DOI: 10.31793/1680-1466.2024.29-2.101

Effect of Ki67 labeling index on clinicohistopathological characteristics of radiogenic and sporadic papillary thyroid carcinoma with regard to the BRAF^{V600E} mutational status

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Abstract. In papillary thyroid carcinomas (PTCs), Ki67 labeling index (LI) is a prognosticator of metastases and recurrence, but it is not clear whether this extends to patients exposed to radiation in childhood. The aim of this work was to determine whether certain associations exist between Ki67 LI and clinicohistopathological characteristics of radiogenic and sporadic PTCs removed from patients of different ages and whether possible associations depend on the BRAFV600E status. **Material and methods.** Analysis of clinical histopathological and immunohistochemical (IHC) data on 552 PTCs (416 radiogenic and 136 sporadic) was performed using univariate tests and multivariate statistical modeling. **Results.** In radiogenic PTCs from patients aged up to 29 years, an increase in Ki67 LI was associated with the higher frequency of dominant papillary growth pattern (odds ratio (OR)=1.208, p=5.34E-04), *BRAF^{V600E}* mutation (OR=1.183, p=0.007) and oncocytic changes (OR=1.120, p=0.044), and with the risk of recurrence (hazard ratio (HR)=1.249, p=0.033). An increase in Ki67 LI in the BRAF^{V600E}-positive tumors did not lead to significant changes in pathological and clinical PTCs characteristics, whereas in the BRAF^{V600E}-negative tumors it was associated with the risk of recurrent metastases (HR=1.227, p=0.038), including the radioiodine refractory ones (RAI-R, OR=1.551, p=0.037). The effect of Ki67 LI on

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the mentioned characteristics of sporadic PTC in the same age group was similar for most variables, but was absent for the risk of recurrences both in the whole group and in the $BRAF^{V600E}$-positive or $BRAF^{V600E}$-negative PTCs. In patients aged up to 49 years with radiogenic PTC, the effects of Ki67 LI were in line with those determined in younger patients. **Conclusions.** In radiogenic PTCs, unlike in sporadic tumors, an increase in Ki67 LI was associated with a worse postoperative prognosis, namely with an increase in the risk of recurrent metastases, including RAI-R, which, in turn, was associated exclusively with the $BRAF^{V600E}$-negative status. In the $BRAF^{V600E}$-positive PTCs, regardless of their etiology, an increase in Ki67 LI did not affect the clinical and histopathological indicators and the prognosis.

Keywords: papillary thyroid carcinoma, Chornobyl accident, Ki67 proliferative index, *$BRAF^{V600E}$* mutation, immunohistochemical study.

Ki67 (MIB-1) is the most common marker of proliferative activity of various tumors widely used in diagnostic pathology. As for PTCs, a number of studies have shown that their proliferative activity, namely the Ki67 LI, is associated with a larger tumor size [1-4] and has a significant prognostic power for regional metastases and recurrence [5-8]. However, whether these associations hold true for PTCs from patients exposed to ionizing radiation in childhood remains unknown.

In our previous works [9-11], which involved IHC study of the $BRAFV^{600E}$ mutant protein expression in radiogenic and sporadic PTCs from patients of different ages, the frequency of RAI-R recurrent metastases was found to be increased in older patients with the $BRAF^{V600E}$-positive PTCs [11]. The available literature data regarding the relationship of Ki67 LI with *$BRAF^{V600E}$* are contradictory. Some groups claimed a higher Ki67 LI in the $BRAF^{V600E}$-positive PTCs [1, 12], whereas others observed no difference in Ki67 LI between the $BRAF^{V600E}$-positive and the $BRAF^{V600E}$-negative PTCs [13]. To date, no reports addressing the relationship between Ki67 LI and BRAF mutational status are available for patients with a history of radiation exposure.

In the current study, we aimed to determine whether there are associations between Ki67 LI and clinicohistopathological characteristics of radiogenic and sporadic PTCs removed from patients of different ages and whether such possible associations depend on the $BRAF^{V600E}$ status.

Material and methods

Patients

Histopathological and IHC studies were performed on PTCs removed from 552 patients aged up to 49 years (416 radiogenic and 136 sporadic) operated on at the State Institution «V.P. Komisarenko Institute of Endocrinology and Metabolism of the National Academy of Medical Sciences of Ukraine» (IEM) during the period from 1990 to 2017, and which were IHC stained with antibodies to the mutated $BRAF^{V600E}$ protein (VE1) and Ki67 (MIB-1).

In the first two parts of the study, 236 radiogenic PTCs (patients aged up to 4 years at the time of the Chornobyl accident who lived in the most contaminated by ^{131}I Kyiv, Chernihiv and Zhytomyr regions) and 136 sporadic PTC (patients who lived in the same regions but born after January 1st, 1987, thus not exposed to ^{131}I) were analyzed, respectively. Considering the fact that patients with sporadic PTCs at the beginning of the study were aged up to 29 years, the radiogenic group in the first part of the work also included only patients of a similar age group.

For the third part of the study, 180 cases from patients aged 29 to 49 years old, operated in the IEM from 2015 to 2017, who were at the time of the accident in childhood and lived in the northern, most contaminated by ^{131}I regions of Ukraine, were added to the 236 radiogenic PTCs from the first part; in total, there were 416 cases of radiogenic PTCs.

The study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the Bioethics Committee of IEM (protocols N 22-KE of April 26, 2018, and N 31-KE of February 27, 2020), the Chornobyl Tissue Bank (CTB, project N001-2020), and the Ethics Committee of Nagasaki University (protocol 20130401–7 of July 1, 2021, the latest update). Informed consent was obtained from all patients enrolled in the study or their guardians.

Histopathology

As in previous studies [9-11], the initial analysis of hematoxylin and eosin-stained histological speci-

mens, and establishment of PTC diagnosis was performed in the IEM by two experienced pathologists (TB and LZ). The pathological diagnosis was based on the 4th edition of the WHO Histological Classification [14]. The vast majority of cases were additionally reviewed by a pathology panel of the Chornobyl tissue bank project [15, 16]. The diagnosis of PTC was confirmed in all cases. pTNM categories were determined according to the latest 8th edition of tumor TNM classification [17].

PTCs were evaluated by size, dominant histological growth pattern, oncocytic changes, main invasive and clinical characteristics, as described in previous publications [9-11, 18]. To assess overall invasiveness, we used an integrative variable, the «invasiveness score» [11, 19], which included N1, M1, extrathyroidal extension (ether minimal or to the muscle), multifocal tumor growth and lymphatic/vascular invasion. The invasiveness score determined in this way ranged from «0» (no sign of invasiveness) to «5» (all the above signs present). Recurrent metastases examined after reoperation not earlier than 6 months after the 1st surgery were evaluated for being radioiodine-avid or RAI-R according to the recommendations of the American and European Thyroid Associations [20-22].

Immunohistochemistry

IHC studies were performed at Nagasaki University according to the protocol developed in the Department of Radiation Molecular Epidemiology of the Atomic Bomb Diseases Institute of the Nagasaki University (LZ, TR). IHC staining to determine $BRAF^{V600E}$ expression was performed as previously described [9-11]. A positive IHC reaction with antibodies to $BRAF^{V600E}$ was consistent with the presence of the *$BRAF^{V600E}$* mutation [23].

The proliferative activity of tumors was evaluated by IHC using a Ki67 antibody (clone MIB-1; DAKO, Glostrup, Denmark, 1:100 dilution) in a Ventana BenchMark ULTRA instrument. The Ki67 LI (Ki67 LI) was determined with the image-analyzing software (CountoCell, Ki67 antigen Semi-Auto Counter, Seiko Tec LTD, Fukuoka, Japan) in a total of approximately 1,000 PTC cells per case (LZ).

Thyroid dosimetry

Individual thyroid radiation doses due to ^{131}I ingestion after the Chornobyl accident were calculated for each patient at the Radiation Protection Laboratory of the State Institution «National Scientific Center of Radiation Medicine, Hematology and Oncology of the National Academy of Medical Sciences of Ukraine» using an ecological and dosimetric model which combines the system of ecological transport of iodine and biokinetic models of iodine [24].

Probability of causation due to radiation

The probability of causation (POC) of a tumor by exposure to a known radiation dose of an individual of a given sex and age after a certain period of latency was determined using the US NIH/NCI Division of Cancer Epidemiology and Genetics' Interactive RadioEpidemiological Program – Probability of Cancer Causation from Radiation Version 5.7.1 software [25, 26]. This software, as mentioned in our previous works [11, 27], uses «Personal Information» and «Dose Exposure Information» and calculates POC ranging from the 1st to the 99th percentile based on 10,000 random-seeded simulations. POC estimate in this study is the 50th percentile value. The higher POC value reflects the higher likelihood of cancer development due to radiation exposure.

Statistical analysis

Logistic regression models were adjusted for age at operation and sex. Models with very small numbers of outcomes (<5 per cell) were conducted using Firth's approach to bias-reducing penalized maximum likelihood fit. To plot estimated probabilities obtained in the logistic regression models (SAS PROC LOGISTIC), the PROC LOESS was used for smoothing. Multivariable linear regression models were applied to continuous dependent variables. Effect of Ki67 LI on disease-free survival was estimated using the Kaplan-Meier method and the proportional hazard models.

