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The usefulness of determining the presence of BRAF ^{V600E} mutation in fine-needle aspiration cytology in indeterminate cytological results

Przydatność określania obecności mutacji BRAF ^{V600E} w biopsji aspiracyjnej celowanej cienkoigłowej w zmianach niezdeterminowanych

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Abstract

Introduction: Fine-needle aspiration biopsy (FNAB) is regarded as the gold standard method for the diagnosis of thyroid nodules, but it has its limitations. Additional methods that would improve sensitivity and specificity in the diagnosis of thyroid cancer (TC), especially in indeterminate lesions. Molecular tests seem to be such a tool. *BRAF* ^{V600E} mutation (the most common in TC) can be detected in FNAB and can be potentially a very useful ancillary marker for FNAB practice.
The aim of our study was to evaluate the usefulness of the detection of the *BRAF* ^{V600E} mutation in FNAC in the early diagnosis of TC in patients with indeterminate cytology.
Material and method: 2290 FNAB were performed and 147 indeterminate results (group 3, 4, and 5 of the Bethesda system) were obtained. Material from these groups was submitted for molecular tests for the occurrence of *BRAF* ^{V600E} mutation. Sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of the tests were calculated.
Results: Determining the presence of *BRAF* ^{V600E} mutation in FNAC material in groups 3 and 4 together and in group 5 is associated with sensitivity of TC diagnosis of 37.5% and 81.8%, respectively. In all cases the detection of *BRAF* ^{V600E} mutation was associated with histopathologically proving the presence of TC (specificity of the test — 100%).
Conclusions:
1. The presence of *BRAF* ^{V600E} mutation in FNAC material is always associated with the presence of TC.
2. The usefulness of determining the presence of *BRAF* ^{V600E} in FNAC in cytological groups 3 and 4 is associated with low sensitivity in the diagnosis of thyroid cancer.
3. Due to its high specificity *BRAF* ^{V600E} study may be useful in determining the scope of surgery in patients in cytological group 5.
(Endokrynol Pol 2016; 67 (1): 41–47)

Key words: *BRAF V600E; thyroid cancer; FNAC*

Streszczenie

Wstęp: Biopsja aspiracyjna celowana cienkoigłowa (BACC) jest uznawana za złoty standard w diagnostyce guzków tarczycy. Ma ona jednak swoje ograniczenia. Poszukiwane są więc dodatkowe metody, które poprawiłyby czułość i specyficzność diagnozowania raka tarczycy, zwłaszcza w przypadku zmian niezdeterminowanych w BACC. Badania molekularne wydają się być takim narzędziem. Mutacja *BRAF* ^{V600E} (najczęstsza w raku tarczycy) może być wykrywana w materiale z biopsji i może wspomagać BACC w rozpoznawaniu raka tarczycy.
Celem pracy była ocena przydatności wykrywania mutacji *BRAF* ^{V600E} w BACC w zmianach niezdeterminowanych we wczesnej diagnostyce pacjentów ze zmianami ogniskowymi w tarczycy.
Materiał i metody: Przeprowadzono 2290 BACC, uzyskując w 147 próbkach wyniki niezdeterminowane (grupy 3, 4 i 5 wg klasyfikacji Bethesda). W grupie tej przeprowadzono badania molekularne w kierunku występowania mutacji *BRAF* ^{V600E}. Obliczono czułość, swoistość, wartość predykcyjną dodatnią, wartość predykcyjną ujemną i dokładność testu.
Wyniki: Obecność mutacji *BRAF* ^{V600E} w grupach cytologicznych 3 i 4 łącznie oraz w grupie 5 wiązała się z czułością w rozpoznawaniu raka tarczycy odpowiednio 37,5% i 81,8%. W każdym przypadku wykrycia mutacji *BRAF* ^{V600E} w badaniu pooperacyjnym rozpoznano raka tarczycy (specyficzność testu —100%).

