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Professor Krzysztof Krzemieniecki Award for the best case report accepted for publication

Case Report Contest Policies

This policy defines the scope, requirements and regulations regarding **The Krzysztof Krzemieniecki Award** for the best case report published in "Oncology in Clinical Practice" (OCP) Fifth Edition.

1. The aim of the contest is to encourage submission of quality case reports related to oncological practice and to promote them in the scientific deliberations.
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5. The first author of the winning paper will be eligible for a prize of gross 1000,00 Euro gross (one thousand euro).
6. Results will be announced during the XXIV National Congress of The Polish Society of Clinical Oncology and subsequently at the Journal website.
7. Winner will be notified via email.
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ORIGINAL ARTICLE

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Evaluation of the efficacy of chemotherapy with capecitabine and oxaliplatin in patients with disseminated colorectal cancer. The impact of primary cancer focus on treatment efficacy

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ABSTRACT

Introduction. Colorectal cancer is an increasingly common cancer, and due to the possibility of using many drugs nd combination therapy, it bears the hallmarks of a chronic disease. Improving the quality of life is important.

Material and methods. The following analysis applies to the oxaliplatin and capecitabine (CAPOX) regimen in a group of 305 patients. This chemotherapy was used as part of palliative treatment lines I, II or III.

Results. The work proved the effectiveness of the scheme despite the reduction of drug doses in about 50% of patients, and toxicity grade 3 was only present in 5% (grade 4 complications were not observed). The group of patients in which CAPOX was used as the first treatment line was considered representative, and the effectiveness of the treatment depending on the location of the primary tumour was evaluated.

Conclusion. Differences in overall survival of patients after stratification were observed relative to the location of the primary tumour. Survival was longer in patients with left-sided primary tumour compared to right-sided localisation and was, respectively, 20.4 (95% CI, 17.5–23.4) and 12.1 months (95% CI, 10.5–13.8) (P = 0.014).

Key words: metastatic colorectal cancer, oxaliplatin, capecitabine, primary tumour location

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Introduction

Palliative chemotherapy has been used in patients with generalised colorectal cancer (CRC) for many years. At the time of diagnosis, a generalised disease is found in approximately 15–20% of patients.

Even after radical surgery (R0 resection), approximately 50% of patients will develop metastatic lesions, including 30–35% having only liver metastases. In this cohort, 10–25% are eligible for surgical treatment, and 75–90% of patients will be offered palliative chemotherapy. The use of chemotherapy allows objective response to be obtained in 50% of cases in imaging tests, and extended progression-free survival (PFS) to 10 months and overall survival (OS) to 20–24 months [1].

In about 15–20% of patients, generalised colorectal cancer is an asymptomatic or slow-growing disease; therefore, aggressive treatment is not required [2]. It should be emphasised that the quality of life of patients receiving palliative treatment, apart from the toxicity of treatment, is significantly influenced by the frequency and length of hospitalisation.

Currently, an important argument in choosing a treatment regimen is patients' quality of life, which includes — among others — the frequency and route of drug admini-

Table 1. Characteristics of patients in the observed group

Treatment line	First-line treatment	Second-line treatment	Third-line treatment	
Number of patients	222	66	17	
Gender	Male 183 (60%)	Female 122 (40%)		
Age	Mean	Range	≥ 65 years	< 65 years
	64.4	32–87	146 (48%)	159 (52%)
Prior adjuvant treatment	YES 139 (46%)	NO 166 (54%)		
WHO performance status	0–1 278 (91%)	2 27 (9%)		

stration. The generalised stage of colorectal cancer often requires lengthy treatment, and the use of oral medications significantly improves the comfort of such treatment. These regimens include: CAPOX, XELIRI, and capecitabine alone.

The CAPOX regimen includes capecitabine and oxaliplatin. Capecitabine is administered orally at a dose of 1000 mg/m^2 twice daily for 14 days, and oxaliplatin is administered on the first day of the cycle at a dose of 130 mg/m^2 as a two-hour intravenous infusion. The cycle is repeated every 21 days.

Nonetheless, the most common therapeutic option proposed for patients with stage IV colorectal cancer is systemic treatment, which improves the quality of life and often extends the survival. The most commonly used anticancer drugs (in monotherapy or multi-drug regimens) for colorectal cancer include fluorouracil, irinotecan, oxaliplatin, capecitabine, bevacizumab, aflibercept, cetuximab, panitumumab, and regorafenib. The main goal is to achieve the greatest effectiveness with the least toxicity of treatment.

A regimen containing a combination of capecitabine and oxaliplatin is used in the first-, second-, or third-line treatment, depending on the genetic characteristics.

Currently, the growing importance of primary tumour location in the biology of colorectal cancer is underlined. The location of the primary tumour on the right side is associated with a worse prognosis. More and more publications are devoted to the impact of tumour location on response to targeted therapy with anti-epidermal growth factor receptor (EGFR) or anti-vascular endothelial growth factor (VEGF) antibodies, while there is little data on the effect of tumour location on the effectiveness of chemotherapy.

The aim of the study was to evaluate the effectiveness of chemotherapy with capecitabine and oxaliplatin in patients with generalised colorectal cancer and to compare treatment results depending on the tumour's original location.

Material and methods

We carried out a retrospective analysis of consecutive patients diagnosed with generalised colorectal cancer treated at the Colon Cancer Clinic and the Gastrointestinal Cancer Clinic between March 2008 and April 2011. The inclusion criteria included: histopathological diagnosis of colorectal adenocarcinoma, good general condition (WHO 0–2), locally advanced or metastatic colorectal cancer, the use of chemotherapy according to CAPOX scheme (I, II, or III line), and the presence of a measurable lesion. Table 1 presents the characteristics of 305 patients included in the analysis.

On average, six CAPOX cycles were used in each treatment line. A retrospective analysis of response to CAPOX treatment was performed (including disease control rate [DCR], time to progression [TTP], and overall survival), taking into account dose reductions and treatment toxicity. A retrospective analysis of clinical outcome in patients with metastatic colorectal cancer depending on the location of the primary tumour was also made. This analysis included only the group of patients treated with the CAPOX regimen in the first and second line of treatment.

Results

There were no statistically significant differences in disease control rates (DCR) between the analysed groups. In the entire study group, regardless of the treatment line in which the CAPOX regimen was used, DCR was 75.9%; in individual lines: I — 77.3% (n = 167), II — 72.2% (n = 47), and III — 69.2% (n = 9) (P = 0.604).

Table 2 presents the distribution of response to the treatment according to the RECIST 1.1 criteria.

The median overall survival (OS) in the first-line treatment is 19.3 months (95% CI, 17.06–23.5), in the second-line treatment 14.2 (95% CI, 11.61–17.83), and in the third-line treatment 13.96 (95% CI, 11.78–16.73).

There was no grade 4 haematological or non-haematological toxicity in the study group. Grade 3 leukopaenia and neutropaenia were only observed in patients receiving CAPOX regimen in third-line treatment (5.9% — grade 3 leukopaenia, 2.9% — grade 3 neutropaenia). A statistically significant difference in complications

Table 2. Assessment of response to treatment with the CAPOX regimen in individual treatment lines

Treatment line	Complete response (%)	Partial response (%)	Stable disease (%)	Progressive disease (%)
First-line treatment	4.2	35.2	38	22.6
Second-line treatment	4.6	24.6	43.1	27.7
Third-line treatment	0	7.7	61.5	30.8

Table 3. Results of treatment of metastatic colorectal cancer with the CAPOX regimen (capecitabine + oxaliplatin) depending on the primary tumour location

	Right side	Left side	Total
DCR (%)	68.3	76.7	74.7
Median PFS (months, 95% CI)	3.9 (3.4–4.5)	4.2 (3.9–4.5)	4.1 (3.9–4.4)
Median OS (months, 95% CI)	12.0 (10.0–14.0)	18.7 (16.4–21.1)	16.9 (14.9–18.8)
First-line mOS [(months, 95% CI)	12.1 (10.5–13.8)	20.4 (17.5–23.4)	19.3 (15.6–23.1)
Second-line mOS (months, 95% CI)	7.2 (6.2–8.2)	16.1 (12.0–20.1)	14.2 (11.3–17.1)

CI — confidence interval; DCR — disease control rate; OS — overall survival; PFS — progression-free survival

according to the treatment line was found only in case of leukopaenia after CAPOX used in third-line treatment ($P < 0.001$). Grade 3 vomiting occurred in 0.9% of patients in first-line treatment and 1.5% in second-line. Grade 3 hand-foot syndrome was observed in 0.5% of patients in first-line treatment. Grade 3 sensory neuropathy was found in 2.8% of patients in first-line treatment and 3% in second-line treatment.

There was a statistically significant increase in the frequency of oxaliplatin dose reduction in subsequent treatment lines (I — 53.5%, II — 69.7%, III — 82.4%; $P = 0.008$) as well as drug withdrawal (I — 12.5%, II — 16.7%, III — 35.3%; $P = 0.034$). No similar difference was found for capecitabine, for which dose reduction rates were similar in all treatment lines (I — 56%, II — 66%, III — 58.8%).

The clinical benefit obtained in the study did not depend on the chemotherapy line in which CAPOX regimen was used in patients with stage IV colorectal cancer.

We did not observe any relationship between the results of CAPOX treatment and primary tumour location in patients treated in the first line (Tab. 3). The percentage of patients achieving disease control was 68.3% for right-sided and 74.7% for left-sided tumour location ($p = 0.188$).

Similarly, the median progression-free survival (PFS) did not differ and was for right-sided and left-sided location 3.9 (95% CI, 3.4–4.5) and 4.2 months (95% CI, 3.9–4.5; $P = 0.443$), respectively. Median PFS for the whole cohort was 4.1 months (95% CI, 3.9–4.4) and was shorter than in published randomised clinical trials for CAPOX regimen (7.1–10.3 months for CAPOX in first-line treatment; 4.7 months for CAPOX in second-line treatment) [3, 4]. The reason for the difference between our results and data from clinical trials is uncertain, but it is probably due to patient selection for randomised trials.

Median OS in the study population was 16.9 months (95% CI, 14.9–18.8). This value is similar to the results obtained in randomised clinical trials, in which (depending on the study) it was from 16.0 to 24.6 months, average 17–19 months [3, 4]. In one study with use of CAPOX regimen in second-line treatment the median OS was 11.9 months, compared to 14.2 months (95% CI, 11.3–17.0) in an analogous group in our population [6].

However, we observed a statistically significant difference in overall survival for patients stratified according to the primary tumour location. If the tumour was located on the right side of the colon, the median OS was 12.1 months (95% CI, 10.5–13.8), compared to 20.4 months (95% CI, 17.5–23.4) for the disease with left-sided location ($P = 0.014$). In one retrospective study of patients with metastatic colorectal cancer receiving polychemotherapy without targeted drugs, results similar to ours were achieved: for right and left-sided disease the median OS was 13.0 and 17.8 months, respectively [6].

Based on this data, it is difficult to determine whether this difference in OS is to any extent the result of differences in the effectiveness of CAPOX regimen in these two subgroups. It has been reported in many studies, however, that the difference in overall survival is certainly greatly influenced by the more aggressive course of right-sided colorectal cancers [7, 8]. Poorer prognosis of colorectal cancers located on the right side was also confirmed in the group of patients receiving CAPOX regimen in second-line treatment [9].

Discussion

Currently, there are many data related to the effectiveness and toxicity of chemotherapy, but it is worth emphasising the effectiveness of the CAPOX regimen with a significant dose reduction. No grade 4 toxicity was observed, and grade 3 only in 5% of patients.

Particularly interesting is the importance of primary tumour location in the biology of colorectal cancer, and this observation is discussed below. In this context right-sided tumours (proximal to the splenic flexure) and left-sided tumours (distal to this structure) are distinguished.

The biological explanation for differentiating these locations is, among others, distinct embryogenesis of right and left segments of the large intestine (developing from the middle and posterior intestine, respectively), separate vascularisation (superior and inferior mesenteric artery, respectively), and differences in the intestinal microbiome and alternative carcinogenesis pathways occurring in these sections (right-sided cancers more often develop from serrated adenomas or traditional serrated polyps harbouring *BRAF* mutations and/or microsatellite instability; left-sided cancers typically evolve from classic adenomas with *APC* gene mutations) [8].

The distinction between these locations of colorectal cancers also has great prognostic justification. The results of several studies and meta-analyses indicate that cancers located on the left side have a lower risk of death (relative risk in the large meta-analysis 0.82, 95% CI 0.79–0.84) regardless of the presence of other prognostic factors (e.g. clinical stage, chemotherapy, cancer histology, and *BRAF* mutation) [8]. Attention is also paid to the predictive significance of primary tumour location, which can be of great importance when choosing the method of palliative therapy. In patients with left-sided tumours, unequivocal benefit from using anti-EGFR antibodies (e.g. cetuximab, panitumumab) has been proven; in turn, in patients with a primary tumour located on the right side, the use of anti-VEGF antibodies (e.g. bevacizumab) is preferred [11].

Unfortunately, there are very little data available on the impact of colorectal cancer location on response to chemotherapy, including fluoropyrimidines. Based on experimental data, it appears that fluorouracil may be more active in right-sided cancers due to the higher expression of thymidine phosphorylase and lower expression of gamma-glutamyl hydrolase, which promotes higher folic acid levels in cancer cells and higher fluoropyrimidine cytotoxicity [12]. Thymidine phosphorylase is also required to convert the prodrug capecitabine to the active form, fluorouracil [13]; thus, it appears that a higher level of this enzyme in right-sided tumours [14] may contribute to higher capecitabine activity. However, there is no direct evidence confirming this hypothesis.

The negative prognostic value of right-side location of colorectal cancer persists regardless of the treatment used [6, 7]. This does not mean, however, that patients with right-sided tumours do not benefit from chemotherapy; probably the opposite is true: in stage III cancers, adjuvant treatment with fluorouracil or capecitabine with oxaliplatin has a relatively greater benefit in terms of disease-free survival (DFS) in patients with right-sided cancer [15, 16].

Unfortunately, there are also scarce data on the impact of tumour location on chemotherapy results for stage IV cancers. In one study, Negri et al. did not observe differences in objective response rate (ORR) between originally left- and right-sided cancers during treatment with fluorouracil alone or in combination with mitomycin and interferon, although right-sided location was associated with 1.6-times higher risk of death [17].

In the FIRE-1 study comparing FuFIRI (irinotecan, fluorouracil infusion, leucovorin) and mIROX (irinotecan, oxaliplatin) regimens in the first-line treatment, it was found that using the FuFIRI regimen leads to a higher ORR in patients with primary left-sided tumour (33% and 47% for right-sided and left-sided cancer, respectively); however, such differences were not observed for the mIROX regimen (ORR 40% for both locations) [18]. A tendency towards longer OS was also observed when the FuFIRI scheme was used for left-side primary tumour location and the mIROX scheme for right-sided cancer, but these results did not reach statistical significance [18]. Unfortunately, there are no such studies for chemotherapy regimens currently most commonly used in first- and second-line palliative treatment (FOLOX/CAPOX, FOLFIRI/XELIRI), especially taking into consideration the fact that these regimens are nowadays frequently associated with biological drugs for which the location of the primary tumour is a strong predictive factor (i.e. as described above).

An additional issue is the molecular differences between right- and left-sided cancers, which can affect the response to chemotherapy. Particularly important are the differences in the occurrence of microsatellite instability-high (MSI-H) and *BRAF* gene mutations, which are more frequent in cancers originally located on the right side. In the presence of *BRAF* gene mutation (18.4–22.4% of right-sided cancers and 1.3–7.8% of left-sided cancers), which is a poor prognostic factor, patients do not benefit significantly from chemotherapy with fluoropyrimidine, oxaliplatin, or irinotecan [19–22]. In turn, the presence of MSI-H, which is typical for sporadic *BRAF* mutant cancers (52% of patients with *BRAF* mutation also indicate MSI-H), are found in about 5% of metastatic colorectal cancers, almost exclusively right-sided [7]. Tumours with MSI-H are characterised by markedly reduced sensitivity to fluoropyrimidines,

as seen in preclinical studies [22, 23] and confirmed in a number of clinical studies [24–27]. Similarly, the lack of efficacy of fluoropyrimidines is observed in CpG island methylator phenotype (CIMP) cancers, and this is typical for sporadic MSI-H cancers and in cancers of mucocellular histology, which is a manifestation of MSI-H presence [7, 28]. In summary, primary right-sided cancers show a number of molecular features such as MSI-H, CIMP, and *BRAF* mutations that promote resistance to fluorouracil and capecitabine. Molecular aberrations responsible for reduced effectiveness of fluoropyrimidines are found in the absolute minority of right-sided cancers. It remains an open question to what extent these relationships can be extrapolated to all right-sided colorectal cancers.

Conflict of interest

The authors report no conflicts of interest.

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The study of genetic and clinicopathological characterisation of Turkish bilateral breast cancer patients

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ABSTRACT

Introduction. Although bilateral breast cancers are a rare condition in the general population, the incidence has increased significantly in *BRCA1* and *BRCA2* gene carrier breast cancer patients. Besides the genetic susceptibility, many risk factors such as age, first breast cancer diagnosis age, lifestyle, and environmental factors may be effective in the development of this type of cancer. This study aimed to determine *BRCA1/2* gene carriage in patients with bilateral breast cancer and to find out the risk factors that may lead to contralateral cancer formation.

Material and methods. From 2016 to 2018, in Turkey, we grouped 31 women diagnosed with bilateral breast cancer synchronously and metasynchronously. Analysis of *BRCA1* and *BRCA2* genes of these women evaluated for clinical and pathological tumour characteristics was performed using the NGS technique.

Results. No significant difference was found between the metachronous (MBBC) and synchronous (SBBC) groups in terms of clinical and pathological tumour characteristics. MBBC patients' age at first diagnosis of breast cancer was lower than SBBC. Also, there was a statistically significant relationship between chronic diseases and MBBC cancers ($\chi^2 = 11.519$; $p = 0.001$). In our study, disease-related variants were found only in three patients, and two of these variants were identified the first time in the literature.

Conclusion. The risk of bilateral breast cancer of BRCA1/2 carriers increases when the first breast cancer is diagnosed at a young age and there is a significant family history of cancer. MBBC is associated with chronic diseases, and large-scale research will contribute to clarifying this relationship.

