

# The influence of the vascularisation of the follicular thyroid nodules on the proliferative activity of the follicular cells

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[Received 11 September 2003; Accepted 5 January 2004]

*The enlargement of the thyroid is, in general, benign in origin and due to nodular goitre. Follicular cellular proliferation of thyroid nodules has been increasingly observed recently. With fine needle aspiration biopsy (FNAB) this is classified as a follicular tumour. These lesions present various patterns of vascularisation in ultrasound examination. The aim of the study was to establish the relation between follicular nodule vascularisation and the proliferative activity of various types of follicular cell. According to the manner of proliferation, patients were divided into groups as follows: (I) patients with hyperplastic nodules (46 cases), (II) patients with follicular adenoma (42 cases), and (III) patients with follicular cancer (9 cases). In each case B-mode sonography, Power Doppler, sonographically guided FNAB (S-FNAB), morphological examination and morphometry were performed. The proliferative activity was detected with immunohistochemical methods (PCNA, Ki 67 and MPM2) to determine the so-called "proliferative index". The study revealed increased proliferative activity in tumours of malignant origin and increased vascularisation in coexistence with increased proliferation of the follicular cells. As assessed by Power Doppler, an increased flow pattern in the centre of the nodules correlates with increased proliferative activity. The results suggest that Power Doppler examination could be helpful in selecting nodules for FNAB, especially in multinodular goitre.*

**Key words: thyroid, follicular tumour, Power Doppler, vascularisation, proliferation**

## INTRODUCTION

The most common diagnostic method is ultrasound (US). However, this does not provide any definitive solution to the question of whether the lesion is benign or malignant [8]. The most important role in preoperative thyroid examination is played by fine needle aspiration biopsy (FNAB) [3], especially when ultrasonographically guided (S-FNAB) [4].

The microscopic examination of tissue obtained in this way often reveals follicular cell proliferation, so-called follicular tumours.

With the use of Doppler sonography various degree of vascularisation can be observed within the thyroid nodules. New ultrasound techniques such as Power Doppler (PD) enable us to visualise tissue vascularisation [5]. Power Doppler provides the oppor-

tunity to establish the slow flow within the small vessels and also to visualise the morphological nature of the vessel. Tumours classified as follicular with FNAB present various degree of vascularisation. In these cases the morphological nature of the tumour may finally be established by morphological examination [2].

The aim of the study was to evaluate the relationship between the degree of vascularisation of follicular thyroid nodules and the proliferative activity of follicular cells.

## MATERIAL AND METHODS

The group under investigation consisted of 82 females and 15 males of mean age  $41 \pm 14.5$  years (18–67 years) qualified for thyroid surgery. The surgery was determined upon when FNAB presented a proliferation of follicular cells characteristic for follicular tumours. In each case B-mode real time sonography of the thyroid gland was performed and, additionally, examination by Power Doppler. The ultrasound examination was conducted with a linear 7.5 MHz probe. The flow pattern was subjectively classified with the naked eye and the tumours were divided into two subgroups: A — nodules with no internal flow and flow around the nodule, and B — nodules well vascularised within with potential flow around the lesion. The nodules were also classified according to the diameter:  $\leq 1$  cm, and  $> 1$  cm. According to the manner of proliferation, the group under investigation was divided into the following subgroups: (I) cases with hyperplastic nodules within the goitre, (II) cases with follicular adenoma and (III) cases with follicular cancer. The proliferative activity of the follicular cells was evaluated by immunohistochemical methods using antibodies against the antigen proteins observed in the various phases of the cell cycle, PCNA, Ki-67 and MPM2, LSAB + Kit HRP as a detection kit and DAB as a chromogen (DAKO). The proliferative index (PI) was determined as a percentage of follicular cells with a positive nuclear reaction in relation to all the cells. In each case the degree of vascularisation was evaluated on the basis of vessel localisation (central, peripheral, intermediate focal), and the relation of vessel localisation to the tumour surface in the microscope field with magnification  $240 \times$ . The examination was performed with the Olympus MicroImage System equipment for morphometric examination. The Mann-Whitney U test was used for statistical purposes with  $p$  values  $< 0.05$  considered as significant. Data are

presented as means  $\pm$  SD. The study was accepted by the Local Bioethical Committee.

