The importance of inferior-superior thyroid veins sampling in the diagnosis of thyroid carcinomas

Znaczenie badania krwi pobranej z żył tarczowych górnych i dolnych w rozpoznawaniu raka tarczycy

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Abstract
Introduction: We aimed to determine whether levels of thyroglobulin measured in blood from the inferior-superior thyroid veins and the peripheral antecubital vein could predict the presence of thyroid carcinoma in patients undergoing surgery for thyroid diseases.

Material and methods: Sixty-one patients were prospectively enrolled in the study. Levels of thyroglobulin were analysed. Sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) of these markers were investigated.

Results: Twenty-six out of 61 patients (42.6%) with malignancy were diagnosed. The levels of thyroglobulin in the inferior-superior thyroid veins were higher than those in the peripheral antecubital vein (p = 0.001). The levels of thyroglobulin in the blood taken from the antecubital vein and the inferior-superior thyroid veins did not differ between benign and malignant thyroid disorders. For thyroglobulin, sensitivity was 33.3%, specificity 60.6%, PPV 27.8%, and NPV 66.7% respectively.

Conclusion: Thyroglobulin levels in the antecubital vein compared to the inferior-superior thyroid veins were not significant either in benign or malignant disorders.

Key words: thyroglobulin, thyroid vein sampling, thyroid cancer

Introduction
Thyroid cancers represent the commonest malignancy of the endocrine system, and the second commonest malignancy in the head and neck region (excluding skin cancers) [1]. The initial evaluation of the thyroid nodule continues to rely chiefly on fine needle aspiration (FNA) and ultrasound imaging. Despite the improvements in imaging methods and the wide usage of FNA, there is also a group of patients who cannot be diagnosed as differentiated thyroid carcinoma preoperatively [2]. Two of the advances in optimising operative strategy in thyroid disorders are intraoperative frozen section analysis and intraoperative touch imprint cytology [3]. They might improve outcomes in patients with suspicious nodular disorders of the thyroid gland.

Recent clinical guidelines have attempted to settle various controversies, but many inherent errors of clinical testing result in delayed diagnosis and unnecessary surgery. A better solution may ultimately involve the use of molecular markers of thyroid carcinogenesis, but further research is still needed regarding the basic biology of thyroid cancer [4]. A very well known marker for medullary thyroid carcinoma, calcitonin, is the...
most reliable marker in the course of the disease. We hypothesised that the concentrations of thyroglobulin for thyroid carcinoma would be higher in blood taken from the thyroid vein, relative to their concentrations in systemic blood.

The purpose of this study was therefore to test the hypothesis as to whether blood levels of the markers differ between the inferior thyroid vein and the peripheral circulation, and also to test whether these markers are useful in predicting the diagnosis made via microscopic exams of tissue specimens.

**Material and methods**

Sixty-one consecutive patients with an FNA diagnosis of suspicious for malignancy or malignant nodular goitre were prospectively enrolled in the study. The study was approved by the local ethics committee and written informed consent was obtained from each patient. Our research complied with the principles of the Helsinki Declaration.

Of the 61 patients, 48 were female (78.7%) and 13 were male (21.3%). Overall mean age was 47 years (range 23–78 years). Patients with thyroid cancers with distant metastases or a history of previous radiation exposure were excluded. All patients were preoperatively evaluated by physical examination, monitoring of serum thyroglobulin and serum thyrotropin, neck ultrasonography, and chest radiography. Thyroid scans were performed in all patients. Ultrasound-guided FNA was successfully performed using a 22-G needle prior to surgery. Smears of the FNA samples were stained by May-Grünwald-Giemsa stain and evaluated immediately by the cytologist. Patients with suspicious nodules were re-evaluated with intraoperative frozen section. Final diagnoses were confirmed postoperatively with permanent histopathological sections.

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The samples were centrifuged at 3,500 rpm for 5 minutes in order to separate the plasma. After separation, all samples were frozen at −30°C and levels of thyroglobulin were measured with a Liaison kit (Byk-Songtec Diagnostica, Liaison thyroglobulin, 2001 USA) by immunometric assay. Normal levels for thyroglobulin were 1.5–4 ng/mL respectively. All malignant patients were separated into two groups. The separation was performed in two different ways to find out the positive predictability of independent variables (levels of thyroglobulin) for subjects due to postoperative histopathologic diagnosis. Re-arranged end results of the patients and their groups: prevalence of malignant cases; correlation of the levels of samples from benign cases (n = 35) with those of malignant patients (n = 26) according to postoperative histological examination.

Sensitivity, specificity and predictivity were assessed for thyroglobulin as follows: sensitivity (definition of the thyroid cancers detected with high marker levels) was defined as true positive / [true positive + false negative]; specificity (definition of benign thyroid diseases detected with normal levels of markers) was defined as true negative / [true negative + false positive]; positive predictive value (PPV) (probability of diagnosing the disease if the FNA is positive) and negative predictive value (NPV) (probability of diagnosing normal subjects if the FNA is negative) were calculated as follows:

- **PPV:** \((\text{Prevalence}) \times (\text{Sensitivity}) / (\text{Prevalence}) \times (\text{Sensitivity}) + ((1-\text{Prevalence}) \times (1-\text{Specificity}))\)
- **NPV:** \(((1-\text{Prevalence}) \times (\text{Specificity}) / (1-\text{Prevalence}) \times (\text{Specificity})) + ((\text{Prevalence}) \times (1-\text{Sensitivity}))\)
- Numeric variables between the groups were analysed using Mann-Whitney U test. The significance level was taken at \(p\) below 0.05.