Key words: *BRCA*, bilateral breast cancer, metachronous, synchronous, chronic disease

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Introduction

Breast cancer is the most common type of cancer in women all over the world and is one of the first causes of deaths due to female sex cancers [1]. Although this type of cancer is mostly seen in the unilateral breast, approximately 2% to 11% of all events are bilaterally detected, and the second most common malignancy in breast cancer patients is located in the contralateral breast [2]. The development of diagnostic, screening, and treatment techniques in cancer and increased survival of cancer patients are thought to lead to the more frequent observation of bilateral breast cancer. However, the causes of invasive or in situ histological types of lobular breast cancer, gene mutation, early detection of breast cancer, and a history of radiation exposure in previous cancer treatment are thought to increase the risk of BBC development [3, 4]. While the risk of BBC developmental cumulative incidence is 3.4% in 10 years in patients with unilateral breast cancer, this rate increases to 13–40% in women with *BRCA* mutation [5, 6]. BBC patients, according to the time elapsed between the detection of tumors in both breasts (although many authors have not yet reached a consensus), can be grouped as synchronous (SBBC) or meta-synchronous (MBBC) [7–10]. The number of studies investigating the clinical and pathological characteristics of both groups is not sufficient in the literature. In this study, we aimed

Table 1. Age parameters of SBBC and MBBC groups

Variable	Synchronous (n = 14)		Metasynchronous (n = 17)		Statistical analysis* Probability
	$\bar{X}$ ± S.S.	Median [Min–Max]	$\bar{X}$ ± S.S.	Median [Min–Max]	
Age (years)	52.93 ± 8.75	54.5 [39.0–67.0]	54.29 ± 8.91	53.0 [42.0–73.0]	t = –0.428 p = 0.672
Age at first ca dia. (years)	48.43 ± 8.83	49.5 [36.0–66.0]	40.88 ± 9.47	40.0 [19.0–61.0]	T = 2.276 **p = 0.030**
Age at sec ca dia. (years)	48.64 ± 8.78	49.5 [36.0–66.0]	51.94 ± 8.61	49.0 [40.0–67.0]	t = –1.053 p = 0.301

*Independent Samples t-test (T-table value) statistics were used to compare the measurement values of two independent variables in the normal distribution data

to evaluate the demographic and clinical characteristics, pathological details of tumours, and *BRCA1/2* mutation status of patients we grouped as MBBC and SBBC.

Methods

We performed this study in 31 patients who were diagnosed with bilateral breast cancer and referred to the genetics department of Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital between June 2016 and January 2018 in order to clarify the genetic aetiology. All patients who participated in the study were in accordance with the National Comprehensive Cancer Network (NCCN) guidelines for BRCA1/BRCA2 test standards [11]. Of the patients' demographic characteristics, background and family history, age at first and second cancer diagnosis, tumour-node-metastasis (TNM) staging, oestrogen receptor (ER)/progesterone receptor (PR), C-erbB-2 status, etc. were obtained from the patients themselves, their medical records, and the electronic database of the hospital during genetic counseling. Bilateral breast cancer of patients was grouped into SBBC or MBBC based on the interval between the first and contralateral tumours (≤ 12 and > 12 months, respectively) [12]. All patients included in the study were informed about this study and gave written, informed consent for publication. The independent Ethics Committee of the Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital approved this descriptive case series study (Document No. 2020-02/536).

Genetic materials obtained from peripheral blood samples of patients were tested by next-generation sequencing methods to detect germline variants of *BRCA1* and *BRCA2* genes. In the genetic analysis of the patients, the Oncomine™ BRCA Research Assay commercial kit was used, and this analysis was performed on the Ion S5™ System (Ion Torrent™) platform. In this analysis, all exonic regions and the part up to 20 base pairs of exon-intron boundaries were examined. The sequence results were compared with the human genome of hg19, and Ion Reporter Software Version 5.4 (Thermo Fisher Scientific) was used for bioinformatics analysis. The in silico analysis for the gene variants was performed using SIFT, PolyPhen2, DANN, PROVEAN, GERP, MPC, Mutation Assessor, Fathmm, and Mutation Taster. In this study, genomic changes were identified according to ACMG criteria [13].

SPSS (IBM SPSS Statistics 24) was used for all statistical calculations. Independent sample t-test (t-table value) and Mann-Whitney U test (Z-table value) statistics were used to compare the measurement values of two independent variables. χ^2 cross tables were used in the study of the relations of the two qualitative variables. A p-value less than or equal to 0.05 was considered statistically significant.

Results

Of the 31 patients who were treated and followed up for bilateral breast cancer between January 2016 and December 2017, 14 (45.16%) presented with SBBC and 17 (54.84%) were diagnosed with MBBC, and all of them underwent *BRCA1/2* genes analyses. The median age of all patients was 53 (range 39–73 years) years, SBBC patients were 49.5 (range 36–66) years old, and MBBC patients were 40 (range 1–61) years old. There was a statistically significant difference between breast cancers in terms of breast cancer diagnosis age (t = 2,276; p = 0.030). The time interval between cancers, breast CA first diagnosis age of metasynchronised patients was statistically significantly lower than synchronised patients (Tab. 1).

In terms of the 31 bilateral breast cancer patients, patient demographics and tumour-related general factors are shown in Table 2. In the assessment of smoking status, women who smoked at least 10 cigarettes a day for 10 years or more were included in the positive group. Chronic diseases were recognised as conditions requiring periodic monitoring and supportive care (hypertension, diabetes mellitus, goiter, familial Mediterranean

Table 2. Baseline patient demographics and clinical details

Characteristic	n (%)	Characteristic	n (%)	Characteristic	n (%)
Level of education		**Age at first labour**		**Mass size (left)**	
Elementary school	9 (29.0)	Nulliparity	1 (3.2)	≤ 2 cm	9 (49.9)
High school	15 (48.4)	< 20	3 (9.7)	2–4 cm	6 (33.4)
University	7 (22.6)	20–30	23 (74.2)	Multifocal	3 (16.7)
		> 30	4 (12.9)		
Working condition		**First breast ca laterality**		**Mass size (right)**	
Yes	7 (22.6)	Left	11 (35.6)	≤ 2 cm	9 (45.0)
No	24 (77.4)	Right	10 (32.2)	2– ≤ 4 cm	7 (35.0)
		Bilateral	10 (32.2)	> 4 cm	2 (10.0)
				Multifocal	2 (10.0)
Residence		**Time between cancers**		**Metastasis (at the first diagnosis)**	
Rural	7 (22.6)	Simultaneously	10 (32.2)	None	22 (71.0)
City	24 (77.4)	Simultaneously – ≤ 1 year	4 (13.0)	Axillar	7 (22.6)
Smoking		1 year – ≤ 5 years	3 (9.6)	Bone	1 (3.2)
Yes	7 (22.6)	5 years – ≤ 10 years	3 (9.6)	Lung	1 (3.2)
No	24 (77.4)	More than 10 years	11 (35.6)		
BMI		**The first breast ca diagnosis**		**Metastasis (at the contralateral breast diagnosis)**	
Normal	9 (29.0)	Palpable mass	14 (41.1)	None	28 (90.4)
Overweight	13 (42.0)	Swelling and disfigurement	7 (20.6)	Lung	2 (6.5)
Obese	9 (29.0)	Nipple discharge	2 (5.9)	Lung and bone	1 (3.1)
Menarche age		Routine check	11 (32.4)		
< 12	3 (9.7)				
12–14	26 (83.8)				
> 14	2 (6.5)				
Menstrual periods				**Histopathology (left)**	
Regular	28 (90.3)	**The second breast ca diagnosis**		Ductal carcinoma in situ	2 (7.1)
Irregular	3 (9.7)	Palpable mass	5 (15.6)	Invasive ductal carcinoma	18 (64.3)
Relation between diagnosis and menopause		Swelling and disfigurement	2 (6.2)	Invasive lobular carcinoma	5 (17.8)
Pre-menopausal	16 (51.6)	Routine check	25 (78.2)	Mixed invasive carcinoma	1 (3.6)
After menopause	15 (48.4)			Musinoz carcinoma	1 (3.6)
				Metaplastic carcinoma	1 (3.6)
Breast feeding duration		**Number of relatives with cancer**			
No	2 (6.5)	1	8 (44.4)	**Histopathology (right)**	
≤ 1 year	20 (64.5)	2	5 (27.8)	Ductal carcinoma in situ	1 (3.4)
1–2 years	7 (22.5)	3	3 (16.7)	Lobular carcinoma in situ	1 (3.4)
More than 2 years	2 (6.5)	4 and more	2 (11.1)	Invasive ductal carcinoma	20 (69.0)
Chronic disease/Surgical history		**Relative with breast and over Ca**		Invasive lobular carcinoma	6 (20.8)
Yes	19 (61.3)/17 (54.8)	Breast	17 (85)	Invasive apocrine carcinoma	1 (3.4)
No	12 (38.7)/14 (45.2)	Over	3 (15)		

fever, asthma, etc). Surgical history group; appendectomy, cholecystectomy, haemorrhoidectomy, etc. Patients undergoing surgeries were included. In the evaluation of cancer history in relatives, all cancers diagnosed in many organs such as breast, ovary, colon, and brain were included. Relatives diagnosed with breast and ovarian cancer were grouped separately. Metastasis status was determined in all patients after both the initial diagnosis and one-year follow-up. When calculating BMI (kg/m^2), 25.0–29.9 was considered as overweight, 30 and over as

obese, and 18.5–24.9 as healthy. While patients were mostly diagnosed with the first breast cancer because of a palpable mass complaint, the second breast cancer was diagnosed in many patients during their routine checks. The most common breast cancer histopathological type of both breasts was invasive ductal carcinoma, and the mass size was ≤ 2 cm.

For *BRCA1* and *BRCA2* gene analysis, the accession numbers of these genes were accepted as NM_007294.3 (*BRCA1*) and NM_000059.3 (*BRCA2*), respectively. Genomic changes in *BRCA1* and *BRCA2* were detected in only nine (29%) of 31 patients (Tab. 3). Seven of these gene changes were in the *BRCA2* (77.8%) gene, and two were in the *BRCA1* (22.2) gene and were heterozygous contitions. Of these genomic changes, two were pathogenic, one was probably pathogenic, and the remaining seven were variants of uncertain significance (VUS).

In these genes, two variants which were not reported in the literature and classified as pathogenic by us were detected. The variants NM_007294.3 (*BRCA1*): c.2131_2132delAA (p.Lys711Valfs*6) in patient P28 and NM_000059.3 (*BRCA2*):c.1773_1776delTTAT (p.Ile591Metfs*22) in patient P13 were formed in the exonic regions of the genes. These variants caused a loss of function in the gene by means of the frameshift mutation mechanism. Various insilico predictive analysis programs support that these variants have a deleterious effect on the gene or gene product. The variant c.8954-5a> G, detected in the *BRCA2* gene of P14, was previously reported as a likely pathogenic variant in the literature [14, 15]. P13 and P14 patients were grouped as MBBC, and P28 patients were grouped as SBBC. These three patients had their first breast cancer diagnosis in their 40s and their cancer was first detected in the right breast. These patients had numerous cancerous relatives. Patients first consulted a doctor for a palpable mass in the right breast. After the analysis, these three patients were diagnosed with hereditary breast and ovarian cancer syndrome (HBOC) associated with BRCA1 and BRCA2 and were given genetic counseling. Because BRCA disease-related variants were seen in a small number of patients in our sample, it was not possible to compare them statistically with others in this group.

VUS variants were detected in *BRCA1/2* genes of the remaining six patients, two of which were reported in the literature [16–19]. The variants detected in patients P2, P23, P24, and P26 had not been previously reported in the literature. Among the patients with VUS variant, P9 was remarkable because she was diagnosed as Hodgkin's disease when she was 32 years old. In this patient, two separate VUSs were detected: NM_000059.3 (*BRCA2*):c.3310A > C (p.Thr1104Pro) and NM_000059.3 (*BRCA2*):c.3503T > A (p.Met1168Lys). The patient was 54 years old, was first diagnosed with MBBC in the left breast, and her family cancer history was not significant for HBOC. In patients with other VUSs, breast cancer was diagnosed almost exclusively in the left breast (except P24) and grouped as MBBC. In addition to giving genetic counseling to these patients, it was also planned to reevaluate all VUSs determined according to ACMG once every six months. In 10 (58.8%) patients with MBBC, the first cancer was detected in the left breast. The first application of patients in this group was usually due to a mass complaint addressed in the breast.

As a result of the comparison of demographic data of the patients grouped as SBBC and MBBC, a statistically significant relationship was found in the breast where the cancer was first localised ($\chi^2 = 18.850$; $p = 0.000$). There was also a statistically significant relationship between the time interval of cancers and chronic disease ($\chi^2 = 11.519$; $p = 0.001$) (Tab. 4). There was no significant relationship between the two groups in the other demographic data. In addition, histopathological data of tumors in both breasts were compared but no statistically significant result was obtained in the groups. We performed a statistical analysis of the number of children and the number of relatives with cancer variables by grouping according to the first diagnosis age of the patients as ≤ 40 years and > 40 years. The result was not statistically significant ($p > 0.05$).

Discussion

The NCCN guideline recommends the analysis of *BRCA1* and *BRCA2* genes in individuals with bilateral breast cancer [20]. In detecting multiple primer breast cancer of patients, the diagnostic criteria that Warren and Gates first determined in 1932 were used. These criteria include the following: that each tumour is malignant, it has its own pathological features and its own metastatic pathway and the diagnosis of metastatic or recurrent tumours can be excluded, and tumours occur in different parts or organs and are not continuous with each other [21].

In this study of *BRCA1/2* gene analysis findings, demographic characteristics of 31 patients with bilateral breast cancer were investigated, and disease-causing gene variants were identified in three patients. In this way, the aetiology of the disease became clear in these patients. Of these gene variants, the frameshift ones were first described in our patient in the literature. These patients were diagnosed as HBOC and therefore were given genetic counseling. In addition, genetic counseling was given to six patients in whom the VUS variants were identified. VUS classification means that there is insufficient or conflicting evidence regarding a molecular alteration's role in the disease, and hence a periodic

Table 3. *BRCA1* and *BRCA2* gene analysis results and details

Patient ID	Gene	Nucleotid change/ AA change	Exon/ intron	Function	ACMG scoring	Age	Age at first/second diagnosis	First diagnosis location in breast	SBBC/ MBBC	Background	Cancer history on relatives		References
P13	*BRCA2*	c.1773_1776delTTAT (p.Ile591Metfs*22)	Exon 10	Frameshift	PAT	57	40/52	RIGHT	MBBC	Thrombosis, Steatosis hepatis	1^0	2 Lung, 1 Stomach, 1 Larynx	Novel
											2^0	2 Lung, 1 Breast	
											3^0	2 Lung, 1 Breast, 1 Endometrium, 1 Brain	
P14	*BRCA2*	c.8954–5A > G (p.?)	Intron22	Splice Acceptor	L.PAT	61	43/61	RIGHT	MBBC	Ovarian cyst, Diabetes	1^0	1 Breast	De Garibay et al. (2014), Santos et al. (2014)
											2^0	2 Breast	
											3^0	1 Endometrium,	
P28	*BRCA1*	c.2131_2132delAA (p.Lys711Valfs*6)	Exon 10	Frameshift	PAT	43	40/41	RIGHT	SBBC	–	1^0	1 Ovary	Novel
											2^0	–	
											3^0	2 Breast, 1 Ovary, 1 Leukaemia	
P2	*BRCA1*	c.694G > T (p.Asp232Tyr)	Exon 10	Missense	VUS	43	38/42	LEFT	MBBC	Haemorrhoid, Hypertension	1^0	1 Breast	Novel
											2^0	1 Stomach	
											3^0	1 Lung, 1 Breast, 1 Stomach	
P7	*BRCA2*	c.9364G > A (p.Ala3122Thr)	Exon 25	Missense	VUS	61	45/59	LEFT	MBBC	Tonsillectomy, Steatosis hepatis	1^0	–	Tavtigian et al. (2008), Tazzite et al. (2012)
											2^0	–	
											3^0	–	
P9	*BRCA2*	c.3310A > C (p.Thr1104Pro)/ c.3503T > A (p.Met1168Lys)	Exon 11	Missense	VUS	65	54/64	LEFT	MBBC	Hodgkin's disease (Diagnosis: 32), Hypertension	1^0	1 Brain	DE Silva et al. (2011), Karbassi et al. (2016)
											2^0	–	
											3^0	–	
P23	*BRCA2*	c.1160T > C (p.Val387Ala)	Exon 10	Missense	VUS	42	19/40	LEFT	MBBC	Haemorrhoid, Goitre	1^0	1 Melanoma	Novel
											2^0	1 Colon	
											3^0	–	
P24	*BRCA2*	c.8474C > T (p.Ala2825Val)	Exon 19	Missense	VUS	61	39/39	SAME	SBBC	Cholecystectomy, Appendectomy, FMF, Glaucoma	1^0	–	Novel
											2^0	3 Breast	
											3^0	1 Breast	
P26	*BRCA2*	c.670G > A (p.Asp224Asn)	Exon 8	Missense	VUS	49	40/47	LEFT	MBBC	Hypertension, Goitre, Myomectomy	1^0	–	Novel
											2^0	1 Prostate	
											3^0	1 Endometrium, 1 Breast, 1 Colon	

Table 4. Significant parameters of SBBC and MBBC groups

Variable	SBBC (n = 14)		MBBC (n = 17)		Statistical analysis* Probability
	n	%	n	%	
First Ca					
Right	3	21.4	7	41.2	$\chi^2 = 18.850$
Left	1	7.2	10	58.8	**p = 0.000**
Simultaneous	10	71.4			
Chronic disease					
No	10	71.4	2	11.8	$\chi^2 = 11.519$
Yes	4	28.6	15	88.2	**p = 0.001**

*χ^2 cross tables were used to examine the relationships between the two qualitative variables

re-evaluation of the VUS identified in patients in the genetic test was planned.

Although there are many studies in the literature regarding the increase in breast cancer risk in individuals carrying *BRCA1/2* gene mutations, there are fewer reports that determine this risk in contralateral breast cancer. In BRCA carriers, the risk of developing breast cancer until the age of 70 years is approximately 50–87%. These carriers have a 32–64% risk for the development of contralateral breast cancer. In the literature some authors claim that the overall risk for contralateral MBBC is approximately 0.5%, and this risk may reach up to 3% of women with BRCA1/2 carriers, and even a 10-year risk of 13–40% can be reached [22, 23]. In another study, 10-year contralateral breast cancer risk in BRCA1 carriers was reported to be 24%, and the same risk for BRCA2 carriers was 19% [6]. The unquestionable joint consequence obtained as a result of research in the literature is that BBC risk increases in carrier women with disease-related variants of the *BRCA1/2* genes. Weitzel et al. searched women in detecting the first breast cancer diagnose age. And they determined that the diagnosis age of first cancer for BRCA1 and BRCA2 was on average 38.6 and 43.6 years old, respectively. In their study, they also examined the time interval between the two cancer diagnoses and found an average of 5.1 years for BRCA1 carriers and 5.2 years for BRCA2 carriers [24]. In another study, individuals with the *BRCA1* mutation were shown to have a 1.6-fold risk of contralateral breast cancer compared to those with *BRCA2* mutations [23]. Rogozińska-Szczepka et al. determined that the age at first diagnosis of bilateral cancer with BRCA carriers and BRCA non-carriers was at the age of 42 and 49 years, respectively [25].