Calculations were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA) or IBM SPSS Statistics Version 24 software (International Business Machines Corp., Armonk, NY, USA). All tests were two-sided; $p<0.05$ was considered indicative of statistical significance.

Results and discussion

1. The effect of Ki67 LI on radiogenic PTCs from patients aged up to 29 years

All data collected or generated for young patients with radiogenic PTCs are shown in **Fig. 1**. Among the 236 analyzed radiogenic PTCs, the median Ki67 LI was 2.6% (IQR=1.5-4.5%) with a significant prevalence of the frequency of tumors

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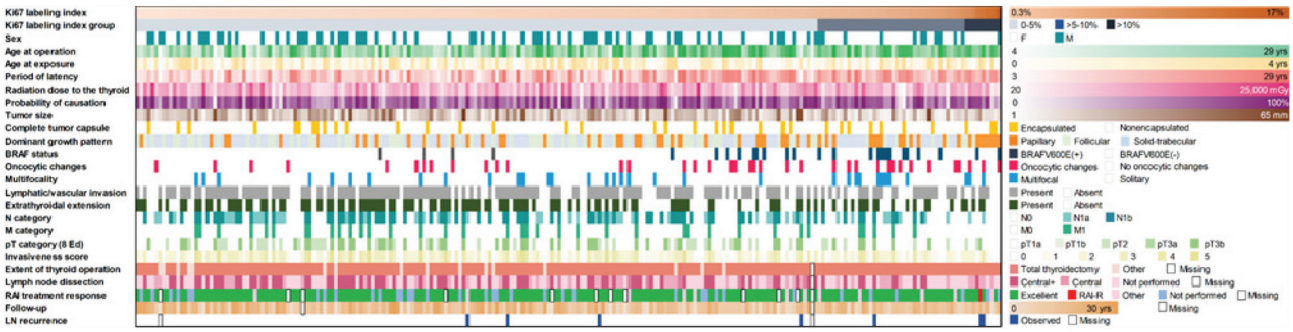

Fig. 1. Characteristics of 236 radiogenic PTCs from patients aged up to 29 years.

in which Ki67 LI⩽5% (78.8% *vs* 21.2% with Ki67 LI>5%, p<0.001).

Descriptive characteristics of the group of radiogenic PTCs were as follows: the median age of patients at the time of surgery was 16.0 (IQR=12.8-22.8) years, the median tumors size was 16 (IQR=12-30) mm, the most frequent dominant solid-trabecular growth pattern was observed was in 44.1% of tumors, the frequency of the *BRAF*^{V600E} mutation was rather low, 11.0%, the median invasiveness score was 2 (IQR=1-3). Most patients (93.2%) underwent total thyroidectomy and postoperative radioiodine therapy (RIT, 79.7%). The frequency of recurrent metastases was low, 3.3%, and they were removed 0.8-9.4 years after the primary operation (**Table 1**).

The analysis of Ki67 LI effect on the clinicohistopathological characteristics of radiogenic PTCs, which included univariate logistic, linear, and Cox proportional hazard regression models, established that Ki67 LI was statistically significantly associated with an increase in patient age at the time of surgery (b=0.169, p=9.48E-06), more frequent dominant papillary (OR=1.208, p=5.34E-04) and less frequent follicular growth pattern pat-

Table 1. Characteristics of 236 radiogenic PTCs from patients aged up to 29 years by Ki67 LI.

Parameters	Number (%) or median (range; IQR)	OR, b or HR (95%CI)[a]	p-value
Sex F/M (%M, F:M ratio)	156/80 (33.9%; 2.0:1)	0.942 (0.846-1.048)[b]	0.271
Age at operation, years	16.0 (4.2-28.9; 12.8-22.8)	0.169 (0.095-0.242)[c]	9.48E-06
Age at exposure, years	2.0 (0.1-4.0; 1-3)	-0.013 (-0.052-0.025)[c]	0.495
Period of latency, years	14.0 (3.8-28.2; 10.0-20.7)	0.294 (0.168-0.420)[c]	6.81E-06
Radiation dose to the thyroid, mGy	319 (28-24110; 160-712)	-0.012 (-0.037- 0.014)[c]	0.381
POC, %	70.7 (8.1-99.5; 53.5-84.4)	-0.248 (-1.181-0.684)[d]	0.6
Tumor size, mm	16 (1-65; 12-30)	-0.031 (-0.076-0.014)	0.179
Complete tumor capsule	36 (15.3%)	1.004 (0.886-1.137)	0.955
Dominant growth pattern		1.146 (1.072-1.224)	5.57E-05
papillary	54 (22.9%)	1.208 (1.085-1.334)	5.34E-04
follicular	78 (33.1%)	0.889 (0.791-0.999)	0.048
solid-trabecular	104 (44.1%)	0.939 (0.848-1.039)	0.221
Ki67 LI, %	2.6 (0.3-16.6; 1.5-4.5)	NA[e]	NA
0–5%	186 (78.8%)	NA	NA
>5–10%	40 (16.9%)	NA	NA

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Parameters	Number (%) or median (range; IQR)	OR, b or HR (95%CI)[a]	p-value
>10%	10 (4.2%)	NA	NA
$BRAF^{V600E}$-positive	26 (11.0%)	1.183 (1.046-1.338)	0.007
Oncocytic changes	38 (16.1%)	1.120 (1.003-1.250)	0.044
Multifocality	34 (14.4%)	1.016 (0.897-1.151)	0.803
Lymphatic/vascular invasion	230 (55.3%)	0.952 (0.861-1.052)	0.335
Extrathyroidal extension (any)	160 (67.8%)	0.946 (0.857-1.044)	0.27
N category (N1)	124 (52.5%)	0.907 (0.820-1.003)	0.057
M category (M1)	37 (15.7%)	0.947 (0.811-1.106)	0.489
Invasiveness score	2 (0-5; 1-3)	0.938 (0.863-1.020)	0.133
Thyroid surgery volume, n=235			
total thyroidectomy	219 (93.2%)	1.810 (1.143-2.866)	0.011
organ-preserving operation	16 (6.8%)	0.552 (0.349-0.875)	0.011
Lymph node dissection, n=235	136 (57.9%)	0.984 (0.895-1.082)	0.74
RIT performed	188 (79.7%)	1.026 (0.907-1.159)	0.686
RIT response, n=188		0.799 (0.680-0.939)	0.006
RAI-R recurrence *vs* other	1 (0.5%)	1.509 (1.055-2.159)[f]	0.024
excellent *vs* other	175 (93.1%)	0.818 (0.693-0.966)	0.018
Follow-up, years, n=234	13.2 (0.01-27.9; 8.5-18.2)	0.076 (-0.184-0.336)	0.566
LN recurrence, n=214	7 (3.3%)	1.249 (1.018-1.532)[g]	0.033
LN recurrence, n=214	7 (3.3%)	1.259 (1.078-1.470)[h]	0.004
Time to recurrence, years	n=7; 3.0 (0.8-9.4; 1.0-8.3)	0.002 (-1.191-1.194)	0.997

Note: [a] – adjusted for age at operation and sex unless otherwise specified; [b] – adjusted for age at operation; [c] – adjusted for sex; [d] – non-adjusted; [e] – not available; [f] – Firth's penalized logistic regression; [g] – Cox proportional hazard model adjusted for patient age, sex, thyroid surgery volume and lymph node dissection; [h] – non-adjusted Cox proportional hazard model.

terns (OR=0.889, p=0.048), $BRAF^{V600E}$-positivity (OR=1.183, p=0.007) and oncocytic changes in PTC cells (OR=1.120, p=0.044). At the same time, Ki67 LI did not significantly affect any indicator of invasiveness. Of importance, Ki67 LI reduced the frequency of complete remission of the disease in response to RIT (OR=0.818, p=0.018) and increased the chance of recurrence (HR=1.249, p=0.033, **see Table 1**).

At the next step, we subdivided radiogenic PTCs by their $BRAF^{V600E}$ status. First of all, it should be noted that Ki67 LI in the $BRAF^{V600E}$-positive tumors (4.8%, IQR=3.8-6.3%) significantly exceeded that in the $BRAF^{V600E}$-negative tumors (2.3%, IQR=1.3-3.9%, p=7.64E-06, the Mann-Whitney test).

Analysis of the effect of Ki67 LI in the $BRAF^{V600E}$-positive and -negative subgroups of radiogenic PTCs (**Table 2**) did not detect Ki67 LI association with structural, invasive and clinical characteristics in the former, while in the latter it was statistically significantly associated with such important clinical indicators as the probability of developing recurrent metastases (HR=1.227, p=0.038), including the RAI-R metastases (OR=1.551, p=0.037).

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2. The effect of Ki67 LI on sporadic PTCs from patients aged up to 29 years

All data collected or generated for young patients with sporadic PTCs are shown in **Fig. 2**.

Among the 136 sporadic PTCs, the median Ki67 LI was 4.7% (IQR=2.8-7.2%) with a slight prevalence of tumors with Ki67⩽5%, 55.9% *vs.* 44.1% of those with Ki67 LI>5%.

