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Occurrence of BRAF mutations in a Polish cohort of PTC patients — preliminary results

Występowanie mutacji BRAF u chorych na zróżnicowane raki tarczycy w populacji polskiej — doniesienie wstępne

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Abstract

Introduction: Genetic alterations involving the mitogen-activated protein kinase (MAPK) pathway are frequently demonstrated in papillary thyroid cancer (PTC). BRAF(V600E), the most frequent mutation in adult patients, is present in approximately 50% of PTC. Most clinical studies have demonstrated an association of BRAF(V600E) mutation with aggressive clinicopathological characteristics and high tumour recurrence, although the results are controversial.
In this study we present the preliminary results of BRAF mutation frequence in a group of 88 Polish patients with papillary thyroid cancer (PTC) and relate it to the outcome all DTC patients operated in 2004 and 2005. BRAF (V600E) mutation was diagnosed in 38 (43%) of cases.
Material and methods: The presence of BRAF mutation was evaluated in 88 PTC tumours. DNA was isolated from tissue parafin blocks, and the mutation V600E was evaluated by sequence analysis with an AbiPrism 377 and 3130 xl genetic analyzer (Life Technologies). Statistical analysis was carried out with the use of SPSS 12 software. The χ^2 and Kaplan-Meyer survival analysis were performed.
Results: From all analyzed clinico-pathological factors, only older age positively correlated with BRAF mutation frequency ($p = 0.0017$). Lymph node/distant metastases, multifocality, and extra-thyroid extension did not correlate with BRAF status. One cancer related death and two reccurences were observed in the BRAF+ group while one relapse was diagnosed in the BRAF– group.
Conclusions: Although many studies document BRAF mutation as a prognostic factor in PTC our results underline that it is too early to consider it as a routine clinical predictive factor.
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Key words: *papillary thyroid carcinoma, BRAF mutation, prognostic and predictive significance*

Streszczenie

Wstęp: Mutacje dotyczące genów na szlaku kinaz MAP (kinazy aktywowane mitogenem)/kinaz ERK (kinazy regulowane sygnałem zewnątrzkomórkowym) są najczęstszymi zdarzeniami molekularnymi w raku brodawkowatym tarczycy (PTC, *papillary thyroid cancer*). Mutację genu BRAF (V600E) stwierdza się u około 50% dorosłych chorych z PTC. W większości dotychczas opublikowanych badań występowanie mutacji *BRAF* (V600E) wiąże się z agresywnym przebiegiem klinicznym PTC i wysokim odsetkiem nawrotów choroby. Jednak nie wszyscy autorzy podzielają tę opinię.
Celem pracy była ocena częstości mutacji BRAF (V600E) i wstępna ocena jej znaczenia predykcyjnego u 88 chorych na raka brodawkowatego tarczycy.
Materiał i metody: Obecność mutacji BRAF (V600E) oceniono w 88 guzach (rakach brodawkowatych). DNA izolowano z bloczków parafinowych z użyciem sekwencjonatora AbiPrism 377 i 3130xl Genetic Analyzer (Applied Biosystems).W analizie statystycznej wykorzystano: test χ^2, test U Mann-Whitney oraz ocenę przeżycia metodą Kaplana-Meier.
Wyniki: Mutację BRAF (V600E) stwierdzono u 38 (43%) chorych. Spośród przeanalizowanych czynników kliniczno-patologicznych jedynie starszy wiek korelował z częstością mutacji. Nie wykazano korelacji pomiędzy obecnością mutacji a przerzutami do węzłów chłonnych/odległymi, wieloogniskowością czy naciekaniem torebki tarczycy. Stwierdzono jeden zgon i dwa przerzuty raka w grupie BRAF+ i jeden nawrót raka w grupie BRAF–.
Wnioski: Mimo wykazania w wielu pracach mutacji BRAF jako czynnika prognostycznego w PTC wstępne wyniki opisywanego badania wskazują na potrzebę dalszych badań zanim mutacja BRAF (V600E) stanie się powszechnie stosowanym czynnikiem predykcyjnym w raku brodawkowatym tarczycy.
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Słowa kluczowe: *rak brodawkowaty tarczycy, mutacja BRAF, znaczenie prognostyczne i predykcyjne*