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Wnioski:
1. Obecność mutacji *BRAF* ^{V600E} w materiale BACC jest zawsze związana z obecnością RT.
2. Przydatność określenia obecności *BRAF* ^{V600E} w BACC w grupach cytologicznych 3 i 4 jest związana z niską czułością rozpoznania RT.
3. Ze względu na wysoką specyficzność *BRAF* ^{V600E} badania mogą być przydatne w określaniu zakresu operacji u pacjentów z grupy cytologicznej 5. **(Endokrynol Pol 2016; 67 (1): 41–47)**

Słowa kluczowe: *BRAF V600E; rak tarczycy; BACC*

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Introduction

The incidence of thyroid nodules detected by ultrasonography (US) exceeds 50% in the adult population [1]. Most of them are benign and only 1–5% turn out to be thyroid cancer [2]. The incidence of thyroid cancer is increasing globally, as well as in Poland where it has quintupled over the past 20 years [3]. Fine-needle aspiration biopsy (FNAB) is regarded as the gold standard method for the diagnosis of thyroid nodules (TN), but it has its limitations — for example, inadequate specimens or indeterminate results. This occurs in approximately 15–30% of cases [4]. Consequently there is increasing demand for an accurate initial diagnosis of TN to avoid unnecessary operation and to correctly and quickly qualify the patient for the operation. More than 80% of TCs are papillary thyroid cancers (PTC) [5]. A point mutation of *BRAF* ^{V600E} results in consecutive activation of serine/threonine kinase RAF type B and the whole signalling pathway of MAPK (MAPK kinase), which is important in the process of oncogenesis in the development of TC. The *BRAF* ^{V600E} mutation frequency ranges from 30 to 80% of cases of PTC [2, 6]. *BRAF* ^{V600E} mutation can be detected in FNAC, and potentially can be a very useful ancillary marker for FNAC practice.

The aim of our study was to evaluate the usefulness of the detection of the *BRAF* ^{V600E} mutation in FNAC in the early diagnosis of TC in patients with indeterminate cytology (Bethesda groups 3, 4, and 5) in a population with high prevalence of *BRAF* ^{V600E} mutation in TC.

Material and methods

Patients

1590 patients were examined (1375 females and 215 males; mean age 51.6 ± 12.6 years) from October 2013 to October 2014, and there were 2290 FNABs performed. Patients were qualified for biopsy from the Endocrinology Outpatient Clinic in Holycross Cancer Centre, Kielce, Poland according to "AACE/AME/ETA medical guidelines for clinical practice and management of thyroid nodules" [7] and according to "Diagnosis and treatment of thyroid cancer — Polish guidelines" [8]. The smears were evaluated according to Bethesda Thyroid Cytology Classification (Bethesda system). Molecular testing aimed at the identification of *BRAF* ^{V600E} mutation was performed in the patients with cytological group 3, 4, and 5. Written, informed consent was obtained from each patient prior to the study.

Fine-needle aspiration biopsy (FNAB)

Fine-needle aspiration biopsy was obtained by a pathologist under ultrasonographic (US) guidance of a radiologist. Either the radiologist or the pathologist was certified to perform the thyroid biopsies. Neck ultrasonography was performed with the use of devices featuring a colour Doppler function: Siemens Versa pro and Hitachi EUB-6500, with a high frequency linear probe (7.5 MHz). The smears were obtained by using 27-gauge needles fitted to a 2-mL syringe. In each case, two smears were prepared and fixed in 86% ethanol and stained with H-E stain. In our institution a minimum of six clusters of 10 epithelial cells must be found on two smears, based on two separate aspirations, for the specimen to be considered satisfactory. These smears were evaluated according to the Bethesda system. Most of the material (about two thirds) from the biopsy needle was used for cytological examination. The remaining material was stored frozen for potential further molecular examination. Only patients with Bethesda cytological results in groups 3, 4, and 5 were submitted to molecular tests for occurrence of *BRAF* ^{V600E} mutation.

