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Serum ROBO4 and CLEC14A: preliminary evaluation as diagnostic and progression biomarkers in colorectal cancer patients

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Introduction. Colorectal cancer (CRC) is an important global burden, and the discovery of biomarkers for screening and monitoring is a current challenge. The present study aimed to determine the serum concentration of ROBO4 and CLEC14A in CRC patients and assess the diagnostic and progression value of these biomarkers in CRC.

Material and methods. Serum samples were collected from 32 patients with CRC and from 16 healthy individuals. Blood serum of CRC patients were tested before and after surgery. Serum concentration of ROBO4 and CLEC14A were measured using ELISA tests.

Results. The serum concentrations of ROBO4 and CLEC14A were significantly higher in CRC patients than non-cancer controls. The sensitivitiy and specificity of ROBO4 and CLEC14A in distiguishing cancer patients from controls ranged from 71.9% to 100% and from 84.5% to 100%, respectively. The serum ROBO4 concentration was associated with the TNM stage, depth of invasion, and lymph node and distant metastases. The level of ROBO4 was statistically lower 3 months after the surgery, compared to the level noted prior to the operation.

Conclusions. Our preliminary study has provided evidence that ROBO4 and CLEC14A seem to be suitable biomarkers for clinical diagnostic purposes in colorectal cancer.

Key words: ROBO4, CLEC14A, biomarker, colorectal cancer, angiogenesis

Introduction

Cancer is an important problem in terms of public health. In developed countries with the western diet and lifestyle, cancer causes nearly a quarter of all deaths [1, 2]. Among cancers, colorectal cancer (CRC) is the fourth malignancy most commonly detected worldwide and represents 9.4% of all cancer incidences in men and 10.1% in women. In 2018, there were approx. 1.9 million new CRC cases diagnosed worldwide and approx. 0.9 million deaths from colorectal cancer were evidenced [3]. An alarming trend is that CRC patients are shifting younger, e.g. the median age in 2001–2002 *vs.* 2015–2016 was 72 *vs.* 66 years at diagnosis [4]. Since colorectal cancer presents clear symptoms only in advanced stages and there are no sensitive and accurate diagnostic methods, the detection of CRC in early stages is problematic and difficult [5]. The main therapies applied for CRC are surgical treatment, chemotherapy, and radiotherapy. Unfortunately, the survival rate is still poor in distant metastatic patients [6]. Even if combined treatments are used,

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a recurrence occurs in approx. 1/3 of cases, and the median survival after surgery with the best supportive care of chemotherapy is up to 24 months [7]. Therefore, the identification of sensitive, reliable, and noninvasive biomarkers as screening tests for CRC would be of great advantage, improving patient outcomes and declining the mortality rate [8]. In particular:

- diagnostic biomarkers indicating the early stage of the disease,
- predictive biomarkers that are crucial for the assessment of the risk of cancer development,
- prognostic biomarkers of the risk of cancer progression are required [6]. However, regardless of many efforts, there are still no adequate biomarkers for accurate prediction and diagnosis of CRC [9].

A critical phase for tumor development and further spread is angiogenesis. Angiogenesis supports tumor growth by the influx of essential nutrients and oxygen to the cancer mass [10, 11]. It is widely documented that, without new vasculature formation, the maximum size of 1–2 mm is recognized as the limit for neoplastic expansion [12]. Tumor blood vessels are irregular and differ in their morphology (shape and size) and function from normal vessels. The endothelial cells of tumor blood vessels exhibit overexpression of molecules named tumor endothelial markers (TEMs) [12–14]. Several investigations have indicated that two proteins (ROBO4 and CLEC14A) among TEMs are overexpressed on the surface of tumor endothelial cells in a wide range of solid tumors (ovary, prostate, breast, liver, bladder, kidney, and lung) [15, 16].

The ROBO4 (magic roundabout 4) protein has been extensively expressed in endothelial cells of various cancer cell lines, including breast and colon cancer, but was not identified in such cell lines as fibroblasts and endometrial stromal cells [17]. Moreover, as shown by immunohistochemistry analysis, ROBO4 expression was restricted to sites of active formation of new blood vessels [18]. It was found that the ROBO4 molecule serves a crucial function in tumor neovascularization by initiating vascular endothelial cell migration via specific interaction with ligands (i.e. glycoprotein SLITs) [19, 20]. The involvement of the ROBO4 protein in pathological angiogenesis indicates that this molecule is a mediator of the tumor growth process [21]. Indeed, it has been proved that blocking ROBO activity can cause inhibition of tumor mass [22].

C-type lectin domain family 14 member A (CLEC14A) is considered to be a TEM due to its overexpression in tumor vasculature, compared to adjacent nontumor blood vessels. High expression of CLEC14A was observed in head and neck squamous cell carcinoma, breast cancers, and clear cell renal cell carcinoma [23,24]. Additionally, studies with CLEC14A (–/–) mice proved the promoting role of CLEC14A in tumor growth [24].

Although numerous studies have revealed that activation of ROBO4 and CLEC14A proteins contributes to angiogenesis and plays a decisive role in tumor growth and metastasis, there are limited reports on the expression of these molecules in tissue or blood in colorectal cancer patients [19–24].

The objective of the present research was to determine the serum concentration of ROBO4 and CLEC14A in colorectal cancer patients. Besides, we tried to assess the relationship between the levels of the biomarkers in serum and the clinicopathological features of CRC patients. The clinical value of ROBO4 and CLEC14A in diagnosis and progression of colorectal cancer was also evaluated by comparison with the CEA and CA 19.9 markers commonly used in clinical practice.

Materials and methods

Patients, clinical diagnosis, ethics

The study group comprised 48 patients divided into two groups: 32 patients with colorectal cancer (CRC group) and 16 healthy individuals (control group). All CRC patients were diagnosed and underwent cancer surgery between March 2018 and April 2019. The mean age of the CRC patients was 66.14 ± 9.17 years (range: 47–82) After surgery, all resected tissues underwent histopathological examination, and the pathologist confirmed CRC in all tissue samples. The primary tumour location was the colon in 18 cases (56%) and the rectum in 14 cases (44%). The advancement of the tumour stages was assessed by a highly specialized pathologist according to the staging system (AJCCS) developed by the American Joint Commission on Cancer. Preoperative radiotherapy, chemotherapy, or chemoradiotherapy excluded patients from the examination.

Healthy volunteers (mean age 61 ± 4.59 years, range: 44–79 years) were recruited from the patients of the Outpatient Clinic of our hospital during a routine colonoscopy screening. The control participants did not take any medical treatment during the study period. In addition, the colonoscopy did not reveal any pathological changes. The characteristics of the patients enrolled in the study are presented in table I.

The study was performed according to the Helsinki Declaration 1964 with later amendments and approved by the Ethical Committee (decision no. KE-0254/180/2017). In accordance with the ethical policy, all participants were adequately informed about the aim and methods of the study. As part of the procedure, all patients signed a written consent form before the initiation of the research.

Sample preparation, biomarker assay

Venous blood samples (~10 ml) were collected into commercially available anticoagulant-treated tubes (EDTA). Blood was taken from the CRC patients at two time points: before the surgery (point 0) and postoperatively (point 1), i.e. during the control visit 3 months after the operation. Blood from healthy individuals was sampled only once. The samples were immediately centrifuged at 1000 × g for 10 min at 4°C and the sera were stored at –80°C until further analysis. The concentrations of ROBO4 and CLEC14A in the serum samples were quantified with the use of sandwich ELISA (enzyme-linked immuno-

Table I. Characteristics of the colorectal cancer (CRC) patient group

Colorectal cancer group		Number of patients
gender	male	17
	female	15
tumor location	colon	18
	rectum	14
tumor size	<5.0 cm	16
	≥5.0 cm	16
TNM stage	I + II	18
	III + IV	14
depth of tumor invasion (T-stage)	T1	5
	T2	8
	T3	10
	T4	9
lymph node metastases (N-stage)	N0	24
	N1 + N2	8
distant metastases (M-stage)	M0	26
	M1	5
lymphovascular invasion	absent	20
	present	12

TNM – tumor nodes metastases

sorbent assay) according to the manufacturer's instructions (MyBioSource, San Diego, USA).

The CEA and CA 19.9 serum markers were measured routinely in the CRC patients and controls using a Cobas 6000 analyzer (Roche Diagnostic, North America). CEA and CA 19.9 in the CRC patients were measured at two time points: before and 3 months after the surgery.

Statistical analysis

The data were shown as descriptive statistics (mean ± SD; median with minimum and maximum values). Statistical calculations were performed using SPSS software (SPSS 15.0, Chicago, IL, USA) and XLSTAT 2018; Data Analysis and Statistical Solution for Microsoft Excel (Addinsoft, Paris, France, 2017). Prior to the analyses, the data were tested for normal distribution using the Kolmogorov-Smirnov test. As the data indicated non-normal distributions, non-parametric tests were applied to compare the serum biomarker levels between the studied groups and the serum biomarker levels and clinicopathological paramteres. The Mann-Whitney *U* test was applied to assess the difference between two variable groups, while comparisons among multiple groups were performed using the Kruskal-Wallis test. Receiver-operating characteristic (ROC) curves were used to determine the sensitivity and specificity of serum ROBO4, CLEC14A, CEA, and Ca 19.9. Differences between serum biomarker levels from point 0 to point 1 were evaluated with the Wilcoxon match-pairs signed ranks test. In all analyses, the differences were considered statistically significant when $p < 0.05$.

Results

Serum levels of ROBO4 and CLEC14A in CRC patients

The serum concentration of ROBO4 and CLEC14A was significantly higher in the CRC patients than in the healthy individuals (fig. 1). The mean ROBO4 concentration was approx. 2-fold higher in the CRC group, compared to the control (675.50 ± 275.28 pg/ml *vs.* 339.15 ± 103.27 pg/ml, respectively), while the mean CLEC14A serum level was approx. 4-fold higher in the CRC patients than in the non-cancer individuals (50.91 ± 11.28 ng/ml *vs.* 12.45 ± 5.20 ng/ml, respectively).

Next, the serum levels of ROBO4 and CLEC14A in early-stage (TNM I+II) CRC patients were compared with healthy individuals. The mean serum concentrations of both studied biomarkers were significantly higher in the TNM stage I+II CRC patients than in the controls (fig. 1).

Evaluation of serum ROBO4 and CLEC14A as potential diagnostic biomarkers for CRC

We used ROC analysis to evaluate the ROBO4 and CLEC14A power in discrimination between patients with CRC and healthy controls (tab. II and fig. 2). The ROBO4 protein provided 71.9% sensitivity, 84.5% specificity, and an AUC of 0.873 (95% CI: 0.778–0.968) in diagnosing CRC. The AUC for CLEC14A for discrimination between CRC patients and healthy controls was 1.0; the cutoff value of 23.69 ng/ml contributed to 100% sensitivity and specificity. The cutoff value for CEA was 6.89 ng/ml and provided sensitivity and specificity of 62.5 and 77.0%, respectively (AUC: 0.801; 95 CI: 0.679–0.992). In the case of CA 19.9, the sensitivity and specificity were 81.3% and 91.4%, respectively, at the cutoff point of 11.45 ng/ml (AUC: 0.823; 95 CI: 0.667–0.979).

Relationship between serum levels of ROBO4 and CLEC14A and clinicopathological features in CRC patients

Table III shows the correlation between serum ROBO4 and CLEC14A levels and clinicopathological characteristics in CRC patients. The serum ROBO4 concentration was associated with the TNM stage ($p < 0.001$), depth of invasion (T stage; $p < 0.001$), and lymph node (N stage; $p = 0.015$), distant metastases (M stage; $p = 0.041$) and the presence of the lymphovascular invasion ($p = 0.034$). No significant relationship was observed between the CLEC14A concentration in the serum and the clinopathological features (tumor site, lymph node

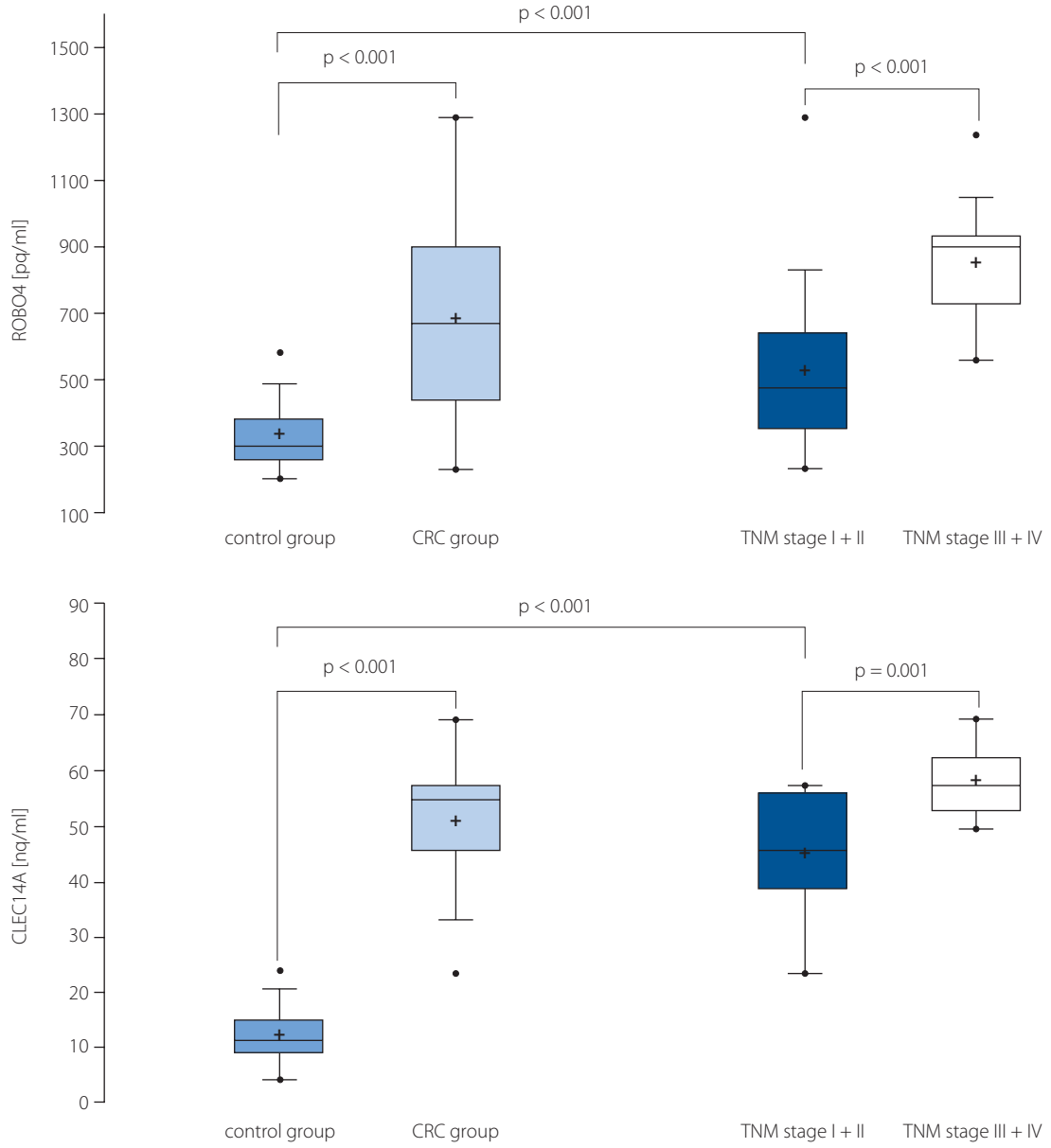

Figure 1. Serum ROBO4 and CLEC14A concentrations in CRC patients and healthy controls

Table II. Diagnostic value of serum ROBO4, CLEC14A, CEA, and CA 19.9 in CRC patients

Factor	Cutoff value	Sensitivity (%)	Specificity (%)	95% CI	AUC
ROBO4	498.76	71.9	84.5	0.778–0.968	0.873
CLEC14A	23.69	100.0	100.0	1.0–1.0	1.0
CEA	6.89	62.5	77.0	0.679–0.992	0.801
CA 19.9	11.45	81.3	91.4	0.667–0.979	0.823

ROBO4 – roundabout4; CLEC14A – C-type lectin family 14 member A; CEA – carcinoembryonic antigen; CA 19.9 – carbohydrate antigen; CI – confidence interval; AUC – area under the curve

and distant metastases – N and M stages; in all cases $p > 0.05$). However, the increased CLEC14A levels were associated with the tumor size ($p = 0.015$), TNM stage ($p = 0.001$), and depth of invasion (T stage; $p = 0.002$).

Postoperative changes in serum ROBO4, CLEC14A, CEA, and CA 19.9 concentrations in CRC patients

Changes of the serum level of ROBO4, CLEC14A, CEA, and CA 19.9 proteins in the postoperative period were assessed

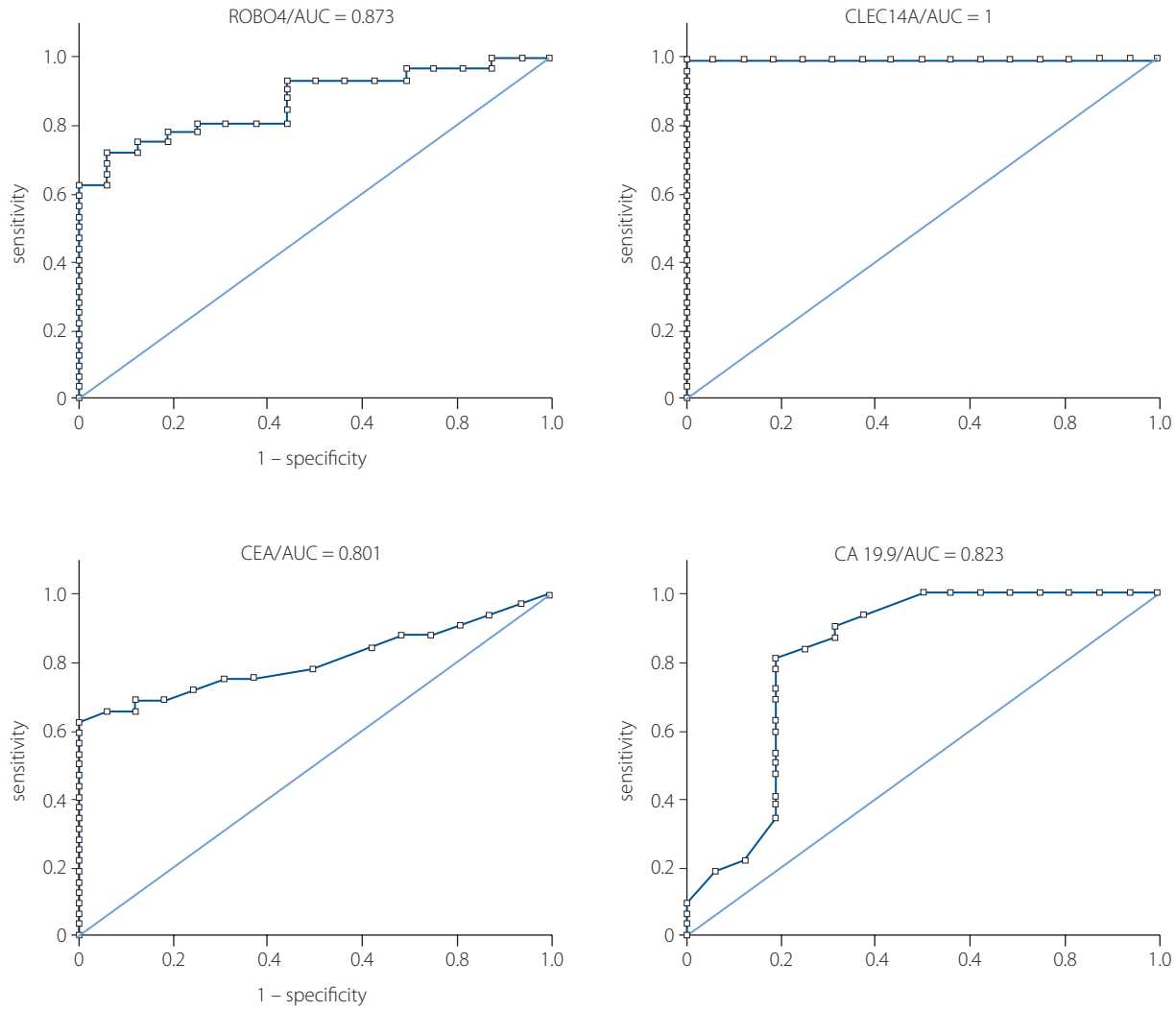

Figure 2. Receiver-operating curve (ROC) for ROBO4, CLEC14A, CEA, and Ca19-9

Table III. Serum concentration of ROBO4 and CLEC14A in relation to the clinicopathological features of CRC patients

