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Analysis of clinical significance of equivocal thyroid cytology with a special consideration for FLUS category — five years of new classification of FNA results

Analiza znaczenia klinicznego niejednoznacznych wyników biopsji tarczycy ze szczególnym uwzględnieniem kategorii ZPBN na podstawie 5 lat stosowania nowej klasyfikacji rozpoznań cytologicznych

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Abstract
Introduction: The diagnostic category of follicular lesion of undetermined significance (FLUS) was intended to allow selection of cases with low risk of malignancy from all smears with indeterminate, suspicious cytology (ISC), which can potentially take advantage from repeat fine-needle aspiration (rFNA).
Aim of the study was a comparison of the risk of malignancy related to FLUS nodules and other nodules with ISC: suspected follicular neoplasm (SFN) and suspected malignancy (SM), as well as analysis of the usefulness of assessing ultrasonographic malignancy risk features (UMRF) in nodules with ISC.
Material and methods: We analysed UMRF, rFNA, and results of histopathological examination (H) in 441 FLUS, 135 SFN, and 72 SM nodules.
Results: The frequency of exposing cancer in H in FLUS nodules was 5.9%, and when cytological follow up was also included it was 2.9%. rFNAs made the diagnosis more precise in 72.7% of FLUS, and in 5.2% it was diagnosis/suspicion of cancer. The incidence of cancer in SFN nodules was 8.2%, in SM nodules with suspicion of papillary cancer — 61.1%, and in nodules with suspicion of other or unspecified malignancy — 53.8% ($p < 0.0001$ FLUS *vs.* both groups).
The presence of calcifications is the only independent UMRF for nodules with ISC (OR 4.7). Features of importance are also microcalcifications (OR 3.8), especially in the SM group, and taller-than-wide-shape (OR 2.2). FLUS and SFN nodules are characterised by particularly low value of assessing suspicious margins; analysis of hypoechogenicity is of low value in SFN nodules, like suspected vascularisation in SFN and SM nodules.
Conclusions: The risk of cancer in FLUS and SFN nodules is lower than in SM nodules. rFNAs of FLUS nodules make the diagnosis more precise in more than 70% of cases and are effective in revealing cancers. UMRFs present variable diagnostic value depending on the subcategory of ISC. **(Endokrynol Pol 2016; 67 (1): 23–34)**

Key words: *thyroid cancer; FNA; FLUS*

Streszczenie
Wstęp: Rozpoznanie zmiana pęcherzykowa bliżej nieokreślona (FLUS) miało wyodrębnić spośród rozmazów z niejednoznaczną, podejrzaną cytologią (ISC) przypadki z niskim ryzykiem złośliwości i potencjalną korzyścią z wykonania powtórnej FNA (rFNA).
Celem pracy było porównanie ryzyka złośliwości guzków FLUS i innych guzków z ISC: podejrzenie nowotworu pęcherzykowego (SFN) i podejrzenie złośliwości (SM) oraz analiza przydatności oceny ultrasonograficznych cech ryzyka złośliwości (UMRF) w guzkach z ISC.
Materiał i metody: Analizowano UMRF, rFNA i wyniki badania histopatologicznego (H) 441 guzków FLUS, 135 SFN i 72 SM.
Wyniki: Częstość ujawniania raka w H w guzkach FLUS wynosiła 5,9%, a uwzględniając także cytologiczny *follow up* 2,9%. rFNAs uściśliły rozpoznanie w 72,7% guzków FLUS, w 5,2% ich wynik zawierał rozpoznanie/podejrzenie raka. Częstość raka w guzkach SFN wynosiła 8,2%, w guzkach SM z podejrzeniem raka brodawkowatego 61,1%, a z podejrzeniem innego nowotworu złośliwego lub bez określenia jego typu 53,8% ($p < 0,0001$ *vs.* FLUS w obu przypadkach).
Obecność zwapnień jest jedyną niezależną UMRF dla guzków z kategorii ISC (OR 4,7). Ponadto znaczenie mają ocena mikrozwapnień (OR 3,8), szczególnie w grupie SM, i podejrzanego kształtu (OR 2,2). W grupach FLUS i SFN szczególnie niską wartość ma ocena podejrzanych granic, ponadto w grupie SFN analiza hipoechogeniczności, a w grupie SFN i SM — podejrzanego unaczynienia.

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Wnioski Ryzyko raka w guzkach FLUS i SFN jest niższe niż w kategorii SM. rFNA guzków FLUS uściśla rozpoznanie w ponad 70% przypadków i jest skuteczna w ujawnianiu raków. UMRF mają zróżnicowaną wartość diagnostyczną w zależności od podkategorii ISC.
(Endokrynol Pol 2016; 67 (1): 23–34)

Słowa kluczowe: *rak tarczycy; biopsja aspiracyjna cienkoigłowa; zmiana pęcherzykowa bliżej nieokreślona*

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Introduction

Results of fine-needle aspiration biopsy (FNA) of the thyroid equivocal in respect to diagnosis or exclusion of cancer constitute a significant problem in clinical practice. They include mainly follicular lesions (FL). In such cases the routine cytological examination does not allow us to distinguish benign from malignant nodules, and the cytological result indicates only suspicious for follicular neoplasm (SFN) nodules. Indeterminate cytology may also be a consequence of the presence in a smear of features typical of thyroid cancer, but their degree is insufficient for unequivocal diagnosis. In such case the result indicates a suspicious of malignancy (SM) nodule [1].

