR, especially in genotype 1. This association mandates larger prospective multicentre trials to conclusively address this hypothesis if thyroxine is to be considered a potential adjuvant to current treatment regimen.

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Authors' contributions
HAT conceived the study, participated in its design, assisted with data collection and statistical analysis, and coordinated and helped to draft the manuscript. GEMR contributed to the statistical and meta-analytical methods, and participated in the discussion and drafting of the manuscript. TLJ, RG gathered, provided the data, and participated in the discussion and drafting of the manuscript. All authors read and approved the final revised manuscript.

Competing interests
The authors declare that they have no competing interests.

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References

1. World Health Organisation: **Hepatitis C. Factsheet No. 164.** 2000 [http://www.who.int/mediacentre/factsheets], accessed 20-May-2011.
2. Third National Hepatitis C Strategy 2010-2013: 2011 [http://www.health.gov.au/internet/main/publishing.nsf/Content/ohp-national-strategies-2010-hcv/$File/hcv.pdf].
3. Armstrong GL, Wasley N, Simard EP, McQuillan GM, Kuhnert WL, Alter MJ: **The prevalence of hepatitis C virus infection in the United States, 1999 through 2002.** *Ann Intern Med* 2006, **144**:705-714.
4. Dienstag JL: **Hepatitis C: A bitter harvest.** *Ann Intern Med* 2006, **144**:770-771.
5. Shephard CW, Finelli L, Alter MJ: **Global epidemiology of hepatitis C virus infection.** *Lancet Infect Dis* 2005, **5**:558-567.
6. Tran HA: **Hepatitis C infection, treatment regimens, and thyroid function abnormalities.** *The Endocrinologist* 2007, **17**:231-235.
7. Yan KK, Guirgis M, Ding T, George J, Dev A, Lee A, Zekry A: **Treatment responses in Asians and Caucasians with chronic hepatitis C infection.** *World J Gastroenterol* 2008, **14**:3416-3420.
8. Patel K, Muir AJ, McHutchinson JG: **Diagnosis and treatment of chronic hepatitis C infection.** *BMJ* 2006, **332**:1013-7.
9. Tran HA, Reeves GEM, Gibson R, Attia JR: **Development of thyroid diseases in the treatment of chronic hepatitis C with α-interferon may be a good prognosticator in achieving a sustained virologic response: A meta-analysis.** *J Gastroenterol Hepatol* 2009, **24**:1163-1168.
10. Tran HA, Reeves GEM, Jones TL: **The natural history of interferon-alpha2b-induced thyroiditis and its exclusivity in a cohorte of patients with chronic hepatitis C infection.** *QJM* 2009, **102**:117-22.
11. Manns MP, Wedemeyer H, Cornberg : **Treating viral hepatitis C: efficacy, side effects and complications.** *Gut* 2006, **55**:1350-1359.
12. Rotondi M, Minelli R, Magri F, Leporati P, Romagnani P, Baroni MC, Delsignore R, Serio M, Chiovato L: **Serum CXCL10 levels and occurrence of thyroid dysfunction in patients treated with interferon-alpha therapy for hepatitis C virus-related hepatitis.** *Eur J Endocrinol* 2007, **156**:409-14.
13. Lin HY, Thacore HR, Davis PJ, David FB: **Thyroid hormone potentiates the antiviral action of interferon-gamma in cultured human cells.** *J Clin Endocrinol Metab* 1994, **79**:62-5.
14. Rehermann B: **Hepatitis C virus versus innate and adaptive immune responses: a tale of coevolution and coexistence.** *J Clin Invest* 2009, **119**:1745-1754.
15. Hiroishi K, Eguchi J, Ishii S, Hiraide A, Sakaki M, Doi H, Omori R, Imawari M: **Immune response of cytotoxic T lymphocytes and possibility of vaccine development for hepatitis C virus infection.** *J Biomed Biotechnol* 2010, **2010**:263810.
16. Aghemo A, Grazia M, Colombo M: **Pegylated interferons α2a and α2b in the treatment of chronic hepatitis C.** *Nat Rev Gastroenterol Hepatol* 2010, **7**:485-494.
17. Zeuzem S: **Do differences in pegylation of interferon alfa matter?** *Gastroenterology* 2010, **138**:34-36.
18. Awad T, Thorlund K, Hauser G, Stimac D, Mabrouk M, Gluud C: **Cochrane Hepato-Biliary Group. Peginterferon alpha-2a may achieve higher sustained response than peginterferon alpha-2b in chronic hepatitis C: a Cochrane systematic review of randomized clinical trial.** *Hepatology* 2010, **51**:1176-1184.
19. Ghany MG, Strader DB, Thomas DL, Seeff LB: **Diagnosis, Management, and Treatment of Hepatitis C: An Update.** *Hepatology* 2009, **49**:1335-1374.
20. Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, Nakagawa M, Korenaga M, Hino K, Hige S, Ito Y, Mita E, Tanaka E, Mochida S, Murawaki Y, Honda M, Sakai A, Hiasa Y, Nishiguchi S, Koike A, Sakaida I, Imamura M, Ito K, Yano K, Masaki N, Sugauchi F, Izumi N, Tokunaga K, Mizakami M: **Genome-wide association of IL28B with response to pegylated interferon-α and ribavirin therapy for chronic hepatitis C.** *Nat Genet* 2009, **41**:1105-1109.
21. Ge D, Fellay J, Thompson A, Simon J, Shianna K, Urban T, Heizen E, Qiu P, Bertelsen A, Muir A, Sulkowski M, McHutchinson J, Goldstein D: **Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance.** *Nature* 2009, **461**:399-401.
22. Thomas D, Thio C, Martin M, Dongliang Ge Y, O'hUigin C, Kidd J, Kidd K, Khakoo S, Alexander G, Goedert J, Kirk G, Donfield S, Rosen H, Tobler L, Busch M, McHutchinson J, Goldstein D, Carrington M: **Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature.** 2009, **461**:798-801.
23. Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate M, Bassendine M, Spengler U, Dore G, Powell E, Riordan S, Sehridan D, Smedile A, Fragomeli V, Muller T, Bahlo M, Stewart G, Booth D, George J, for the Hepatitis C Study: **IL28B is associated with response to chronic hepatitis C interferon-α and ribavirin therapy.** *Nat Genet* 2009, **41**:1100-1104.
24. Ahlenstiel G, Booth DR, George J: **IL28B in hepatitis C virus infection: translating pharmacogenomics into clinical practice.** *J Gastroenterol* 2010, **45**:903-910.
25. Vezali E, Elefsiniotis I, Mihas C, Konstantinou E, Saroglou G: **Thyroid dysfunction in patients with chronic hepatitis C: Virus- or therapy-related?** *J Gastroenterol Hepatol* 2009, **24**:1024-1029.
26. Kee KM, Lee CM, Wang J-H, Tung HD, Changchien CS, Lu SN, Wang P-W: **Thyroid dysfunction in patients with chronic hepatitis C receiving a combined therapy of interferon and ribavirin: Incidence, associated factors and prognosis.** *J Gastroenterol Hepatol* 2006, **21**:319-326.
27. Moncoucy X, Leymarie F, Delemer B, Levy S, Bernard-Chabert B, Bouche O, Jolly D, Diebold MD, Cadiot G, Thiefin G: **Risk factors and long-term course of thyroid dysfunction during anti-viral treatments in 221 patients with chronic hepatitis C.** *Gastroenterol Clin Biol* 2005, **29**:339-345.
28. Mallet V, Gilgenkrantz H, Serpaggi J, Verkarre V, Vallet-Pichard A, Fontaine H, Pol S: **Effective treatment of hepatitis C often results in regression of cirrhosis and a reduced risk for cirrhosis-related complications.** *Ann Intern Med* 2008, **149**:399-403.

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CHAPTER VIII. SUMMARY AND CLINICAL MANAGEMENT STRATEGIES

Figure 2 summarises our current understanding and appreciation of thyroid diseases occurring in this particular clinical setting. A formal management recommendation for the development of thyroid disease in this setting was proposed and has been accepted for publication (Appendix II). The accepted version of the manuscript has therefore been included below.

Publication:

Tran HA, Jones TL, Ianna EA, Foy A, Reeves GEM. THYROID DISEASE IN CHRONIC HEPATITIS C INFECTION TREATED WITH INTERFERON-A: MANAGEMENT STRATEGIES AND FUTURE PERSPECTIVE. Endocr Pract, September-2012: *In Press*.

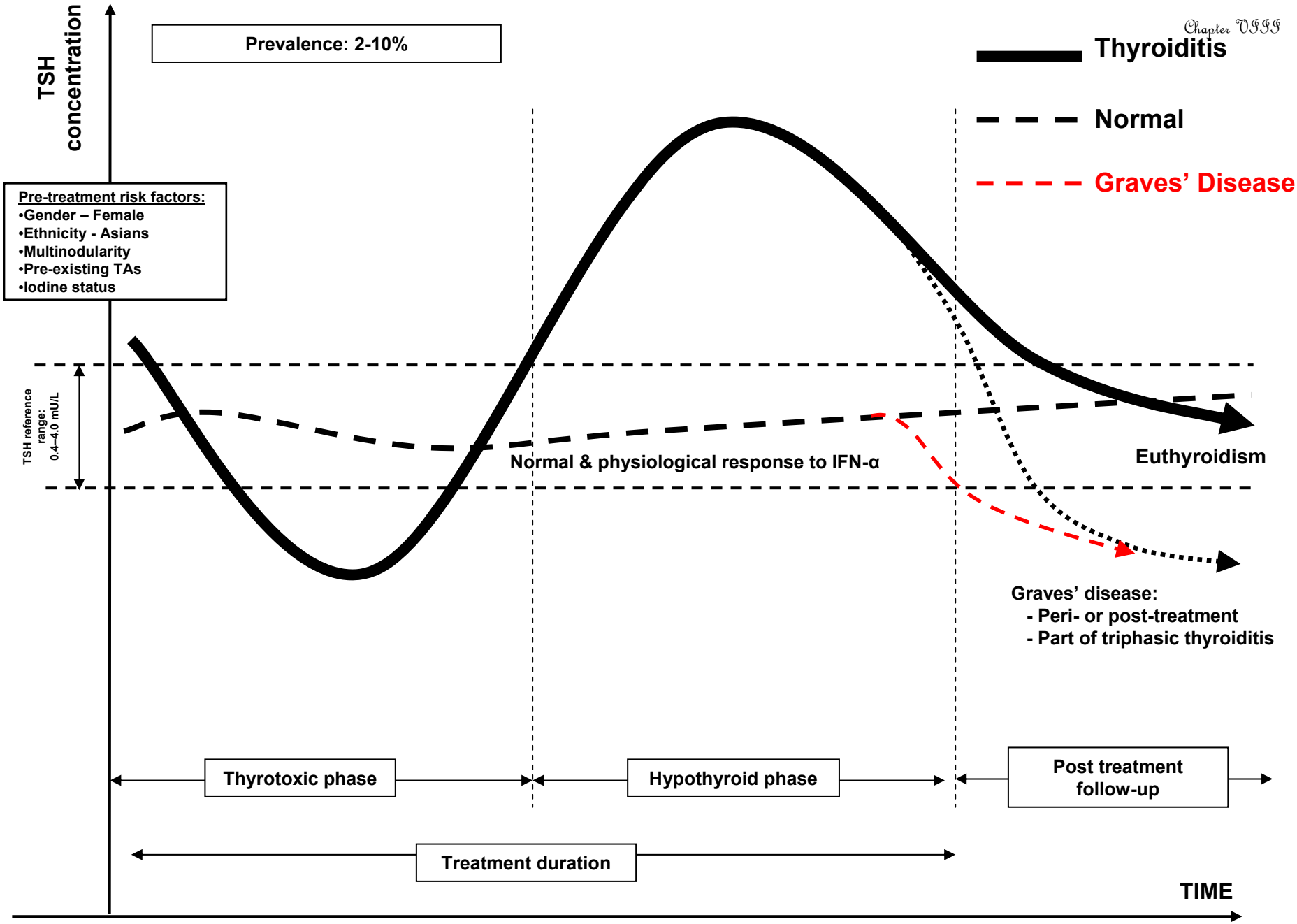

Figure 2. Summary of the pathophysiology of thyroid disease in the treatment of hepatitis C with IFN-α and RBV

***ENDOCRINE PRACTICE* Rapid Electronic Article in Press**

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Review Article EP12195.RA

THYROID DISEASE IN CHRONIC HEPATITIS C INFECTION TREATED WITH COMBINATION INTERFERON-α AND RIBAVIRIN: MANAGEMENT STRATEGIES AND FUTURE PERSPECTIVE

Running title: Hepatitis C, Interferon-α and thyroid disease

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ABSTRACT

Objective: Hepatitis C infection is one of the major epidemics afflicting young people in both the developed and developing countries in the 21st century. The commonest endocrine disorder associated with this infection, especially in conjunction with interferon-α based therapy is thyroid disease. This manuscript aims to review the development of thyroid disease before, during and after the completion of treatment with combination interferon-α and ribavirin for chronic hepatitis C infection. We also aim to summarise the current understanding of the natural history of the condition and propose management and follow-up guidelines.