Colorectal cancer group			ROBO4	CLEC14A
tumor location	colon n = 18	mean ± SD	678.00 ± 249.05	52.28 ± 10.61
		median	765.72	55.92
		min	234.57	23.69
		max	933.59	69.37
	rectum n = 14	mean±sd	672.27 ± 315.56	49.16 ± 12.25
		median	615.20	52.60
		min	318.65	25.44
		max	1286.69	64.73
	Mann-Whitney *U* test		0.613	0.464
tumor size	<5.0 cm n = 16	mean ± SD	615.73 ± 257.57	45.80 ± 13.07
		median	643.23	45.92
		min	279.14	23.69
		max	10.47.06	69.37

Table III. cont. Serum concentration of ROBO4 and CLEC14A in relation to the clinicopathological features of CRC patients

Colorectal cancer group			ROBO4	CLEC14A
tumor size	≥5.0 cm n = 16	mean ± SD	735.26 ± 287.49	56.02 ± 6.02
		median	744.64	56.28
		min	234.57	39.71
		max	1286.69	66.78
	Mann-Whitney *U* test		0.341	0.015
TNM stage	I + II n = 18	mean ± SD	538.92 ± 260.75	45.28 ± 11.20
		median	476.38	45.92
		min	234.57	23.69
		max	1286.69	58.04
	III + IV n = 14	mean ± SD	851.09 ± 181.01	58.16 ± 6.22
		median	904.37	57.60
		min	566.96	49.78
		max	1239.95	69.37
	Mann-Whitney *U* test		<0.001	0.001
depth of tumor invasion (T-stage)	T1 n = 5	mean ± SD	329.16 ± 70.40	32.96 ± 8.35
		median	354.82	33.51
		min	234.57	23.69
		max	406.52	42.47
	T2 n = 8	mean ± SD	513.01 ± 144.74	48.25 ± 10.24
		median	508.28	47.39
		min	318.65	32.67
		max	752.71	62.62
	T3 n = 10	mean ± SD	699.20 ± 163.19	54.25 ± 3.90
		median	727.10	56.01
		min	339.32	46.07
		max	933.59	59.09
	T4 n = 9	mean ± SD	986.00 ± 179.82	59.55 ± 6.56
		median	926.47	58.33
		min	710.54	49.78
		max	1286.69	69.37
	Kruskal-Wallis test		<0.001	0.002
lymph node metastases (N-stage)	N0 n = 24	mean ± SD	606.45 ± 261.68	48.90 ± 12.15
		median	603.46	51.40
		min	234.57	23.69
		max	1286.69	69.37
	N1 + N2 n = 8	mean ± SD	882.65 ± 212.62	56.94 ± 4.82
		median	922.37	56.27
		min	594.44	49.78
		max	1239.95	64.73
	Mann-Whitney *U* test		0.015	0.094

→

Table III. cont. Serum concentration of ROBO4 and CLEC14A in relation to the clinicopathological features of CRC patients

Colorectal cancer group			ROBO4	CLEC14A
distant metastases (M-stage)	M0 n = 26	mean ± SD	640.51 ± 262.93	50.23 ± 11.78
		median	643.23	54.65
		min	234.57	23.69
		max	1286.69	69.37
	M1 n = 5	mean ± SD	920.38 ± 263.58	55.67 ± 5.54
		median	923.56	55.42
		min	594.44	49.78
		max	1239.95	62.03
	Mann-Whitney *U* test		0.041	0.457
lymphovascular invasion	absent n = 20	mean ± SD	659.75 ± 301.20	47.87 ± 12.46
		median	683.66	50.12
		min	234.57	23.69
		max	1239.95	66.78
	present n = 12	mean ± SD	746.27 ± 264.06	52.99 ± 10.21
		median	696.49	56.23
		min	439.32	25.44
		max	1286.69	69.37
	Mann-Whitney *U* test		0.044	0.195

ROBO4 – roundabout4; CLEC14A – C-type lectin family 14 member A; TNM – tumor nodes metastases; SD – standard deviation; min – minimum; max – maximum

(fig. 3). The serum level of ROBO4 and CEA was statistically lower at point 1 (3 months after the surgery) compared to the level noted at point 0 – prior to the operation (point 0 *vs.* point 1; ROBO4: 675.50 ± 275.28 *vs.* 419.21 ± 166.98 pg/ml, CEA: 12.07 ± 8.25 *vs.* 7.22 ± 4.70 ng/ml). The serum concentrations of CLEC14A and CA 19.9 decreased in the postoperative time period, compared to the preoperative level; however, the declines were not statistically significant.

Discussion

In the recent years, there has been increasing interest in identification of CRC with the use of noninvasive biomarkers [8]. The expression of ROBO4 and CLEC14A proteins in tumor neovasculature makes these molecules a potential target for use as a diagnostic and prognostic indicators of cancer, including CRC [17, 23, 24].

To the best of our knowladge, the present study investigated the serum level of ROBO4 and CLEC14A in colorectal cancer (CRC) patients for the first time. We found that the mean ROBO4 and CLEC14A concentrations in the serum of CRC patients were significantly higher than in the non-cancer controls. Previous literature reports based on immunohistochemical methods evidenced specific endothelial expression of ROBO4 and CLEC14A in various cell lines, i.e. in MCF-7 breast carcinoma and SY-SH-5Y-neuroblastoma cells [15, 17, 19]. Up-regulation of these biomarkers was also proved in human tissues, i.e. in vessels of colorectal liver metastases, bladder and breast carcinoma, and liver and kidney cancer [15, 19, 26]. Moreover, the expression of ROBO4 and CLEC14A proteins was dominant at sites of active angiogenesis and in regions exposed to hypoxia [19, 27, 28]. In CRC, up-regulation of ROBO4 mRNA was detected in more than 70% of carcinoma tissues and this protein was exclusively present in the endothelium of cancer vessels [29].

In our study, the ROBO4 and CLEC14A serum levels increased already in early-stage CRC, in comparison to the control samples. Moreover, we found that ROBO4 and CLEC14A had high power to discriminate between CRC patients and cancer-free individuals. Interestingly, the diagnostic sensitivity and specificity of serum CLEC14A reached 100% at the level of 23.98 ng/ml, which is higher than values noted for CEA (sensitivity: 62.5% and specificity: 77.0%) and CA 19.9 (sensitivity: 81.3% and specificity: 91.4%), i.e. biomarkers that are currently commonly used in clinical practice. The high predictive ability of CLEC14A was previously described by Robinson et al., who performed ROC curve analysis of CLEC14A staining scores

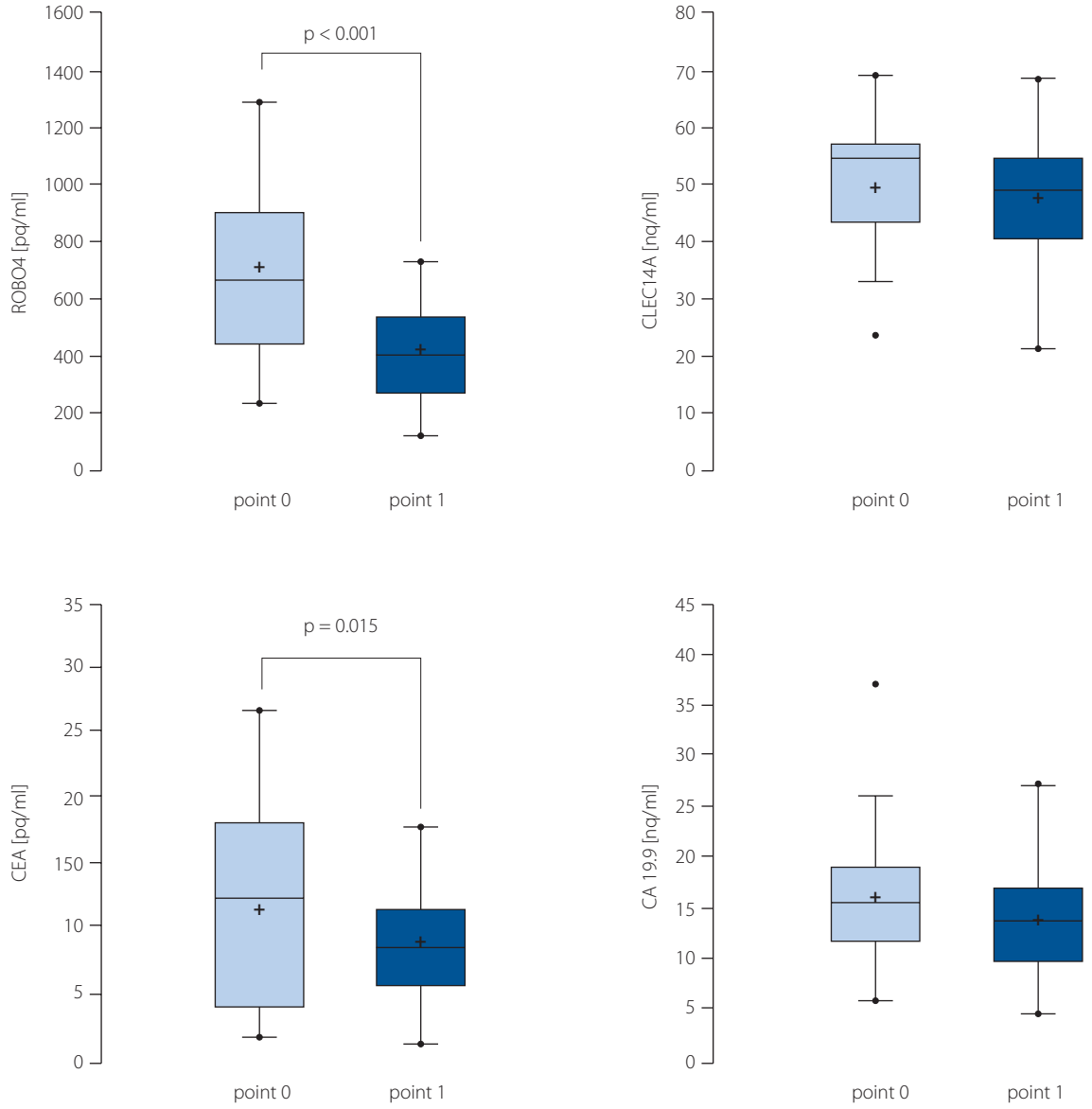

Figure 3. Postoperative changes of serum ROBO4, CLEC14A, CEA, and CA 19.9 concentrations in CRC patients

in various tumor tissues and evidenced their high sensitivity (75%) and specificity (85%) in distinguishing between cancer and non-cancer tissue status [30]. The results of our study, together with literature data evidencing that ROBO4 and CLEC14A molecules dominate in tumor endothelial cells, suggest that these biomolecules have diagnostic potential in cancers, presumably including CRC [15, 17, 19, 30, 31].

Further, we analyzed the association between the ROBO4 and CLEC14A serum concentrations and clinicopathological features of the CRC patients. In our study, the increased ROBO4 levels were related to the depth of tumor invasion as well as lymph node and distant metastases. In contrast, the high concentration of CLEC14A was not associated with the presence of lymph node and distant metastases. There is scarce information on the association between ROBO4 or CLEC14A expression and cancer advancement and prognosis. In prostate cancer, a higher histological tumor (Gleason) score was related to overexpression of ROBO4 [32]. In acute myeloid leukemia patients, overexpresion of ROBO4 was a poor prognostic factor and was corelated with shorter disease-free survival and overall survival [33]. Contrasting results were reported by Zhao et al., who evidenced that endothelial overexpression of ROBO4 suppressed breast cancer angiogenesis and reduced the speed of tumor growth [34]. Simmilary, in non-small lung cancer, high ROBO4 tissue expression was related to good prognosis and was connected with normalization of endothelial cells and reduction of cancer spread [16]. Considering CLEC14A, recent reports indicate that elevated levels of this molecule can inhibit carcinogenesis and progression of lung adenocarcinoma [35]. The expression of ROBO4 or CLEC14A molecules in various cancers tissues

(up- or down-regulation) suggests that these proteins may act as important modulators of tumorgenesis and tumor progression. Indeed, ROBO4 and CLEC14A are known as angiogenic factors with an essential role in tumor growth. It was revealed that blocking anti-ROBO4/CLEC14 antibodies induced reduction of the formation of new vessels and led to inhibition of cancer mass [25, 31]. Currently, the pro-angiogenic properties of CLEC14A and its involvement in tumor growth are well documented [24, 25]. For example, the CLEC14A protein promotes filopodia formation and activates cell migration, which is detrimental for tumor cell proliferation [15]. Furthermore, the inhibition of the interaction between CLEC14A and multimerin 2 (MMRN2) by a blocking antibody reduces tumor vessel sprouting and hinders the growth of the tumor mass [25].

As a novel observation, we found that the ROBO4 serum concentrations decreased significantly within 3 months after the surgical removal of CRC. In the case of CLEC14A, we documented a tendency of the serum concentration to decline after the operation. Therefore, we hypothesized that the level of circulating forms of ROBO4 and CLEC14A is associated with the tumor mass. However, we did not find any literatre data to support this hypothesis. We can only speculate that resection of solid tumor mass and removal of existing new vessels that are known to express ROBO4 and CLEC14A proteins result in a decline in the concentrations of these biomarkers in blood. Prevoiusly, Krishna et al. observed reduction of tumor microvessel CLEC14A expresion after preoperative chemotherapy administered to patients with epithelial ovarian cancer [36]. It is accepted that chemotherapy performed prior to surgical cancer excision contributes to reduction of tumor mass, down staging, and a decrease in the expression of cancer-specific molecules, including tumor endothelial markers [37, 38].

Conclusions

In this preliminary study, higher serum levels of ROBO4 and CLEC14A were observed in the CRC patients. Especially, the relationships between ROBO4 and CLEC14A serum levels and TNM stages were assesed and a signinficant post-operative decrease in the serum levels of these biomarkers was demonstrated.

Therefore, ROOB4 and CLEC14A seem to be suitable biomarkers for clinical diagnostic purposes. Nevertheless, due to the preliminary character of our findings, the results have to be taken with caution. In the future, more extensive and prospective studies with a larger CRC patient population seem to be required to validate our results.

Conflict of interest: none declared

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Prognostic significance of sex in patients with primary tracheal tumors – a retrospective, single-center study

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Introduction. We aimed to assess the prognostic significance of the sex of patients with primary tracheal tumors based on our own results as well as the literature review.

Material and methods. We carried out a retrospective analysis of 89 patients with primary tracheal tumors treated at the Maria Sklodowska-Curie National Research Institute of Oncology in Warsaw, Poland, between January 2000 and December 2016. Men and women were compared in terms of overall survival, disease-free survival, and progression-free survival.

Results. In the entire study group, the median overall survival was 61.30 months in women and 8.55 months in men ($p < 0.0001$). 5-year overall survival rates were 2.1% in men versus 50.6% in women ($p < 0.0001$). Among those receiving radical treatment, women had improved survival rates compared with men. Sex was an independent prognostic factor in both the total study population and among those undergoing radical treatment.

Conclusions. According to our results, women with primary tracheal tumors have significantly better survival than men. Because female sex is an independent prognostic factor in patients with primary tracheal tumors, the ratio of women to men should be taken into consideration in reports comparing the outcomes of different treatments.

Key words: tracheal tumors, adenoid cystic carcinoma of the trachea, squamous cell carcinoma of the trachea, sex

Introduction

Primary tracheal tumors are rare, and therefore remain poorly understood. They represent 0.2% of all respiratory cancers and 0.02–0.04% of all malignancies [1], with an annual incidence of approximately 0.1 per 100 000 people. The most common types are squamous cell carcinoma (SCC) and adenoid cystic carcinoma (ACC), which together account for more than two-thirds of primary tracheal tumors in adults [2].

Squamous cell carcinoma of the trachea usually presents as multiple and often ulcerative lesions growing into the tracheal lumen, with histology identical to that of SCC of the lung [3]. Squamous cell carcinoma can occupy any part of the trachea, and a third of patients have mediastinal or pulmonary metastases at diagnosis [2]. Of the trachea is 2–4 times more common in men than in women and develops primarily in the sixth and seventh decades of life [2–5]. It is strongly associated with tobacco smoking [3, 4], and 30–40% of patients with SCC of the trachea have concurrent metachronous or synchronous primary smoking-related cancer of the oropharynx, larynx, or lung [2, 3].

Adenoid cystic carcinoma of the trachea occurs with similar frequency in men and women, and is most common in the fourth and fifth decades of life [2–5]. The etiology of ACC is unknown; however, unlike SCC, it is not associated with tobacco smoking [3, 4, 6]. Adenoid cystic carcinoma is characterized by submucosal and perineural spread [7].

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It often develops slowly, but can be more aggressive in some cases, with a tendency to local infiltration and, less frequently, lymph node metastases. Moreover, local or systemic recurrences may occur beyond 10 years after primary treatment [2, 3].

The prognosis of patients with primary tracheal tumors is determined by several factors. Histological diagnosis of ACC [4, 8–20], better performance status [14, 16, 21–23], and radical surgery [4, 5, 8, 16, 17, 19, 24–26] have been identified as favorable prognostic factors. Literature on the influence of sex on overall survival (OS) in different tumors has been increasing. An Australian study showed that men had lower 5-year OS than women for all cancers (47.1% [95% confidence interval (CI): 46.9–47.4] versus 52.0% [95% CI: 51.7–52.3]). Specifically, poorer survival for men was observed for 11 cancers (head and neck, esophagus, colon, pancreas, lung, bone, melanoma, mesothelioma, kidney, thyroid, and non-Hodgkin lymphoma) [27]. Several studies on the most common respiratory cancer – non-small cell lung cancer – have shown that women have a lower risk of disease progression and death than men [28–31]. Better prognosis for women with lung cancer has also been shown in Polish studies [32, 33]. In an American study based on the Surveillance, Epidemiology, and End Results (SEER) database, women with ACC of the head and neck had better OS than men in multivariate analyses (HR 0.73; 95% CI: 0.65–0.82) [34]. Data on the influence of sex on the survival of patients with primary tracheal tumors are lacking. In this study we therefore aimed to examine the prognostic significance of sex in patients with primary tracheal tumor.

Material and methods

This retrospective analysis included patients with primary tracheal tumors treated at the Maria Sklodowska-Curie National Research Institute of Oncology in Warsaw, Poland, between January 2000 and December 2016. Patients were identified by searching the institution's cancer registry. We enrolled adults (≥18 years) diagnosed with primary tracheal tumors for whom complete data were available. Patients with tumors that may have originated from the larynx, main bronchus, or other organs (e.g., thyroid or esophagus) were excluded.

Overall, the records of 89 actively treated patients with primary tracheal tumors were included. Data on demographics, clinicopathological variables (symptoms, smoking history, performance status, histological diagnosis, location, and extent of the tumor), and type of treatment were extracted from traditional (paper-based) and electronic medical records. The follow-up ended on December 31, 2019.

Differences in distribution were determined using one-way analysis of variance for normally distributed variables and the Kruskal-Wallis test for other continuous variables. Fisher's exact test was applied to assess the independence between categorical variables.

The Kaplan-Meier estimator, log-rank test, and Cox proportional hazards model were used to analyze survival. For all tests, statistical significance was set at $p < 0.05$. Variables for which the p value was less than 0.10 were included in the multivariate Cox models.

