VILNIUS UNIVERSITY

Augustas Beiša

THE SIGNIFICANCE OF THE BRAF V600E MUTATION AND THE CYTOMORPHOLOGICAL FEATURES FOR THE OPTIMIZATION OF PAPILLARY THYROID CANCER DIAGNOSTICS

Summary of Doctoral Dissertation

Biomedical Sciences, Medicine (06 B)

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VILNIAUS UNIVERSITETAS

Augustas Beiša

BRAF V600E MUTACIJOS IR CITOMORFOLOGINIŲ POŽYMIŲ REIKŠMĖ PAPILINIO SKYDLIAUKĖS VĖŽIO DIAGNOSTIKOS OPTIMIZAVIMUI

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Abbreviations

BRAF – *B-type Rapidly Accelerated Fibrosarcoma*
FNA – fine needle aspiration biopsy
PCR – polymerase chain reaction
PTC – papillary thyroid cancer
TC – thyroid cancer
US – ultrasound
VUH SK – Vilnius University Hospital Santaros klinikos

Introduction

Thyroid nodules are detected in about 50% of adults who live in regions deficient in iodine. Although thyroid cancer is the most common oncological endocrine disorder, only 5% of the thyroid nodules are malignant. Papillary thyroid cancer (PTC) comprises 85 to 90% of cases of all histological types of thyroid cancer. Although PTC has a slow progression and good survival rates, 20% to 30% of patients have localized disease recurrences or distant metastases and about 1% of patients die from this disease.

A fine needle aspiration biopsy (FNA) performed under ultrasound (US) guidance and a cytological examination of nodules remains the gold standard for the differential diagnostics of thyroid cancer. However, due to a well-differentiated nature of most thyroid cancers, 10 to 26% of samples are ascribed to cytologically indeterminate diagnostic categories (categories III, IV, V, according to the Bethesda system). In order to determine the exact diagnosis, diagnostic surgery is usually proposed for these patients; however, a histological examination of resected tissues confirms the diagnosis of thyroid cancer in only one-third of patients. In the absence of reliable preoperative diagnostic criteria, there is a risk of general, surgical intervention-related and thyroid surgery-specific complications including temporary or permanent vocal cord paralysis and temporary or permanent decrease in blood calcium levels. After surgery, replacement therapy with thyroxine is usually required for the rest of patients' lives. Besides, psychological discomfort due to a 4 to 6 cm long scar in the neck may be experienced.

These causes lead to high socioeconomic costs and encourage to search for new options of diagnostics and prediction that could potentially optimize patient selection for surgical treatment.

Molecular genetic tests are among the most promising methods for the diagnostics of thyroid cancer and for the predictions of the disease course. The first works on the impact of *BRAF* mutations in oncological diseases were performed in studies of skin, colon and lung tumors. The

link between *BRAF* mutations and PTC was confirmed in publications in 2003. A high V400E mutation specificity for PTC was noticed at that time. Therefore, Xing et al. had proposed BRAF V600E mutation testing for presurgical PTC diagnostics already in 2004. Although many publications on the applications of BRAF V600E mutation testing for PTC diagnostics and prognostication have appeared since that time, published data and recommendations remain controversial. Since the prevalence of BRAF V600E mutation in PTC tumors ranges from 30 to 80% depending on the geographical region, it can be stated that only the detection of mutation prevalence in the population under study can determine the applicability of this testing in clinical practice. The prevalence of BRAF V600E mutations in PTC in Lithuania has not been investigated so far.

Aim of the Study

The aim of this dissertation is to evaluate the significance of the Bethesda system, the cytomorphological features, the BRAF V600E mutation and the BRAF V600E mutation combined with the cytomorphological features for the optimization of papillary thyroid cancer diagnostics. Further aims are:

1. To define indications for surgical treatment vs. an active surveillance of thyroid nodules;
2. To evaluate the possibilities of predicting signs of histological PTC aggressiveness prior to surgery.

Tasks of the Study

The tasks of the study are the following:

1. To evaluate the significance of the Bethesda system for the diagnostics of PTC;
2. To determine the prevalence of the BRAF V600E mutation in the population of Lithuanian patients with PTC;
3. To evaluate the significance of the cytomorphological features for the diagnostics of PTC in cytologically indeterminate diagnostic categories;

4. To evaluate the significance of the BRAF V600E mutation for the diagnostics of PTC in cytologically indeterminate diagnostic categories;
5. To evaluate the significance of the BRAF V600E mutation combined with the cytomorphological features for the diagnostics of PTC in cytologically indeterminate diagnostic categories;
6. To evaluate the significance of the Bethesda system for the prognostication of signs of histological PTC aggressiveness;
7. To evaluate the significance of BRAF V600E for the prognostication of signs of histological PTC aggressiveness.

Novelty of the Study

The following arguments that are laid out are in support of this study's novelty:

1. For the first time, the prevalence of the BRAF V600E mutation was determined in the population of patients with PTC in Lithuania;
2. The significance of the BRAF V600E mutation, combined with the cytomorphological features for the diagnostics of PTC in indeterminate cytological categories, was determined, and a PTC risk calculator, adapted for use in clinical practice, was developed;
3. The significance of the Bethesda system for the prognostication of signs of histological PTC aggressiveness and PTC staging was determined.

Practical Significance

This study contributes to the worldwide search for the diagnostic and prognostic biomarkers of thyroid cancer and the evaluations of applications of established biomarkers in clinical practice. More accurate diagnostics of thyroid cancer have the potential to reduce the number of diagnostic surgeries and provide indications for an active surveillance of patients. The prognostication of PTC aggressiveness and disease staging has a potential to provide means for the estimation of the extent of surgical treatment. Individualized treatments may directly affect the physical health and psychological well-being of patients and reduce socioeconomic costs.

Statements to be Defended

The following two statements stand as those to be defended by this study:

1. The BRAF V600E mutation testing, combined with the analysis of the cytomorphological features, may improve the diagnostics of PTC in cytologically indeterminate diagnostic categories;
2. Based on the Bethesda system, it is possible to predict signs of histological PTC aggressiveness.

Methodology of the Study

A permission of the Lithuanian Bioethics Committee (No. 158200-04-475-137) was obtained on April 26, 2012. The location of the study was Vilnius University Hospital Santaros Klinikos (VUH SK). Patients who applied to VUL SK and met the inclusion criteria of the study were invited to participate in this study.

Criteria for Inclusion into the Study

The inclusion of patients into the study was based on the following criteria:

1. Patients were 18-years-old or older;
2. Thyroid nodules with signs of malignancy were detected on thyroid US;
3. A US-guided FNA of thyroid nodules with signs of malignancy was performed;
4. Cytological features, classified as categories III, IV, V, VI according to the Bethesda system, were determined.

Exclusion Criteria

Patients were excluded from the study based on the following criteria:

- Pregnancy;
- Cytological features classified as categories I or II according to the Bethesda system were determined in an FNA aspirate.
- The indication for surgery was not a suspected oncological disorder.

Testing and Diagnostics

All patients with a suspected thyroid disease underwent a US scan examination in accordance with the diagnostic standards of the VUH SK. When thyroid nodules with signs of malignancy were detected on a US scan, a routine US-guided FNA of the nodule with signs of malignancy was performed. Every FNA aspirate was sent for a cytological examination (all samples were evaluated by the same investigator). For every sample, 22 cytological features were evaluated and all samples were classified according to the Bethesda System for Reporting Thyroid Cytopathology. All patients were referred for endocrinological consultation. Patients with nodular cytological features classified as categories III, IV, V, VI were also referred for surgical consultation. For all patients having indications for surgery, surgical treatment was recommended. Patients who agreed to be operated on and met the inclusion criteria were invited to participate in the study and given informed consent forms approved by the Lithuanian Bioethics Committee. Responses to any additional questions raised by patients were given in verbal by researchers of VUH SK. Upon the familiarization with study conditions and informed consent forms and agreements to participate in the study, patients signed informed consent forms. These forms were also signed and left for storage by the senior researcher. A cytology laboratory researcher was informed about all the patients who agreed to participate in the study and the residues of FNA aspirates of these patients were transferred to the genetic testing laboratory for BRAF V600E mutation testing (all samples were tested by the same investigator). Patients were admitted to the Center of Abdominal Surgery for surgical treatment. Surgically removed histological tissues were sent for a histological examination (all preparations were examined by the same investigator).

The staging of oncological disorders was performed according to the 7th ed. of TNM classification issued in 2009, as recommended by the American Joint Committee on Cancer (AJCC). When the final histological

examination revealed PTC but no BRAF V600E mutation was detected in the cytological sample, an additional testing for a BRAF V600E mutation from histological samples was performed.