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Table I. *Disease stage in 195 studied PTC patients*
Tabela I. *Stopień zaawansowania choroby w badanej grupie 195 chorych na raka zróżnicowanego tarczycy*

T	Number of patients	Frequency	N1	Freguency	M1	Freguency
T1	116	60%	22	19%	1	1%
T2	30	15%	8	27%	1	3%
T3	35	18%	18	51%	2	9%
T4	14	7%	9	64%	6	43%
Total	195	100%	57	29%	10	5%

Introduction

The prognostic impact and predictive value of clinical and histological features in differentiated thyroid cancers (DTC) [1–5], as well as an optimal treatment related to disease stage [3, 6–14], are still discussed. Recent molecular investigations constitute a new approach. One of the candidates as a predictive marker of PTC (papillary thyroid cancer) is BRAF mutation, which leads to constitutive activation of the mitogen-activated protein kinase (MAPK) pathway. Many papers describing the relation between the presence of BRAF mutation and cancer stage, as well as its unfavourable course, have been published [15–23, for review see 15]. Since BRAF mutation can be quickly diagnosed preoperatively in the fine needle aspiration biopsy material, its diagnosis could be of great value in pre-operative prognostication of PTC. However, the question arises whether the predictive potential of BRAF status is sufficient to guide the extent of surgery.

The aim of this study was a preliminary evaluation of the frequency of BRAF mutation in our PTC patients for further investigation of its putative predictive significance.

Material and methods

A group of 195 DTC patients primarily operated in the Clinical Surgery Clinic in Gliwice in 2004–2005 were assessed for their staging and disease course and related to the results of BRAF investigation in paraffin-embedded tumour fragments if available.

The study group consisted of 166 (85%) women and 29 men (15%), mean age 46 years (median 47 years). Mean time of follow-up was 4 years (median 4 years). Follicular thyroid cancer (FTC) was diagnosed in 8 (4%) patients, whereas in the remaining subjects papillary thyroid cancer (PTC) was diagnosed by postoperative histopathology.

All patients were treated by total thyroidectomy and various extents of lymphadenectomy (unilateral modified cervical lymphadenectomy was performed in 18 cases (20%), bilateral in 4 patients (5%)) and referred for complementary radioiodine therapy. The details of our thyroid surgical algorithm are described elsewhere [24].

The presence of BRAF mutation was evaluated in 88 PTC tumours. DNA was isolated from tissue paraffin blocks, and the mutation V600E was evaluated by sequence analysis with an AbiPrism 377 and 3130 xl genetic analyzer (Life Technologies).

Statistical analysis was carried out with the use of SPSS 12 software. The chi^2 test, Mann-Whitney U test, and Kaplan-Meyer survival analysis were performed.

Results

There were 146 (75%) pT1–pT2 patients and 49 (15%) pT3 subjects in the study group. Lymph node metastases were diagnosed in 57 (29%) cases, whereas distant metastases were diagnosed in 10 (5%) subjects (Table I). The 5-year overall and disease-free survival ratios were 98% and 95%, respectively (Fig. 1).

The frequency of BRAF mutation in the analyzed group of 88 PTCs was 43%. The mean tumour diameter in the BRAF+ group was 21 mm (the smallest was 8 mm and the largest was 10 cm). In 11% cases the tumour diameter was < 1 cm. Among 50 BRAF negative (BRAF-) PTCs, mean tumour diameter was 13 mm (range 4–100 mm). In 30 (60%) cases the tumour was not greater than 1 cm. Neither the difference in tumour diameter nor in the frequency of pT1 tumours was statistically significant.