Methods: PubMed was searched up to June 30th, 2011 for English-language publications that contained the search terms "hepatitis C virus", "chronic hepatitis C", "HCV", "thyroid disease", "thyroiditis", "autoimmunity", "interferon-alpha" and "ribavirin". Additional publications were identified from the reference lists of papers identified by this search. All studies must be original research publications and included combination interferon-α and ribavirin use in whom thyroid disease developed. All available manuscripts were reviewed and critically analysed.

Results: The prevalence of thyroid disease before combination IFN-α and Ribavirin therapy ranges from 4.6 to 21.3%; during therapy 1.1 to 21.3% and after therapy 6.7 to 21.3%. The commonest thyroid disease is thyroiditis. The frequency of thyroid testings and diagnostic criteria for the various thyroid conditions are not standardised and many publications are retrospective.

Conclusion: Patients undergoing this therapy should have a strictly standardised protocol in order to detect and manage developed thyroid disease appropriately. However, current

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published reports are heterogeneous and inconsistent. Based on current available literature, we recommend monthly screening test with thyrotropin level whilst receiving combination interferon-α and ribavirin therapy. Free thyroid hormone parameters can be sequentially be performed if thyrotropin levels are abnormal.

INTRODUCTION

Hepatitis C infection is one of the major epidemics afflicting young people, in Australia and worldwide. Fortunately, the incidence of Hepatitis C Virus (HCV) infection has either declined or plateaued in recent years (1, 2). The latter has occurred in Australia in the last 2-3 years due to better education and the needle exchange program (3). Nevertheless, this remains a problem worldwide with more than 80% of intravenous drug users being positive for HCV, totalling 10 million cases world wide (4). In Southeast Asia and Africa, the respective population prevalences are 2.2 and 5.3% (5). The latest World Health Organisation estimates that 170 million people are infected with HCV worldwide (2). Although the prevalence of chronic HCV infection in the United States is 1.3%, it results in a substantial 3.2 million patients (5). Furthermore and contrary to the overall decline in HCV prevalence, the incidence in the younger age group continues to increase, predominantly due to intravenous drug use (IVDU) (6). As a result, the number of patients seeking treatment and its attendant complications is likely to increase. The expected increase in the surveillance for thyroid disease (TD) will also be reflected in the number of thyroid function tests (TFTs) and subsequent by the cost to the community health services.

It has been observed that HCV and related treatment regimens result in an increased incidence of autoimmunity including the generation of auto-antibodies and some autoimmune

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diseases such as type I diabetes mellitus, systemic lupus erythematosus and crescentic glomerulonephritis (7). The most common endocrine manifestation of HCV infection is autoimmune thyroid disease. The management of TD in the setting of interferon-α-based (IFN-α) therapy is poorly defined and remains controversial. The current European Association in the Study of Liver clinical practice guidelines cursorily recommends 12 weekly thyrotropin (TSH) levels whilst on therapy (8). Similarly, The American College of Gastroenterology recommends thyroid function monitoring every 12 weeks whilst on treatment (9). The National Academy of Clinical Biochemistry guidelines makes no management recommendations for HCV (and treatment) associated TD and its monitoring (10). Consequently, it is vital that the association between HCV and TD and their natural histories are better understood so that the appropriate management strategies can be implemented. This report examines in details the natural history of TD which developed before, during and after treatment with combination ribavirin (RBV) and IFN-α. It excludes previous IFN-α monotherapy and any combinations other than IFN-α and RBV because this combination constitutes the current state of the art HCV treatment. However, it includes both standard IFN-α (sIFN-α) and pegylated IFN-α (pIFN-α).

PATHOGENESIS

The prevalence of TD in untreated HCV patients is not well documented and depends on the frequency and definition of thyroid parameters. Therefore, the exact prevalence of TD in this setting remains controversial. A previous comprehensive review revealed a prevalence ranging from 2.3 to 12.3% in untreated HCV patients (11). In this report, the prevalence before treatment ranges from 1.9 to 21.2% (Table 1). It is also interesting to note that 10% of patients with autoimmune hepatitis has concurrent autoimmune thyroiditis (12). During therapy with combination IFN-α and RBV, it is from 1.1 to 21.3% (*Table 2*). The reason for the similarity of

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the prevalence between before and during therapy is most likely to be related to the difference in the definition of thyroid disease in various publications. For other non-HCV treatments using IFN-α therapy such as renal cell carcinoma, the prevalence is not known, probably due to the low frequency of its use.

The theory regarding the causality of HCV infection and the treatment regimen on TD has been expressed in terms of immuno-modulation and/or immuno-dysregulation (7, 13, 14) ~~12, 13)~~. The exact mechanism remains uncertain. In the presence of HCV infection (without IFN-α based treatment), there is only a weak association with thyroid autoimmunity but only in females, not males (15). This was subsequently supported by Betterle et al in 2000 who found that there was increase in frequency of clinical of latent autoimmune diseases in patients with chronic HCV infection (16). Additionally, and because HCV infection is insidious and silent, there is no reported study on the biochemical profile of thyroid physiology anteceding treatment with IFN-α. Since HCV particles induced the production of IFN-α, it is of interest to observe its influence on thyroid parameters. Acutely, TSH levels tend to decrease whilst free levothyroxine (LT4) and free triiodothyronine (fT3) levels remain unchanged in the first 24 hours with an increase in Interleukin-6 (IL-6) level and suppressed Tumour Necrosis Factor (TNF) and IL-1 (17). In chronic exposure, TSH levels remain fairly stable, dropping to a nadir at 4 months and then return to pre-treatment levels. All thyroid parameters remain within reference range however (18). It is hypothesised that in an otherwise normal and untreated thyroid gland, the presence of the HCV particles will result in an induction of IFN-α and IFN-β production as part of the innate immune response (19). Interferon has also resulted in the activation of natural killer cells, maturation and proliferation of dendritic cells, proliferation of memory T cells, and prevention of T-cell apoptosis (20). These will induce a rise in the thyroid auto-antibody titre

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(21), which will in turn cause destructive changes in the thyroid gland in certain cases, depending upon the genetic predisposition. Furthermore, the additions of treatment regimens, which always include IFN-α, add another insult to the already vulnerable thyroid gland. It has been suggested that IFN *in vitro* may cause hypothyroidism (Hypo), at least in part, by an abnormal expression and function of key proteins involved in iodine uptake and organification (22). However, it is clear that with each insult to the thyroid gland in the appropriate clinical setting, TD is likely to occur (*Figure 1*). For TD to develop there must be additional factors causing deviation from this normal physiological adjustment. Environmental factors such as preexisting thyroid nodularity, high thyroid autoantibody titre and smoking have been thought to potentiate the effects of IFN on thyroid tissue. Iodine status does not appear to potentiate the effect of IFN on thyroid function (23). Genetic and constitutional factors such as Human Leucocyte Antigen-DR3 (HLA-DR3), Cytotoxic T-Lymphocyte Antigen-4 (CTLA-4), female gender, increased age and a family history of autoimmune thyroid disease (AITD) all confer an increased risk of developing TD (11, 24). Repeated treatments with IFN-α also increase the incidence of TD (13). It should be noted however that the majority of patients undergoing combination IFN therapy do not develop TD (15, 25). Beside the α form, IFN-β has also been reported to result in TD, although not to the same frequency (26). This is because IFN-β is used exclusively in the treatment of Multiple Sclerosis and not in those with HCV infection where HCV is known to have an immuno-stimulating effect.

Despite its potential adverse effects, IFN-α in combination with RBV remains the treatment of choice for chronic HCV infection as previously mentioned. As treatment progressed, IFN-α was pegylated to improve its half-life and improved the frequency of its delivery to weekly. Our previous study indicated that the pegylated forms of IFN-α appears to

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have a similar thyroid outcome compared with standard IFN (27). Thus this report includes studies using either IFN-α preparations, the pegylated and unpegylated forms.

THYROID DISEASE AND IFN-α BASED THERAPY

Thyroid Disease Before IFN Therapy

The prevalence of TD before treatment initiation in patients with chronic HCV infection is not known. Only a few number of studies performed pre-treatment thyrotropin screenings but often do not specify the exact nature of the TD, whether it be Hypo or thyrotoxicosis (TTX). The prevalence varies considerably from 2.3 to 13% in various series (11). Andrade et al (28) had 3 TD patients (1 with overt Hypo, 1 subclinical Hypo and one subclinical thyrotoxicosis (TTX) and 4 with positive for anti-TPO antibodies out of 65 cases. Costelloe et al (29) had 5 preexisting TD excluded out of 265 pure HCV cases prior to starting treatment. Dabrowska et al (30) has the prevalence at 21.4%, 19 out of 89 cases. Rodriguez-Torres et al (31) has it at 15%, 15 out of 100 screened cases prior to treatment. Moncoucy et al (32) had 24 out of 292 (8.2%) patients with 'dysthyroidism' before treatment. Carella et al (33), put the prevalence at 4.8%, 7 out of 147. These are summarised in Table 1.

As previously mentioned, these studies often do not clearly define the criteria to determine the exact thyroid diagnoses. This makes it difficult to compare the prevalence of TD with those in the general population with HCV infection. Anti-viral therapy is relatively contraindicated in the presence of TD and therefore should be fully delineated and managed before starting therapy. (9). However, there are no current data to suggest that pre-existing TD or its treatment alter the HCV treatment response.

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Thyroid Disease During IFN Therapy

Combination pegylated IFN-α and RBV therapy has become the gold standard treatment for patients with HCV. Beside regular IFN-α's which are given thrice weekly, the pIFN-α's involve the addition of a Polyethylene Glycol (PEG) moiety to the IFN molecule which results in a longer half-life with increased therapeutic efficacy (34). The medication thus can be conveniently given on a weekly basis which markedly improved adherence. Despite this difference, the pegylated form of IFN-α appears to have a similar, but not greater effect than that of regular IFN-α on TD although supportive data are still inadequate for certainty (27).

Because of the similarity between regular IFN-α and pegylated IFN-α, this manuscript has included reports that use both forms in combination with RBV. *Table 2* summarises the available publications, notwithstanding the inadequate data on frequency and lack of diagnostic criteria of the various thyroid conditions.

Thyroid disease which develops during treatment tends to be mostly bi-phasic thyroiditis and occurs on average on the 18th week of therapy (43). Graves' like TTX has been reported but these occur late in the course of treatment. The number of Graves' subjects is too small to make any definitive conclusion to distinguish it from those arising de novo (44). Many reports are plagued by the inconsistencies in the definition of TD making it difficult to append an accurate diagnosis be it bi-phasic thyroiditis or TTX for prognostication and follow-up (32, 40). However, it is critical that TFTs are performed regularly, ideally every 4 weeks, in order to detect TD which is often unpredictable (43). Additionally, symptoms are often subclinical and masked by the effects of IFN therapy. Thyroid disease detection is important in order to counsel patients effectively for any atypical or unusual symptoms that develop during therapy. Of more importance was the recent suggestion that treatment-associated TD heralds a good prognostic

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marker for sustained virologic response (SVR) (45, 46). This is not necessarily causal but the presence of thyroid hormones *in vitro* potentiates the antiviral action of interferon (albeit γ- rather than α-) in cultured human cells (47).

It is therefore strongly recommended that TFTs be performed monthly during treatment as symptoms are non-specific. Furthermore, this regimen will correctly delineate thyroiditis from primary de novo Hypo which is an uncommon occurrence in this setting. It is also important that that the hyperthyroid phase of bi-phasic thyroiditis is distinguished from primary Graves' like TTX.

Thyroid Disease After IFN Therapy

In patients who had developed thyroiditis during the course of treatment, there are very few follow up studies and hence the natural history of this condition remains contentious. Of the few available studies, the number of patients and duration of follow-up are variable. Similarly, they also suffer from the unstandardized classifications of TD mentioned in the previous section. However, it appears that a certain percentage of these patients sustain permanent Hypo although few were given the opportunity of a trial without LT4 to determine its permanency, *Table 3*.

In those who did not develop TD during therapy, as distinct from above, the data regarding the prevalence of this issue in the general treated group *following* the completion of treatment is lacking. Tran et al (48) performed a 3 year follow up in 190 patients and found thyroid disease a rare occurrence in this clinical setting. Others have studied their whole group who had TD during treatment for a variable duration. In spite of the dearth of data, it appears satisfactory to follow these cases to 6 months after the completion of treatment, coinciding with the SVR review episode. Thereafter, TD is unlikely and any development is likely to be

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independent of IFN based therapy. Where hepatitis C infection has been eradicated, the development of TD is less likely as HCV on its own has also been implicated in TD. The data are summarized in Table 4.

Notwithstanding the relative lack of information on this important topic, the following recommended thyroid surveillance protocol, based on current limited available data and the authors' experience, offers the most suitable strategy to manage TD before, during and after treatment for chronic HCV infection.

