

Research paper

Effect of Restoration of Euthyroidism on Peripheral Blood Cells and Erythropoietin in Women with Subclinical Hypothyroidism

Mirjam Christ-Crain¹, Christian Meier¹, Peter Huber², Henryk Zulewski¹, Jean-Jacques Staub¹, Beat Müller¹

¹Division of Endocrinology, Diabetes, and Clinical Nutrition, Department of Internal Medicine, and ²Department of Central Laboratories, University Hospitals, CH-4031 Basel, Switzerland

Supported by grants from the Swiss National Science Foundation (32.27866.89, 32.37792.93, and 32.37792.98) and unconditional research grants from Henning Berlin, Novartis, Roche Research, Nora van Meeuwen-Hölfliger, and Krokus Foundations and the "Sonderprogramm zur Förderung des akademischen Nachwuchses der Universität Basel" (to BM)

ABSTRACT

Background: In overt hypothyroidism (OH) anemia is common, while less frequently basophilia has been described. In subclinical hypothyroidism (SCH), however, data on the distribution of peripheral blood cells are lacking. Therefore, we evaluated the effects of L-T4 replacement therapy on peripheral blood elements in female patients with SCH before and after restoration of euthyroidism in a randomized, double-blind and placebo-controlled study. **Patients and Methods:** Sixty-six women with SCH (TSH 12.9 ± 8.2 mU/L) were randomly assigned to receive L-thyroxine or placebo for 48 weeks. 63 of the 66 women completed the study. Peripheral blood cells were measured at baseline and 48 weeks after L-thyroxine or placebo treatment, respectively. **Results:** The percentage of lymphocytes decreased ($p < 0.05$), whereas percent of monocytes ($p < 0.05$) and eosinophiles ($p < 0.05$) increased significantly upon restoration of euthyroidism after 48 weeks. Hemoglobin and hematocrit remained unchanged throughout the study period. However, erythropoietin levels increased significantly ($p < 0.01$) during L-T4 treatment. In the placebo group all parameters remained unchanged throughout the study. **Conclusions:** Overall, we observed subtle alterations of the leuco-lympho-monocytic distribution of the peripheral blood cells upon restoration of euthyroidism in patients with SCH. Hemoglobin and Hematocrit remained unchanged; however, the increasing level of erythropoietin during L-T4 treatment suggests an already stimulated, yet compensated erythropoietic system in mild thyroid failure.

Key Words: blood, hypothyroidism, thyroxine

Address correspondence and requests for reprints to:

Dr. M. Christ, Division of Endocrinology, Department of Internal Medicine, University Hospitals, Petersgraben 4, CH-4031 Basel, Switzerland, Phone: +41 - 61 - 265 5078, Fax: +41 - 61 - 265 5100, E-mail: christmj@bluewin.ch

Received 30-06-03, Revised 25-08-03, Accepted 12-09-03

INTRODUCTION

Different forms of anemia have been reported in patients with overt hypothyroidism (OH) with a prevalence of up to 47%¹. Even if vitamin B12, folic acid and iron concentrations are normal, anemia that normalizes in response to thyroxine replacement is found in up to 25% of hypothyroid patients¹. An impaired erythropoietin (Epo) production leads to normochromic normocytic anemia, the most common form of anemia in thyroid failure². Less frequent forms of anemia in hypothyroidism are hypochromic microcytic anemia associated with iron deficiency and, in patients with Hashimoto's thyroiditis and autoimmune gastritis, macrocytic anemia induced by vitamin B12³ or folic acid deficiency¹. In contrast, hemolytic anemia is very rare in primary hypothyroidism⁴, though patients with beta thalassemia carry a high risk for development of hypothyroidism due to iron deposition in the thyroid gland⁵.

Granulocyte and lymphocyte counts are described as being normal in OH, with the exception of isolated reports of basophilia in circulating blood cells⁶. However, a more recent study found no difference in basophil counts between normal subjects and patients with OH⁷.