The median age of patients with sporadic PTC at the time of surgery was 17.5 (IQR=14.5-22.3) years, tumors size was 16 (IQR=10-28) mm. Almost a half (47.1%) of PTCs had a dominant papillary growth pattern. The frequency of the *BRAF*^{V600E} mutation was 29.4%, oncocytic changes in tumor cells were observed in 45.6% of tumors, which appears to be higher than in radiogenic PTCs. Invasiveness score was somewhat lower that in the radiogenic PTCs, of 1 (IQR=0-3). Most patients, as in the radiogenic group, underwent total thyroidectomy (85.3%) and postoperative RIT (79.4%). The recurrence rate was low, 2.9%; recurrent metastases were removed 0.7-6.3 years after the primary operation (**Table 3**).

Ki67 LI in sporadic PTCs, in contrast to radiogenic PTCs, was statistically significantly associated with a decrease in patient age at the time of surgery (b=-0.245, p=0.029), but similarly to radiogenic PTCs, it was associated with a decrease in tumor size (b=-0.310, p=0.001), an increase in the frequency of dominant papillary growth pattern (OR=1.133, p=0.018), *BRAF*^{V600E} mutation (OR=1.179, p=0.003) and oncocytic changes in

Table 2. Effect of Ki67 LI on clinicopathological characteristics in the BRAF^{V600E}-positive and BRAF^{V600E}-negative radiogenic PTCs from patients aged up to 29 years.

Parameters	BRAF^{V600E}-positive, n=26		BRAF^{V600E}-negative, n=210	
	b, OR or HR (95%CI)[a]	p-value	b, OR or HR (95%CI)[a]	p-value
Tumor size, mm	0.016 (-0.124-0.156)	0.813	-0.023 (-0.073-0.028)	0.375
Dominant growth pattern	1.031 (0.723-1.471)	0.865	0.901 (0.817-0.993)	0.036
Oncocytic changes	1.083 (0.810-1.449)	0.591	1.107 (0.977-1.255)	0.110
Multifocality	1.252 (0.861-1.822)	0.24	0.992 (0.859-1.145)	0.912
Lymphatic/vascular invasion	0.754 (0.502-1.130)	0.172	1.011 (0.901-1.134)	0.858
Extrathyroidal extension (any)	0.834 (0.491-1.416)	0.501	0.984 (0.883-1.096)	0.766
N category (N1)	0.651 (0.378-1.122)	0.122	0.946 (0.848-1.056)	0.321
N1a	0.863 (0.580-1.284)	0.467	0.007 (0.881-1.150)	0.923
N1b	0.224 (0.031-1.595)	0.135	0.930 (0.819-1.055)	0.257
M category (M1)	0.770 (0.198-2.998)	0.706	0.974 (0.831-1.143)	0.749
Invasiveness score	0.802 (0.573-1.122)	0.198	0.978 (0.892-1.072)	0.634
Total thyroidectomy			1.713 (1.063-2.759)	0.027
Lymph node dissection	0.921 (0.680-1.246)	0.593	1.018 (0.915-1.132)	0.742
RIT performed	1.117 (0.826-1.512)	0.472	1.061 (0.917-1.228)	0.423
RAI-R recurrence vs other	NA[b] (n=0)	NA	1.551 (1.027-2.341)	0.037
Excellent RIT response vs other	1.068 (0.598-1.906)	0.824	0.804 (0.675-0.958)	0.014
LN recurrence	NA	NA	1.227 (1.005-1.499)[c]	0.038
LN recurrence	NA	NA	1.343 (1.114-1.609)[d]	0.001

Note: [a] – adjusted for age at operation and sex unless otherwise specified; [b] – not available; [c] – Cox proportional hazard model adjusted for patient age, sex, thyroid surgery volume and lymph node dissection; [d] – non-adjusted Cox proportional hazard model.

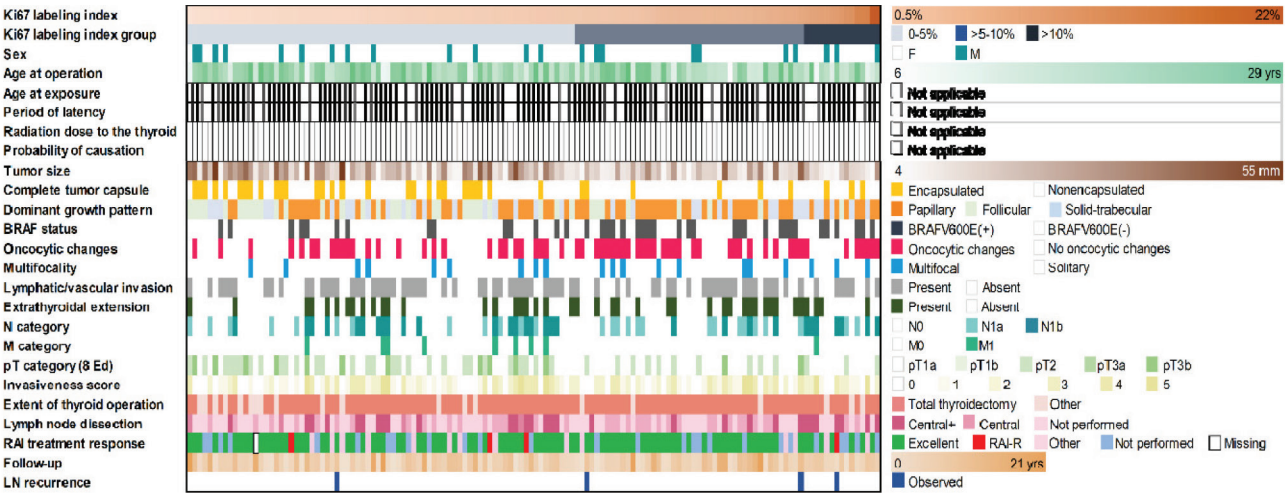

Fig. 2. Characteristics of 136 sporadic PTCs from patients aged up to 29 years

Table 3. Characteristics of 136 sporadic PTCs from patients aged up to 29 years by Ki67 LI.

Parameters	Number (%) or median (range; IQR)	OR, b or HR (95%CI)[a]	p-value
Sex F/M (%M, F:M ratio)	113/23 (16.9%; 4.9:1)	0.996 (0.883-1.125)[b]	0.955
Age at operation, years	17.5 (6.8-28.0; 14.5-22.3)	-0.245 (-0.465-0.026)[c]	0.029
Tumor size, mm	16 (4-55; 10-28)	-0.310 (-0.486-0.135)	0.001
Complete tumor capsule	37 (27.2%)	0.853 (0.739-0.985)	0.030
Dominant growth pattern		0.928 (0.847-1.016)	0.108
papillary	64 (47.1%)	1.133 (1.021-1.257)	0.018
follicular	37 (27.2%)	0.749 (0.626-0.896)	0.002
solid-trabecular	35 (25.7%)	1.018 (0.921-1.126)	0.721
Ki67 LI, %	4.7 (0.5-22.2; 2.8-7.2)	NA[d]	NA
0–5%	76 (55.9%)	NA	NA
>5–10%	45 (33.1%)	NA	NA
>10%	15 (11.0%)	NA	NA
$BRAF^{V600E}$-positive	40 (29.4%)	1.179 (1.056-1.316)	0.003
Oncocytic changes	62 (45.6%)	1.137 (1.024-1.263)	0.016
Multifocality	20 (14.7%)	1.081 (0.958-1.220)	0.206
Lymphatic/vascular invasion	75 (55.1%)	0.964 (0.873-1.064)	0.465
Extrathyroidal extension (any)	43 (31.6%)	0.991 (0.896-1.097)	0.867
N category (N1)	57 (41.9%)	1.057 (0.961-1.162)	0.256
M category (M1)	9 (6.6%)	0.846 (0.660-1.085)	0.189
Invasiveness score	1 (0-4; 0-3)	1.013 (0.933-1.099)	0.761
Thyroid surgery volume			
total thyroidectomy	116 (85.3%)	1.102 (0.937-1.295)	0.240
organ-preserving operation	20 (14.7%)	0.907 (0.772-1.067)	0.024
Lymph node dissection	73 (53.7%)	1.033 (0.936-1.139)	0.519

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Parameters	Number (%) or median (range; IQR)	OR, b or HR (95%CI)[a]	p-value
RIT performed, n=135	108 (79.4%)	1.060 (0.932-1.206)	0.372
RIT response, n=108		1.056 (0.900-1.240)	0.501
RAI-R recurrence vs other	4 (3.7%)	1.076 (0.876-1.322)	0.486
excellent vs other	90 (83.3%)	1.060 (0.901-1.247)	0.482
Follow-up, years	5.4 (0.01-21.0; 2.6-8.8)	0.112 (-0.654-0.878)	0.774
LN recurrence, n=126	4 (2.9%)	1.202 (0.908-1.536)[e]	0.135
LN recurrence, n=126	4 (2.9%)	1.174 (0.926-1.490)[f]	0.186
Time to recurrence, years	n=4; 1.3 (0.7-6.3; 0.8-4.0)	0.109 (NA-NA)	NA

Note: [a] – adjusted for age at operation and sex unless otherwise specified; [b] – adjusted for age at operation; [c] – adjusted for sex; [d] – not available; [e] – Cox proportional hazard model adjusted for patient age, sex, thyroid surgery volume and lymph node dissection; [f] – non-adjusted Cox proportional hazard model.