RECOMMENDATIONS

The following are our evidence-based recommendations for the surveillance and management of TD during combination treatment with IFN-α and RBV for chronic hepatitis C infection:

For case detection, it is recommended that all patients undergoing combination IFN-α and RBV therapy for HCV infection have a baseline TSH level, followed by monthly TSH levels for the duration of treatment (39). All patients should have a follow-up TSH at the time of SVR review (48).

For confirmation of TD, a specialist Endocrinology unit should be consulted. Patients with TSH <0.05mIU/L should proceed to have LT4 and fT3 levels. Hypothyroidism is confirmed where TSH is >4.0mIU/L. Free T4 level should also be performed at the same time. In order to further delineate the diagnosis, human thyrotropin receptor antibody, anti-thyroglobulin antibody and anti-thyroperoxidase antibody titres should be performed in all confirmed cases, a nuclear thyroid uptake scan should also be performed in all cases.

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With regards to management, observation is recommended with monthly TFTs in cases of thyroiditis (43). Beta-blockade is indicated where there are excessive adrenergic symptoms. Thionamides are not indicated in the thyrotoxic phase. Levothyroxine supplement is not indicated in the hypothyroid phase unless profoundly symptomatic. For Graves' like TTX, thionamides are indicated with beta-blockers for adrenergic symptom control where appropriate (44). Levothyroxine supplement is indicated in the presence of confirmed and symptomatic primary Hypo. De novo Hypo in this setting is very uncommon.

In terms of prognosis, bi-phasic thyroiditis is benign and thyroid function returns to normal in the long term (44). In Graves' like TTX, the prognosis is guarded with limited available data (44). It is therefore recommended that these patients be treated identical to those in whom Graves' disease arises de novo. In primary Hypo, the prognosis is unknown. It is recommended that a trial off LT4 three months after the completion of IFN-α therapy to determine if life long LT4 supplement is required.

For post-treatment follow-up and review, screening for TD with a one off TSH level is recommended at the time of SVR review. Routine monthly TSH values following the completion of therapy is not recommended due to the low prevalence of TD in this group (50). Case detection during this period should be guided by symptomatology.

FUTURE DIRECTIONS

Although the incidence of HCV infection has plateaued, if not declined (1), the number of patient requiring treatment is expected to increase, at least in the near future, resulting in an increased prevalence of associated TD. Furthermore, as the infection is likely to be transmitted by IVDU mostly in the young adolescents and young adults (6), it is expected these patients are more likely to seek treatment and hence the likely increase in TD. The introduction of a new class of

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medication, the serine protease inhibitors, is not expected to result in a reduction of TD prevalence because combination therapy with IFN and RBV will remain the backbone of chronic hepatitis C treatment (51). Most trials involving protease inhibitors so far include IFN and RBV in their treatment protocols (52). In addition, because of a high response rate in patients with genotype 3, it is likely that combination IFN and RBV therapy will continue to be the treatment of choice in the near future. It is therefore important that TD associated with IFN therapy is fully understood as it can contribute significantly to comorbidities. It is equally important to determine whether adjunctive LT4 itself can be an important adjuvant therapy in the treatment of this chronic and debilitating HCV condition although supporting evidence is still in its embryonic stage with case reports and nested case study (45, 46). For this to be achieved, firstly a pilot study is recommended, in which supraphysiological exogenous LT4 is given to HCV subjects to mimic the thyroid hormonal profile, as in our previous study (43). Secondly and depending on the pilot study outcome, a randomized and controlled trial can then be performed to truly ascertain the beneficial effect of LT4 supplementation.

CONCLUSION

In patients receiving combination IFN-α and RBV therapy, there is a strong association with TD although the literature is sparse, lacking clarity and the nomenclature not standardised. Patients undergoing this therapy should have a strictly standardised protocol in order to detect and manage associated TD appropriately, especially where there is preliminary evidence that TD heralds a favourable prognostic outcome with regards to curing HCV infection (45, 46).

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DISCLOSURE

No competing financial interests exist.

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REFERENCES

1 **Williams IT, Bell BP, Kuhnert W, Alter MJ.** Incidence and transmission patterns of acute hepatitis C in the United States, 1982–2006. *Arch Intern Med.* 2001;171:242-248.

2 WHO website. Hepatitis C. www.who.int/mediacentre/factsheets/fs164/en. July 2012. Accessed for verification September 13, 2012.

3 **Australian Government, Department of Health and Ageing.** Third National Hepatitis C Strategy 2010–2013. www.health.gov.au/internet/main/publishing.nsf/content/ohp-national-strategies-2010-hcv/$File/hcv.pdf. Accessed for verification September 18, 2012.

4 **Nelson PK, Mathers BM, Cowie B, et al.** Global epidemiology of hepatitis B and hepatitis C in people who inject drugs: results of systematic reviews. *Lancet.* 2011;378:571-583.

5 **Armstrong GL, Wasley A, Simard EP, McQuillan GM, Kuhnert WL, Alter MJ.** The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. *Ann Intern Med.* 2006;144:705-714.

6 CDC, from the centers for disease control and prevention 2011. Hepatitis C virus infection among adolescents and young adults – Massachusetts, 2002–2009. *JAMA.* 2011;305:2511-2513.

7 **Wilson LE, Widman D, Dikman SH, Gorevic PD.** Autoimmune disease complicating antiviral therapy for hepatitis C virus infection. *Semin Arthritis Rheum.* 2002;32:163-173.

8 **European Association for the Study of Liver.** EASL Clinical practice guidelines: Management of hepatitis C virus infection. *J Hepatol*. 2011;55:245-264.

9 **Ghany MG, Strader DB, Thomas DL, Seeff LB.** Diagnosis, management, and treatment of hepatitis C: An update. *Hepatology*. 2010;49:1335-1374.

DOI:10.4158/EP12195.RA

10 **Demers LM, Spencer CA.** Laboratory support for the diagnosis and monitoring of thyroid disease, 2003. www.nacb.org/lmpg/thyroid_lmpg_pub.htm. Accessed for verification September 13, 2012.

11 **Tran HA.** Hepatitis C infection, treatment regimens, and thyroid function abnormalities. The Endocrinologist 2007;17:231-235.

12 **Tuefel A, Weinmann A, Kahaly GJ, et al.** Concurrent autoimmune diseases in patients with autoimmune hepatitis. *J Clin Gastroenterol.* 2010;44:208-213.

13 **Carella C, Mazziotti G, Amato G, Braverman LE, Roti E.** Interferon-α-related thyroid disease: Pathophysiological, epidemiological, and clinical Aspects. *J Clin Endocrinol Metab.* 2004;89:3656-3661.

14 **Prummel MF, Laurberg P.** 2003. Interferon-α and autoimmune thyroid disease. *Thyroid.* **2003;13**:547-551.

15 **Huang M-J, Tsai S-L, Huang B-Y, et al.** Prevalence and significance of thyroid autoantibodies in patients with chronic hepatitis C virus infection: a prospective controlled study. *Clin Endocrinol.* 1999;50:503-509.

16 **Betterle C, Fabris P, Zanchetta R, et al.** Autoimmunity against pancreatic islets and other tissues before and after interferon-a therapy in patients with hepatitis C virus chronic infection. *Diabetes Care.* 2000;23:1177–1181.

17 **Corssmit EPM, Heyligenberg R, Endert E, Sauerwein HP, Romijn JA.** Acute effects of Interferon-alpha administration on thyroid hormone metabolism in healthy men. *J Clin Endocrinol Metab.* 1995;80:3140-3144.

18 **Tran HA, Jones TL, Batey RG.** The spectrum of thyroid disease treated with alpha-2-beta interferon and ribavirin in an Australian population. *BMC Endocr Disord.* 2005;**5**:8.

DOI:10.4158/EP12195.RA

19 **Lloyd AR, Jagger E, Post JJ, et al.** Host and viral factors in the immunopathogenesis of primary hepatitis C virus infection. *Immunol Cell Biol.* 2007;85:24-32.

20 **Tilg H.** New insights into the mechanisms of interferon alpha: an immunoregulatory and anti-inflammatory cytokine. *Gastroenterology.* 1997;112:1017-1021.

21 **Antonelli A, Ferri C, Pampana A, Fallahi P.** Thyroid disorders in chronic hepatitis C. *Am J Med.* 2004;117:10-13.

22 **Caraccio N, Guannini R, Cuccato S, et al.** Type I interferon modulate the expression of thyroid peroxidase, sodium/iodide symporter, and thyroglobulin genes in primary human thyrocyte cultures. *J Clin Endocrinol Metab.* 2005;90:1156-1162.

23 **Minelli R, Braverman LE, Biuberti T, et al.** Effects of excess iodine administration on thyroid function in euthyroid patients with a previous episode of thyroid dysfunction induced by interferon-alpha treatment. *Clin Endocrinol (Oxford).* 1997;47:357-361.

24 **Tomer Y, Davies TF.** Searching for the autoimmune thyroid disease susceptibility genes. From gene mapping to gene function. *Endocrin Rev.* 2003;24:694-717.

25 **Kontorinis N, Garas G, Young J, et al.** Outcome, tolerability and compliance of compassionate use interferon and ribavirin for hepatitis C infection in a shared care hospital clinic. *Int J Med.* 2003;33:500-504.

26 **Durelli L, Ferrero B, Oggero A, et al.** Betaferon Safety Trial Study Group 2001. Thyroid function and autoimmunity during Interferon beta-1b treatment: A multicenter prospective study. *J Clin Endocrinol Metab.* 2001;86:3525-3532.

27 **Tran HA, Attia J, Jones TL, Batey RL.** Pegylated interferon-alpha-2-beta in combination with ribavirin does not aggravate thyroid dysfunction in comparison to regular interferon-alpha-2-beta in a hepatitis C population: Meta-analysis. *J Hepatol Gastroenterol.* 2007;22:472-476.

DOI:10.4158/EP12195.RA

28 **Andrade LJ, Atta AM, Atta ML, Mangabeira CN, Parana R**. Thyroid disorders in patients with chronic hepatitis C using interferon-alpha and ribavirin therapy. *Braz J Infect Dis.* 2011;15:377-381.

29 **Costelloe SJ, Wassef N, Schulz J, et al.** Thyroid dysfunction in a UK hepatitis C population treated with interferon-α and ribavirin combination therapy. *Clin Endocrinol.* 2010;73:249-256.

30 **Dabrowska MM, Panasiuk A, Flisiak R.** Thyroid dysfunction in antiviral therapy of chronic hepatitis C. *Hepatogastroenterol.* 2010;57:826-831.

31 **Rodriguez-Torres M, Rios-Bedoya CF, Ortiz-Lasanta G, Marxuach-Cuetara AM, Jimenez-Rivera J**. Thyroid dysfunction (TD) among chronic hepatitis C patients with mild and severe hepatic fibrosis. *Ann Hepatol.* 2008;7:72-77.

32 **Moncoucy X, Laymarie F, Delemer B, et al.** Risk factors and long-term course of thyroid dysfunction during antiviral treatments in 221 patients with chronic hepatitis C. *Gastroenterol Clin Biol.* 2005;29:339-345.

33 **Carella C, Mazziotti G, Morisco F, et al.** The addition of ribavirin to interferon-α therapy in patients with hepatitis C virus-related chronic hepatitis does not modify the thyroid autoantibody pattern but increases the risk of developing hypothyroidism. *Eur J Endocrinol.* 2002;**146**:743-749.

34 **Moreno-Otero R.** Therapeutic modalities in hepatitis C: challenges and development. *J Viral Hepat.* 2005;12:10-19.

35 **Jamil KM, Leedman PJ, Kontorinis N, et al.** Interferon-induced thyroid dysfunction in chronic hepatitis C. *Gastroenterol Hepatol.* 2009;24:1017-1023.

DOI:10.4158/EP12195.RA

36 **Vezali E, Elfsiniotis I, Mihas C, Konstantinou E, Saroglou G.** Thyroid dysfunction in patients with chronic hepatitis C: Virus- or therapy-related? *Gastroenterol Hepatol*. 2009;24:1024-1029.

37 **Gelu-Simon M, Burlaud A, Young J, Pelletier G, Buffet C** 2009. Evolution and predictive factors of thyroid disorder due to interferon alpha in the treatment of hepatitis C. *World J Gastroenterol.* 2009;15:328-333.

38 **Masood N, Ghori R, Memon A, et al**. Frequency of thyroid disorders during intereferon and ribavirin therapy in chronic hepatitis C infection. *J Coll Phys Surg Pakistan.* 2008;18:347-351.

39 **Kee K-M, Lee C-M, Wang J-H, et al.** Thyroid dysfunction in patients with chronic hepatitis C receiving a combined therapy of interferon and ribavirin: Incidence, associated factors and prognosis. *Jrn Gastroenterol Hepatol.* 2006;21:319-326.