Subclinical hypothyroidism (SCH) is characterized by the finding of elevated TSH levels in the presence of normal circulating thyroid hormones⁸. It is not clear whether SCH affects the hematopoietic system at all and in particular the concentration of hemoglobin and the distribution of peripheral blood elements. Therefore, the aim of our study was to investigate the effect of L-thyroxine treatment on peripheral blood elements in patients with confirmed SCH analyzing data from a prospective double-blind placebo-controlled study. Specifically, we evaluated the effect of thyroid hormone replacement on hemoglobin, hematocrit and Epo levels, and on the distribution of different subpopulations of circulating white blood cells.

MATERIALS AND METHODS

The present analysis was part of a prospective, double blind, placebo-controlled study, whose design and patient characteristics have been described previously⁹. Briefly, 66 women with SCH were enrolled in the study. Patients between 18 and 75 years old, with TSH levels more than 5.0 mIU/L on two consec-

utive blood tests, free T4 concentration within the normal range, and good general health were included. A total of 63 women (mean age 58.5 ± 1.3 years) completed the study according to the study protocol, with no serious adverse events reported. The underlying thyroid disorders leading to SCH consisted of autoimmune thyroiditis (n=32), Graves' disease (n=21; treated with radioiodine, surgery or carbimazole), toxic multinodular goiter (n=1, treated with radioiodine), surgically resected goiter (n=6) and idiopathic SCH (n=3). The frequencies of underlying thyroid disorders were equally distributed in the L-thyroxine and placebo groups. No patient in any of these groups reported a recent or chronic infection or had known arthritic inflammation. Similarly, no patient had any known allergic disease or skin disease. No patient took medicaments such as antibiotics or corticosteroids, known to influence the number of neutrophils, eosinophils or lymphocytes. The patient groups were well balanced regarding aspirin use, estrogen replacement therapy as well as serum thyroid hormone concentrations at baseline. The local Ethics Committee for Human Studies approved the study. All patients gave their written informed consent to participate in the trial.

Hormone measurements were assessed at baseline, and at the end of the study after 48 weeks. The serum TSH concentration (reference range, 0.3-4mU/L) was measured with an immunometric assay (Delfia, Wallac, Inc., Turku, Finland). Free T4 (8.0-23.0 pmol/L) and total T3 (1.2-3.1 nmol/L) were determined by microparticle enzyme immunoassays (Imx, Abbott Laboratories, Inc., Chicago, IL). TPO antibodies were measured by Elisa (Vita Diagnostica GmbH, Merzhausen, Germany). Hemoglobin (12.0-16.0g%), hematocrit (36.0-46.0%) and blood cells (leucocytes, $3.5-10.0 \times 10^9/L$; neutrophils, 40-74%; lymphocytes, 19-48%; monocytes, 3.4-9%; eosinophils, 0.0-7.0%; basophils 0.0-1.5%) were measured by Advia (Bayer Diagnostics, Zürich, Switzerland). Epo (13-23 IU/L) was measured with Immulite (Diagnostic Products Corporation, Los Angeles, CA, USA).

Circulating vitamin B12 (reference range 193-982 pmol/l) and folic acid levels (reference range 3-17 nmol/l) were measured with the Immulite 2000 Analyzer (Diagnostic Products Corporation, Los Angeles, CA, USA). Serum potassium (3.7-4.7mmol/L) and lactat dehydrogenase levels (135-214 IU/L) were mea-

sured by Hitachi 917 (Roche, Rotkreuz, Switzerland).

STATISTICAL ANALYSES

All data are expressed as mean $\pm$ standard deviation (SD) in text and tables and as mean $\pm$ standard error of the mean (SEM) in figures. Mann-Whitney *U* test was used to show differences among the groups. Treatment effects in the L-thyroxine or placebo group were analyzed by Wilcoxon matched pair test. *P* values <0.05 were considered statistical significant. Data were analyzed using Statistica for Windows (version 5.0, StatSoft, Inc., Tulsa, Okla.).

RESULTS

At baseline the two groups of women with SCH (L-thyroxine, *n*=31; placebo, *n*=32) were similar with respect to thyroid hormone levels, age and body mass index. At baseline, basal TSH levels were mildly to

markedly elevated (range 5.0-50 mU/L) with an exaggerated TSH response of more than 35 mU/L after TRH administration. Peripheral thyroid hormone concentrations (FT4 and T3) were within the lower reference range.