PTC cells (OR=1.137, p=0.016). Ki67 LI in sporadic PTCs, in agreement with observations in radiogenic tumors, did not affect any of the indicators of invasiveness, but in contrast to radiogenic PTCs, it did not reduce the frequency of complete remission (excellent *vs* other) in response to RIT (OR=1.060, p=0.482) and did not statistically significantly increase the chance of recurrence (HR=1.202, p=0.135, see **Table 3**).

Similarly to radiogenic PTCs, Ki67 LI in sporadic tumors was statistically significantly higher in the $BRAF^{V600E}$-positive PTCs (5.9%, IQR=4.3-8.7) than in the BRAFV^{600E}-negative tumors (4.1%, IQR=2.5-6.9, p=0.003).

Analysis of the Ki67 LI effects in the $BRAF^{V600E}$-positive and -negative subgroups of sporadic PTCs (**Table 4**) demonstrated that it was not associated with the structural, invasive and clinical characteristics, which is concordant with results in the radiogenic PTCs. Ki67 LI was also not associated with recurrence both in the $BRAF^{V600E}$-positive or in $BRAF^{V600E}$-negative PTCs despite the fact that three of the four recurrent sporadic PTCs, as shown in our previous studies [9, 10], were $BRAF^{V600E}$-positive. The only parameter that depended on Ki67 LI in sporadic PTCs was the increasing frequency of oncocytic changes in the $BRAF^{V600E}$-negative PTC cells (OR=1.160, p=0.047).

Table 4. Effect of Ki67 LI on clinicopathological characteristics in the $BRAF^{V600E}$-positive and $BRAF^{V600E}$-negative sporadic PTCs from patients aged up to 29 years.

Parameters	$BRAF^{V600E}$-positive, n=40		$BRAF^{V600E}$-negative, n=96	
	b, OR or HR (95%CI)[a]	p-value	b, OR or HR (95%CI)[a]	p-value
Tumor size, mm	-0.702 (-1.418-0.014)	0.054	-0.491 (-1.268-0.284)	0.211
Dominant growth pattern	0.860 (0.647-1.098)	0.225	0.989 (0.884-1.108)	0.854
Oncocytic changes	1.019 (0.866-1.199)	0.819	1.160 (1.002-1.342)	0.047
Multifocality	0.989 (0.791-1.237)	0.923	1.123 (0.942-1.139)	0.194
Lymphatic/vascular invasion	0.898 (0.747-1.078)	0.248	1.085 (0.934-1.261)	0.284
Extrathyroidal extension (any)	0.924 (0.751-1.137)	0.453	1.013 (0.886-1.157)	0.855
N category (N1)	0.942 (0.783-1.133)	0.526	1.127 (0.987-1.286)	0.077
N1a	0.962 (0.777-1.192)	0.723	0.999 (0.840-1.190)	0.995
N1b	0.924 (0.686-1.245)	0.604	1.139 (0.997-1.302)	0.056
M category (M1)	0.972 (0.746-1.268)[b]	0.706	0.872 (0.669-1.135)	0.307

End of the table 4

Parameters	$BRAF^{V600E}$-positive, n=40		$BRAF^{V600E}$-negative, n=96	
	b, OR or HR (95%CI)[a]	p-value	b, OR or HR (95%CI)[a]	p-value
Invasiveness score	0.909 (0.785-1.054)	0.207	1.069 (0.958-1.194)	0.233
Total thyroidectomy	0.736 (0.482-1.101)	0.136	1.204 (0.939-1.542)	0.143
Lymph node dissection	0.895 (0.741-1.082)	0.253	1.111 (0.967-1.277)	0.138
RIT performed	0.856 (0.666-1.102)	0.228	1.079 (0.915-1.273)	0.366
RAI-R recurrence vs other	0.987 (0.748-1.302)	0.928	1.547 (0.831-2.878)	0.168
Excellent RIT response vs other	1.168 (0.816-1.671)	0.396	1.005 (0.826-1.223)	0.957
LN recurrence	0.978 (0.545-2.052)	0.941	1.395 (0.808-5.039)[c]	0.432
LN recurrence	1.051 (0.679-1.411)[c]	0.778	3.981 (0.864-93.085)[d]	0.249

Note: [a] – adjusted for age at operation and sex unless otherwise specified; [b] – Firth's penalized logistic regression; [c] – Firth's penalized Cox proportional hazard model; [d] – Firth's penalized non-adjusted Cox proportional hazard model.

3. The effect of Ki67 LI on radiogenic PTCs from patients aged up to 49 years

All data collected or generated for patients aged up to 49 years with radiogenic PTCs are shown in **Fig. 3**.

Expansion of the radiogenic group size to 416 individuals by inclusion of patients aged at the time of surgery from 29 to 49 years (**Table 5**) led to an increase in their median age at operation to 26.5 (IQR=15.3-36.5) years, age at the time of the accident to 3.0 (IQR=1.0-7.0) years, latency period to 24.2 (IQR=13.0-29.6) years, and also to a decrease in ^{131}I thyroid dose to 172 (IQR=63-454.0) mGy and POC level to 53.8 % (IQR=23.9-77.8%) as compared to the radiogenic group of younger patients (see **Table 1**). In line with our previous studies [11, 28], the longer latency period was associated with a higher frequency of PTCs with the dominant papillary growth pattern, oncocytic changes and the *$BRAF^{V600E}$* mutation (up to 32.5%), and with a lower frequency of invasive features. Thyroidectomy (92.5%) followed by RIT (79.3%) remained the main treatment approach. The recurrence rate remained virtually unchanged (3.4%), metastases were removed 0.7-9.4 years after primary surgery. Note however that the frequency of RAI-R metastases increased from 0.5% to 2.4% (see **Tables 1, 5**).

Analysis of the Ki67 LI effects on the clinicohistopathological characteristics of radiogenic PTCs from patients aged up to 49 years is shown in **Table 5** and **Fig. 4-6**. Ki67 LI was positively associated with age at the time of surgery, namely with adult patients aged 25 and older (**Fig. 4A**) and/or with adolescent and adult age groups (**Fig. 4B**), with age over 7 years at the time of the Chornobyl accident (**Fig. 4C**) and period of latency of 15-30 years (**Fig. 4D**). The probability of detecting radiogenic PTCs with Ki67 LI>10% in childhood cases was extremely low, approximately 5% (see **Fig. 4A, B**). Note that the probability of detecting PTCs in children aged up to 15 years in the sporadic group with Ki67 LI>10% was substantially higher – up to 60% (data

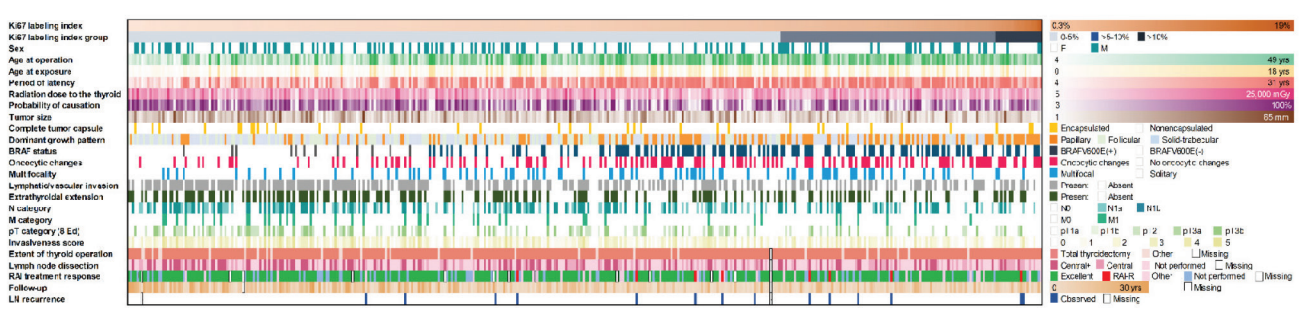

Fig. 3. Characteristics of 416 radiogenic PTCs from patients aged up to 49 years

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Table 5. Characteristics of 416 radiogenic PTCs from patients aged up to 49 years by Ki67 LI.