40 **Wirth S, Boustani-Pieper H, Lang T, et al.** 2005. Peginterferon alfa-2b plus ribavirin treatment in children and adolescents with chronic hepatitis C. *Hepatology.* 2005;41:1013–1018.

41 **Bini EJ, Mehandru S.** 2004. Incidence of thyroid dysfunction during interferon Alfa-2b and ribavirin therapy in men with chronic hepatitis C. *Arch Intern Med.* 2004;164:2371-2376.

42 **Adinolfi LE, Utili R, Tonziello A, Ruggiero G.** Effects of alpha interferon induction plus ribavirin with or without amantadine in the treatment of interferon non-responsive chronic hepatitis C: a randomized trial. *Gut.* 2003;52:701-705.

43 **Tran HA, Reeves GEM, Jones TL.** 2009. The natural history of interferon-a2b-induced thyroiditis and its exclusivity in a cohort of patients with chronic hepatitis C infection. *Q J Med.* 2009;102:117-122.

DOI:10.4158/EP12195.RA

44 **Tran HA, Reeves GEM.** Characteristics of Graves' disease in a cohort of chronic hepatitis C patients treated with interferon-α and ribavirin. *J Endocrinol Metab.* 2011;1:14-20.

45 **Tran HA, Ianna EA, Jones TL, Reeves GEM.** The adjuvant role of thyroxine in the treatment of chronic hepatitis C infection. *Q J Med.* 2012;105:683-687.

46 **Tran HA, Jones TL, Gibson R, Reeves GEM.** 2011. Thyroid disease is a favourable prognostic factor in achieving sustained virologic response in chronic hepatitis C undergoing combination therapy: A nested case control study. *BMC Endocrine Disorders.* 2011;11:10.

47 **Lin Y-H, Thacore HR, David FB, David PJ.** Thyroid hormone analogues potentiate the antiviral action of interferon-gamma. *J Cell Physiol.* 1996;167:269-276.

48 **Tran HA, Reeves GEM, Ianna EA, Leembruggen N**. Thyroid function outcomes after pegylated interferon-α and ribavirin therapy for chronic hepatitis C. *Endocr Pract.* 2010;16:934-939.

49 **Carella C, Mazziotti G, Morisco F, et al.** The addition of ribavirin to interferon-α therapy in patients with hepatitis C virus-related chronic hepatitis does not modify the thyroid autoantibody pattern but increases the risk of developing hypothyroidism. *Eur J Endocrinol.* 2002;146:743-749.

50 **Tran HA, Jones TL, Ianna EA, Reeves GEM.** The natural history of interferon-a induced tiroiditis in chronic hepatitis C patients: a long term study. Thyroid Research 2011;4:2.

51 **Jensen DM.** A new era of hepatitis C therapy begins. *N Engl J Med*. 2011;364:1272-1274.

52 **Sherman KE, Flamm SL, Afdhal NH, et al.** Response-guided telaprevir combination treatment for hepatitis C virus infection. *N Engl J Med.* 2011;365:1014-1024.

DOI:10.4158/EP12195.RA

Authors	- Publication origin - Year - Study type	Number of patients F : M	Age (years)	Ethnicity	Number of thyroid patients (%)
Andrade et al (28)	- Brazil - 2011 - Pros	65 38 : 27	49.6 ± 11.8	Unspec	3 (4.6%) * 4 with anti-TPO positivity
Costelloe et al (29)	- England - 2010 - Pros	265 88 : 172	47.0 ± 10.5	White Caucasian: 142 Asian: 30 Unknown: 72	5 (1.9%)
Dabroska et al (30)	- Poland - 2010 - Retro	89 32 : 57	42 ± 11	Unspec	19 (21.3%)
Rodriguez-Torres et al (31)	- Puerto Rico - 2008 - Retro	100 35 : 65	52.7 (median)	Unspec	15 (15%)
Moncoucy et al (32)	- France - 2005 - Retro	292	** Analysis was done for patients with TD only. No other data was available*		24 (8.2%)
Carella et al (33)	- Italy - 2002 - Pros	147 48 : 99	48 (median)	Unspec	7 (4.8%)
MEAN PREVALENCE: ~9.3%					

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Table 1. The prevalence of thyroid *disease* before starting combination treatment including the mean. Unspec: Unspecified; Pros: Prospective; Retro: Retrospective.

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Authors	- Publication origin - Year - Study type	Number of patients F : M	Age (years)	Ethnicity	No. of TD patients (%)	TFT criteria	pIFN & RBV	Regular IFN & RBV	Type of thyroid disease	Frequency of monitoring
Andrade LJ et al (28)	- Brazil - 2011 - Pros	65 38 : 27	49.6 ± 11.8	Unspec	4 (4.6%)	Not stated	0	65	1 TTX and 2 Hypo	3 monthly
Costelloe et al (29)	- England - 2010 - Pros	260 88 : 172	47.0 ± 10.5	C: 142 A: 30 Unknown: 72	58 (2.6%)	TSH < 0.27 and TSH > 4.2	0	58	27 TTX and 31 Hypo	4 weekly
Dabrowska et al (30)	- Poland - 2010 - Retro	114 32 : 57	42 ± 11	Unspec	12 (13.5%)	Not stated	0	89	12 TTX and 4 Hypo	3 monthly
Jamil et al (35)	- Australia - 2009 - Retro	346 Gender – unspecified	43.2 in subgroup analysis	C: 81% A: 17 % in subgroups	37 (8.8%)	TSH > 4 or fT4 <10 and TSH < 4.0 or > 23	25	12	Not stated	3 monthly
Vezali et al (36)	- Greece - 2009 - Retro	61 28 : 33	39.5 ± 13.1	Unspec	13 (21.3%)	TSH > 4.0 or < 0.3 irrespective of fT4/fT3 levels	50	0	Not stated	3 monthly
Gelu-Simeon et al (37)	- France - 2009 - Retro	264	** Study included monotherapy subjects, hence no age, ethnicity or gender can be deciphered.*		27 (10.0%)	TSH <0.3 or >4.0 on 2 successive tests	182	82	Not stated	2 monthly
Masood et al (38)	- Pakistan - 2008	100 77 : 23	35.3 ± 7.8	Unspec	18 (18.0%)	"Below or above normal range"	0	100 for 24 wks	Not stated	3 monthly

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	- Pros							*irrespective of HCV genotype*		
Kee et al (39)	- Taiwan - 2006 - Pros	461 199 : 262	50.6 ± 11.3	Unspec	58 (12.6%)	TSH > 5.0, fT4 < 10.2 and TSH < 0.1, fT4 > 25.9	9	49	Not stated	3 monthly

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Wirth et al (40)	- Germany - 2005 - Pros	62 33 : 29	10.6 (median)	Unspec	5 (10.3%)	TSH elevation, no other criteria stated	61	0	Elevated TSH	Not stated
Moncoucy et al (32)	- France - 2005 - Retro	67	** Analysis was done for patients with TD only. No other data was available*		4 (7.0%)	Increase or decrease in TSH on 2 occasions (0.23-4.0)	Not stated	Not stated	Not stated	Every 2-3 months
Tran et al (18)	- Australia - 2005 - Retro	272 122 : 150	42 ± 8	C: 75% A: 8%	18 (5.5%)	TSH < 0.1, fT4 > 26.0 or TSH > 4.0		272	15 Hypo and 3 TTX	Monthly
Bini et al (41)	- USA - 2004 - Pros	225 0 : 225	49.7 ± 6.4	White: 43.1% A/A: 34.2% Hisp: 20.4%	15 (10.7%)	TSH > 5.5, fT4 < 10.3 or TSH<0.4, fT4>34.7		15	12 Hypo and 3 TTX	Monthly
Kontorinis et al (25)	- Australia - 2003 - Pros	81 28 : 53	43.9 (median)	Unspec	8 (10.0%)	Not stated		8	6 Hypo and 2 TTX	Not stated
Adinofi et al (42)	- Italy - 2003 - Pros	114 38 : 76	51 (median)	Unpsec	1 (1.1%)	Not stated		1	Thyroiditis	Every 2-4 weeks
Carella et al (33)	- Italy - 2002 - Pros	72 48 : 99	48 (median)	Unspec	11 (15.3%)	TSH>3.5, fT4 < 9.0, no definition for TTX		11	Not stated	Before and after 6 months of therapy
MEAN PREVALENCE: ~10.8%										

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Table 2. Summary of published of thyroid disease studies, their definition and frequency of thyroid testing including the mean prevalence in the setting of RBV and IFN-α. Unspec: Unspecified; Pros: Prospective; Retro: Retrospective; Hypo: Hypothyroidism; TTX: Thyrotoxic; C: Caucasians; A: Asians; A/A: African-Americans; Hisp: Hispanics.

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Authors	- Publication origin - Year - Study type	Number of patients F : M	Age (years)	Ethnicity	Number of patients with TD (%)	Follow-up duration	Outcomes
Jamil et al (35)	- Australia - 2009 - Retro	346 Gender unspecified	43.2 (median) in subgroup analysis	C: 81% A: 17% in subgroup analysis	Only 45 follow ups	Periods 4 to 113 months, mean 40	16 (93%) has permanent hypothyroidism; 93% permanent hypothyroidism. 2 out of six became hypothyroid and 1 remained hyperthyroid after 13 months.
Vezali et al (36)	- Greece - 2009 - Retro	61 28 : 33	39.5 ± 13.1	Unspec	13 (21.3%)	Follow up 6-96 months, mean 39.4 months	9 out of 13 did not reverse after stopping treatment and needed ongoing treatment of unknown duration. 2 cases within 1 month of stopping treatment, 3 cases in 6, 6.5 and 26 months of therapy stopping.
Gelu-Simeon et al (37)	- France - 2009 - Retro	264 – Study include monotherapy subjects hence no age, ethnicity or gender can be deciphered			27 (10.0%)	Follow up at 41.6 ± 15.4	Outcomes not stated
Kee et al (39)	- Taiwan - 2006 - Pros	461 199 : 262	50.6 ± 11.3		58 (12.6%)	6 month follow-up	After 6 months, 37 (63.8%) spontaneously normal. 16 followed up for 18 months: 6 recovered after 30.5 months, 2% persisted at 18 months, 1% has persistent symptoms requiring long-term treatment
Moncoucy et al (32)	- France - 2005 - Retro	268 - Analysis was done for patients with TD only. No other data was available		Unspec	15 (7.0%)	Follow-up at 15-90 months	2 Hyperthyroid and 13 hypothyroid, 7 out of 13 has permanent hypothyroidism.
Tran et al (18)	- Australia - 2005 - Retro	272 122 : 150	42 ± 8	C: 75% A: 8%	18 (6.7%)	Follow-up at 12 months	13 still hypothyroid.
Bini et al (41)	- North America - 2004 - Pros	225 0 : 225	49.7 ± 6.4	C: 43.1% A/A: 34.2% His: 20.4%	15 (10.7%) -	Follow up at 24 weeks	12 Hypo; 10 out of 12 resolved after 24.4 follow-up and 2 permanent hypothyroid. 3 TTX: 2 resolved, 1 GD requiring long term treatment at 22.7 month follow-up.

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Table 3. Long term outcomes of TD which had developed *during* combination treatment. Unspec: Unspecified; Pros: Prospective; Retro: Retrospective; Hypo: Hypothyroidism; TTX: Thyrotoxic; GD: Graves' Disease; C: Caucasians; A: Asians; A/A: African-Americans; Hisp: Hispanics.

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Authors	- Publication origin - Year - Study type	Number of patients F : M	Age (years)	Ethnicity	Number of thyroid patients (%)	Follow-up duration (months)	Thyroid cases
Tran et al (48)	- Australia - 2010 - Pro	190 78 : 112	49 ± 7		2 (1.1%)	6 months with thyroid tests at 0, 1, 3 and 6	2 cases: 1 thyroiditis and 1 with TSI blocking antibody related hypothyroidism
Vezali et al (35)	- Greece - 2009 - Retro	61 28 : 33	39.5 ± 13.1	Unspec	5 (8.2%)	Follow up 6-96 months, mean 39.4 months	2 cases within 1 month of stopping treatment 3 cases after 6, 6.5 and 26 months of stopping therapy
Gelu-Simeon et al (37)	- France - 2009 - Retro	264 – Study include monotherapy subjects hence no age, ethnicity or gender can be deciphered			27 (10.0%)	Follow up at 41.6 ± 15.4	Outcomes not stated
Kee et al (39)	- Taiwan - 2006 - Pros	461 199 : 262	50.6 ± 11.3	Unspec	58 (12.6%)	6 month follow-up	No thyroid disease mentioned
Bini et al (41)	- North America - 2004 - Pros	225 0 : 225	49.7 ± 6.4	C: 43.1% A/A: 34.2% His: 20.4%	15 (10.7%)	6 month follow-up	No new cases were detected at the end of treatment
Carella et al (49)	- Italy - 2001 - Pros	114 48 : 99	48 (median)	Unspec	7 (6.1%)	74 months (6.2 years)	7 (unspecified) cases at the end of follow-up
MEAN PREVALENCE: ~8.1%							

Table 4. Outcomes of TD developed *after* combination treatment. Unspec: Unspecified; Pros: Prospective; Retro: Retrospective; Hypo: Hypothyroidism; C: Caucasians; A: Asians; A/A: African-Americans; Hisp: Hispanics.