Thyroid Ultrasound (US) Examination and Fine Needle Aspiration Biopsy (FNA). Validated Procedures of VUH SK

The thyroid US examination was performed on patients lying on their back, shoulders elevated with a pillow, head reclined. Ultrasound equipment with a high frequency linear sensor (7–15 MHz), high resolution (0.7–1 mm) and high penetration was used. Images were evaluated in a gray real-time and color Doppler scales. The thyroid gland parenchyma, the size of the lobes, the size of nodules, the localization of nodules, nodular US characteristics as well as the changes of the central and lateral lymph nodes were evaluated. All thyroid nodules were classified as having high, moderate, low or very low risk of malignancy and being non-malignant according to US characteristics.

Nodules with a High Risk of Malignancy

Hypoechoic, solid nodules or nodules with small amounts of fluid with 1 or more signs of malignancy according to US characteristics:

- Uneven nodule margins;
- Microcalcifications;
- Breaks in calcified rings;
- Taller-than-wide shape of nodules;
- An extension of the nodule beyond the margins of the thyroid gland.

Nodules with an Intermediate Risk of Malignancy

These nodules are hypoechoic, solid or with small amounts of fluid, with even margins and without other US signs of malignancy.

Nodules with a Low Risk of Malignancy

These nodules are isoechoic or hyperechoic, solid or partially solid with the solid part lying excentrically, without US signs of malignancy.

Nodules with a Very Low Risk of Malignancy

These nodules are spongiform or partially cystic, without any US signs of malignancy.

Non-malignant nodules – cystic thyroid nodules without any solid components.

Indications for punction are based on the US characteristics and size of the nodules:

- Thyroid nodules of 1 cm or more in size, with a high risk of malignancy (without extrathyroidal extension);
- Thyroid nodules of any size with a high risk of malignancy and extrathyroidal extension;
- Thyroid nodules of 1 cm or more in size with an intermediate risk of malignancy;
- Thyroid nodules of 1.5 cm or more in size with a low risk of malignancy;
- Thyroid nodules of 2 cm or more in size with a very low risk of malignancy.

Remarks:

- Multiple thyroid nodules of 1 cm or more in size were punctured based on the same indications as solitary nodules.
- In cases of a multinodular goiter, thyroid nodules with the highest risk of malignancy according to US signs or the largest nodules were punctured.
- In cases of multiple thyroid nodules with a low or very low risk of malignancy, the FNA was not performed and patients were offered active surveillance (an endocrinologist was responsible for the choice

of the monitoring mode); in other cases, the largest (more than 2 cm in size) thyroid nodule was punctured.

When a nodule with indications for US-guided FNA was detected during the US examination of a thyroid gland, after the preparation of the hands, the field and the tools according to the approved procedure, a US sensor was placed on the neck in the projection of the thyroid gland, and the image of a to-be- suspicious nodule or pathological tissue of the thyroid gland was placed onto the screen. The procedure was performed by holding a US sensor in one hand and a syringe with a 21-27G needle in the other hand. The puncture was performed close to the sensor, perpendicularly or transversaly to the ultrasound beam. Following the tip, the needle was inserted into the desired location and punctured in the mode of pendulum. An aspirate was obtained by vacuuming the syringe. The nodules were punctured twice and more times if necessary. A part of the thyroid tissue obtained from the FNA was injected into a liquid-based citology vial, and the remaining part of the aspirated material was spread evenly in a thin layer onto the slide. Smears were air-dried fixed.

Cytological Examination of the Aspirate

FNA aspirates of every patient were examined as smears stained with the Papanicolau and automatic BD Prep Stain system. The smears were examined using a bright-field microscope Olympus CH30/31. Initially, the whole smear was examined with a 10x magnifying lens. The smear was considered to be suitable for further examination if at least six groups of thyroid epithelial cells (7 to 10 cells in each group) were present. The cytomorphological features of cells were evaluated with 40 or 100x magnifying lenses. A special form was created for the systematic collection and analysis of cytological data. Cases were classified according to the Bethesda System for Reporting Thyroid Cytopathology.

Genetic Testing of the BRAF V600E Mutation

Liquid specimens of a thyroid FNA aspirate (treated with an erythrocyte lysis solution) or material from the histological preparation were transferred to 1.5 ml tubes. Genomic DNA was isolated using the GeneJet Genomic DNA Purification Kit (Thermo Scientific, Vilnius, Lithuania). In order to detect a BRAF V600E mutation (GTG > GAG), a real-time PCR assay was developed. The first reaction was used to amplify a sequence flanking the BRAF codon V600 of both mutated and non-mutated codons (internal reference sample control). The second reaction was designed to specifically target the GTG > GAG mutation. Primers were predesigned manually and investigated *in silico* using the OligoCalc web tool. A BRAF V600E mutation specific forward primer was edited manually by introducing a relevant mismatch nucleotide at the 3' end according to the Amplification Refractory Mutation System primer design approach. The reverse primer was the same for both reactions. Primer sequences were as follows: BRAF_V600E_ Forward GTGATTTTGGTCTAGCTACGGAG; BRAF_Forward TAGGTGATTTTGGTCTAGCTACAG; BRAF_Reverse CATCCACAAAATGGATCCAGAC. Real-Time PCR reactions were performed in duplicates using a Maxima™ SYBR Green qPCR Master Mix, supplemented with 0.4 units per reaction of UNG (both Thermo Scientific, Vilnius, Lithuania), 300 nmol of each primer (Metabion, 82152 Planegg/ Steinkirchen, Germany), and 0.005-0.05 μg of DNA in a 20 μl reaction volume. The Real-Time PCR reaction conditions on a Bio-Rad CFX96 system (Bio-Rad Laboratories, Hercules, CA, US) were as follows: 2 minutes at 50°C, 10 minutes at 95°C, 37 cycles at 95°C for 15 seconds, and 1 minute at 60°C.

The first 20 BRAF V600E-positive and 20 wild type allele (Braf V600E-negative) cases were confirmed by sequencing histological samples according to the method described earlier. The analytical sensitivity of the Real-Time PCR assay was 2.5 log (or 0.2%). It was tested by serial dilutions of previously sequenced positive BRAF V600E DNA in negative DNA.

Surgical Treatment

All patients received timely and appropriate treatment according to the existing treatment standards of VUH SK. Participation in the biomedical study had no effect on the course, treatment or outcomes of their disease.

The operating surgeon was not familiar with the results of the BRAF V600E mutation testing and these results could not influence decisions on the indications for surgery or extent of surgical treatment. Patients underwent a resection of isthmus, one lobe, one lobe with isthmus or a resection of the entire thyroid gland. An urgent histological examination of resected tissues was usually performed during the operation. Part of the patients' cohort underwent prophylactic lymph node dissection (VI level) on one or both sides. When pathological US signs in the lateral lymph nodes were detected prior to surgery, a modified radical lymph node dissection (II-V levels) was also performed.

Statistical Analysis of Results

The sample size was calculated based on the number of newly diagnosed thyroid cancer cases per year in the Cancer Registry database, with a confidence level of 95% and a margin of error 5%. A database of study participants was created for the analysis of study results. All statistical analyses were performed using IBM SPSS STATISTICS, version 23.0. The significance of the Bethesda system, the cytomorphological features, the BRAF V600E mutation, the BRAF V600E mutation combined with cytological features for the diagnostics of papillary thyroid cancer were assessed in this study. The significance of the Bethesda system and the BRAF V600E mutation for predicting the histological aggressiveness of papillary thyroid cancer were also evaluated. The absolute values and percentages for categorical variables and mean averages with standard deviations or medians with ranges for continuous variables were given in the descriptions of patients' comparative groups. Continuous variables were checked for normal