Parameters	Number (%) ormedian (range; IQR)	OR, b or HR (95%CI)[a]	p-value
Sex F/M (%M, F:M ratio)	292/124 (29.8%; 2.4:1)	0.960 (0.889-1.037)[b]	0.302
Age at operation, years	26.5 (4.2-49.1; 15.3-36.5)	0.261 (0.176-0.347)[c]	4.39E-09
Age at exposure, years	3.0 (1-18.6; 1.0-7.0)	0.040 (0.000-0.080)[c]	0.051
Period of latency, years	24.2 (3.8-31.2; 13.0-29.6)	0.407 (0.290-0.525)[c]	2.97E-11
Radiation dose to the thyroid, mGy	172 (6-24110; 63-454)	-0.036 (-0.060-0.011)[c]	0.004
POC, %	53.8 (1.3-99.5; 23.9-77.8)	-1.391 (-2.355-0.427)[d]	0.005
Tumor size, mm	15 (1-65; 10-24)	-0.054 (-0.085-0.023)	0.001
Complete tumor capsule	36 (8.7%)	1.029 (0.916-1.156)	0.626
Dominant growth pattern		1.146 (1.072-1.224)	5.57E-05
papillary	134 (32.2%)	1.210 (1.120-1.306)	1.00E-06
follicular	112 (26.9%)	0.855 (0.775-0.944)	0.002
solid-trabecular	170 (40.9%)	0.933 (0.867-1.003)	0.061
Ki67 LI, %	3.3 (0.3-18.7; 1.9-5.3)	NA	NA
0–5%	297 (71.4%)	NA	NA
>5–10%	98 (23.6%)	NA	NA
>10%	21 (5.0%)	NA	NA
$BRAF^{V600E}$-positive	135 (32.5%)	1.162 (1.069-1.263)	4.01E-04
Oncocytic changes	138 (33.2%)	1.154 (1.069-1.245)	2.27E-04
Multifocality	85 (20.4%)	0.971 (0.889-1.060)	0.508
Lymphatic/vascular invasion	230 (55.3%)	0.935 (0.871-1.005)	0.068
Extrathyroidal extension (any)	169 (40.6%)	0.939 (0.872-1.012)	0.099
N category (N1)	183 (44.0%)	0.933 (0.868-1.004)	0.063
M category (M1)	41 (9.9%)	0.935 (0.808-1.082)	0.365
Invasiveness score	2 (0-5; 0-3)	0.927 (0.872-0.985)	0.014
Thyroid surgery volume, n=415			
total thyroidectomy	384 (92.5%)	1.250 (1.029-1.518)	0.024
organ-preserving operation	31 (7.5%)	0.800 (0.659-0.971)	0.024
Lymph node dissection, n=415	220 (53.0%)	1.004 (0.928-1.075)	0.902
RIT performed	330 (79.3%)	1.085 (0.987-1.193)	0.091
RIT response, n=330		0.914 (0.826-1.011)	0.081
RAI-R recurrence vs other	8 (2.4%)	1.100 (0.899-1.346)	0.352
excellent vs other	291 (88.2%)	0.907 (0.819-1.005)	0.061
Follow-up, years, n=414	5.4 (0.01-27.9; 3.1-14.1)	-0.466 (-0.672-0.261)	1.10E-05
LN recurrence, n=398	14 (3.4%)	1.192 (1.030-1.381)[e]	0.019
LN recurrence, n=398	14 (3.4%)	1.186 (1.049-1.341)[f]	0.006
Time to recurrence, years, n=14	1.7 (0.7-9.4; 0.9-3.0)	0.824 (-0.916-2.564)	0.316

Note: [a] – adjusted for age at operation and sex unless otherwise specified; [b] – adjusted for age at operation; [c] – adjusted for sex; [d] – non-adjusted; [e] – Cox proportional hazard model adjusted for patient age, sex, thyroid surgery volume and lymph node dissection; – n on-adjusted Cox proportional hazard model.

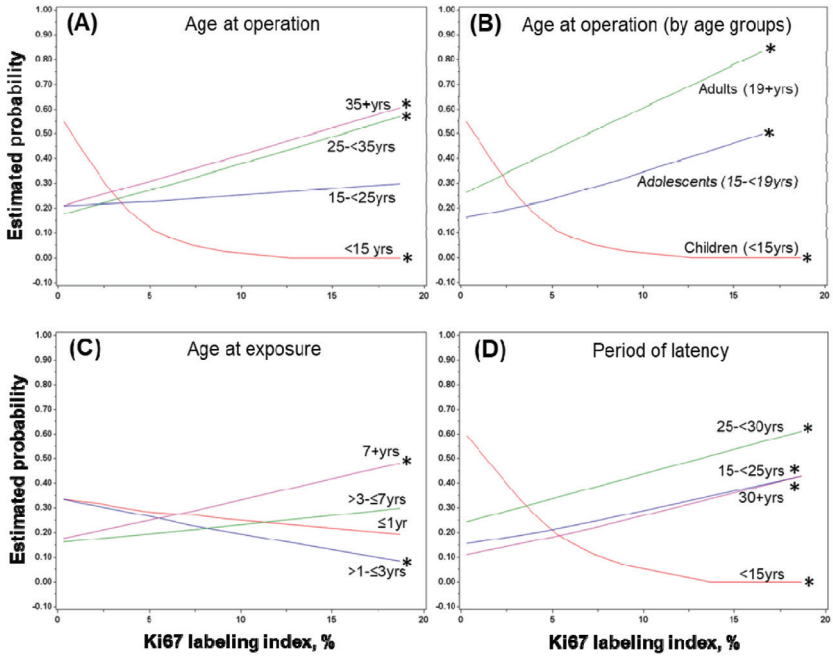

Fig. 4. Association of Ki67 LI with (A, B) age at operation, (C) age at exposure and (D) period of latency in 416 patients aged up to 49 years with radiogenic PTCs.

Note: Asterisks indicate statistical significance.

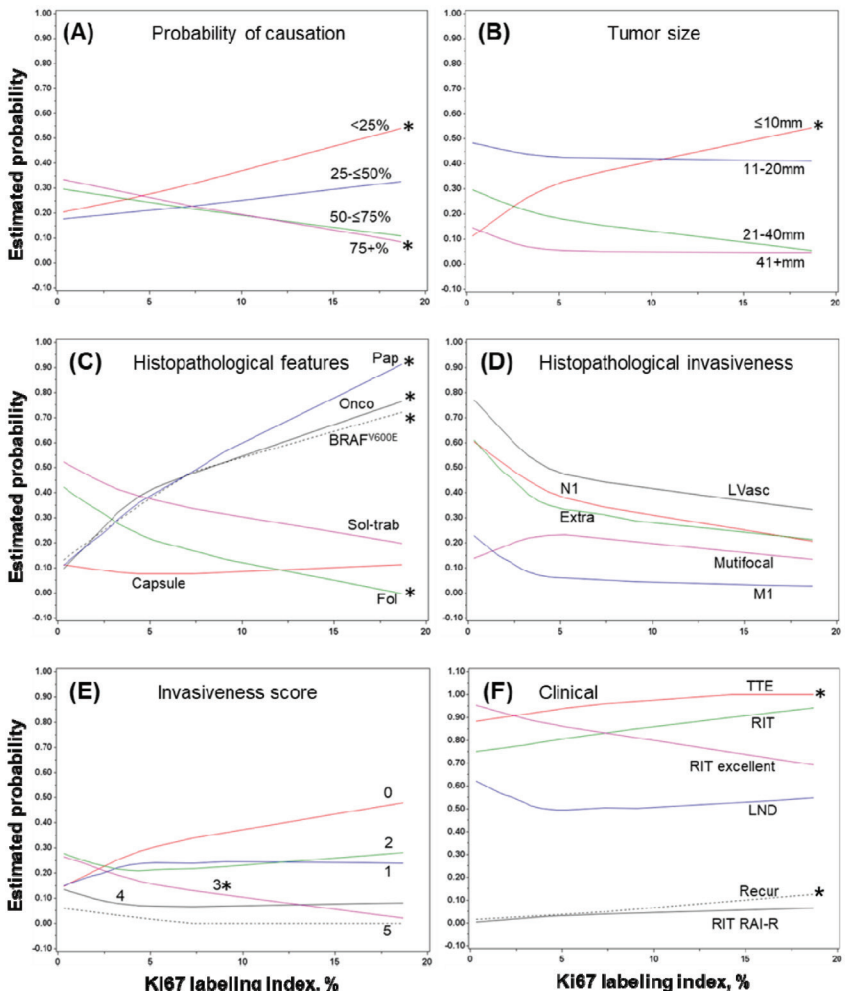

Fig. 5. Association of Ki67 LI with (A) probability of tumor causation due to radiation exposure, (B) tumor size, (C) histopathological features, (D) histopathological invasiveness, (E) invasiveness score and (F) clinical parameters of 416 radiogenic PTCs from patients aged up to 49 years.

Note: Asterisks indicate statistical significance.

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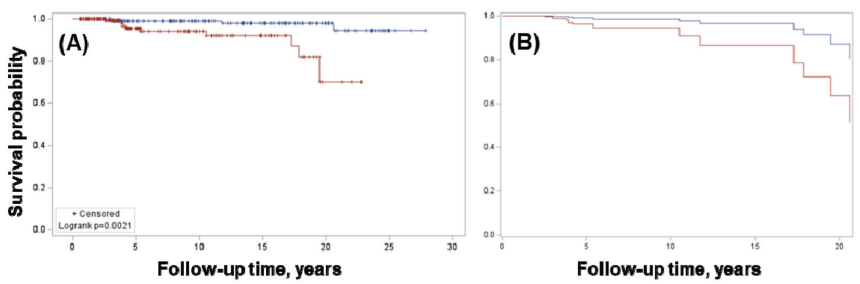

Fig. 6. Effect of Ki67 LI on disease-free survival in the Ki67 ≤3.5% (blue lines) and >3.5% (red lines) subgroups of 398 patients with radiogenic PTCs aged up to 49 years with available follow-up data. (A) The Kaplan-Meier survival estimates (p=0.002, the logrank test), vertical dashes indicate censored observations. (B) Disease-free survival functions computed using a proportional hazard model adjusted for age at surgery and sex (HR=4.444 (1.334-18.035), p=0.022).

not shown). As presented in **Table 5**, with a Ki67 LI increase, a decrease in POC level was observed (b=-0.029, p=0.044), likely due to the changes in its components, namely, an increase in patient age at the time of surgery (b=0.261, p=4.39E-09) and a decrease in ^{131}I thyroid dose (b=-0.036, p=0.004).

