Histometry of lymphoid infiltrate in the thyroid of primary thyrotoxicosis patients

Relation of extent of thyroiditis to preoperative drug treatment and postoperative hypothyroidism

R. J. YOUNG, M. B. SHERWOOD¹, J. G. SIMPSON, A. G. NICOL, W. MICHIE, AND J. SWANSON BECK

From the Departments of Pathology, Universities of Dundee and Aberdeen, Aberdeen Royal Infirmary and Department of Anatomy, University of St. Andrews

SYNOPSIS The thyroids of primary thyrotoxicosis patients prepared for partial thyroidectomy with propranolol contained much more lymphoid infiltrate than those prepared with carbimazole. No relation was found between the extent of lymphoid infiltrate in the thyroid and the development of postoperative hypothyroidism either between or within the two drug treatment groups. This study has shown that the extent of thyroid infiltrate should not be used as the major factor in predicting hypothyroidism after subtotal thyroidectomy for primary thyrotoxicosis.

Lymphoid infiltrate has long been recognized as a component of the thyroid gland in primary thyrotoxicosis (Wegelin, 1926), and it has been established that the preoperative titre of circulating antithyroid antibodies is related to the extent of lymphoid infiltration in the thyroid (Goudie *et al*, 1959; Buchanan *et al*, 1962; Irvine *et al*, 1962; Irvine and Stewart, 1967).

It has been claimed that the incidence of postoperative hypothyroidism after subtotal thyroidectomy is higher in those patients whose thyroids show more extensive lymphoid infiltrate (Whitesell and Black, 1949; Greene, 1950 and 1953; Levitt, 1951; Hargreaves and Garner, 1968). Since these reports were based on semiquantitative measurement methods, we have re-investigated this problem using the more accurate histometric technique. This paper reports our findings on the relation of the extent of thyroiditis to the preoperative drug treatment regime and to the incidence of postoperative hypothyroidism.

Material and Methods

CLINICAL INVESTIGATIONS

This study was undertaken on 95 female primary

¹Present address: Medical Student, University of Manchester
Correspondence: Professor J. Swanson Beck, Department of Pathology, Ninewells Hospital and Medical School, Dundee DD2 1UB

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thyrotoxicosis patients for whom subtotal thyroidectomy was regarded as the treatment of choice. The diagnosis of primary thyrotoxicosis was based on the criteria of Young *et al* (1975). Two groups of patients were studied:

(a) Propranolol group

Twenty-six thyroids were removed in the years 1970-72 from patients who were prepared for operation with propranolol. All patients had received the drug for at least six weeks up to and including the day of operation and none had received carbimazole or other antithyroid drug during the previous six months.

(b) Carbimazole group

Sixty-nine patients treated with carbimazole were studied. There were 17 thyroids from patients coming to operation during the period 1970-72, and 52 thyroids from patients treated by operation during the period 1960-68 before the introduction of propranolol treatment in Aberdeen Royal Infirmary. The latter group of patients was selected retrospectively so that they matched the propranolol patients for decade of age and month of operation to avoid any bias that may have arisen in the assignment of patients to either of the preoperative drug treatment groups during the period 1970-72. All patients were euthyroid at the time of operation but there was considerable variation in the duration of pre-

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operative treatment with carbimazole: those patients referred directly to the Thyroid Clinic at Aberdeen Royal Infirmary by general practitioners generally received a relatively short period of preoperative drug treatment (2-4 months), whereas those referred by other physicians generally had a more extended course of preoperative drug therapy (up to four years).

All patients received potassium iodide for 10 days before operation. During this preoperative period, treatment with carbimazole was stopped whereas propranolol was continued through the operation.

Postoperative hypothyroidism was assessed by the criteria used in our previous study (Young *et al*, 1975).

PATHOLOGICAL METHODS

Resected thyroids were weighed and then the tissue was fixed in 4% neutral buffered formaldehyde. After fixation the thyroids were cut into 5 mm slices; blocks were taken from any areas of unusual naked-eye appearance in addition to a minimum of two representative blocks from each lobe. Sections (5 μ m) prepared from paraffin blocks were stained with haematoxylin and eosin with standard techniques.

Sections were examined with a 40 $\times$ objective and a 10 $\times$ focusing eyepiece containing a 36-intersection graticule (Leitz code number 519902). The volume proportion of the lymphoid tissue (% L) and of epithelium (% E) in the thyroid was measured with the point-counting technique (Dunnill, 1968). In preliminary studies it was shown (1) that the standard error of the observations was less than 3% when 20 fields were selected for counting using random numbers to define the co-ordinates of the Vernier scale of the mechanical stage, and (2) that examination of one section with this method gave results closely comparable to those obtained from examination of several sections from the same gland.