Until recently, indeterminate, suspicious cytology (ISC) was considered as an indication for surgical treatment, which could solve diagnostic uncertainty. But the high variability of the cancer risk (between 5 and 30% for FL and from 30 to 70% for SM) related to such diagnoses raised marked clinical doubts [2–3]. The problem was aggravated by the lack of uniform formulation of diagnostic conclusion in such cases. Thus, the attempt to improve classification of cytological results undertaken by the National Cancer Institute (NCI) in 2008 was accepted with much hope by centres focused on diagnostics of the thyroid nodules [4–5]. In Poland the new classification of thyroid biopsy results, based on NCI proposal, has been in full operation for five years [6]. The classification introduced the new subgroup of the results within ISC "follicular lesion of undetermined significance" (FLUS). The goal of that modification was to delineate FL with low malignancy risk, in which it is justified to perform repeat FNA within 6 to 18 months in order to obtain more precise diagnosis. That additional diagnostic category was meant to be formulated mainly when there were features of both benign lesion and follicular neoplasm coexisting in a smear, and in that way it was adopted into Polish recommendations for diagnostics of the thyroid nodules [6]. However, the definition of FLUS adopted by the NCI is wider. It includes also the borderline results when characteristic features of benign and malignant lesions coexist in a smear and the cellularity of aspirate is scant [4–5]. It was assumed that FLUS diagnosis should be rarely formulated (< 7%) and the incidence of cancer among nodules with such diagnosis would not exceed 15%. Another assumption was that FLUS diagnosis would not be a direct indication for surgical treatment, in contrast to other ISC categories (SFN and SM). However, numerous reports indicate that FLUS may be related to higher than assumed risk of malignancy [7–14]. It is also unclear whether implementation of the new FLUS category does not significantly affect the malignancy risk related to other ISC categories (SFN and SM). That risk was assumed for SFN category as 5–20% and for SM category — 30–50%, according to Polish Recommendations [6]. Thus, the aim of the paper was to determine the actual risk of malignancy in the case of FLUS diagnosis and to compare it to the cancer risk in cases of SFN and SM, as assessed by analysis of repeat FNA and postoperative histopathological examinations. An additional aim was the comparison of ultrasonographic characteristics of FLUS, SFN, and SM nodules in relation to the final histopathological diagnosis.

Material and methods

At the first step all FNA outcomes were selected, which had been classified as FLUS, SFN, or SM category in the period between May 2010 and May 2015. The patients diagnosed for suspicion of cancer recurrence were excluded from the analysis.

Then the results of repeat FNA (rFNA) of FLUS nodules were analysed. The frequency was determined of formulation of particular diagnostic categories included in the current classification: non-diagnostic (ND), benign lesion (BL), FLUS, SFN, SM, and malignant neoplasm (MN). Additionally, the frequency was assessed of results indicating the necessity for surgical treatment (SFN, SM, and MN) as well as of BL outcome — suggesting conservative treatment.

For the next step the results of postoperative histopathological examinations were analysed in patients treated surgically. The incidence of cancer in FLUS nodules was compared between patients operated after rFNA (in relation to the final cytological diagnosis) and patients subjected directly to the surgical treatment (without rFNA). Also, the incidence of cancer revealed by histopathological examination was determined in nodules diagnosed cytologically as SFN or SM.

Finally, the size and ultrasonographic features of FLUS, SFN, and SM nodules were compared in relation to the final histopathological diagnosis: benign nodule *vs.* malignant neoplasm. The presence of the following ultrasonographic malignancy risk features (UMRF) were assessed: 1) hypoechoic (compared with the surrounding thyroid or strap muscles) and solid (< 10% cystic) echostructure, 2) calcifications with separate consideration for microcalcifications, 3) irregular or blurred margins, 4) more-tall-than-wide shape (measured in a transverse view), and 5) chaotic intranodular vascular spots. Also, the occurrence was analysed of: 1) > 1 UMRFs, 2) presence of at least 1 UMRF recognised as significant in examined ISC nodules on the basis of logistic regression analysis, 3) presence of hypoechoic solid nodule with at least one other risk feature (with exception of suspicious vascularisation) — which is the criterion for high risk of malignancy adopted in the new American Thyroid Association (ATA) recommendations [15], and 4) presence of a nodule with at least one risk feature enumerated by ATA independently on its hypoechogenicity.

The FNAs were carried out in patients referred by endocrinologists from outpatient clinics. All the biopsies were US-guided. The US examinations were performed with the use of two high-resolution sonographs with a 7.5–13 MHz linear transducer and power Doppler capability, before September 2011 (Siemens Elegra Advanced, Siemens Medical Systems, Inc., Issaquah, WA, USA and then Aloka Prosound Alpha 7, ALOKA Co. Ltd., Tokyo, Japan). FNAs were performed on nodules with a diameter of at least 5 mm, which were palpable or had at least one malignancy risk factor (ultrasonographic or clinical). Smears were fixed in 95% ethanol solution and stained with haematoxylin and eosin. A detailed description of the FNA procedure was presented in our earlier work [16–17].

Specimens showing a population of thyroid follicular cells (TFC) arranged in three-dimensional sheets, groups, and microfollicles (sometimes with nuclear overlapping and crowding) with scant or no colloid in background, or containing single cell population of oncocytic TFC arranged in sheets, and groups were classified as SFN or SFN oxyphilic type (SFN-O), respectively. A diagnosis of FLUS (or FLUS-O respectively) was made when the specimen showed features of both benign lesion and follicular neoplasm or showed low cellularity with the presence of architectural or so-called nuclear atypia. The SM category included nodules, aspirates from which showed morphological features of malignant neoplasm, but they did not meet all criteria necessary for such diagnosis. In that category the subgroup of nodules raising suspicion of papillary cancer (SM-pap), as well as the subgroup with features suggesting other malignant neoplasm or not allowing to specify its histogenesis (SM-non-pap), were created. Another analysed subgroup included very scant aspirates but with single cancer features and a cytological result that showed a low diagnostic value of aspirates and necessity of performing repeat FNA as soon as possible (SM-low diagnostic).

Surgical thyroidectomy specimens were processed by standard procedures. If necessary, immunohistochemical analysis was applied. Histopathological results were formulated according to the WHO Histological Classification of Thyroid Tumours.

Continuous variables (like the age of patients) were analysed with ANOVA and Newman–Keuls test. The comparison of frequency distributions was performed with chi2 test (or with Yates corrected chi2 test). Associations between US features and malignancy were evaluated by using logistic regression analysis, and odds ratios (OR) with relative 95% confidence intervals (95% CI) were calculated to determine the relevance of all potential predictors of outcome. Sensitivity (SEN), specificity (SPC), positive predictive value (PPV), and negative predictive value (NPV) were analysed based on established UMRF sets. The value of 0.05 was assumed as the level of significance.

The study design was approved by the Local Bioethics Committee.