DNA isolation

The material for the isolation of DNA was washed from the needle after the FNAC obtained with 500 ul of the phosphate buffer. DNA was isolated using the Micro Genomic Blood AX Gravity (A & A Biotechnology, Gdańsk, Poland) according to the manufacturer's instructions. For cytological smears DNA isolation was performed using a DNA FFPE Kit (Qiagen) according to the manufacturer's instructions.

Test used for detection of BRAF V600E mutation

The following tests were used to detect *BRAF* ^{V600E} mutation: Sanger sequencing with sensitivity of 10–20% of mutated alleles in combination with allele-specific PCR (ASA-PCR) with a sensitivity of 5% of mutated alleles (from 1.10.2013 to 31.03.2014) and qPCR (from

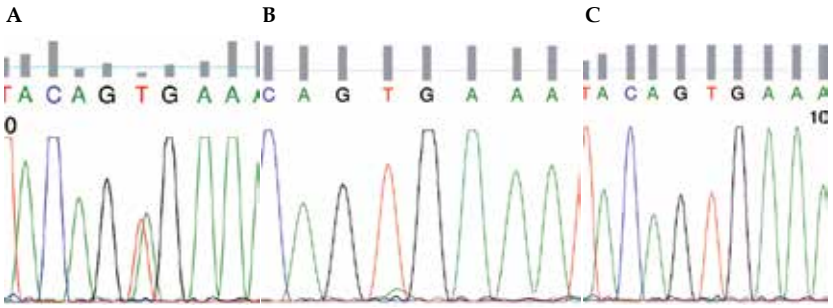

Figure 1. *Examples of the results of Sanger Sequencing:* **A.** *The BRAF V600E mutation;* **B.** *Inconclusive; C. Wild type — WT*
Rycina 1. *Przykłady wyników sekwencjonowania Sangerowskiego:* **A.** *Mutacja BRAF V600E;* **B.** *Wynik niejednoznaczny;* **C.** *WT*

1.04.2014 to 30.09.2014) with a sensitivity of about 1%. Finally each genetic sample was examined with a highly sensitive method. The change of method during the study was caused by the diagnostic capabilities of our genetics department.

Details concerning molecular techniques have previously been described in detail elsewhere [9, 10]

Sequencing

We used the following PCR primers: *BRAF*ek15f 5′-TCATAATGCTTGCTCTGATAGGA-3′ and *BRAF*ek15r 5′-GGCCAAAAATTTAATCAGTGGA-3′ for the amplification of 224 bp of DNA segment containing codon 600 of *BRAF*. Sequencing was performed using ABI 3130 Automatic Capillary DNA Sequencer and BigDye Terminator v1.1 Cycle Sequencing kit (Life Technologies, Warsaw, Poland) according to the manufacturer's instruction (Fig. 1 A–C).

Allele–specific PCR

Allele–specific PCR was performed using the *BRAF*ek15f, *BRAF*ek15r, and a p.V600E mutation–specific primer and (*BRAF*ek15mutA 5′-GGTGATTTTGGTCTAGCTACAGA-3′) primers. The *BRAF*ek15f and *BRAF*ek15r create a 224bp control band, and *BRAF*ek15mutA with *BRAF*15r create a mutation band of 126bp. When the control band was not visible, the results were uninterpretable (Fig. 2).

qPCR

A qPCR assay targeting 68 bp of *BRAF* exon 15 was performed using Rotor-Gene Q (Qiagen, Syngen-Biotech, Poland) with the following probes: *BRAF*mut (6FAM-CTACAGAGAAATC-MG-BNFQ), *BRAF*-WT (VIC-CTACAGTGAAATC-MGB-NFQ) and primers: forward 5′ agacctcacagtaaaaataggtgattttgg 3′ and reverse 5′ gatgggacccactccatcg 3′ (Fig. 3).