Analysis of the relationship between mutation, clinical, and pathological risk factors showed a strong association of BRAF mutation with patient age ($p = 0.0017$). There was no correlation between other clinical and pathological risk factors and BRAF presence (Table II).

One cancer-related death and two recurrences were observed in the BRAF+ group, whereas in the BRAF- group only 1 relapse was diagnosed. The 5-year disease-free survival ratio was higher in the BRAF- group than in the BRAF+ group (98% and 94%, respectively); however, the difference was statistically insignificant (Fig. 2).

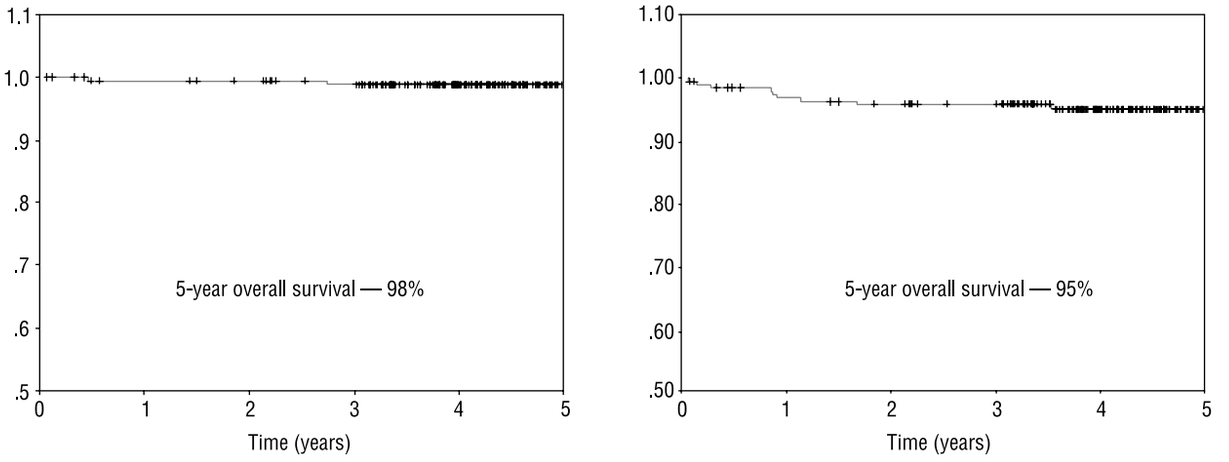

Figure 1. *Overall and disease-free survival in studied PTC patients*

Rycina 1. *Prawdopodobieństwo przeżycia całkowitego i bezobjawowego w badanej grupie chorych*

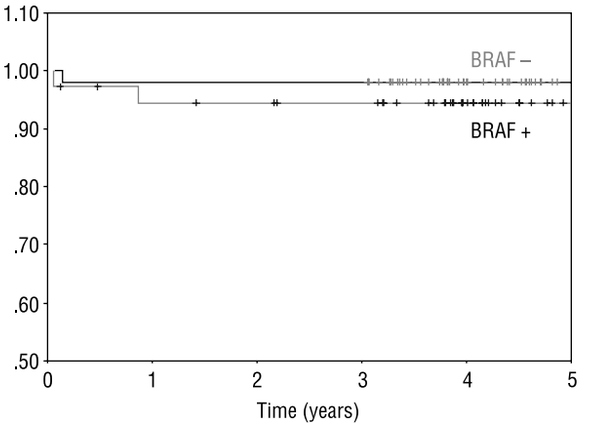

Figure 2. *Five-year disease-free survival according to BRAF status: BRAF– 98%, BRAF+ 94% (non significant)*

Rycina 2. *Prawdopodobieństwo przeżycia bezobjawowego w zależności od występowania mutacji BRAF. Pięcioletnie przeżycie bezobjawowe w grupie BRAF– 98% 5-letnie przeżycie bezobjawowe w grupie BRAF+ 94% (nz)*