Results

Table I shows the characteristics of patients with FNA results classified into particular ISC categories. Diagnoses of FLUS/FLUS-O were formulated in the case of 753 nodules. That category constituted between 3.7% and 5.1% of all FNA results in subsequent years. The results belonging to the SFN/SFN-O category were formulated

Table I. ***Characteristics of the patients in analysed groups of cytological diagnoses***

Tabela I. ***Charakterystyka pacjentów w poszczególnych grupach rozpoznań cytologicznych***

	FLUS/FLUS-0	SFN/SFN-0	SM
Number of patients	722	181	85
Age — mean ± SD (years)	59.7 ± 13.4	58.2 ± 15.5	57.7 ± 19.7
Gender			
Females	653 (90.4%)	155 (85.6%)	71 (83.5%)
Males	69 (9.6%)	26 (14.4%)	14 (16.5%)[a]
Number of nodules	753	183	85
Volume of nodules mean ± SD [cm^3]	5.5 ± 12.8	5.8 ± 11.9	6.4 ± 10.5

[a]percentage of males $p < 0.05$ *vs.* FLUS/FLUS-0. FLUS — follicular lesion of undetermined significance; SFN — suspicious for follicular neoplasm; SM — suspicious for malignancy

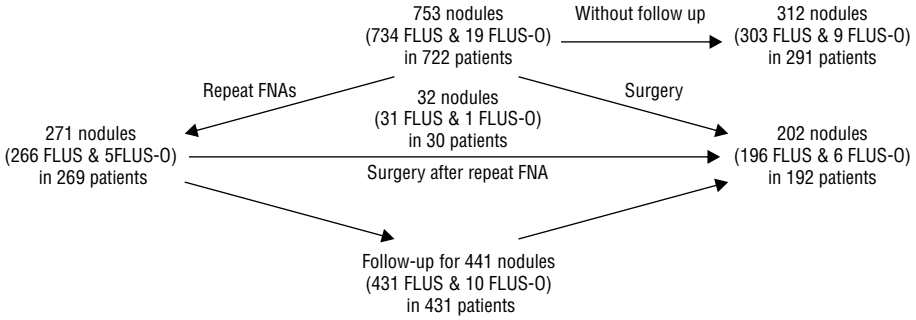

Figure 1. *Characteristics of analysed FLUS/FLUS-O nodules*
Rycina 1. *Charakterystyka badanych guzków FLUS/FLUS-O*

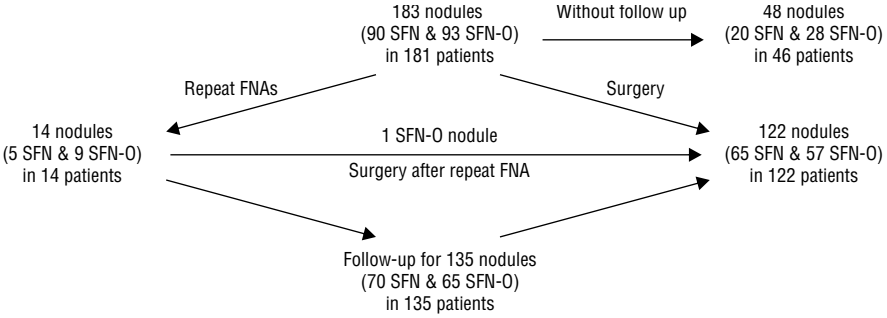

Figure 2. *Characteristics of analysed SFN/SFN-O nodules*
Rycina 2. *Charakterystyka badanych guzków SFN/SFN-O*

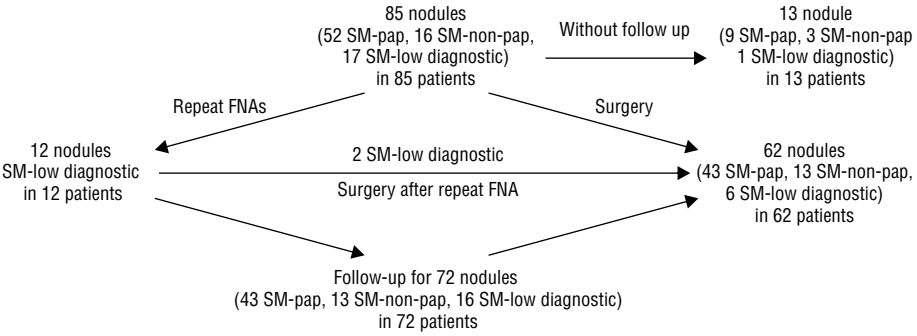

Figure 3. *Characteristics of analysed SM nodules*
Rycina 3. *Charakterystyka badanych guzków SM*

in 183 nodules, with frequency between 1.0 and 1.5%. FNA results categorised as SM constituted less than 1% of all diagnoses, and were observed in 85 patients. No significant differences were noted between those categories in the mean age of patients or the mean volume of nodules. The percentage of males in the SM group was significantly higher than in the FLUS/FLUS-O group (16.5 *vs.* 9.6, $p < 0.05$).

Figures 1–3 show data on cytological and histopathological follow up of the nodules of the examined ISC groups. In total, the follow up included 441 nodules of FLUS/FLUS-O category (431 and 10 nodules, respectively), 135 nodules of SFN/SFN-O category (70 and 65, respectively), and 72 nodules of SM category (43 SM-pap, 13 SM-non-pap, and 16 SM-low diagnostic).

Table II shows the results of rFNA of nodules diagnosed as FLUS/FLUS-O in the first cytology, and Table III summarises final cytological diagnoses of those nodules. Eventually rFNA allowed us to obtain more precise diagnosis in 197 out of 271 (72.7%) nodules, (in 176 — 64.9% —cases first rFNA allowed the for-

Table II. *Results of subsequent FNA in patients with cytological diagnosis of FLUS/FLUS-O, SFN/SFN-O, SM (the results of eventually operated patients are shown in parentheses)*

Tabela II. *Wyniki kolejnych FNA pacjentów z cytologicznym rozpoznaniem FLUS/FLUS-O, SFN/SFN-O, SM (wyniki osób następnie operowanych wskazane w nawiasach)*