OS was defined as time from diagnosis to death from any cause. Disease-free survival (DFS) was defined as time from initiation of radical treatment to recurrence or death from any cause, and progression-free survival (PFS) as time from initiation of palliative treatment to disease progression or death.

Results

Clinicopathological characteristics

Among the total study population (n = 89), men slightly predominated (48 men and 41 women). The median age at diagnosis was 62 years. SCC was the most common histological type, identified in 50 (56.2%) of 89 patients. 19 patients were diagnosed with ACC (21.3%). The remaining histological findings were classified for statistical purposes as "other". Men were more frequently diagnosed with SCC (66%), whereas ACC predominated among women (73.7%). The majority (78%) of patients with SCC were over 60 years of age and none were under 35 years of age. ACC was diagnosed in all age groups (36.8% of patients were under 35 years of age). Among the 43 patients for whom data on smoking history were available, 100% of those diagnosed with SCC were current or former smokers. Only women were never-smokers. The most commonly reported symptoms were dyspnea (37.1%) and hemoptysis (36%). 68% of women and 56% of men had a WHO performance status of 0–1. Among the patients who underwent radical treatment, 28 (62.2%) were women and 17 (37.8%) were men, compared with 13 (29.5%) women and 31 (70.5%) men among those receiving palliative treatment. 13 (28.9%) of 45 patients receiving radical treatment underwent surgical resection, of whom 11 (85%) were women and two (15%) were men.

Survival analyses

For the entire study group, the median OS was 61.30 months in women and 8.55 months in men ($p < 0.0001$). 5-year OS rates were also significantly higher in women than in men (50.6% *vs.* 2.1%) (fig. 1). Among those who underwent radical treatment, 5-year OS rates were 5.9% in men, compared with 70.6% in women ($p < 0.0001$). Median OS in this group was 16.9 months in men versus 129.4 months in women ($p < 0.0001$) (fig. 2). 5-year DFS rates were 10.9% in men versus 33.6% in women ($p = 0.0023$). Sex was an independent prognostic factor in both the total study population and among those receiving radical treatment. We found no differences in OS and PFS between sexes in patients receiving palliative treatment.

Discussion

Data on the influence of sex on the survival of patients with tracheal tumors are scarce and inconsistent. In a retrospecti-

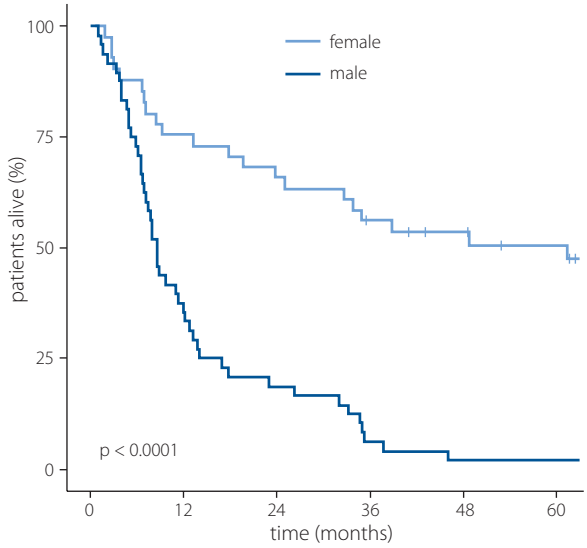

Figure 1. Cumulative probability of overall survival by sex in the total study group

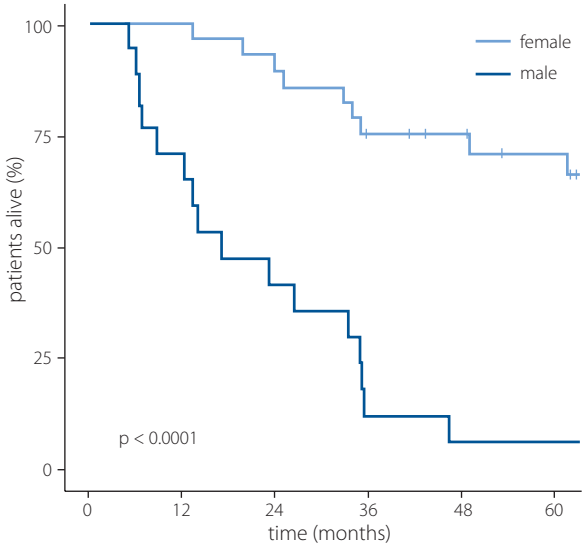

Figure 2. Cumulative probability of overall survival by sex in patients receiving radical treatment

ve analysis of 30 patients with ACC of the trachea, 5-year OS rates were 92% in men and 77% in women (p = 0.345) [25]. Moreover, the only available meta-analysis did not identify a statistically significant effect of sex on PFS or OS [5]. Another study showed that women had better disease-specific survival, defined as time from the initiation of treatment to death from the tracheal tumor (p = 0.044); however, no effect on OS was identified (p = 0.467) [10]. In a study of surgically treated patients with ACC of the trachea, female sex was a favorable prognostic factor for DFS, but not for OS [35]. Only one study, by Hetnał et al. [16] reported a favorable OS for women compared with men (5-year OS rates of 7% in men versus 32% in women, p = 0.04); however, multivariate analysis did not confirm sex to be an independent prognostic factor.

In our study, female sex was associated with favorable OS. OS and DFS benefits were also seen for women among patients who underwent radical treatment. Sex was an independent prognostic factor in both the overall study population and among those who underwent radical treatment. Various factors were considered to identify the underlying causes of such profound differences in survival by sex.

Analysis of other favorable prognostic factors in men and women showed that, in many cases, women predominated among groups with favorable characteristics. We found that:

- the group of never-smokers consisted of only women,
- the proportion of women and men who had a WHO performance status of 0/1, T1, and N0 were as follows: 68% *vs.* 56%, 39% *vs.* 19%, and 61% *vs.* 33%,
- 73.6% of patients with ACC were women,
- 62% of women were eligible for radical treatment compared with 38% of men,
- among those undergoing primary surgical treatment, 11 (85%) were women and two (15%) were men.

Attention was also paid to the difference between male and female life expectancy. Women live longer than men. According to Statistics Poland, the average life expectancy was 73.8 years for men in 2018, compared to 81.7 years for women. Few studies (and none on tracheal tumors) have taken life expectancy into account. A study on surgically treated patients with non-small cell lung cancer that accounted for expected lifetime found that women had better survival than men [36].

Other factors that may be associated with the above relationship include the patient's age at diagnosis of the tumor and comorbidities. A study in small-cell lung cancer indicated that the relationship between the patient's sex and age was important. A favorable prognostic effect of female sex was observed in younger patients, whereas prognosis in men was independent of age. The median OS in patients under 60 years was 13.3 months for women and 10.1 months for men (p = 0.0001); however, no significant difference between sexes was seen in older patients (p = 0.12) [37]. Another study also demonstrated improved prognosis in younger individuals (especially among women) [33]. In our study population, 100% of patients under 35 years of age were women. We found no data in the literature on the correlation between the survival of patients with tracheal tumors and their sex and age.

Sex-specific differences in comorbidities may translate into differences in survival between women and men. Some studies suggest that men have more comorbidities than women at cancer diagnosis and that there is a relationship between comorbidities and poor survival (e.g. in lung cancer) [38]. We did not analyze the presence of comorbidities in our study, nor did we find any data concerning the influence of comorbidities on OS in patients with tracheal tumors in the literature.

Other studies evaluating the effect of female sex on survival highlighted that women seek health care more often

and sooner than men, which contributes to earlier diagnosis of cancer [39, 40]. Women may also be more likely than men to take the proposed treatment. Furthermore, women more frequently adhere to the treatment plan and better tolerate treatment [34, 41].

Differences in molecular, endocrine, and metabolic abnormalities may be another factor. In other cancers, men and women were found to vary in terms of genetic disorders. For example, the *EGFR* mutation in non-small cell lung cancer is more common among women than men [42, 43]. Studies on the aforementioned factors could provide relevant information regarding differences in the biological behavior of tracheal cancers and explain disparities in survival.

Conclusions

This study suggests that women with primary tracheal tumors have significantly better survival than men, in both univariate and multivariate analysis. Since female sex is an independent prognostic factor for tracheal tumors, the ratio of women to men should be taken into consideration in reports comparing the outcomes of different treatments. The reasons why women with tracheal tumors live longer than men remain unexplained. Studies on genetic, hormonal, and metabolic factors could help explain sex-specific differences in survival rates.

Conflict of interest: none declared

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Radiation dose in CT-guided microwave liver tumor ablation

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Introduction. Ablation is one of most important methods of liver tumor treatment. However, radiation is one of disadvantages of CT-guided procedures including ablation. The purpose of this study is to assess the factors that have impact on radiation doses during CT-guided microwave liver tumor ablation.

Material and methods. Radiation doses of CT-guided liver tumor ablations were collected in 127 patients. They were then compared in terms of number of lesions, lesion size and depth, use of additional localization needles and hydrodissection as well as tumor location.

Results. The median radiation doses of ablations of multiple tumors (2348 mGy*cm) were significantly higher (p = 0.03) than those of single tumors (1784 mGy*cm). No statistically significant differences were noted when other factors (lesion size, depth, location, use of localization needles and hydrodissection) were taken into consideration.

Conclusions. The number of lesions is the most important factor in terms of expected radiation doses in CT-guided microwave liver tumor ablations.

Key words: microwave ablation, radiation dose, CT-guided ablation

Introduction

Thermal ablation is an established method of liver tumor treatment [1, 2]. It is frequently performed with CT-guidance due to its high spatial resolution as well as the ability to precisely visualize needles and organs [3]. However this method of guidance is associated with radiation that can potentially increase the risk of malignancy [4, 5]. The risk is low but not negligible and, according to the ALARA concept, the radiation should always be kept as low as reasonably possible. This study is an attempt to estimate those factors affecting radiation doses during CT-guided liver ablation procedures.

Materials and methods

The institutional bioethical committee waived the need for formal consent due to retrospective nature of this study. 127 consecutive patients (85 males, 42 females) underwent liver tumor ablations between 2018 and 2019; 88 of them had single tumor, while 39 patients had multiple (89) tumors. Among the tumors there were 43 hepatocellular carcinomas (HCCs) and 134 metastases: breast cancer (n = 4), neuroendocrine tumors (NET) (n = 4), colorectal cancer (n = 126). The mean age of the patients was 69 years (range 25–91).

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Table I. Radiation doses (dose length product – DLP) in ablations of single *vs.* multiple tumors

	n total	Mean	Standard deviation	Minimum	Median	Maximum	p value
DLP for single tumors [mGy*cm]	88	2377	1697	450	1784	7518	0.03
DLP for multiple tumors [mGy*cm]	39	2333	746	967	2348	3839	

All procedures were performed percutaneously with a microwave ablation device (Solero, Angiodynamics, Lantham, NY, USA), under general anesthesia. The ablations were done under CT-guidance using 320 slice Toshiba Aquilion One scanner (Toshiba/Canon, Nasu, Japan). Ultrasound was done just before every procedure to make sure no new lesions were visible and the tumor was still ablatable. Non-enhanced CT was performed at the beginning of every procedure to visualize the tumor. Then 3-slice (quick-check) scans were done during the procedure, every time the needle was advanced into the tumor.

After the ablation needle was removed, a 3-phase CT scan was done to estimate the ablation zone size and location, with a special focus on oncological margins of at least 5–10 mm. The following parameters were used for spiral CT scans: 120 kV and 300 mA for spiral scans or 50 mA (quick-check scans). No real-time CT-fluoroscopy was used during the procedures.

In 48 patients who had large tumors (>20 mm), one or two localization needles were used (Chiba, 21G, Cook, Bloomington, IN, USA). Those needles were placed to mark the borders of the tumors that required multiple ablation sessions. Hydrodissection was performed in 5 patients. A thin (22 G) needle was placed under CT guidance in a narrow (1–3 mm) space between the liver and adjacent stomach, colon or kidney. Between 50 and 200 ml of normal saline was then injected to isolate these structures from the heat produced during ablation and to prevent thermal damage to those organs.

Data on radiation doses in terms of dose length product were collected from the dose report generated by the scanner. The effective dose in mSv was calculated by multiplying by a factor of 0.015 [6]. The carcinogenic effect of the procedure, defined as excess risk of malignancy, was calculated at 5% per sievert [7].

The CT images from the procedures were retrospectively reviewed and the following data were collected: number of lesions, lesion size, number of localization needles inserted, hydrodissection application, lesion depth (from the entry point on the skin), location of the lesion (liver segment).

Statistical analysis

The Shapiro-Wilk test was used to assess the normality of distribution of the investigated parameters. Differences were tested by the Wilcoxon rank-sum test and Kruskal-Wallis test. Pearson's correlation was used to analyze the association between DLP versus depth and DLP versus diameter. The values $p < 0.05$ were considered statistically significant. Statistical analysis was done using R environment (version 3.3.2, The R-Foundation, Austria).

Results

Ablations of multiple tumors were associated with higher radiation doses than single tumors in terms of DLP. Median DLP (mGy*cm) for single tumors was 1784 (range: 450–7518) while for multiple tumors it was 2348 (967–3839) and the difference was statistically significant ($p = 0.03$) (tab. I). The median effective doses were calculated at 26.8 mSv and 35.2 mSv respectively.

There was no statistical significance ($p = 0.23$) (fig. 1) in DLP increase in patients in whom localization needles were

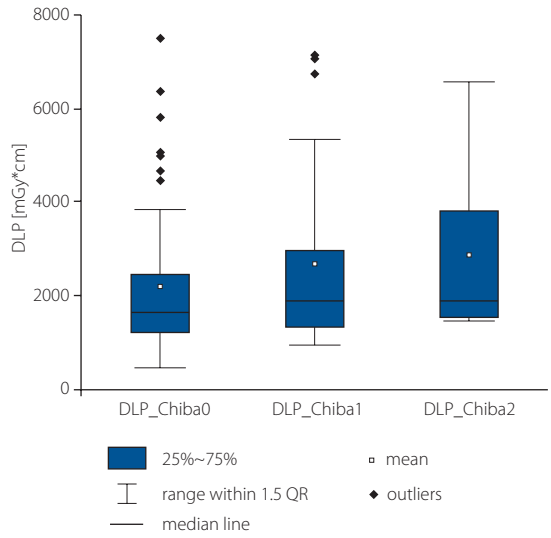

Figure 1. Radiation doses (DLP) by a number of localization needles used

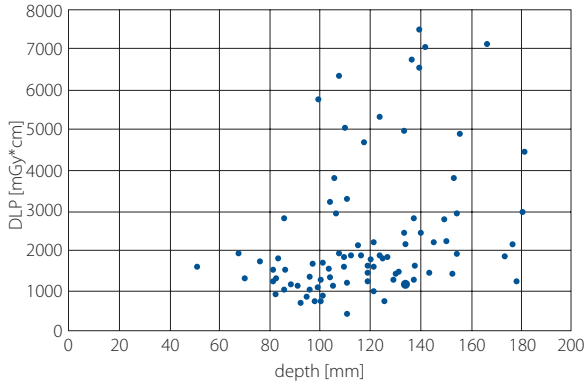

Figure 2. Radiation doses (DLP) by lesion depth

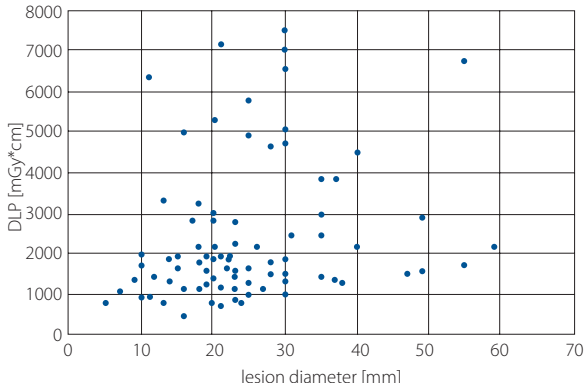

Figure 3. Radiation doses (DLP) by lesion diameter

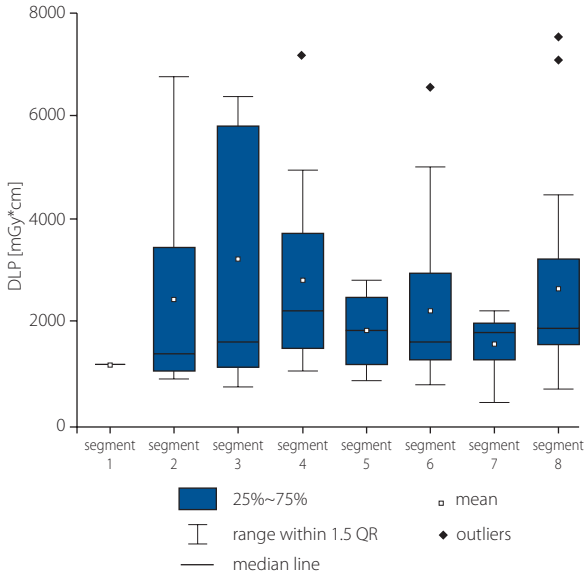

Figure 4. Radiation doses (DLP) in ablations of lesions in particular liver segments

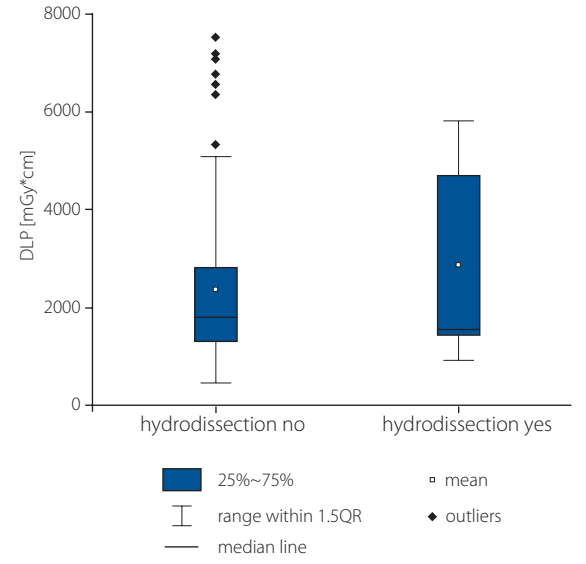

Figure 5. Radiation doses (DLP) during ablation without and with hydrodissection

used. The correlation between DLP and lesion depth or size was very weak and was not statistically significant (fig. 2 and fig. 3). Similarly, the location (by liver segment, fig. 4) of the lesion and the use of hydrodissection (fig. 5) did not have a statistically significant impact on the radiation doses. The estimated lifetime excess risk of malignancy was calculated at 0.10% for ablations of single lesions and 0.14% for ablations of multiple lesions.

Discussion

CT-guidance is frequently used in percutaneous liver tumor ablation due to its excellent spatial resolution and ability to visualize organs and needles with high quality. In many cases ultrasound is not able to show all tumors, especially in a cirrhotic liver or after chemotherapy. Additionally, ultrasound is not a reliable way to show the ablation zone and margin size which is an independent predictor of local tumor progression [8].

Radiation is one of the disadvantages of this method and doses should be kept as low as reasonably achievable (ALARA). The radiation doses in terms of DLP had quite a wide range (450–7518 mGy*cm). Out of several parameters, the number of ablated lesions was a factor that had a significant impact on the radiation dose. Ablation of multiple tumors caused higher radiation than procedures done on single lesions (median 2348 *vs.* 1784 mGy*cm which corresponds to 35 *vs.* 26 mSv).

The results are comparable to other studies. In a publication by Hu et al. [9] the radiation doses acquired during CT-guided ablation were slightly higher and estimated at 41.1 mSv. Similar results were reported by McCarthy et al. [10] where the estimated radiation dose was 30.7 mSv. It is worth noting that the results are similar in many aspects even though the procedures were performed in different centers on different CT scanners.