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Figure 1. A stepwise hypothesis regarding the causality of TD in relation to HCV infection, IFN related regimen and modifying factors. The TSH level declines in a stepwise fashion with each additional risk factor culminating in the development of thyroid disease, predominantly thyroiditis and less commonly Graves' like disease. TA: Thyroid autoantibodies; HCV: Hepatitis C virus; IFN: Interferon; RBV: Ribavirin.

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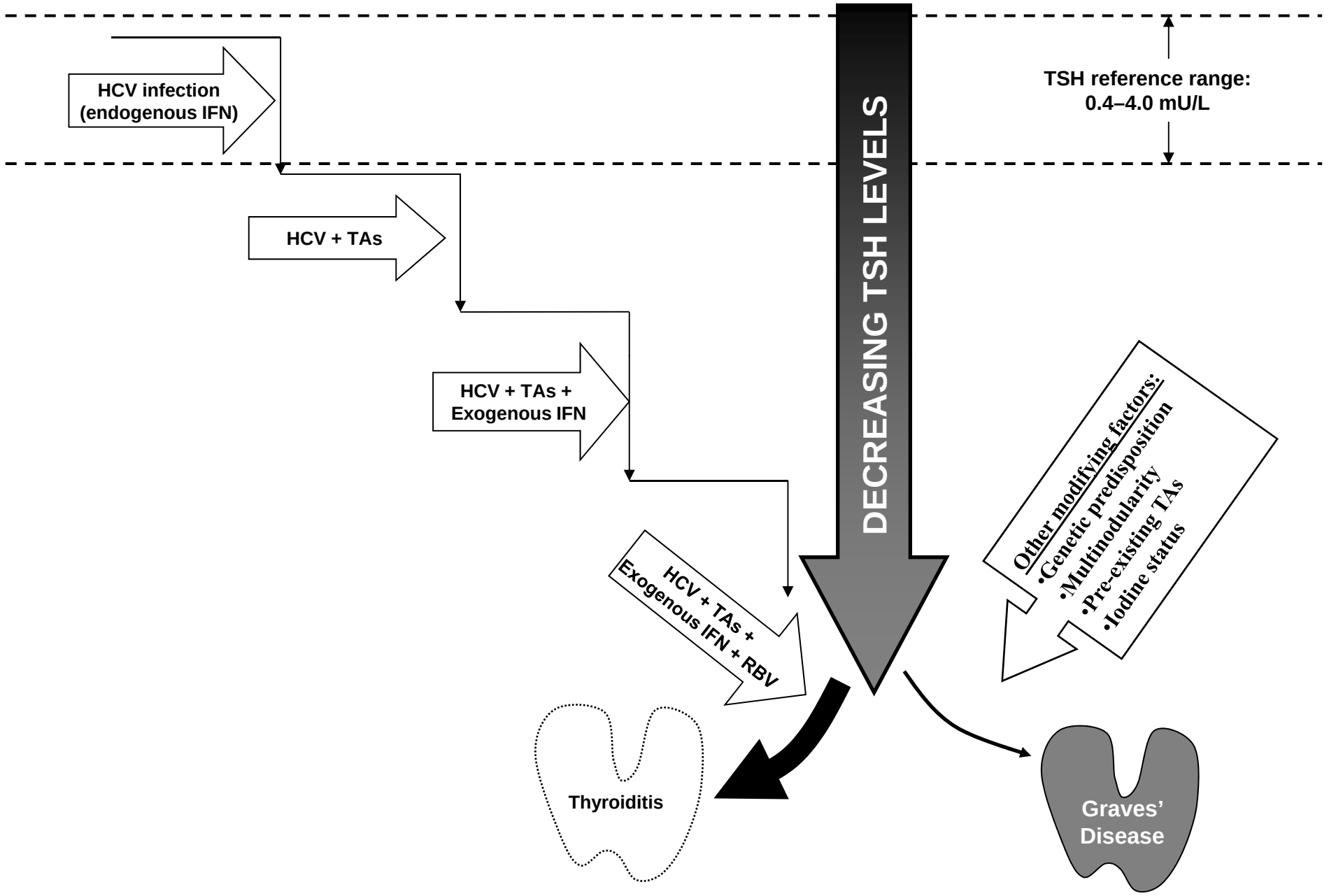
HCV infection
(endogenous IFN)
HCV + TAs
HCV + TAs +
Exogenous IFN
HCV + TAs +
Exogenous IFN + RBV
DECREASING TSH LEVELS
TSH reference range:
0.4–4.0 mU/L
Other modifying factors:
•Genetic predisposition
•Multinodularity
•Pre-existing TAs
•Iodine status
Thyroiditis
Graves'
Disease

CHAPTER IX. CONCLUSION

The findings from this dissertation have improved the understanding of thyroid disease occurring during and after combination therapy with IFN-alpha and RBV. The prevalence of TD proper approximates 5-8% and is consistent with our findings over an extended period. The assessment of TD should be done in a specialized endocrinology unit where a clear diagnosis could be assigned to each case. The majority of cases develop thyroiditis of biphasic nature with an initial phase of hyperthyroidism which is often symptomatic and compounded the psycho-physical adverse effects of IFN. The second phase entails hypothyroidism with eventual recovery back to normality. Whilst appearing benign, the long term outcome of these patients beyond 3 years, which was the limit of our research, is not known.

Rarely, patients developed Graves' like thyrotoxicosis in the latter half of treatment, either de novo or a second 'turn' of the bi-phasic disease in which it becomes tri-phasic. These patients require conventional treatment with thionamides. The duration of treatment is uncertain but it appears shorter than those arising de novo at 12-18 months. The prevalence of this condition is rare however, approximates <0.01% as we encounter 1 of these cases per 200-250 treated cases. Hypothyroidism is uncommon except for the second phase of the thyroiditis as aforementioned.

Treatment for HCV, even when TD has developed, should continue independently as any reduction or cessation of dosage may compromise therapeutic outcome and eventual SVR. This applies mainly to biphasic thyroiditis as Graves' disease commonly occurs in the post-therapy window.

CHAPTER X. BIBLIOGRAPHYY

A] REFERENCES BY TRAN, H. A. (M.D. Applicant)

1. **Tran HA**. THE UNCERTAIN NATURAL HISTORY OF THYROTOXIC PATIENTS TREATED WITH COMBINATION INTERFERON-α2β AND RIBAVIRIN. *Arch Intern Med*, 2005; 165: 1072.

2. **Tran HA.** THYROTOXICOSIS DURING PEGYLATED INTERFERON THERAPY IN A PATIENT WITH CHRONIC HEPATITIS C VIRUS. Endocr Pract, 2006; 12: 231–2.

3. **Tran HA**. HEPATITIS C INFECTION, TREATMENT REGIMENS, AND THYROID ABNORMALITIES. *The Endocrinologist*, 2007; 17: 231–235.

4. **Tran HA**, Jones TL, Batey RG. THE SPECTRUM OF THYROID DYSFUNCTION IN AN AUSTRALIAN HEPATITIS C POPULATION TREATED WITH COMBINATION INTERFERON-α2β AND RIBAVIRIN. *BMC Endocr Disord*, 2005; 5: 8.

5. **Tran HA**, Reeves GEM, Lyons TJ, Attia JR. HISTOPATHOLOGICAL FINDINGS OF AUTOIMMUNITY IN THYROID, PITUITARY AND ADRENAL DISEASES IN CHRONIC HEPATITIS C POST-MORTEM CASES. Endocr Pract, 2010; 16: 566-569.

6. **Tran HA**, Attia JR, Jones TL, Batey RG. PEGYLATED INTERFERON-α2β IN COMBINATION WITH RIBAVIRIN DOES NOT AGGRAVATE THYROID DYSFUNCTION IN COMPARISON TO REGULAR INTERFERON-α2β IN A HEPATITIS C POPULATION: META-ANALYSIS. *J Hepatol Gastroenterol*, 2007; 22: 472–6.

7. **Tran HA**, Reeves GE, Jones TL. THE NATURAL HISTORY OF INTERFERON- α2β-INDUCED THYROIDITIS AND ITS EXCLUSIVITY IN A COHORT OF PATIENTS WITH CHRONIC HEPATITIS C INFECTION. Q J Med, 2009; 102: 117–122.

8. **Tran HA**. THE SWINGING THYROID IN HEPATITIS C INFECTION AND INTEFERON THERAPY. Q J Med, 2010; 103: 187–191.

9. **Tran HA**, Reeves GEM. CHARACTERISTICS OF GRAVES' DISEASE IN A COHORT OF CHRONIC HEPATITIS C PATIENTS TREATED WITH INTERFERON-α AND RIBAVIRIN. J Endocrinol Metab, 2011; 1: 14–20.

10. **Tran HA**, Jones TL, Ianna EA, Reeves GE. THE NATURAL HISTORY OF INTERFERON-α INDUCED THYROIDITIS IN CHRONIC HEPATITIS C PATIENTS: A LONG TERM STUDY. Thyroid Res, 2011; 8: 4 (1): 2.

11. **Tran HA**. HEPATITIS C INFECTION AND THYROTOXIC PERIODIC PARALYSIS – A NOVEL USE OF AN OLD DRUG. Am J Med Sci, 2008; 336: 515–518.

12. **Tran HA**, Song S, Lojewski R, Reeves GE. EXACERBATION OF HEPATITIS C INDUCED SUBCLINICAL HYPOADRENALISM BY INTERFERON α2β: A CASE REPORT. Cases J, 2008; 1: 157.

13. **Tran HA, Reeves GEM.** THE INFLUENCE OF HEPATITIS C INFECTION AND INTERFERON-α THERAPY ON THYROTROPIN BLOCKING AND STIMULATING ANTIBODIES IN GRAVES' OPHTHALMOPATHY: A CASE REPORT. Thyroid Res, 2010; 2: 12.

14. **Tran HA, Crock PA, Reeves GEM.** PITUITARY DISEASE IN CHRONIC HEPATITIS C INFECTION AND INTERFERON-α RELATED THERAPY: TWO CASE REPORTS. *J Endocrinol Metab, 2012; (In Press).*

15. **Tran HA**, Reeves GEM, Gibson R, Attia JR. THE DEVELOPMENT OF THYROID DISEASES IN THE TREATMENT OF CHRONIC HEPATITIS C WITH INTERFERON-α MAY BE A GOOD PROGNOSTICATOR IN ACHIEVING A SUSTAINED VIROLOGICAL RESPONSE: A META-ANALYSIS. J Hepatol Gastroenterol, 2009; 24: 1163–1168.

16. **Tran HA,** Reeves GEM, Ianna EA. THE ADJUVANT ROLE OF THYROXINE IN THE TREATMENT OF CHRONIC HEPATITIS C. Q J Med, 2012; 105: 683–687.

17. **Tran HA,** R Gibson, Reeves GEM. THYROID DISEASE IS A FAVORABLE PROGNOSTIC FACTOR IN ACHIEVING SUSTAINED VIROLOGIC RESPONSE IN CHRONIC HEPATITIS C UNDERGOING COMBINATION THERAPY: A NESTED CASE CONTROL STUDY. BMC Endocr Disord, 2011; 11: 10.

18. **Tran HA,** Reeves GEM. THE SPECTRUM OF AUTOIMMUNE THYROID DISEASE IN THE SHORT TO MEDIUM TERM FOLLOWING INTERFERON-α THERAPY FOR CHRONIC HEPATITIS C. Int J Endocrinol, 2009; 2009: 241786. Epub 2009 Aug 31.

19. **Tran HA**, Reeves GEM, Ianna EA, Leembruggen N. THYROID FUNCTION OUTCOMES AFTER PEGYLATED INTERFERON-A AND RIBAVIRIN THERAPY FOR CHRONIC HEPATITIS C. Endocr Pract, 2010; 16: 934-939.

20. **Tran HA,** Jones TL, Ianna EA, Foy A, Reeves GEM. THYROID DISEASE IN CHRONIC HEPATITIS C INFECTION TREATED WITH INTERFERON-A: MANAGEMENT STRATEGIES AND FUTURE PERSPECTIVE. Endocr Pract, September-2012: *In Press*.

B] REFERENCES PUBLISHED BY OTHER AUTHORS

21. Vilcek J. Novel interferons. Nat Immunol, 2003; 4: 8–9.

22. Talpaz M, Kantarjan HM, McCredied K, Trujillo JM, Keating MJ, Gutterman JU. Haematologic remission and cytogenetic improvement induced by recombinant human interferon alpha A in chronic myelogenous leukemia. N Engl J Med, 1986; 314: 1065–9.