The importance of the first diagnosis age in breast cancer was emphasised in a study conducted by Metcalfe et al., who found that 'women diagnosed with breast cancer under the age of 40 had a 42% risk of developing contralateral breast cancer for 15 years and an annual risk of 2.8%. The same risk decreased to 19% in women who had their first diagnosis after 50 years of age and the annual risk was 1.3%' [6]. Graeser et al. conducted a similar study in relatives of *BRCA1* mutation carriers and found that 'those who received first diagnosis with breast cancer younger than 40 years of age had an increased 25-year contralateral breast cancer risk compared to those older than 50 years (63% and 20%, respectively). The annual risk ratios were 2.5% in the young group and 0.8% in the other group' [23].

In our study, we first examined 31 patients demographically. We then grouped all patients as SBBC and MBBC and compared them for tumour characteristics. Fourteen of the patients (45.16%) were grouped as SBBC, and 17 (54.84%) were grouped as MBBC. The median age was 53 years for all patients, 49.5 years for SBBC patients, and 40 years for MBBC patients, and all values were the same as those in the literature [26]. 32.4% of the patients were diagnosed with first breast cancer and 78.2% with second breast cancer during routine controls. For this reason, the fact that both healthy and breast cancer women are subject to routine checks plays an important role in the early diagnosis and determination of treatment options for this disease. In the literature, it has been illustrated that young patients are vulnerable to MBCC [7].

There was a statistically significant relationship between chronic disease and MBBC in our patient group (p = 0.001). The mean age of first and second cancer diagnosis in the MBCC group was 40 and 49 years, respectively. The time interval between the two cancer diagnoses was 11 years (35.6) or more. It was found that 10 (71.4%) of the patients with SBBC had no chronic disease and 15 (88.2%) patients with MBBC had a chronic disease. Among these diseases, goitre, hypertension, diabetes mellitus, migraine, and some inflammatory diseases such as Behcet's disease and familial Mediterranean fever can be considered. All these diseases require periodic monitoring, medical supportive care, and/or drug therapy. In order to investigate this relationship in more detail, it is important to divide chronic diseases into subgroups in larger

patient groups and to question the relationship between the time of diagnosis of these subgroups and the duration of diagnosis of first and second breast cancer. In fact, chronic diseases diagnosed after the treatment of cancer, which is itself a kind of chronic disease, may be triggered by the long-term side effects of this treatment. Also, a chronic disease in the organism may provide a basis for facilitating the development of contralateral breast cancer due to itself and/or treatment. In the literature, a study showing an increased relationship of MBCC compared to SBBC in chronic diseases has not been published. The results of this study, which was carried out for the first time in a small group of patients in Turkey, should be confirmed in a large number of patient groups, and the underlying cause of this condition should be discovered.

In our patient groups, following the literature, there was a statistically significant difference between breast cancers in terms of breast cancer diagnosis age ($p = 0.030$). An aspect of the time interval between cancers was that the breast cancer first diagnosis age of MBBC patients was statistically lower than that of SBBC patients. A clear difference was not found between the tumour characteristics of both groups clinicopathologically [12].

Conclusion

Women with bilateral breast cancer who have a *BRCA* mutation carrier receive their first breast cancer diagnosis at an early age and have a remarkable family history of cancer. MBBC patients receive their first diagnosis at an earlier age than those with SBBC. For the first time in the literature, this study demonstrated a significant association between MBBC with chronic diseases and SBCC. Increasing the number of patients and conducting larger-scale studies will help clarify the uncertainties in the relationship between chronic diseases and MBBC.

Ethical compliance

The independent Ethics Committee approved this study.

Conflicts of interest

The authors declare to have no conflict of interest.

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VIA MEDICA

REVIEW ARTICLE

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Bevacizumab or standard chemotherapy in previously treated patients with metastatic colorectal cancer — a systematic review

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ABSTRACT

Introduction. The *BRAF V600E* mutation (*BRAF*mt) occurring in the metastatic colorectal cancer (mCRC) patients is associated with poorer prognosis, in comparison to the wild-type variant of the *BRAF* gene (*BRAF*wt). Aim of this work was to assess the clinical efficacy of bevacizumab (BEVA) or standard chemotherapy (ChT) in the 2nd or further lines of treatment in mCRC *BRAF*mt population.

Material and methods. MEDLINE/PubMed, Embase and Cochrane CENTRAL databases were systematically searched. The reference lists of relevant studies were also checked.

Results. 6 eligible trials were identified: MOMA (BEVA ± ChT), allowing for limited overall survival (OS) assessment, WJOG 6210G (BEVA + FOLFIRI), RAISE and 20050181 (FOLFIRI), PICCOLO and Spindler 2013 (irinotecan monotherapy). None of those trials were designed for the treatment evaluation in *BRAF*mt population. Available evidence was restricted to limited analyses in small subgroups (from a few to several dozens of patients), occasionally comprising *RAS* gene mutation (*RAS*mt) as well. Based on the identified studies, the comparison of BEVA ± ChT *vs.* ChT or among different ChTs in *BRAF*mt population was not feasible.

In case of BEVA (MOMA), OS hazard ratio (HR) for *BRAF*mt *vs.* *BRAF*wt was 1.52 (95% CI: 0.79–2.89) with difference in medians equal to 12.1 months (19.2 *vs.* 31.3 months, respectivelly), and *BRAF*mt or *RAS*mt patients had median OS lower by 7.9 months and median progression free survival (PFS) by 3.0 months in WJOG 6210G trial. In case of ChT, median PFS was lower in *BRAF*mt by 12–67% (HRs range: 1.01–5.3), and median OS by 34–73% (HRs range: 1.05–5.00).

Conclusions. Due to limited clinical evidence, assessment of further lines of treatment in *BRAF*mt mCRC patients is uncertain, however existing data consistently suggest lower effectiveness of BEVA ± ChT or ChT in *BRAF*mt, than in *BRAF*wt subgroup. Hopefully, combining anti-EGFR therapies with BRAF/MEK inhibitor is expected to improve prognosis of those patients.

Key words: BRAF, colorectal cancer, systematic review, bevacizumab, chemotherapy

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Introduction

Substitution of valine (Val) with glutamic acid (Glu) in codon 600 (*V600E*) of the proto-oncogenic BRAF kinase gene that is part of the mitogen-activated protein kinase (MAPK or RAS-RAF-MEK-ERK) signalling pathway, is present in 8–12% of metastatic colorectal cancer (mCRC) cases, more often with right-sided primary tumor location [1, 2]. This pathway plays an essential role in the regulation of cell proliferation,

differentiation, survival, and apoptosis, it is also responsible for signal transduction from growth factor receptors, including epidermal growth factor receptor (EGFR) [1–3]. This point mutation leads to constitutive kinase phosphorylation, which drives sustained activation of MAPK signalling pathway. The mechanism of this process has not been fully understood, but it seems that cancers with such a genetic abnormality constitute a distinct phenotypic group [2, 4–6]. The V600E *BRAF* mutation is detected in 40–60% of sporadic cancers with microsatellite instability but almost never in Lynch syndrome (about 1%) and in tumors with *KRAS* and *NRAS* mutations [1, 2, 7]. However, the co-occurrence of *BRAF* aberration and microsatellite instability may be associated with a better prognosis by abolishing the opposing effects of both genetic changes [2, 7, 8]; this mechanism is not fully understood [2]. It is widely accepted that the presence of *BRAF V600E* mutation in patients with colorectal cancer is associated with a poor prognosis at any stage of the disease [1, 2], and the median overall survival may be up to three times lower compared to patients with a wild-type gene variant [9]. *BRAF* mutations other than V600 occur much less frequently and most likely bear no adverse prognostic significance [1, 2].

Most available data in mCRC patients relate to first-line treatment; there are no clear differences in progression-free survival (PFS) when chemotherapy alone is used, however, overall survival is markedly shorter in *BRAF*mt group [2, 10]. Despite the limited scientific evidence, bevacizumab added to FOLFOXIRI chemotherapy is currently the recommended molecularly targeted drug in the first-line treatment of advanced disease [9–14]. However, according to available clinical data [15, 16], a response to anti-EGFR therapy (cetuximab, panitumumab) is unlikely, and the occurrence of the *V600E* mutation is a contraindication to such treatment unless it is combined with anti-BRAF therapy [7, 14]. There is scarce data concerning the clinical efficacy of further treatment lines. The aim of this study was to systematically review the clinical trials assessing bevacizumab or irinotecan- or oxaliplatin-based chemotherapy in second and further treatment lines of mCRC with *BRAF* mutation.

Methods

A systematic search of MEDLINE, Embase and Cochrane CENTRAL databases was conducted on August 5, 2019. The search strategy included all types of studies, i.e. secondary and primary, including both randomized and non-randomized clinical trials, as well as non-controlled ones, assessing the use of bevacizumab or chemotherapy containing irinotecan or oxaliplatin in second or further treatment lines in advanced CRC. Studies assessing clinical efficacy (OS, PFS, objective response rate — ORR) in patients with the *BRAF V600E* gene mutation were included, encompassing comparative assessment between sought interventions in the target population or assessment in relation to patients without the *BRAF* mutation. The defined strategy also allowed to find secondary studies. Detailed information on the search strategy and systematic review is provided in the Supplemental materials (Tab. S1, S2, Fig. S1).

Two-stage publication selection (titles and abstracts analysis followed by full texts analysis) in accordance with the defined PICOS scheme (Tab. S2) as well as the assessment of study quality and risk of bias in the ROB 2.0 [17] and ROBINS-I [18] scales were performed by two independently working researchers (W.S., M.H.) (Tab. S3). Data extraction was carried out in pairs in which one of the persons checked the correctness of the data. Doubts were discussed with the third person (M.K.) until consensus was reached. The above assumptions were pre-determined before the actual review. Presenting the results, the data for *BRAF*mt subgroup were extracted, referring to *BRAF*wt group when possible. In some cases, the necessary calculations were made to present the result for *BRAF*mt *vs. BRAF*wt comparison and based on available data the relative benefit (RB), response rate and a difference in median survival were estimated. The systematic review was carried out in accordance with current Health Technology Assessment (HTA) guidelines of the Agency for Health Technology Assessment and Tariffs (AOTMiT, Agencja Oceny Technologii Medycznych i Taryfikacji) [19].

Results

As a result of the systematic review (Tab. S1), six primary trials (presented in six publications) were found: MOMA [20], WJOG 6210G [21], RAISE [22], 20050181 [23], PICCOLO [24] and Spindler 2013 [25] (Fig. S1). The results of additional analysis of data from the PICCOLO — Seligmann 2016 study were also taken into account [10]. Five of the included studies [20–24] were randomized clinical trials (RCT), but none of them was specifically targeted at the population with *BRAF* mutation — determination of this mutation was not required by inclusion criteria, and the assessment of the significance of *BRAF* mutation was exploratory and included only a subgroup of patients with available material and genotyping results. Furthermore, each study in one arm used intervention not included in the criteria of the presented review — the combination of anti-EGFR or anti-VEGF drug with chemotherapy. In one study [20] only limited assessment of OS was pos-

sible, including the use of BEVA with chemotherapy in the next treatment line after disease progression in the majority of patients. Only the observational study Spindler 2013 [25] was aimed at assessing the impact of *BRAF* mutations. Available results were sufficient only for analysis of clinical efficacy within a small *BRAF*mt subgroups (from several to several dozen patients), sometimes including *RAS*mt [21] and referring them to *BRAF*wt population. The identified studies did not allow for comparative assessment of BEVA with chemotherapy *vs.* CHT or various CHTs within the *BRAF*mt population. No systematic reviews were found assessing the use of the given intervention in further treatment lines. The characteristics of the included trials are presented in Table S4 and the main results are summarized in Table 1. No meta-analyzes of the results were performed due to high clinical heterogeneity.

Bevacizumab (BEVA) + chemotherapy

In the MOMA trial (Cremolini 2019) [20], 232 patients with mCRC were randomized to one of two protocols: 8 cycles of first-line induction therapy with FOLFOXIRI + BEVA, followed by maintenance therapy continued to disease progression — BEVA or BEVA + metronome chemotherapy (capecitabine and cyclophosphamide). Central determination of *BRAF* (exon 15 [*V600E*] assessment with use of Matrix-Assisted Laser Desorption/Ionization Time-of-Flight MassARRAY system or *RAS* gene mutations was performed in 203 patients, and in 20 (10%) patients mutated *BRAF* status was detected. During a median follow-up of 47.8 months, a total of 210 patients progressed and 152 (72%) received next treatment line, of which 91 (60%) were re-treated with BEVA + FOLFOXIRI, and 31 (20%) — BEVA + FOLFIRI/FOLFOX, and 3 (2%) — BEVA + fluorouracil. In total, BEVA was used in 82% of patients receiving the subsequent treatment line. Therefore, overall survival (OS) analysis also included the use of BEVA in the second treatment line, however, it can be assumed that the observation concerned a maximum of approximately 11 *BRAF*mt patients who had progressed and received BEVA again in the next line. In the *BRAF*mt population, the median OS was 19.2 months and was significantly lower than in *RAS*wt and *BRAF*wt patients (N = 36) — 31.3 months (difference of 12.1 months), similarly to *RAS*mt (N = 150) — 24.9 months (difference of 6.4 months). In the whole group, the risk of death at a given time point was higher for *BRAF*mt compared to *BRAF*wt and *RAS*wt, but the difference did not reach the statistical significance threshold: HR = 1.52 (95% CI: 0.79–2.89), P = 0.208 [20] (Fig. 1.).

The evaluation of bevacizumab in further treatment lines was also carried out in a randomized West Japan Oncology Group (WJOG 6210G) study (Shitara 2016) [21], which included patients with mCRC or inoperable, locally advanced CRC, with clinically or radiologically confirmed progression during or up to 3 months after the last dose of first-line chemotherapy with fluoropyrimidine, oxaliplatin and bevacizumab. In addition, it was required to exclude *KRAS* gene mutation (*KRAS*wt) in exon 2 (codon 12 or 13) in the central or local evaluation of paraffin-embedded tumor tissue. The study included 121 patients who were randomized to receive BEVA + FOLFIRI or panitumumab + FOLFIRI. Two patients in each group were excluded from further efficacy analysis due to failure to meet inclusion criteria. After progression, 77.8% of patients received another line of treatment, of which 34.1% received bevacizumab. In addition, 109 patients underwent extended genetic profiling covering *KRAS* and *NRAS* gene mutations — exon 2 (codons 12 and 13), exon 3 (codons 59, 61, 117 and 146) and *BRAF* — exon 15 (codon 600) using next-generation sequencing (NGS) of circulating tumor DNA in serum. *BRAF* gene mutation was detected in 5 (4.6%) patients and *RAS* genes mutations in 14 (12.8%) patients. The results are presented in a way that allows comparison of the combined subgroup with *BRAF* or *RAS* mutation with tumors without mutations in the tested genes (wild-type). Among *BRAF*mt or *RAS*mt patients receiving BEVA + FOLFIRI treatment in the second line (N = 11), the median OS was 8.2 months (95% CI: 6.0–13.7) and was 7.9 months lower compared to wild type subgroups (N = 44) — 16.1 months (95% CI: 12.7–21.1). The median PFS was lower by approximately 3 months in the *BRAF*mt and *RAS*mt groups: 3.7 months (95% CI: 1.8–6.0) *vs*. 6.7 months (95% CI: 5.4–9.4), respectively. The authors also reported that among patients with measurable disease receiving BEVA + FOLFIRI, the objective response rate in the *BRAF*mt or *RAS*mt subgroup was 18.2% and 2.6% in non-mutated patients, respectively. The available data did not allow further calculations, and when interpreting the results it should also be considered that in the BEVA + FOLFIRI group only 3 patients achieved an objective response [21].

Chemotherapy

The assessment of chemotherapy in further treatment lines in patients with mCRC harboring *BRAF* mutation was based on four clinical trials, two of which enabled the evaluation of FOLFIRI scheme: RAISE [22] and 20050181 [23]; and another two irinotecan monotherapy: PICCOLO [24] and Spindler 2013 [25].

The RAISE study evaluated the efficacy and safety of ramucirumab combined with FOLFIRI compared to placebo + FOLFIRI in patients with progression of mCRC during or within 6 months after the last dose of

Table 1. The main results of studies enabling the assessment of clinical efficacy in patients with *BRAF V600E*-mutated mCRC

Study	Sample size	OS	PFS	ORR	RoB
Bevacizumab ± chemotherapy					
MOMA [20]	*BRAF*mt *vs. BRAF*wt and *RAS*wt[1]: 20 *vs.* 36	Median (–39%*): 19.2 *vs.* 31.3 months HR = 1.52 (95% CI: 0.79–2.89); P = 0.208	–	–	Critical
WJOG 6210G [21]	*BRAF*mt or *RAS*mt *vs. BRAF*wt and *RAS*wt[2, 3]: 11 *vs.* 44	Median (–49%*): 8.2 (95% CI: 6.0–13.7) *vs.* 16.1 (95% CI: 12.7–21.1) months	Median (–45%*): 3.7 (95% CI: 1.8–6.0) *vs.* 6.7 (95% CI: 5.4–9.4) months	18.2% *vs.* 2.6%[4]	Critical
FOLFIRI					
RAISE [22]	*BRAF*mt *vs. BRAF*wt and *RAS*wt[5]: 21 *vs.* 143	Median (–73%*): 4.2 *vs.* 15.5 months	Median (–53%*): 2.7 *vs.* 5.7 months	–	Critical
20050181 [23]	*BRAF*mt and *RAS*wt *vs. BRAF*wt and *RAS*wt[6]: 23 *vs.* 190	Median (–63%*): 5.7 *vs.* 15.4 months HR* = 5.00 (95% CI: 3.03–7.69)	Median (–67%*): 1.8 *vs.* 5.5 months HR* = 3.23 (95% CI: 1.96–5.26)	–	Critical
Irinotecan monotherapy					
PICCOLO [24]	*BRAF*mt and *KRAS*wt *vs. BRAF*wt and *RAS*wt and *PIK3CA*wt[7]: 31 *vs.* 163	HR = 1.56 (95% CI: 1.03; 2.37); P = 0.035	–	n/N: 6.5%* (2/31) *vs.* 12.3%* (20/163) RB* = 0.53 (95% CI: 0.13–2.14); P = 0.3688	Critical
PICCOLO — additional analysis [10]	*BRAF*mt *vs. BRAF*wt: 40 *vs.* 419	Median (–34%*): 6.7 (95% CI: 3.9–18.6) *vs.* 10.2 (95% CI: 5.4–18.1) months HR[8] = 1.21 (95% CI: 0.84–1.76); P = 0.31	Median (–12%*): 3.5 (95% CI: 2.6–7.3) *vs.* 4.0 (95% CI: 2.7–8.0) months HR[8] = 1.01 (95% CI: 0.69–1.49); P = 0.93	5.0% *vs.* 8.1% OR[8] = 0.56 (95% CI: 0.13–2.49); P = 0.45	Serious
Spindler 2013 — prospective cohort [25]	*BRAF*mt *vs. BRAF*wt: 8 *vs.* 89	HR* = 3.33 (95% CI: 0.96–11.11)	HR* = 3.57 (95% CI: 0.99–12.50)	0% *vs.* 14% (NS)	Critical
Spindler 2013 — retrospective cohort [25]	*BRAF*mt *vs. BRAF*wt: 8 *vs.* 101	HR* = 1.05 (95% CI: 0.45–2.50)	HR* = 1.79 (95% CI: 0.70–4.55)	0% *vs.* 15% (NS)	Critical
Spindler 2013 — multivariate analysis[9] [25]	–	HR = 4.3 (95% CI: 1.7–10.6); P = 0.002	HR = 5.3 (95% CI: 2.1–13.0); P = 0.0002	–	Critical

OS — overall survival; PFS — progression-free survival; ORR — objective response rate; RoB — risk of bias; HR — hazard ratio; RB — relative benefit; OR — odds ratio; NS — not significant

RoB is the lowest rating among the ROBINS-I scale domains. For almost all included studies, this arises from a critical risk assessment resulting from the presence of interfering factors in the study population (the exception is PICCOLO — additional analysis, where this risk was assessed as serious).