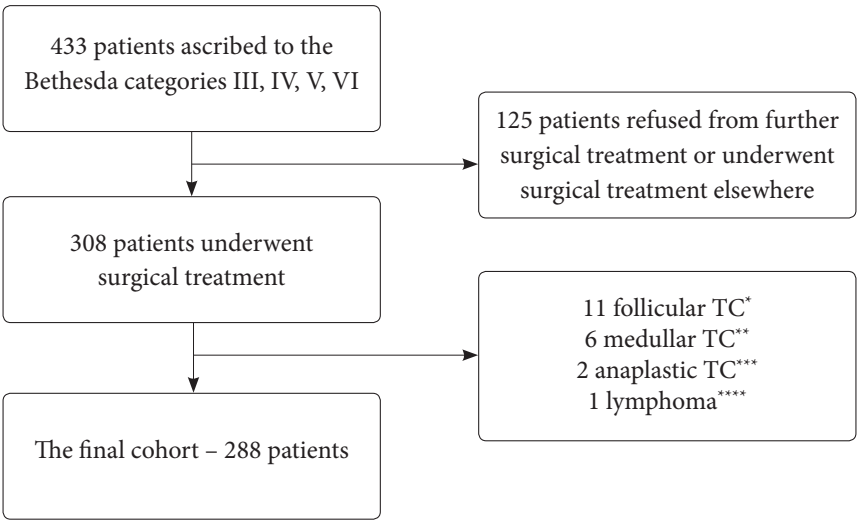
distribution using the Kolmogorov-Smirnov and the Shapiro-Wilk test. The statistical significance of differences between two groups of non-parametric data with non-normal distribution was assessed using the Mann-Whitney U test. The statistical significance of differences between the nominal and categorical variables was assessed using the Pearson χ^2 Chi-Square criterion; otherwise, the Fisher's Exact Test was used when the observed frequency of at least one value of the variable was <5. After establishing a statistically significant correlation between the factors under analysis, the strength of this correlation was also calculated using the correlation coefficient r. Cramer's V correlation coefficient was calculated for the assessment of the correlation between the nominal variables. For assessments regarding the strength of the correlation, when one of the variables was nominal or categorical and the other variable was non-parametric, the Eta factor was calculated. For predictions of histological examination results when the results of other testings were known, a binary logistic regression model was developed. The cutoff of 0.5 was selected and patients were divided into two groups of PTC risk: the low risk group (when PTC risk <0.5) and the high risk group (when PTC risk ≥0.5). The statistical significance level $\alpha = 0.05$ was chosen, and the results were statistically significant when the p-value of the two-sided hypothesis was less than 0.05 ($p < 0.05$).

Results of the study

Composition of the Patients' Cohort

From January 1, 2012 to February 1, 2016, 433 patients were included into the biomedical study, while 145 patients were excluded from the study.

The final cohort consisted of 288 patients who participated in the study until its end; these patients underwent surgery because of suspected thyroid cancer and met the criteria for inclusion into the study. The scheme of patient inclusion and exclusion from the study is presented in Figure 1.



* 5 cases were ascribed to category IV, 5 – V, 1 – VI.
** 1 case was ascribed to category IV, 2 – V, 3 – VI.
*** 1 case was ascribed to category III, 1 – V.
**** 1 case was ascribed to category III.
All cases were negative for the BRAF V600E mutation.

Figure 1. The scheme of patient inclusion and exclusion from the study.

General Characteristics of Study Participants

A total of 288 patients participated in the study. There were 251 female (87.2%) and 37 male (12.8%) patients. The mean age of patients was 54.4 ± 13.1 years. An FNA of thyroid nodules prior to surgery was performed on all patients. All patients had the cytological examination of a thyroid FNA sample specimen; in every sample, 22 cytomorphological features were evaluated and all samples were classified according to the Bethesda System for Reporting Thyroid Cytopathology. Besides, a BRAF V600E mutation testing from the FNA aspirate was performed in all patients, and an additional BRAF V600E mutation testing was also performed on the

histological specimens when the mutation was not detected in the initial FNA sample specimen testing. All study participants underwent surgical treatment. A histological examination of resected tissues was performed for all patients.

Characteristics of the Comparative Groups of Patients

Based on the results of histological examination, the patients were divided into two comparative groups: 143 patients (49.7%) entered the PTC group, 145 patients (50.3%) entered the benign nodular thyroid disease group.

General Data

Statistically significant differences were observed in the distribution of males and females in the abovementioned comparative groups: in the PTC group, there were 131 females (91.6%) and 12 males (8.4%), in the benign nodular thyroid disease group, there were 120 females (82.8%) and 25 males (17.2%), $p = 0.025$ ($p < 0.05$). There were no statistically significant differences in the distribution of patients by age: in the PTC group, the mean age of patients was 53.8 ± 12 years, in the benign nodular thyroid disease group, the mean age of patients was 54.9 ± 14.1 years, $p = 0.309$ ($p \geq 0.05$).

Cytomorphological Features

A high anisokaryosis, a streaked chromatin, a finely dispersed chromatin, nucleoli, a dark cytoplasm, papillary structures, atypia, intranuclear cytoplasmic inclusions, psammoma bodies, an absence of colloid, a dense colloid and eosinophilic colloid bodies were statistically significantly more frequent in the PTC group as compared to the benign nodular thyroid disease group ($p < 0.05$).

A low or intermediate anisokaryosis, a homogeneous chromatin, Hurthle cells, a normofollicular/macrofollicular structure, a microfollicular structure, a abundant amount of colloid and liquid colloid were statistically significantly more frequent in the benign nodular thyroid disease group

as compared to the PTC group (p <0.05). The data on the frequencies of cytomorphological features and correlations between the comparative groups are presented in Table 1.

Table 1. Data of the cytological analysis of comparative groups, n = 288.

Cytomorphological features	Papillary thyroid cancer, n=143	Benign nodular thyroid disease, n=145	*p* value
Neuclei:			
Monomorphic	1 (0.7%)	1 (0.7%)	0,992*
Polymorphic	142 (99.3%)	144 (99.3%)	
Anisokaryosis:			
Not detected	0 (0%)	2 (1.4%)	0,498*
Low	10 (7%)	36 (24.8%)	**0,000****
Intermediate	74 (51.7%)	94 (64.8%)	**0,024****
High	59 (41.3%)	13 (9%)	**0,000****
Homogenous chromatin:			
Absent	88 (61.5%)	14 (9.7%)	**0,000****
Present	55 (38.5%)	131 (90.3%)	
Streaked chromatin:			
Absent	31 (21.7%)	89 (61.4%)	**0,000****
Present	112 (78.3%)	56 (38.6%)	
Coarsely dispersed chromatin:			
Absent	87 (60.8%)	103 (71%)	0,068**
Present	56 (39.2%)	42 (29%)	
Finely dispersed chromatin:			
Absent	61 (42.7%)	88 (60.7%)	**0,002****
Present	82 (57.3%)	57 (39.3%)	
Nucleoli:			
Absent	13 (9.1%)	28 (19.3%)	**0,013****
Present	130 (90.9%)	117 (80.7%)	
Amount of cytoplasm:			
Sufficient	76 (53.1%)	84 (57.9%)	0,414**
Abundant	67 (46.9%)	61 (42.1%)	

Table 1 (Continuation). Data of the cytological analysis of comparative groups, n = 288.

Cytomorphological features	Papillary thyroid cancer, n=143	Benign nodular thyroid disease, n=145	*p* value
Intensity of cytoplasm:			
Light	61 (42.7%)	116 (80%)	**0,000****
Dark	82 (57.3%)	29 (20%)	
Degeneration:			
Absent	80 (55.9%)	83 (57.2%)	0,824**
Present	63 (44.1%)	62 (42.8%)	
Hurthle cells:			
Absent	119 (83.2%)	94 (64.8%)	**0,000****
Present	24 (16.8%)	51 (35.2%)	
Lymphocytes:			
Absent	63 (44.1%)	73 (50.3%)	0,285**
Present	80 (55.9%)	72 (49.7%)	
Makrophages:			
Absent	54 (37.8%)	66 (45.5%)	0,182**
Present	89 (62.2%)	79 (54.5%)	
Normofollicular/ Macrofollicular structures:			
Absent	109 (76.2%)	82 (56.6%)	**0,000****
Present	34 (23.8%)	63 (43.4%)	
Microfollicular structures:			
Absent	55 (38.5%)	38 (26.2%)	**0,026****
Present	88 (61.5%)	107 (73.8%)	
Papillary structures:			
Absent	43 (30.1%)	131 (90.3%)	**0,000****
Present	100 (69.9%)	14 (9.7%)	
Atypia:			
Absent	5 (3.5%)	34 (23.4%)	**0,000****
Present	138 (96.5%)	111 (76.6%)	
Cytoplasmic inclusions:			
Absent	43 (30.1%)	128 (88.3%)	**0,000****
Present	100 (69.9%)	17 (11.7%)	

Table 1 (Continuation). Data of the cytological analysis of comparative groups, n = 288.