1. FNA	2. FNA	3. FNA	4. FNA	5. FNA	6. FNA
FLUS — 266	ND-36	ND-5 (1)	ND-1		
			BL-2	ND-1	
		BL-12	ND-1 (1)		
			BL-2		
			FLUS-1	FLUS-1	
		FLUS-6 (1)			
		SFN-1 (1)			
		SM-2 (2)			
	Zł-163 (7)	ND-3	BL-1	BL-1	
		BL-32 (1)*	BL-8	BL-1	BL-1
			FLUS-2	BL-1	
		FLUS-8 (1)	BL-3		
	FLUS-56 (9)	BL-13	BL-3		
			FLUS-4	FLUS-1	
		FLUS-5	BL-1		
		SM-1 (1)			
	FLUS-O-2				
	SFN-4 (2)				
	SM-3 (2)				
	MN-2 (2)				
FLUS-O — 5	ND-1				
	BL-3	BL-1	FLUS-O-1	BL-1	
	SFN-O-1 (1)				
271	271 (23)	89 (8)	30 (1)	7	1
SFN — 5	ND-1	ND-1	BL-1		
	BL-2	FLUS-1			
	FLUS-2	BL-1			
		FLUS-1			
SFN-O — 9	ND-1	SFN-O-1 (1)			
	BL-3	BL-2			
	FLUS-O-2				
	SFN-O-3	SFN-O-1			
14	14	7 (1)	1		
SM-low diagnostic	BL-9 (1)				
	FLUS-2 (1)				
	SM-pap-1				
12	12 (2)				

*MN in nodules other then previous FLUS. BL — benign lesion; FLUS — follicular lesion of undetermined significance; ND — non-diagnostic; MN — malignant neoplasm; SFN — suspicious for follicular neoplasm; SM — suspicious for malignancy

Table III. *Final cytological diagnosis in repeat FNA (in the case of non-diagnostic aspiration the former FNA result was accepted)*

Tabela III. *Ostateczne rozpoznanie cytologiczne w powtórnych FNA (w przypadku wyniku niediagnostycznego przyjmowano wynik wcześniejszej FNA)*

First FNA category	Final cytological diagnosis — number (%)
FLUS — 266	ND — 13 (4.9%)
	BL — 180 (67.7%)
	FLUS/FLUS-O — 60 (22.6%)*
	SFN — 5 (1.9%)
	SM — 6 (2.2%)
	MN — 2 (0.8%)
FLUS-O — 5	ND-1 (20.0%)
	BL-3 (60.0%)
	SFN-O — 1 (20.0%)

*including 2 cases of FLUS-O. BL — benign lesion; FLUS — follicular lesion of undetermined significance; MN — malignant neoplasm; ND — non-diagnostic; SFN — suspicious for follicular neoplasm; SM — suspicious for malignancy

mulation of a more precise result). The BL diagnosis was formulated in 183 cases (67.5% of FLUS/FLUS-O nodules examined with rFNA), SFN/SFN-O — in 6 cases (2.2%), SM — also in 6 cases (2.2%), and MN in 2 cases (0.7%). In the case of 74 (27.3%) nodules FLUS/FLUS-O was sustained or rFNA did not change the diagnosis because of a lack of cellular material. All 14 (5.2%) diagnoses related to the risk of malignancy higher than that of FLUS/FLUS-O, i.e. SFN, SM, and MN, were formulated in two first rFNA (10 in the second FNA and 4 in the third FNA) (Table II). Only one of those nodules increased its volume, but the increase was less than 50%.

Postoperative histopathological examination of FLUS/FLUS-O nodules revealed 12 (5.9%) thyroid cancers: 11 (5.6%) in FLUS nodules (10 papillary cancers, 1 undifferentiated cancer) and 1 (16.7%) in FLUS-O nodule (follicular cancer of oxyphilic type) (Table IV). Additionally, in eight patients papillary cancers were revealed in other nodules: in seven cases the nodules were incidentaloma of several millimetres in diameter, and in one case the cancer was diagnosed with FNA in the other lobe of the thyroid.

In the group of 30 patients with 32 FLUS/FLUS-O nodules operated after rFNA 6 cancers were found (18.8%); all of them were diagnosed with rFNA (diagnoses of MN or SM). In the group of 162 patients operated without preceding rFNA cancers were found less frequently — in 6 out of 170 (3.5%, $p < 0.001$)

Table IV. *Results of histopathological examinations of the thyroid in relation to the category of FNA result*
Tabela IV. *Wyniki badań histopatologicznych tarczycy w zależności od kategorii wyniku FNA*

Category of FNA result (number of nodules)	Result of histopathological examination		
	Benign nodule		Malignant neoplasm
	Non-neoplastic lesions	Benign neoplasm	
FLUS (196)	164 (83.7%)	21 (10.7%)	11 (5.6%)
FLUS-O (6)	5 (83.3%)	0	1 (16.7%)
FLUS/FLUS-O (202)	169 (83.7%)	21 (10.4%)	12 (5.9%)
	190 (94.1%)		
SFN (65)	53 (81.5%)	8 (12.3%)	4 (6.2%)
SFN-O (57)	43 (75.4%)	8 (14.0%)	6 (10.5%)
SFN/SFN-O (122)	96 (78.7%)	16 (13.1%)	10 (8.2%)
	112 (91.8%)		
SM-ca pap (43)	19 (44.2%)	2 (4.7%)	22 (51.1%)[a, b] (22/36 — 61.1%)*)
SM-non-pap (13)	5 (38.5%)	1 (7.7%)	7 (53.8%)[a, b]
SM — low diagnostic (6)	5 (83.3%)	0	1 (16.7%)
SM (62)	29 (46.8%)	3 (4.8%)	30 (48.4%)
	32 (51.6%)		

*after exclusion of 7 cases of SM-pap-low risk of malignancy; [a]$p < 0.0001$ *vs.* FLUS, SFN, FLUS/FLUS-O, SFN/SFN-O; [b]$p < 0.0005$ *vs.* SFN-O. FLUS — follicular lesion of undetermined significance; FNA — fine-needle aspiration; SFN — suspicious for follicular neoplasm; SM — suspicious for malignancy

nodules. Considering both histopathological and cytological follow-up of FLUS/FLUS-O nodules, thyroid cancers were revealed in 13 out of 441 nodules (2.9%), including 12 out of 431 FLUS (2.8%) and 1 out of 10 FLUS-O (10.0%). Detailed analysis of cytological reports with FLUS/FLUS-O in conclusion showed that cancers were diagnosed in 1 out of 3 (33.3%) nodules with single grooves or intranuclear vacuoles present in aspirate, in 5 out of 90 (5.5%) nodules with anisokaryosis of TFC in the smear, and in 6 out of 109 (5.5%) nodules with monomorphic TFC with features of architectural atypia.