*Estimated based on available data; [1]overall survival (OS) analysis included the use of BEVA in 2nd treatment line (for *BRAF*mt population it can be assumed that the follow-up ultimately involved a maximum of approximately 11 patients who had progressed and re-received BEVA in the next treatment line); [2]in BEVA + FOLFIRI subgroup; [3]including three *BRAF*mt patients, taking into account that in total in *BRAF*mt or *RAS*mt group they accounted for 26%; [4]the total number of patients with objective response in the BEVA + FOLFIRI group was 3; [5]in placebo + FOLFIRI subgroup; [6]in FOLFIRI subgroup; [7]in irinotecan monotherapy subgroup; [8]results adjusted to response to previous treatment, performance status, presence of peritoneal metastases, primary tumor resection, and tumor location; [9]multivariate analysis taking into account age, performance status and *BRAF* and *KRAS* genes status

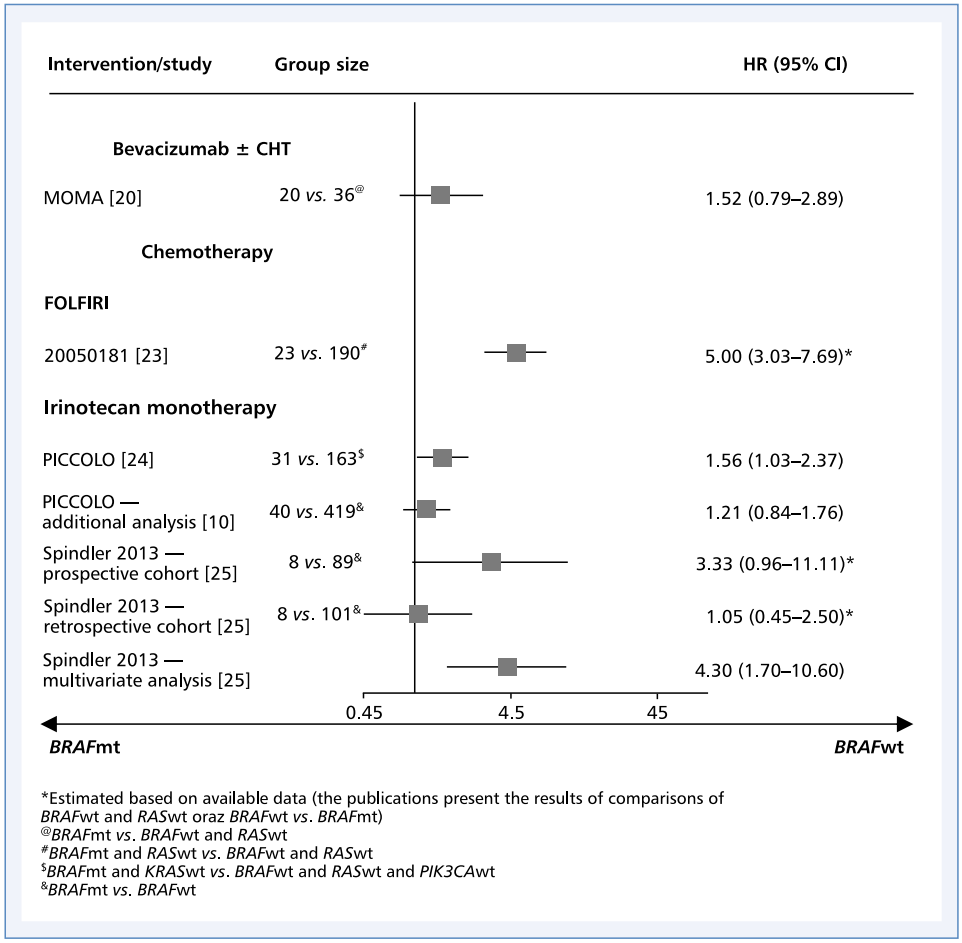

Figure 1. Hazard ratio (HR) for overall survival (OS)

first-line therapy of metastatic disease, including bevacizumab, oxaliplatin and fluoropyrimidine, if they received at least one cycle of therapy [22]. In total, 1,072 patients were included, of which exploratory assessment of the effect of *RAS* and *BRAF* mutations in the tumor tissue on the clinical effectiveness of the intervention was possible in 912 patients, and *BRAF*mt (*V600E*) was detected in 41 (4.5%) patients. Among patients receiving FOLFIRI chemotherapy alone, the median OS (4.2 months) was 11.3 months lower in *BRAF*mt patients (N = 21), compared to *BRAF*wt and *RAS*wt groups (N = 143) — 15.5 months, and 7.3 months compared to *RAS*mt patients (N = 294) — 11.5 months. Similarly, the median PFS was 2.7 months in *BRAF*mt patients compared to 5.7 months in *BRAF*wt and *RAS*wt patients and 4.3 months in *RAS*mt patients [22].

The 20050181 trial was another study enabling the evaluation of the FOLFIRI regimen in further treatment lines in patients with mCRC harboring *BRAF* mutation [23]. A total of 1,186 patients who progressed during or within 6 months after completing the first line FU-containing chemotherapy were randomized to panitumumab + FOLFIRI or FOLFIRI. Of these, 1,014 (85%) patients had assessed *RAS* mutations, and then among 421 *RAS*wt patients, 45 (11%) were found to have *BRAF*mt. A total of 638 (54%) patients had *RAS* or *BRAF* mutations. Extended genetic diagnostics of paraffin-embedded tumor tissue in patients with normal exon 2 of the *KRAS* gene included Sanger sequencing of exon 3 (codons 59/61) and 4 (codons 117/146) of the *KRAS* gene; exon 2 (codons 12/13), 3 (codons 59/61) and 4 (codons 117/146) of the *NRAS* gene and exon 15 (codon 600) of the *BRAF* gene.

The authors of 20050181 study performed an exploratory analysis of clinical efficacy depending on the *BRAF* status. Among patients treated with FOLFIRI, the median OS was lower by 9.7 months in patients with *BRAF*mt and *RAS*wt tumors (N = 23) — 5.7 months, compared to *BRAF*wt and *RAS*wt (N = 190) — 15.4 months: HR = 5.00 (95% CI: 3.03–7.69) (Fig. 1). Similarly, the median PFS in *BRAF*mt and *RAS*wt group was 1.8 months, e.g. 3.7 months lower than in *BRAF*wt and *RAS*wt groups — 5.5 months: HR = 3.23 (95% CI: 1.96–5.26) (Fig. 2). In both cases, the observed diffe-

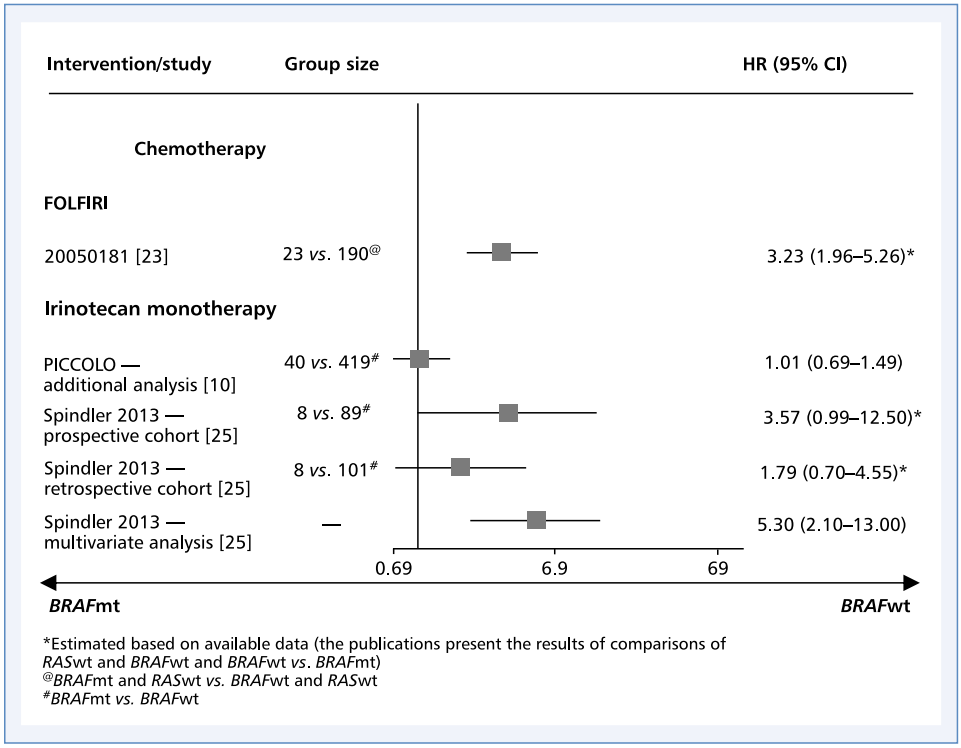

Figure 2. Hazard ratio (HR) for progression-free survival (PFS)

rences depending on the presence of *BRAF* mutations were statistically significant.

Another two trials evaluated irinotecan-based chemotherapy in the further treatment lines of advanced CRC with *BRAF* gene mutation. PICCOLO RCT included patients with inoperable, locally advanced, or metastatic CRC with prior progression during or after fluoropyrimidine-containing chemotherapy. Almost all patients had previously received oxaliplatin. After protocol amending, only patients with wild-type 12, 13 and 16 codons of *KRAS* gene were recruited (no prior anti-EGFR therapy was required). Finally, the analysis included 460 patients randomly assigned to receive panitumumab + irinotecan or irinotecan alone [24]. Further pyrosequencing of available paraffin-embedded tumor tissue was also carried out including codon 146 of *KRAS* gene, codon 12, 13 and 61 of *NRAS* gene, codon 542, 545, 546 and 1047 of *PIK3CA* gene and codon 600 of *BRAF* gene. Mutations in the *BRAF* gene were detected in 68 (14.8%) patients. OS in patients with *BRAF* gene mutation receiving irinotecan alone (N = 31) was significantly shorter compared to the group with wild type of all of the genes listed (*BRAF*wt, *RAS*wt and *PIK3CA*wt) (N = 163): HR = 1.56 (95% CI: 1.03–2.37), P = 0.035. This study also assessed objective response rate (ORR) according to RECIST criteria, which was twice lower in *BRAF*mt patients compared to all-wt: 2 (6.5%) *vs.* 20 (12.3%); RB = 0.53 (95% CI: 0.13–2.14), however with no statistical significance (P = 0.3688) [24].

In addition, Seligmann et al. [10] assessed the effect of *BRAF* mutation on the effectiveness of irinotecan monotherapy based on patients-level data from the PICCOLO study, considering the entire population, regardless of *KRAS* mutation status. The analysis included 459 patients with available results of *BRAF* mutation assessment, among which in 40 patients *V600E* mutation was found. Median OS in *BRAF*mt individuals was 3.5 months lower compared to the *BRAF*wt group: 6.7 *vs.* 10.2 months, but the difference did not reach statistical significance: HR = 1.21 (95% CI: 0.84–1.76). PFS medians were similar: 3.5 *vs.* 4.0 months; HR = 1.01 (95% CI: 0.69–1.49), while ORR probability was lower by 44%, but not reaching statistical significance: 5.0% *vs.* 8.1%, OR = 0.56 (95% CI: 0.13–2.49); P = 0.45.

The last analyzed trial was the non-randomized study — Spindler 2013 [25], evaluating the effect of *KRAS* and *BRAF* gene mutations on the outcomes in mCRC patients receiving irinotecan monotherapy in the second line (in prospective and retrospective cohort). The study included 110 patients in the prospective cohort, of which in 97 patients *BRAF* mutation status of tumor tissue was evaluated, with 8 (7%) positive results; and 111 patients in the retrospective cohort, among whom 109 were genotyped and *BRAF*mt was detected in 8 (8%) subjects. Assessment of mutation in 600 codon of *BRAF* gene was performed with the use of Amplification Refractory Mutation System-Quantitative PCR of DNA isolated from paraffin-embedded tumor tissue.

In the prospective cohort, HR for OS in *BRAF*mt (N = 8) *vs*. *BRAF*wt (N = 89) was 3.33 (95% CI: 0.96–11.11), while in the retrospective cohort — 1.05 (95% CI: 0.45–2.50) in 8 and 101 patients, respectively. Similarly, the risk of progression or death (PFS analysis) was higher in *BRAF*mt patients in both the prospective (HR = 3.57 [95% CI: 0.99–12.50]) and the retrospective cohort (HR = 1.79 [95% CI: 0.70–4.54]), however with no statistical significance. In the multivariate analysis considering age, performance status (PS) and *BRAF* and *KRAS* genes mutational status, the presence of *BRAF* mutations was associated with significantly worse prognosis: HR = 4.3 (95% CI: 1.7–10.6), P = 0.002, for comparison *BRAF*mt *vs. BRAF*wt in OS analysis (Fig. 1) and HR = 5.3 (95% CI: 2.1–13.0), P = 0.0002 in PFS analysis (Fig. 2). No *BRAF*mt patient achieved the objective response compared to 14% of *BRAF*wt patients in the prospective cohort and 15% in the retrospective cohort, but these differences did not reach statistical significance [25].

Risk of bias assessment

Five RCTs were included in the systematic review [20–24], however, the randomization did not refer to the subject of this review: *BRAF* gene mutational status was neither an inclusion criterion nor a stratification factor of randomization, genetic analysis was performed only in part of included patients, and the analysis of *BRAF* mutation impact was exploratory. These studies were not designed to compare interventions that *BRAF*mt patients were randomized to, and one of the trial arms included intervention whose assessment was not the purpose of this review (panitumumab + FOLFIRI [21], ramucirumab + FOLFIRI [22], panitumumab + irinotecan [24]). In one study [20] only limited inference based on OS assessment was possible due to the fact that observation within this endpoint also included BEVA in the subsequent treatment line after disease progression used in the majority of patients. Ultimately, in these RCTs, it was only possible to assess clinical efficacy within one study arm among patients with a known *BRAF* mutation and to refer these results to patients with a wild genotype. Accordingly, it was considered that in the context of presented study it would be appropriate to assess the risk of bias using a scale for non-randomized trials, as it will allow taking into account the baseline differences in demographic and clinical characteristics resulting from the lack of effective randomization. Table S3 presents the result of the risk of bias assessment of all 6 publications included in the systematic review in the ROBINS-I scale. The risk of bias was generally high, and most of the limitations found resulted from the analysis of outcomes only in subgroups distinguished based on *BRAF* gene mutational status, for which many significant data were not presented in publications yet. Considering the construction of the ROBINS-I scale, such a severe limitation in the interfering factors domain translates into a critically high risk in the overall assessment of the likelihood of endpoints reliability, regardless of the result of the assessment in other domains in the scale. An exception was the additional analysis in the PICCOLO study [10], in which the use of appropriate statistical adjustments allowed to partially eliminate the risk of bias associated with the uneven distribution of prognostic factors between groups — therefore the cumulative risk of bias was assessed as high.

Discussion

According to published reports, the *BRAF* mutation is associated with a significantly reduced survival of colorectal cancer patients receiving chemotherapy, both in the early and advanced stages [26]. While some evidence is available on the efficacy of 1st line treatment in advanced disease, there is limited data regarding further treatment. To our knowledge, this is the first published systematic review of available evidence assessing the efficacy of bevacizumab and chemotherapy in 2nd and further treatment lines of advanced colorectal cancer with the *BRAF V600E* mutation.

Although the presented review included predominantly RCTs, the available results only allowed for assessment of clinical efficacy within particular treatment arms, and the comparison did not relate to different interventions in the *BRAF*wt population (presence of *BRAF* mutation or even a requirement for genetic evaluation of this genetic abnormality were not an inclusion criterion in any of the RCTs), but only a reference of the outcomes observed in subjetcs receiving the same intervention with the mutation to those with the wild gene. Therefore, the assessment of the impact of *BRAF* mutation on treatment outcomes had an exploratory nature and was only possible in some patients with available material and genetic tests performed. The analysis of the effect of *BRAF* mutation on the effectiveness of irinotecan monotherapy was a goal of only Spindler 2013 observational study [25]. To WJOG 6210G [21] and PICCOLO [24] (after protocol amendment) trials only patients with non-mutated *KRAS* gene were enrolled. Similarly, in study 20050181 [23], only patients with *KRAS*wt underwent extended genetic diagnostics, including *BRAF* gene assessment. In general, control groups in RCTs included patients with wild genotype, according to both *BRAF* and *RAS* mutations (and additionally *PIK3CA* [24]). Only in one study, the extended genetic profiling was carried out using peripheral blood circulating tumor DNA [21], in others, they were performed using paraffin-embedded

tissue specimens. Patients with metastatic CRC were included in most clinical trials, and only two enrolled patients with inoperable, locally advanced tumors [21, 24].

Severely limited data was found to assess the efficacy of bevacizumab (± CHT) in 2nd and subsequent treatment lines in patients with advanced CRC harboring *BRAF* mutation. In one study (MOMA) with bevacizumab in the 1st treatment line of metastatic disease only limited OS analysis was possible because this observation also included subsequent treatment lines, and most patients received re-treatment with bevacizumab [20]. However, it is estimated that up to app. 11 patients with the *BRAF* mutation were subjected to such analysis. On the other hand, in another study (WJOG 6210G), the assessment included a total of 11 patients with either *BRAF* or *RAS* mutation and it can be assumed that the former one occurred only in about 3 patients [21]. Nevertheless, in both studies, the median OS was consistently reduced in patients with the *BRAF* mutation, by 39% and 49%, respectively [20, 21], and the risk of death was 1.5 times higher [20]; similarly, the median PFS was reduced by 45% [21]. The data on the objective response rates in the bevacizumab group were insufficient, which makes impossible to draw plausible conclusions.