As opposed to the results of the study by McCarthy et al. [10], hydrodissection was not a factor that would cause a statistically significant increase in radiation dose. The small number of patients that had this additional measure applied in our study could be the reason for such results. However, this result corresponds to other data in our study, especially the application of localization needles as both techniques (hydrodissection and localization needles) require additional punctures and should have a similar impact on the radiation dose.

The lack of statistical significance between radiation doses in the ablation of small and large lesions was somewhat unexpected since large lesions require more needle repositioning and thus more scans. Radiation doses for patients with additional localization needles did not show statistically significant differences. Higher radiation doses in such procedures were expected since they required additional scans to insert the needles precisely into the tumor's border. Moreover, there was a lack of statistical significance when lesion size, depth or location (liver segment) were taken into consideration.

The range of radiation dose values was fairly wide so it remains possible that factors other than the number of lesions have a significant impact. If the effects of lesion size, depth, location (liver segment), hydrodissection and additional needles on radiation doses exist, they seem to have been dominated by other, unknown factors. The effect of "difficulty" of the procedure could be such a factor. Some tumors are more difficult to ablate than others, but no clear parameters have been defined so far. It is possible that the difficulty of the procedure depends on many factors and such complexity makes it hard to clearly define it. That said, the search for such parameters could be a subject of further studies.

This study did not include contrast injections as a factor potentially affecting the radiation dose [10] since all patients had a contrast enhanced CT after needle removal. This step is necessary to assess margin size which predicts the risk of local tumor progression [11]. The majority of radiation doses in CT-guided procedures comes from helical scans [12]. Limiting such scans by replacing some of them with quick-check scans can significantly reduce the radiation dose in CT-guided procedures [13]. However, it can be difficult in such complex procedures as ablations where the operator needs to have high quality visualizations of large volumes of liver tissue. While limiting radiation in CT-guided procedures is important, it should not be done at the cost of reducing the effectiveness of precise needle placement.

The excess risk of malignancy was calculated at 0.10 (single lesions) or 0.14% (multiple lesions) which compares favorably with 0.43% of children and young adults who underwent regular CT scans [14]. The radiation doses acquired by patients who underwent liver tumor ablations correspond to doses acquired during 2–4 multiphase abdominal CT scans.

Liver tumor ablation is a safe procedure with very low major complication rates, from 1.1% [15] to 5% [16], with practically no post-procedural mortality. This compares favorably to liver tumor resection where complications tend to be more frequent, e.g. 27.5% [16]. The results of our study show that excess risk of malignancy in liver tumor ablation is low and in our opinion it should not be a major factor when making decisions on liver tumor treatment. Considering the high efficacy of ablation in liver tumor treatment [17] and its low carcinogenic effect, the potential health gains outweigh the risks of the procedure. The retrospective nature of this study is one of its limitations. Variations in ablation technique between the operators may have also affected the outcomes. Also, the applied conversion factor that was derived from ICRP [7] is designed to estimate the risk to the general population more than individual patients.

Conclusions

The radiation doses and excess risk of malignancy in CT-guided liver ablation are low. The risks are higher in ablations of multiple tumors, however lesion size, depth and location or application of hydrodissection or additional needles do not have a significant impact on radiation dose.

Conflict of interest: none declared

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Secondary prevention and treatment of cervical cancer – an update from Poland

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Introduction. Cervical cancer is the 4th most common cancer in terms of incidence and mortality in women worldwide. The aim of the study was to investigate and analyze the effects of Poland's publicly funded cervical cancer screening and treatment programs.

Material and methods. The study analyzed the financial and epidemiological data provided by the Polish National Health Fund and the Polish National Cancer Registry on the prevention and treatment of cervical cancer in Poland in 2011–2017.

Results. Our study identified a systematic reduction in the number of patients undergoing cervical cytology. Despite high levels of financial expenditure, no correlation was found between the total cost of benefits in PLN million ($W = 0.911$; $p = 0.404$) and mortality expressed by the standardized coefficient ($W = 0.884$; $p = 0.243$).

Conclusions. Despite decreasing mortality rates in cases of cervical cancer in Poland, the organization and delivery of prevention and treatment programs should be considered insufficient.

Key words: cervical cancer, screening, treatment, public health

Introduction

Cervical cancer (CC) is the fourth most common cause of morbidity and mortality among women worldwide. Worldwide, in 2020, incidence and mortality were 604,000 and 342,000, respectively [1]. In Poland in 2018, there were 2360 new cases, representing a world age-standardized rate (ASW) of 7.1 per 100,000 women annually, making it the 8th most common cancer in the female population. The mortality figure was 1593 women, representing an ASW of 4.0 per 100,000. It is worth noting that the mortality rate for CC has recently been decreasing [2].

The Polish cervical screening program consists of a Pap smear (slide cytology) taken every 3 years. When lesions are detected, referred to as either atypical squamous cells of undefined significance (ASCUS) or low-grade squamous intraepithelial lesions (LSIL), a cytologic evaluation is repeated. A colposcopy with the possibility of a biopsy is performed for the following results: atypical squamous cells, cannot exclude a high-grade lesion (ASCH); high-grade squamous intraepithelial lesions (HSIL); atypical glandular cells (AGH); and in some cases, LSIL. Polymerase chain reaction (PCR) testing for the presence of HPV is not included in the program [3].

In Poland, reduced mortality is due to the introduction of secondary prophylaxis in the 1980s based on Pap smear testing (cervical cytology). In 2006, cervical screening beca-

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me a national program. The program involves regular Pap smears repeated at three-yearly intervals in women aged 25 to 59. Until 2015, the administration of the screening program included sending personal invitation letters to women [4]. Following screening, the program's diagnostic and therapeutic steps include a colposcopy with cervical biopsy, surgical intervention (conization, hysterectomy), chemotherapy, and radiotherapy [5].

Despite a steady decrease in CC mortality, it is alarming that Poland's 5-year survival rate is the lowest (56.4%) of all European countries where the European average is 62.4% [6, 7].

Cervical cancer is an important health and economic issue. Prophylaxis against CC and the treatment of patients with CC are considerable burdens on public health funding. We believe that a systematic evaluation of the effect of screening programs can lead to the improved organization of resources. The aim of the study was to investigate and analyze the costs of Poland's publicly funded CC screening program and treatment for the period of 2011 to 2017, considering the latest data from CC statistics. The intention of the authors is that the results of the data analyses, regardless of the final conclusions, will be useful for future CC screening planning.

Material and methods

Our study used data on the screening program carried out in specialist outpatient clinics (*ambulatoryjna opieka specjalistyczna* – AOS [in Polish]), and CC treatments undertaken in public hospitals in Poland between 2011 and 2017, shared by the Polish National Health Fund (NHF) at the authors' inquiry. The NHF is the primary funder of the Polish healthcare system, and thus it collects extensive data on patient demographics, the number and type of services provided, costs generated, and the duration of hospital stays. In addition, we used epidemiological data on CC mortality rates published by the Polish National Cancer Registry (http://onkologia.org.pl). Among the screening data up to 2015 is a group of women obtained from sending personalized invitations. Treatment data is a separate statistic. It is not limited to the cases screened in 2011–2017.

We analyzed the results of all cervical cytology tests conducted in specialist outpatient clinics in the public healthcare system between 2011 and 2017, including the number of women tested, the percentage of the general population included in the program, the cost of the services provided, and the standardized mortality rate due to CC. We considered each case qualified for further in-depth diagnosis and / or treatment as an abnormal Pap test result (ASCUS, ASCH, LSIL, AGH or HSIL). Similarly, we evaluated the treatment of CC patients in Poland, without analyzing the proportion of the general population. Patients receiving medical services encoded with the C53 (malignant neoplasm of cervix uteri) code according to the ICD-10 classification were enrolled in the study in the treatment analysis.

To test for normality of distribution, we used the Shapiro-Wilk test. The direction and strength of linear correlations between two variables were evaluated using the Pearson correlation coefficient, and the t-test was used to evaluate the statistical significance of correlations. The significance level was set at $\alpha = 0.05$. We conducted our analysis using the R statistical program (v4.01).

Results

Cervical cancer screening

The data on cytological screening between 2011 and 2017 is shown in table I. In 2011, 793,400 women underwent screening in outpatient settings (AOS). Over subsequent years, the numbers decreased. By 2017, 463,000 women presented for screening, 41.6% fewer than in 2011. A similar trend was observed in the annual percentages of the general population included in the screening program. The rate of abnormal screening test results requiring further diagnostic procedures was found to correlate significantly with the percentage of patients included in screening in the general population ($r = 0.961$; $p = 0.019$).

Table I. Cervical cytology studies between 2011 and 2017

Criterion	2011	2012	2013	2014	2015	2016	2017	2017/2011 (%)
number of patients tested in outpatient specialty care (AOS)	793,398	726,548	665,520	691,682	652,258	538,273	462,970	58.4
cost of procedures (million PLN)	40.15	39.49	36.03	28.50	27.07	22.69	20.21	50.3
% of general population	24.4	23.75	22.91	22.34	21.72	20.5	18.73	–
mortality rate – ASW	4.84	4.83	4.63	4.46	4.2	4.1	4.2	–

Table II. The number of patients having cervical cytology positive test result and the costs of detecting single positive case (qualified for further in-depth diagnosis and /or treatment) between 2014 and 2017

Criterion	2014	2015	2016	2017	2017/2014 (%)
number of patients with positive test results	19,940	18,521	15,075	13,702	68.7
costs of detecting a single positive case (PLN)	1429.50	1461.79	1505.16	1475.05	103.2

Table III. Costs of cervical cancer treatment between 2011 and 2017

Criterion	2011	2012	2013	2014	2015	2016	2017	2017/2011 (%)
number of patients	22,478	22,829	22,850	22,377	21,730	21,113	20,511	91.2
total costs of procedures (million PLN)	130.85	137.51	140.05	140.05	122.91	123.64	130.89	100.0
average cost of treatment of a single patient (PLN)	5821.33	6023.67	6128.96	6258.63	5656.35	5855.96	6381.57	109.6
total cost of surgery (million PLN)	14.32	15.07	14.86	13.76	14.08	12.91	13.02	90.9
total cost of radiotherapy (million PLN)	56.70	63.24	65.34	66.74	58.79	60.42	61.61	108.7
total cost of chemotherapy (million PLN)	25.83	26.63	26.18	27.46	25.23	24.95	23.40	90.6

The number of patients with abnormal screening test results and the costs of detecting a single positive case (qualified for further in-depth diagnosis and / or treatment) in the years 2014–2017 are presented in table II. The data of the NHF register do not contain information from the previous years. In 2014, the number of abnormal cytology tests results reached nearly 20,000. In the following years, this number decreased. In 2017, there were 13,700 abnormal tests, which was 31.3% fewer than in 2014. The cost of detecting one case over that period was similar to previous periods with mean cost M = 1467.86 PLN (SD = 31.37 PLN). Despite fewer women presenting for screening and fewer abnormalities requiring further in-depth diagnosis and / or treatment being detected, there was a systematic decline in the age-standardized mortality rate (ASW) for CC, from 4.84% in 2011 to 4.2% in 2017 (a decrease of 0.64 percentage points).

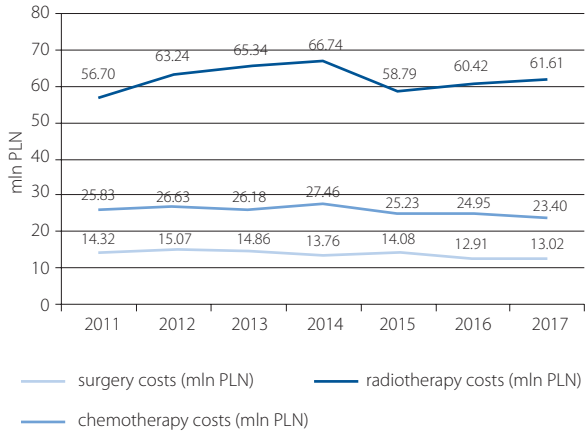

Figure 1. Costs of cervical cancer treatment divided by treatment modalities

Cervical cancer treatment

The costs of treatment of patients with CC between 2011 and 2017, with respect to different types of treatment, are summarized in table III. Between 2011 and 2017, the overall cost of CC treatment was relatively constant, with the average per year cost being 132.3 million PLN (SD = 7.24 million PLN). Relatively minor differences were observed year-by-year with respect to the cost per patient, and also with respect to the costs of different treatment modalities (fig. 1). Despite the average annual cost of treatment (132 million PLN during the analyzed period), no statistically significant correlation has been observed between the overall cost (W = 0.911; p = 0.404) and mortality expressed as the standardized coefficient (W = 0.884; p = 0.243). Also, the correlation between the treatment costs per patient (W = 0.975; p = 0.929) and mortality rates was not significant (tab. IV). The above-listed correlations showed no statistical significance even though there was an increased number of cytology study results and an increased number of services provided (r = 0.886; p = 0.008). For a complete evaluation of hospital treatments over the studied period, we

Table IV. Correlation between total cost of procedures and cost of treating one patient with respect to mortality rate

Costs	Mortality rate – ASW			
	N	r	CI	p
cost of procedures (million PLN)	7	0.641	[–0.22–0.94]	0.121
cost of treating one patient (PLN)	7	0.008	[–0.75–0.76]	0.986

Table V. Mean and maximum hospital stay between 2014 and 2017

Criterion	2014	2015	2016	2017	2017/2014 (%)
mean hospital stay (days)	4.73	4.55	4.49	4.38	92.6
maximum hospital stay (days)	104.38	95.88	101.38	96.56	92.5

used both mean and maximum durations of the hospital stays (tab. V). In 2014, the mean hospital stay was 4.73 days. Over the following years, this index consistently decreased, reaching 4.38 days of hospital stay in 2017. The maximum hospital stay in 2014 was 104.38 days. Over the following years, this parameter's value also declined, reaching 96.56 days in 2017.

Discussion

Cervical cancer is the fourth most common cause of morbidity and mortality among women worldwide. However, its prevalence is inversely proportional to a country's medical resources, with incidence rates being highest in those countries where no cytology screening program is available at all [8]. Cervical cancer has been the focus of public health programs in Poland for the past 15 years. The result of this is that in the last three years alone, approximately 72% of women have undergone cervical cytology, according to the Organization for Economic Co-operation and Development (OECD) data [9]. Notwithstanding these and other countries' efforts, and despite the possibility of primary prophylaxis by vaccination against the human papillomavirus (HPV), it is expected that in the next 15 years, cases of CC worldwide will increase by 42% annually on average, while an 11% increase is expected in developed economies [10]. Poland's recent promotion to the rank of developed country in terms of capital markets does not correlate with its health ranking. One area where this lack of correlation is revealed is in the health outcomes resulting from cervical cancer screening tests.

Our study found a decline in the number and proportion of women enrolled in the national screening program in AOS over the 2011 to 2017 observation period. This is a negative connotation since the number of lesions requiring further diagnostics detected in cervical cytology correlates strongly with numbers of patients tested in outpatient specialty care (AOS) and the percentage of the national population covered by the screening program. In addition, Turkot et al., in their 2018 audit of cytology laboratories in Poland, found there was a significant increase in Pap testing outside the national healthcare program, that is, in the private healthcare sector [11]. Both our results and those of the authors mentioned above may suggest that Pap smear tests are performed in private healthcare. There is an open question about the reasons for the decline in patients' interest in examinations financed by the national screening program. The cessation of sending personalized invitations in 2015 can be considered to be one of the reasons. This action was dictated by low cost-effectiveness considerations [3]. The significantly positive correlation shown between the number of patients tested in outpatient specialty care (AOS) and the value of benefits in PLN million, calls into question the advisability of stopping the sending of personal invitations to cervical screening tests as an activity aimed at cost optimization of the process. Referring to the second argument concerning the ineffectiveness of invitations, it is necessary to cite the studies of independent centers, which say that the use of personalized methods of contact targeted at specific age groups, combined with setting the date of the examination, significantly increases participation in the screening program [12–14]. The increased interest the private healthcare sector has shown in performing screening tests may result, in part, from the availability of improved diagnostic methods, including the possibility of testing for the presence of HPV [15].

The current state of knowledge indicates that almost all CCs are caused or co-caused by persistent high-risk HPV (hrHPV) infection. Two genotypes (16 and 18) are responsible for 70% of CC and 50% of HSIL cases [4]. High-risk HPV tests are characterized by a 20–50% higher sensitivity than routine cervical cytology which means that the risk of overlooking a malignant transformation from precancer to cancer is minimized when compared with a Pap smear slide evaluation (when, in both cases, a negative screening test result is compared) [3].

Our study shows that the declining number of women screened under the national screening program is accompanied by a slight reduction in the national mortality rate from cervical cancer (ASW decreased by 0.64 percentage points over the studied 7-year period). Analysis over a broader time period showed that the annual percentage change (APC 1990–2017) in the mortality rate in Poland accounted –2.3. By comparison, in the countries of the so-called old European Union (EU15), the APC was –2.5, with a low ASW rate of 1.9 [16].

Considering our results and those of other researchers, the slight decrease in mortality observed in Poland should be considered unsatisfactory and indicates the need to make changes in the overall approach of the preventive program. Moreover, the observed decrease in mortality may be partly attributed to the activities of the private healthcare sector in Poland, but we do not have sufficient data to test this hypothesis. We suggest creating a national cervical cancer prevention register that encompasses the combined data of both the NHF and the private sector.

In studies assessing the Standard Expected Years of Life Lost per death (SEYLLd), for every woman's death in Poland from CC in 2011, the SEYLLd was 25.8 years lost, while in 2015 it was 23.7 years. Despite this decrease, when analyzed according to education level, the SEYLLd in women with only primary school education, was 5.8 times greater than in women with higher education [17]. This relationship is another reason to suggest that reintroducing personalized invitations for specific social groups ("Poland says STOP cervical cancer") may be beneficial. Another interesting option especially for young people starting sexual activity can be Instagram influencers spots to encourage vaccination against HPV.

Across the analyzed period, the average annual cost of detecting one lesion requiring further treatment remained at the relatively constant level of 1,467.86 PLN, and the average annual cost of treatments also remained constant at 132.3

million PLN. This funding level places a heavy burden on the public health system. The large number of women treated for CC and the high costs of prophylaxis and treatment constitute a significant challenge for the healthcare budget in Poland and worldwide as well [18, 19]. Various cost-saving measures undertaken so far, including the inclusion of primary healthcare midwives in the cytology collection process, have not produced the expected results [20]. An effective solution may be the introduction of HPV screening tests [21, 23]. Recent research results suggest that replacing a triennial program of cytology with screening for HPV every 5 years has benefits [24, 25].

The CC mortality rate decreased insignificantly over the period of our study, despite high, though relatively constant, levels of treatment cost. According to OECD data, even though Poland's 5-year survival results (55%) for CC treatment have improved slightly, they are still below the European average of 63% [9, 26]. It should therefore be assumed that if CC treatment in Poland is operating below the average European effectiveness, there is room for improvement.

Apart from the ethical aspect, Poland's relatively low 5-year survival rate of CC has an economic context. In 2012 alone, CC incidence and the consequent mortality caused the loss of approximately 957,678 working days in Poland, and this resulted in production losses of EUR 111.4 million, 66% of which was related to mortality [27].

Our study has shown that in Poland, public sector CC treatment costs and the duration of hospitalization have both remained at constant levels during our study period. In comparison, Western European countries have seen a decrease in the cost of treatment with accompanying reductions in morbidity and mortality [28]. Those countries are seeing the long-term effects of the introduction of the HPV vaccination, which is not yet common in Poland [29, 30]. It is worth emphasizing that for the period we analyzed, our study did not identify any significant statistical relationships between the cost of treatment services provided and the mortality rate expressed by ASW. This may suggest a relationship between the decline in mortality and the level of preventive measures only, and not the quality of treatment services. However, in the context of CC, there are no reports in the current literature that would challenge what our study discovered. In the absence of a relationship between the cost of treatment and mortality rates, the hypothesis that mortality rates are influenced by preventative measures rather than by hospital services remains.

