Thyroid autoantibodies in thyroid cancer: 
Incidence and relationship with tumour outcome

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Abstract. In the present investigation we studied serum anti-thyroglobulin and anti-thyroid microsomal autoantibodies, measured by hemagglutination technique, in 600 patients with thyroid cancer seen by us from 1975 to 1985 (mean follow-up 46 months). Positive thyroglobulin antibodies and/or microsomal antibodies were found in 138 (23%) patients (23.9% with papillary, 25% with follicular, 16.1% with anaplastic, and 4.1% with medullary thyroid carcinomas). The incidence of positive tests was similar in each decade of life (ranging between 21.9% and 27.9%), whereas in a normal sex-matched population with no evidence of thyroid disease, the frequency of positive tests was very low in young people and increased to 23% in people older than 60. In 64 patients with no evidence of residual or metastatic thyroid tissue after surgery and radioiodine, initially positive antibody titres became negative in 54.6%, decreased in 32.8%, did not change in 3.1%, and increased in 9.3%. On the contrary, antibody titres of patients with persistent disease became undetectable in 8.3%, decreased in 16.6%, remained unchanged in 25%, and increased in 50%. The clinical course of differentiated thyroid cancer was unaffected by the presence of thyroid antibodies and no difference was found in the death rate between antibody-positive and antibody-negative patients (11.5% and 13.6%, respectively). In conclusion, our data indicate that: 1) autoimmune phenomena are not an infrequent finding in thyroid cancer; 2) as in non-malignant thyroid diseases, positive-antibody tests are more frequently observed in females than in males; 3) at variance with normal controls, no age-dependent increase in serum anti-thyroid antibodies was found in thyroid cancer; 4) the presence of metastatic thyroid tissue seems to be necessary to perpetuate the autoantibody synthesis, and 5) anti-thyroid autoantibodies are not a protective or worsening factor in the tumour outcome.

The relationship between thyroid autoimmunity and thyroid cancer is still unclear. No evidence of increased incidence of antithyroid-microsomal autoantibodies was found by Kornstad (1974) in a large series of thyroid cancer patients using the complement fixation technique. In a few similar studies using the more sensitive passive hemagglutination method, a significant increase in the incidence of anti-thyroglobulin and microsomal autoantibodies has been reported in thyroid malignancies, with positivity ranging from 2.3 to 15% for thyroglobulin antibodies and from 9.1 to 21% for microsomal antibodies (Kornstad 1974; Amino et al. 1975; DeGroot et al. 1976; Pinchera et al. 1977; Mariotti et al. 1978). The main limitation of most of the above investigations was the rather small number of patients studied and the lack of adequate follow-up.

In this study we re-evaluated the incidence of thyroglobulin antibodies and microsomal antibodies in a large series of patients with thyroid cancer. We also correlated the behaviour of serum antibodies with several clinical parameters and with the tumour outcome.
Patients and Methods

Patients
We studied 600 patients with histologically confirmed thyroid carcinoma seen in our clinic from 1975 to 1985 (mean follow-up 46 months). There were 449 females and 151 males with a female/male ratio (F:M) of 3:1, aged 7–85 years. Histology showed papillary carcinoma in 401 patients (66.8%), follicular in 144 (24%), anaplastic in 31 (5.2%) and medullary in 24 (4%). In all patients initial treatment was total thyroidectomy, followed in the case of almost all differentiated tumours by ablation of thyroid residues with radioiodine therapy, also in patients with papillary cancer without extrathyroidal extension. Subsequent treatment was L-thyroxine in suppressive doses, radioiodine for functioning metastases, and surgery with or without radiotherapy and chemotherapy for non-functioning metastases and for anaplastic and medullary tumours. Eighteen per cent of the patients were seen by us before any treatment, all the others were sent to us soon after thyroidectomy. On clinical and laboratory findings a concomitant diagnosis of Hashimoto’s thyroiditis had been made in 7 patients (all with antibody titres > 1:6400) and of Graves’ disease in 3 patients. During follow-up, 79 patients died of their disease: all those with anaplastic cancer (N = 31), 9 with medullary, 24 with papillary and 15 with follicular carcinomas.