Studies on the efficacy of chemotherapy in further treatment lines (FOLFIRI or irinotecan monotherapy) also had significant limitations but evaluated *BRAF*mt population was greater and included from 16 to 31 (40, taking into account the alternative analysis of PICCOLO study data [10]) patients in particular studies [22–25], a total of 91 patients (100 including [10]). When using FOLFIRI in one study, the median OS was 73% lower in the *BRAF*mt group and PFS by 53% [22], while in the other by 63% and 67%, respectively [23]. The risk of death at a given time point was several times higher if the mutation was present — five times [23], about 1.5 times [10, 24] and more than four times (multivariate analysis [25]), and the differences were statistically significant. Similarly, the risk of death or disease progression was more than three and four times higher ([23] and [25] — multivariate analysis, respectively), although in an alternative estimation of the PICCOLO study results there was no significant difference in PFS (median 12% lower, HR = 1.01 [10]).

Regarding the objective response rate, available data was markedly limited, in one study the incidence of this endpoint was almost twice lower in *BRAF*mt patients (statistically insignificant difference) [10, 24], while in the other study no patient with *BRAF* mutation ORR was reported [25].

Seligmann et al. [10] assessed the effect of *BRAF* mutation on the results of treatment of advanced CRC with standard chemotherapy using patient-level data from RCTs: COIN [27, 28] and FOCUS [29] (oxaliplatin and fluorouracil in the 1st line) and PICCOLO [24] (irinotecan in the 2nd line). The results of this additional analysis regarding the PICCOLO are presented in the main part of this publication. For the 1st line treatment of advanced disease, the authors found that the presence of the *BRAF* mutation is a significant OS prognostic factor (cumulative data for both RCTs: 10.8 *vs.* 16.4 months [HR = 1.49 (95% CI: 1.23–1.80); P < 0.001)], also after matching with respect to baseline characteristics. However, no clear impact of the mutation on PFS and ORR was observed. Survival after progression was also assessed, defined as the time from progression to death among patients with disease progression; when the date of progression was unknown, the date of the last chemotherapy cycle was taken into account. Patients with the *BRAF* mutation had a shorter survival after progression compared to those with the wild-type gene in both 1st line studies (COIN and FOCUS), the results for both clinical trials: 3.2 *vs*. 8.6 months; HR = 1.72 (95% CI: 1.35–2.19), P < 0.001 [10]. It is worth noting that significantly fewer *BRAF*mt patients received subsequent treatment line: 33% *vs*. 51%, P < 0.001; and a significantly higher percentage of *BRAF*mt patients with rapid progression (< 6 months) was observed in both the 1st and 2nd treatment line — 36.5% compared to — 21.9% in non-mutated patients; P < 0.001 [10].

It should be noted that inference based on the collected data is subject to uncertainty, due to the small size of *BRAF*mt population, and on the other hand with methodological limitations of included trials. In addition, the generally high risk of systematic error in the included studies greatly limits conclusions of the analysis. This is mainly due to the nature of the analyzes that were only possible when the included studies were treated as single-arm. It should be noted that the result of the assessment in the other domains of the ROBINS-I scale was better, although this does not change the overall assessment of systematic error risk. The clinical heterogeneity of the trials (especially in terms of interventions used) prevents proper data synthesis and may affect the interpretation and the ability to relate the review results to the target population of metastatic colorectal cancer patients.

Despite the aforementioned numerous limitations, the analysis quite clearly indicates lower effectiveness of evaluated interventions (bevacizumab ± chemotherapy or chemotherapy) in *BRAF*mt patients. In this group, none of the studied therapies were as effective as in *BRAF*wt population. The advantage of this systematic review is the extended search, which was carried out in 3 databases and also included non-randomized studies to comprehensively assess the effectiveness of the examined intervention. However, gray literature not being indexed in medical databases and ongoing research were not included which could

affect the scope of the evidence described. Only full-text articles in Polish or English were selected, and there were few studies, and some of them did not fully answer the clinical question, which is also a limitation of this analysis.

Combination of BRAF inhibitors with other medications may be more effective than monotherapy, which may lead to resistance by secondary activation of the MAPK pathway — this mechanism may be due to increased EGFR and MEK/ERK pathway signalling activity [1, 2, 30, 31]. Therefore, particular hope is given in the combination of BRAF inhibitors with drugs directed against parallel signalling pathways [1, 6]. There are promising recently published results of phase III BEACON study, in which the combination of cetuximab (anti-EGFR drug), encorafenib (BRAF inhibitor) and binimetinib (MEK inhibitor), as well as cetuximab with encorafenib in the 2nd or 3rd line of treatment significantly increased overall survival of patients with *BRAF V600E* mutation compared to standard therapy [32]. Some hope is also raised by a treatment regimen containing dabrafenib (BRAF inhibitor), panitumumab (anti-EGFR) and trametinib (MEK inhibitor), which has shown promising efficacy in patients with the *BRAF V600E* mutation [33]. Both of these regimens are currently recommended in the US NCCN guidelines for the treatment of patients with mCRC with the current *BRAF* mutation in 2nd and further treatment lines [7].

Clinical comment (P.P.)

When colorectal cancer progresses to metastatic disease, the *BRAF V600E* mutation, which is detected in about 10% of patients, becomes a factor that significantly worsens prognosis. The median survival of patients enrolled in current phase III clinical trials is over 30 months, but if the *BRAF* mutation is present, this value is two or three times lower [36].

It has been demonstrated that patients with *BRAF* mutation undergoing chemotherapy receive systemic treatment of subsequent lines less frequently than other patients, which results from the rather dynamic progression often causing symptoms and deterioration of the general condition preventing further cancer treatment. Until recently, this molecular abnormality has not been routinely studied in patients enrolled in clinical trials, so data on the efficacy of various chemotherapy and biological treatment regimens are based largely on retrospective analyses and are inevitably burdened with selection bias. However, these data quite consistently indicate that, apart from adverse effect on prognosis, the *BRAF V600E* mutation is a determinant of ineffectiveness or very little benefit from the use of anti-EGFR antibodies, especially in monotherapy.

For the reasons given above, it is suggested that in patients with this molecular disorder, 1st line systemic treatment should be as intensive as possible (at least doublet or triplet chemotherapy, i.e. FOLFOXIRI regimen) preferably with the addition of bevacizumab.

The data on the value of subsequent line therapies are extremely scarce, but the efficacy of chemotherapy and anti-angiogenic drugs appears to be low, as confirmed by this systematic review.

After successes in melanoma patients, BRAF tyrosine kinase inhibitors appeared to be the natural choice for next-line therapy, especially in combination with MEK inhibitors, but early phase clinical trials were disappointing [33]. Some optimism was brought only by attempts to use triple therapy additionally containing anti-EGFR antibody. The results of the BEACON phase III study dedicated to previously treated patients with *BRAF V600E* mutation have been recently published. Combination of cetuximab with a BRAF inhibitor encorafenib, as well as triple therapy containing an additional MEK inhibitor binimetinib, have been shown to increase overall survival and time to the quality of life deterioration compared to cetuximab combined with irinotecan-based chemotherapy [32, 37].

Another potentially very effective treatment method may be anti-PD1 immunotherapy or a combination of anti-PD1 and anti-CTLA4 because *BRAF V600E* mutation quite often coexists with microsatellite instability, which is a favorable predictor for this treatment [38]. Available data, however, come from phase II non-controlled studies, and patients with *BRAF V600* mutation were a minority [39].

Conflict of interest

The authors have no conflict of interest to declare.

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Appendix

Table S1. Search strategy

#	Query
PubMED	
#2	advanced OR metastatic
#3	colon cancer OR colorectal cancer
#4	(#2 AND #3)
#7	randomized controlled trial[pt]
#8	random allocation[mh]
#9	random*[tiab]
#10	controlled[tiab]
#11	(#7 OR #8 OR #9 OR #10)
#13	BRAF
#16	(bevacizumab OR FOLFOXIRI OR FOLFIRI OR FOLFOX OR oxaliplatin OR irinotecan)
#17	(#4 AND #16)
#18	(#17 AND #11)
#20	(#13 AND #17)
#22	(#18 OR #20)
Cochrane	
#1	advanced OR metastatic in Trials
#2	[mh „colorectal neoplasms"] OR „colon cancer" in Trials
#3	#1 AND #2 in Trials
#4	bevacizumab OR FOLFOXIRI OR FOLFIRI OR FOLFOX OR oxaliplatin OR irinotecan in Trials
#5	#3 AND #4 in Trials
#6	BRAF in Trials
#7	#6 AND #5 in Trials
#8	#7 OR #5 in Trials
Embase	
#1	(advanced:de OR metastatic:de) AND [embase]/lim
#2	(‚colon cancer':de OR ‚colorectal cancer'/exp) AND [embase]/lim
#3	(‚bevacizumab':de OR folfoxiri:de OR ‚folfiri':de OR ‚folfox':de OR ‚oxaliplatin':de OR ‚irinotecan':de) AND [embase]/lim
#4	#1 AND #2
#5	#3 AND #4
#6	[randomized controlled trial]/lim AND [embase]/lim
#7	random*:ab,ti AND [embase]/lim
#8	controlled:ab,ti AND [embase]/lim
#9	randomization:de AND [embase]/lim
#10	#6 OR #7 OR #8 OR #9
#11	#5 AND #10
#12	braf AND [embase]/lim
#13	#5 AND #12
#14	#11 OR #13

Table S2. PICOS scheme

Parameter	Inclusion criteria
Population	Adults with advanced CRC and assessed *BRAF* (V600E) status, progression after first-line treatment of advanced disease
Intervention	Bevacizumab (± CHT) in 2nd or further treatment line due to advanced disease Oxaliplatin- or irinotecan-based chemotherapy regimens in 2nd or further treatment line due to advanced disease
Comparison	As above or none
Outcomes	Overall survival (OS) Progression-free survival (PFS) Objective response rate (ORR) Studies enabling comparative assessment of sought interventions in the *BRAF*mt population or relating their effectiveness to *BRAF*wt patients were included
Study design	Randomized, controlled clinical trials, controlled or non-controlled non-randomized studies, published in full-text in English or Polish Systematic reviews published in English or Polish

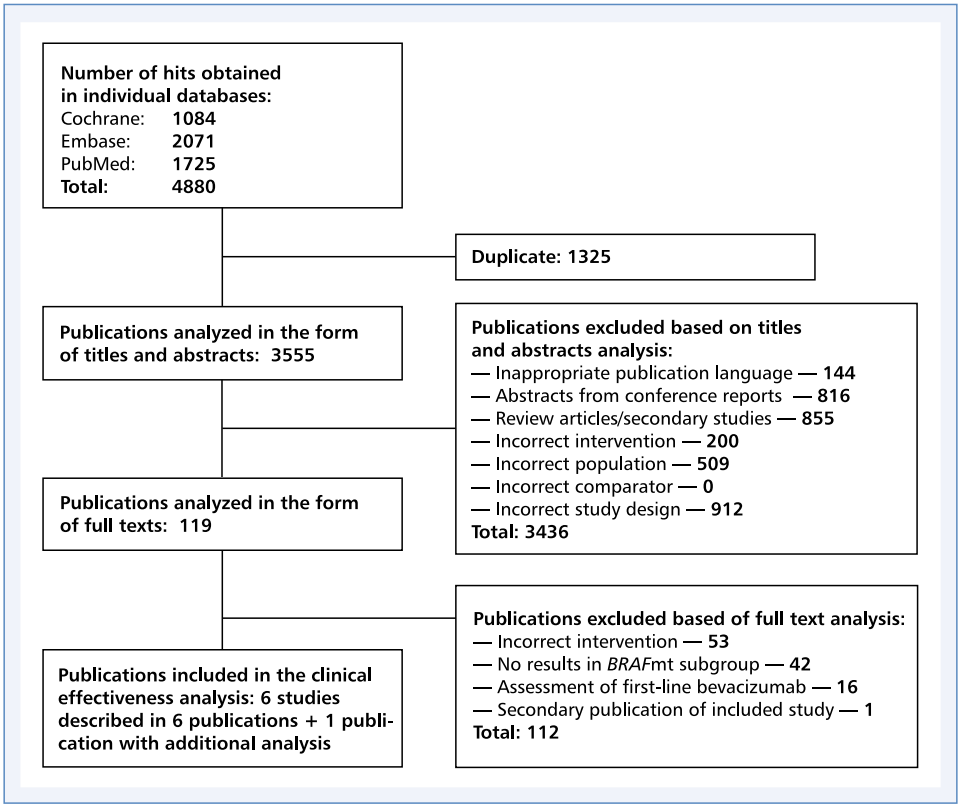

Figure S1. Search results. List of excluded studies at the stage of full texts analysis along with the reasons for exclusion is available on request

Table S3. Assessment of risk of bias in the included studies using the ROBINS-I tool

Study name	Domain	Disturbing factors	Patients selection	Intervention classification	Deviations from planned interventions	Missing data	Outcomes	Selection of described results	Total rating
MOMA [20]	OS	Critical	Low	Low	Low	Moderate	Low	Low	Critical
WJOG 6210G [21]	OS	Critical	Low	Low	Low	Moderate	Low	Low	Critical
	PFS						Serious		
RAISE [22]	OS	Critical	Low	Low	Low	Moderate	Low	Low	Critical
	PFS						Moderate		
20050181 [23]	OS	Critical	Low	Low	Low	Serious	Low	Low	Critical
	PFS						Serious		
PICCOLO [24]	OS	Critical	Low	Low	Low	Moderate	Low	Low	Critical
	PFS						Serious		
	ORR						Serious		
PICCOLO — additional analysis [10]	OS	Serious	Low	Low	Low	Moderate	Low	Low	Serious
	PFS						Serious		
	ORR						Serious		
Spindler 2013 — prospective cohort, multivariate analysis [25]	OS	Critical	Low	Low	Low	Moderate	Low	Low	Critical
	PFS						Serious		
Spindler 2013 — retrospective cohort [25]	OS	Critical	Low	Low	Low	Low	Low	Low	Critical
	PFS						Serious		

The risk of bias on the ROBINS-I scale can be assessed as (in order from lowest to highest): low, moderate, serious and critical, and in the absence of relevant information: unspecified. The total risk error rating is not higher than the lowest among the results in individual domains.

Table S4. Characteristics of included clinical trials

Study name	Study type and location	Intervention	Comparator	Population[a]	Total number/ /number analyzed for mutation/ /*BRAF*mt number	Observation period, endpoints	Funding
MOMA [20]	RCT, non-blinded 16 cancer centers in Italy	After 8 cycles of **FOLFOXIRI** induction therapy (irinotecan 165 mg/m^2 iv + next oxaliplatin 85 mg/m^2 iv + levofolinic acid 200 mg/m^2 + next fluorouracil 3200 mg/m^2 in continuous 48 h infusion) + **BEVA** (5 mg/ kg iv) (q2w) randomized to BEVA (7.5 mg/kg iv) or **BEVA** (7.5 mg/kg iv) + **metronomic chemotherapy** (capecitabine [500 mg 3 × daily] and cyclophosphamide [50 mg daily])	–	mCRC	232/206/20	Median 47.8 months I: PFS II: OS, objective response rate[b], resection rate, safety	GONO Foundation, the ARCO Foundation and F. Hoffmann--La Roche
WJOG 6210G [21]	RCT, non-blinded, multicenter (Japan)	**Panitumumab** (6 mg/kg) + **FOLFIRI** (doses not described) (q2w)	**BEVA** (5 mg/kg) + **FOLFIRI** (doses not described) (q2w)	mCRC or locally advanced inoperable CRC, progression after treatment with fluoropyrimidine, oxaliplatin and bevacizumab, *KRAS*wt in exon 2	117/109/5	15.4 months *vs*. 13.4 months I: OS II: PFS, objective response rate[b], safety, biomarkers analysis	West Japan Oncology Group (WJOG)
RAISE* [22]	RCT, double-blinded (blinded: patients, sites personnel involved, study sponsor), multicenter, international	**Ramucirumab** (8 mg/kg in 60 min) + next **FOLFIRI** (irinotecan 180 mg/m^2 in 90 min iv + next or simultaneously folinic acid 400 mg/m^2 in 120 min iv + next fluorouracil 400 mg/m^2 in 2–4 min bolus iv + next fluorouracil 2400 mg/m^2 in continuous 48 h infusion) (q2w)	**Placebo** (in 60 min) + next **FOLFIRI** (irinotecan 180 mg/m^2 in 90 min iv + next or simultaneously folinic acid 400 mg/m^2 in 120 min iv + next fluorouracil 400 mg/m^2 in 2–4 min bolus iv + next fluorouracil 2400 mg/m^2 in continuous 48 h infusion) (q2w)	mCRC, progression after treatment with fluoropyrimidine, oxaliplatin and bevacizumab	1072/912/41	– I: OS II: PFS, objective response rate[b], disease control, safety, PROs (PROs questionnaires: EORTC QLQ-C30 version 3.0, EQ-5D)	Eli Lilly and Company

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Table S4 cont. Characteristics of included clinical trials

Study name	Study type and location	Intervention	Comparator	Population[a]	Total number/ /number analyzed for mutation/ /*BRAF*mt number	Observation period, endpoints	Funding
20050181* [23]	RCT, non-blinded multicenter, international	**FOLFIRI** (irinotecan 180 mg/m^2 iv + folinic acid 400 mg/m^2 iv or levofolinate 200 mg/m^2 iv + fluorouracil 400 mg/m^2 bolus iv + fluorouracil 2400 mg/m^2 as a continuous infusion over 2 days)	**Panitumumab** (6.0 mg/kg in 60 min + next in 30 min in subsequent infusions) + next **FOLFIRI** (irinotecan 180 mg/m^2 iv + folinic acid 400 mg/m^2 iv or levofolinate 200 mg/m^2 iv + fluorouracil 400 mg/m^2 bolus iv + fluorouracil 2400 mg/m^2 as a continuous infusion over 2 days)	mCRC, progression after fluoropyrimidine-based CTH	1186/421[c]/45	Median 48 wks. I: OS, PFS II: objective response rate[b] and duration, safety, PROs	Amgen Inc
PICCOLO [24]	RCT, non-blinded 60 sites in the UK	**Panitumumab** (9 mg/kg iv) + **irinotecan** (350 mg/m^2; if age > 70 years or performance status 2 — 300 mg/m^2) (q3w)	**Irinotecan** (350 mg/m^2; if age > 70 years or performance status 2 — 300 mg/m^2) (q3w)	mCRC or locally advanced inoperable CRC, progression after fluoropyrimidine-based CTH, no previous anti-EGFR therapy	460/bd./68 (in all $KRAS_{c.12–13,61}$ wt) Additional analysis — irinotecan [10]: 511/459/40	Median of treatment cycles: 4 (range: 0–28) I: OS II: PFS, objective response rate[b], PROs, safety (questionnaire: EORTC QLQ-C30)	Cancer Research UK, Amgen Inc.
Spindler 2013 [25]	Non-RCT, prospective--retrospective, single center (Denmark)	**Irinotecan** (both cohorts: 350 mg/m^2) (q3w)	–	mCRC, 2nd line treatment	Prospective cohort — 110/97/8 Retrospective cohort — 111/109/8	Prospective cohort: median of treatment cycles 4 (1–15); Retrospective cohort: median of treatment cycles 6 (2–15) OS, PFS, objective response rate, safety	Tryg Fonden and the Research Counsil Hospital Lillebaelt

RCT — randomized controlled trial; BEVA — bevacizumab; q2w/q3w — dose administered every 2 and 3 weeks, respectively; PROs — patient-reported outcomes, CTH — chemotherapy

*Detailed data on the research methodology come from the main publications that were not included at the stage of research selection due to the lack of results related to the analysis — RAISE [34], 20050181 [35]

[a]Detailed population data for the subgroups analyzed was not available

[b]Tumor assessment according to the RECIST criteria 1.1

[c]*RAS*wt patients

VIA MEDICA

REVIEW ARTICLE

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Diagnosis and treatment of lymphangioleiomyomatosis (LAM) from the PEComa group

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ABSTRACT

Lymphangioleiomyomatosis (LAM) is a rare, proliferative lung disease, leading to progressive damage of their structure and is a member of the PEComa neoplasm family (perivascular epithelioid cell tumors). In the patients, solid-cystic masses described as lymphangioleiomyoma or extrapulmonary LAM (E-LAM) can occur. E-LAM foci have been described in the mediastinum, supraclavicular lymph nodes, the liver, walls of the small and large intestine, the pancreas, mesentery. E-LAM masses can attain very large sizes — tumors 15–22 cm long have been described. On the basis of positive results of clinical trials sirolimus, a drug from the group of mTOR kinase inhibitors, was registered by the Food and Drug Administration (FDA) in May 2015 as the first and currently only drug for systemic LAM therapy. Sirolimus use is recommended in patients with LAM, accompanied by rapidly progressing deterioration of respiratory system function or $FEV_1 \leq 70\%$ predicted value and in patients with pleural lymph exudate before applying invasive methods of treatment.