Statistical analysis

In order to define clinically performed molecular tests in the group of patients with indeterminate cytology (Bethesda group 3, 4, 5) the obtained results were defined as: 1. true positive (TP) — the presence of *BRAF* ^{V600E} mutation in FNAB and post-operation confirmed thyroid cancer; 2. true negative (TN) — neither *BRAF* ^{V600E} mutation found nor evidence of malignancy in post-operative histopathology nor the lack of a malicious nature of the changes found in the patients who were not operated on but left under active observation the observation period was from 6 months to 12 months; 3) false positive (FP) — confirmed *BRAF* ^{V600E} in the absence of confirmation of cancer in post-operative material; and 4) false negative (FN) — absence of *BRAF* ^{V600E} in FNAB and the confirmation of TC in the post-operative material. This enabled the determination of: sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of the used method.

For statistical analysis the results of group 3 and 4 were combined because the diagnosis of the risk of cancer occurrence is lower than in the fifth group. In addition, further proceedings in groups 3–4 are similar: either observation can be considered or diagnostics operation (usually to remove the lobe with isthmus). However, with cytological diagnosis in group 5 — the treatment of choice is surgery of the total (total thyroidectomy) or partial removal of the thyroid gland (subtotal thyroidectomy).

The obtained results (sensitivity, specificity, positive predictive value PPV, negative predictive value NPV, and accuracy) were given in percentages and its 95% confidence intervals (CI) were calculated. The software used for calculations was MEDCALC (www.medcalc.org/calc/diagnostic_test.php)

Results

2290 cytological smears were obtained from 1590 consecutive patients. Obtained cytological results were classified according to Bethesda system. In cytological group: 1 (nondiagnostic or unsatisfactory) there were 163 (7.12%) smears; 2 benign — 1965 (85.8%), 3 — AUS/