Patient groups were balanced regarding peripheral blood cells with levels well within the normal range (Table 1).

In all T4-treated patients, TSH concentrations decreased in the first weeks of treatment and were within the reference range at least for the last 24 weeks, mean serum TSH level at the end of the study was 3.1 ± 1.6 mU/L. No patient had a blunted or absent TSH response to thyrotropin-releasing hormone, thereby excluding over-treatment. Peripheral thyroid hormone concentrations (FT4 and T3) remained within the respective reference ranges. As expected, no significant changes in any variable of thyroid function could be seen in patients with placebo.

Table 1. Parameters before and 48 weeks after treatment with L-T4 or placebo (mean $\pm$ SD)

Variable	Treatment with L-thyroxine (n=31)			Treatment with placebo (n=32)		
	Before treatment	After 48 weeks	p	Before treatment	After 48 weeks	p
Thyrotropin (mU/L)	14.1 $\pm$ 9.8	1.1 $\pm$ 1.6	0.001	11.4 $\pm$ 5.8	9.9 $\pm$ 3.3	ns
Free thyroxine (pmol/L)	11.8 $\pm$ 1.6	18.2 $\pm$ 3.4	0.001	12.2 $\pm$ 1.4	12.5 $\pm$ 2.5	ns
Triiodothyronine (nmol/L)	2.0 $\pm$ 0.5	1.7 $\pm$ 0.1	0.001	1.9 $\pm$ 0.3	1.9 $\pm$ 0.1	ns
Blood elements						
Hemoglobin (g%)	13.3 $\pm$ 0.9	13.3 $\pm$ 0.8	ns	13.5 $\pm$ 1.1	13.3 $\pm$ 1.1	ns
Hematocrit (%)	40.4 $\pm$ 2.5	40.9 $\pm$ 2.6	ns	40.0 $\pm$ 3.2	40.4 $\pm$ 2.8	ns
MCV (fl)	88.2 $\pm$ 4.7	89.7 $\pm$ 4.8	ns	88.6 $\pm$ 5.0	89.8 $\pm$ 5.8	ns
MCH (pg)	29.1 $\pm$ 2.1	29.2 $\pm$ 1.4	ns	29.8 $\pm$ 1.7	29.4 $\pm$ 2.1	ns
MCHC (%)	33.0 $\pm$ 1.3	32.6 $\pm$ 1.0	ns	33.7 $\pm$ 1.2	32.8 $\pm$ 1.0	ns
Erythropoietin (IU/L)	9.8 $\pm$ 4.5	12.2 $\pm$ 7.3	0.006	12.6 $\pm$ 11.8	12.4 $\pm$ 7.1	ns
LDH (IU/L)	149.5 $\pm$ 34.8	158.2 $\pm$ 42.0	ns	152.3 $\pm$ 38.0	153.0 $\pm$ 43.4	ns
Potassium (mmol/L)	3.7 $\pm$ 0.3	3.9 $\pm$ 0.3	0.01	3.8 $\pm$ 0.3	3.8 $\pm$ 0.3	ns
Vitamin B12 (pmol/L)	357.4 $\pm$ 225.4	337.8 $\pm$ 232.2	ns	377.7 $\pm$ 112.6	371.2 $\pm$ 151.1	ns
Folic acid (nmol/L)	4.8 $\pm$ 2.4	4.3 $\pm$ 2.2	ns	4.5 $\pm$ 1.6	4.3 $\pm$ 2.5	ns
Leukocytes ($\times 10^9$ /L)	5.2 $\pm$ 1.5	5.3 $\pm$ 1.4	ns	6.2 $\pm$ 2.0	6.2 $\pm$ 1.6	ns
Eosinophils (%)	2.4 $\pm$ 1.9	3.0 $\pm$ 2.4	0.01	2.8 $\pm$ 1.3	2.8 $\pm$ 1.3	ns
Basophils (%)	0.8 $\pm$ 0.4	0.7 $\pm$ 0.3	ns	0.7 $\pm$ 0.2	0.7 $\pm$ 0.3	ns
Neutrophils (%)	57.8 $\pm$ 7.0	59.3 $\pm$ 7.0	ns	58.7 $\pm$ 10.0	57.1 $\pm$ 8.0	ns
Lymphocytes (%)	30.5 $\pm$ 6.4	27.8 $\pm$ 5.9	0.01	29.8 $\pm$ 9.2	30.6 $\pm$ 7.4	ns
Monocytes (%)	6.0 $\pm$ 1.6	6.6 $\pm$ 1.9	0.04	6.0 $\pm$ 1.2	6.4 $\pm$ 1.5	ns