Key words: lymphangioleiomyomatosis, sirolimus

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Introduction

Lymphangioleiomyomatosis (LAM) is a rare, proliferative lung disease leading to progressive damage to their structure with the formation of numerous small cysts and lymph accumulation in the pleural cavity [1]. At its basis is the multifocal proliferation of smooth muscle cells and perivascular epithelioid cells, (PECs); in LAM they are also designated LAM cells in the lung interstitium and is therefore included in the family of PEComa type neoplasms (perivascular epithelioid cell tumors) [2]. The PEComa group also includes angiomyolipoma (AML), clear-cell sugar tumor (CCST) — pulmonary and extrapulmonary forms (primary extrapulmonary sugar tumor, PEST), clear-cell myomelanocytic tumor (CCMMT) and primary cutaneous PEComa, CCCMT — cutaneous clear cell myomelanocytic tumor and PEComa NOS (not otherwise specified) [3]. Pulmonary *LAM* (P-LAM) occurs in two clinical forms: associated with tuberous sclerosis [Bourneville-Pringle disease, tuberous sclerosis complex (TSC)] — a genetic syndrome caused by inactivating germline mutations in the *TSC1* and *TSC2* genes, characterized by the occurrence of many tumors of the hamartoma type, perturbations of the nervous system, including epileptic seizures, autism and intellectual disability of various degrees [4], and as a sporadic form — sporadic LAM (S-LAM), in women with no predisposing factors [5]. In addition to pulmonary manifestation of LAM, in these patients, numerous irregularities are observed in the lymph system outside the chest such as perturbations of the patency of the thoracic duct, lymph exudate in the retro-orbital space and pelvis, or lymphadenopathy [5]. Moreover, in rare cases, there is a proliferation of pathological lymph vessels infiltrated by LAM cells, leading to an obstruction of their lumen and accumulation of lymph resulting in the formation of solid-cystic masses described as lymphangioleiomyoma or extrapulmonary LAM (E-LAM) [6].

Frequency of occurrence

The frequency of occurrence of lung LAM differs depending on the clinical type — pulmonary LAM is found in 30–50% women and approximately 10% men with tuberous sclerosis [7, 8], whereas the sporadic form occurs in one woman out of 200–400 thousand [5, 7]. Lymphangioleiomyomatosis concerns almost exclusively women of reproductive age — the median age at diagnosis is in the range of 35–41 years [9– 12]. Pleural LAM is rarely diagnosed in postmenopausal patients – of note here is the often occurring information about the use of hormonal replacement therapy [12, 13]. Single cases have been described of the occurrence of the disease in children and men without predisposing genetic factors [14– 16]. Extrapulmonary perturbations within the lymphatic system are found often in patients with the pulmonary LAM form — perturbations in the patency of the thoracic duct are found in over 70%, lymph exudate in the retroperitoneal space is observed in 30% of the cases [17]. Lymphangioleiomyoma is less common — in 10–21% of patients with pulmonary S-LAM [12, 17– 19] and is often accompanied by a swelling of lymph nodes within the abdominal cavity, ascites and a broadening of the thoracic duct [18]. It seems that the frequency of occurrence of lymphangioleiomyoma in patients with the pulmonary form of LAM increases with the severity of its course [18], but they are not more common in patients with tuberous sclerosis — in a retrospective analysis from the Mayo Clinic, among 403 patients with TSC, E-LAM occurred in only 3 [20]. In the analysis of autopsy material or material obtained from gynecological surgery in 10 female patients with the pulmonary form of LAM, the presence of small LAM foci in the uterus, adnexa or broad ligament of the uterus was observed in as many as 90% , and in 80% — the occupation of the lymph nodes in the retroperitoneal space [21]. It is not clear whether the presence of E-LAM foci predisposes to the development of the pulmonary form of LAM. In a large retrospective analysis of material obtained from 1732 patients without a history of LAM (median age 56 years), who underwent gynecological surgery with lymphadenectomy, extrapulmonary LAM foci were found in 8 patients [22]. In one of them a pulmonary form of LAM was diagnosed, 7 years after the gynecological surgery whereas the remaining women did not have a LAM relapse in any form (median observation 26 months) [22]. In a similar analysis among 19 patients in whom asymptomatic extrapulmonary LAM had been detected occupying the uterus and pelvical and paraortal lymph nodes during an average 33 month observation none developed the pulmonary form [23]. Besides the description of cases concerning women with LAM foci in paraaortal and pelvical lymph nodes, in whom pulmonary LAM did not occur nor tuberous sclerosis [24, 25], descriptions are also available in which extrapulmonary LAM was the first symptom of the development of the pulmonary LAM form, which if it occurs is most commonly diagnosed within about 2 years from the diagnosis of the extrapulmonary form [6, 26–31].

Anatomy and clinical picture

Pulmonary LAM leads to progressive destruction of the lung parenchyma and its replacement by numerous small cysts, causing a progressive respiratory deficiency [7]. In the course of LAM exercise tolerance is progressively limited as well as everyday physical activity to a degree greater than in for example chronic obstructive pulmonary disease [32]. Among the most common early symptoms of the disease are: dyspnea, persistent coughing and hemoptysis [7, 11, 12]. Dyspnea at rest and the need for oxygen therapy appears in most patients within 10 years from the diagnosis [33]. The course of the disease is complicated by recurring pneumothorax, occurring in about 5% of the patients per year from the moment of diagnosis; additionally, in about one-half of the cases they are the first symptom of developing LAM [3, 12, 34]. Moreover, because of the perturbation of lymphatic vessel patency, there are lymph exudates in the pleural cavity causing an intensified dyspnea and chest pain [35]. LAM is also a rare cause of occurrence of pulmonary hypertension (about 7.6% patients LAM) [9].

Lymphangioleiomyoma is most commonly localized in the retroperitoneal space and the uterus and in surrounding lymph nodes [6, 24, 27]. Moreover, E-LAM foci developing in the mediastinum [36], supraclavicular lymph nodes [37, 38], liver [39], walls of the small and large intestine [40, 41], pancreas [26, 42], mesentery [43] have been described. E-LAM masses can reach very large sizes — cases of tumors with the greatest length 15–22 cm have been described [18, 44–46] — and can extend along lymph vessels — coming from the retroperitoneal space to the chest [19], and further even along the neck along with the sternocleidomastoid muscle [47].

The symptoms of lymphangioleiomyoma presence occur in about 55–60% patients [6, 18] and the most commonly are: pain in the vicinity of the tumor [26, 29, 46] and bleeding from the reproductive system in the case of LAM localized in the uterus [24, 48, 49]. Symptoms associated with the pressure of the tumor mass on neighboring organs are also frequently observed: dyspepsia, bloating and symptoms of obstruction of the alimentary tract [46], hydronephrosis or edema and paresthesia of the lower extremities [46, 50]. Lymphangioleiomyoma localized in the mediastinum may cause Horner syndrome, lymph exudates to the pleural cavity and heart rhythm perturbations [6, 18]. If LAM cells infiltrate the ureters, chyluria occurs [51], whereas the occupation of Vater's papilla may lead to cholestasis [45]. In the case of large lymphangioleiomyomas, ascites

linked to a large lymph volume may be observed [6] or bleeding from the tumor to the abdominal cavity [52]. Symptoms due to pressure on neighboring organs such as: bloating, pain in the vicinity of the tumor, pollakiuria, edema and paresthesia of the lower extremities or constipation are more intense during the day [18]. This is associated with an increase in tumor volume during the day due to greater flow of lymph through the abdominal cavity and the pelvis after meals and during daily activity, and an increase in hydrostatic pressure in the erect position [18, 27]. The increase in volume during the day is 140% on average [18]. Changes in lymphangioleiomyoma volume during the menstrual cycle have also been described [53].

Diagnosis

According to the recommendations of the European Pulmonological Society, the gold standard in LAM diagnosis is a lung biopsy and high-resolution computer tomography of the chest [7]. Characteristics additionally favoring this diagnosis are: angiomyolipoma currently/in the patient's history, lymph exudates within the chest and abdominal cavity, tuberous sclerosis, the presence of lymphangioleiomyoma or microscopically detected occupation of lymph nodes by LAM cells [7]. In the recommendations of the American Thoracic Society/Japanese Respiratory Society from 2017, the following are also included among these properties: plasma concentration of vascular epithelial growth factor D (VEGF-D) ≥ 800 pg/mL and the presence of LAM cells in a cytological examination of the lymph exudates in the pleural cavity [54]. Because LAM is exceedingly rare in males, the final diagnosis should be made in each case on the basis of a typical result of a lung biopsy [7]. A transbronchial biopsy has been shown to be a relatively safe method in the group of LAM patients [55].

High-resolution computer tomography is the examination of choice both in the diagnostic process as well as to observe disease progression [7]. Among typical radiological changes observed in all patients are numerous, small round cysts in the lung (2–5 mm diameter), equally distributed within the lung parenchyma [11]. Because of the frequent co-occurrence of LAM and renal angiomyolipoma computer tomography with an abdominal cavity and pelvic contrast is recommended, and in the case of contraindications for contrast use — a magnetic resonance analysis [7].

Lymphangioleiomyoma in computer tomography most commonly takes the form of well-defined, solid-cystic lesions, with walls of different thickness and numerous septs [26, 27, 46]. Less commonly a solid or only cyst-like character is observed [27]. The lesion is generally delimited but a few lymphangioleiomyomas with an infiltrating type of growth are observed [18]. Solid tumors have a density similar to the liver (59–71 HU), cyst-like lesions a lower one (3–25 HU), corresponding to lymph accumulation [18]. The cyst walls may become more pronounced after contrast [17]. Lymphangioleiomyoma under magnetic resonance shows differentiated values of signal both in T1 and in T2 exposure [38, 44, 49, 50]. In PET a small uptake of glucose is observed [26]. In ultrasound E-LAM can give the appearance of an isoechogenic, hypo- and hyperechogenic lesion, not permitting its differentiation from ovarian neoplasms or lymphomas [27]. However, changes in the structure during the day are characteristic: solid changes in the morning hours, can in the evening have a solid-cystic character and change their echogenicity [27]. Moreover, lymphangioleiomyomas have been found to increase their volume during the day which differentiates them from other neoplastic lesions localized in the retroperitoneal space and the pelvis [27].

LAM is also associated with an increased risk of meningioma development, therefore in the case of symptoms from the central nervous system magnetic resonance of the head is justified [7]. Patients with LAM should also be under the care of a genetic counselling facility — TSC is characterized by a broad range of phenotypes and poorly clinically expressed characteristics of this syndrome may lead to incorrect classification of the case as sporadic [7].

Morphology

The typical appearance for LAM encompasses the occurrence of numerous cysts in the parenchymal tissue of the lungs and multifocal proliferation of immature smooth myocytes and perivascular epitheliod cells (PECs), known in the case of lymphangioleiomyoma as LAM cells [7]. Foci of proliferation for LAM cells and myocytes are most commonly located around lymph vessels, interlobar septae and the pleura [3]. Within LAM cysts smaller fusiform cells are localized more centrally, whereas epithelioid cells with abundant cytoplasm are localized mainly at the periphery [56]. Cytological atypia and division patterns are generally not present [57].

On the basis of the intensity of two morphological changes typical for LAM: the occurrence of cysts and intensification of LAM cell proliferation within lung biopsy material, Matsui et al. (2001), elaborated a division into III histologic severity grades (LHS, histologic severity of LAM) [34]. The percentage of lung tissue occupied by the described lesions viewed under a small magnification was qualified as follows: LHS1 < 25%; LHS-2: 25–50% and LHS-3 > 50% [34]. The authors also showed a strong correlation of the grade of LHS severity with 10-year overall survival, which was: 100%,

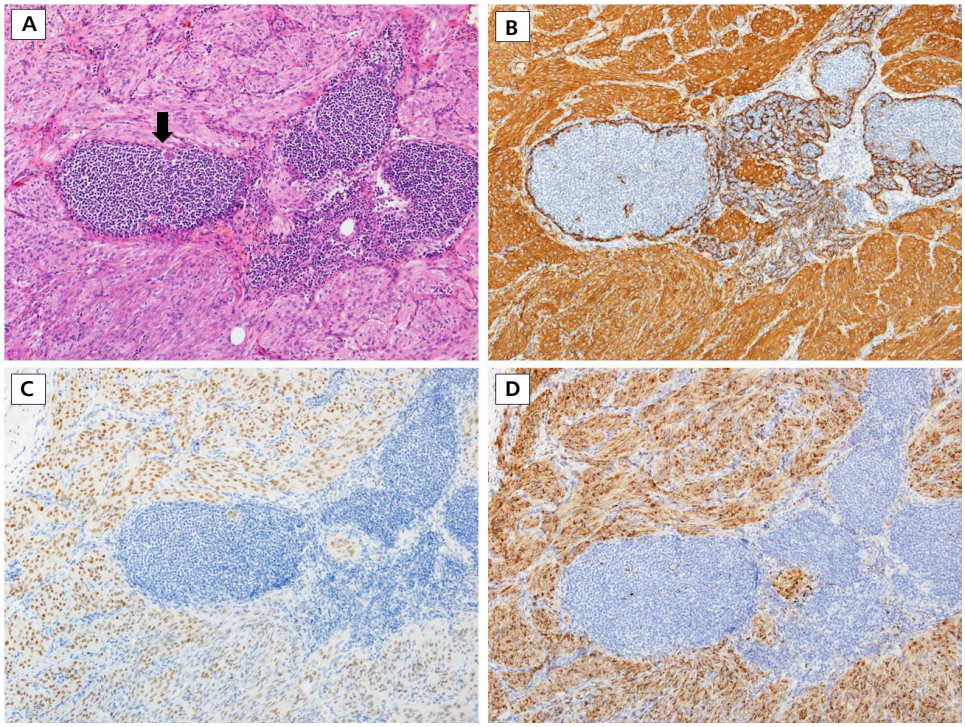

Figure 1A–D. Retroperitoneal LAM with infiltration of lymph node — in the order of staining HE, SMA, HMB-45, progesterone receptors [100×]

74.4% and 52.4%, respectively in w LHS-1, LHS-2 and LHS-3 [34]. LHS is currently an acknowledged prognostic factor in pulmonary LAM [58].

Lymphangioleiomyoma is generally described as lesions well delimited from surrounding tissues [21, 26], with the presence of a fibrous capsule [57]. Infiltration of LAM cells beyond the capsule is rare [6]. LAM foci in the uterus are an exception, they are frequently poorly separated from unaffected smooth uterine muscle coat [21, 24]. Differential diagnosis of E-LAM within the uterus encompasses myoma, leiomyosarcoma, endometrial stromal sarcoma or paraganglioma [48]. Lymphangioleiomyomas similarly as the pulmonary form is composed of fusiform and epithelioid LAM cells with round and oval nuclei with an abundant slightly acidophilic cytoplasm [6, 24, 50]. These cells form nests — resembling the so-called „zellballen" described in phaeochromocytomas [26], separated by slot-like vessels with thin walls lined with endothelial cells [50], which may undergo hyalinization [21, 24, 26]. Typically necrosis and blood extravasation to the tumor are not observed [6, 21, 26, 46]. In most cases the mitotic activity is low [6, 21], in some cases, up to 4 figures for 10 visual fields were observed [24, 37]. A fairly common finding are small clusters of reactive lymphocytes, resembling lymph nodules [6].

LAM cells are characterized by the simultaneous expression of melanocyte (HMB-45, Melan A, MART1) and smooth muscle (SMA, desmin, actin) markers (Fig. 1) [59, 60]. The expression of HMB-45 is observed in all cases, and the percentage of cells showing the expression of this marker is variable — it is in general 20–40% and concerns predominantly epithelioid cells [11, 56, 60, 61]. In almost all cases the expression of the estrogen (ER) and progesterone (PR) receptor also occurs — mainly within fusiform cells [56], and in general PR expression is stronger [62]. Other markers useful in the differential diagnosis are β-catenin and E-cadherin and cathepsin K, whose expression has been observed in all analyzed cases [56, 63, 64]. The co-occurrence of the expression of lymph vessel endothelium markers is also characteristic: podoplanin (D2-40), PROXI, VEGFR-3 and LYVEI, which occurs both in cells lining lymph vessels and in the LAM cells themselves [56]. Among less typical markers is the epithelial growth factor receptor (EGFR), whose expression was observed in about one-half of cases [56]. Kobayashi et al. (2018) have also demonstrated the cytoplasmic expression of EGFR/ErbB-1 and HER4/ErbB-4 in LAM cells [65]. Maisel et al. (2018) described the strong expression of the programmed death-ligand 1 (PD-L1) in preparations from lung biopsies from 6 patients with LAM, which was greater in preparations from pulmonary nodules than in tissue from healthy lungs [66]. They also found the presence of T lymphocytes showing the expression of programmed death-ligand 1 (PD-1) receptor, infiltrating

LAM foci [66]. In the case of lymphangioleiomyoma, LAM cells show the expression of a similar marker profile: HMB45 — particularly in epithelial cells [6], Melan A, calponin, SMA, desmin, nuclear expression of ER and PR [6, 24, 46, 50]; moreover expression of β-catenin [35], E-cadherin [67], MiiTF [25] and in the lymph vessel endothelium: CD34, podoplanin, CD31, and VEGFr-3 [21, 24, 26, 43].