Cytomorphological features	Papillary thyroid cancer, n=143	Benign nodular thyroid disease, n=145	*p* value
Psammoma bodies:			
Absent	125 (87.4%)	143 (98.6%)	**0,000**[*]
Present	18 (12.6%)	2 (1.4%)	
Colloid:			
Absent	39 (27.3%)	23 (15.9%)	**0,018**[**]
Small amount	88 (61.5 %)	80 (55.2%)	0,273[**]
Intermediate amount	9 (6.3%)	19 (13.1%)	0,051[**]
Abundant	7 (4.9%)	23 (15.9%)	**0,002**[**]
Consistency of colloid:			
No colloid	39 (27.3%)	23 (15.9%)	**0,018**[**]
Liquid	34 (23.8%)	104 (71.7%)	**0,000**[**]
Dense	70 (49%)	18 (12.4%)	**0,000**[**]
Eosinophilic colloid bodies:			
Absent	98 (68.5%)	124 (85.5%)	**0,001**[**]
Present	45 (31.5%)	21 (14.5%)	

[*] Fisher's Exact Test

[**] Pearson's Chi-Squared Test (χ^2)

Values in bold represent statistically significant correlations.

Bethesda System Categories

A comparison of the distributions of the Bethesda system categories in the PTC and the benign nodular thyroid disease groups showed statistically significantly higher frequencies of categories III and IV in the benign nodular thyroid disease group ($p < 0.05$). Conversely, V and VI categories were statistically significantly more frequent in the PTC group ($p < 0.05$). The frequencies of the Bethesda system categories and correlations between comparative groups are presented in Table 2.

Table 2. The distribution of the Bethesda system categories in the comparative groups, n = 288.

Bethesda system categories	Papillary thyroid cancer, n=143	Benign nodular thyroid disease, n=145	*p* value
III	14 (9.8%)	28 (19.3%)	**0,022**[*]
IV	22 (15.4%)	104 (71.7%)	**0,000**[*]
V	41 (28.7%)	10 (6.9%)	**0,000**[*]
VI	66 (46.2%)	3 (2.1%)	**0,000**[**]

[*] Pearson's Chi-Squared Test (χ^2)

[**] Fisher's Exact Test

Values in bold represent statistically significant correlations.

BRAF V600E mutation Testing

The testing performed in cytological specimens or in both cytological and histological specimens showed that the BRAF V600E mutation was statistically significantly more frequent in the PTC group ($p < 0.05$). The results of the BRAF V600E mutation testing and correlations between the groups are presented in Table 3.

Table 3. Results of the BRAF V600E mutation testing in the comparative groups, n = 288.

Results of mutation testing:	Papillary thyroid cancer, n=143	Benign nodular thyroid disease, n=145	*p* value
BRAF V600E (cytological specimens):			
Negative	52 (36.4%)	145 (100%)	**0,000**[*]
Positive	91 (63.6%)	0 (0%)	
BRAF V600E (cytological and histological specimens):			
Negative	33 (23.1%)	145 (100%)	**0,000**[*]
Positive	110 (76.9%)	0 (0%)	

[*] Fisher's Exact Test

Values in bold represent statistically significant correlations.

A BRAF V600E mutation was detected in 8 (57.1%) PTC cases of the Bethesda III category, 11 (50%) of Bethesda IV, 33 (80.5%) Bethesda V and 58 (87.9%) PTC cases of the Bethesda VI category.

In a classical PTC variant, a BRAF V600E mutation was detected in 88 (83%) cases, in an oncocytic PTC variant – 11 (78.6%), follicular PTC – 11 (47.8%) cases.

Data of Surgical Treatment

Results of the urgent intraoperative histological examination, types of surgeries and lymph node dissection modes were not compared. Detailed data of surgical treatments are shown in Table 4.

Table 4. Data of surgical treatments, n = 288.

Feature	Papillary thyroid cancer, n=143	Benign nodular thyroid disease, n=145
Report of urgent intraoperative histological examination:		
Performed	113:	145:
Non-informative	2 (1.8%)	1 (0.7%)
Benign nodular thyroid disease	12 (10.6%)	144 (99.3%)
Papillary thyroid cancer	99 (87.6%)	0 (0%)
Type of surgery		
Thyroidectomy	141(98.6%)	75 (51.7%)
Lobisthmectomy	2 (1.4%)	67 (46.2%)
Isthmectomy	0 (0%)	3 (2.1%)
Type of lymph node dissection:		
Performed	116:	4:
Central unilateral	99 (85.3%)	4 (100%)
Central bilateral	11 (9.5%)	0 (0%)
Modified radical unilateral	3 (2.6%)	0 (0%)
Central bilateral and modified radical unilateral	3 (2.6%)	0 (0%)

Results of the Final Histological Examination in the Group of Papillary Thyroid Cancer (PTC)

The mean tumor size in the final histological examination of the 143 patients with PTC (87.7% of all thyroid cancer cases) was 1.5 (0.3–7) cm. A tumor extension beyond the thyroid capsule was observed in 59 patients (41.3%). Of the 116 patients in whom a lymph node dissection was performed, 49 patients (42.2%) had metastases in the neck lymph nodes. A lymphovascular invasion was detected in 52 patients (36.4%). Multifocal thyroid cancer was detected in 55 patients (38.5%). An encapsulated tumor was detected in 47 patients (32.9%). All cases were classified according to the TNM Classification of Malignant Tumors. According to the evaluation of tumor size and the extension to the surrounding tissues, 39 cases (27.3%) were classified as T1a, 34 (23.8%) – as T1b, 11 (7.7%) – as T2, 59 (41.3%) – as T3. According to the evaluation of tumor spread into the regional lymph nodes, 27 cases (18.9%) were classified as Nx (no lymph node dissection), 67 (46.9%) – as N0, 45 (31.5%) – as N1a, 4 (2.8%) – as N1b. Distant metastases (M1) were not detected in any patient. According to clinical and histological data, 83 cases (58%) were diagnosed with stage I, 3 (2.1%) – stage II, 24 (16.8%) – stage III, 33 (23.1%) – IVA disease stage. 106 patients (74.1%) had a classical PTC variant, 23 (16.1%) – a follicular PTC, 14 (9.8%) – an oncocytic PTC variant.

Significance of the Bethesda System for the Diagnostics of PTC

Based on the results of 288 cytological analyses, all cases were classified according to the Bethesda System for Reporting Thyroid Cytopathology; 42 cases (14.6%) were classified as belonging to category III, 126 (43.8%) – to category IV, 51 (17.7%) – to category V, 69 (24%) – to category VI.

Out of 42 patients ascribed to category III according to the cytological analysis, PTC in the final histological examination was detected in 14 (33.3%) patients, while 28 (66.7%) patients had benign nodular thyroid disease.

Out of 126 patients ascribed to category IV, PTC in the final histological examination was detected in 22 (17.5%) patients, while 104 (82.5%) patients had benign nodular thyroid disease. Of the 51 patients ascribed to category V, 41 (80.4%) patients had PTC and 10 (19.6%) patients had benign nodular thyroid disease. Out of 69 patients classified as category VI, 66 (95.7%) patients had PTC, 3 (4.3%) patients had benign nodular thyroid disease (Figure 2).

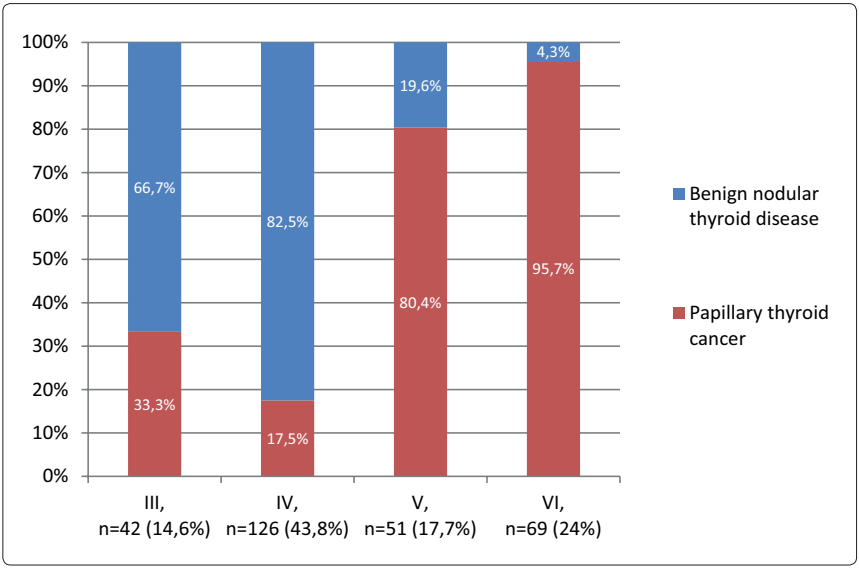


Figure 2. The Significance of the Bethesda System for PTC diagnostics, n = 288.