## RESULTS AND DISCUSSION

The morphological examination revealed 46 cases with hyperplastic nodules within the goitre (I), 42 with follicular adenoma (II), and 9 cases with follicular cancer (III). Thirty nine nodules were smaller or equal to 1 cm in diameter and 58 nodules were greater than 1 cm. The morphometric examination showed numerous tiny vessels in the centre of the nodules in the cases of follicular cancer. The vascularised area was  $15.8 \pm 2.7\%$  in nodules greater than 1 cm and  $14.3 \pm 3.1\%$  in smaller lesions. The cases of follicular cancer presented significantly lower peripheral vascularisation than occurred in the nodule centre. In the cases of hyperplastic nodules of less than 1 cm in diameter no flow was revealed in the central part of the lesion, while in larger nodules ( $> 1$  cm) it was present in  $8.1 \pm 2.2\%$ . In these cases the vessels were localised between the centre and the peripheral part of the nodule. The vascularisation in the nodule centre indicates a relationship to follicular cancer ( $p < 0.05$ ). In thyroid adenomas a number of vessels were found in peripheral part of the nodules and significantly more than in the centre (Table 1). With regard to the Power Doppler pattern, 44 (28 nodules  $\leq 1$  cm, 16 nodules  $> 1$  cm) nodules were classified as A and 53 (11 nodules  $\leq 1$  cm, 42 nodules  $> 1$  cm) nodules as B. The proliferative activity of the follicular cells of nodules is presented in Table 2. The data show increased proliferative activity in adenomas in comparison with hyperplastic nodules and greater in cancer than in adenomas. Statistically significant differences were found between the IP of cancer and of adenoma, and between the IP of cancer and hyperplastic adenoma. In Power Doppler examination 53 cases of vascularisation within the nodules were detected, corresponding to focal and central vascularisation in morphometry. Our results are evidence of the high sensitivity but less favourable specificity of this method and are in line with the results presented by Cerbone et al. [1]. It is well recognised that the diagnostics of follicular thyroid tumours presents a number of problems. The proliferative activity should therefore be established in these kinds of thyroid lesion [6]. The relation between vascularisation and follicular cell proliferation was studied by Salabe, who concluded that hyperplastic thyroid nodules present no genetic and/or epigenetic mechanism [7].

**Table 1.** Vascularisation in morphometric evaluation

	N	Nodule diameter [cm]	No. of cases (ratio: vascularised field/tumour field) [%]					
			N	Peripheral	N	Intermediate focal	N	Central
Hyperplastic nodule (I)	26	≤ 1	8	7.9 ± 1.8	18	8.4 ± 2.1		
	20	> 1	4	10.2 ± 2.7	14	7.8 ± 1.9	2	8.1 ± 2.2
Follicular adenoma (II)	10	≤ 1	8	12.4 ± 3.1			2	8.6 ± 2.8
	32	> 1	22	13.2 ± 3.7	6	10.7 ± 2.3	4	11.3 ± 2.5
Follicular cancer (III)	3	≤ 1	1	12.2 ± 1.9			2	14.3 ± 3.1
	6	> 1	1	14.3 ± 1.8	1	12.6 ± 3.1	4	15.8 ± 2.7

**Table 2.** Proliferative activity of follicular cells

		N	IP PCNA	IP Ki67	IP MPM2
Hyperplastic nodule (I)	≤ 1 cm	26	38.5 ± 3.7	34.3 ± 2.6	32.9 ± 3.1
	> 1 cm	20	42.3 ± 2.8	39.4 ± 4.1	29.3 ± 2.8
Adenoma (II)	≤ 1 cm	10	41.2 ± 4.2	40.6 ± 3.7	34.3 ± 1.9
	> 1 cm	32	43.8 ± 2.9	42.1 ± 3.2	36.9 ± 2.7
Cancer (III)	≤ 1 cm	3	64.3 ± 2.8	62.1 ± 3.7	61.4 ± 2.9
	> 1 cm	6	68.4 ± 3.9	63.9 ± 2.8	59.8 ± 4.1

## CONCLUSIONS

1. Increased proliferative activity occurs in nodules of greater morphological malignancy.

2. Increased vascularisation of the thyroid nodules coexists with increased proliferative activity of the follicular cells.

3. Nodules with increased vascularisation in the centre (Power Doppler) present increased proliferative activity. The Power Doppler examination could be helpful in selecting nodules for FNAB, especially in multinodular goitre.

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