Genetics

Similarly, as other proliferative diseases from the PEComa family, lymphangioleiomyoma has a higher frequency of occurrence in patients with tuberous sclerosis — characterized by the presence of germline inactivating mutations and loss of heterozygosity (LOH) in the 16p13.3 region, in the *TSC2* locus, encoding the tuberin protein and in the 9q34 region, locus *TSC1*, encoding the hamartin protein [8, 68]. Tuberin has an inhibiting action on the signalling protein Rheb — a homolog of the Ras protein (Ras homolog enriched in the brain — Rheb), which in turn is a known activator of the mTOR serine-threonine kinase [69]. Hamartin forms a complex with tuberin, stabilizing it and protecting it from degradation in proteosomes [70]. Excessive activation of the Rheb protein due to the loss of function of one of these two proteins leads to activation of the mTORC1 pathway, increased synthesis of proliferation stimulating proteins and angiogenesis resulting in the presence of numerous tumors of the PEComa type in patients with tuberous sclerosis [69]. The pulmonary form of LAM is found in approximately 30–50% women and in approximately 10% men with tuberous sclerosis, these are more commonly patients with mutations within *TSC2* [8, 71]. Similarly, somatic inactivating mutations and loss of heterozygosity in the *TSC1* and *TSC2* genes are observed in LAM cells obtained from patients with the sporadic LAM form though as this form is rare there are no analyses of large groups of patients [72]. Badri et al. (2013) analyzed material obtained from 10 patients with sporadic LAM, showing that in as many as 8 of them LAM cells showed perturbations within the *TSC2* locus, moreover, in 4 of them complete loss of tuberin occurred because of loss of heterozygosity and a mutation in the second allele (3 cases) and two inactivating mutations in both alleles simultaneously in one case [73]. In the paper by Fujita et al. (2015), inactivating somatic *TSC1*/*TSC2* mutations were detected in LAM cells in 6 out of 9 patients with LAM [74]. Among other genetic changes observed in patients with lymphangioleiomyoma are: germline mutations within *BARD1*, *BLM* and *BRCA2* [14] and *EGFR* amplification [56], however, their role in LAM pathogenesis has not been fully analyzed.

Evaluation of the stage of the pulmonary form of LAM and factors affecting the severity of its progression

Among analyses of the functioning of the respiratory system whose results correlate with the irregularities observed in radiological and histopathological analyses and which change with disease progression are: analysis of the coefficient of lung transfer for carbon monoxide (TLco) and a spirometric analysis — especially the measurement of the forced expiratory volume in 1 second (FEV_1) [58]. In the first evaluation of the degree of progression of the disease TLco and spirometric analysis with a bronchodilatory test are recommended [7]. Moreover, in patients from whom a lung biopsy was obtained a histological evaluation of the progression in the LHS scale, which was described earlier, is performed [58]. FEV_1 and TLco measurements should be repeated every 3–6 months in order to evaluate the progression of the disease and the response to treatment and in the case of stable results, the control analyses can be reduced to annual ones [7]. In patients in the initial stage of the disease in general deviations are not observed in gasometric blood analysis, it is used as an indication of indications for oxygen therapy and lung transplantation in patients with advanced disease [7]. An exercise test and a 6-minute marching test (6MWT) find application in the evaluation of the effect of the disease on general performance and response to treatment [58]. The pulmonary LAM form is characterized by a milder course in patients with tuberous sclerosis, in comparison to the sporadic form [7, 58]. Moreover, patients in whom the first LAM symptoms are hemoptysis and dyspnea have a worse disease than those in whom LAM was diagnosed because of pneumothorax, which may be associated with the delay in diagnosis in the first group [58]. A tendency of LAM to progress more slowly in post-menopausal women has been indicated [75]; in the analysis by Gupta et al. (2019) the change in FEV_1 in pre-menopausal women was on the average –118 mL/year, whereas in postmenopausal women it was –74 mL/year ($p = 0.003$) [76]. A recently described prognostic factor in lymphangioleiomyoma is the plasma level of vascular endothelial growth factor D (VEGF-D), which is much higher in patients than in the healthy population, especially in patients with tuberous sclerosis [77]. A high level of VEGF-D (over 800 pg/mL) was correlated with a more rapid rate of deterioration of FEV_1 values [78] and the presence of lymph exudates to the pleural cavity and the number of pulmonary cysts [77]. Other markers which have been associated with the severity of the course of pulmonary LAM are: the concentration of extracellular matrix metalloproteinases (MMP) in urine [58], the plasma concentration of vitamin D binding protein (VTDB) [79, 80], VEGF-3 receptor and chemokine CCL21 [81]

or the expression of the receptor of the human epithelial growth factor 3 (HER3) w LAM cells [61].

Pulmonary LAM treatment

Respiratory rehabilitation encompassing aerobic exercises and exercises for strengthening the breathing muscles exerts a proven, favorable effect on the evaluation of life quality, the 6MWT result, the feeling of dyspnea and the level of everyday activity of LAM patients [82]. By analogy to the procedure in chronic obstructive pulmonary disease, it is recommended that LAM patients be covered by prophylactic vaccinations against pneumonia diplococcus and influenza and because of the frequent occurrence of osteoporosis, patients with LAM should undergo periodic densitometric analyses [7]. LAM is associated with an increased risk of pneumothorax, which occurs in about one-half of patients at the moment of diagnosis and in over 60% of patients in the course of the disease, with a tendency to frequent recurrences [7]. Factors additionally increasing the risk of pneumothorax are pregnancy and flying [7, 83]. Pleurodesis is effective in preventing recurring pneumothorax; the analysis by Gonano et al. (2018) of a group of 145 patients indicated that pleurodesis prevented its occurrence with a probability of 82%, 68% and 59% after one, 5 and 10 years, respectively after the procedure in comparison with 55%, 46% and 39% among patients who did not undergo the procedure ($p = 0.026$) [83]. In the case of abundant lymph exudates to the pleural cavity a diet low in fat is recommended, and if clinical symptoms occur (dyspnea, coughing, chest pain) — traditionally draining of the pleural cavity through thoracentesis is applied [7], however, in the light of new evidence before starting treatment by invasive methods an attempt to control the symptoms by systemic treatment with sirolimus is recommended [5, 35, 84]. In advanced stages of LAM, in patients considered to be class III–IV on the NYHA scale with hypoxemia at rest, lung transplantation can be applied [7]. Average time from diagnosis to the transplantation varies in papers from various centers in the range of 4–5 years [85, 86]. In a large retrospective analysis of data concerning the course of the disease in 138 patients with LAM who had undergone a lung transplantation 1-year, 5-year and 10-year overall survival after the procedure were: 94%, 73% and 56%, respectively, and the causes of death were most commonly: bronchiolitis obliterans and chronic transplant rejection [87]. Such parameters as the presence of lung hypertension and the 6MWT distance before the procedure, the patient's age, time of organ ischemia during the procedure or transplantation of one or both lungs did not affect overall survival after the procedure [87]. In the analysis by Reynaud-Gaubert et al. (2008), summing up the results of lung transplantation of several French transplantation centers 1-year, 5-year and 10-year overall survival after the procedure were 79.6%, 74.4% and 64.7%, respectively [85]. Baldi et al. (2017) obtained similar results in an analysis encompassing 11 patients with LAM, the probability of surviving one, three and five years after lung transplantation was 90%, 90% and 75%, respectively [86]. There are rare descriptions of patients with a LAM recurrence in the transplanted lung [85, 88].

In recent years a breakthrough has occurred in systemic lymphangioleiomyomatosis treatment, which for many years was resistant to anti-estrogen therapy (removal of ovaries, use of medroxyprogesterone or selective estrogen receptor modulators) whose efficacy had finally not been proven [89]. No efficacy was demonstrated for treating LAM using doxycycline [5] or an aromatase inhibitor — letrozole [90]. On the basis of positive results of clinical trials, sirolimus, a drug from the group of mTOR kinase inhibitors was registered by the Food and Drug Administration (FDA) in May 2015 as the first and currently the only drug for systemic LAM treatment [5]. According to the guidelines of the American Thoracic Society/Japanese Respiratory Society, sirolimus is indicated in patients with LAM, accompanied by a rapidly deteriorating function of the respiratory system or $FEV_1 \leq 70\%$ wn. and in patients with symptomatic pleural lymph exudates, before using invasive treatment methods [5]. Clinical trials concerning the use of mTOR inhibitors (sirolimus, everolimus) in treating lymphangioleiomyomatosis are summarized in Table 1 — without taking retrospective analyses into consideration.

A randomized, multicenter, placebo-controlled, double-blind phase III clinical trial MILES (Multicenter International Lymphangioleiomyomatosis Efficacy And Safety Of Sirolimus), is so far the largest clinical trial concerning the use of sirolimus in LAM [91]. 89 patients were randomly divided into 43 receiving placebo and 46 sirolimus p.o. at an initial dose of 2 mg/d, and then established to maintain the serum concentration of the drug at a level of 5 to 15 ng/mL [91]; this dosage was accepted as a standard in successive trials [84, 92, 93]. After 12 months of treatment in the group receiving sirolimus FEV_1 stabilization was observed ($+1 \pm 2$ mL/month) with worsening FEV_1 at 12 ± 2 mL/month in the placebo group ($p < 0.001$). In the treatment group improvement was also observed in forced vital capacity (FVC), exercise tolerance, evaluation of the quality of life and a decrease in the serum concentration of VEGF-D in comparison with the placebo group. After finishing taking sirolimus, FEV_1 decreased at the same rate in both groups, which suggests that sirolimus therapy does not stop the progression of the disease when therapy is terminated, but also does not speed up this process [94].

Table 1. Clinical trials concerning the use of mTOR inhibitors mTOR (S-sirolimus, E-everolimus) in LAM

Author, year	Trial type	N	Age-years	TSC %	Drug	Drug concentration/ /dose	Length	FEV_1 changes	Other effects
Bissler et al. 2008 *[105]	Single center, open	25 (18 LAM)	–	66.7	S	10–15 ng/mL	12 months + 12 months observation	At the end of treatment: +118 ± 330 mL *vs.* wp At the end of observation: + 62 ± 411 mL *vs.* wp	↑ FVC, ↓AML volume
Dabora et al. 2011 *[106]	Multicenter, open	36 (21 LAM)	34	100	S	9–15 ng/mL	52 weeks	No changes *vs.* wp	↓AML volume, ↓VEGF–D, ↓number of skin changes
Davies et al. 2011 *[107]	Multicenter, open	16	–	33	S	3–10 ng/mL	24 months	–76 ± 52 mL/r	↓AML volume, ↓FVC, ↓Dlco
McCormack et al. 2011 *[91]	Multicenter, randomized, double blind, placebo control	89	45.4	9	S	5–15 ng/mL	12 months + 12 months observation	Sirolimus: +1 ± 2 mL/month placebo: –12 ± 2 mL/month	↑FVC, ↑QoL, ↓VEGF–D, ↓FRC
Neurohr et al. 2011 [93]	Single center, open/observational	10	42.4	20	S	5–10 ng/mL	12.1 ± 2.81 months	After 6 months: + 345 ± 58 mL	↑ FVC, ↑Dlco, ↑6MWD
Taveira-DaSilva et al. 2011 [84]	Single center, open/observational	19	41	0	S	5–15 ng/mL	2.5 years	Before treatment: –2.8% ± 0.8% wn/r sirolimus: + 1.8% ± 0.5% wn/time of observation	↑FVC, ↑ΔDLco, ↓chylothorax
Bissler et al. 2013*[108]	Multicenter, randomized, double-blind, placebo-control	118 (29 LAM)	31	96	E	10 mg/d	E: average 38 weeks placebo: average 34 weeks	Everolimus: –1% wp placebo: –4% wp	↓AML volume, ↓ number of skin changes
Yao et al. 2014 [109]	Single center, open	38	40.9	13	S	8.0 ± 2.5 ng/mL	Average 40.8 months	Before treatment: –2.3 ± 0.1% wn/r sirolimus: +1 ± 0.3% wn/r	↑DLco
Goldberg et al. 2015* [110]	Multicenter, open	24	42.5	5	E	10 mg/d	26 weeks	+ 114 mL (95% CI: 11–217)	↑ 6MWD, ↓VEGF–D
Takada et al. 2016* [92]	Multicenter, open	63	41.5	3.2	S	5–15 ng/mL	24 months	FEV_1 ↓ FEV_1↑ in the group with chylothorax in history	–
Bee et al. 2018* [111]	Prospective, cohort	47	43.6	19	S	1–2 mg/d	Average 35.8 months	In the whole group: +11 mL/r (od +254 do −148 mL/r) In a group of 21 patients in whom Δ FEV_1 before the trial was known: −150 mL/r before *vs.* +35 mL/r during	–
Taveira-DaSilva et al. 2018 [112]	Single center, open	25	40,6	8	S	5–15 ng/mL	54 ± 19.2 months	Before treatment: –7.4% ± 1.4% wn/r sirolimus: –0.3% ± 0.5% wn/r	↓ AML diameter, ↓VEGF–D, ↓Dlco, ↓chylothorax

BP — blood pressure; DLCO — lung diffusion capacity for carbon dioxide; DN — adverse effects; DDO — lower respiratory tract; DO — respiratory tract; GDO — upper respiratory tract; FEV_1 — first second forced vital capacity; FVC — forced vital capacity; OO — peripheral edema; N — number of trial participants; TC — total cholesterol; Qo — the quality of life; TG — triglycerides; WBC — white blood count; ZUM — infection of the urinary tract; wn — predicted value; wp — initial value; *trial included in meta-analysis by Gao et al. (2018) [98]

In the MILES trial, VEGF-D serum concentration was indicated as a negative prognostic factor but at the same time as a positive predictive factor for response to sirolimus treatment [95]. A higher VEGF-D level at the beginning of the trial was associated with a better response to treatment in the group receiving sirolimus (improvement in FEV_1 and FVC values), but at the same time a more rapid decrease in the value of these parameters in the placebo group [95]. In the last performed analyses a positive effect of sirolimus on burdensome LAM complications such as lymph exudates to the pleural cavity and recurring pneumothorax was also observed. In the trial by Zhou et al. (2018) in 5 analyzed patients with recurring pneumothorax in spite of pleurodesis, taking sirolimus in doses ensuring the maintenance of the drug concentration in serum at a level 3–10 ng/mL, no recurrences of pneumothorax were observed during the whole time of treatment [96]. However, interruption of therapy or a decrease of the blood concentration of the drug below 3 ng/mL resulted in recurrence of pneumothorax in 2 patients during 2 and 3 year-long observation [96]. In an observational trial by Taveira-DaSilva et al. (2011) planned to evaluate the benefit of using sirolimus in patients with a severe course of LAM and abundant lymph exudates in the pleural cavity in all 12 patients a complete or almost complete reduction of the volume of the accumulating liquid took place which allowed draining of the pleural cavity to be stopped in 2 of them [84]. In two retrospective analyses the effectiveness of sirolimus at a lower dose (target drug concentration in serum below 5 ng/m) in comparison with a standard dose (drug concentration in serum 5–15 ng/mL on the basis of the MILES trial), was evaluated, yielding contradictory results [10, 97]. Ando et al. (2013) showed an improvement in the function of the respiratory system and withdrawal of lymph exudates in patients treated with low doses, to a degree comparable with the results of trials using the higher dose [10]. However, Yoon et al. (2018) showed lower effectiveness of lower doses of sirolimus, whose use at the same time did not lead to a decreased frequency of undesirable adverse effects [97]. In a meta-analysis by Gao et al. (2018), encompassing 7 clinical trials concerning the use of sirolimus in LAM, a significant improvement of FEV_1 and FVC was confirmed in treated patients – the weighted average of differences was: 0.15 l (95% CI: 0.08–0.22, $p < 0.01$, $I_2 = 0\%$) for FEV_1 and 0.22 l (95%: 0.11–0.32, $p < 0.01$, $I_2 = 0\%$) for FVC [98]. However, no improvement of the 6-minute walk test nor the diffusion capacity of the lungs for carbon dioxide was observed. The accumulated frequency of occurrence of adverse events was: 50% for stomatitis, 40% for hyperlipidemia, 23% for headaches, 20% for bone marrow suppression and 19% for diarrhea [98]. Among frequently mentioned adverse effects of sirolimus are also respiratory tract infections, but sirolimus was not shown to increase their frequency with respect to the population of patients with LAM not using systemic therapy [99].

Sirolimus is currently the only drug whose use in LAM is recommended in the guidelines of the American Thoracic Society/Japanese Respiratory Society, in the light of the lack of convincing evidence for the effectiveness of other substances [5]. Preclinical and clinical trials are also being conducted on autophagy inhibitors [100], statins [101], hydroxychloroquine [102], a synthetic flavonoid — Proxison — an antioxidant normalizing mitochondrial metabolism, which showed synergy with sirolimus in inhibiting LAM cell growth *in vitro* [103], drugs targeting signal pathways connected with the receptor for vascular endothelial growth factor — VEGFR [104] or PD-1 inhibitors, prolonging survival in mouse models of LAM [66].