Control population
The control population consisted of 654 healthy subjects (488 females, 166 males) with no clinical or biochemical evidence of thyroid dysfunction, collected during the last 6 years and matched for sex and age (range 10–80 years).

Methods
Serum thyroglobulin antibodies and microsomal antibodies were measured by passive hemagglutination (Mariotti et al. 1978; Fulthorp et al. 1961) as part of the routine procedure any time the patient was seen in the in-patients or out-patients clinic. In this way, each patient had at least one determination every year. Tests were done using commercial kits (Sera-Tek Microsomal Antibody Test and Thyroglobulin Antibody Test, Miles Laboratories Inc, Elkhart, IN). Serial four-fold dilutions starting with 1:100 were performed by means of the Takatsy microtitration apparatus (Scientific Shau'don Instruments Co, London, UK). Since sera with positive hemagglutination titres corresponding to the lowest dilution employed (1:100) often gave inconsistent results in repeated tests, only titres > 1:400 were considered as indicative of unequivocally positive antibody levels.

Since most of our patients came to our department soon after thyroidectomy, antibody tests before any treatment were available only in a minority of them (18%).

Results

Incidence of thyroid autoantibodies in relation to sex and histology
As shown in Fig. 1, positive thyroglobulin antibodies and/or microsomal antibodies were present in 96 (23.9%) patients with papillary, 36 (25%) with follicular, 5 (16.1%) with anaplastic, and 1 (4.1%) with medullary thyroid cancer, corre-
Per cent distribution of thyroglobulin antibodies, microsomal antibodies or both in 138 thyroid cancer patients with positive thyroid autoantibodies (■ anti-thyroglobulin antibodies; □ antimicrosomal antibodies; both □) according to histology.

Responding to a total incidence of 23% (138 patients). This incidence was statistically higher (Chi-Square = 52.4, P < 0.001) than that found in 654 sex- and age-matched normal subjects, in whom the frequency of positive thyroid autoantibodies was 8.25% (54 subjects). In differentiated tumours, positive antibodies were more frequently found in women (25.8% and 26.5% of women with papillary and follicular cancer, respectively) than in men (18.4% and 19.3%), whereas in anaplastic cancer, thyroid antibodies were detected in 22.2% of men and 13.6% of women. In antibody-positive patients, the F:M ratio was 4.3:1 for differentiated and 1.5:1 for anaplastic cancer with no significant difference to patients with negative antibody tests.

As shown in Fig. 2, microsomal antibodies alone were found in 92 out of 138 positive patients

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**Fig. 2.**
Per cent distribution of thyroglobulin antibodies, microsomal antibodies or both in 138 thyroid cancer patients with positive thyroid autoantibodies (■ anti-thyroglobulin antibodies; □ antimicrosomal antibodies; both □) according to histology.

**Fig. 3.**
Thyroid antibody titres (□ anti-thyroglobulin; □ antimicrosomal) among patients with positive tests at the first observation.
(66.6%), thyroglobulin antibodies and microsomal antibodies in 43 (31.1%) and thyroglobulin antibodies alone only in 2 (2.2%). This corresponded to an overall incidence of 23% for microsomal antibodies and 7.5% for thyroglobulin antibodies. The pattern of distribution was similar for papillary and follicular carcinomas; anaplastic tumours had higher positivity for both thyroglobulin antibodies and microsomal antibodies, but in this type of cancer positivity of thyroglobulin antibodies alone was never observed.

The distribution of antibody titres observed in all cancer patients is reported in Fig. 3. The titre refers to that found at the first observation: before any treatment in 19 patients, 30–90 days after thyroidectomy in 98, and later on during follow-up in 40 patients. Although the majority of patients had low antibody titres (< 1:1600), a substantial number had moderately to extremely elevated antibody titres.