Significance was determined by Wilcoxon matched pair test.

EFFECT OF TREATMENT ON PERIPHERAL BLOOD CELLS

In all women, peripheral blood elements were measured at baseline and after 48 weeks of L-thyroxine and placebo treatment. Values of all measured parameters were well within the reference range, at baseline and after 48 weeks of L-thyroxine and placebo treatment. Hemoglobin and hematocrit, overall, showed no significant treatment effect throughout the study in both the placebo and T4-treatment group. Serum Epo levels, which tended to be lower in the L-thyroxine group at baseline, increased significantly during L-T4 treatment ($p < 0.01$), whereas no change could be seen in the placebo group. Circulating vitamin B12, folic acid and LDH remained unchanged, both, in the L-T4 and in the placebo treated group, respectively. Circulating potassium levels increased significantly during L-T4 treatment ($p < 0.05$).

Interestingly, the number of lymphocytes decreased

significantly after 48 weeks of treatment ($p < 0.05$), whereas the number of monocytes increased significantly ($p < 0.05$). In the placebo group, the respective changes were not significant (Table 1). We separated patients with autoimmune thyroiditis ($n = 17$) and non-autoimmune thyroiditis ($n = 14$). Thereafter, the latter finding was restricted to patients with autoimmune thyroiditis characterized by mean circulating anti TPO of 468.9 ± 700.1 U/ml (reference range > 100 U/ml) (Figure 1). The distribution of lymphocytes and monocytes in the placebo group was unchanged in patients with autoimmune and non-autoimmune thyroiditis. The percentage of eosinophile granulocytes increased significantly from 2.4 ± 1.9 to $3.0 \pm 2.4\%$ ($p < 0.05$) in the L-thyroxine group. In the placebo group the eosinophile count tended to be higher at baseline, remaining, however, unchanged throughout the study. The total granulocyte count was not altered during therapy and there was no change in percent of basophil granulocytes. Overall, no change in the distribution