The highest frequency of PTC, 95.7%, was observed in the cytological category VI, and statistically significant differences as compared to the frequencies of PTC in the other three categories of the Bethesda system were observed. In comparison of PTC and the benign nodular thyroid disease in categories III and VI, $p = 0.000$ ($p < 0.05$), IV and VI, $p = 0.000$ ($p < 0.05$), V and VI, $p = 0.015$ ($p < 0,05$).

Following these results, it was decided that there are no diagnostic problems with Bethesda category VI; therefore, a further analysis of PTC diagnostics included only indeterminate diagnostic categories of PTC (Bethesda III, IV, V).

Out of 219 patients ascribed to indeterminate diagnostic categories according to the results of the cytological analysis, the final histological examination revealed PTC in 77 (35.2%) patients and the benign nodular thyroid disease in 142 (64.8%) patients (Figure 3).

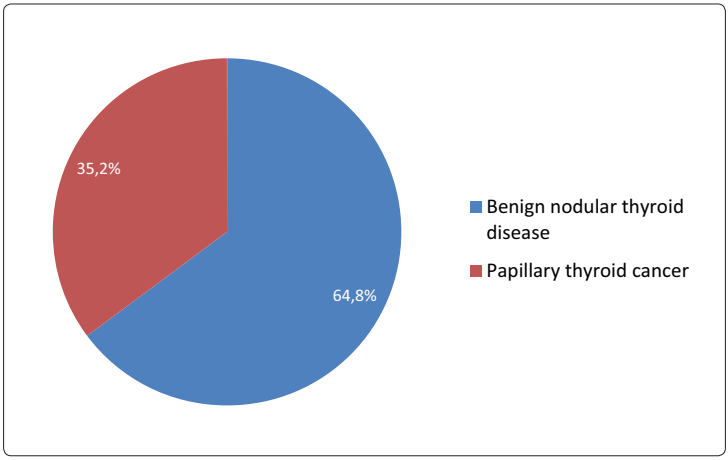


Figure 3. The distribution of the histological examination results in the cytologically indeterminate diagnostic categories, n = 219.

The Significance of the Cytomorphological Features for PTC Diagnostics in the Indeterminate Diagnostic Categories

In order to differentiate PTC from the benign nodular thyroid disease in the cytologically indeterminate diagnostic categories, a binary logistic regression model that analyzes the effect of cytomorphological features on the results of the final histological examination was developed. The final binary logistic regression model was expressed by the equation:

z (probability ratio logit function) = 0,84 + (-1,14 × homogeneous chromatin) + (-1,04 × liquid colloid) + (1,13 × eosinophilic colloid bodies) + (1,73 × papillary structures) + (1.74 x cytoplasmic inclusions).

Here the homogeneous chromatin, the liquid colloid, eosinophilic colloid bodies, papillary structures and cytoplasmic inclusions are expressed as the numerical values obtained in the study, with two values being 0 (absent) or 1 (present).

The logistic model was well-suited for the analyzed data and, in a way, was optimal, too: all coefficients significantly differed from 0, the adding of an additional variable did not improve the model, the log probability function of the model was the highest. The suitability of the model for the analyzed data was confirmed by the χ^2 statistics criterion of the probability ratio of the model, equal to 99.43, $p = 0.000$ (<0.05). The χ^2 statistics of the Hosmer-Lemeshow test was 2.72, $p = 0.744$ (> 0.05). The Cox & Snell R Square determination coefficient was 0.37, while Nagelkerke was equal to 0.50. According to the Wald criterion, all regressors were statistically significant, i.e., for all regressors, $p < 0.05$.

Out of 219 patients, the regression analysis of 154 (70.3%) patients classified them as having a low risk of PTC, while 65 (29.7%) were classified as having a high risk of PTC.

Out of 154 patients who were classified by the regression model as having a low risk of PTC, the final histological examination revealed PTC in 24 (15.6%) of the patients, and the benign nodular thyroid disease in 130 (84.4%) of the patients. Of the 65 patients who were classified by the regression model as having a high risk of PTC, the final histological examination revealed PTC in 53 of the (81.5%) patients, and the benign nodular thyroid disease in 12 (18.5%) of the patients (Figure 4).

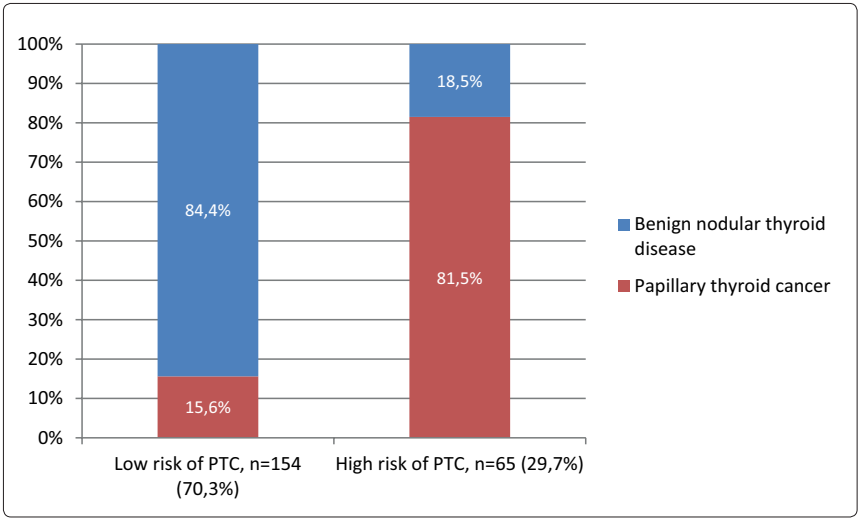


Figure 4. The diagnostic value of the regression model based on the analysis of cytomorphological features, n = 219.

According to the risk of having PTC and the results of the final histological examination, all cases were clasified as true positive, true negative, false positive and false negative. Fifty three cases were considered to be true positive, 130 were true negative, 12 were false positive, 24 were false negative. Calculated diagnostic accuracy measures of the model are shown in Table 5.

Table 5. The diagnostic accuracy measures of the regression model based on the analysis of cytomorphological features, n = 219.

Measure	Value	95% confidence interval
Sensitivity	68.8%	57,3-78,9
Specificity	91.6%	85,7-95,6
Positive predictive value	81.5%	71,6-88,6
Negative predictive value	84.4%	79,5-88,3
Accuracy	83.6%	78-88,2

The Significance of the BRAF V600E Mutation Testing for PTC Diagnostics in the Indeterminate Diagnostic Categories

Of the 219 patients ascribed to indeterminate diagnostic categories, a BRAF V600E mutation was detected in 52 patients (23.7%). Of these, 37 patients (71.2%) had a BRAF V600E mutation in FNA specimens, 15 (28.8%) – in the histological specimens. A BRAF V600E mutation was not detected in 167 (76.3%) patients.

Of the 52 patients with a BRAF V600E mutation, the final histological examination revealed PTC in all 52 patients (100%). Of the 167 patients without a BRAF V600E mutation, the final histological examination revealed PTC in 25 (15%) patients and the benign nodular thyroid disease in 142 (85%) patients. Statistically significant differences of the BRAF V600E mutation distribution in the comparative groups were found, $p = 0.000$ ($p < 0.05$) (Figure 5).

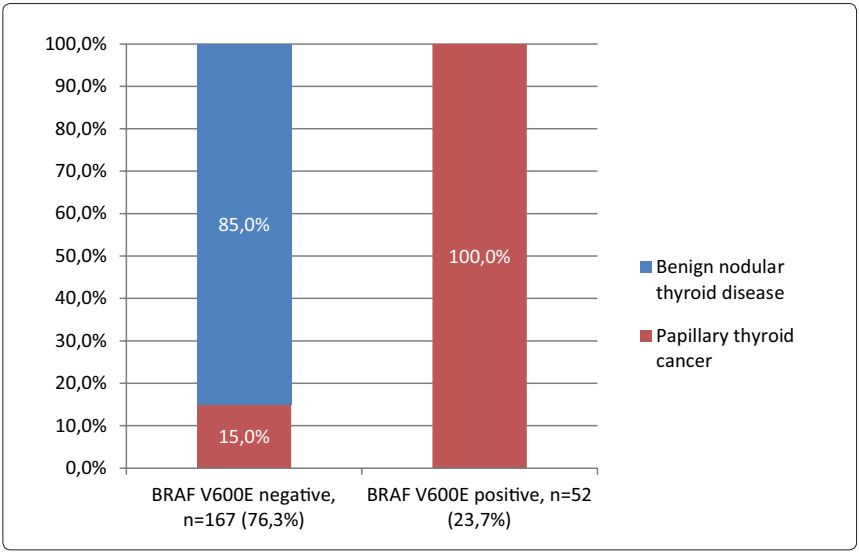


Figure 5. The diagnostic value of a BRAF V600E mutation status, n = 219.