IMMUNOLOGICAL METHODS

Antithyroglobulin antibody and anticytoplasmic antibody were estimated by the tanned red cell haemagglutination (Fulthorpe *et al*, 1961) and immunofluorescence (Beck, 1971) techniques respectively.

Results

The amount of lymphoid tissue in the thyroids of the two groups is shown in the figure. There is considerable variation between patients in each drug treatment group, but it is clear that the lymphoid infiltrate is more frequent and generally more abundant in thyroids from the propranolol group than in those from the carbimazole group (Wilcoxon test

Figure Extent of lymphoid infiltrate in thyroids of primary thyrotoxicosis patients prepared for subtotal thyroidectomy with carbimazole or propranolol.

$T_{\text{propranolol}} = 1921.5$; $P < 0.0001$). The findings in the recent and retrospective groups of carbimazole-treated patients did not differ significantly.

There was no qualitative difference either in the anatomical localization, in the relative amounts of large and small aggregates of lymphoid cells, or in the incidence of germinal centres between the two drug treatment groups.

Despite the marked differences in extent of lymphoid tissue infiltrate between the thyroids of the two drug treatment groups, the incidence of positive tests for antithyroid antibodies was similar in the groups; antithyroglobulin antibody was positive in 23% and 19% and anticytoplasmic antibody in 40% and 46% in the carbimazole and propranolol prepared patients respectively. The table shows that there is a significant relation between the extent of lymphoid infiltrate and the positive tests for anticytoplasmic antibody in both drug treatment groups. A significant relation between extent of lymphoid infiltrate and positive tests for antithyroglobulin antibody was established for the carbimazole group; only five out of 26 patients in the propranolol group had positive tests for antithyroglobulin and the lack of correlation in this group is possibly a consequence

Hypothesis that Observation A greater if Condition B satisfied		Treatment Group			
		Carbimazole (69 patients)		Propranolol (26 patients)	
A	B	U Statistics	P	U Statistics	P
%L	Anticytoplasmic antibody positive	379	<0.01	38.5	<0.025
%L	Antithyroglobulin antibody positive	246	<0.001	29.0	NS
%L	Postoperative hypothyroidism	367	NS	63.0	NS
%E	%L > 1.0	436	NS	49.0	NS

Table Results of Mann-Whitney ranking tests on histometric measurements of volume proportions of lymphoid tissue in thyroids of primary thyrotoxicosis patients treated with iodide after a course of carbimazole or propranolol

NS = not significant at the 5% level

%L = volume proportion of lymphoid tissue in resected thyroid

%E = volume proportion of epithelium tissue in resected thyroid

of the small number of patients studied and the lower incidence of the antithyroglobulin antibody.

The incidence of postoperative hypothyroidism is similar in patients in the two drug treatment groups—19 out of 69 treated with carbimazole (28%) and eight out of 26 treated with propranolol (30%). Moreover, the extent of lymphoid infiltrate was not related to the development of postoperative hypothyroidism in either of the drug treatment groups (table).

Discussion

In this study we have compared the effects of two preoperative treatment regimes employing different drugs on the extent of lymphoid infiltrate in the thyroids of patients with primary thyrotoxicosis. Our findings must not be interpreted as a direct comparison of the iatrogenic effects of carbimazole and propranolol on the thyroid since patients in the carbimazole group had been under treatment for longer periods than those who had received propranolol. Furthermore, propranolol treatment was continued through the operation, whereas carbimazole was stopped 10 days before operation, and both groups of patients received preoperative iodide.

The amount of lymphoid tissue in the thyroid glands of the primary thyrotoxicosis patients treated with propranolol is substantially greater than in those treated with carbimazole, and an analogous difference has been seen in the extent of hyperplasia in the thymus of these patients (Simpson *et al*, 1975). Both findings may be related to the pharmacological action of these drugs. The conventional antithyroid drugs, such as carbimazole, reduce thyroid hormone synthesis and release and so make the patient euthyroid. By contrast, propranolol is a potent specific inhibitor of cardiac and peripheral β -receptors, which reduces tachycardia and peripheral vasodilatation but does not affect circulating thyroid

hormone levels. Many animal experiments have shown that thyroxine can induce marked hyperplasia of the lymphoid tissues (Fisher, 1964). Consequently the most plausible explanation of our findings would be that the lymphoid tissues in carbimazole-treated patients have been released from excessive thyroid hormone stimulation and have partly reverted to normal by the time of operation, whereas the lymphoid tissues in the propranolol-treated patients continue to be subjected to excessive thyroid hormone stimulation. Two other explanations for the findings should be considered. First, it is possible that the conventional antithyroid drugs have immunosuppressive properties in view of their chemical similarity to uracil, but this pharmacological action has not been investigated; if this were so then it could induce lymphoid tissue atrophy in the patients treated with carbimazole. Secondly, it is remotely possible that propranolol could cause hyperplasia of lymphoid tissues by direct stimulation, but this explanation is unlikely since the appearances of the lymphoid tissues in propranolol-treated patients are similar to the historical pathological description of untreated primary thyrotoxicosis (Wegelin, 1926).