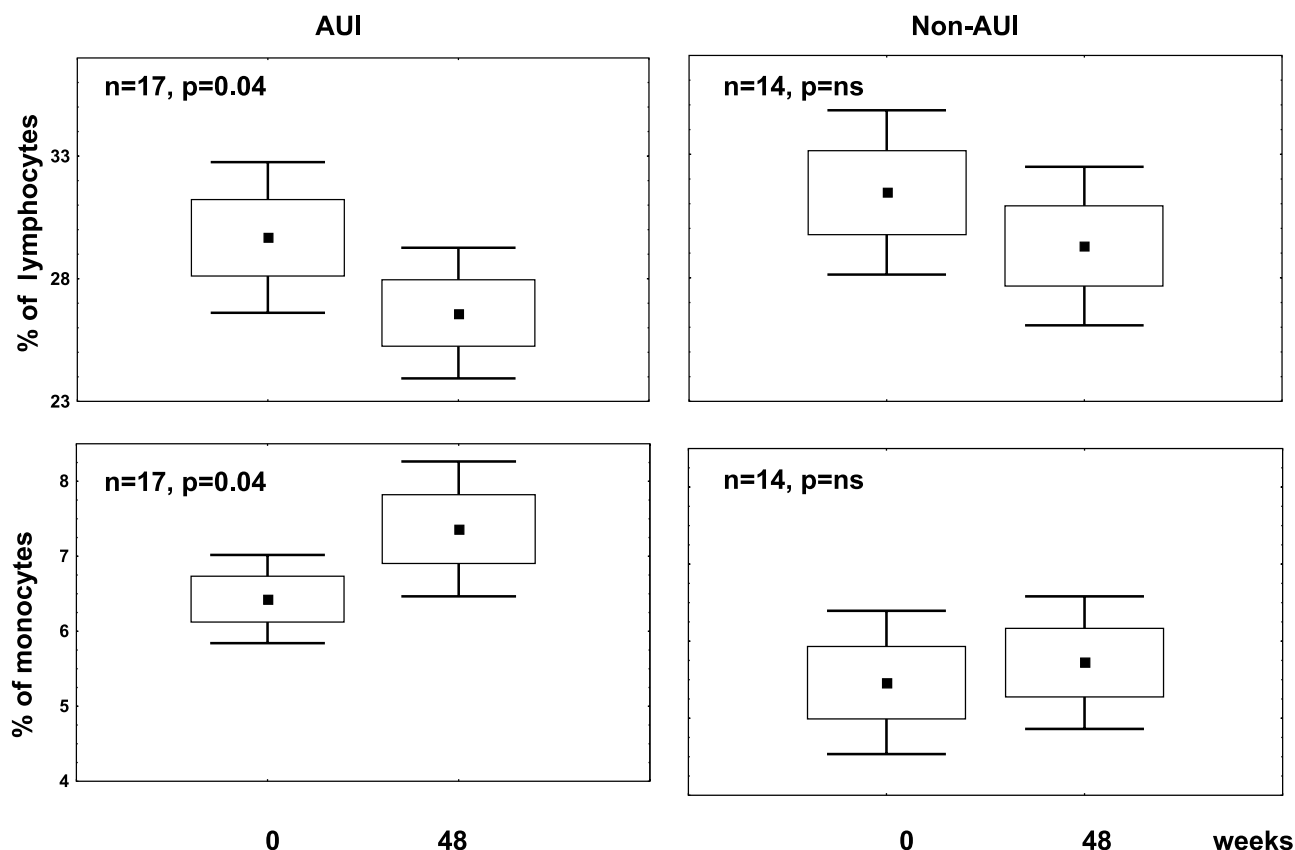


Figure 1. Number of circulating lymphocytes and monocytes before and after L-thyroxine treatment in patients with autoimmune thyroiditis (AUI) and in patients without autoimmune disease (non AUI). Two group comparison was applied by Mann-Whitney-U test. Diamonds represent means, boxes SEM and whiskers 1.96.SEM of the combined data.

of peripheral blood cells could be observed in the placebo treated group (Table 1).

DISCUSSION

Anemia is a frequent finding in OH^{1,3} and may participate in the clinical picture of hypothyroidism, that affects many target organs^{9,10}, but changes in the hematopoietic system in general have never been evaluated.

The results of this prospective, placebo controlled study in female patients with SCH show peripheral blood cells within the reference range with subtle changes upon restoration of euthyroidism, mainly in patients with underlying autoimmune thyroiditis. Serum hemoglobin and hematocrit levels are in the normal range without change during L-T4 treatment, but with a significant increase of circulating Epo levels during therapy. This finding suggests a relatively decreased individual level of Epo in SCH. Thus, people with SCH metabolically seem to compensate with lower Epo levels, in contrast to patients with overt disease. In hypothyroidism, the basal cellular metabolism is diminished and the oxygen consumption of the tissues is correspondingly reduced. As a consequence, synthesis and release of Epo is diminished. Thus, anemia in hypothyroidism with decreased Epo synthesis may be an adaptive process to the diminished oxygen demand of the tissues². Since mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), vitamin B12 and folic acid, lactate dehydrogenase (LDH) as well as creatinine and urea values in our patients were within the reference range and did not change during L-thyroxine treatment, we have no evidence of any other type of anemia. Iron deficiency in hypothyroidism is ascribed to menorrhagia that can accompany hypothyroidism and none of our patients had menorrhagia during the study. Thus, in agreement with previous studies in patients with OH, we postulate that decreased Epo levels may be the main mechanism of anemia in hypothyroidism.