The verification of the hypothesis that the results of the final histological examination depend on the mutation status for a BRAF V600E mutation revealed a statistically significant correlation between a BRAF V600E mutation and PTC detected in the final histological examination: r = 0.76, p = 0.000 (p <0,05).

According to the mutation status for a BRAF V600E mutation and the results of the final histological examination, all cases were categorized to true positive, true negative, false positive and false negative. Fifty-two cases were true positive, 142 were true negative, 0 were false positive, 25 were false negative. Calculated diagnostic accuracy measures are presented in Table 6.

Table 6. The diagnostic measures of a BRAF V600E mutation testing.

Measure	Value	95% confidence interval
Sensitivity	67.5%	55,9–77,8
Specificity	100%	97,4–100
Positive predictive value	100%	–
Negative predictive value	85%	80,5–88,7
Accuracy	88.6%	83,6–92,5

The Significance of BRAF V600E Mutation Testing Combined with the Analysis of Cytomorphological Features for PTC Diagnostics in the Indeterminate Diagnostic Categories

In order to refine the diagnostics of PTC in the cytologically indeterminate diagnostic categories, we added the results of the BRAF V600E mutation testing and pre-operative clinical data (gender, age, the relevant Bethesda system category) to the results of the cytological analysis and developed an additional binary logistic regression model. The final binary logistic regression model was expressed by the equation:

z (probability ratio logit function) = 1.82 + (-1.46 × liquid colloid consistency) + (1.57 × eosinophilic colloid bodies) + (2.16 × papillary structures) + (22.79 × BRAF V600E mutation).

Here liquid colloid consistency, eosinophilic colloid bodies, papillary structures, and the BRAF V600E mutation are expressed as the numerical values obtained in the study, with two values being 0 (absent) or 1 (present).

The logistic model was well suited for the analyzed data and, in a way, was optimal, too: all coefficients significantly differed from 0, the adding of an additional variable did not improve the model, the log probability function of the model was the highest. The suitability of the model for the analyzed data was confirmed by the χ^2 statistics criterion of the probability ratio of the model, equal to 177,2, p=0,000 (<0,05). The χ^2 statistics of the Hosmer-Lemeshow test was 0,14, p=0,998 (>0,05). The Cox & Snell R Square determination coefficient was 0,56, while Nagelkerke was equal to 0,76. According to the Wald criterion, all regressors were statistically significant, i.e., for all regressors, p <0.05, except for the BRAF V600E mutation, which was exempted due to its high significance for the final result of the regression equation.

Of the 219 patients, the regression model classified 156 patients (71.2%) as having a low risk of PTC, and 63 (28.8%) patients as having a high risk of PTC.

Of the 156 patients who were classified by the regression model as having a low risk of PTC, the final histological examination revealed PTC in 15 (9.6%) patients and the benign nodular thyroid disease in 141 (90.4%) patients. Of the 63 patients who were classified by the regression model as having a high risk of PTC, the final histological examination revealed PTC in 62 (98.4%) patients and the benign nodular thyroid disease in 1 (1.6%) patient (Figure 6).

According to the risk of having PTC and the results of the final histological examination, all cases were categorized into true positive, true negative, false positive and false negative. Sixty two cases were considered to be true positive, 141 – true negative, 1 – false positive, 15 – false negative. Calculated diagnostic accuracy measures are presented in Table 7.

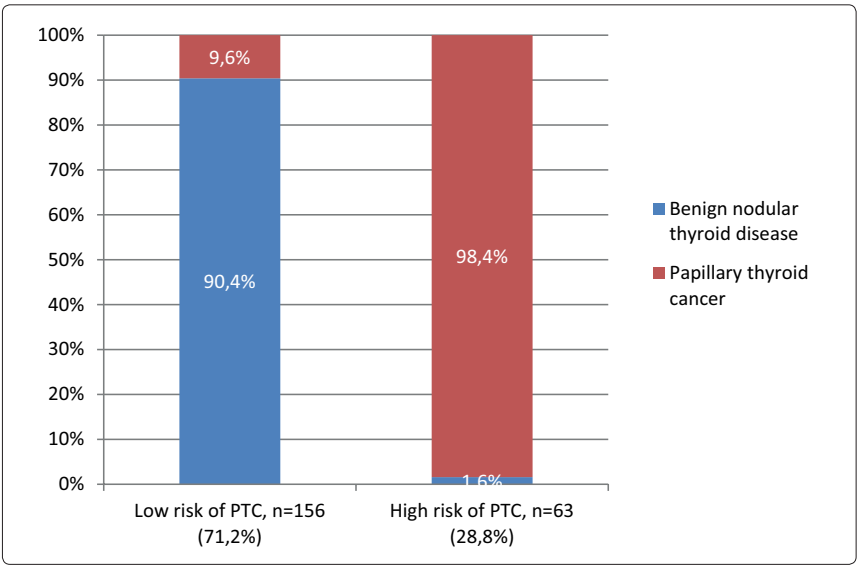


Figure 6. The diagnostic value of the regression model based on a BRAF V600E mutation and the analysis of cytomorphologic features, n = 219.

Table 7. The diagnostic accuracy measures of the regression model based on the analysis a BRAF V600E mutation and the analysis of cytomorphological features, n = 219.

Measure	Value	95% confidence interval
Sensitivity	80.5%	69,9–88,7
Specificity	99.3%	96,1–100
Positive predictive value	98.4%	89,8–99,8
Negative predictive value	90.4%	85,7–93,7
Accuracy	92.7%	88,4–95,8

The Significance of the Bethesda System for Predicting Signs of Histological PTC Aggressiveness

The comparisons of signs of histological aggressiveness in indeterminate diagnostic categories (III, IV, V) and category VI revealed that in group VI, tumors were statistically significantly larger, an extrathyroid tumor

extension and a higher stage of cancer was observed more often ($p < 0.05$). There were no other statistically significant differences between the groups. Frequencies of signs, reliability levels and correlation indices are given in Table 8.

Table 8. The significance of the Bethesda system for predicting signs of aggressiveness, n = 143.

Feature	Bethesda III-V, n=77	Bethesda VI, n=66	*p* value	*r* correlation coefficient
Size (cm):	1,1 (0,3-3,8)	1,5 (0,3-7)	**0,008***	**0,24*****
Extrathyroid extension:				
Absent	54 (70.1%)	30 (45.5%)	**0,003****	**0,25******
Present	23 (29.9%)	36 (54.5%)		
Metastases in lymph nodes (n=116):				
Absent	34 (61.8%)	33 (54.1%)	0,401**	-
Present	21 (38.2%)	28 (45.9%)		
Lymphovascular invasion:				
Absent	53 (68.8%)	38 (57.6%)	0,163**	
Present	24 (31.2%)	28 (42.4%)		
Multifocality:				
Absent	52 (67.5%)	36 (54.5%)	0,112**	-
Present	25(32.5%)	30(45.5%)		
Encapsulated tumor:				
Absent	47 (61%)	49(74.2%)	0,094**	-
Present	30 (39%)	17 (25.8%)		
Stage:				
I-II	53 (68.8%)	33 (50%)	**0,022****	**0,19******
III-IVA	24 (31.2%)	33 (50%)		

* Mann-Whitney-Wilcoxon Test

** Pearson's Chi-Squared Test (χ^2)

*** Eta coefficient

**** Cramer's V coefficient

Values in bold represent statistically significant correlations.

The Significance of a BRAF V600E Mutation for Predicting the Signs of PTC Histological Aggressiveness

Comparisons of signs of the histological aggressiveness in BRAF V600E negative and BRAF V600E positive cases did not reveal any statistically significant differences ($p \geq 0.05$). Frequencies of signs and reliability levels are given in Table 9.

Table 9. The significance of a BRAF V600E mutation for predicting signs of PTC aggressiveness, n = 143.