Estimated probability of detecting PTC with Ki67 LI greater than 10% and POC level greater than 75% was less than 20% (**Fig. 5A**). A higher Ki67 LI was also associated with a decrease in tumor size (b=-0.054, p=0.001) in parallel with a significant increase in the probability of detecting PTCs up to 10 mm in size, i.e. microcarcinoma (**Fig. 5B**), with a higher frequency of tumors with the dominant papillary growth pattern (OR=1.210, p=1.00E-06), oncocytic changes (OR=1.154, p=2.27E-04) and the *BRAF*^{V600E} mutation (OR=1.162, p=4.01E-04 (**Fig. 5C**). At the same time, the invasive properties of PTC did not change significantly with increasing Ki67 LI (see **Table 5** and **Fig. 5D, E**), except for a statistically significant lower probability of detecting tumors with an invasiveness score of «3». Among the clinical characteristics (**Fig. 5F**), there was a positive association of a higher Ki67 LI with thyroidectomy (OR=1.250, p=0.024) and the chance of recurrence (HR=1.1929, p=0.019, **see Table 5**).

Our analysis of disease-free survival using the Kaplan-Meier method (**Fig. 6A**) and the proportional hazard model (**Fig. 6B**) showed that, despite the relatively low frequency of recurrences, the Ki67 LI level higher than 3.5% in the given group of radiogenic PTCs was a statistically significant risk factor.

Ki67 LI in the BRAF^{V600E}-positive tumors (4.6%, IQR=3.6-6.3%, very close to that in patients aged up to 29 years), significantly exceeded KI67 LI in the BRAF^{V600E}-negative tumors (2.7%, IQR=1.6-4.6%, p=1.43E-13). Analysis of the Ki67 LI effects in the BRAF^{V600E} status subgroups showed that the increase in proliferative activity in the BRAF^{V600E}-positive tumors (**Table 6; Fig. 7A, 7C, 8A, 8C, 8E; 9A, 9C**) did not lead to statistically significant changes in their structural, invasive and clinical characteristics (similarly to the effects in patients aged up to 29 years, see **Table 2**), except for a lower probability of the dominant follicular growth pattern (see **Fig. 7C**), and invasiveness score of «3» (**Fig. 8C**).

In contrast, an increase of Ki67 LI in the BRAF^{V600E}-negative PTCs (see **Table 6, Figs. 7B, 7D; 8B, 8D, 8F; 9B, 9D**) significantly contributed to the behavior of radiogenic tumors (see **Table 5, Figs. 4, 5, 6**), similarly to the changes in the whole groups of young/middle age patients (see **Table 1**). Specifically, in the BRAF^{V600E}-negative PTCs, a higher Ki67 LI was associated with an increased probability of detection of microcarcinomas (see **Fig. 7B**), with a higher frequency of tumors with the dominant papillary growth pattern and oncocytic changes (see **Fig. 7 D**), with a higher risk of recurrence and the decreased frequency of excellent response to RIT (see **Fig. 8F**), and with a lower disease-free survival of patients with radiogenic PTC under the age of 49 years at the Ki67 LI cut-off of 3.7% (see **Figs. 9B, 9D**).

The results of our study demonstrate that Ki67 LI level, both in the radiogenic and sporadic PTCs from young patients aged up to 29 years, was associated with a smaller tumor size, more frequent papillary growth pattern, oncocytic changes in tumor cells and the *BRAF*^{V600E} mutation, but did not affect the probability of more frequent appearance of invasive PTC features such as extrathyroidal extension, lymph node metastases, multifocality, and lymphatic/vascular invasion (see **Tables 1, 3**), which is at variance with the literature data on sporadic PTCs in older patients [29-31].

Note that, as our previous studies showed [32-34], radiogenic PTCs in young patients who were

Table 6. Effect of Ki67 LI on clinicopathological characteristics in the BRAF^{V600E}-positive and BRAF^{V600E}-negative radiogenic PTCs from patients aged up to 49 years.

Parameters	BRAF^{V600E}-positive, n=135		BRAF^{V600E}-negative, n=281	
	b, OR or HR (95%CI)[a]	p-value	b, OR or HR (95%CI)[a]	p-value
Tumor size, mm	-0.014 (-0.224-0.196)	0.897	-0.078 (-0.195-0.039)	0.191
Dominant growth pattern	0.904 (0.792-1.031)	0.134	0.891 (0.822-0.965)	0.005
Oncocytic changes	1.042 (0.919-1.181)	0.52	1.180 (1.069-1.303)	0.001
Multifocality	0.873 (0.746-1.021)	0.089	0.996 (0.889-1.117)	0.948
Lymphatic/vascular invasion	0.917 (0.800-1.052)	0.217	0.973 (0.889-1.065)	0.552
Extrathyroidal extension (any)	0.939 (0.813-1.084)	0.387	0.955 (0.872-1.047)	0.326
N category (N1)	0.936 (0.817-1.072)	0.337	0.945 (0.864-1.035)	0.222
N1a	0.980 (0.833-1.152)	0.804	0.985 (0.874-1.109)	0.798
N1b	0.910 (0.753-1.100)	0.329	0.946 (0.854-1.048)	0.289
M category (M1)	1.093 (0.733-1.629)	0.662	0.936 (0.800-1.096)	0.413
Invasiveness score	0.905 (0.809-1.013)	0.084	0.947 (0.878-1.021)	0.156
Total thyroidectomy	1.101 (0.752-1.614)	0.621	1.254 (1.004-1.566)	0.046
Lymph node dissection	1.000 (0.885-1.129)	0.997	1.010 (0.926-1.101)	0.825
RIT performed	1.128 (0.937-1.358)	0.203	1.085 (0.967-1.217)	0.166
RAI-R recurrence *vs* other	0.910 (0.634-1.306)	0.609	1.268 (0.968-1.660)	0.085
Excellent RIT response *vs* other	1.015 (0.836-1.234)	0.878	0.865 (0.762-0.981)	0.024
LN recurrence	0.970 (0.642-1.467)[b]	0.886	1.225 (1.023-1.466)[b]	0.027
LN recurrence	0.948 (0.697-1.289)[c]	0.733	1.312 (1.111-1.551)[c]	0.001

Note: a – djusted for age at operation and sex unless otherwise specified; [b] – Cox proportional hazard model adjusted for patient age, sex, thyroid surgery volume and lymph node dissection; [c] – non-adjusted Cox proportional hazard model.

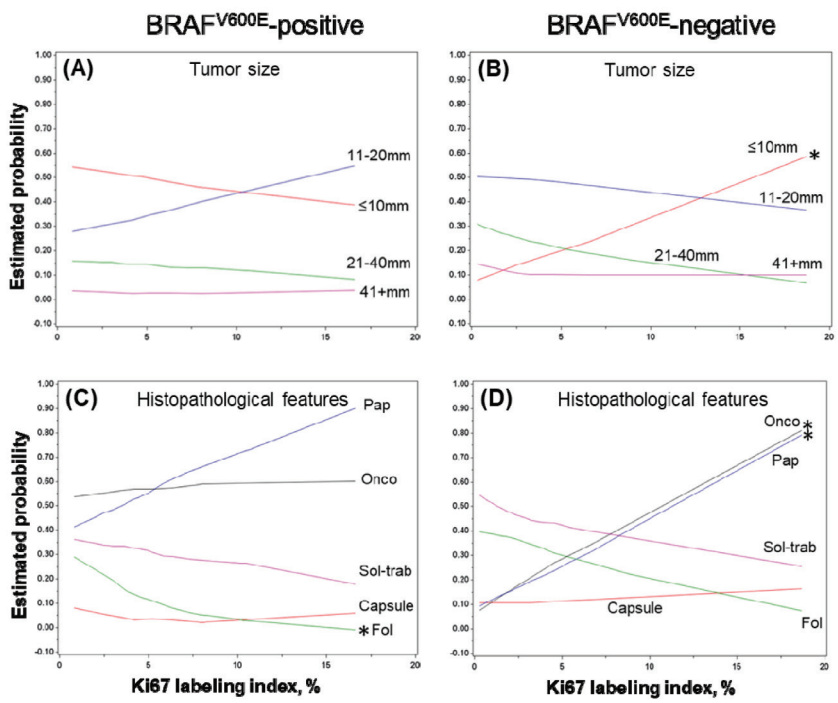

Fig. 7. Association of Ki67 LI with (A, B) tumor size and (C, D) histopathological features of (A, C) 135 BRAFV600E-positive and (B, D) 281 BRAFV600E-negative radiogenic PTCs from patients aged up to 49 years.