23. Arnason BG. Interferon beta in multiple sclerosis. Neurology; 1993: 43: 641.

24. Gleave ME, Elhilali M, Fradet Y, Davis I, Venner P, Saad F, Klotz LH, Moore MJ, Paton V, Bajmonde A. Interferon gamma-1b compared with placebo in metastatic renal-cell carcinoma. Canadian Urologic Oncologic Group. N Engl J Med, 1998; 338: 1265.

25. Ghany MG, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: An update. Hepatology, 2009; 49: 1353–1374.

26. Hoofnagle JH, Mullen KD, Jones DB. Treatment of chronic non-A, non-B hepatitis with recombinant human alpha interferon. A preliminary report. N Engl J Med, 1986; 315: 1575–1578.

27. David GL, Balart LA, Schiff ER. Treatment of chronic hepatitis C with recombinant interferon alfa: A multicenter randomized, controlled trail. N Engl J Med, 1989, 321: 1501-1506.

28. Di Bisceglie AM, Martin P, Kassianidies. Recombinant interferon alfa therapy for chronic hepatitis C. A randomized, double-blind, placebo-controlled trial. N Engl J Med, 1989; 321: 1506-1510.

29. Lau JYN, Tam RC, Liang TJ, Hong Z. Mechanism of action of ribavirin in the combination treatment of chronic HCV infection. Hepatology, 2002; 35: 1002–1009.

30. Glue P, Fang JWS, Sabo R, Rouzier-Panis R, Raffanel C, Rabo R, Gupta SK, Jacobs S, Geraund JJ, Albrecht JK. Peg-interferon-a2B: pharmacokinetics, pharmacodynamics, safety, and preliminary efficacy data [Abstract]. Hepatology, 1999; 30 (Suppl): A189.

31. Jensen DM. A new era of hepatitis C therapy begins. N Engl J Med, 2011; 364: 1272–1274.

32. Fentiman IS, Thomas BS, Balkwill FR, Rubens RD, Hayward JL. Primary hypothyroidism associated with interferon therapy of breast cancer. Lancet, 1985; 8348: 1166.

33. Koh LK, Greenspan FS, Yeo PP. Interferon-alpha induced thyroid dysfunction: three clinical presentations and a review of the literature. Thyroid, 1997; 7: 891–896.

34. Williams IT, Bell BP, Kuhnert W, Alter MJ. Incidence and transmission patterns of acute hepatitis C in the United Stated, 1982–2006. Arch Intern Med, 2011; 171: 242–248.

35. De Groot LJ. Dangerous dogmas in medicine: The nonthyroidal illness syndrome. J Clin Endocrinol Metab, 1999; 84: 151–164.

36. Andrade LJ, Atta AM, Atta ML, Mangabeira CN, Parana R. Thyroid disorders in patients with chronic hepatitis C using interferon-alpha and ribavirin therapy. Braz J Infect Dis, 2011; 15: 377–381.

37. Costelloe SJ, Wassef N, Schulz J, Vaghijiani T, Morris C, Whiting S, Thomas M, Dusheiko G, Jacobs M, Vanderpump MPJ. Thyroid dysfunction in a UK hepatitis C population treated with interferon-α and ribavirin combination therapy. Clin Endocrinol, 2010, 73: 249–256.

38. Dabrowska MM, Panasiuk A, Flisiak R. Thyroid dysfunction in antiviral therapy of chronic hepatitis C. Hepatogastroenterol, 2010; 57: 826–831.

39. Jamil KM, Leedman PJ, Kontorinis N, Tarquino L, Nazareth S, McInerney M, Connelly C, Flexman J, Burke V, Metcalf C, Cheng W. Interferon-induced thyroid dysfunction in chronic hepatitis C. Gastroenterol Hepatol, 2009; 24: 1017–1023.

40. Vezali E, Elfsiniotis I, Mihas C, Konstantinou E, Saroglou G. Thyroid dysfunction in patients with chronic hepatitis C: Virus- or therapy-related? Gastroenterol Hepatol, 2009; 24: 1024–1029.

41. Gelu-Simon M, Burlaud A, Young J, Pelletier G, Buffet C. Evolution and predictive factors of thyroid disorder due to interferon alpha in the treatment of hepatitis C. World J Gastroenterology; 2009: 15: 328–333.

42. Masood N, Ghori R, Memon A, Memon S, Memon KI, Memon I, Jaffri M, Baloch GH. Frequency of thyroid disorders during intereferon and ribavirin therapy in chronic hepatitis C infection. J Coll Phys Surg Pakistan, 2008; 18: 347–351.

43. Kee K-M, Lee C-M, Wang J-H, Tung H-D, Changchien C-S, Lu S-N, Wang P-W. Thyroid dysfunction in patients with chronic hepatitis C receiving a combined therapy of interferon and ribavirin: Incidence, associated factors and prognosis. Jrn Gastroenterol Hepatol; 2006; 21: 319-326.

44. Wirth S, Boustani-Pieper H, Lang T, Ballauff A, Kullmer U, Gerner P, Wintermeyer P, Jenke A. Peginterferon alfa-2b plus ribavirin treatment in children and adolescents with chronic hepatitis C. Hepatology, 2005; 41: 1013–1018.

45. Moncoucy X, Laymarie F, Delemer B, Levy S, Bernard-Chabert B, Bouche O, Jolly D, Diebold M-D, Cadiot G, Thiefin G. Risk factors and long-term course of thyroid dysfunction during antiviral treatments in 221 patients with chronic hepatitis C. Gastroenterol Clin Biol, 2005; 29: 339–345.

46. Bini EJ, Mehandru S. Incidence of thyroid dysfunction during interferon Alfa-2b and ribavirin therapy in men with chronic hepatitis C. Arch Intern Med, 2004; 164: 2371–2376.

47. Kontorinis N, Garas G, Young J, Speers D, Chester BP, MacQuillan GC, De Boer B, Chapman MD, Forster E, Jeffrey GP. Outcome, tolerability and compliance of compassionate use interferon and ribavirin for hepatitis C infection in a shared care hospital clinic. Int J Med, 2003; 33: 500–504.

48. Adinolfi LE, Utili R, Tonziello A, Ruggiero G. Effects of alpha interferon induction plus ribavirin with or without amantadine in the treatment of interferon non-responsive chronic hepatitis C: a randomized trial. Gut, 2003; 52: 701–705.

49. Carella C, Mazziotti G, Morisco F, Rotondi M, Cioffi M, Tuccillo C, Sorvillo F, Caporaso N, Amato G. The addition of ribavirin to interferon-α therapy in patients with hepatitis C virus-related chronic hepatitis does not modify the thyroid autoantibody pattern but increases the risk of developing hypothyroidism. Eur J Endocrinol, 2002; 146: 743–749.

50. Kryczka W, Brojer E, Kowalska A, Zarebska-Michaluk D. Thyroid gland dysfunctions during antiviral therapy of chronic hepatitis C. Med Sci Monit, 2001; 7 (Supp. 1): 221–225.

51. Hamadah I, Binamer Y, Sanai FM, Abdo AA, Alajlan A. Interferon-induced vitiligo in hepatitis C patients: a case series. Int J Dermatol, 2010; 49: 829-833.

52. Fabbri C, Jaboli MF, Giovanelli S, Azzaroli F, Pezzoli A, Accogli E, Liva S, Nigro G, Miracolo A, Festi D, Colecchia A, Montagnani M, Roda E, Mazzella G. Gastric autoimmune disorders in patients with chronic hepatitis C before, during and after interferon-alpha therapy. World J Gastroenterol, 2003; 9: 1487-1490.

53. Uto H, Matsuoka H, Murata M, Okamoto t, Miyata Y, Hori T, Ido A, Hirono S, Hayashi K, Tsubouchi H. A case of chronic hepatitis C developing insulin-dependent diabetes mellitus associated with various autoantibodies during interferon therapy. Diabetes Res Clin Pract, 2000; 49: 101-106.

54. Betterle C, Fabris P, Zanchetta R, Pedini B, Tositti G, Bosi E, de Lalla F. Autoimmunity against pancreatic islets and other tissues before and after interferon-alpha therapy in patients with hepatitis C virus chronic infection. Diabetes Care, 2000; 23: 1177-1781.

55. Williams IT, Bell BP, Kuhnert W, Alter MJ. Incidence and transmission patterns of acute hepatitis C in the United States, 1982–2006. Arch Intern Med, 2011; 171: 242–248.

56. Jensen DM. A new era of hepatitis C therapy begins. N Engl J Med, 2011; 364: 1272–1274.

57. Sherman KE, et al. Response-guided telaprevir combination treatment for hepatitis C virus infection. N Engl J Med, 2011; 365: 1014–1024.

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STATEMENT I

This statement explains the contribution of all authors in the article listed below:

Tran HA. THE UNCERTAIN NATURAL HISTORY OF THYROTOXIC PATIENTS TREATED WITH COMBINATION INTERFERON-α2β AND RIBAVIRIN. *Arch Intern Med*, 2005; 165: 1072.

Table 1: Author contribution percentage and description of contribution to the article below

Author	Contribution (%)	Description of contribution	Signature
Tran, HA	100%	Designed, literature searched, executed and completed the manuscript.	

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Publication:	Archives of Internal Medicine
Publisher:	American Medical Association
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STATEMENT II

This statement explains the contribution of all authors in the article listed below:

Tran HA. THYROTOXICOSIS DURING PEGYLATED INTERFERON THERAPY IN A PATIENT WITH CHRONIC HEPATITIS C VIRUS. Endocr Pract, 2006; 12: 231-2.

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Tran, HA	100%	Designed, literature searched, executed and completed the manuscript.	

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Phone: 61249214005
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STATEMENT III

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Tran HA. HEPATITIS C INFECTION, TREATMENT REGIMENS, AND THYROID ABNORMALITIES. *The Endocrinologist*, 2007; 17: 231-235.

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STATEMENT IV

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Table 4: Author contribution percentage and description of contribution to the article below

Author	Contribution (%)	Description of contribution	Signature
Tran, HA	85%	Designed, literature searched, data gathering, statistical analysis, executed and completed the manuscript.	
Jones, TL	10%	Data gathering review and commented on the manuscript.	
Batey, RG	5%	Contributed to the statistical analysis, critically review the manuscript.	

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Table 5: Author contribution percentage and description of contribution to the article below

Author	Contribution (%)	Description of contribution	Signature
Tran, HA	80%	Designed, literature searched, data gathering, statistical analysis, executed and completed the manuscript.	
Reeves, GEM	5%	Statistical analysis, commented and review of the manuscript.	
Lyons, TJ	10%	Data gathering, perform histopatholgical review of samples and review the manuscript.	
Attia, JR	5%	Statistical analysis, commented and critical review of the manuscript.	

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Organization/Company: Hunter Area Pathology Service, Locked Bag 1, HRMC, Newcaslte, New South Wales, Australia
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STATEMENT VI

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Table 6: Author contribution percentage and description of contribution to the article below

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Tran, HA	75%	Designed, literature searched, data gathering, statistical analysis, executed and completed the manuscript.	
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STATEMENT VII

This statement explains the contribution of all authors in the article listed below:

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Table 7: Author contribution percentage and description of contribution to the article below

Author	Contribution (%)	Description of contribution	Signature
Tran, HA	90%	Designed, literature searched, data gathering, statistical analysis, executed and completed the manuscript.	
Reeves, GEM	10%	Commented and critical review of the manuscript.	

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STATEMENT VIII

This statement explains the contribution of all authors in the article listed below:

Tran HA, Reeves GE, Jones TL. THE NATURAL HISTORY OF INTERFERON-α2β-INDUCED THYROIDITIS AND ITS EXCLUSIVITY IN A COHORT OF PATIENTS WITH CHRONIC HEPATITIS C INFECTION. Q J Med, 2009; 102: 117-122.

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Tran, HA	85%	Designed, literature searched, data gathering, statistical analysis, executed and completed the manuscript.	
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STATEMENT IX

This statement explains the contribution of all authors in the article listed below:

Tran HA, Jones TL, Ianna EA, Reeves GE. THE NATURAL HISTORY OF INTERFERON-α INDUCED THYROIDITIS IN CHRONIC HEPATITIS C PATIENTS: A LONG TERM STUDY. Thyroid Res, 2011; 8: 4 (1): 2.

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STATEMENT XII

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STATEMENT XIII

This statement explains the contribution of all authors in the article listed below:

Tran HA, Song S, Lojewski R, Reeves GE. EXACERBATION OF HEPATITIS C INDUCED SUBCLINICAL HYPOADRENALISM BY INTERFERON α2β: A CASE REPORT. *Cases J, 2008; 1: 157.*

Table 13: Author contribution percentage and description of contribution to the article below

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STATEMENT XIV

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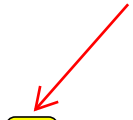

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STATEMENT XVI

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Tran HA, Reeves GEM, Ianna EA, Leembruggen N. THYROID FUNCTION OUTCOMES AFTER PEGYLATED INTERFERON-A AND RIBAVIRIN THERAPY FOR CHRONIC HEPATITIS C. Endocr Pract, 2010; 16: 934-939.