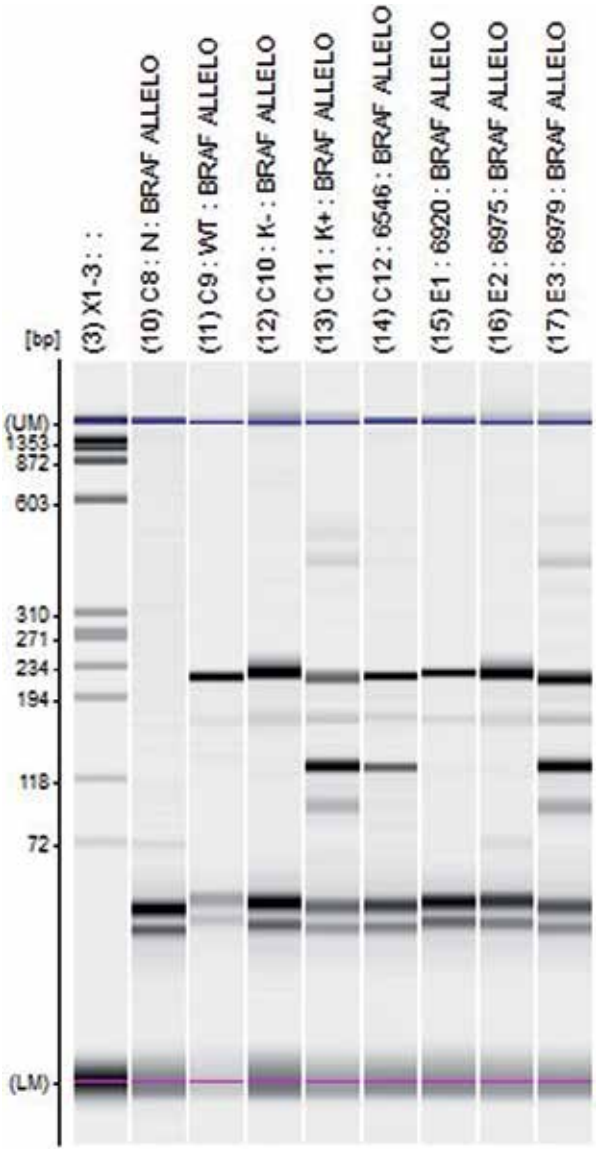

Figure 2. *Genotyping BRAF V600E mutation using ASA-PCR method. Visualisation by chip electrophoresis Multina (Shimadzu, Japan). X1 — weight marker, C8 — no template control, C9 — negative control — sample without the BRAF V600E mutation — WT, C10 — negative control sample without mutation, C11 — control sample with BRAF V600E mutation; C12, E1, E2 and E3 tested samples. 224pz - reaction control band, 126pz — band, BRAF V600E mutation*

Rycina 2. *Genotypowanie mutacji BRAF V600E za pomocą metody ASA-PCR. Wizualizacja za pomocą elektroforezy chipowej Multina (Shimadzu, Japan). X1 — marker masy, C8 — kontrola bez matrycy, C9 — kontrola, próbka bez mutacji BRAF V600E, C10 — próbka kontrolna bez mutacji, C11 — próbka kontrolna z mutacją BRAF V600E; C12, E1, E2 i E3 próbki badane. 224pz — prążek kontrolny reakcji, 126pz — prążek świadczący o obecności mutacji BRAF V600E*

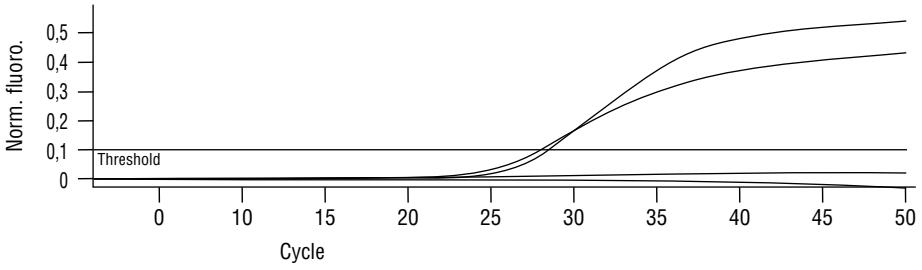

Figure 3. *Genotyping BRAF V600E mutation using qPCR. black colour — no template control sample, the green colour — the control sample without the BRAF V600E mutation, red — the control sample with mutations in the BRAF V600E, blue colour — samples tested*

Rycina 3. *Genotypowanie mutacji BRAF V600E za pomocą metody qPCR. Kolor czarny — próbka kontrolna bez matrycy, Kolor czerwony — próbka kontrolna bez mutacji BRAF V600E, kolor czerwony — próbka kontrolna z mutacją BRAF V600E, kolor niebieski — próbki badane*

/FLUS (atypia of undetermined significance or follicular lesion of undetermined significance) — 106 (4.63%); 4 — FN/SFN (follicular neoplasm or suspicion for a follicular neoplasm) — 29 (1.27%); 5 — SFM (suspicious for malignancy) — 12 (0.5%), 6 — malignant 15 (0.66%).

147 indeterminate results were obtained (Bethesda groups 3, 4, 5). Material from these groups was submitted for molecular tests for the occurrence of *BRAF* ^{V600E} mutation. *BRAF* ^{V600E} was revealed in only 12 nodules (3 in cytological group 3 and 9 in cytological group 5).

In cytological group 3 (AUS/FLUS) there were 106 smears: 103 with wild type and 3 with *BRAF* ^{V600E} mutation. Out of the 103 patients without the *BRAF* ^{V600E} mutation 62 were operated on, and in post-operative pathological examination nodular goitre (NG) was revealed in 59 patients, in 2 FTC, and PTC in 1. PTC, however, was an accidental discovery: a papillary micro carcinoma of 4 mm dimension was found in a patient with a NG — a lesion that was not subjected to biopsy. All three remaining patients with *BRAF* ^{V600E} mutation were operated on. On histopathological examination, in two of them classical variant of PTC (cvPTC) and in one follicular variant of PTC (fvPTC) were revealed.