The finding that lymphoid infiltrate is more abundant in patients with antithyroid antibodies confirms and extends the work of Goudie *et al* (1959), Buchanan *et al* (1962), Irvine *et al* (1962), and Irvine and Stewart (1967). The nature of the lymphoid cells in thyroid infiltrate is not yet decided. In a preliminary report, Farid *et al* (1973) claim that T-lymphocytes are more numerous in the thyroids of patients with Graves' and Hashimoto's disease than in normal individuals, but the histopathological observation that plasma cells are common in the areas of lymphoid infiltrate suggests that at least some of the lymphocytes must be B-cells. Shevach *et al* (1973), using a more discriminating technique, have shown that in Sjögren's syndrome, another putative 'autoimmune' disease, there are considerable num-

bers of B-cells in the chronic inflammatory infiltrate.

In the light of Volpé's hypothesis of a stimulatory role for lymphocytes in the pathogenesis of primary thyrotoxicosis (Volpé *et al*, 1974) it is of interest that no relation was established between the extent of thyroid epithelial hyperplasia (as measured by the volume proportion of epithelial cells in the thyroid) and that of lymphoid infiltration in either drug group (table).

In this histometric study no relation was found between the extent of lymphoid infiltrate in the thyroid and postoperative hypothyroidism either between or within the two drug treatment groups. The incidence of hypothyroidism after partial thyroidectomy is similar for both treatment groups (28% for carbimazole-prepared patients and 30% for propranolol-prepared patients) even though the extent of lymphoid infiltrate was so much more abundant in the propranolol group. These are surprising findings since it is widely held that there is a high incidence of postoperative hypothyroidism in primary thyrotoxicosis patients who had an appreciable amount of lymphoid infiltrate in the resected thyroid specimen. Three sources are commonly cited. (a) Whitesell and Black (1949) assessed the extent of thyroiditis as the mean of visual estimates of the extent of lymphoid infiltrate from 150 low-power fields. They examined 86 thyroids with thyroiditis and all but five had greater than 10% of lymphoid infiltrate; the values for the majority lay in the range 10%-40% (these are much greater than the values found in the present series). They found a strong correlation between the degree of thyroiditis and postoperative hypothyroidism but the overall incidence of hypothyroidism in their series was only 29%, similar to that of the present series. Furthermore, they noted that myxoedema failed to develop in some patients in spite of advanced thyroiditis and that myxoedema occurred not uncommonly in patients with lesser degrees of thyroiditis. (b) Greene (1950) studied 161 thyrotoxicosis patients and reported that postoperative hypothyroidism occurred only when the lymphoid infiltrate contained germinal centres. However, since 88% of these glands were described as nodular, it is difficult to accept that his study was restricted to patients with primary thyrotoxicosis. (c) Levitt (1951) reviewed thyroid sections from 2114 patients who had come to partial thyroidectomy; 1410 of these were considered to be thyrotoxic and the histological appearances were classified into six categories. It was shown that the incidence of postoperative hypothyroidism was markedly greater if the histological appearance was that of diffuse lymphoid hyperplasia or fibrolymphoid hyperplasia, but his definition of these categories appears to correspond very closely with the

currently accepted appearance of Hashimoto's disease. No information was given in any of these reports on criteria for the diagnosis of either hyper- or hypothyroidism, preoperative drug treatment, the findings at operation, the size of the thyroid remnant or the method of postoperative follow-up. Moreover, the methods used in these previous reports to estimate lymphoid tissue are at the best semiquantitative, as also was the recent study of van Welsum *et al* (1974), the only report which disputes the relation between lymphoid infiltrate and postoperative hypothyroidism. It seems fair to conclude, therefore, that while the association between the extent of lymphoid infiltrate and postoperative hypothyroidism has gained wide acceptance, the evidence for this has never been firmly based. The findings of the present study based on more accurate histometric methods suggest that the extent of thyroid lymphoid infiltrate is not by itself a major factor in anticipating hypothyroidism after subtotal thyroidectomy for primary thyrotoxicosis.

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