In SFN/SFN-O nodules thyroid cancers were found in postoperative histopathological examination in 10 out of 122 nodules (8.2%) (NS *vs.* FLUS), including 4 out of 65 (6.2%) SFN nodules (2 papillary cancers, 1 follicular cancer, 1 undifferentiated cancer) and in 6 out of 57 (10.5%) SFN-O nodules (1 papillary cancer and 5 follicular cancers, 2 of them of oxyphilic type) (Table IV). The result did not indicate higher risk of malignancy (SM or MN) in any of the rFNA of SFN/SFN-O nodules (Table II). Only one patient was operated after rFNA (which confirmed SFN-O), and in histopathological examination the hyperplastic nodule was found. Considering nodules followed up histopathologically or cytologically, thyroid cancers were found in 7.4% of cases — more frequently than in the FLUS/FLUS-O group ($p < 0.05$), in 4 out of 70 (5.7%) SFN nodules, and in 6 out of 65 (9.2%) SFN-O nodules.

In the group of SM nodules thyroid cancers were revealed histopathologically in 30 out of 62 (48.4%) nodules, with similar frequency in SM-pap nodules — 51.1% (22 out of 43 nodules, papillary cancers only) and SM-non-pap nodules — 53.8% (7 out of 13 nodules, 3 papillary cancers, 1 medullary cancer, 1 adenoid cystic carcinoma, 2 malignant neoplasms of unclear histogenesis). No thyroid cancer was found in any of the 7 nodules of the SM-pap subgroup, in case of which the cytological report contained a commentary indicating low cancer probability; after exclusion of those nodules the incidence of malignancy in the SM-pap subgroup was 61.1% (22 out of 36 nodules) (Table IV). In both mentioned SM subgroups (SM-pap and SM-non-pap) the incidence of cancer was higher than in the FLUS/FLUS-O and SFN/SFN-O group ($p < 0.0001$ in both cases). In the SM-low diagnostic subgroup cancer (papillary) was found in 1 out of 6 (16.7%) nodules; 2 patients in that subgroup were operated after rFNA, the result of which corresponded to BL and FLUS, and histopathological examination confirmed benign lesion. rFNA was also performed in 12 non-operated patients — of SM-low diagnostic subgroup only: in one patient the result implying

Table V. *Characteristics of ultrasonographic images in particular ISC categories in relation to the results of postoperative histopathological examination (the nodules without full data on ultrasonographic examination were excluded)*

Tabela V. *Charakterystyka obrazu ultrasonograficznego w poszczególnych kategoriach ISC z uwzględnieniem wyników pooperacyjnego badania histopatologicznego (wyłączono guzki z brakiem pełnych danych z badania US)*

Sonographic feature	FLUS/FLUS-O 189		SFN/SFN-O 111		SM 51		ISC 351		ISC group OR (95% CI) p value			
	B 177	M 12	B 101	M 10	B 28	M 23	B 306	M 45	Univariate analysis		Multivariate analysis	
Solid hypoechoic	88	8	63	6	10	12	161	26	1.2 (0.7–2.3)		1.2 (0.6–2.3)	
	49.7%	66.7%	62.4%	60.0%	35.7%	52.2%	52.6%	57.8%	0.517		0.624	
Taller-than-wide shape	17	3	16	2	6	6	39	11	2.2 (1.0–4.7)		1.8 (0.8–4.1)	
	9.6%	25.0%	15.8%	20.0%	21.4%	26.1%	12.7%	24.4%[a]	0.039		0.166	
Pathological vascularisation	35	4	25	2	5	3	65	9	0.8 (0.4–0.9)		0.9 (0.4–2.1)	
	19.8%	33.3%	24.8%	20.0%	17.9%	13.0%	21.2%	20.0%	0.810		0.844	
Suspicious margin	17	1	10	0	5	7	32	8	1.9 (0.8–4.3)		1.2 (0.5–3.1)	
	9.6%	8.3%	9.9%	0.0%	21.4%	30.4%	10.5%	17.8%	0.154		0.671	
Calcifications (micro or macro or rim type calcifications)	16	4	16	4	6	10	38	18	4.7 (2.4–9.4)		4.1 (1.8–9.6)	
	9.0%	33.3%[b]	15.8%	40.0%	21.4%	43.5%	12.4%	40.0%[c]	0.000		0.000	
Microcalcifications	6	1	7	1	1	5	14	7	3.8 (1.5–10.2)		1.1 (0.3–3.6)	
	3.4%	8.3%	6.9%	10.0%	3.6%	21.7%	4.6%	15.6%[d]	0.006		0.882	
									SEN	SPC	PPV	NPV
> 1 UMRFs	44	5	37	5	6	10	87	20	44.4	71.6	18.7	89.8
	24.9%	41.7%	36.6%	50.0%	21.4%	43.5%	28.4%	44.4%[a]				
Taller-than-wide shape or calcifications	16	4	16	4	9	11	41	19	42.2	86.6	31.7	91.1
	9.0%	33.3%[b]	18.8%	40.0%	32.1%	47.8%	13.4%	42.2%[c]				
Solid hypoechoic nodule with: taller-than-wide shape or suspicious margin or microcalcifications	22	3	17	1	4	9	43	13	28.9	85.9	23.2	89.2
	12.4%	25.0%	16.8%	10.0%	14.3%	39.1%[e*]	14.1%	28.9%[a]				
Taller-than-wide shape or suspicious margin or calcifications	41	5	30	5	12	13	83	23	51.12	72.9	21.7	91.0
	23.2%	41.7%	29.7%	50.0%	42.9%	56.6%	27.1%	51.1%[d]				

[a]$p < 0.05$ M *vs.* B in ISC group; [b]$p < 0.05$ M *vs.* B in FLUS/FLUS-O group; [c]$p < 0.0001$ M *vs.* B in ISC group; [d]$p < 0.005$ M *vs.* B in ISC group; [e]$p < 0.0886$ M *vs.* B in SM group (*when calcifications were considered $p < 0.05$). FLUS — follicular lesion of undetermined significance; ISC — indeterminate, suspicious cytology; NPV — negative predictive value; PPV — positive predictive value; SEN — sensitivity; SFN — suspicious for follicular neoplasm; SM — suspicious for malignancy; SPEC — specificity

higher risk —SM-pap (the patient has not been operated yet) (Table II). Considering nodules followed up histopathologically or cytologically, thyroid cancers were diagnosed in 2 out of 16 (12.5%) SM-low diagnostic nodules.