**Results**

Preoperatively, eight patients (13.1%) were diagnosed with thyroid malignancy by FNA. According to the postoperative pathology reports, an additional 18 patients were diagnosed with malignancy. Thus in total, 26 patients (42.6%) with malignancy were diagnosed. All cases underwent surgery after achieving a euthyroid state. All nodules presented with a solid or mixed (solid-cystic) pattern. Mean nodule size was 21.1 mm (min 1.2 mm, max 9.5 cm). Mean size of the nodule was 29.4 mm in benign, and 21.3 mm in malignant, subjects. There were 42 cases with multinodular goitre, and 12 cases with single solitary nodule. We detected seven malignancies among 12 cases (58.33%) with single solitary nodule and 19 malignancies among 42 cases (45.23%) with multinodular goitre. This difference was not significant \((p = 0.27)\). The difference in mean size of the nodules between the two groups was not significant \((p = 0.11)\). In thyroid scans, there were only three cases of hot nodule.

Less than total thyroidectomy was performed in 32 patients (52.4%) and total thyroidectomy in 29 patients (47.6%). Papillary thyroid carcinoma was detected in 20 patients (32.8%), and follicular thyroid carcinoma in six patients (9.8%). Six of 20 cases with papillary thyroid cancer had occult carcinoma. None of the patients had enlarged cervical lymph nodes.

Mean values of thyroglobulin in blood taken from antecubital vein, inferior-superior thyroid vein, and
Thyroid vein sampling in thyroid carcinomas

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Discussion

Surgical treatment is still the treatment of choice for most thyroid diseases, especially for malignant disorders. Often, surgery is carried out in the presence of a suspicious nodule [5]. Preoperative diagnosis of thyroid malignancies predicts the limits of the surgical procedure. Furthermore, early detection of asymptomatic malignancy leads to better prognosis and outcome. Cost-effective screening methods used in other solid cancers like PSA, NSE, and β-HCG are still lacking in the early diagnosis of thyroid malignancies [6]. In experienced hands, diagnostic accuracy of about 90% is obtained by FNA [7]. In general, 20–30% of patients are referred for surgery on the basis of the FNA features [8]. Approximately one third (17% to 51%) of these cases have a thyroid malignancy. False-negative rates range from 1% to more than 11% [9]. The sensitivity is about 95%, and the specificity is 95% [10]. In our series, we detected lower rates of sensitivity and specificity in cases investigated for selective venous sampling with respect to thyroglobulin levels, thereby preventing their usage as a diagnostic or screening tool. However, like FNA, detection of TSH mRNA yielded encouraging results for discrimination between benign and malignant disorders of the thyroid [11]. In the cited article, the authors found better rates of sensitivity, specificity, and PPV, even in cases with indeterminate FNA. They further concluded that detection of TSH mRNA might be beneficial in the postoperative follow-up of patients with thyroid malignancies.

Levels of calcitonin are beneficial in the prediction and follow-up of patients with medullar thyroid carcinoma [12], but similar correlation between differentia ted thyroid carcinomas and biochemical markers is still lacking. Thyroid carcinomas constitute a heterogeneous group of malignancy with ambiguous outcomes. There has been a demand for molecular markers to identify thyroid cancer pathogenesis and distinguish them from benign disorders of the thyroid [13]. Thyroglobulin has been extensively investigated from this aspect. Selective sampling has been the mainstay of some studies in cases with medullar thyroid cancers.

It has been previously stated that selective venous sampling in patients with medullar thyroid carcinoma might be an effective tool in the detection of early recurrences [14]. In another study by Mohammed et al. [15], the role of calcitonin in 77 patients with medullar thyroid cancers was studied by selective venous cannulation of the thyroid, cervical, and mediastinal regions. These two studies formed the basis of the idea of cannulating the inferior thyroid vein.

Since the first demonstration by Van Herle et al. that thyroglobulin, the main iodo-protein of the thyroid gland, was detectable in the circulation of normal subjects by using specific radioimmunoassay, an impressive number of papers have been produced describing several clinical applications of Tg measurements, which is higher in some diseases such as follicular thyroid cancer [16]. Thyroglobulin (Tg) is a large molecule containing 2,750 amino acids with a molecular weight of 330 kD and 20 putative N-linked glycosylation sites. Tg gene

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<th>Thyroglobulin [ng/mL]</th>
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<tr>
<td>Benign</td>
<td>Malignant</td>
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<tr>
<td>ACV 9.94 ± 17.43</td>
<td>9.92 ± 16.34</td>
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<td>ITV-STV 72.37 ± 34.98</td>
<td>68.4 ± 30.42</td>
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<td>ACV/ITV-STV ratio 0.18 ± 0.29</td>
<td>0.31 ± 0.42</td>
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ACV — antecubital vein; ITV — inferior thyroid vein; STV — superior thyroid vein
expression is regulated by thyroid transcription factor 1 and human paired box 8. Iodinated Tg is stored in the lumen of the thyroid follicles and is released in response to specific hormonal stimulation by thyroid stimulating hormone (TSH). Following Tg reabsorption by thyrocytes and subsequent degradation, thyroid hormones triiodothyronine (T3) and thyroxine (T4) are secreted in the bloodstream [17].

Now, because of its higher levels in follicular cancer, the measurement of Tg is a mainstay of the post-surgical follow-up of follicular thyroid cancer. After total thyroid ablation by surgery and radioiodine, the Tg level should be measured. It must be undetectable, i.e. any detectable level should alert the clinician [18].

Ringel et al. [19] observed the importance of the levels of thyroglobulin in the early diagnosis of residual and recurrent thyroid carcinomas. They investigated the m-RNA of thyroglobulin by radioimmunoassay, and concluded that positive scans result in a dismal prognosis.

Conclusively, positive scans result in a dismal prognosis. Positive scans result in a dismal prognosis.