*Relationship between thyroid cancer, anti-thyroid antibodies and age*

As shown in Fig. 4, the incidence of anti-thyroid antibodies in thyroid cancer patients was almost equally distributed in all age groups, ranging from a minimum of 21.9% (21–40 years) to a maximum of 27.9% (1–20 years). This pattern of positivity was completely different from that found in 654 sex-matched normal controls, in whom the incidence of positive anti-thyroid antibodies was very low in young people (4%) and increased linearly to reach a maximum of 23% in subjects older than 60 years. The comparison between cancer patients and normal subjects showed a significant difference in the incidence of anti-thyroid antibodies in all age groups with the exception of subjects older than 60 years (Chi-Square = 16.1, P < 0.01).

It is well known that differentiated thyroid cancer in the general population has a typical age-distribution with a maximal incidence between the 3rd and 5th decade of life for papillary and after the 5th decade for follicular cancer. This age-dependency was confirmed in our series with no difference between patients with or without circulating antibodies (Fig. 5).

*Anti-thyroid antibodies and tumour outcome*

In the 5 patients with anaplastic cancer and in the one with medullary thyroid cancer, initially positive antibody titres declined, but remained detectable after surgery and subsequent chemotherapy and/or external radiotherapy.

Of the 132 patients with differentiated thyroid carcinoma and positive antibodies, 98 were positive since the first observation (19 before and the others 30–90 days after thyroidectomy). At the time of writing 76 of these patients had a long-term follow-up (more than 5 years), shown in Fig. 6, which allowed evaluation of the outcome. Successful treatment was achieved in 64 patients,
whereas persistent or progressive metastatic disease occurred in 12. As shown in Fig. 7, initially positive antibody titres became negative in 54.6%, decreased in 32.8% did not change in 3.1% and increased in 9.3% of the successfully treated patients. In contrast, titres of patients with persistent disease became undetectable in 8.3%, decreased in 16.6%, did not change in 25% and increased in 50% (Chi-Square = 23.4; P < 0.001).

Thirty-four additional patients with differentiated thyroid carcinoma had negative antibody tests when first seen soon after surgery, but became positive during follow-up (months or even years later). Nine of these patients had lung and/or node metastases, 7 had thyroid residues and 18 were free of disease. In this group antibody tests remained positive in 5 of those with metastatic involvement and became negative in all the others in subsequent tests.

The clinical course of differentiated thyroid carcinoma was unaffected by the circulating thyroid antibodies; in fact, similar remission or progression rates were found in patients with or without positive antibody tests. Furthermore, no difference was found in the death rate of the two groups of patients, 16/138 (11.5%) in the antibody-positive and 63/462 (13.6%) in the antibody-negative group (P > 0.5 by Chi-Square).
Changes of thyroid antibody titres in 76 patients with adequate follow-up according to the outcome of the tumour (successfully treated  ■ , persistent disease □).

Discussion

The precise incidence of thyroid autoantibodies in patients with thyroid tumours is still debated. The incidence of thyroglobulin antibodies by passive hemagglutination has generally been found slightly increased when compared with that observed in healthy controls, with percentages ranging between 2.3 (Pinchera et al. 1977) and 15% (Kornstad 1974; Amino et al. 1975; DeGroot et al. 1976). A higher prevalence of thyroglobulin antibodies has been observed using more sensitive assays, such as radioimmunoassays (Feldt-Rasmussen et al. 1983; Ericsson et al. 1985; Reiners & Hufner 1987) or enzyme-linked immunosorbent assays (ELISA) (Høier-Madsen et al. 1984).

More scanty data are available for microsomal antibodies. No significant increase in microsomal antibodies was found by Kornstad (1974) in a large series of thyroid cancer using the rather insensitive complement fixation test, whereas a higher incidence (13%) of this type of antibody was reported by Amino et al. (1975) using the indirect immunofluorescence technique, and by others (DeGroot et al. 1976; Mariotti et al. 1978) using passive hemagglutination, with percentage ranging between 13–21. All these studies, except the one by Kornstad were carried out in a small number of patients and no follow-up data were provided.