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17. **Tran HA**, Reeves GEM, Gibson R, Attia JR. THE DEVELOPMENT OF THYROID DISEASES IN THE TREATMENT OF CHRONIC HEPATITIS C WITH INTERFERON-α MAY BE A GOOD PROGNOSTICATOR IN ACHIEVING A SUSTAINED VIROLOGICAL RESPONSE: A META-ANALYSIS. J Hepatol Gastroenterol, 2009; 24: 1163–1168.

STATEMENT XVII

This statement explains the contribution of all authors in the article listed below:

Tran HA, Reeves GEM, Gibson R, Attia JR. THE DEVELOPMENT OF THYROID DISEASES IN THE TREATMENT OF CHRONIC HEPATITIS C WITH INTERFERON- α MAY BE A GOOD PROGNOSTICATOR IN ACHIEVING A SUSTAINED VIROLOGICAL RESPONSE: A META-ANALYSIS. J Hepatol Gastroenterol, 2009; 24: 1163-1168.

Table 17: Author contribution percentage and description of contribution to the article below

Author	Contribution (%)	Description of contribution	Signature
Tran, HA	80%	Designed, literature searched, statistical analysed, executed and completed the manuscript.	
Reeves, GEM	5%	Commented and reviewed the manuscript.	
Gibson, R	5%	Commented and reviewed the manuscript.	
Attia, JR	10%	Assisted with data gathering, statistical analysed, and commented on the final manuscript	

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18. **Tran HA,** Reeves GEM, Ianna EA. THE ADJUVANT ROLE OF THYROXINE IN THE TREATMENT OF CHRONIC HEPATITIS C. Q J Med, 2012; 105: 683-687.

STATEMENT XVIII

This statement explains the contribution of all authors in the article listed below:

Tran HA, Ianna EA, Jones TL, Reeves GEM. THE ADJUVANT ROLE OF THYROXINE IN THE TREATMENT OF CHRONIC HEPATITIS C INFECTION. Q J Med, 2012; 105: 683-687.

Table 18: Author contribution percentage and description of contribution to the article below

Author	Contribution (%)	Description of contribution	Signature
Tran, HA	85%	Designed, literature searched, executed and completed the manuscript.	
Ianna, EA	5%	Commented and reviewed the manuscript.	
Jones, TL	5%	Commented and reviewed the manuscript	
Reeves, GEM	5%	Commented and reviewed the manuscript.	

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STATEMENT XIX

This statement explains the contribution of all authors in the article listed below:

Tran HA, R Gibson, Reeves GEM. THYROID DISEASE IS A FAVORABLE PROGNOSTIC FACTOR IN ACHIEVING SUSTAINED VIROLOGIC RESPONSE IN CHRONIC HEPATITIS C UNDERGOING COMBINATION THERAPY: A NESTED CASE CONTROL STUDY. BMC Endocr Disord, 2011; 11: 10.

Table 19: Author contribution percentage and description of contribution to the article below

Author	Contribution (%)	Description of contribution	Signature
Tran, HA	85%	Designed, literature searched, executed and completed the manuscript.	
Gibson, R	5%	Commented and reviewed the manuscript.	
Reeves, GEM	10%	Provided and assisted with gathering data, statistical analysed and commented on the manuscript	

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STATEMENT XX

This statement explains the contribution of all authors in the article listed below:

Tran HA, Jones TL, Ianna EA, Foy A, Reeves GEM. THYROID DISEASE IN CHRONIC HEPATITIS C INFECTION TREATED WITH INTERFERON-A: MANAGEMENT STRATEGIES AND FUTURE PERSPECTIVE. Endocr Pract, September-2012: *In Press.*

Table 20: Author contribution percentage and description of contribution to the article below.

Author	Contribution (%)	Description of contribution	Signature
Tran, HA	80%	Designed, literature searched, executed and completed the manuscript.	
Jones, TL	5%	Commented and reviewed the manuscript.	
Ianna, EA	5%	Commented and reviewed the manuscript.	
Foy, A	5%	Commented and reviewed the manuscript.	
Reeves, GEM	5%	Provided and assisted with gathering data, statistical analysed and commented on the manuscript	

Huy Tran

From: Lori Clawges [lclawges@aace.com]
Sent: Wednesday, 31 October 2012 8:29 AM
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Subject: RE: Form submission from: COPYRIGHT/PERMISSION REQUEST FORM

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Thanks,
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Sent: Monday, October 22, 2012 9:10 PM
To: Lori Clawges
Subject: RE: Form submission from: COPYRIGHT/PERMISSION REQUEST FORM

Dear Lori,

Thank you so much for the below. I would like to follow up on two more articles please, for the same purpose:

1. Tran HA, et al. Thyroid Function Outcomes After Pegylated Interferon-alpha And Ribavirin Therapy For Chronic Hepatitis C. Endocr Pract, 2012; 16: 934-939.

2. Tran HA, et al. Thyroid Disease In Chronic Hepatitis C Infection Treated With Inteferon-alpha: Management Strategies And Future Perspective. Endocr Pract, September-2012: In Press.

I would be very grateful for the approval of the above two articles which I believe were requested through the Endocrine Practice Web Site.

Please let me know of any more requirement.

With kind regards

HUY TRAN

-----Original Message-----
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Thyrotoxicosis during pegylated interferon therapy in a patient with chronic hepatitis C virus.
Histological Evidence of Autoimmunity in Thyroid, Pituitary and Adrenal disease in Chronic Hepatitis C Post-Mortem Cases Thyroid Function Outcomes after Pegylated Interferon-alpha and Ribavirin Therapy for Chronic Hepatitis C

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ERRATA

1. Statistical anomalies in Chapter 7 (Page 135).

   a) Article entitled *"Development of thyroid diseases in the treatment of chronic hepatitis C with α-Interferon may be a good prognosticator in achieving a sustained virological response: A meta-analysis".*

   The p values in Table 1 are incorrect (page 139). Similarly the results for non-TD group in Figure 1 are also incorrect and clearly an arithmetic error (page 140). These should be identical to that in Table 1. All of the above have been revised in *Addendum I* for review. The relevant corrections are highlighted in yellow for ease of reference. The revised results show a marginally higher odds ratio in favour of the hypothesis although it remains statistically insignificant.

   These errata have been submitted to the relevant journal and the Editorial Office's response is eagerly awaited.

   b) Article entitled *"Thyroid disease is a favourable prognostic factor in achieving sustained virologic response in chronic hepatitis C undergoing combination therapy: a nested case-control study"*.

   There has been a gross transcription error and corruption of data with regards to the subsets of numerical values in Table 2 (page 153). This revised table is in *Addendum II* for review with the corrections highlighted in yellow as in (a). The published SVR rates, page 152, column 1, paragraph 2, remain unchanged.

   The extremely high SVR in genotype 3 groups, especially the control (non-TD group), is incorrect and is indeed lower upon revision.

   In addition and as a result of the revised data in Table 2, the following wording changes have been made:

   i. Page 153, column 2, line 21: The sentences "*However, end of treatment responses (ETR) appear to confirm previous observation that it is often a reliable predictor of SVR. Most patients who achieved ETR also completes SVR except for the two genotype 1 patients who relapsed as defined by failure to achieve SVR*" have been removed.

   ii. Page 154, column 1, line 6: The sentence "*Although our control cohort has a SVR rate of 100%, the overall reported and accepted rate approximates 80%, perhaps suggesting an additional synergistic effect in this genotype subgroup*" should read "*Our control cohort has a SVR rate of 81.3%, consistent with the overall reported and accepted rate of approximately 80.0%.*"

The study conclusion, and specifically all the conclusions in the Results section stand unchanged.

Similar to (a), these errata have been submitted to the relevant journal for consideration.

ADDENDUM I

	All Patients N = 201	Thyroid Dysfunction N = 11	No thyroid dysfunction N = 190	P-value
Female gender – number (%)	97 (48%)	7 (63%)	90 (47%)	0.35
Mean age (years)	49 ± 8	48 ± 9	50 ± 8	0.42
Weight (kg)	74 ± 18	76 ± 21	74 ± 18	0.36
Body Mass Indices (kg/m^2)	27 ± 7	26 ± 9	28 ± 8	0.42
Asian origin – no. (%)	36 (18%)	0	36 (19%)	0.53
Caucasians – no. (%)	151 (75%)	11 (100%)	140 (74%)	0.80
Genotypes – no. (%) • 1 • 2 • 3 • 4	 100 (50%) 16 (8%) 76 (38%) 9 (4%)	 4 (36%) 3 (27%) 2 (18%) 2 (18%)	 95 (50%) 13 (7%) 74 (39%) 8 (4%)	***0.01***
Viral Load • At baseline (log IU/mL) • Positive EVR at 12 weeks – (this is only applicable to genotypes 1 & 4) * • Positive ETR (at 24 weeks for genotypes 2&3 and 48 weeks for genotypes 1&4) # • SVR (24 weeks post therapy)	 5.97 ± 0.74 75/109 (69%) 161/201 (80%) 110/201 (54%)	 5.92 ± 0.80 6/6 (100%) 11/11 (100%) 11/11 (100%)	 5.98 ± 0.79 70/103 (68%) 143/190 (75%) 99/190 (53%)	 0.59 0.75 ***<0.001***
Albumin (36–48 g/L)	36 ± 2	36 ± 3	35 ± 8	0.68
Serum Bilirubin (2–20 µmol/L)	15 ± 5	14 ± 7	16 ± 4	0.13
Alanine Aminotransferase (< 45 U/L)	79 ± 22	65 ± 18	72 ± 20	0.26

γ-Glutamyl Transpeptidase (1–30 U/L)	53 ± 14	62 ± 11	54 ± 15	0.08
Prothrombin time (11–18 seconds)	15 ± 2	14 ± 3	15 ± 3	0.28
Haemoglobin (115–165 g/L)	154 ± 12	154 ± 13	153 ± 10	0.75
White cell counts (4.0–11.0 x 10^6/mL)	5.8 ± 1.9	6.9 ± 2.5	5.9 ± 2.2	0.15
Platelets (150–400 x 10^9/mL)	166 ± 37	181 ± 29	168 ± 38	0.27

Table 1. Characteristics of 201 patients including those *with and without* thyroid diseases. *; defined as ≥ 2 $\log_{10}$ viral load reduction compared with baseline by quantitation, #; no viral RNA is detected at the completion of treatment. EVR; Early Virological Response, ETR; End of Treatment Response, SVR; Sustained Virological Response, N/SS; Not Statistically Significant. Due to the small number in the TD group, there is inadequate statistical power to arrive at any definitive conclusions.

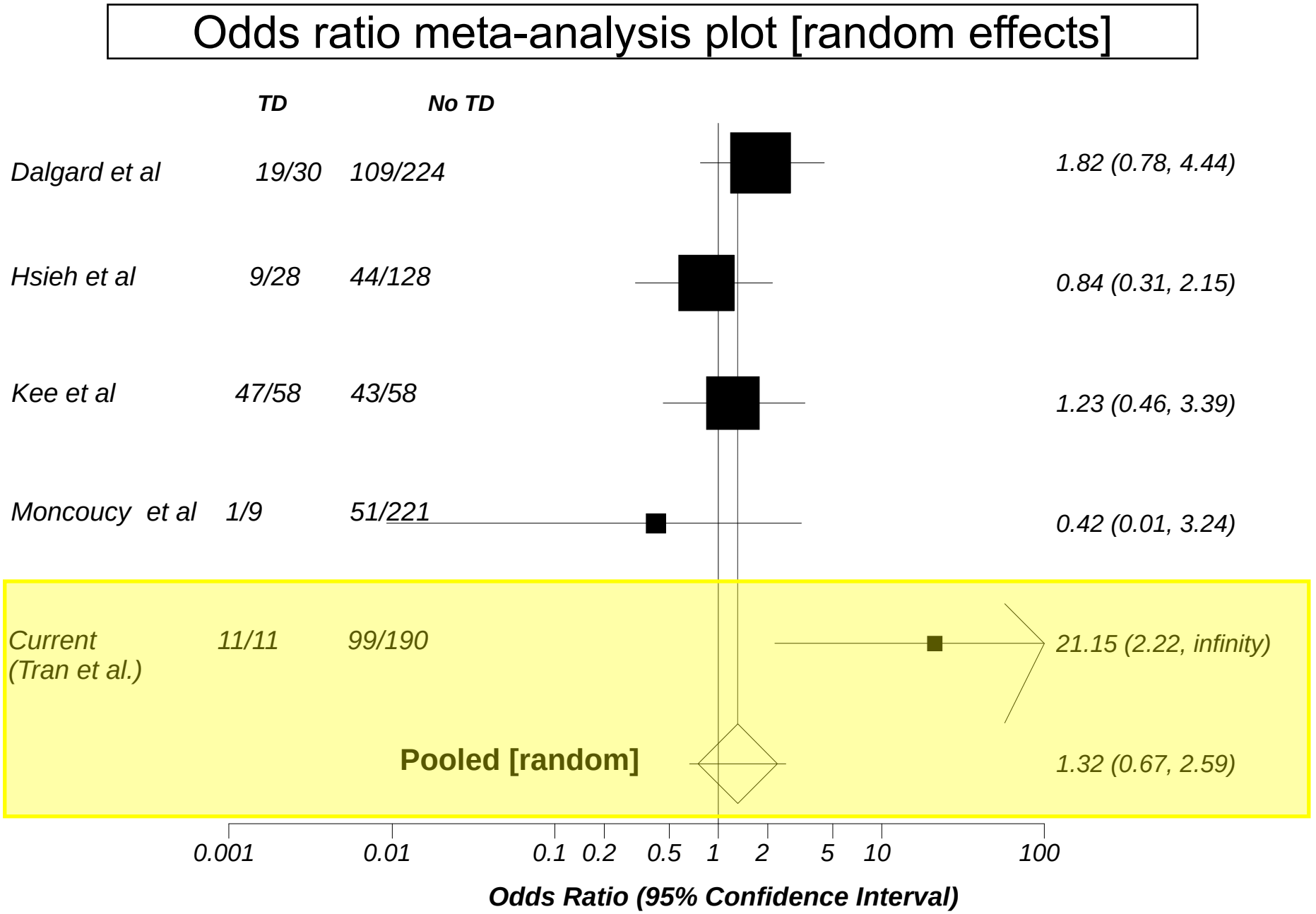

Figure 1. Meta-analysis from 5 reported studies, including this report, showing a null effect. TD; thyroid disease.