Note: Asterisks indicate statistical significance.

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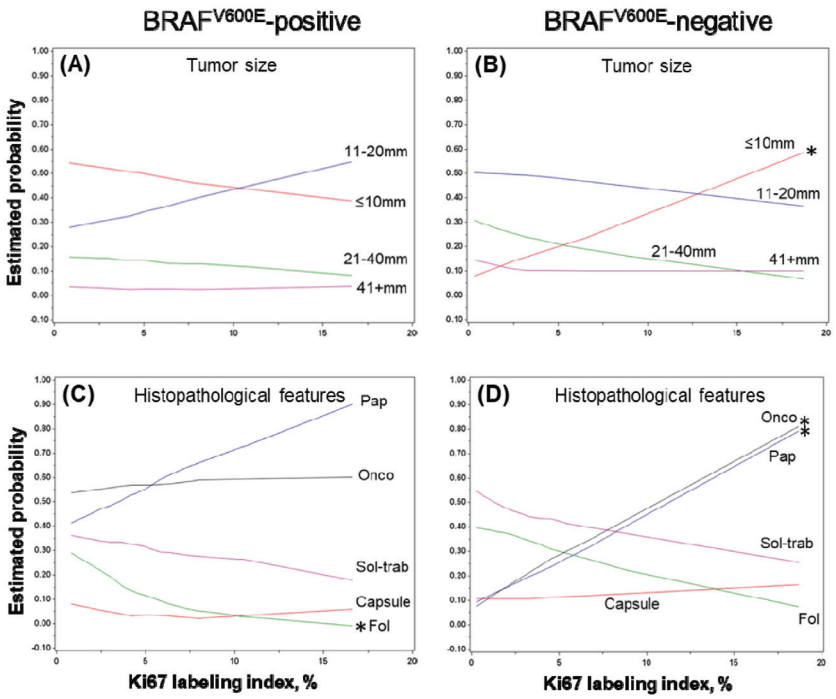

Fig. 7. Association of Ki67 LI with (A, B) tumor size and (C, D) histopathological features of (A, C) 135 BRAFV600E-positive and (B, D) 281 BRAFV600E-negative radiogenic PTCs from patients aged up to 49 years.

Note: Asterisks indicate statistical significance.

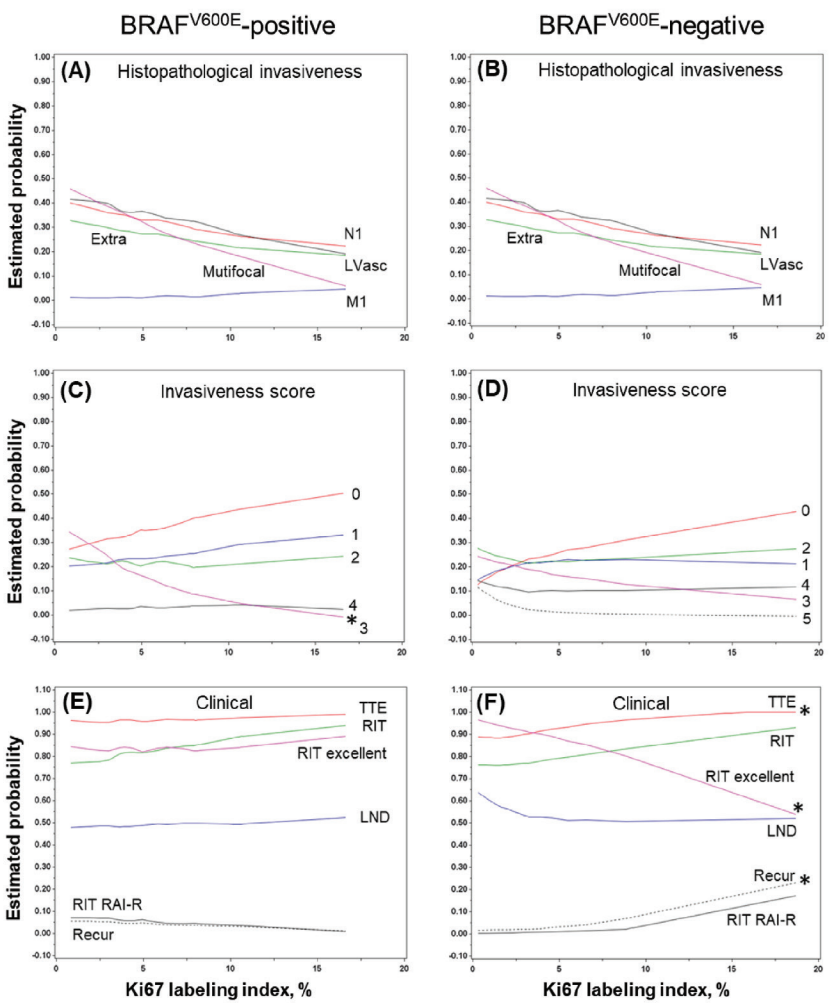

Fig. 8. Association of Ki67 LI with (A, B) histopathological invasiveness, (C, D) invasiveness score and (E, F) clinical parameters of the (A, C, E) 135 BRAFV600E-positive and (B, D, F) 281 BRAFV600E-negative radiogenic PTCs from patients aged up to 49 years.

Note: Asterisks indicate statistical significance.

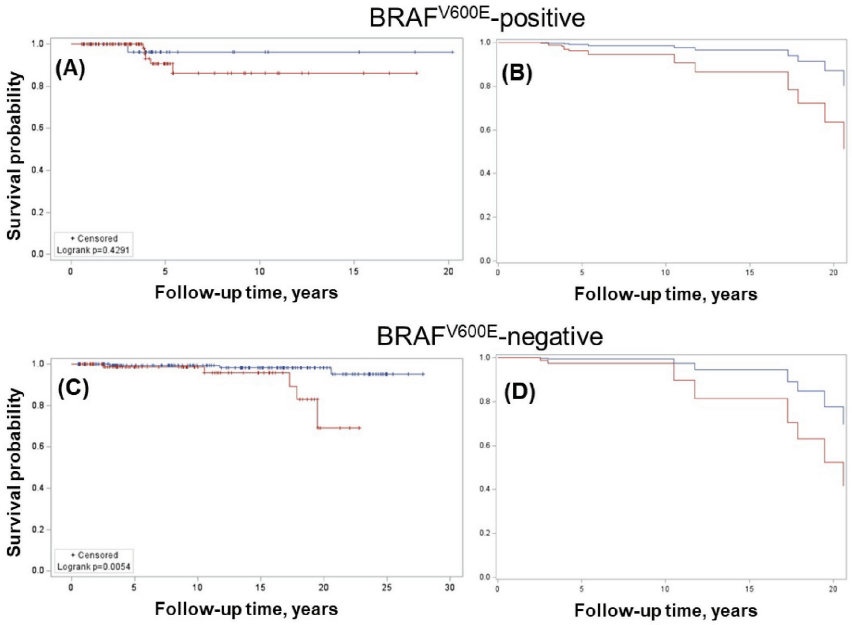

Fig. 9. Effect of Ki67 LI on disease-free survival in the Ki67 ≤3.7% (blue lines) and >3.7% (red lines) subgroups of (A, B) 132 patients with the BRAF^{V600E}–positive and 266 BRAF^{V600E}–negative radiogenic PTCs aged up to 49 years with available follow-up data. (A, C) The Kaplan-Meier survival estimates (p=0.429 for the BRAFV600E–positive PTCs and p=0.005 for the BRAF^{V600E}–negative PTCs, the logrank test), vertical dashes indicate censored observations. (B, D) Disease-free survival functions computed using proportional hazard models adjusted for age at surgery and sex (HR=3.118 (0.473-61.234), p=0.310 for the BRAF^{V600E}–positive PTCs and HR=4.717 (1.040-25.640, p=0.049 for the BRAF^{V600E}–negative PTCs).

exposed to Chornobyl fallout in childhood differed from sporadic tumors in terms of more aggressive behavior, but as evidenced by the newly obtained data, this was not associated with an increase in Ki67 LI. Nevertheless, an increase in Ki67 LI in radiogenic PTCs, in contrast to that in sporadic ones, did affect the probability of disease recurrence (see **Tables 1, 3**), and, of importance, only in the BRAF^{V600E}-negative PTCs (see **Tables 2, 4**). Note that the BRAF^{V600E}-negative PTCs display a more aggressive behavior as compared with the BRAF^{V600E}-positive tumors in PTCs of either etiology [9, 10].