Discussion

Differentiated thyroid cancers, and papillary and follicular thyroid carcinomas constitute the commonest endocrine malignancies, which, among other endocrine neoplasms, are characterized by the most favourable prognosis. However, about 10% DTC patients die of cancer and another 7–10% develop local recurrence or distant metastases [1, 3]. These data have remained unchanged for years, and the discussion concerning the optimal treatment strategy is still ongoing [2]. Supporters of the radical surgical approach believe that these operations reduce the risk of cancer recurrence, increase the effectiveness of radioiodine ablation, enable the use of thyroglobulin as a sensitive marker of local and distant metastases, and eliminate the possibility of DTC conversion to anaplastic thyroid carcinoma [3, 7]. Opponents of this strategy propose operation limited only to the tumour lobe with isthmus and/or subtotal resec-

Table II. ***Relation between BRAF mutation and clinico-pathological factors***

Tabela II. ***Obecność mutacji BRAF w guzie nowotworowym, a kliniczno-patologiczne czynniki ryzyka***

Factor	BRAF +38	BRAF –50	p
Women	35 (92%)	42 (84%)	ns
Men	3 (8%)	8 (16%)	
Mean age at time of surgery (years)	**51**	**42**	**p = 0.017**
Median	**55**	**40**	
Mean time of follow-up (years)	4	4	ns
Lymph node metastases	13 (34%)	18 (36%)	ns
Distant metastases	3 (8%)	1 (2%)	ns
Multifocality	11 (29%)	15 (30%)	ns
Extrathyroidal infiltration	11 (29%)	13 (26%)	ns

tion of the second lobe in low-risk patients. Moreover, some of them resign from the complementary radioiodine therapy [4, 8]. This conservative approach is related to the relatively indolent natural history of PTC and minimally invasive FTC to the possibility of the use of radioiodine ablation after less extensive surgery as well as to the decreased risk of complications.

Similar controversies concern the extent of neck lymphadenectomy in PTC [10, 11]. In recent years an increasing number of papers suggesting the safety of routine central lymph node dissection abandonment in low-risk patients have been published. The justification of this approach is connected to the increased probability of postoperative complications (higher than previously reported) and the lack of increased risk of relapse or cancer-related death, depending on the extent of the operation [11, 12]. The indications for lateral neck lymphadenectomy are also equivocal. Modified selective lymphadenectomy (after confirmation of metastases) are carried out most commonly. However, some centres suggest elective lymphadenectomy [14].

Currently, the most commonly recommended extent of surgery is total thyroidectomy and resection of central compartment neck lymph nodes in almost all patients [15]. Only reliable pre-operative prognostic factors allowing the selection of DTC cases with favourable/unfavourable prognosis would allow appropriate risk-orientated treatment tailoring. In this setting, one of the most intensively discussed molecular markers with both diagnostic and prognostic relevance in PTC is V600E BRAF mutation, which is present in 30–80% of papillary cancers (mean: 49% [16]).

Our preliminary data, although based on a relatively small group of 88 PTC patients, fit well into this range. According to the data published in recent years, the presence of BRAF mutation is associated with poorer disease prognosis [15]. A meta-analysis carried out by Lee et al. [16] including 1168 patients demonstrated the correlation of BRAF mutation and histological PTC subtype, extrathyroidal invasion, and clinical stage of the disease. However, what is very interesting, the relationship between BRAF mutation and these factors was not reported in other studies (for review see Handkiewicz et al. [15]). Age at PTC diagnosis was the most consistent clinical factor associated with BRAF status, according to this analysis. This was also confirmed in our study. The power and significance of an individual risk factor depended on the type on analysis (uni- or multivariate). In our univariate analysis we did not observe any correlation between BRAF status and lymph node metastases, but the number of our cases could be insufficient. While this correlation was reported by many groups, up to now no study has demonstrated the relationship between BRAF and the strongest unfavourable risk factors such as distant metastases [17–23]. Until now the impact of BRAF status on disease-free survival has been observed in a few studies only. One of them, including 102 patients with a median follow-up > 10 years, reported the association of BRAF mutation and overall survival [23].