Therefore, the aim of this study was not only to assess the incidence of circulating autoantibodies in a large series of thyroid cancer patients, but also to evaluate whether thyroid antibodies can affect the outcome of the tumour or have any prognostic meaning. This goal has been achieved taking advantage of routine measurements of thyroid autoantibodies in all our patients, which allowed collection of a sufficient number of subjects with adequate follow-up for a detailed analysis.

The overall incidence of thyroglobulin antibodies and/or microsomal antibodies in our series was 23%. In agreement with previous studies carried out using passive hemagglutination (DeGroot et al. 1976; Mariotti et al. 1978), microsomal antibodies were more frequently found (23%) than thyroglobulin antibodies (7.5%). The incidence of thyroglobulin antibodies is lower than that reported in some recent studies (Feldt-Rasmussen et al. 1983; Ericsson et al. 1985; Reiners & Hufner 1987; Høier-Madsen et al. 1984) performed using radioimmunoassays or ELISA techniques for antibody detection. This discrepancy could be due to the lower sensitivity of the passive hemagglutination or to the interference of serum thyroglobulin in the thyroglobulin antibodies determination by different methods (Pinchera et al. 1977; Bayer & Kriss 1979).

In our series, the F:M ratio was higher, but not significantly, in antibody-positive (4.1:1) than in antibody-negative (2.7:1) patients. This finding may reflect the higher prevalence in thyroid cancer of thyroid autoimmune diseases in females (Doniach 1975; Volpé 1986). It is also known that the frequency of autoimmune phenomena increases in apparently normal elderly people (Doniach 1975). This finding has been confirmed by our results in sex-matched controls; interestingly, however, the incidence of thyroid autoantibodies was almost identical in all age groups of thyroid cancer patients.

It is well established that papillary thyroid cancer is more frequent among young adults and almost 50% of the cases occur before the age of 40–50, whereas follicular neoplasms usually occur in an older age group (Ingbar 1985). In our series, this typical age-distribution of thyroid carcinoma was confirmed both in antibody-positive and antibody-negative patients.

In 76 patients with positive antibodies and follow-up of more than 5 years, it was possible to assess that antibody production declines or ceases with the removal of residual or metastatic thyroid...
tissue, while continues or even increases in the case of persistent or progressive disease. Our finding suggests that the presence of metastatic tissue may play an important role in perpetuating the activity of lymphocyte clones producing thyroglobulin or microsomal antibodies.

Since treatment of thyroid cancer involves the use of radioactive iodine (131I), one can speculate that the release of thyroid antigens in the circulation owing to the radiation damage (Pacini et al. 1980) could initiate the channel of events leading to the production of autoantibodies. This phenomenon has been implicated in the transient increase in serum thyroid autoantibody titres after radioiodine treatment in Graves' disease (Kriss et al. 1967; Pinchera et al. 1969; Einhorn et al. 1965). In our series most of the antibody-positive patients were positive before they received 131I treatment, whereas only 5 developed positive antibodies after one or more radioiodine doses. Furthermore, many patients with thyroid cancer who received 131I therapy, even in high cumulative doses (up to 1 Ci) never became antibody-positive. These data are in agreement with the results of previous studies (Kriss et al. 1967; Pinchera et al. 1969; Einhorn et al. 1965) showing that most hyperthyroid patients with undetectable basal serum thyroid antibodies did not develop positive tests after 131I. Thus, it would appear that 131I per se generally has no major role in determining persistent thyroid humoral autoimmune reaction in our patients. However, our data cannot exclude that radioiodine treatment may be responsible for transient exacerbation of pre-existing thyroid autoimmune phenomena.

Finally, as far as the clinical outcome is concerned, the presence of circulating thyroid autoantibodies does not represent a protective or worsening factor in the history of thyroid cancer, as shown by the similar clinical course and death rate among antibody-positive and antibody-negative patients.

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