No significant differences in the size of nodules were found between benign lesions and cancers in particular subgroups of ISC nodules (mean volume ± SD in all ISC — 6.4 ± 12.2 *vs.* 5.0 ± 4.8 cm^3), and in each subgroup the mean volume of cancers was slightly lower than that of benign nodules. No significant differences were found in the mean age of the patients with cancers and benign nodules (mean age ± SD of the ISC group for cancers and benign nodules: 53.5 ± ± 16.2 *vs.* 53.0 ± 13.5 years, respectively). Noticeable differences were observed only in the SFN/SFN-O group (respectively, 47.7 ± 17.7 *vs.* 53.0 ± 13.5 years, NS). The percentages of males with cancer and benign nodule were also similar in ISC nodules: 13.3% *vs.* 11.1%, and the most visible differences were in the SFN/SFN-O group: 20.0% *vs.* 12.9% (NS).

Table V shows ultrasonographic characteristics data of the nodules of examined ISC subgroups in relation to the category of final histopathological diagnosis. Multivariate analysis of regression indicated that the only independent factor pointing to malignancy in an

ISC nodule was the presence of calcifications (OR 4.1). That feature was present in 33.3% to 43.5% of cancers in particular ISC subgroups and was the only independent malignancy risk factor in the FLUS/FLUS-O subgroup (OR 8.5, 95% CI 1.7–42.4, $p < 0.01$). Univariate analysis of regression suggested that suspicious shape (OR 2.2) and microcalcifications (OR 3.8) might also be useful predictors of malignancy. However, the detailed analysis in the subgroups indicated their low SEN (suspicious shape — 20–26% in the particular subgroups, microcalcifications — 21.7% in SM subgroup, and in FLUS/FLUS-O and SFN/SFN-O subgroups — not exceeding 10%). The presence of suspicious margins was rare in the cancers in the FLUS/FLUS-O and SFN/SFN-O groups (< 5%), and in the SM group it reached 30%, but this feature was also present in 21.4% of benign nodules in that subgroup. Hypoechogenicity was observed with similar frequency in cancers and benign nodules of the SFN/SFN-O group (60.0% *vs.* 62.4%). In the other subgroups that feature dominated in cancers but without reaching the border of significance. Internal vascularisation had no diagnostic meaning in any ISC subgroup, and in the SFN/SFN-O and SM subgroups it was even less frequent in cancers than in benign lesions.

Analysis of the presence of at least two of any UMRFs (which was also a significant factor according to the univariate logistic regression analysis: OR 2.0, 95% CI 1.1–3.8, $p < 0.05$) did not significantly increase SEN in comparison to the evaluation of calcifications only (Table V). The limitation of the features in the analysed set to calcifications and suspicious shape only (OR 4.7, 95% CI 2.4–9.3, $p < 0.0001$) increased PPV, but not SEN. When the set of features recommended by the ATA (hypoechoic nodule with suspicious shape or suspicious margins or microcalcifications) (OR 1.8, 95% CI 1.3–2.6, $p < 0.0001$) was applied the SEN dropped to 28.9% because of its insufficiency in the SFN/SFN-O (SEN 10%) and FLUS/FLUS-O (SEN 25%) subgroups. Better results could be obtained with the set of features based on the presence of any single feature from those recommended by the ATA independently on echogenicity of the nodule and including all calcifications and not only microcalcifications (OR 2.8, 95% CI 1.5–5.3, $p < 0.001$) (SEN for FLUS/FLUS-O — 41.7%, for SFN/SFN-O 50%, and for SM — 56.6%).

Table VI shows a comparison of the FLUS/FLUS-O nodules' volume and the incidence of UMRFs in those nodules in relation to the applied procedures after first FNA, i.e. rFNA *vs.* surgery. It was found that the mean volume of FLUS nodules operated directly after first FNA was higher than that of the nodules subjected to rFNA (6.8 ± 12.8 *vs.* 3.9 ± 11.6 cm^3, $p < 0.05$). On the other hand, the nodules subjected to rFNA more frequently showed calcifications (18.5% *vs.* 7.6%, $p < 0.005$). The incidence of other UMRFs was similar. The mean age of the patients with FLUS nodule treated surgically was lower than that of the patients subjected to rFNA (51.2 ± 12.4 *vs.* 60.0 ± 12.8, $p < 0.0001$).

Table VI. *Characteristics of ultrasonographic image of FLUS nodules subjected to repeat FNA or to surgical treatment without control cytological examination*

Tabela VI. *Charakterystyka obrazu ultrasonograficznego guzków FLUS poddawanych powtórnym FNA i kierowanych na leczenie operacyjne bez kontrolnej cytologii*

Sonographic feature	Decision after first FNA (number/% of nodules)	
	Repeat FNA	Surgery
	271	157*
Solid hypoechoic	139	76
	51.3%	48.4%
Taller-than-wide shape	37	15
	13.7%	9.6%
Pathological vascularisation	45	29
	16.6%	18.5%
Suspicious margin	36	12
	13.3%	7.6%
Calcifications	50	12
	18.5%	7.6%[a]
Microcalcifications	18	4
	6.6%	2.5%
> 1 URFs	80	36
	29.5%	22.9%
Mean volume ± SD	3.9 ± 11.6	6.8 ± 12.8[b]

*the nodules without full data on ultrasonographic examination were excluded; [a]$p < 0.005$ surgery *vs.* Rfna; [b]$p < 0.05$ surgery *vs.* rFNA. FNA — fine-needle aspiration