In cytological group 4 (FN/SFN) there were no *BRAF* ^{V600E} mutations revealed. Twenty out of 29 patients from this group were operated on because of the clinical status of the tumours. Postoperatively: two patients had FTC, and the remaining 18 had nodular goitre (NG).

Forty-one patients from group 3 and 9 patients from group 4 were not operated on — and left for active observation (repeated FNAC or US) — without evidence of thyroid cancer during follow-up from 6 to 12 months.

In cytological group 5 (SFM) there were 12 patients — all of them according to guidelines were operated on (diagnostic operation). Nine of them had *BRAF* ^{V600E} mutation. Postoperatively cvPTC was revealed in this group of patients. In the remaining three patients with wild type: diagnosis of fvPTC was seen in one, anaplastic thyroid carcinoma in one, and NG in one patient was established after the operation.

Fifty patients were left under active observation. In the TP group there were 12 patients (3 from group 3 and 9 from group 5). In the TN group there were 127 patients (41 not operated on and 59 operated on from group 3; 9 not operated on and 18 operated on from group 4). There were no FP patients in the examined group — all patients with preoperative diagnosis of *BRAF* ^{V600E} in FNAC had confirmed thyroid cancer postoperatively. In the FN group there were five patients (three from group 3 [2-FTC and 1 PTC] and two with FTC from group 4). All of the results are presented in Figure 4.

Based on the obtained results of sensitivity, specificity, PPV, NPV, and accuracy were calculated for the occurrence of *BRAF* ^{V600E} mutation — together for groups 3 and 4 and for group 5. The results are shown in Table I.

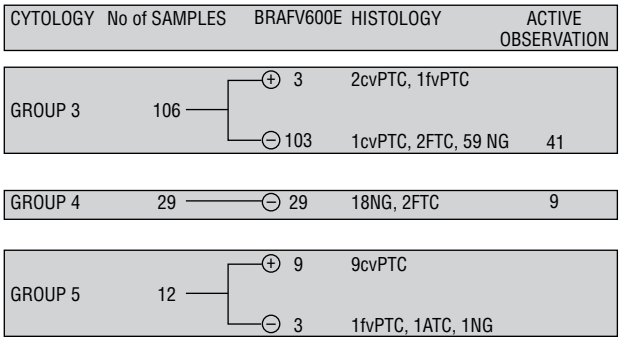

Figure 4. *Study scheme and results. cvPTC — classical variant of papillary thyroid carcinoma, fvPTC — follicular variant of papillary thyroid carcinoma, FTC — follicular thyroid carcinoma, NG — nodular goitre, ATC — anaplastic thyroid carcinoma*

Rycina 4. *Schemat badania i wyniki. cvPTC — wariant klasyczny raka brodawkowatego, fvPTC — wariant pęcherzykowy raka brodawkowatego, FTC — rak pęcherzykowy, NG — wole guzkowe, ATC — rak anaplastyczny*

Determining the presence of *BRAF* ^{V600E} mutation in FNAC material in groups 3 and 4 together and in group 5 is associated with sensitivity of TC diagnosis of 37.5% and 81.8%, respectively. In all cases the detection of *BRAF* ^{V600E} mutation was associated with histopathologically proving the presence of TC (specificity of the test — 100%).