Unexpectedly, our study shows increasing potassium levels during L-T4 treatment. Fommei et al, investigating the role of thyroid hormones on blood pressure, recently showed significantly increased aldosterone levels in humans with OH¹¹. An activation of the plasma renin-aldosterone system in patients with SCH could explain the lower potassium levels¹².

Previous studies on human and animal lymphocyte count and function during hypo- and hyperthyroid states produced conflicting results¹³⁻¹⁶. Case reports in humans show an increased susceptibility towards infections in autoimmune hypothyroidism^{17,18}. In addition, a general disturbance in monocyte function in thyroid autoimmune disease has been described¹⁹. Our results show a significant decrease in the lymphocyte and increase in monocyte count in the patients with autoimmune thyroiditis during L-T4 treatment, whereas patients with hypothyroidism of other origin show no change in lymphocyte or monocyte count. Thus, we do not believe that the change in number of lymphocytes is due to the lack of thyroid hormone, we rather suppose that the changes in lymphocytes are due to the autoimmune process *per se*. Accordingly, similar changes in the number of lymphocytes are also observed in autoimmune hyperthyroidism²⁰. We did not assess lymphocyte and monocyte function in our study. Possibly, the elevated number of lymphocytes in SCH of autoimmune nature may demonstrate a nonspecific activation of the immune system as it is found in some immunodeficient states, AIDS, gravidity and other autoimmune diseases²¹. Alternatively, it is tempting to speculate that the relatively increased number of lymphocytes in autoimmune thyroiditis could be the result of a polyglandular autoimmune syndrome with concomitant impaired adrenal function. Renin-activity or circulating antibodies against adrenal cells were not measured. Nevertheless, our observation of a relative eosinopenia in SCH, which is reversible upon restoration of euthyroidism, argues against this hypothesis.

Data on the occurrence and extent of basophilia in primary thyroid failure are controversial. Hypothyroidism has been considered to be a cause of basophilia⁶, however, this could not be confirmed in other studies⁷, which is in accordance with the result of this randomized placebo-controlled trial. Therefore, we do not believe that primary thyroid failure before or after T4-replacement significantly influences basophil count.

Overall, one might argue that because the observed changes in white blood cell distribution and circulating Epo levels are relatively small, they might represent only an unfortunate result of randomization. However, in comparison to all previous reports on the topic in the literature, our study was a carefully de-

signed, prospectively randomized double-blind study. As each patient is his own control, we believe that our findings are significant and noteworthy. In addition, in the L-thyroxine group post-therapy concentrations for TSH were in the upper normal range 3.1 ± 1.6 mU/L. Thus, a further decrease of TSH could have resulted in even more pronounced treatment effects. Nevertheless, the clinical implications of the subtle changes observed should be interpreted cautiously. For example, whether the changes of the white blood cell distribution result in functional alterations of the host immune defense remains speculative at present.

In conclusion, L-thyroxine treatment with restoration of euthyroidism in patients with SCH results in increasing circulating Epo levels. Since hemoglobin and hematocrit levels remain unchanged, patients with SCH seem to be able to compensate lower Epo levels. Furthermore, thyroid hormone therapy in SCH affects the leuco-lympho-monocytic distribution of the peripheral blood cells especially in autoimmune thyroiditis.

Importantly, most patients with SCH in the general community discovered by screening or case finding have autoimmune thyroiditis. Possibly, during restoration of euthyroidism, the autoimmune process in these patients might be silenced, reflected in a decreased lymphocyte count.

Acknowledgments

We thank S. Alscher and M. Kunz for their excellent technical assistance and M. Guglielmetti for statistical help.