Limitations

This study has several limitations. Actual recommended tools for an analysis of health care systems in the context of cost-effectiveness, including cervical cancer prevention are the Incremental Cost-Effectiveness Ratio (ICER), Quality Adjusted Life Year (QALY), and Quality-Adjusted Life Expectancy (QALE). These parameters were not used in the study due to the lack of current data from Poland in the literature. The results of other scientists refer to years earlier than presented in our study.

Conclusions

We want to draw attention to the systematic decrease in the number of patients undergoing cytological examinations funded by the state, which also translates into a reduction of the percentage of the population covered. The recommended solution is to return to personalized invitations, but instead of using letters as before, we suggest that invitations be made through "new media" – e-mail/SMS under the administration of a primary care physician and midwife. The results of our research suggest that patients may be undergoing cytological testing in private healthcare settings. This situation significantly impedes public access to complete statistical data and a comprehensive assessment of the effectiveness of cervical cancer preventive measures and resourcing in Poland. To enable a full analysis in the future, we propose the creation of a national cervical screening registry to include all National Health Funded providers and private healthcare sector providers.

The issue that our report raises, regarding the falling number of women undergoing Pap smear testing within public healthcare settings, also results from the difficulty of public healthcare providers accessing modern diagnostic methods such as liquid-based cytology or molecular diagnostics for the presence of HPV; these observations also indicate possible avenues for changes. The slow decrease in mortality due to cervical cancer described in our study can be accelerated by introducing mandatory vaccination against HPV. Currently, the limited spread of mandatory vaccination programs, funded by some municipalities, has not had a noticeable effect on population-wide data. Poland's unsatisfactory 5-year cervical cancer survival rates may be a result of the phenomena described above: declining prophylactic examination rates across the whole population, diagnostics primarily based on cytological smears, and the limited availability of vaccination against HPV.

We believe that the current processes of diagnosis and treatment of cervical cancer in Poland require a change of approach in line with the recommendations presented in our study.

Conflict of interest: none declared

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Off-label use of medicinal products in oncology: exercising due diligence or experimental activity?

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One of the primary responsibilities of a physician is to diagnose and treat diseases with due diligence. Exercising due diligence in treatment process involves, among others, the use of optimal diagnostic, therapeutic and follow-up management in accordance with the current state of medical knowledge. Each medicinal product has the Summary of Product Characteristics which defines, among others, registered indications, the age group for which the product is registered, the dosing scheme, and route of administration of the product. Polish law does not refer directly to the admissibility of products that use off-label nor does it include regulations forbidding such activities. Considering a number of problems associated with products which use off-label and, on the other hand, commonness of such activities, it is necessary to introduce legal regulations defining the legitimacy and admissibility of such methods of proceeding.

Key words: off-label use, oncology treatment, medical experiment, due diligence, reimbursement of drugs

Introduction

According to article 4 of the Act on the Profession of Doctor and Dentist [1], one of the fundamental responsibilities of a physician is to diagnose and treat diseases with due diligence. Within due diligence, a physician is obliged to apply available methods and means of preventing, diagnosing, and treating diseases, including especially, those being optimal procedures in time offering the best chances of treatment success. Often it requires the use of medicinal products discordant with the provisions of the Summary of the Product Characteristics (SPC), for instance, due to the lack of medicinal products registered in a particular indication or in a specified age group. Exercising due diligence in the treatment process involves, among others, applying optimal diagnostic, therapeutic, and follow-up management in line with the current state of medical knowledge. The authors state that current medical knowledge should be understood as reflecting recent guidelines, management schemes, and treatment standards formulated by scientific societies and groups of experts, as well as applying the elements of evidence based medicine (EBM) as a supplementary factor.

The issue of due diligence is directly referred to article 355 of the Civil Code [41] stating that "the debtor is obliged to perform generally required diligence in relationships of a particular type (due diligence)". In the physician-patient relationship, a physician becomes the stipulated debtor and, at the same time, guarantor of the patient's life and health which binds a physician to undertake any actions focused on the intended objective. The ground for these actions is undoubtedly due diligence understood as treatment implementation based on the current medical knowledge supplemented with EBM. The element of due diligence is, among others, the implementation of pharmacological therapy with the use of medicinal products

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in an optimized manner and adjusted to the individual needs of the patient. According to article 2 item 32 of the Pharmaceutical Law Act [2], a "medicinal product" should be understood "as a substance or a combination of substances presented as owning properties to prevent or treat diseases in humans or animals or administered to diagnose or restore, improve or modify the physiological functions of an organism through pharmacological, immunological or metabolic activities".

Each medicinal product has the Summary of Product Characteristics (SPC) which determines, among others, registered indications, the age group for which the product was registered, dosage regimen, and route of administration of the product. In clinical practice medicinal products are also used beyond SPC provisions (off-label use) which is a result of, among others, constantly developing medical knowledge, a patient's individual needs and strictly formal reasons – i.e. the lack of verification of SPC content which was established several or a dozen or so years earlier. Off-label use of medicinal products implies a number of questions of a legal nature, including the admissibility and legal compliance of such activity, and the responsibility of health care professionals regarding negative effects arising from initiation or continuation of off--label treatment.

Aim

The aim of this paper is to analyze the admissibility of off-label use of medicinal products in oncology and to indicate whether such activity should be identified as exercising due diligence or rather as an experimental activity. The subsidiary aim is to indicate a physician's responsibility to provide information on treatment implemented off-label before its commencement. The paper deliberately omits principles of responsibility associated with the use of medicinal products discordant with the provisions of the Summary of Product Characteristics. Given the extensiveness of the subject associated with physician responsibility due to off-label use of drugs, a separate paper should be dedicated to this issue.

Material and methods

This paper uses analysis of the provisions of the law, the present position of the doctrine, and jurisprudence. The material involves current legal regulations referring to conducting therapeutic experiments, principles of using medicinal products as well as principles of expressing consent to treatment. The fundamental material was complemented by the positions grounded in the doctrine and content of the current Polish Courts' Case Law on the use of medicinal products beyond SPC provisions.

Admissibility of off-label use of medicinal products in oncology

In many fields of medicine, off-label use of medicinal products constitutes a typical and completely acceptable activity in light of current medical knowledge. According to the WHO, half of all drugs available on the worldwide pharmaceutical market is at least incidentally used in a manner not stated in the instructions [3]. In 1997, the FDA defined this method of therapy as "off-label use" referring to the use of drugs in unregistered indications, in a dosage or scheme varying from SPC provisions, or in a population for which the drug was not registered [4].

The Summary of Product Characteristics is created based on the European Parliament and Council Directive 2001/83/WE on community code referring to medicinal products used in humans [5] and the Pharmaceutical Law Act [2]. The information included in SPC is the result of clinical trials conducted for registration of a particular drug. Article 11 section 1 items 1–13 of the Pharmaceutical Law Act [2], includes a list of information necessary to include in the content of the summary of product characteristics. The most important include: clinical data involving indications for use, dosage and route of administration, contraindications, special warnings and precautions for use, interactions with other medicinal products or other forms of interactions, use during pregnancy and breast-feeding, effects on the ability to drive and use machinery, adverse reactions, overdose and antidotes, pharmacological properties, pharmaceutical data on, among others, expiry date, special precautions for storage, name and address of marketing authorization holder. The listing of all SPCs for drugs authorized for use is available on the website of the Office for Registration of Medicinal Products, Medical Devices and Biocidal Products (URPL) [6].

The literature indicates the following off-label drug use amounts: 7.5–15% in typical outpatient internal indication, 30–50% in oncology patients and even 90% in the case of neonatology and pediatric oncology. Off-label use is especially common in the field of pediatrics, oncology, dermatology, hematology, and palliative care [7]. Review of the literature concerning off-label use of drugs in oncology indicates that it is common practice used with various intensity by oncologists around the world.

The authors M.M. Saiyed, P.S. Ong, and L. Chew indicate that the use of drugs beyond registration indications in hospitalized oncology patients varied between 18% and 41% [8]. Among adult patients with cancer, 13–71% received at least one off-label chemotherapy. Major reasons for off-label drug use was the lack of product registration in the treatment of diseases diagnosed in a patient, or the necessity to retreat from the dosage scheme indicated in the SPC [8]. The scale of needs for off-label treatment in oncology is depicted by research conducted by A.K. Herbrand, A.M. Schmitt, M. Briel, et al. in years 2015–2018. Research conducted in a Swiss population demonstrated that 45% of first line treatment cases in a group of 3046 patients treated for cancer was associated with a decision to implement off-label treatment [9]. In a study published in 2021, Japanese researchers demonstrated that diseases most commonly treated off-label were sarcoma, urologic cancers, and gastrointestinal cancers [10]. Research conducted in Peter

MacCallum Cancer Centre in Australia demonstrated that prescribing beyond registered indications is prevalent in patients hospitalized due to acute cancer wherein approximately 22% of all prescriptions concern off-label or unlicensed drugs [11]. Off-label use in oncological treatment is also prevalent in Germany [12] and France [13].

It should be emphasized that, beside oncology, off-label therapy is prevalent in the pediatric and neonatology population. Research conducted in Europe indicates that at least one third of hospitalized children and up to 90% of infants treated in neonatal intensive care units are administered off--label treatment [14].

Polish literature [15] distinguishes four cases of the use of drugs beyond strictly registered indications, involving the use of:

- a medicinal product in a manner or with the route of administration which was not stated in the Summary of Product Characteristics,
- a drug in line with registered indication in patients for whom dosage was not determined,
- a drug in an indication which was not listed in the Summary of Product Characteristics but for which reliable data confirming its safety and efficacy exist,
- a drug in a new indication which is not yet supported by evidence, but for which there are scientific foundations allowing to expect its efficacy and safety.

In the opinion of I. Vrancken, the notion of off-label use should be primarily understood as the use of drugs in the population which was not stated in the SPC as well as discordant with the registered indication [16]. The literature also items out that the use of drugs beyond SPC may stand for the use of a drug in a different age group, other doses or discordant with the purpose [17]. In the opinion of the authors, off-label use of medicinal products should be divided into two categories:

- off-label use of medicinal products in the primary meaning – i.e. the use of products discordant with the registered indications (beyond registered indications), or in an age group for which the drug was not registered,
- off-label use of medicinal products in the secondary meaning – i.e. the use of products in different dosing schemes or route of administration as well as the modification of other SPC provisions.

The literature referring to the legal aspects of the use of medicinal products beyond SPC defines off-label use also as the use of a product discordant with the approved product information, as well as the implementation of treatment in a different manner than that stated in the patient information leaflet (PIL) [18].

Regarding the meaning of the Summary of Product Characteristics, the Court of Appeal in Warsaw in its judgement of 14.02.2014 [19] emphasized that "SPC is one of the crucial documents in marketing authorization procedure for medical products, it contains data of a manufacturer, composition, action, posology and any identified risks associated with the use of a particular product, however it is not of a normative nature, but rather informative one concluding the state of knowledge on this product in a particular moment. Considering continuous development in medical knowledge, a physician must have appropriate license to adjust the use of drugs to current achievements of medicine and the needs of a particular patient".

On the other hand, the Supreme Court in the judgement of 24.11.2011 [20] referred to the relationship of SPC provisions to a physician's decision on drug dosage. The Supreme Court stated that: "a physician's entitlement to prescribe a dosage regimen recognized as appropriate, arises from the fact that he makes therapeutic decisions and is responsible for them, therefore, in any event, he cannot be bound by a dosing regimen determined in the summary of product characteristics. A physician's decision on dosage regimen must consider the individual needs determined by the health state of a particular patient and other professionally assessed circumstances; if it was to be otherwise § 8 section 1 item 2 of the Regulation of the Minister of Health of 17.05.2007 would be completely redundant or would lead to the absurd conclusion that a physician is obliged to automatically duplicate only the dosage regimen determined in the summary of product characteristics".

A similar statement was issued by the Supreme Court in the resolution of 26.10.2011 [21], emphasizing that "article 45 of the Act on the Profession of Doctor and Dentist of 5.12.1996 (…) and article 10 section 1 item 11 and article 11 section 1 item 4 of the Pharmaceutical Law Act of 6.09.2001, do not lay the foundation to assume that a physician is bound by the dosage regimen included in the summary of the product characteristics".

The presented jurisprudence indicates that SPC provisions are only of a formal nature and in each case do not guarantee proceeding in line with the current medical knowledge. The doctrine mentions that the medical knowledge resulting from research must be publicly released in a verifiable form, so as to allow not only control and possible criticism of the accuracy of the applied method, but also the replication of research in line with the proposed method in order to compare the obtained results [22].

At the same time, the literature emphasizes that "no regulation requires that, for valid and efficient execution of a physician's competence to prescribe a drug (in any form), a medical product is prescribed in line with registered indications" and "there are no detailed rules to limit a physician's right to prescribe a medicinal product of his choice, naturally considering the diagnostic and therapeutic findings in a particular case, maintaining the legal and non-legal directives for physician's due diligence. This conclusion also applies to therapies with medicinal products used beyond registered indications" [23].

Although, the law neither excludes nor limits off-label use of drugs, the authors state that SPC provisions should not be

subject to arbitrary and unlimited modification, especially in terms of non-adherence to registered indications incorporated in the SPC. It should be considered inherent to distinguish the primary meaning of off-label use, which should be understood as non-adherence to registered indications or the use of a product in an age group other than that indicated in the SPC, from its secondary meaning which should be identified as a change of dosage regimen, route of administration, or change of other conditions for drug use expressed in the SPC. As far as a change of route of administration or modification of dosage regimen can be justified by the individual specificity of a disease or a patients' individual traits, the use of drugs beyond registered indications should be justified by the need to save life or health. The authors' opinion correlates with the position of American oncologists; they emphasize that in cases when previously used medicinal products registered in oncological treatment do not have the expected outcome, the implementation of off-label treatment is admissible [24]. Moreover, as recent studies have shown, off-label use has not only a positive, but also a negative impact on the health of oncological patients [25]. The authors indicate the following, among others, indications for use of medicinal products beyond the SPC:

- direct threat to the life or health of a patient,
- exhaustion of the available and registered medicinal products and no expected outcome of therapy,
- the lack of medicinal products registered in a particular indication in the specified age group.

Automatic decision-making on the implementation of treatment beyond SPC without consideration. The point was to indicate analysing conditions given in a certain situation can represent the adoption of practice of drug use incompatible with the registration as a rule, as well as an increase of health risks associated with the use of drugs discordant with their formal registration. Admissibility of automatic use of off-label drugs emerges in a situation in which such possibility is foreseen by an announcement of the Minister of Health on the list of reimbursed medicines, foods for medical purposes and medical devices [26]. In specific cases, the refund announcement allows to prescribe a reimbursed medicine, even though the medicine is not registered in the indications concerned. Although it is the exception to the rule, according to which reimbursement corresponds with registered indications, the announcement does not refer to single use of off-label therapy. In the case when column no 13 of the appendix to the reimbursement announcement named "reimbursement indications beyond registration" includes specified units not included in the SPC, a drug can be prescribed with reimbursement in spite of not being registered in these indications [27]. The situation regarding off-label use of medicinal products based on drug programs is similar. Drug programs constitute the appendix to the reimbursement announcement which determines their binding nature. This means that a physician using a medicinal product beyond the SPC, in line with the drug program guidelines, is not subject to liability due to making such a decision and his activity is identified as exercising due diligence and fulfilling his obligations associated with the initiation of treatment of a patient qualified to the drug program [28].

Off-label drug use and regulations for medical experiments

Off-label use of medicinal products in its primary meaning should not be identified with a medical experiment in the true meaning of this concept. According to article 21 section 2 of the Act on the Profession of Doctor and Dentist [1]: "a medical experiment is the implementation of novel or only partially tested diagnostic, medical or prophylactic methods to achieve direct health benefit in a patient. It can be conducted if previously applied methods are not efficient or not sufficiently efficient (…)." The medical experiments category includes also research experiments. According to article 2 section 3 of the Act on the Profession of Doctor and Dentist [1] "a research experiment primarily aims at expanding medical knowledge (…)." Administration of an off-label drug does not have such an aim, however, in practice, it may enrich medical knowledge. Therefore, the use of drugs beyond the SPC in order to achieve optimal therapeutic effect cannot be in principle identified as a research experiment activity.

Regardless of whether a medical experiment is regarded as being of therapeutic or research in nature, eo ipso such activities contribute to the expansion of medical knowledge (especially evident in the case of research experiments). Administrating a particular patient an off-label drug does not have such an aim (although in practice it may enrich medical knowledge).

The fundamental difference between a medical experiment and the use of medicinal products beyond SPC, in the primary meaning, is the fact that activities of an experimental nature are entirely novel or only partially tested. On the other hand, the use of medicinal products discordant with registration indications is, in principle, an activity having its foundations in EBM, medical literature, and guidelines of teams of experts. Due to the safety of use specified by EBM, off-label drug use should be identified with regular medical service which is not a medically experimental. At the same time, it should be remembered that the regular health service, i.e. not experimental in nature, can be associated with an increased risk of a negative impact on a patient's life or health. The literature emphasizes that activity of an experimental nature cannot be identified with regular therapeutic activity [29]. What is more, the literature indicates that: "(…) only research activities conducted in line with generally accepted principles for scientific research, especially in strictly defined, purposefully chosen, precisely controlled conditions allowing for multiple replication, can be called medical experiment. Therefore unplanned, single use of a novel or unconventional treatment method applied by a physician to save a patient's life or health is not

a medical experiment" [30]. In the authors' opinion, the medical experiment catalogue, within the meaning of provisions of chapter 4 of the Act on the Profession of Doctor and Dentist [1] (medical experiments) does not include single activities aiming at the protection of a patient's life or health in urgent cases, understood as all the cases in which the risk of loss of life, severe body injury or severe disorder of health occurs. As literal wording of article 30 of the Act on the Profession of Doctor and Dentist states: "a physician is obliged to provide medical aid in all cases when a delay in its provision could cause a risk of life loss, severe body injury or severe disorder of health" [1]. Therefore, the activities a physician is obliged to undertake in line with article 30 of the Act on the Profession of Doctor and Dentist [1] must not be identified with a medical experiment, which is due to the procedure of their implementation (urgent case), the nature of the activity (prophylactic, medical, and diagnostic activities), and the specifics of risk associated with refraining from the implementation of optimal methods of medical procedure (loss of life, severe disorder of health, severe body injury).

At the same time, urgent cases and measures should not be identified with an experiment carried out in conditions of an urgent cases and measures. In accordance with article 25a item 2 and 5 of the Act on the Profession of Doctor and Dentist [1], a medical experiment can be conducted without the participant's consent if the following conditions are met: "an urgent case occurs and due to the necessity to undertake immediate action, it is impossible to obtain consent for participation in the medical experiment from a legal representative of the participant or judicial authorization within a sufficiently short period of time", and "the experiment's participant [. . .] he and his legal representative will receive all significant information regarding participation in this experiment in the shortest period of time possible." However, it should be emphasized that all actions bearing the marks of a medical experiment in the understanding of the Act on the Profession of Doctor and Dentist can only be conducted after previously obtained positive opinion of the Bioethics Committee – article 29 section 1 of the Act on the Profession of Doctor and Dentist [1]. The above analysis clearly demonstrates that incidental medical activities aiming at saving life or health in urgent cases are not medical experiments, even if their nature is innovative, atypical or uncommon.

Assuming that the use of drugs beyond SPC is not of an experimental nature is crucial, among others, from the perspective of the obligation to conclude liability insurance by the entity conducting the experiment. According to article 23c section 1 of the Act on the Profession of Doctor and Dentist [1], the experimenting entity is obliged to conclude a separate liability insurance agreement covering the experiment's participant and a person who can be directly influenced by the effects of the experiment. Exception from the obligation to conclude liability insurance is defined in article 23c section 2 of the Act on the Profession of Doctor and Dentist [1], stating that conducting a medical experiment in spite of lack of liability insurance is only admissible in the case of a need for the experiment in urgent mode or in the case when the life of the experiment's participant is threatened.