It is also important to emphasize that in the expanded group of patients with radiogenic PTCs (aged up to 49 years at the time of surgery), the frequency of BRAF^{V600E} was nearly 3 times higher than that in young patients (see **Tables 1, 5**). According to our previous study, the *BRAF^{V600E}* mutation was a risk factor for the RAI-R recurrent metastases [11]. Despite the BRAF^{V600E}–positivity strongly associates with elevated Ki67 LI, the current study shows that the latter does not affect the risk of recurrence nor it associates with the RAIR-R recurrent metastases in the BRAF^{V600E}–positive PTCs (see **Table 6, Fig. 8E**). As in the young subjects, the increased Ki67 LI worsened prognostic indicators exclusively in the BRAF^{V600E}-negative PTCs in the middle-aged patients. With this in mind, note that recurrent BRAF^{V600E}-negative PTCs which were described in detail in our previous work [11, Supplementary Table 2] demonstrated a significantly stronger association with radiation exposure than the recurrent BRAF^{V600E}-positive tumors in terms of higher POC level and ^{131}I thyroid dose. It therefore seems appropriate, in order to optimize follow-up of patients who were exposed to Chornobyl radiation in childhood and have BRAF^{V600E}-negative PTCs (especially of the papillary structure with oncocytic changes), to recommend an additional IHC study with an antibody to Ki67 (MIB-1) which is available in most pathological laboratories of Ukraine. This may provide additional information regarding the chance of recurrence and its response to RAI therapy.

Conclusions

Ki67 LI in radiogenic and sporadic PTC of young patients aged up to 29 years was associated with the dominant papillary growth pattern, oncocytic changes and a more frequent BRAF^{V600E} mutation. Similar associations were found in radiogenic PTCs from patients aged up to 49 years.

In radiogenic PTCs, in contrast to sporadic ones, an increase in Ki67 LI was associated with a worse postoperative prognosis, namely with an increase in the chance of recurrence and of development of RAI-R recurrent metastases; this again was observed exclusively in the BRAF^{V600E}-negative tumors.

The BRAF^{V600E}-positive PTCs, regardless of their etiology, were associated with a higher Ki67

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LI as compared to the $BRAF^{V600E}$-negative tumors, but the Ki67 LI increase did not affect the clinico-histopathological characteristics of PTCs and the disease prognosis.

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Abbreviation

HR – hazard ratio
IEM – V.P. Komisarenko Institute of Endocrinology and Metabolism of the National Academy of Medical Sciences of Ukraine
IHC – immunohistochemical
IQR – interquartile range
LI – labeling index
OR – odds ratio
POC – probability of causation
PTC – papillary thyroid carcinomas
RAI-R – radioiodine refractory
RIT – radioiodine therapy

Acknowledgments

The authors gratefully acknowledge the confirmation of Ukrainian diagnoses during 1998-2022 by the International Pathology Panel of the Chornobyl Tissue Bank, which was supported by National Cancer Institute grant number U24CA082102: Professors A. Abrosimov, T. Bogdanova, G. Fadda, J. Hunt, M. Ito, V. Livolsi, J. Rosai, E.D. Williams, Dr. N. Dvinskyh and Dr. L. Zurnadzhy.

Вплив підвищення Ki67 проліферативного індексу на клініко-гістопатологічні показники радіогенних і спорадичних папілярних тиреоїдних карцином залежно від BRAF^{V600E} статусу

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Резюме. У папілярних тиреоїдних карциномах (ПТК) Ki67 проліферативний індекс (ПІ) має прогностичне значення щодо розвитку метастазів та їхніх рецидивів, однак неясно чи розповсюджується це на пацієнтів, опромінених у дитинстві. **Мета** – визначити чи існують певні асоціації між Ki67 ПІ та клініко-гістопатологічними характеристиками радіогенних і спорадичних ПТК, видалених у пацієнтів різного віку та чи залежать такі можливі асоціації від BRAF^{V600E} статусу пухлини. **Матеріал і методи.** Аналіз клініко- гістопатологічних та імуногістохімічних досліджень 552 ПТК (416 радіогенних і 136 спорадичних) з використанням багатофакторних моделей логістичної та лінійної регресії. **Результати.** У радіогенних ПТК пацієнтів віком до 29 років підвищення Ki67 ПІ асоційовано зі збільшенням частот домінантної папілярної будови (відношення шансів (ВШ)=1,208, p=5,34E-04), *BRAF^{V600E}* мутації (ВШ=1,183, p=0,0007) та онкоцитарних змін (ВШ=1,120, p=0,044), а також із ризиком рецидивів (коефіцієнт ризику (КР)=1,249, p=0,033). Збільшення Ki67 ПІ в BRAF^{V600E}-позитивних пухлинах не призводило до суттєвих змін патологічних і клінічних характеристик ПТК, але в BRAF^{V600E}-негативних пухлинах було асоційовано з ризиком розвитку рецидивних метастазів (КР=1,227, p=0,038), зокрема й радіойодрезистентних (ВШ=1,551, p=0,037). Вплив Ki67 ПІ на показники спорадичних ПТК пацієнтів такої ж вікової групи був подібним за більшістю змінних, але був відсутнім щодо ризику рецидивів як у цілому по групі, так і в BRAF^{V600E}-позитивних чи BRAF^{V600E}-негативних ПТК. У пацієнтів віком до 49 років із радіогенною ПТК ефект Ki67 ПІ співпадав із визначеним у молодших пацієнтів. **Висновки.** У радіогенних ПТК, на відміну від спорадичних, підвищення Ki67 ПІ пов'язано з гіршим післяопераційним прогнозом, а саме зі збільшенням ймовірності розвитку рецидивів метастазів, зокрема й радіойодрезистентних, що асоційовано, у свою чергу, виключно з BRAF^{V600E}-негативним статусом пухлини. У BRAF^{V600E}-позитивних ПТК, незалежно від їхньої етіології, підвищення Ki67 ПІ не впливало на клініко-гістопатологічні показники ПТК і прогноз захворювання.

Ключові слова: папілярна тиреоїдна карцинома, Чорнобильська аварія, Ki67 проліферативний індекс, *BRAF^{V600E}* мутація, імуногістохімічне дослідження.

Для цитування: Богданова ТІ, Зурнаджи ЛЮ, Саєнко ВО, Болгов МЮ, Масюк СВ, Бурко СВ, Дегтярьова ТЛ, Чернишов СВ, Гулєватий СВ, Ітo М, Рагуновіч ТІ, Тронько МД. Вплив підвищення Ki67 проліферативного індексу на клініко-гістопатологічні показники радіогенних і спорадичних папілярних тиреоїдних карцином залежно від BRAF^{V600E} статусу. Ендокринологія. 2024;29(2):101-118. DOI: 10.31793/1680-1466.2024.29-2.101.

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Фінансування: дослідження проводилось у рамках бюджетного фінансування за планом науково-дослідних робіт ДУ «Інститут ендокринології та обміну речовин ім. В.П. Комісаренка НАМН України» (№ державної реєстрації: 0122U000544) і за фінансової підтримки Інституту захворювань, пов'язаних із наслідками вибуху атомної бомби університету Нагасакі (Японія) та Програми спільного використання мережевого типу Науково-дослідного центру медичної науки про радіаційні катастрофи (Японія).

Декларація з етики: автори задекларували відсутність конфлікту інтересів і фінансових зобов'язань.

Стаття: надійшла до редакції 24.05.2024 р.; перероблена 31.05.2024 р.; прийнята до друку 24.06.2024 р.; надрукована 30.06.2024 р.

For citation: Bogdanova TI, Zurnadzhy LYu, Saenko VO, Bolgov MYu, Masiuk SV, Burko SV, Degtyaryova TL, Chernyshov SV, Gulevatyi SV, Ito M, Rogounovitch TI, Tronko MD. Effect of Ki67 labeling index on clinicohistopathological characteristics of radiogenic and sporadic papillary thyroid carcinoma with regard to the $BRAF^{V600E}$ mutational status. Endokrynologia. 2024;29(2):101-118. DOI: 10.31793/1680-1466.2024.29-2.101.

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Personal contribution: Bogdanova T.I. – idea, research design, analysis of results, writing, design, preparation for publication of the paper; Zurnadzhy L.Yu. – participation in the development of the concept of the paper, review and analysis of histological specimens, IHC study and analysis of results, participation in the writing of the manuscript; Saenko V.O. – statistical analysis of results, design and preparation of the figures, discussion of the results obtained, editing of English manuscript; Bolgov M.Yu. – automated preparation of the spreadsheet of clinical characteristics according to a self-developed algorithm; Masiuk S.V. – dosimetric support of the study; Burko S.V. – histological study; Degtyaryova T.L. – histological study; Chernyshov S.V. – analysis of clinical data; Gulevatyi S.V. – analysis of RIT results; Ito M. – discussion of the results obtained; Rogounovitch T.I. – development of IHC detection of mutated BRAFV600E protein, participation in IHC study; Tronko M.D. – discussion of the results obtained.

Funding: the study was carried out within the budget funding according to the plan of research work of the State Institution «V.P. Komisarenko Institute of Endocrinology and Metabolism of the National Academy of Medical Sciences of Ukraine», registration number of the study: 0122U000544; the study also was supported in part by the Atomic Bomb Diseases Institute, Nagasaki University, Japan, and the Program of the Network-Type Joint Usage / Research Center for Radiation Disaster Medical Science.

Declaration of ethics: The authors have declared no conflicts of interest or financial obligations.

The article: received May 24, 2024; revised May 31, 2024; accepted June 24, 2024; published 30 June 2024.