Discussion

In the era of wide availability of US, thyroid lesions are more often recognised. It is estimated that in the adult population, more than 50% of thyroid lesions are detected by ultrasound [1]. Thyroid cancer occurs in only approx. 1–5% of the total amount of the changes shown by ultrasound examination [2]. FNAC performed under US guidance is still the best method to diagnose TC. However, it has limitations referring to, in particular, follicular lesions (cytological group 3 and 4) and lesions suspected of malignancy (cytological group 5) according to Bethesda Thyroid Cytology

Table I. ***Determining the presence of BRAF ^{V600E} mutation in fine-needle aspiration cytology***
Tabela I. ***Określenie obecności mutacji BRAF V600E w biopsji aspiracyjnej cienkoigłowej***

Cytological group	Sensitivity 95% (CI)	Specificity 95% (CI)	PPV 95% (CI)	NPV 95% (CI)	Accuracy 95% (CI)
Group 3 + 4	37.5%	100%	100%	96.2%	96.3%
	0.123–0.375	0.984–1.000	0.984–1.000	0.947–0.962	0.933–0.963
Group 5	81.8%	100%	100%	33.3%	83.3%
	0.732–0.818	0.056–1.000	0.895–1.000	0.019–0.333	0.676–0.833

CI — confidence intervals; PPV — positive predictive value; NPV — negative predictive value

Classification [4]. In the case of cytological group 3, repeated FNAC under US guidance is recommended within 3–12 months and a possible diagnostic operation is advisable. In the case of cytological group 4 diagnostic operations (usually subtotal thyroidectomy) are recommended to be considered. However, in the case of cytological group 5 a diagnostic operation — total or subtotal thyroidectomy — is recommended [8]. Unnecessary operations (benign histology) occur most frequently in patients with cytological groups 3 and 4 because of the low prevalence of TC in these groups (10–25% in group 3 and 15–30% in group 4) [11]. Additional methods that would improve sensitivity and specificity in diagnosis of TC, especially in indeterminate lesions. Molecular tests seem to be such a tool. More than 80% of thyroid cancers are PTC, and the most common genetic alteration in PTC is *BRAF* ^{V600E}. Its frequency ranges from 30 to 80% of cases of PTC — in the Polish population it occurs in 68–70 % of PTC [9, 12, 13]. Because of the high prevalence of this mutation it was feasible to use *BRAF* mutation in FNAC as a marker of TC in our study.

The prevalence of *BRAF* ^{V600E} mutation in thyroid cancer depends on different factors: geographic area, ethnicity, and iodine consumption [14, 15]. It is much higher in Korea - present in 80% of PTC [16], while it occurs only in 30–50% in Western countries [2, 15]. The prevalence of *BRAF* ^{V600E} mutation depends on the type of thyroid cancer. It occurred in about 80-100% of tall cell variant (rare variant of PTC), in 45–68% of classical variant (most frequent), and in up to 12–18% (15%) of follicular variant of PTC [17, 18]. *BRAF* ^{V600E} mutation can occasionally be present in lymphoma as well as anaplastic thyroid carcinoma.

Sensitivity markers of *BRAF* ^{V600E} mutation in material obtained from FNAB in the diagnosis of TC in different studies range from about 32% (Cohen [19]) to 82 % (Kim [20]).

The cited works differ in groups of patients evaluated with the use of molecular techniques: the proportion of PTC in the whole group TC and the way of assessing analysis of sensitivity referring to PTC or to all TC. In the Kim study the molecular tests were performed in 961 patients with a high risk of TC. Sonographically the thyroid lesions characterised as suspicious or indeterminate features. FNAC was not performed in sonographically benign lesions. As many as 181 (18.3%) FNACC results were in group 6, and postoperatively on histopathology in 180 cases of PTC [162 *BRAF* ^{V600E} (+), and among *BRAF* ^{V600E} (–): 18-PTC and in one-MTC] were revealed. This is probably the reason for such high sensitivity: 82.5% obtained in this study [20]. A high (75%) sensitivity was also obtained in detection of *BRAF* ^{V600E} mutation in the study by Jo et al. In this study there were prospectively evaluated 101 thyroid nodules with FNAB (43 benign, 30 malignant, 24 indeterminate or suspicious, 4 nondiagnostic). On final histopathological diagnosis PTC occurred in 54 patients (from cytological groups malignant and indeterminate). Among 30 of them *BRAF* ^{V600E}(+) mutation was present in 22 patients with cytological group 6 (malignant), in 7 indeterminate nodules, and in 1 from the non diagnostic cytology group [21].