According to § 2 of the Ordinance of the Minister of Finance, Funds and Regional Policy on compulsory civil liability insurance of the body carrying out the medical experiment [31], third party liability insurance is covered by the civil liability of the body carrying out the medical experiment for damage caused by its action or negligence to the participant and the person whose effects may be directly affected by the experiment, in connection with the medical experiment being carried out. As is indicated in the doctrine "both – the Pharmaceutical Law Act (article 37 b section 2 item 6) and the Act on Medical Devices (article 40 section 4 item 6) introduced a requirement to conclude mandatory liability insurance for damages caused due to conducting clinical trials. This resolution can be justified by the protection of participants' rights, for whom in case of a damage due to experimental activities, compensation would be guaranteed. However, application of these regulations is limited to research activities regulated pursuant to current acts. Therefore, it was demanded to unify these solutions and introduce them to chapter 4 of the Act on the Profession of Doctor and Dentist. Such a regulation was placed in the added article 23 c which imposes such an obligation" (. . .) [32].

The use of medical products incompatible with SPC records does not constitute a medical experiment, if implementation of treatment aims at protecting the life or health of patients, instead of, for example, only observation of drug activity. In case of medical off-label use of medicinal products, provisions of the Act on the Profession of Doctor and Dentist on medical experiments have no appropriate application, therefore no obligation for concluding liability insurance by the entity which initiates and conducts such therapy occurs.

It should be emphasized that the use of medicinal products discordant with SPC represents exercising due diligence, provided that such activity constitutes optimal therapeutic management.

The issue of the lack of due diligence in treatment process was addressed by the Court of Appeal in Krakow in its judgement of 12.10.2007 [33], emphasizing that "it is a physician's fault not to exercise the highest degree of due diligence which is possible at currently used methods of treatment of a particular disease (. . .)." The use of medicinal products beyond SPC constitutes due diligence, provided that such activity is commonly accepted and applied, as well as being in line with the current state of medical knowledge.

According to P. Kwinta "continuous development in medical sciences (. . .) leads to the situation in which the information included in SPC, being a primary document required for drug

registration, in some circumstances can be out-of-date" [34]. Both doctrine and judicature refer to the issues of the use of medicinal products discordant with registration provisions, however these issues are not treated in a uniform manner. Also, they are not directly regulated by the law.

Obligation to provide information in case of off-label drug use

In case of decision on off-label use of a drug a physician is obliged to inform a patient on possible results and complications of planned procedure, including alternative types of therapeutic management.

The obligation to provide information specified in article 31 section 1 of the Act on the Profession of Doctor and Dentist [1] requires that a physician provides, among others, information on the potential negative effects associated with a proposed treatment, the available alternative methods of proceeding, as well as the possible negative effects resulting from treatment initiation withdrawal. This issue becomes especially significant in cases where a physician proceeds discordant with SPC provisions, for the patient must know that such a method will be used and why a physician has decided to apply it. In the case where a patient accepts the offered nonstandard therapeutic management, they assume the risk associated with off-label drug administration. However, it is crucial to clarify the predictable consequences (favorable and unfavorable for the patient) of such a method of prescribing drugs [42–45]. The current case law states that once a patient is properly informed, he assumes the risk associated with the undertaken activities, under the condition that a "medical error", resulting in negative consequences, is not made by the physician [46, 47].

The literature emphasizes that the obligation to provide information is not limited to the level of information considered important by a physician, but a patient [35]. Due to the specificity and scope of results possibly occurring in treatment beyond the SPC, such activities should be identified as high-risk activities which highlights the importance of the obligation to provide information. In the authors' opinion, the risk of off--label treatment can be identified as typical (average) only in cases, when the use of a product incompatible with SPC is a common and schematic activity.

The doctrine emphasizes that "the lack of due diligence (provided for a professional) in the case of a physician, can involve the use of a drug in a defective way or to an inappropriate patient as well as the lack of possibility to predict adverse reactions of a drug as a result of a physician's insufficient knowledge regarding its properties or side effects when they possessed or should have possessed such knowledge. Responsibility for damage caused this way can be assigned to the physician (…), as lack of due diligence is a physician's fault. A physician (…) will be also responsible in case of prescribing or administering a patient drugs which harm the patient and the physician possessed or should have possessed knowledge on the properties of these drugs" [36]. In terms of the use of medicinal products beyond the SPC, prediction of all and even typical effects of their application is impossible due to the lack of previous drug assessment in terms of the risks associated with its use.

In view of the article 31 section 1 of the Act on the Profession of Doctor and Dentist, prior to off-label treatment initiation, a physician should deliver, among others, any information on effects and complications that may be predictable in light of the current medical knowledge, including these of casuistic occurrence. Case law indicates that an obligation to provide information covers normal, predictable, events even of rare occurrence, but impossible to exclude (…), especially those of a dangerous nature for life or health [37].

The delivery of understandable and comprehensive information on off-label treatment provides grounds for patient's informed consent for treatment [38]. In the judgement of 9.04.2019, the Court of Appeal in Warsaw itemed out that "the right for information – beside the right for consent – is in fact one of the most important elements of the relationship between the health care professional and the patient. Guaranteeing a patient the right to information is condition *sine qua non* of protection of his autonomy. Thereby the right for information should be treated as an instrument of significant importance (…)" [39]. On the other hand, the Court of Appeal in Warsaw, in the judgement of 19.02.2019, adopted a position that "(…) obligation to provide appropriate information is in fact integrally associated with a physician's obligations concerning the treatment process alone. Properly fulfilled obligation to inform is a necessary condition for a patient's expression of legally binding consent (termed as "informed") for determined treatment; the ineffectiveness of consent due to the lack of delivery of appropriate information affirms the unlawful activities of a physician (…)" [40].

Conclusions

Polish law does not refer directly to the admissibility of off-label use of medicinal products, nor includes regulations forbidding to undertake such activities. The physician's obligation to exercise due diligence should be identified with, among others, the necessity to implement optimal pharmacological therapy in accordance with current medical knowledge. In some cases, optimal therapeutic management is associated with the necessary off-label use of medicinal products. However, the use of treatment beyond SPC provisions should not be the rule, but rather it should be justified by strictly defined conditions. The occurrence of conditions in the form of protection of health and life has particular relevance in a case when off-label products use consists of implementation or continuation of treatment with a product which was not registered in an indication in which it is used, or it is used in an age group not listed in the SPC. Off-label use of drugs should not be identified with an experiment in the understanding of the provisions of the Act on the Profession of Doctor and Dentist due to the

different specificity of both activities, as well as the criteria to be met to undertake each activity.

Due to the nature of off-label therapy, its initiation or continuation requires particularly careful communication to the patient on the possible consequences of the action of the drug administered against the SPC provisions. Considering the number of problems associated with off-label use of drugs and, on the other hand, commonplace nature of such activities, the introduction of legal regulations defining the legitimacy and admissibility of such methods of proceeding is necessary. At the same time, it is necessary to initiate education of health professionals regarding the legal possibilities concerning off-label use of drugs, as well as prescribing beyond registration in line with the guidelines of reimbursement announcement.

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The Poly(ADP-ribose) polymerase inhibitors in pancreatic cancer

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Genome instability and mutations are the hallmarks of cancer. Mutations within *BRCA* genes increase the risk of pancreatic cancer (PC) development. Poly(ADP-ribose) polymerase inhibitors (PARPi) show the synthetic lethality phenomenon in tumoral cells with *BRCA* mutation and improve outcomes in patients with breast, prostate and ovarian cancer. Olaparib was the first PARPi registered for the patient with metastatic PC with a deleterious or suspected deleterious germline *BRCA*-mutation. The POLO phase III clinical trial shows that olaparib in PC increases progression-free survival, however it does not prolong the overall survival. Currently, many clinical trials are ongoing to determine the clinical utility of PARPi in monotherapy or polytherapy of PC. The role of PARPi in PC has not been well established and many questions remain unanswered. This review aims to summarise the rationales behind the use of PARPi and current clinical data.

Key words: PARP inhibitors, olaparib, pancreatic cancer, *BRCA* mutation

Introduction

It is estimated that 60,430 (31,950 men and 28,480 women) cases of pancreatic cancer (PC) will be diagnosed and 48,220 people (25,270 men and 22,950 women) will die in 2021 in the USA according to the American Cancer Society [1]. PC is the fourth leading cause of cancer death in men as well as women. The prognosis of PC is unfavorable and life expectancy is about 5% at 5 years [2]. The majority of patients at the time of diagnosis present unresectable tumours due to either local extension or distant metastases. The current treatment options for patients with metastatic PC include fibrinolysin, gemcitabine with nab-paclitaxel, or erlotinib regimens which significantly improved the clinical outcomes in comparison to gemcitabine monotherapy that was the standard therapy for many years [3, 4].

Advances in molecular biology and genetics allow designing poly(ADP-ribose) polymerase inhibitors (PARPi), which are a new class of drugs based on molecular profiling, including *BRCA* mutational status assessment. PARP belongs to a group of enzymes involved in DNA repair, which are activated by DNA damage [5, 6]. It includes olaparib, niraparib, talazoparib and rucaparib. PARPi improved treatment outcomes in patients with breast, prostate and ovarian cancer [7–12].

Currently, they are being tested in monotherapy or polytherapy in PC and may potentially improve the therapeutic armamentarium for that population of patients. In December

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2019, the Food and Drug Administration (FDA) approved olaparib as a maintenance treatment for patients with deleterious or suspected deleterious germline *BRCA*-mutated metastatic pancreatic adenocarcinoma [13]. Recently, in the phase III POLO trial, it was shown that olaparib increases the median of progression-free survival (mPFS), however without improving the median of overall survival (mOS) [14]. Nevertheless, PARPi are a promising new class of drugs that need further studies. This review aims to summarise the preclinical and clinical data on PARPi in PC.

The role of *BRCA* genes and BRCAness in PC

One of the hallmarks of cancer is genomic instability which leads to DNA alterations and predisposes to cancer development [15]. Two types of genetic alterations which lead to tumorigenesis can be distinguished – germline mutations and somatic – a somatic acquired mutation that arises spontaneously as a result of environmental factors like smoking [16]. The majority of PC, approximately 80%, do not have any associations with either positive family history, or inherited genetic causes. 5.2% are associated with an inherited component without positive family history and about 8% of patients with PC have a positive family history [17]. The most common mutation is in the *KRAS* (Kirsten rat sarcoma virus) gene whereas germline and somatic mutations in genes *BRCA* (breast cancer) *1/2, ATM* (ataxia–teleangiesctasia mutated) and *PALB2* (partner and localizer of *BRCA2*) occurs less common of cases [15]. The incidence of germline and somatic mutations in PC is presented in table I. BRCA1 and BRCA2 are proteins that are involved in DNA repair and transcriptional regulation in response to DNA damage. They also take part in replication fork protection and are important factors responsible for resistance to the activity of numerous nucleases, including MRE11, DNA2, EXO1 and MUS81 [20, 21]. Importantly, both proteins are involved in the homologous recombination repair (HRR) process, in which a homologous DNA sequence is used to guide repair that results in restoring the DNA sequence to its original form [22, 23]. Cells with dysfunction in *BRCA 1/2* genes have deficits in HRR and must use less accurate mechanisms to repair double-strand breaks, increasing the risk of cancer development [24]. In unselected populations, a pathogenic mutation in *BRCA1* is found in less than 1% and *BRCA2* mutation in up to 2% of PC cases [17]. Identifying the *BRCA* mutation status in patients is clinically relevant because the mutation provides the data on other possible cancer risks associated with the *BRCA* mutation, like breast, ovarian and prostate cancers. Additionally, identifying the *BRCA* mutation status allows for testing at-risk family members for the same mutation with limited cost [25].

The mOS of patients with PC and *BRCA1* and *BRCA2* mutations is approximately 15 and 13 months, respectively [24]. Among approximately 13 hereditary genes associated with PC development, *BRCA1* and *2* mutations are the most frequent genetic alteration responsible for FPC, which are diagnosed in 2.7% of patients with PC [17]. It has been reported that in about 3,9% of unselected patients, somatic *BRCA1/2* mutations drive the PC [28]. The mOS for patients who carry mutations in *HRR* genes (*ATM, BARD1* [*BRCA1-associated RING domain protein 1*], *BRCA1, BRCA2, BRIP1* [*BRCA1 interacting protein 1*], *PALB2, RAD51C, RAD51D*) associated with PC is 14.6 months, whereas mOS for patients without mutations was 11.7 months [26].

Apart from *BRCA1/2* mutations, the other mutations related to PC are alterations within other HRR genes like *ATM, CDKN2A* (cyclin-dependent kinase inhibitor 2a), *MLH1* (mutL homolog 1) [17]. As opposed to breast cancer and prostate cancer, mutations in *CHEK2* (checkpoint kinase 2) and *PALB2* have no si-

Table I. The incidence of germline and somatic mutations in PC

Gene – germline mutation	Incidence in PC	Incidence in patients with a positive family history of PC	Gene – somatic mutation	Incidence in PC	Reference
BRCA1 *BRCA2* *PALB2*	2.4% 26.2% 2.4%	– – –	*KRAS* *TP53* *SMAD4* *CDKN2A* *SMARCB1* *RB1*	88.1% 33.3% 16.7% 4.8% 2.4% 2.4%	[73]
ATM *BRCA1* *BRCA2* *PALB2* *RAD51*	2.1% 0.6% 2.2% 0.4% 0.2%	– – – – –	–	–	[26]
ATM *BRCA1* *BRCA2* *CDKN2A* *MSH2* *PALB2*	2.6% 0.7% 3.6% 1.3% 0.3% 0.3%	3.2% 1.1% 4.3% 2.2% 0.5% 0.5%	–	–	[33]

gnificant correlation to pancreatic cancer [17, 29]. The mOS for patients treated with FOLFIRINOX chemotherapy in metastatic PC, who have somatic or germline mutations in *BRCA1*, *BRCA2*, *PALB2*, *MSH2*, *FANC* (the Fanconi anemia) complementation group was 14 months in comparison to 5 months in patients without mutations [30].

BRCAness is a phenomenon referred to as the existence of a HRR defect despite the absence of a germline *BRCA1/2* mutation in tumour, which leads to oversensitivity to DNA damage as a result of increased genomic instability. The most common mutation in the HRR repair gene that contributes to the BRCAness phenotype is a somatic defect in *BRCA1* and *BRCA2*, however, BRCAness is also related to other genes involved in HRR, such as *ATM*, *PALB2*, *ATR* (ataxia teleangiectasia and Rad3 related), *CHEK1/2*, *RAD51*, *NBS1* (Nijmegen breakage syndrome) and *FANC* family of genes [19, 31]. The incidence of HRR mutations in PC is shown in table II.

The data describing the role of genes other than *BRCA* are limited. Among the HRR genes, one of the most relatively known mutations related to inherited and sporadic PC is the *ATM* mutation [32]. The incidence of *ATM* mutations in patients with a positive family history of PC is approximately 3.2% [30]. ATM serine/threonine kinase controls cells' survival, death, cell cycle arrest, apoptosis and DNA repair. Pathogenic germline *ATM* mutation increases the risk of PC [34–37]. However, *ATM* mutational status may be also important in predicting radiation and chemotherapy response [38, 39]. *ATM*-deficient PC cells are more sensitive to fractionated radiation than wild-type pancreatic cancer [38]. *ATM*-mutated PC cells treated with olaparib significantly enhance suppression of the PC proliferation *in vivo* and *in vitro* [40].

Furthermore, it has been demonstrated that tumours with BRCAness have similar therapeutic vulnerability as tumours with germline *BRCA* gene mutations. For that reason, it is considered as a potentially significant factor in PARPi therapy [41, 42].

Table II. Frequency of BRCAness mutations among patients with a positive family history of PC [17]

BRCAness	Prevalence in PC
BRCA1	0.6%
BRCA2	2.10%
ATM	3.29%
PALB2	0.6%
ATR	–
CHEK1	–
CHEK2	2.4%
RAD51	0%
NBS1	0.3%
FANC	0.3%

DNA damage response and PARP involvement in synthetic lethality

DNA damage occurs constantly in cells due to exogenous and endogenous stressors leading to genome instability. DNA damage response (DDR) is a central mechanism responsible for detecting DNA lesions and promoting their swift repair. In the process of DDR, a great amount of different intra- and extracellular signalling pathways and enzyme activities are activated. In suboptimal or lack of activity of DDR, an exaggerated level of genomic instability arises – a characteristic feature of cancers. In human cells, two major forms of DNA damage could occur, either a single-strand break (SSB) or double-strand breaks (DSB), whereby SSB occurs more often. Different forms of DNA damage bring responses by proper signalling pathways and repair mechanisms [43, 44]. There are four known repair pathways involved in SSB: base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR) and trans-lesional synthesis. HRR and non-homologous end joining (NHEJ) are two pathways responsible for repair DSBs. The HRR process involves BRCA1/2, PALB2, ATM, RAD51, CHEK1/2, ATR, p53 proteins and MRN complex composed of Mre11, Rad50 and NBS1/NBN proteins [45–47]. When DSB occurs, it is detected by the MRN complex and the ATM and ATR – the cell cycle regulatory kinases are activated. Subsequently, ATM activates CHK2, which arrests cell cycle progression, contributes to regulating BRCA1 in DNA repair, and interacts with TP53, which is responsible for cell cycle and apoptosis control. The MRN complex also recruits BRCA1/2 and PALB2 to the DNA damage site. These proteins form a new complex, which finally activates RAD51 that is responsible for binding single-stranded DNA segments and invading the homologous sequences in the sister chromatid.

PARP enzymes are known as DNA damage sensors. This nuclear deoxyribonucleic acid-binding protein contains an N-terminal double zinc-finger DNA-binding domain, a nuclear localization signal, a central automodification and a C-terminal catalytic domain. Its basal enzymatic activity is low but the variety of allosteric activators, for example, damaged DNA, nucleosomes and a variety of protein-binding partners, strongly stimulates it. When SSBs occur, the PARP enzymes are activated and binds to the site of single-DNA damage using its zinc-finger DNA-binding domain. It cleaves NAD+ into nicotinamide and ADP-ribose. The latter cleavage product is covalently attached to glutamate or aspartate residues of nuclear acceptor proteins in the form of long branching ADP-ribose polymers. This results in a highly negatively charged polymer and subsequently leads to the unwinding and repair of the damaged DNA through the BER [48–52]. PARPi interfere with base excision repair by binding to the catalytic domain of PARP, which prevents PARylation, traps PARP to the SSB, and prevents repair. Consequently, an accumulation of SSB occurs, which degenerate into DNA DBS. As a result, cancer cells undergo cell cycle arrest and apoptosis when exposed to these agents.

Inhibition of PARP-1 in PC cells increases the caspase-3 activity, and by increasing the p53 protein expression suppresses BCL-2 (B-cell lymphoma 2), as a consequence leading to apoptosis and suppression of PC cell proliferation [53]. Except for SSB, in cells PARP enzymes also take part in HRR-mediated DSB repair [54]. Inhibition of these enzymes in cancer cells could cause cell death which is based on a phenomenon called synthetic lethality (fig. 1). It is defined as the situation when two or more separate genes are simultaneously mutated which lead to cell death. The product of one of these genes is crucial to the survival of the cell, whereas another gene is used as an alternative. In a situation when the gene is mutated, it is replaced by a second one that is involved in an alternative pathway of the same process. In cells with *BRCA* biallelic mutation, cells become incapable to properly perform HRR. In case of DNA damage, these disorders are repaired with PARP and BER repair. The use of olaparib in the presence of the mutation disrupts both repair mechanisms, leading to cell death, because inhibition of PARP activity leads to the accumulation of single-strand breaks, which can lead to double-strand breaks properly repaired by HRR [55–58]. The synthetic lethality in *BRCA*-mutated cancers caused by selective inactivation of PARP enzymes cells are unable to successfully repair DNA damaged, which consequently cause its death [59, 60].