In our dataset, the 5-year disease-free survival was slightly worse in the BRAF+ group; however, the differences between the groups were not significant. The most important limitation is the rare occurrence of disease relapse in our group of patients. That is why we reported not only the BRAF-analyzed subgroup, but the whole group of patients treated in the years 2004 to 2005. In the whole 2004–2005 cohort, the 5-year recurrence rate approached 5%, similarly to our earlier data [25]. Of course, it would be of benefit to have molecular support for prediction of which patients are at risk of lymph node metastases. However, the power of this prediction should exceed 90% to be of clinical value.

Summing up the analysis of the impact of BRAF mutation, it should be emphasized that actual published data have demonstrated its significance as a negative prognostic factor [19–23]. However, from a clinical point of view, the question of whether this mutation is a good predictive factor seems to be more appropriate. It is essential because more aggressive therapeutic approach — total thyroidectomy with central neck dissection followed by radioiodine treatment — is recommended for BRAF positive patients even if the tumour diameter is less than 1 cm. Nevertheless, at this low stage of the disease the prognosis is excellent, and 99% of cases showed no evidence of cancer recurrence even after incomplete thyroidectomy [25]. In our opinion, taking into consideration BRAF status only, the risk of inadequate, overly aggressive treatment in low risk DTC patients is high [15]. It is noteworthy that BRAF mutation may be diagnosed in up to 60–70% of microcarcinomas [26]. In our data the frequency of BRAF mutation in papillary microcancer is lower, at about 30% (data not shown).

On the other hand, the opposite question is whether in BRAF negative patients less extensive surgery is safe and not related to increased risk of cancer recurrence or cancer-related death. Certainly, the answer is negative in children, in whom the presence of BRAF mutation is rare [17] and the risk of distant metastases is high — even 20%. Consequently, it is currently too early to establish a therapeutic decision on the basis of BRAF status only. Probably, in order to create a good prognostic factor in PTC, not a single gene but a group of genes (gene signature) should be evaluated, and further studies are necessary.

Conclusions

Many studies document that BRAF mutation is a good prognostic factor in papillary thyroid cancer. However, our results indicate that it is too early to consider it as a routine clinical predictive factor.