Feature	BRAF V600E negative, n=33	BRAF V600E positive, n=110	*p* value
Size (cm):	0,9 (0,3-7)	1,3 (0,3-5)	0,073*
Extrathyroid extension:			
Absent	23 (69.7%)	61 (55.5%)	0,145**
Present	10 (30.3%)	49 (44.5%)	
Metastases in lymph nodes (n=116):			
Absent	13(59.1%)	54 (57.4%)	0,888**
Present	9 (40.9%)	40 (42.6%)	
Lymphovascular invasion:			
Absent	24 (72.7%)	67 (60.9%)	0,216**
Present	9 (27.3%)	43 (39.1%)	
Multifocality:			
Absent	19 (57.6%)	69 (62.7%)	0,594**
Present	14 (42.4%)	41(37.3%)	
Encapsulated tumor:			
Absent	18 (54.5%)	78 (70.9%)	0,079**
Present	15 (45.5%)	32 (29.1%)	
Stage:			
I-II	23 (69.7%)	63 (57.3%)	0,201**
III-IVA	10 (30.3%)	47 (42.7%)	

* Mann-Whitney-Wilcoxon Test

** Pearson's Chi-Squared Test (χ^2)

Conclusions

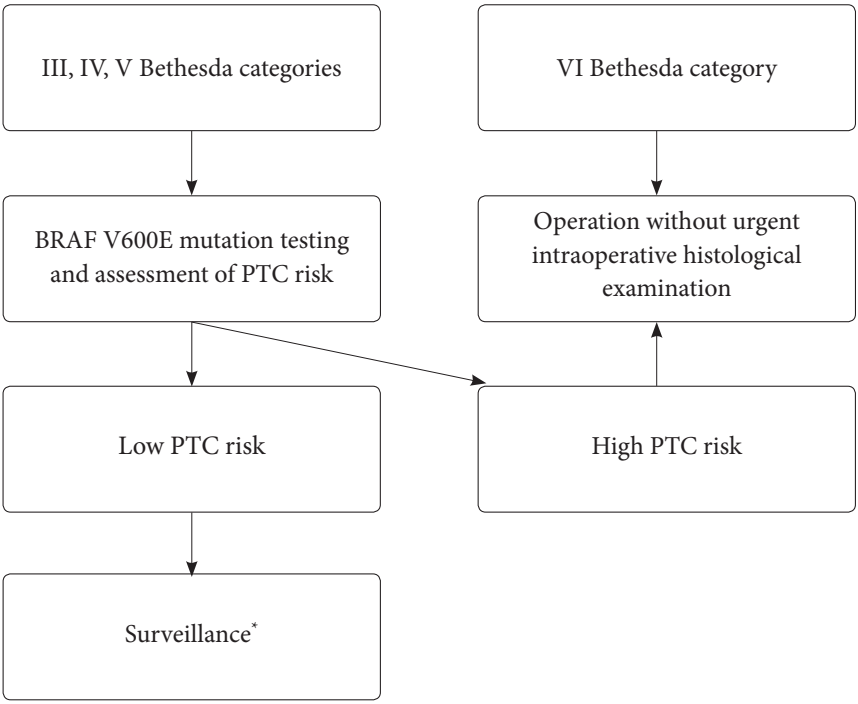
1. Patients' data analysis shows that the frequency of PTC in category III is 33.3%, in category IV – 17.5%, in category V – 80.4%, in category VI – 95.7%;
2. A BRAF V600E mutation was detected in 76.9% of the patients with PTC;
3. The PTC diagnostic sensitivity of the logistic regression model that is based on the analysis of the cytomorphological features and includes five cytolomorphological features, applied in the indeterminate diagnostic categories, is 68.8%, the specificity is 91.6%, the positive predictive value is 81.5%, the negative predictive value is 84.4%, accuracy is 83.6%;
4. The PTC diagnostic sensitivity of a BRAF V600E mutation in the indeterminate diagnostic categories is 67.5%, specificity is 100%, positive predictive value is 100%, negative predictive value is 85%, accuracy is 88.6%;
5. The PTC diagnostic sensitivity of the logistic regression model that is based on the BRAF V600E mutation testing combined with the analysis of the cytomorphological features and includes three cytomorphological features, applied in the indeterminate diagnostic categories, is 80.5%, specificity is 99.3%, positive predictive value is 98.4%, negative predictive value is 90.4%, accuracy is 92.7%;
6. The categories of the Bethesda system have a prognostic value. Statistically significant differences were found with a weak or very weak direct correlation between category VI and tumor size ($p = 0.008$, $r = 0.24$), extrathyroid extension ($p = 0.003$, $r = 0.25$) and the stage of cancer ($p = 0.022$, $r = 0.19$);
7. A BRAF V600E mutation is not suitable for predicting histological aggressiveness. There were no statistically significant differences in the histological findings in BRAF V600E negative and BRAF V600E positive patient groups.

Recommendations

- For patients who are classified as category VI according to the Bethesda system, we recommend surgical treatment. Due to the high risk of the PTC in this category, an urgent, intraoperative, histological examination of resected tissues is not recommended in this category of patients.
- When samples are categorized as indeterminate diagnostic categories (III, IV, V) according to the Bethesda system, we recommend to test them additionally for a BRAF V600E mutation. The risk of PTC in these patients should be assessed by using a calculator based on the BRAF V600 mutation testing combined with the analysis of the cytomorphological features. A calculator developed for clinical applications may be found on the internet at http://ptc-calc.we2host.lt/.
- Active surveillance is recommended for patients who are ascribed to indeterminate diagnostic categories and in whom:
  1. On the US scan, there are no signs of disease spread;
  2. Calcitonin levels in the blood do not exceed normal ranges;
  3. There is a low risk of PTC based on the results of the calculator that combines the results of the BRAF V600 mutation testing and the analysis of the cytomorphological features;

  In these patients, indications for an ultrasound examination with a fine needle biopsy and a BRAF V600E mutation testing should be reevaluated based on disease progression.
- For patients who are ascribed to the group of high PTC risk according to the calculator that combines the BRAF V600E mutation testing and the analysis of the cytomorphological features, we recommend surgical treatment. Due to the high risk of the PTC in this category, an urgent, intraoperative, histological examination of resected tissues is not recommended in this group of patients.

An algorithm of diagnostic recommendations is presented in Figure 7.



* There are no signs of disease spread on the US scan; calcitonin levels in the blood do not exceed normal ranges.

Figure 7. The algorithm of diagnostic recommendations.

The Presentation and Approbation of the Results

Publications:

1. Beiša A, Beiša V, Stoškus M, Ostanevičiūtė E, Griškevičius L, Strupas K. "The Value of the Repeated Examination of BRAF V600E Mutation Status in Diagnostics of Papillary Thyroid Cancer." *Endokrynol Pol.* 2016;67(1):35–40.
2. Beiša A, Kvietkauskas M, Beiša V, Stoškus M, Ostanevičiūtė E, Jasiūnas E, Griškevičius L, Strupas K. "The Utility of the Bethesda Category and Its Association with BRAF Mutation in the Prediction of Papillary Thyroid Cancer Stage." *Langenbecks Arch Surg*. 2017 Mar;402(2):227–234.
3. Beiša A, Kvietkauskas M, Beiša V, Stoškus M, Ostanevičiūtė E, Jasiūnas E, Griškevičius L, Šeinin D, Šileikytė A, Strupas K. "Significance of BRAF V600E Mutation and Cytomorphological Features for the Optimization of Papillary Thyroid Cancer Diagnostics in Cytologically Indeterminate Thyroid Nodules." *Exp Clin Endocrinol Diabetes*, DOI: 10.1055/a-0588-4885.

Presentations:

1. Beiša A, Beiša V, Stoškus M, Ostanevičiūtė E, Juškevičius D, Petroška D, Griškevičius L, Strupas K. BRAF V600E. "Opportunity to Decrease the Amount of Thyroid Surgery." The 5th Workshop of the European Society of Endocrine Surgery (ESES). May 23–25, 2013. Berlin, Germany.
2. Beiša A, Beiša V, Ostanevičiūtė E, Stoškus M, Juškevičius D, Petroška D, Griškevičius L, Strupas K. "Cancer Papillaire de la Thyroid. Expression des Mutations BRAF V600E. Évaluation et de Leurs Methode de Determination." 13th Congrés de l' Association Francophone de Chirurgie Endocrinienne (AFCE). June 14, 2013. Lyon, France.