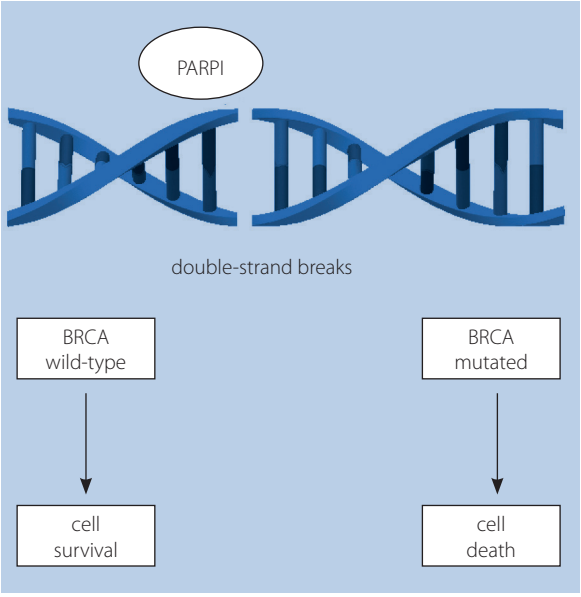

Figure 1. Synthetic lethality and PARPi

Pathobiology of PC

The expression and localization of PARP-1 in the pancreas and PC are different. In the human pancreas, only nuclear PARP-1 (nPARP-1) expression was shown, contrary to nPARP-1 and cytoplasmic PARP-1 (cPARP-1) expression in PC. In the pancreas, the expression of nPARP-1 is enough to maintain the cell's homeostasis by triggering apoptosis in response to DNA damage due to its proapoptotic activity; whereas in PC tissue, the lower expression of nPARP-1 prevents it. PARP-1 takes part in regulating TRAIL (TNF-related apoptosis-inducing ligand) induced apoptosis. Inhibition of PARP-1 may sensitize TRAIL resistant PC cells to TRA-8-induced apoptosis [57]. PARP expression was studied as a new potential prognostic factor in PC. Immunohistochemical analysis of cPARP and nPARP among 178 PC show that high nPARP was associated with a better prognosis (mOS14.5 *vs.* 9.6 months, p = 0.004), however, it did not show a statistically significant correlation with clinicopathological parameters [61]. FeiXu et al. in their studies focused on cPARP-1 and compared the frequency of cPARP-1 in well, moderately and poorly differentiated PC. Initially, they suggest potential relations between cPARP-1 expression on PC pathogenesis and progression, similar to recent breast cancer reports where the correlation between aggressiveness, higher risk of relapse and the death of patients were seen [57, 62]. In their studies, the expression of cPARP-1 was higher in moderately and poorly differentiated than well-differentiated pancreatic tumours. Furthermore, they linked PARP-1 in the cytoplasm to the extrinsic pathway of apoptosis [57].

Table III. Ongoing trials with PARPi in monotherapy in patients with PC

Name of the study	Phase	Indication/ tumour type	Study drug	Control arm	Mutational status	Primary outcome measure	Secondary outcome measure
NCT04005690	early I	stage I–IV PC	olaparib	cobimetinib	–	proportion of all feasibility – evaluable participants that have a measurable change in post-treatment tumour biology from baseline	• incidence of ≥ grade 3 toxicities for each assigned window treatment • proportion of feasibility – evaluable participants within each study arm that have a measurable change in post-treatment tumour biology from baseline
NCT01078662	II	ovarian, breast, prostate, pancreatic advanced tumours	olaparib	–	*BRCA1/2* mutation	tumour response rate	• ORR* • PFS • OS • duration of response

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Table III. cont. Ongoing trials with PARPi in monotherapy in patients with PC

Name of the study	Phase	Indication/ tumour type	Study drug	Control arm	Mutational status	Primary outcome measure	Secondary outcome measure
NCT02184195 (POLO)	III	PC	olaparib	placebo	germline *BRCA1/2* mutation	PFS	• OS • time from randomisation to second progression • time from randomisation to first and second subsequent therapy or death • ORR* • quality of life (QoL) • AEs
NCT02677038	II	metastatic PC	olaparib	–	• mutation in germline *BRCA1/2* negative • BRCAness pheno-type	ORR*	• OS • PFS • change in CA19-9 • AEs
NCT04858334 (APOLLO)	II	resectable PC	olaparib	–	*BRCA1/2, PALB2*	improvement in relapse-free survival	• RFS • OS • efficacy after chemotherapy • differences in survival
NCT03601923	II	advanced PC	niraparib	–	• *BRCA1* • *BRCA2* • *PALB2* • *CHEK2* or *ATM* mutation	PFS	• ORR** • OSR • AEs
NCT04171700 (LODESTAR)	II	solid tumours	rucaparib	–	• *BRCA1* • *BRCA2* • *PALB2* • *RAD51* • *RAD51* • *BARD1* • *BRIP1* • *FANC* • *NBN* • *RAD51* or *RAD51B* mutation	best ORR **	• ORR ** • PFS • AEs
NCT03140670	II	metastatic locally advanced PC	rucaparib	–	*BRCA1/2* or *PALB2* mutation	AEs	–
NCT04550494	II	malignant solid neoplasm including PC	talazoparib	–	germline or somatic aberrations in genes involved in DNA damage response	percent of patients who demonstrate simultaneous Rad51 activation	• ORR** • tumour genomic alterations potentially associated with sensitivity to talazoparib
NCT04182516	I	• locally advanced/ metastatic HER2 negative breast cancer • epithelial ovarian cancer • castration-resistant prostate cancer • PC	NMS –03305293	–	–	number of participants with first-cycle dose-limiting toxicity	AEs

AE – adverse events; ORR* – objective response rate; ORR** – overall response rate; OS – overall survival; OSR – overall survival rate; PC – pancreatic cancer; PFS – progression free survival; RFS – relapse-free survival

Table IV. Ongoing clinical trials with PARPi in polytherapy

Name of the study	Phase	Tumour type	Experimental arm	Control arm	Mutational status	Primary outcome measures	Main secondary outcome measures
NCT02498613	II	• PC • lung cancer • breast cancer	olaparib + cediranib	–	–	ORR*	• AEs • PFS
NCT03682289	II	• PC • renal cell carcinoma • urothelial carcinoma • other solid tumours	olaparib + AZD6738	AZD6738	–	• ORR* • composite prostate cancer • patient response • rate ORR for other solid tumours	• DOR • PFS • AEs
NCT04548752	II	metastatic PC	olaparib + pembrolizumab	olaparib	germline mutation in *BRCA 1/2*	PFS	• AEs • OS • ORR**
NCT04493060	II	metastatic PDAC pancreatic cancer	niraparib + dostarlimab	–	• *BRCA1/2* • *PALB2*	DCR – 12 weeks	• ORR* • time to next treatment • OS • PFS and AEs
NCT04673448	I	• PC • breast cancer • ovarian cancer • fallopian tube or primary peritoneal cancer	niraparib + dostarlimab	–	mutation in *BRCA1* or *BRCA2*	best objective response	• Aes • PFS • DOR • DCR • OS
NCT03404960 (Parpvax)	I/II	PC after platinum-based therapy	1 : niraparib + nivolumab	niraparib + ipilimumab	–	PFS	• the proportion of tumours with HRD, ORR*, DOR, OS, AEs • Immune activation prior/ during treatment
NCT03337087	I/II	metastatic PC	rucaparib + irinotecan liposome + leucovorin + fluorouracil	–	selected (*BRCA1* or *BRCA2* or *PALB2* mutation) and unselected	• number of participants with dose-limiting toxicities • objective response • best response rate	• DCR • OS • PFS • AE
NCT02890355	II	metastatic PC	veliparib + mFOLFIRI	FOLFIRI	–	OS	• AEs • PFS • ORR* • DCR
NCT01585805	II	• metastatic PC • recurrent PC • stage III PC	1: veliparib + gemcitabine + cisplatin 2: veliparib	gemcitabine + cisplatin	*BRCA1/2* or *PALB2* mutation	• the optimal dose of drugs • the response rate to gemcitabine hydrochloride and cisplatin with versus without veliparib • response rate of single-agent veliparib	• PFS • Aes • DCR • OS
NCT00576654	I	metastatic tumours or tumours that cannot be removed by surgery	veliparib + irinotecan	–		• optimal biologic dose • maximum administered dose of study drugs • maximally tolerated dose • recommended phase II dose	• AE • tumour response
NCT04228601	Ib/II	advanced PC	fluzoparib + mFOLFIRINOX	placebo + mFOLFIRI-NOX	mutation in germline *BRCA1/2* or *PALB2*	• number of participants with a dose limited toxicity • maximum tolerated dose • ORR*	• AEs • DCR • OS • PFS

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Table IV. cont. Ongoing clinical trials with PARPi in polytherapy

Name of the study	Phase	Tumour type	Experimental arm	Control arm	Mutational status	Primary outcome measures	Main secondary outcome measures
NCT04644068 (PETRA)	I	PC ovarian cancer breast cancer prostate cancer	AZD5305	AZD5305 + paclitaxel AZD5305 + carboplatin with or without paclitaxel	–	• the number of subjects with adverse events/ serious adverse events • the number of subjects with dose-limiting toxicity	• ORR* • PFS
NCT04503265	I/II	• PC • advanced malignant neoplasm • breast cancer • ovarian cancer • homologous recombination deficiency • prostate cancer	AMXI-5001	–	–	maximum-tolerated dose	recommended phase 2 dose

AE – adverse events; DCR – disease control rate; DOR – duration of response; HRD – homologous recombination deficits; ORR* – objective response rate; ORR** – overall response rate; OS – overall survival; PC – pancreatic cancer; PFS – progression free survival

The results of clinical trials in patients with PC

Currently, the PARPi (olaparib, niraparib, rucaparib and talazoparib) are being tested in monotherapy (tab. III) and polytherapy (tab. IV) on different stages of PC, however, the results of clinical trials are limited. Olaparib remains the most studied drug.

The NCT01078662, phase II trial assessed the efficacy of olaparib in 298 patients with many solid tumours, including PC. 23 patients with PC were enrolled. 74% of them had the *BRCA2* mutation. The primary outcome measure was the tumour response rate. The main secondary outcome measure was the objective response rate, progression-free survival (PFS) and overall survival (OS). Eligible patients had a deleterious or suspected deleterious germline *BRCA* mutation. The tumour response rate in the PC was 21.7% (5–23; 95% CI: 7.5–43.7). Stable disease (≥8 weeks) was observed in 35% (95% CI: 16.4–57.3) of PC patients. The median PFS was 4.6 months. The mOS was 9.8 months. The most common adverse event involved fatigue, nausea and vomiting [63]. Olaparib is also studied in phase II trials in U.S and Israel (NCT02677038, NCT02511223) among 32 patients with metastatic PC and the BRCAness phenotype but without the germline *BRCA1/2* mutation, who received at least one prior therapy. The antitumour activity was seen only in platinum-sensitive patients. The median PFS varies between 14 weeks (range: 5.7–40 weeks) in the Israel part of the study and 24.7 weeks (range: 3.9–41.1 weeks) in the U.S. group [64].

The POLO, a randomized, placebo-controlled phase III trial (NCT02184195), evaluated the role of olaparib as a maintained treatment among 154 enrolled patients with metastatic PC and deleterious/suspected deleterious germline *BRCA1/2* mutation that had not progressed within 16 weeks during the first-line platinum-based chemotherapy (mainly folfirinox). The patients were divided into two groups, the first was given olaparib 300 mg twice a day (n = 92), the second received a placebo (n = 62). The primary endpoint measure was PFS. The main secondary endpoint measure was the OS, time from randomization to the second progression, safety and tolerability. Initially, it was published that olaparib treatment significantly prolonged PFS in comparison to the placebo (7.4 *vs.* 3.8 months; HR = 0.53, p = 0.0038). Recently, on the ASCO Gastrointestinal Cancers Symposium 2021, the newest result data were shown. The OS analysis shows that the OS for the olaparib group was 19 *vs.* 19.2 months for placebo, which failed to be statistically significant (HR: 0.83; p = 0.3487), however, 33.9% of patients who received PARPi survived 3 years *vs.* 17.8% in the placebo group. The most common (≥15%) adverse events in the olaparib group across all grades were nausea, fatigue and diarrhoea. Anemia was the most common AE grade 3 in the study group [14, 65].

The NCT03140670 phase II study is evaluating Rucaparib among patients with metastatic or locally advanced PC and germline, somatic *BRCA1/2*, or *PALB2* mutation. The primary outcome measure is the number of adverse events. The initial results showed that the median PFS was 9.1 months and the ORR of 36.8% [66].

Veliparib was studied, in phase II trials in patients with germline *BRCA1/2* or the *PALB2* mutation and stage III and IV PC. The enrolled patients were treated with 1–2 previous chemotherapy regimen. The response rate was not confirmed. The mPFS was 1.7 ms (95% CI: 1.57–1.83) and mOS was 3.1 ms [67].

The results of clinical studies with drugs other than olaparib are limited. The currently ongoing clinical trials try to determine the biomarkers, the role of genes other than *BRCA* mutated genes and proper sequention of treatment. Among them, one of the most interesting studies is the APOLLO trial (NCT04858334) a phase II, randomized trial that determines the RFS benefit from the maintenance of olaparib therapy following chemotherapy in patients with resected PC and a pathogenic germline or somatic *BRCA1/2, PALB2* mutation.

The LODESTAR, a phase II study (NCT04171700) is evaluating the rucaparib in patients with solid tumours and with deleterious mutations in HRR genes. Patients enrolled to the study had solid tumors with the *BRCA1/2, PALB2, RAD51C, RAD51D, BARD1, BRIP1, FANC, NBN, RAD51*, or *RAD51B* mutation. The primary outcome measure is the best overall response rate. Niraparib is also being studied in a phase II trial (NCT03601923) among patients with the *BRCA1, BRCA2, PALB2, CHEK2*, or *ATM* mutation and advanced PC that is not curable with standard approaches. Talazoparib in monotherapy is studied in two clinical trials. The NCT04550494 trial is the II phase trial that evaluates the pharmacodynamic of PARPi in patients with advanced cancers and mutations in DDR genes. The NCT01286987 trials are a phase I study that evaluates the number of participants with objective response among patients with advanced or recurrent tumours.

PARPi are also being tested in polytherapy with other drugs. It has been hypothesized that combined therapy, especially with chemotherapy, may provide a synergistic therapeutic strategy for patients with PC. The rationale of this combination with a platinum is based on e.g. increased DNA damage by chemotherapy [68]. Initial results come from a phase I trial which assessed the combination of veliparib, gemcitabine and cisplatinin patients with *BRCA1/2* mutated and wild-type PC. The response rate within the *BRCA* mutated cohort was 77.8%. The mOS of patients with *BRCA1/2*-mutated PC and patients with wild-type PC was 23,3 months and 11 months respectively [69]. These promising results led to a phase II, randomized trial. Patients with *BRCA1/2* or *PALB2*-mutated PC were treated with gemcitabine and cisplatin chemotherapy with or without veliparib. The authors found non-significant benefit in the response rate between these two groups (74.1% in arm with veliparib *vs.* 65.2% in chemotherapy arm; $p = 0.55$) [70]. The trials did not show a survival benefit in mPFS (10.1 months for arm with veliparib (95% CI: 6.7–11.5 months) *vs*. 9.7 months for chemotherapy (95% CI: 4.2–13.6 months; $p = 0.73$). Median OS for veliparib and chemotherapy cohort was 15.5 months (95% CI: 12.2–24.3 months) *vs.* 16.4 months for chemotherapy (95% CI: 11.7–23.4 months; $p = 0.6$).

Currently, there are more clinical trials testing PARPi with chemotherapy mainly based on irinotecan-based chemotherapy regimens like (NCT03337087, NCT02890355, NCT00576654, NCT04228601) and cisplatin (NCT01585805). The PARPi are being tested with targeted therapy like cediranib (inhibitor of vascular endothelial growth factor receptor tyrosine kinases; NCT02498613), AZD6738 (ATR kinase inhibitor; NCT03682289), immunotherapy: pembrolizumab (anti-PD1 inhibitor; NCT04548752), dostarlimab (anti-PD1 inhibitor; NCT04493060, NCT04673448), nivolumab (anti-PD1 inhibitor; NCT03404960), ipilimumab (anti-CTLA4; NCT03404960). In addition, the new PARPi are being tested like AMXI-5001, an orally available dual PARP and microtubule polymerization inhibitor (NCT04503265), AZD5305 (NCT04644068) or NMS-03305293 (NCT04182516).

Conclusions

Pancreatic cancer remains one of the deadliest neoplasms with poor survival rates. There is a high need for new therapeutic regimens which improve the clinical outcomes of patients. In recent years, thanks to a deeper understanding of the molecular and genetic landscape of PC, PARPi has also emerged as a novel class of targeted therapy for patients with PC.

PARPi is a new class of drugs based on gene profiling that is currently being studied in PC. Many clinical trials are ongoing to determine the role of drugs in monotherapy and polytherapy. Despite that, the POLO trial did not show that olaparib increases the OS, yet many questions remain regarding the genetic status, role of other HRR genes in PC treatment and sequential treatment strategy. The new direction in PC treatment is signalling pathway inhibitors, immunotherapy agents, drugs targeting the metabolism of tumours and drugs targeting the tumour microenvironment, which could be studied as polytherapy with PARPi [71]. A better understanding of the action and responses at the molecular level of PC cells and the implementation of routine genetic testing in patients have the potential to reveal novel treatment opportunities and thus may broaden the treatment for patients with actionable aberrations [71]. NCCN recommends gene profiling for patients with locally advanced/metastatic PC. The testing should be performed to identify fusions (*ALK* [anaplastic lymphoma kinase], *NRG1* [neuregulin1], *NTRK* [neurotrophic receptor tyrosine kinase 1], *ROS1* [c-Ros Oncogene 1]), mutations (*BRAF, BRCA1/2, HER2* [human epidermal growth factor receptor 2], *KRAS, PALB2*), and MMR deficiency [72]. The recommended material for study is the tumour tissue or, if not available, the cell-free DNA. The preferred technique includes immunohistochemistry, polymerase chain reaction, or next-generation sequencing. Molecular tumour profiling is the future of personalized therapy in pancreatic cancer treatment, which may finally improve the survival rates of patients.

Conflict of interest: none declared

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Consensus on methods of development of clinical practice guidelines in oncology under the auspices of Maria Sklodowska-Curie National Research Institute of Oncology and the Agency for Health Technology Assessment and Tariff System

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Introduction. As the changes leading to improvement of cancer care in Poland have shown the need to introduce clinical practice guidelines into the health care system, it has become clear that no methodological standard of the process for guidelines preparation has been established so far. The following process aims to present a unified and comprehensive clinical practice guidelines (CPGs) development methodology.

Materials and methods. A review of globally recognised methods used by guideline development groups was prepared, informing the discussion during three plenary meetings and extensive consultations in writing. The resulting document was unanimously approved by a group of 24 methodologists and clinical experts, and has been formally recognized as a standard for CPGs development by the management of the National Institute of Oncology and the Agency for Health Technology Assessment and Tariff System.

Results. Within the process, 43 recommendations were formulated to create unified and comprehensive rules for guideline development within the Polish healthcare system.

Conclusions. The presented methods are consistent with the globally recognized tools and methods of guideline development, such as GRADE and ADAPTE, and follow quality criteria described by AGREE II. The process supports the development of high-quality guidelines within a resource-constrained setting by allowing to choose between adoption, adaptation, or *de novo* development of either the whole document of guidelines or particular recommendations.

Key words: practice guidelines, oncology, guidelines development, consensus

Introduction

Cancer is one of the leading causes of morbidity and mortality worldwide, with an estimated 19.3 million new cancer cases and almost 10.0 million cancer deaths in 2020 [1]. In Europe, the total economic toll from cancer was €199 billion in 2018 [2]. Thus, it is crucial to provide sufficient expert guidance and resources to address this burden, especially in resource-constrained settings. Worldwide, attempts have been made to help improve access to cancer control, inter alia, by developing clinical practice guidelines (CPGs).