References

1. Eustatia-Rutten CF, Crossmit EP, Biermasz NR et al. Survival and death causes in differentiated thyroid carcinoma. J Clin Endocrinol Metab 2006; 91: 313–319.
2. Clark OH, Duh QY. Thyroid cancer. Med Clin North Am 1991; 75: 211–234.
3. Mazzaferri EL. An overview of the management of papillary and follicular thyroid carcinoma. Thyroid 1999; 9: 421–426.
4. Hay ID, Bergstralh J, Goellner JR et al. Predicting outcome in papillary thyroid carcinoma: Development of a reliable prognostic scoring system in a cohort of 1779 patients surgically treated at one institution during 1940 through 1989. Surgery 1993; 114: 1050–1058.
5. Mazzaferri EL. Thyroid cancer and Graves' disease: the controversy ten years later. Endocr Pract 2000; 6: 221–225.
6. Mazzaferri EL, Kloos RT. Clinical review 128: current approaches to primary therapy for papillary and follicular thyroid cancer. J Clin Endocrinol Metab 2001; 86: 1447–1463.
7. Kebebew E, Clark OH. Differentiated thyroid cancer: „complete" rational approach. World J Surg 2000; 24: 942–951.
8. Beenken S, Roye D, Weiss H et al. Extent of surgery for intermediate-risk well-differentiated thyroid cancer. Am J Surg 2000; 179: 51–56.
9. Mann B, Buhr HJ. Lymph node dissection in patients with differentiated thyroid carcinoma — who benefits? Langenbecks Arch Surg 1998; 383: 355–338.
10. Mathonnet M. Lymph node dissection in non-medullary differentiated thyroid carcinoma Ann Chir 2006; 131: 361–368.
11. Palestini N, Borasi A, Cestino L et al. Is central neck dissection a safe procedure in the treatment of papillary thyroid cancer? Our experience. Langenbecks Arch Surg 2008; 393: 693–698.
12. Vorländer C, Lienenlüke RH, Wahl RA. Lymph node dissection in papillary and follicular thyroid cancer Chirurg 2008; 79: 564–5670.
13. Gimm O, Dralle H. Lymphadenectomy for thyroid and lymph node carcinomas Chirurg 2007; 78: 182, 184–188, 190–193.
14. Diagnostyka I leczenie raka tarczycy. Rekomendacje Polskiej Grupy do spraw Nowotworów Endokrynnych. Med Prak 2006; 11: 133–157
15. Handkiewicz-Junak D, Czarniecka A, Jarząb B. Molecular prognostic markers in papillary and follicular thyroid cancer: Current status and future directions. Molecular and Cellular Endocrinology 2010; 322: 8–28.
16. Lee JH, Lee ES, Kim YS. Clinicopathologic significance of BRAF V600E mutation in papillary carcinomas of the thyroid: a meta-analysis. Cancer 2007; 110: 38–46.
17. Rosenbaum E, Hosler G, Zahurak M et al. Mutational activation of BRAF is not a major event in sporadic childhood papillary thyroid carcinoma. Mod Pathol 2005; 18: 898–902.
18. Kim J, Giuliano AE, Turner RR et al. Lymphatic mapping establishes the role of BRAF gene mutation in papillary thyroid carcinoma. Ann Surg 2006; 244: 799–804.
19. Kebebew E, Weng J, Bauer J et al. The prevalence and prognostic value of BRAF mutation in thyroid cancer. Ann Surg 2007; 24: 466–470, discussion 470–471.
20. Lupi C, Giannini R, Ugolini C et al. Association of BRAF V600E mutation with poor clinicopathological outcomes in 500 consecutive cases of papillary thyroid carcinoma. J Clin Endocrinol Metab 2007; 92: 4085––4090.
21. Elisei R, Ugolini C, Viola D et al. BRAF(V600E) mutation and outcome of patients withpapillary thyroid carcinoma: a 15-year median follow-up study. J Clin Endocrinol Metab 2008; 93: 3943–3949.
22. Costa AM, Herrero A, Fresno MF et al. BRAF mutation associated with other genetic events identifies a subset of aggressive papillary thyroid carcinoma Clin Endocrinol 2008; 68: 618–634.
23. Ito Y, Yoshida H, Maruo R et al. BRAF mutation in papillary thyroid carcinoma in a Japanese population: its lack of correlation with high-risk clinicopathological features and disease-free survival of patients. Endocr J 2009; 56: 89–97.
24. Handkiewicz-Junak D, Wloch J, Roskosz J et al. Total thyroidectomy and adjuvant radioiodine treatment independently decrease locoregional recurrence risk in childhood and adolescent differentiated thyroid cancer J. Nucl Med 2007; 48: 879–888.
25. Czarniecka A, Jarząb M, Krajewska J et al. The impact of the extent and time of operation on the survival in patients with differentiated thyroid carcinoma (DTC). Endokrynol Pol 2006; 57: 347–355.
26. Park YJ, Kim YA, Lee YJ et al. Papillary microcarcinoma in comparison with larger papillary thyroid carcinoma in BRAF(V600E) mutation, clinicopathological features, and immunohistochemical findings. Head Neck 2010; 32: 38–45.
27. Jarząb B, Handkiewicz-Junak D. Differentiated thyroid cancer in children and adults: same or distinct disease? Hormones (Athens) 2007; 6: 200–209.