ADDENDUM II

Table 2. Sustained Virologic Responses (SVR), Odds Ratios (OR) and 95% Confidence Intervals (CI) analyses for *all* patients, genotype 1, 3 and regimen-specific subgroups.

GENOTYPE 1 (N = 55)								
Treatment regimens	Pegylated IFN-α2a and RBV 1000mg		Pegylated IFN-α2a and RBV 1200mg		Pegylated IFN-α2b and RBV 1000mg		Pegylated IFN-α2b and RBV 1200mg	
	Control 16	TD 4	Control 12	TD 3	Control 8	TD 2	Control 8	TD 2
EVR*	Not applicable (see text)							
ETR – number (%)	9 (56.3%)	3 (75.0%)	9 (75.0%)	3 (100.0%)	4 (50.0%)	2 (100.0%)	5 (62.5%)	2 (100.0%)
SVR – number (%)	6 (37.5%)	3 (75.0%)	5 (41.7%)	2 (66.6%)	2 (25.0%)	1 (50.0%)	2 (25.0%)	2 (100.0%)
SVR for genotype 1	**Control (n =44) SVR (n=15): 34.1%**					**TD (n=11) SVR (n=8): 72.7%**		
Odds Ratio, 95% CI	**5.2, 1.2 to 22.3**							

GENOTYPE 3 (N = 40)				
Treatment regimens	Pegylated IFN-α2a and RBV 800mg		Pegylated IFN-α2b and RBV 800mg	
	Control N = 16	TD N = 4	Control N = 16	TD N = 4
EVR*	Not applicable (see text)			

ETR – number (%)	**14 (100.0%)**	**4 (100.0%)**	**12 (100.0%)**	**4 (100.0%)**
SVR – number (%)	**14 (87.5%)**	**4 (100.0%)**	**12 (75.0%)**	**4 (100.0%)**

SVR for genotype 3 — **Control (n = 32)** SVR (n=26): 81.3% — **TD (n =8)** SVR (n=8): 100.0%

Odds Ratio, 95% CI — **TD is a perfect predictor of SVR in the case group**

Treatment related SVR IFN-α2a vs IFNα2b	Non Statistically Significant

OVERALL SVR: **Control (n=76)** SVR (n=41): 53.9% — **TD (n=19)** SVR (n=16): 84.2%

ODDS RATIO, 95% CI 6.0, 1.5 to 24.6

ADDENDUM I

	All Patients N = 201	Thyroid Dysfunction N = 11	No thyroid dysfunction N = 190	P-value
Female gender – number (%)	97 (48%)	7 (63%)	90 (47%)	0.35
Mean age (years)	49 ± 8	48 ± 9	50 ± 8	0.42
Weight (kg)	74 ± 18	76 ± 21	74 ± 18	0.36
Body Mass Indices (kg/m^2)	27 ± 7	26 ± 9	28 ± 8	0.42
Asian origin – no. (%)	36 (18%)	0	36 (19%)	0.53
Caucasians – no. (%)	151 (75%)	11 (100%)	140 (74%)	0.80
Genotypes – no. (%) • 1 • 2 • 3 • 4	 100 (50%) 16 (8%) 76 (38%) 9 (4%)	 4 (36%) 3 (27%) 2 (18%) 2 (18%)	 95 (50%) 13 (7%) 74 (39%) 8 (4%)	***0.01***
Viral Load • At baseline (log IU/mL) • Positive EVR at 12 weeks – (this is only applicable to genotypes 1 & 4) * • Positive ETR (at 24 weeks for genotypes 2&3 and 48 weeks for genotypes 1&4) # • SVR (24 weeks post therapy)	 5.97 ± 0.74 75/109 (69%) 161/201 (80%) 110/201 (54%)	 5.92 ± 0.80 6/6 (100%) 11/11 (100%) 11/11 (100%)	 5.98 ± 0.79 70/103 (68%) 143/190 (75%) 99/190 (53%)	 0.59 0.75 ***<0.001***
Albumin (36–48 g/L)	36 ± 2	36 ± 3	35 ± 8	0.68
Serum Bilirubin (2–20 µmol/L)	15 ± 5	14 ± 7	16 ± 4	0.13
Alanine Aminotransferase (< 45 U/L)	79 ± 22	65 ± 18	72 ± 20	0.26

γ-Glutamyl Transpeptidase (1–30 U/L)	53 ± 14	62 ± 11	54 ± 15	0.08
Prothrombin time (11–18 seconds)	15 ± 2	14 ± 3	15 ± 3	0.28
Haemoglobin (115–165 g/L)	154 ± 12	154 ± 13	153 ± 10	0.75
White cell counts (4.0–11.0 x 10^6/mL)	5.8 ± 1.9	6.9 ± 2.5	5.9 ± 2.2	0.15
Platelets (150–400 x 10^9/mL)	166 ± 37	181 ± 29	168 ± 38	0.27

Table 1. Characteristics of 201 patients including those *with and without* thyroid diseases. *; defined as ≥ 2 $\log_{10}$ viral load reduction compared with baseline by quantitation, #; no viral RNA is detected at the completion of treatment. EVR; Early Virological Response, ETR; End of Treatment Response, SVR; Sustained Virological Response, N/SS; Not Statistically Significant. Due to the small number in the TD group, there is inadequate statistical power to arrive at any definitive conclusions.

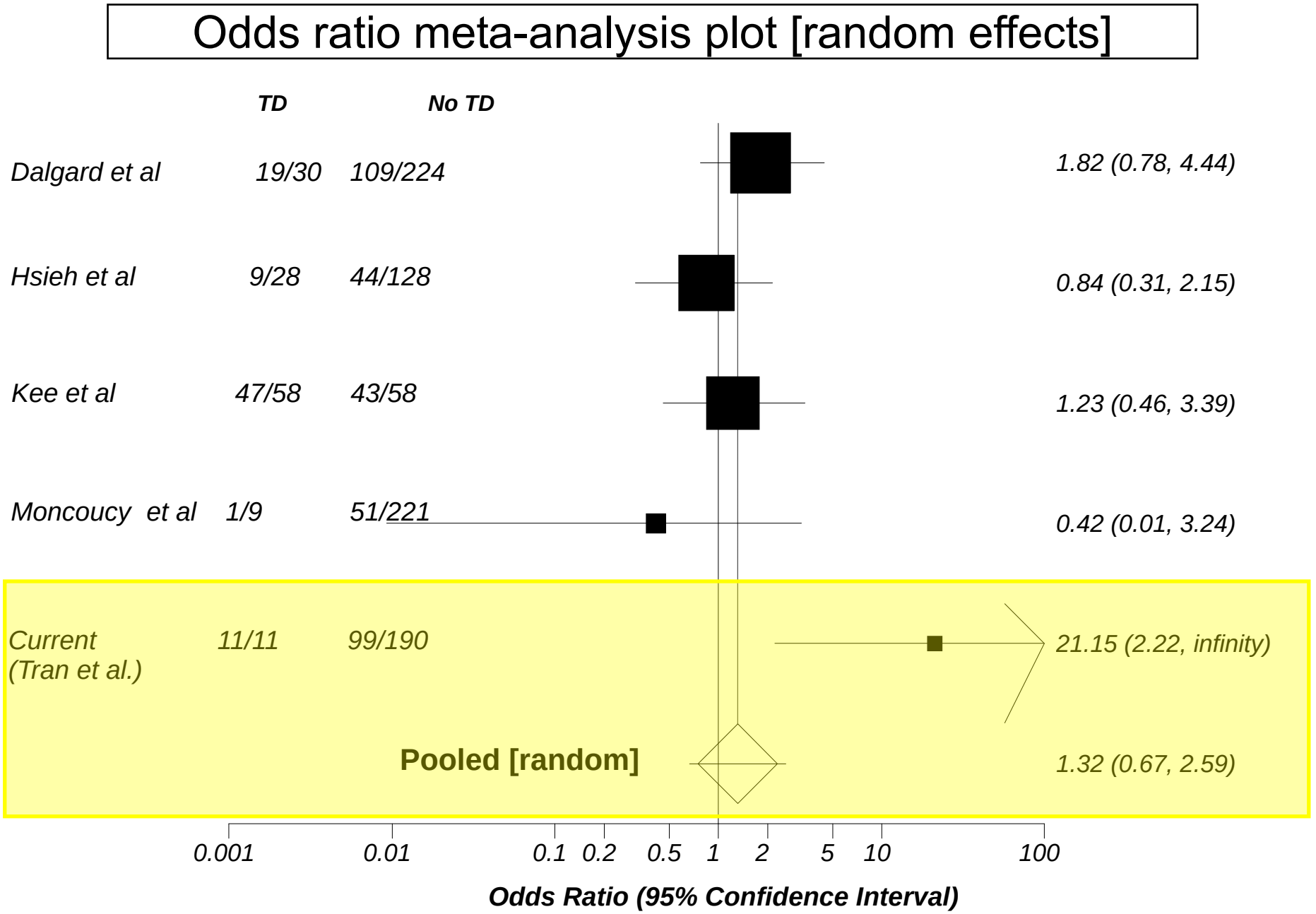

Figure 1. Meta-analysis from 5 reported studies, including this report, showing a null effect. TD; thyroid disease.

ADDENDUM II

Table 2. Sustained Virologic Responses (SVR), Odds Ratios (OR) and 95% Confidence Intervals (CI) analyses for *all* patients, genotype 1, 3 and regimen-specific subgroups.

GENOTYPE 1 (N = 55)								
Treatment regimens	Pegylated IFN-α2a and RBV 1000mg		Pegylated IFN-α2a and RBV 1200mg		Pegylated IFN-α2b and RBV 1000mg		Pegylated IFN-α2b and RBV 1200mg	
	Control 16	TD 4	Control 12	TD 3	Control 8	TD 2	Control 8	TD 2
EVR*	Not applicable (see text)							
ETR – number (%)	9 (56.3%)	3 (75.0%)	9 (75.0%)	3 (100.0%)	4 (50.0%)	2 (100.0%)	5 (62.5%)	2 (100.0%)
SVR – number (%)	6 (37.5%)	3 (75.0%)	5 (41.7%)	2 (66.6%)	2 (25.0%)	1 (50.0%)	2 (25.0%)	2 (100.0%)
SVR for genotype 1	**Control (n =44) SVR (n=15): 34.1%**					**TD (n=11) SVR (n=8): 72.7%**		
Odds Ratio, 95% CI	**5.2, 1.2 to 22.3**							

GENOTYPE 3 (N = 40)				
Treatment regimens	Pegylated IFN-α2a and RBV 800mg		Pegylated IFN-α2b and RBV 800mg	
	Control N = 16	TD N = 4	Control N = 16	TD N = 4
EVR*	Not applicable (see text)			

ETR – number (%)	**14 (100.0%)**	**4 (100.0%)**	**12 (100.0%)**	**4 (100.0%)**
SVR – number (%)	**14 (87.5%)**	**4 (100.0%)**	**12 (75.0%)**	**4 (100.0%)**

SVR for genotype 3 — **Control (n = 32)** **SVR (n=26): 81.3%** — **TD (n =8)** **SVR (n=8): 100.0%**

Odds Ratio, 95% CI — **TD is a perfect predictor of SVR in the case group**

Treatment related SVR IFN-α2a vs IFNα2b	Non Statistically Significant

OVERALL SVR: **Control (n=76)** **SVR (n=41): 53.9%** — **TD (n=19)** **SVR (n=16): 84.2%**

ODDS RATIO, 95% CI **6.0, 1.5 to 24.6**

The End