Slightly lower sensitivity (63.5%) in detection of *BRAF* ^{V600E} in detecting PTC was revealed in the Zatelli study. For molecular analysis, in the study 469 thyroid lesions with sonographic features of malignancy were enrolled. Lesions were of relatively small size: 1.1 ± 0.8 cm. Only the recognition of PTC was analysed. Simultaneous determination of the presence of *BRAF* ^{V600E} mutation and FNAC increased the sensitivity of cytology for PTC from 77% to 87% [22]. In Xing's analysis the presence of *BRAF* ^{V600E} mutation was performed in patients previously submitted for operations based on clinical and sonographic features, and FNACC results obtained a sensitivity of 50% [23].

In the case of separation cytological indeterminate group (3, 4, 5) the sensitivity of the method differs significantly — mostly in cytological group 3. In the study by Lee et al. among 325 smears suspicious for malignancy (SFM) — 205 (63,1%) smears were *BRAF* (+), while in the case of AUS/FLUS only 37 smears among 353, which means 10.5% had *BRAF* (+) mutation [24].

In the presented study the sensitivity of the used method was slightly higher. The total for cytological groups 3 and 4 was 37.5%, and for group 5 it was 81.8%.

It should be emphasised that in the presented study in group 4 *BRAF* ^{V600E} mutation was not revealed in any of the cases. This can be connected with the low prevalence of PTC in this cytological category and higher prevalence of FTC that are *BRAF* ^{V600E} negative. Moreover, in all the studies it was highlighted that high positive predictive value in the detection of cancer can reach 100% [25–27]. Results from the presented study are concordant with cited reports. In all of the patients with mutation *BRAF* ^{V600E} detected in FNAC, postoperatively the diagnosis of TC was established. PPV was 100% for all the examined cytological groups.

It is highlighted that molecular tests have to be adapted to the prevalence of cancer in different cytological categories in different medical centres. So the decision to conduct a test for the presence of *BRAF* mutation — initially dictated by financial considerations and availability – seems to be right.

Detection of *BRAF* ^{V600E} mutation is associated with a specificity of 100% in the detection of cancer. Most

of the TC are PTC, of which about are *BRAF* ^{V600E} positive. These cancers are usually accurately diagnosed in cytology and are most commonly qualified to cytological group 6, and more rarely to cytological group 5 (in our centre postoperative histology reveals TC in 81% of cases). Because of the very high prevalence of TC in this cytological group it is standard procedure to carry out thyroidectomy or diagnostic lobectomy in case of a single nodule. The detection of *BRAF* ^{V600E} mutation in each case resulted in the making of the decision about the total thyroidectomy. Prevalence of cancer in cytological groups 3 and 4 is low, and additionally in these cytological groups FTC are usually present (in this type of cancer *BRAF* ^{V600E} mutation is absent), or follicular variant of PTC (with lower prevalence of *BRAF* ^{V600E} mutation), which limits the usefulness of the detection of the mutation in the diagnosis of TC. In the presented study two cases of undiagnosed TC (*BRAF* ^{V600E} negative) in groups 3 and 4 referred to FTC. In group 5 one undiagnosed TC turned out to be fvPTC (*BRAF* ^{V600E} negative) and one anaplastic cancer.

Conclusions

1. The presence of *BRAF* ^{V600E} mutation in FNAC material is always associated with the presence of TC.
2. The usefulness of determining the presence of *BRAF* ^{V600E} in FNAC in cytological groups 3 and 4 is associated with low sensitivity in diagnosis of thyroid cancer.
3. Due to its high specificity *BRAF* ^{V600E} study may be useful in determining the scope of surgery in patients in cytological group 5.

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