Discussion

Analysis of the clinical significance of equivocal results of fine-needle aspiration biopsy of the thyroid is subjected to several objective difficulties. The results of such analyses are variable also because they markedly depend on the methodological factors. The mentioned difficulties and factors modify the frequency of particular subgroups among ISC nodules and the risk of malignancy related to each subgroup. They also make the data from various centres hardly comparable. The objective difficulties include epidemiological differences between examined populations, usually occurring due to different iodine supply. It modifies a priori risk of malignancy in thyroid nodules and changes the relative frequency of papillary and follicular cancers [18]. In consequence,

it influences the distribution of the subcategories of ISC nodules and affects values of variables describing FNA effectiveness, that are the lower, the higher is the percentage of FL nodules (because of the limitations of FNA in distinguishing their types) is higher [1, 17]. Another difficulty is the heterogeneity of FLUS category. These diagnoses are formulated usually by exclusion of other diagnostic categories and are not a consequence of the presence of specific features in a smear [4–5, 19]. Thus, they are more prone to interobserver discrepancies [13, 20–21]. A particularly controversial problem is the degree of nuclear atypia (polymorphism) that allows the categorisation of aspirate as FLUS and not SM. Some authors even distinguish the separate category for smears with abnormalities in cellular nuclei, such as the presence of occasional nuclear grooves, an abnormal chromatin pattern, or nuclear overlapping and crowding [7–11, 22]. These findings concern the presence of a papillary thyroid carcinoma. Choi et al. (2014) named that subgroup – "AUS - atypia with undetermined significance"; Ho et al. applied the term "AUS/FLUS – cannot rule out PTC" [11, 23]. Rosario defined AUS as cytology demonstrating nuclear atypia, but not diagnostic of suspicious for malignancy or malignant tumour [12]. The next subgroup is intended to include smears that showed predominantly microfollicular pattern with low cellularity and no or minimal colloid – this subgroup is proposed to be still referred to as "FLUS" [11, 12, 22]. These should be follicular nodules in which a distinction between follicular neoplasm and a hypercellular hyperplastic nodule is not possible. Our material included several cases of FLUS that could satisfy the criteria for AUS. It also included a dozen low-cellularity smears with single nuclear features of papillary cancer for which the pathologist formulated the recommendation to repeat FNA in a short time (earlier than recommended for FLUS nodules) and did not supply any additional conclusion. Those smears showed characteristics between SM and ND categories and in consequence were classified in the analysis as SM-low diagnostic. Because of the aforementioned factors the FLUS diagnosis constitutes, according to various authors, between 1% up to nearly 20% of all results [9, 19, 24–34]. In our material these diagnoses are formulated in 4–5% of the patients each year, i.e. with the frequency assumed by the creators of this diagnostic category. As was shown in our earlier study, the introduction of FLUS diagnosis did not cause any significant change in the total number of the results corresponding to FL, but it decreased the frequency of SFN diagnosis [17].

Other problems related to analysis of clinical significance of ISC results are the consequence of drawing conclusions on the risk of malignancy in a nodule directly from the incidence of cancer in postoperative histopathological examination, as is done by some authors. In such circumstances the error is greater, the less obvious indications are for surgical treatment connected to the analysed FNA diagnosis category. In the case of nodules with lower risk of malignancy the surgical treatment is mainly performed in patients with clinical risk factors for malignancy. It can be rationally assumed that in non-operated nodules the actual risk of cancer is lower, but it is hardly assessable on the basis of clinical follow-up only. The other difficulty that limits possibilities of comparison of the published data is various reference levels of the diagnostic centres. Some reports come from consultation centres that deal mainly with especially suspicious and difficult for interpretation nodules, which can falsely increase the observed incidence of cancers in a diagnostic category. Also of high importance is the lack of precise information on exclusion from the analysis of the cancers revealed in nodules other than those examined cytologically — such cancers are usually incidentalomas. Sometimes such exclusion is difficult to perform, when there are numerous nodules in the thyroid and it is difficult to precisely identify in which of the nodules described in an ultrasonographic report the cancer has been diagnosed. As a consequence of the above-mentioned factors the risk of malignancy in FLUS nodules as assessed on the basis of postoperative histopathological examination ranges from 4% up to as much as 50% [12, 22–28, 30–41], so it exceeds by several times the values suggested by NCI recommendations. Many authors indicate that the final malignancy results were observed several times more frequently in AUS than in FLUS [7–14]. In our material, in 1 of 3 cases of FLUS with features of nuclear atypia a papillary cancer was found in postoperative examination. Such low numbers make it difficult to draw any conclusions on the risk of cancer in such cases. However, the low frequency of such aspirates in our material is noticeable, especially in comparison with some reports describing 10-fold higher frequencies [24, 26]. It can be explained by epidemiological circumstances and still high incidence of non-neoplastic thyroid nodules in our patients. But it can also be a consequence of more conservative attitude to the rules for formulation of FLUS diagnosis, which was limited to nodules from the boundary between follicular neoplasms and benign lesions. As a result, the percentage of cancers revealed in FLUS nodules is low at our centre.

As a consequence of different frequencies of cancers diagnosed in FLUS nodules there are variable recommendations on optimal procedures in the case of FLUS. Our data give a rationale for performing rFNA. According to our analysis, it allows more precise diagnosis in more than 70% of cases (and about 64% after first rFNA). Similar data on the efficacy of repeat FNA were shown by Sullivan et al., who

reported that repeat FNA reclassified 56% of AUS/FLUS cases into a definitive category [42]. It should be mentioned that rFNA allowed malignant nodules to be revealed in our material. Similarly, Faquin and Baloch showed higher frequency of revealing cancer in a group of patients operated after repeat FNA than in patients operated directly after obtaining FLUS diagnosis [26]. There are, however, conflicting reports [23, 30, 32].

According to various studies, including our data, in the case of 30% of FLUS nodules rFNA corresponds to the same category again or it does not bring diagnostic material [23, 26, 30, 39–40]. In some other analyses this percentage reaches half of the cases [12, 43]. Some authors believe that the risk of malignancy in nodules with double FLUS diagnosis is higher than in nodules with single FLUS result [12, 39, 42]. Our data did not confirm the increased risk of malignancy in such cases, but the AUS subcategory was not assigned in any of them. It is still debatable how to deal with two FLUS diagnoses. It seems reasonable to think primarily of surgical treatment if the cytological picture corresponds to the AUS subcategory. An additional factor speaking in favour of thyroidectomy may be a suspicious ultrasonographic image of the nodule [12, 43], but some authors did not confirm such conclusions [44]. Currently, molecular studies may be proposed to resolve diagnostic doubts related to ISC nodules (as a replacement for the repeat cytology or as an additional tool), but the results of such methods are still unsatisfactory [15] and the availability of such techniques is limited.