Treatment and prognosis in E-LAM

Lymphangioleiomyomas, even though they attain large sizes and are often non-resectable [29, 45], in general, have a mild clinical course. In a retrospective analysis by Matsui et al. (2001) among 17 patients with E-LAM, only one died because of an aggressive course of a simultaneously occurring pulmonary LAM form, all the others were alive at the moment of publishing the results with an average observation time of 5.5 years [6]. Radical resection allows long-term control of the disease [16, 24, 39, 40, 42, 50], also when laparoscopic techniques are used [43, 113]. There are reports in the literature about local recurrence after E-LAM resection [37, 114], which can, however, be treated with good effects by a repeated resection [115]. Long survival times were also observed in the case of non-resectable disease; the case of an 11-year old girl has been described in whom a non-radically excised mesenterial lymphangioleiomyoma did not undergo progression in spite of lack of treatment for 10 years [116] and a 47-year old woman with uterine E-LAM and metastases to the lungs and ovary who remained in good overall status for 12 years of observation [117]. Because of the lack of unified methods of lymphangioleiomyoma treatment and their relatively mild clinical course, screening of patients with the pulmonary form of LAM for E-LAM is not recommended, if they do not have any clinical symptoms [7]. If cumbersome clinical symptoms occur such as pain in tumor progression, constipation or edema of the lower extremities, attempts to treat are undertaken similar to the pulmonary form of LAM — by hormone therapy [12] and mTOR inhibitors [118] and radiotherapy for the area of the occupied lymph nodes [119]. Radzikowska et al. (2016) performed a retrospective analysis of the effectiveness of sirolimus in 14 patients with pulmonary LAM (including one with TSC) and lymphangioleiomyoma of the retroperitoneal tract [118]. During 10 months of therapy with sirolimus (at

Table 2. E-LAM cases treated with mTOR inhibitors — sirolimus and everolimus available in the literature

Author	Age	TSC	Pulmonary LAM	Localization	Max. tumor diameter	Symptoms from tumor	Drug; Dose	Best response	Time of observation	Progression
Chen et al. 2009 [88]	23	no	yes	PZ	–	–	Sirolimus; 1 mg/d	–	36 months	No
Possekel et al. 2009 [41]	23	no	no	Numerous in liver, mesentery, wall of small and large intestine	10 cm	A feeling of incomplete elimination, stomach pain	Sirolimus; 1.5 Mg/d	PR	3 months	No
Rosenberg et al. 2013 [121]	41	no	yes	PZ	16.5 cm	Stomach pain, early feeling of satiety	Sirolimus; –	PR	9 months	No
Freitas et al. 2015 [122]	26	no	yes	PZ, abdominal lymph nodes	–	–	Sirolimus; 2 mg/d	PR	12 months	No
	37	no	later	PZ	18 cm	Pain in the vicinity of tumor	Sirolimus; 2 mg/d	PR	6 months	No
Hecimovic et al. 2015 [123]	44	no	yes	PZ	7cm	–	Sirolimus; 2–3 mg/d	PR	10 months	No
	33	no	later	PZ, pelvis	10 cm	–	Sirolimus; 1 mg/d	PR (almost CR)	9 months	No
	34	no	later	PZ	8 cm	Stomach pain, increase in stomach diameter, ascites, hemoptysis	Sirolimus; 2 mg/d	PR	3 months	No
Cabeza et al. 2016 [124]	53	no	yes	Numerous in PZ	6.8 cm	Stomach pain	Sirolimus; 2 mg/d	PR (almost CR)	12 months	No
Harrari et al. 2016 [125]	39	no	no	Numerous in PZ	–	–	Sirolimus; 2 Mg/d	PR	18 months	No
Ito et al. 2016 [126]	39	no	yes	PZ, pelvis	6.2 cm;	Edema of lower extremity	Sirolimus; 1mg/d for 2 weeks then 2 mg/d	PR (almost CR)	18 months	No
Wahid et al. 2016 [127]	37	yes	no	pelvis	15.3 cm	Back ache, fainting	Everolimus; 10mg/d	PR	12 months	No
	45	no	no	PZ	8.5 cm	Feeling of satiety, pain in vicinity of tumor	Everolimus; 10mg/d	CR	18 months	No
Lecuelle et al. 2019 [128]	35	no	yes	PZ	8.5 cm	–	Sirolimus; 5mg/d	CR	30 months	No
Ussavarungsi et al. 2019 [129]	26	no	yes	PZ	26 cm	–	Sirolimus; 1 mg/d	SD	14 months	No

CR — complete response; d — day; max. — maximal; PD— disease progression; PR— partial response; PZ — retroperitoneal space

Table 3. E-LAM cases with the use of hormone therapy available in the literature

Author	Age	TSC	Pulmonary LAM	Localization	Max. tumor diameter	Symptoms from tumor	Drug; Dose	Best response	Time of observation	Progression	Drug; Dose
Klein et al. 1992 [130]	36	No	Yes	Mediastinum lymph nodes, pz and pelvis	Numerous — max 5 cm	None	Oophorectomy + ifn-α2b (for 3 months) + t tamoxifen	3 × 10^6 j. 3×/ week + 20 mg/d	CR	18 months	No
de Groot et al. 2008 [45]	23	No	Later	Pz	16 Cm	Pain in the vicinity of tumor	Goserelin + tamoxifen	3.6 mg/month + 40 mg/d	PD	–	–
							Goserelin + medroxyprogesterone + thalidomide	3.6 mg/month + 500 mg/d + 50 mg/d	PD	-	-
							Goserelin + ifn- α2b	3.6 mg/m + 3 × 10^6 j. 3×/week	SD	9 years	No
Yamashita et al. 2011 [131]	30	No	No	Pelvis	-	Recurring stomach pains during menstruation	Leuprorelin	1.88 mg/ month	SD	6 months	No
Szpurek et al. 2014 [117]	47	No	No	Uterus, metastases to ovary and lungs	-	Brak	Tamoxifen	–	SD	5 months	Yes
Basnet et al. 2015 [132]	24	No	No	Pz, pelvis, mesentery	Numerous, approx. 3 Cm	Pain in vicinity of tumor	Medroxyprogesterone	150 mg i.m; 2 doses given with a 3 month interval	PR	6 months	Yes
Yoshizawa et al. 2019 [133]	36	Yes	No	Uterus	-	Stomach pain	Leuprorelin	–	PD	–	–

CR — complete response; d — day; IFN-α2b — interferon α2b; j. — unit; max. — maximum; PD — disease progression; PR — partial response; PZ — retroperitoneal space

a dose of 1–5 mg/d, in order to attain blood concentration of the drug at a level of 5–15 ng/mL), besides improvement of respiratory tract ailments, a significant decrease was observed in the volume of the lesions in comparison to the initial value (1603.85 ± 2437.56 cm^3 *vs.* 198.01 ± 315.43 cm^3; p = 0.00026), and total withdrawal of lymph from the retroperitoneal space and pleura in 13 of then [118]. In the analysis by Mohammadieh et al. (2013), encompassing 5 patients with lymphangioleiomyoma of the abdominal cavity, the effectiveness of everolimus (at a dose 1–1.5 mg/d; in two divided doses) was evaluated [120]. After 6 months of therapy in 4 out of 5 patients a partial or complete response was observed and withdrawal of the ascites [120]. The descriptions in the literature of cases treated with mTOR inhibitors and hormone therapy in E-LAM are presented in Table 2 and Table 3, respectively. It is worth stressing that among the cases presented in Table 2 (mTOR inhibitors), disease progression did not occur in any of the cases during the administration of a drug from this group.

Summary

Lymphangioleiomyomatosis is a member of the PEComa (perivascular epithelioid cell tumors) family [2]. In patients with the pulmonary form of LAM, LAM foci have been described in the uterus, adnexa or broad ligament of the uterus and there is the frequent occupation of lymph nodes in the extraperitoneal space [21]. Lymphangioleiomyoma in computer tomography, in general, has the form of a well-separated solid-cystic lesion, with walls of different thickness and numerous septae [26, 27, 46]. Solid or cystic character is less common [27]. The lesion is generally well separated, but a few lymphangioleiomyomas with infiltrating growth are also observed [18]. Sirolimus is currently the only drug whose use in LAM is recommended by international recommendations [5]. The randomized, multicenter, placebo-controlled, double-blind clinical phase III MILES trial (Multicenter International Lymphangioleiomyomatosis Efficacy And Safety Of Sirolimus), is so far the largest clinical trial concerning the use of sirolimus in LAM [91]. Preclinical and clinical trials also encompass autophagy inhibitors [100], statins [101], hydroxychloroquine [102], anti-VEGFR drugs [104] and PD-1 inhibitors [66].

Conflict of interest

The authors report no conflicts of interest.

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VIA MEDICA

CASE REPORT

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Invasive breast cancer in ectopic axillary breast tissue — case report

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ABSTRACT

Breast cancer is the most commonly diagnosed malignancy in women in Poland. Rare, unusual forms of breast cancer remain a diagnostic problem. The incidence of ectopic breast glandular tissue in the general population varies between 0.4 and 6%. The same abnormalities and diseases as in the anatomical mammary gland may develop in this tissue. Breast cancer may develop as well.

We present the case of a 36-year-old woman, who went to the doctor because of a nodule in the right armpit presented for 2 years. The patient was referred to a surgeon with suspicion of an epidermal cyst. The lesion was surgically removed and in the histopathological examination, the diagnose was: invasive breast cancer in ectopic glandular tissue. After imaging diagnostics, discussion of a multidisciplinary diagnostic and therapeutic team, the patient was offered a surgical procedure - widening of the excision margins to obtain oncological completeness and axillary lymphadenectomy. After the surgery, due to the results of the histopathological examination, complementary systemic treatment (chemo- and hormone therapy) and radiotherapy were used.

Doctors often do not consider the possibility of primary breast cancer occurring elsewhere than in the breast. Breast imaging does not always make it possible to diagnose the disease, and doctors performing and interpreting these tests often do not include primary armpit cancer in the differential diagnosis. This can cause a delay in diagnosis and worsen the prognosis.

Key words: rare cases of breast cancer, ectopic glandular tissue, additional breast, axillary tumor

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Introduction

Breast cancer is the most commonly diagnosed malignancy in women in Poland. It is also one of the biggest threats to the premature mortality of women [1]. The growing awareness of doctors of various specialties not only oncologists, allows more and more often to detect this disease at an early stage, also preclinical. Rare, unusual forms of breast cancer remain a diagnostic problem.

Ectopic breast glandular tissue occurs between 0.4 and 6% of the general human population [2]. Its presence is a consequence of the incomplete disappearance of the so-called mammary creasts (*crestae mammariae)* [3]. Such remaining additional glandular tissue is most often located within the axillary pits, but other locations, such as supra and subclavian region, subcutaneous region, and even anatomically distant locations such as perineum and anus are possible [4]. In such an additional gland, abnormalities and diseases characteristic of the anatomical mammary gland may occur. Cases of adenomas, fibro-cystic lesions, as well as breast cancer are reported [5]. Typically, the patient is not aware of the presence of such an incorrectly located additional glandular tissue until a palpable tumor is found, for example in the armpit.

A case report

A 36-year-old woman, without a significant medical history, was referred by a primary care physician to a general surgeon because of a nodule in the soft tissues of her right armpit for more than 2 years. The lesion has increased over the past few months. Due to the incriminating family history (mother's sister had breast cancer), the patient regularly checked her breasts during gynecological visits. Periodically performed breast ultra-

sound showed no abnormalities. The consulting surgeon found the relative movable tumor with a diameter of 2 cm and redness of the skin above it. The ultrasound examination of the armpit described a hypoechoic, irregular focal lesion with a diameter of 21 mm, involving skin and subcutaneous tissue, as well as, lymph nodes up to 8 mm in diameter next to the principal lesion. There was suspicion of the inflammatory epidermal cyst or inverted acne (*hidradenitis suppurativa*). The lesion was surgically removed.

In histopathological report, invasive cancer no special type (NST) with intermediate grade (G2) was identified, with the presence of angio- and neuro-invasion. The pathologist described the weaving of cancer in the dermis and subcutaneous tissue, and in the vicinity, structures corresponding to the residual weaving of the mammary gland. In summary, it was found that the whole picture corresponds to primary cancer originating from ectopic breast glandular tissue. Along with the major lesion, 4 lymph nodes were removed, in which metastatic lesions up to 3 mm in diameter were found with infiltration outside the lymph node capsule. The degree of pathomorphological severity was defined as pT1cN2a. In immunohistochemistry, the strong expression of estrogen and progesterone receptors, lack of HER2 receptor expression, and Ki67 proliferation index of about 30% were found. The cancer phenotype was defined as luminous B HER2 negative.

After diagnosis, the patient was referred to the Opole Oncology Center, where spectral mammography, chest X-ray and abdominal ultrasound were performed.

In spectral mammography (Fig. 1 and 2) no pathological strengthening foci were found both in breasts and axillary pits (the study was performed after a diagnostic excision). The results of the chest and abdominal imaging were normal. The patient's case was discussed during a multidisciplinary diagnostic and therapeutic team meeting. Due to the presence of cancer stuck to the edges of the surgical incision of the removed lesion and metastases to four lymph nodes with infiltration outside the node capsule, the patient was offered the surgery at first - widening the excision of the lesion with the removal of right axillary lymph nodes. In the pathomorphological examination of the postoperative material, the edges of the preparation were free of neoplastic lesions. There were found postoperative resorptive changes and ductal carcinoma in situ (DCIS) with an intermediate degree of differentiation present in individual ducts in the lodge area. In four of the twelve lymph nodes assessed, metastases of cancer up to 6 mm in diameter with infiltration outside the lymph node capsule were found. After surgery, the patient was offered complimentary treatment as part of a multidisciplinary medical consultation. Chemotherapy based on anthracyclines and taxoids was used, and then, according to the expression of steroid hormone receptors, pharmacological ovarian suppression and treatment with nonsteroidal aromatase inhibitors were included. The patient was also subjected to additional radiation therapy. The breast was irradiated together with the nodal field up to a dose of 50 Gy in 25 fractions with an additional dose of 10 Gy in 5 fractions after the tumor resection using 3D conformal radiotherapy with intensity-modulated radiation therapy (IMRT).

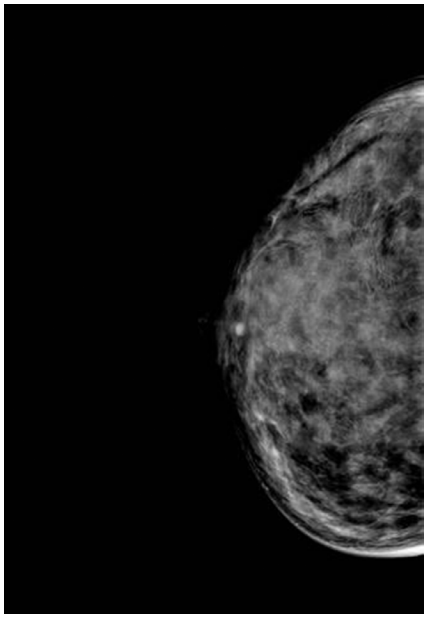

Figure 1. Contrast — enhanced spectral mammography of the right breast

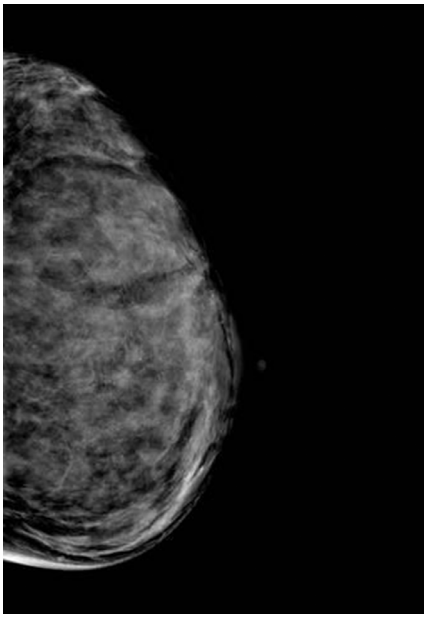

Figure 2. Contrast — enhanced spectral mammography of the left breast

Discussion

In case of a palpable tumor in the armpit, the most often taken into account are enlarged lymph nodes, which have been changed, reacted with inflammation or cancer. In differential, diagnostics should be considered such conditions as inflammation (abscess, boil, lipoma, inverted acne), epidermal cyst, as well as soft tissue tumors or peripheral nerve sheaths tumors [6, 7].

We should also remember about the possibility of ectopic breast glandular tissue. In terms of histological structure, one can distinguish an additional (supernumerary) breast and aberant breast tissue [8]. Additional breasts most often occur in place of embryonic nipple combs, so-called "milky line" from armpits to the groin. The glandular structures of such an additional breast are ordered, with a secretory system, as well as with the possibility of an additional wart and areola. In the breast tissue of the split breast, the glandular structures are chaotic, with no ordered secretory system. They are usually located near the anatomical breast as an "islands" of glandular tissue [8].

Extrapodial glandular tissue undergoes changes under the influence of hormones, analogously to the anatomical mammary gland. It may develop various abnormalities and diseases such as fibro-cystic lesions, adenomas, papillomas, as well as breast cancers [9]. According to various authors, the incidence of breast cancer in ectopic breast glandular tissue accounts for 0.2 to 0.6% of all breast cancers [5]. Because of the rarity of the disease, doctors often do not consider the possibility of primary breast cancer outside the anatomical mammary gland. Imaging breast examinations do not always make it possible to diagnose the disease. This can cause a significant delay in diagnosis and worsen the prognosis.

The treatment of cancer in the ectopic breast glandular tissue within the armpit is generally subject to the same principles as the treatment of cancer of the anatomically located breast. However, some issues remain controversial, especially regarding the extent of surgical treatment. Some authors suggest mastectomy on the same side as the tumor in the ectopic tissue if the axillary lymph nodes are involved [10, 11]. Others argue that removal of the anatomical breast does not bring additional benefits to patients, and the prognosis is the same for local, radical removal of ectopic tissue and amputation [12, 13]. Therefore, the surgical treatment of choice is a wide excision of the lesion with surrounding tissues (including skin). Mastectomy is not indicated if breast imaging does not indicate cancer within it. However, it should be considered if the results of imaging tests are not clear and differential diagnosis does not allow for a reliable diagnosis [14].

In case of a primary lesion located in the ectopic tissue of the breast and clinically unchanged regional lymph nodes (which was not the case in this patient), lymph node surgery is necessary. Does sentinel node biopsy make sense in this case? Some authors show that metastases in axillary lymph nodes occurred in about 50% of the analyzed cases, and therefore very often, which could suggest the legitimacy of lymphadenectomy in such a situation [4, 10, 11]. However, several published papers indicate that lymphoscintigraphy allows accurate localization of sentinel lymph nodes. Therefore, it is possible to perform a sentinel node biopsy procedure and to avoid mutilating lymphadenectomy [16, 17]. This approach is particularly justified in cases of ectopic tissue location outside the armpit, where the absorbent flow down to the armpit on the same side is not obvious [17]. The choice of the site for radioisotope injection remains a technical issue. Will the retroareolar injection on the same side as the axillary tumor be appropriate? Considering lymphatic drainage and axillary location, it appears that peritumoral or surgical site biopsy is more appropriate in this case [17].

The scope of complementary radiotherapy also raises some controversy. Due to the rarity of this form of cancer, there is no clearly defined standard of treatment. Most authors suggest qualifying patients for complementary radiotherapy based on similar criteria as in the case of breast cancer in a typical location. There are differences regarding the scope of radiotherapy. Some authors consider it appropriate to irradiate the lodge after the removed tumor and axillary pit, others suggest covering the breast on the tumor side [18].

Systemic adjuvant treatment should be carried out in accordance with generally accepted recommendations for the treatment of patients with early breast cancer.

In the presented case, after a discussion within the multidisciplinary diagnostic and therapeutic team, a decision was made on supplementary radiotherapy for the lodge after the removed tumor and the armpit due to the involved lymph nodes and skin infiltration. Systemic treatment was adapted to cancer biology and the degree of pathomorphological severity.

Conclusions

Ectopic breast glandular tissue is often not included in routine mammography. Also, some physicians performing breast ultrasound examination do not take into account the possibility of cancer in the ectopic breast gland tissue. This causes delays in the diagnosis and treatment of additional breast cancer.

Conflict of interest

The authors report no conflicts of interest.

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