3. Beiša A, Beiša V, Ostanevičiūtė E, Stoškus M, Griškevičius L, Strupas K. "The Potential Utility of BRAF V600E Status Follow-up in FNA of Thyroid Nodule." The 6th Workshop of the European Society of Endocrine Surgery (ESES). May 28–30, 2015. Varna, Bulgaria.
4. Beiša A, Kvietkauskas M, Beiša V, Stoškus M, Ostanevičiūtė E, Griškevičius L, Strupas K. "The Utility of the BRAF Mutation Status and Bethesda Category in the Prediction of Papillary Thyroid Cancer Stage." The 3rd International Conference of Evolutionary Medicine: Pre-Existing Mechanisms and Patterns of Current Health Issues. June 14–19, 2016. Vilnius, Lithuania.
5. Beiša A, Kvietkauskas M, Beiša V, Stoškus M, Ostanevičiūtė E, Griškevičius L, Strupas K. "The Utility of the Bethesda Category and Its Association with BRAF Mutation in the Prediction of Papillary Thyroid Cancer Stage." The 7th Symposium of the European Society of Endocrine Surgery (ESES). April 6–8, 2017. Oxford, United Kingdom.
6. Beiša A, Kvietkauskas M, Beiša V, Stoškus M, Ostanevičiūtė E, Griškevičius L, Strupas K. "The Value of BRAF V600E Mutation Status Combined with Cytomorphological Features for Surgical Treatment Optimisation of Papillary Thyroid Cancer." A conference for young scientists, organized by the Biological, Medical and Geoscience Division of LAS. Biofuture: the Perspective of Nature and Life Sciences. December 7, 2017. Vilnius, Lithuania.
7. Beiša A, Kvietkauskas M, Beiša V, Stoškus M, Ostanevičiūtė E, Jasiūnas E, Šeinin D, Šileikytė A, Griškevičius L, Strupas K. "The Diagnostic Value of BRAF V600E Mutation Status Combined with Cytomorphological Features for Diagnosis of Papillary Thyroid Cancer in Cytologically Indeterminate Thyroid Nodules." The 8th Biennal Congress of the European Society of Endocrine Surgery (ESES). May 24–26, 2018. Amsterdam, the Netherlands.

Curriculum Vitae

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2013–2018 06	Residency of abdominal surgery at the Faculty of Medicine of Vilnius University.
2015–2018 06	Doctoral (PhD) studies at the Faculty of Medicine of Vilnius University.

Clinical Experience in Healthcare, Social Assistance or Other Institutions or Organizations:

2010 04–2013 06	Nurse assistant at Vilnius University Hospital Santariškių Klinkos. Intensive Care Unit.
2011 01–2013 06	Nurse assistant at the Children's Hospital branch of Vilnius University Hospital Santariškių Klinikos. Department of Anaesthesiology.
2013 07–2015 07	Physician assistant at Vilnius University Hospital Sanatriškių Klinikos. Center of Abdominal Surgery.
2013 09–present	Resident of Abdominal Surgery at Vilnius University.

2016 03–present	Junior private at MD Jonas Basanavičius Military Medical Service.
2017 09–present	Physician assistant at Vilnius University Hospital Sanatros Klinikos. Center of Abdominal Surgery.

Studies and Research Activities:

Posters and Presentations in Local and International Conferences

2013. Congress of European Endocrine Surgery in Berlin, Germany. BRAF V600E. "Opportunity to Decrease the Amount of Thyroid Surgery." Oral presentation.

2013. Conference of Endocrine Surgeons – Phrancophones association in Lille, France. "Cancer Papillaire de la Thyroid. Expression des Mutations BRA F V600E Évaluation et de Leurs Methode de Determination." Poster presentation.

2015. European Congress of Endocrine Surgery in Varna, Bulgaria. "The Potential Utility of BRAF V600E Status Follow-up in FNA of Thyroid Nodule." Poster presentation.

2016. The 3rd International Conference of Evolutionary Medicine: Pre-Existing Mechanisms and Patterns of Current Health Issues in Vilnius, Lithuania. "The Utility of the BRAF Mutation Status and Bethesda Category in the Prediction of Papillary Thyroid Cancer Stage." Oral presentation.

2017. The 7th Symposium of the European Society of Endocrine Surgery (ESES) in Oxford, United Kingdom. "The Utility of the Bethesda Category and Its Association with BRAF Mutation in the Prediction of Papillary Thyroid Cancer Stage." Poster presentation.

2017. A conference for young scientists organized by the Biological, Medical and Geoscience Division of LAS. Biofuture: the Perspective of Nature and Life Sciences in Vilnius, Lithuania. "The Value of BRAF V600E Mutation Status Combined with Cytomorphological Features for Surgical Treatment Optimisation of Papillary Thyroid Cancer." Oral presentation.

2018. The 8th Biennal Congress of the European Society of Endocrine Surgery (ESES) in Amsterdam, the Netherlands. "The Diagnostic Value of BRAF V600E Mutation Status Combined with Cytomorphological Features for Diagnosis of Papillary Thyroid Cancer in Cytologically Indeterminate Thyroid Nodules." Poster presentation.

Publications in Local Journals

1. Beiša V., Lagunavičius K., Beiša A., Strupas K. "Parathyroidectomia: Treatment of Secondary Hyperparathyroidism. Causes and Prophylaxis of Disease Relapse." *Acta Medica Lithuanica*. 2011. Vol. 18. No. 4. P. 170–174

Publications in International Journals

1. Beiša V, Beiša A, Ūselis S, Strupas K. "Parathyromatosis after Parathyroidectomy because of Primary Hyperparathyroidism: A Case Report." *Cent Eur J Med*. 2012;7(3): 371–5.
2. Šileikis A, Beiša V, Beiša A, Samuilis A, Šerpytis M., Strupas K. "Minimally Invasive Retroperitoneal Necrosectomy in Management of Acute Necrotizing Pancreatitis." *Videosurgery Miniinv*. 2013;8(1):29–35.
3. Šilekis A, Beiša A, Zdanytė ES, Jurevičius S, Strupas K. "Minimally Invasive Management of Pancreatic Pseudocysts." *Videosurgery Miniinv*. 2013;8(3):211–5.
4. Beiša V, Kvietkauskas M, Beiša A, Strupas K. "Laparoscopic Approach in the Treatment of Large Epiphrenic Esophageal Diverticulum." *Videosurgery Miniinv*. 2015;10(4):584–8. doi:10.5114/wiitm.2015.56407.
5. Beiša A, Beiša V, Stoškus M., Ostanevičiūtė E, Griškevičius L, Strupas K. "The Value of the Repeated Examination of BRAF V600E Mutation Status in Diagnostics of Papillary Thyroid Cancer." *Endokrynol Pol*. 2016;67(1):35–40. doi:10.5603/EP.2016.0005.

6. Šileikis A, Beiša A, Kvietkauskas M, Stanaitis J, Aleknaitė A, Strupas K. "Minimally Invasive Approach in the Management of Pancreatic Pseudocysts." *JOP. J Pancreas* (Online). 2016 Mar 07; 17(2):222–5.
7. Beiša A, Kvietkauskas M, Beiša V, Stoškus M, Ostanevičiute E, Jasiūnas E, Griškevičius L, Strupas K. "The Utility of the Bethesda Category and Its Association with BRAF Mutation in the Prediction of Papillary Thyroid Cancer Stage." *Langenbeck Arch Surg*. 2017; doi:10.1007/s00423-017-1560-2.
8. Šileikis A, Beiša A, Beiša V, Kvietkauskas M, Kryžauskas M, Strupas K. "Laparoscopic Distal Resection of the Pancreas. Can Be All Resections of Body and Tail of the Pancreas Called the Same?" *Contemp Oncol* (Pozn) 2017; 21 (2): 174–177. DOI: https://doi.org/10.5114/wo.2017.68627
9. Lipnickas V, Beiša A, Makūnaitė G, Strupas K. "Laparoscopic Approach in the Treatment of Large Leiomyoma of the Lower Third of the Esophagus." *Videosurgery Miniinv*. 2017; 12 (4): 437–442.
10. Beiša A, Kvietkauskas M, Beiša V, Stoškus M, Ostanevičiūtė E, Jasiūnas E, Griškevičius L, Šeinin D, Šileikytė A, Strupas K,. "Significance of BRAF V600E Mutation and Cytomorphological Features for the Optimization of Papillary Thyroid Cancer Diagnostics in Cytologically Indeterminate Thyroid Nodules." *Exp Clin Endocrinol Diabetes*, DOI: 10.1055/a-0588-4885.

Experience in Biomedical Research

2012–present: Researcher of the Clinical Study "Diagnostic value of BRAFV600E mutation and other molecular markers in malignant thyroid tumours." Vilnius University.

International Course

2015 ESPEN. The 22th Course of Clinical Nutrition and Metabolic Care. Bucharest, Romania.

2016 Harvard Medical School. Surgery of Thyroid and Parathyroid Glands. Boston, US.

2017 Lille University Regional Hospital Center. Department of Endocrine Surgery. Lille, France.

Ability to Work in the IT Environment:

Excellent skills in working with SPSS and the Microsoft Office service pack, including Excel, Word and Power Point software.

European Union countries Language: *Language*	*Reading*	*Speaking*	*Writing*
Lithuanian	*5*	*5*	*5*
English	*4*	*4*	*4*
French	*2*	*2*	*2*
Non-EU: *Russian*	*4*	*4*	*4*