There are also controversies relating to the situation when the result of rFNA corresponds to a benign lesion. In our material there was no cancer in such patients. Similarly, Faquin and Baloch reported that no case was finally confirmed as malignancy among nodules with initial FLUS cytology result and benign result in repeat FNA [26]. Other authors did not observe an increased risk of cancer in such cases, and they concluded that clinical follow-up instead of surgical excision or continuous repeat FNA may be enough for benign thyroid nodules after FLUS [45]. On the other hand, it was reported in some studies that malignancy risk was higher in patients with benign cytology results after an initial ISC or AUS/FLUS when compared with single benign diagnoses [42, 46]. Undoubtedly this problem needs further investigation.

Some authors indicate that the frequency of cancer is higher in oxyphilic type of FLUS [47]. Others do not support such observations [13–14]. In our material FLUS-O diagnoses were rarely formulated (2.5% of all FLUS), and only six nodules were verified with histopathological examination that revealed one cancer (16.7%). It is difficult to draw any conclusions on increased risk of malignancy from such data. Interestingly, SFN-O diagnoses constituted more than a half of all SFN cases. In that case the incidence of cancer in histopathological examination was 10.5% for SFN-O and 6.2% for SFN, but the difference was insignificant.

Another problem is related to differences in malignancy risk between FLUS and SFN categories. Faquin and Baloch found that risk as assessed by histopathological examination to be similar for both those diagnoses [26], and as reported by Theoharis et al. the incidence of cancers was higher in the FLUS group — 48% than in the SFN group — 34% [24]. Other authors indicate that the FLUS category — according to the assumptions – should select from FL the nodules with lower risk of malignancy: Wu et al. — incidence of cancer in FLUS nodules — 6%, SFN — 22%, Bongiovanni et al. — 14.4% and 32.1%, respectively, Iskander et al. — 13% and 28%, respectively [25, 31, 36]. In our material the difference in the incidence of cancer in postoperative examination of FLUS and SFN nodules was smaller (5.9% *vs.* 8.2%), but if non-operated nodules had been also considered, the risk of malignancy in FLUS nodules would have been three times lower than that in SFN nodules (2.4% and 7.4%, respectively). Considering these data it can be stated that in our centre FLUS category satisfied expectations for selection of low malignancy risk nodules from all follicular lesions.

The last question to discuss is the usefulness of UMRFs analysis in making clinical decisions in patients with FLUS nodules. In our previous study we reported that FLUS nodules showed US features of intermediate values between BL and SFN nodules [16]. In the present study the frequency of particular UMRFs in ISC nodules was evaluated in relation to the final histopathological examination. We found that the only independent feature of malignancy in FLUS nodules was the presence of calcifications of any type. In all ISC nodules, apart from calcifications of all types, suspicious shape and microcalcifications were also significant features. However, the application of the criteria based on the set of those features (calcifications or suspicious shape) or the presence of at least two UMRFs or the features proposed by the ATA as a criterion of high risk of malignancy, shows low SEN in ISC nodules. More than half of cancers were found in FLUS nodules that did not satisfy those criteria. Cuhaci et al. also found that ultrasonographic features alone may be insufficient to predict the malignancy of FLUS nodules [22]. According to their data the predictive features of malignancy are hypoechogenicity and peripheral vascularisation of the nodule. Similarly, Iskandar et al. did not confirm the usefulness of ultrasonographic features in predicting malignancy of

FLUS nodule. They did not find gender nor nodule size to be useful either, and only age < 30 years was associated with an increased risk of malignancy of FLUS [36]. In our study gender, age, and nodule size did not prove to be useful in the prediction of malignancy of ISC nodules, and especially FLUS nodules. It should be mentioned that there are also conflicting reports on the role of UMRFs in patients with ISC. Yoo et al. showed that independent features speaking in favour of malignancy of FLUS nodule included taller-than-wide shape, marked hypoechogenicity, and ill-defined margin [48]. According to their analysis two former features showed very high PPV, and the authors concluded that those features should be regarded as highly suspicious US findings that should suggest surgical treatment instead of repeat cytology. Rosario et al. also reported that analysis of UMRFs was important for making therapeutic decisions in patients with FLUS nodules [12]. Summing up, the data on the usefulness of UMRF assessment in making clinical decisions on FLUS nodules are still unsatisfactory. This could be a consequence of the diversity of the examined populations, different frequencies of particular types of thyroid cancers in the examined groups, different size of these groups, and various proportions of benign and malignant nodules. The large meta-analysis by Remonti et al. [49] was an attempt to summarise analyses published so far. The authors drew the conclusion that in the subgroup of nodules with indeterminate cytology any of the US features was sufficient to determinate the risk of malignancy with an acceptable SEN [49]. It seems that such a view is shared by the majority of clinicians because, as indicated by our data and other published reports, they tended to refer for surgical treatment younger patients or those with larger nodules, but not those with suspicious US image [23, 50].

Conclusions

It should be stressed that the attempt to unify the method of diagnosis formulation in the case of follicular lesions of the thyroid has not been successful. Such consistent diagnoses are a key demand for making studies from different centres comparable. They are also necessary in order to elaborate common recommendations. Currently, it is still important to analyse one's own experience in relation to the risk of cancer in ISC nodules. At our centre the risk of malignancy in FLUS and SFN nodules is visibly lower than in SM nodules. The FLUS category allows the selection from ISC of the nodules with lower risk of malignancy, which should be followed by rFNA. The repeat FNA of FLUS nodules allows the formulation of more precise diagnosis in more than 70% of cases and is effective in revealing cancers. Analysis of UMRFs has a variable diagnostic value depending on the ISC subcategory. It may be an additional aid for making therapeutic decisions, but sole evaluation of UMRFs is insufficient for drawing clinical conclusions.

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