REFERENCES

- Horton L CR, England JM, 1976 The haematology of hypothyroidism. *Quarterly Journal of Medicine New Series*, XLV: 101-124.
- Das KC, Mukherjee M, Sarkar TK, Dash RJ, Rastogi GK, 1975 Erythropoiesis and erythropoietin in hypo- and hyperthyroidism. *J Clin Endocrinol Metab* 40: 211-220.
- Fein HG, Rivlin RS, 1975 Anemia in thyroid diseases. *Med Clin North Am* 59: 1133-1145.
- Nomura S, Eimoto T, Osawa G, Yawata Y, Horino M, 1991 Case report: hypothyroidism as a possible cause of an acquired reversible hemolytic anemia. *Am J Med Sci* 302: 23-27.
- Magro S, Puzzon P, Consarino C, et al, 1990 Hypothyroidism in patients with thalassemia syndromes. *Acta Haematol* 84: 72-76.
- Athens J 1993 Variation of leucocytes in disease In: Lee GR, Bithell TC, Foerster J (eds) *Wintrobe's clinical hematology*, Philadelphia.
- Petrascu SG, Mlynec-Kersjes ML, Haase R, et al, 1993 Basophilic leukocytes in hypothyroidism. *Clin Invest* 71: 27-30.
- Cooper DS, 2001 Clinical practice. Subclinical hypothyroidism. *N Engl J Med* 345: 260-265.
- Meier C, Staub JJ, Roth CB, et al, 2001 TSH-controlled L-thyroxine therapy reduces cholesterol levels and clinical symptoms in subclinical hypothyroidism: a double blind, placebo-controlled trial (Basel Thyroid Study). *J Clin Endocrinol Metab* 86: 4860-4866.
- Zulewski H, Muller B, Exer P, Miserez AR, Staub JJ, 1997 Estimation of tissue hypothyroidism by a new clinical score: evaluation of patients with various grades of hypothyroidism and controls. *J Clin Endocrinol Metab* 82: 771-776.
- Fommei E, Iervasi G, 2002 The role of thyroid hormone in blood pressure homeostasis: evidence from short-term hypothyroidism in humans. *J Clin Endocrinol Metab* 87: 1996-2000.
- Klein I, Ojamaa K, 2001 Thyroid hormone and the cardiovascular system. *N Engl J Med* 344: 501-509.
- Tsai JS, Samuels HH, 1974 Thyroid hormone action: demonstration of putative nuclear receptors in human lymphocytes. *J Clin Endocrinol Metab* 38: 919-922.
- Burman KD, Latham KR, Djuh YY, et al, 1980 Solubilized nuclear thyroid hormone receptors in circulating human mononuclear cells. *J Clin Endocrinol Metab* 51: 106-116.
- Fabris N, 1973 Immunodepression in thyroid-deprived animals. *Clin Exp Immunol* 15: 601-611.
- Kuijpers JL, De Hann-Meulman M, Vader HL, Pop VJ, Wiersinga WM, Drexhage HA, 1998 Cell-mediated immunity and postpartum thyroid dysfunction: a possibility for the prediction of disease? *J Clin Endocrinol Metab* 83: 1959-1966.
- Schoenfeld PS, Myers JW, Myers L, LaRocque JC, 1995 Suppression of cell-mediated immunity in hypothyroidism. *South Med J* 88: 347-349.
- Mayer D, Miller-Hanisch B, Bockh A, Hehrmann R, 1997 Fulminant meningococcal meningitis and sepsis associated with severe hypothyroidism caused by autoimmune (Hashimoto) thyroiditis. *Exp Clin Endocrinol Diabetes* 105: 80.
- Canning MO, Grotenhuis K, De Haan-Meulman M, De Wit HJ, Berghout A, Drexhage HA, 2001 An abnormal adherence of monocytes to fibronectin in thyroid autoimmunity has consequences for cell polarization and the development of veiled cells. *Clin Exp Immunol* 125: 10-18.
- Porter L, Mandel SJ, 2000 The Blood in Thyrotoxicosis In: Brawerman LE, Utiger RD (eds) *Werner & Ingbar's The Thyroid*, Philadelphia, PA; pp, 627-630.
- Straus SE, Tosato G, Armstrong G, et al, 1985 Persisting illness and fatigue in adults with evidence of Epstein-Barr virus infection. *Ann Intern Med* 102: 7-16.