In modern medicine, CPGs play a decisive role in both facilitating the decisions made in specific clinical situations, and influencing the effectiveness and quality of diagnosis and therapy. They constitute a synthesis of the most current, well-founded research that is aimed at identifying the most efficient and safest modes of operating in clinical situations. The CPGs are usually developed by scientific societies, non-governmental organizations as well as governmental institutions.

High-quality guidelines should be based on a transparent process of development and assessment of recommendations, as well as hold the logical connection between alternative therapeutic options and health results, and an appraisal of quality of evidence and strength of recommendations [3, 4]. Thus ensuring the process is systematized, consistent with specific quality criteria, and based on a systematic review of scientific literature, as well as on an assessment of quality and selection of evidence as a basis for the development of recommendations [5].

At the same time, the multitude of organisations that are engaged in guideline production in Poland make it difficult to compare the quality and rigour of CPGs development, as so far no methodological standard of the process has been established. Taking that into consideration and the necessity to introduce a system of quality management into Polish healthcare, the need arose to create a unified and comprehensive guideline development methodology.

Materials and methods

The process was initiated to support the Maria Sklodowska-Curie National Research Institute of Oncology (MSCI) in its statutory activities regarding developing oncology guidelines. It aims to propose the recommended pathway for CPGs development and their effective incorporation into the Polish healthcare system. The process itself is based on the expertise of the clinicians experienced in CPG development, and a review prepared by the Agency for Health Technology Assessment and Tariff System (AOTMiT).

In light of this and in order to propose the best methods, a review of the key solutions for guideline development was prepared by the AOTMiT [6]. The analysis allowed to indicate key areas in the guideline development process, as well as the methods most frequently used by guideline development groups. This served as a basis for further discussion which comprised three on-line meetings and several series of consultations via e-mail. During the meetings, methods for addressing key areas of CPGs development were discussed – both those employed globally by societies producing oncology guidelines, and those recommended by recognized methodological tools and documents (i.e. GRADE, ADAPTE). These allowed the experts to choose – in a series of unanimous votes – solutions in each area best suited to the target conditions.

These decisions allowed for a formulation of the following methodology, which will serve as the basis for the future development of clinical practice guidelines by the National Institute of Oncology. The results of this process were unanimously approved by experts and were formally recognized as a standard by the management of both the National Institute of Oncology and the Agency for Health Technology Assessment and Tariff

System. The presented methods of guideline development respect the principles of evidence-based medicine for guideline development and take into consideration the available resources and organisational context to ensure relevance for local practice. It is designed to transparently communicate the means and solutions used to produce clinical practice guidelines, their adoptions or adaptations. Topic selection within the process is based on health priorities indicated by the Polish Ministry of Health, scientific societies or other institutions depending on the circumstances.

Methods of guideline development

Topic selection

The objective of the guideline should be described in detail including:

- clinical state or health problem,
- population,
- intent (i.e., prevention, screening, diagnosis, treatment, etc.),
- expected benefit or outcome,
- target users.

Guideline development group

Guidelines are developed by an expert group.

- The group is led by a chair appointed by the institution initiating the guideline development process.
- The expert group consists of clinical experts representing fields of medicine relevant to the topic of the guidelines. The chair is responsible for ensuring that all relevant medical specialisations and professions are included.
- The expert group identifies all appropriate stakeholders. If justified, the stakeholders, especially patient representatives, are invited to participate in the work.
- If necessary, EBM analysts are to participate in the process.
- The chair or a designated editor is responsible for editing the document.
- Developed recommendations are subject to approval by the expert group proceeding in full composition of guidelines.
- For each member of the guideline development group, the following information has to be published:
  - discipline/content expertise,
  - institutional affiliation(s),
  - role in the development process, especially the tasks described below.

Conflict of interest

- Conflicting interests are defined as financial or personal involvement, relationship, affiliation or any other activity that could potentially influence the wording of the guidelines. Group members are obliged to disclose all relationships that may constitute a factual or potential conflict of interest.
- Declaration of Interest is to be submitted to the chair using the form provided in the attachment to this document.
- Each group member is obliged to inform other members of any potential or factual conflict of interests that has a bearing upon the developed recommendation.
- The group member suggests how to manage the conflict of interest described above. The possible actions include exclusion from the discussion, exclusion from the consensus or voting or no restrictions at all. The proposed method is submitted for acceptance from other members.
- In case of a substantial conflict of interest, the member is excluded from the process of recommendation development. A substantial conflict of interest is defined as relationships that amount to 20,000 USD (based on NCCN standards) per year in value, not including participation in clinical trials as a research assistant/investigator.
- Information disclosed in the DOI current for the date of finalizing the development process is published as a part of the guidelines and should include the area and institution of conflict.

Criteria for authorship recognition

- The authorship should be ascribed only to persons who fulfil all of the following criteria:
  - substantial contribution in collection, analysis and interpretation of data serving as the basis for the formulation of recommendations;
  - participation in formulation of recommendations or their critical review;
  - final acceptance of the document.

Methods of guideline development

- Guidelines are developed through adoption, adaptation, *de novo* development or a combination of these methods.
- Choice of the method depends on: guideline topic, availability of current high quality guidelines and available resources.
- The choice of the method is made by the expert group.
- The key health question(s) serving as the basis for the recommendations should be specific, preferably in PICO format.
- If either the whole guideline or particular recommendations are developed *de novo*, the relevant body of evidence should be gathered in a systematic review of literature.
- In case of adoption or adaptation of the whole guideline or particular recommendations, the process should be held in compliance with ADAPTE [7] or GRADE-ADOLOPMENT [8] tools, or the methods designated by the authors of the source document.

Formulating and accepting recommendations

- Recommendations are formulated based on the available evidence, taking into account health benefits, side effects and the risk of the intervention.

- The strengths and limitations of the body of evidence should be clearly described in the context of the recommendation it refers to.
- The process aims to achieve unanimous acceptance of the wording of the recommendations.
- If available evidence is limited, inconsistent, of low quality, does not directly concern the target population, or in other justified situations, the recommendation is formulated through formal consensus.
- The modified Delphi method is the preferred consensus technique, involving the following steps:
  - systematic review of evidence for the given health problem,
  - formulation of draft recommendation,
  - collection and summary of group members' appraisal and opinions,
  - a meeting to discuss the results and establish the final wording of the recommendation and level of consensus.
- High level of consensus is considered to have been reached at 85% agreement, and a moderate level of at least 50% (but less than 85%) agreement. Agreement lower than 50% is recognised as a lack of consensus and the recommendation is not to be published.

Quality of evidence and strength of recommendation

- The quality of evidence describes the quality of the overall evidence gathered on the clinical profile of the intervention in relation to the PICO question serving as the basis of the recommendation. It defines the level of certainty that the available scientific evidence reflects the true dimensions and direction of effects.
- The quality of evidence is ascribed to every recommendation in accordance with the grading system presented in table I.
- The strength of recommendation defines the degree of conviction that the content of the recommendation should be considered in clinical practice taken into account the conditions of the target healthcare system. The strength of recommendations is a derivative of i.a. quality of evidence, absolute and relative strength of intervention and the level of consensus with regard to implementation in clinical practice.
- The strength of recommendation is ascribed to every recommendation in accordance with the grading system presented in table II.

Presentation of recommendations

- In order to ensure their unambiguous interpretation, each recommendation should provide a clear and precise description of the population group, clinical description, intervention being recommended, alternative approach(es), and context for which they are intended.

Table I. Quality of evidence

Quality of evidence	
I	evidence from at least one large randomised, controlled trial of good methodological quality (low potential for bias) or meta-analyses well-constructed randomised trials without significant heterogeneity
II	small randomised trials or large randomised trials with a suspicion of bias (lower methodological quality) or meta-analyses of such trials or trials with demonstrated significant heterogeneity
III	prospective cohort studies
IV	retrospective cohort studies or case-control studies
V	studies without a control group, case reports, expert opinions

Source: The ESMO Guidelines Committee. (2021). *Standard Operating Procedures (SOPs) for Authors and templates for ESMO Clinical Practice Guidelines (CPGs) and ESMO-MCBS Scores* [9]

Table II. Strength of recommendation

Strength of recommendation	
1	recommendation based on high-quality evidence and a uniform or high-level consensus among the expert group
2A	recommendation based on lower-level evidence and a uniform or high-level consensus among the expert group
2B	recommendation based on lower-level evidence and a moderate-level consensus among the expert group
3[1]	recommendation based on any level of evidence to which the expert group could not reach consensus

[1] Category 3 was introduced to ensure compliance with NCCN guidelines and should be used only in case of NCCN guidelines adoption/adaptation

Source: Own compilation based on The National Comprehensive Cancer Network [10]

- The recommendations should use standardized wording to maintain consistency throughout the guideline.
- Remarks that describe the context, feasibility and applicability of the recommendation should hold an explicit link to the recommendation it refers to.
- Recommendations are presented in a clear form that is easy to follow. For example, they can be numbered, gathered in thematic sections or a summary section, or, optionally, presented as flow charts (preferably using BPMN2).

Review and quality assessment

- The final draft of guidelines is to be reviewed by all stakeholders mentioned in point: the expert group identifies all appropriate stakeholders. If justified, the stakeholders, especially patient representatives, are invited to participate in the works.
- Quality assessment of guidelines is held using the AGREE II (Appraisal of Guidelines for Research and Evaluation) [4].
- Guidelines should undergo an external peer review by at least two independent reviewers. If the document is to be published in a peer-reviewed journal, this review can substitute for the external peer review.

- The results of reviews and quality assessment are discussed by the guideline development group. The authors should examine every point and indicate any changes in the document that arise from the process, or if no changes are made, they justify the decision.

Updating the guidelines

- The expert group is responsible for constant monitoring whether the guideline needs to be updated.
- If justified, particular recommendations are updated, especially when new significant evidence is available, changes in the health care context take place, or a justified motion from the stakeholders is submitted.
- Formal assessment of guideline validity is held every two years.

Glossary of key terms

- **The quality of evidence for a single study** refers to the impact of methodological structure of a clinical trial upon uncertainty of estimation of intervention results for a specific endpoint in a specific population in a single study [11].
- **The quality of evidence** describes the quality of the overall evidence gathered on the clinical profile of the intervention in relation to the defined endpoint. It defines the level of certainty that the available scientific evidence reflects the true dimensions and direction of effects in the context of the conditions of the target healthcare system. It is also referred to as strength of evidence, trust in estimations, certainty of evidence, or level of evidence, as well as level of strength of evidence [11].
- **The strength of intervention** refers to the effectiveness of the intervention; it illustrates the magnitude of achievable effect of the new intervention in comparison to other available options in the population subject to the recommendation [11].
- **The strength of recommendation** defines the degree of conviction that the content of the recommendation should be considered in clinical practice taking into account the conditions of the target healthcare system. It is a derivative of quality of evidence, absolute and relative strength of intervention and the degree of consensus [11].

Discussion

The approach established within the process allows the development of high-quality guidelines considering the available resources and target healthcare settings, by allowing to choose between adoption, adaptation, as well as *de novo* development of either the whole guidelines document or particular recommendations.

The suggested process has a number of strengths:

1. It is consistent with recognized tools and methods of guideline development.
2. It is flexible in allowing for the use of different guideline development frameworks depending on the subject and available resources. Thus, existing evidence syntheses can be used, if available, avoiding the necessity of conducting full systematic reviews. At the same time, it helps to identify gaps in knowledge, which might necessitate a systematic review.
3. It allows to build locally contextualized recommendations by involving local experts and stakeholders to ensure that the recommendations address local needs and health care system structure.

While developing the presented approach, the authors sought to ensure that the methods comply with international standards as far as possible within the resources. While there are a number of published standards for guideline development methodology, AGREE II [4] is the most recognized and evidence-based of these [12]. Although the presented Guideline Methodology aims to be consistent with AGREE II, it needs to be noted that not all AGREE II items lie within the scope of NIO's statutory activities; that said, these items (or the reasons for not providing the appropriate data) should still be addressed in the clinical practice guidelines developed within the process (tab. III).

Conclusions

The presented Methods of Guideline Development were produced in an attempt to introduce a unified and transparent set of methods of guideline development across each branch of medicine (at least) and, hence, to tackle the uncertainties that arise with regard to the diversity of published standards for guideline development methodology. The suggested approach allows to develop high-quality guidelines within a resource-constrained setting, by allowing to choose between adoption, adaptation, or *de novo* development of either the whole document of guidelines or particular recommendations. At the same time, it is consistent with the recognized tools and methods of guideline development, such as Grading of Recommendations Assessment, Development and Evaluation (GRADE) [13] and ADAPTE [7], and follows key quality criteria described by GIN-McMaster [14] and AGREE II [4].

Conflict of interest: none declared

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Table III. The sections in presented guideline methodology that address AGREE II items

AGREE II items		NIO guideline methodology
Scope and purpose		
1	the overall objective(s) of the guideline is (are) specifically described	1. topic selection
2	the health question(s) covered by the guideline is (are) specifically described	4. methods of guideline development
3	the population (patients, public, etc.) to whom the guideline is meant to apply is specifically described	1. topic selection
Stakeholder involvement		
4	the guideline development group includes individuals from all relevant professional groups	1. guideline development group
5	the views and preferences of the target population (patients, public, etc.) have been sought	1. guideline development group 8. review and quality assessment
6	the target users of the guideline are clearly defined	1. topic selection
Rigour of development		
7	systematic methods were used to search for evidence	4. methods of guideline development
8	the criteria for selecting the evidence are clearly described	4. methods of guideline development
9	the strengths and limitations of the body of evidence are clearly described	5. formulating and accepting recommendations
10	the methods for formulating the recommendations are clearly described	5. formulating and accepting recommendations
11	the health benefits, side effects and risks have been considered in formulating the recommendations	5. formulating and accepting recommendations
12	there is an explicit link between the recommendations and the supporting evidence	5. formulating and accepting recommendations
13	the guideline has been externally reviewed by experts prior to its publication	8. review and quality assessment
14	a procedure for updating the guideline is provided	9. updating the guidelines
Clarity of presentation		
15	the recommendations are specific and unambiguous	7. presentation of recommendations
16	the different options for management of the condition or health issue are clearly presented	7. presentation of recommendations
17	key recommendations are easily identifiable	7. presentation of recommendations
Applicability		
18	the guideline describes facilitators and barriers to its application	5. formulating and accepting recommendations
19	the guideline provides advice and/or tools on how the recommendations can be put into practice	not applicable
20	the potential resource implications of applying the recommendations have been considered	5. formulating and accepting recommendations
21	the guideline presents monitoring and/or auditing criteria	not applicable
Editorial independence		
22	the views of the funding body have not influenced the content of the guideline	not applicable
23	competing interests of guideline development group members have been recorded and addressed	2. conflict of interest

Note: Wherever "not applicable" is used, it is understood as not within the scope of NIO's statutory activities. Particular tasks associated with these quality items are held by other institutions within the Polish healthcare system

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Melanoma metastases to the intestines – presentation and management

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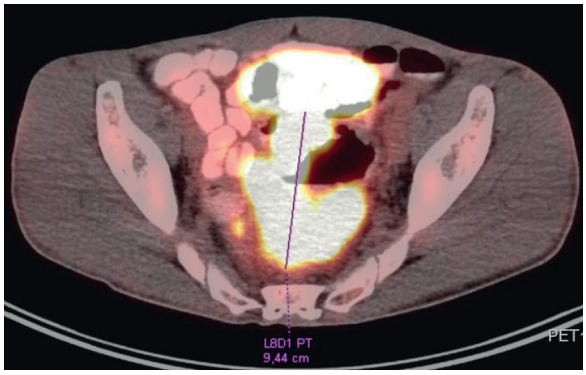

Figure 1. A 49-year-old male with melanoma (pT1N0M1c); a PET scan, a transverse view – a rectosigmoid tumor involving the rectovesical space, 94 × 71 × 67 mm (AP × TR × CC), standardized uptake value (SUV): 11.1

A 49-year-old male, in follow-up due to melanoma on the back (Clark III, Breslow 0.8 mm – pT1N0), 4.5 years after a re-excision with a sentinel lymph node biopsy, presented with a cramping, epigastric pain, nausea, vomiting, and 10 kg weight loss. A PET scan revealed a mass in pelvis (fig. 1). The patient underwent sigmoidectomy and resection of infiltrated loops of the small bowel with adjacent mesentery, followed by a stapled side-to-side ileo-ileal and end-to-end colorectal anastomosis. The pathological report confirmed a metastatic melanoma of the small intestine, infiltrating the sigmoid colon and involving mesenteric lymph nodes (8/20; 0/20 mesorectal LN); *BRAF*(+). The patient received BRAF/MEK inhibitors and anti-PD-L1 immunotherapy (vemurafemib+cobimetinib±atezolizumab). Patient has no sign of disease (9 years after first diagnosis, 4 years after laparotomy). Melanoma may metastasize to the lymph nodes, skin, lungs and pleura, brain, liver, bones, adrenal glands, and gastrointestinal tract. Metastases to the small bowel are rare (1–5%), yet melanoma is the malignancy that most frequently metastasizes to the small intestine (1/3 of all cases). Patients with a newly diagnosed locally advanced melanoma (T4) should undergo an abdominal/pelvic CT to exclude metastases. Melanoma patients who experience abdominal pain and/or distension, nausea/vomiting, hematochezia/melena should be reevaluated with CT/MRI/PET. Patients with isolated bowel metastases or presenting with bowel obstruction, severe bleeding, perforation should be reffered to surgery with metastasectomy (including regional lymph nodes). Adjuvant systemic therapy is advised, with a regimen depending on a *BRAF* gene mutation. Despite intestinal metastases, a prolonged survival is possible with appropriate management [1, 2].

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Hepatocellular cancer and colorectal liver metastasis treatment in the older population

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More than 60% of patients with primary and secondary liver tumors are older than 65 years. Thanks to improvements in radiological staging, anesthesia, surgical technique, and perioperative care it is possible to offer complex liver surgery to older patients. However, chronological age or functional status alone should not be a contraindication for multimodal radical treatment in older patients. Fit patients, according to the Comprehensive Geriatric Assessment, should be qualified for the same treatment as younger patients to ensure the same outcomes. Prefrail patients should undergo prehabilitation, and be reevaluated. Frail patients should be discussed in an oncogeriatric meeting. All patients with liver malignant tumors must be operated on in high-volume hospitals by an experienced surgeon. The introduction of parenchymal sparing surgery (instead of a major resection) in combination with other treatment tools, minimal invasive techniques, and enhanced postoperative recovery demonstrated being beneficial for older patients. In particular, frail, older patients can benefit from the wide variety of treatment options.

Key words: hepatocellular cancer, colorectal liver metastasis, older population, liver resection

The key components of successful oncologic liver surgery are: the ability to achieve an R0 resection, to maintain an appropriate vasculature and biliary system and to leave a sufficient functional liver parenchyma [1]. Thanks to improvements in radiological staging, anesthesia, surgical technique (understanding of segmental liver anatomy, parenchymal preserving surgery, bleeding control), and perioperative care it was possible to offer complex liver surgery to older patients. This is particularly important because, more than 60% of patients with primary and secondary liver tumors are older than 65 years at the moment of diagnosis [2].

At present, there are no treatment guidelines dedicated to older patients. The main reason for this situation is still the underrepresentation of older patients in trials regarding liver resection. In the majority of the published studies, only 15–20% of patients were older than 70 years [2]. Therefore, the extrapolation of such results on the geriatric population can lead to inappropriate treatment decisions.

Normal aging of the liver

Several age-related changes can be observed in liver physiology. The most important are: a decrease in liver weight and volume (up to 25%), decrease in the hepatic blood flow (up to 40%), an increase in the hepatic dense body compartment, shifts in the expression of a variety of proteins, and a decrease in bile flow and bile acid secretion [3–8]. These changes influence the liver's metabolic function, regeneration capacity, and immunity [3–8], which, in turn, may result in an increased risk

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of postoperative liver failure, drug-induced liver damage, and sepsis [3–10]. 25–30% of a total healthy liver volume needs to be preserved after the resection. However, in the case of patients with liver cirrhosis, steatosis or fibrosis, a larger remnant of the organ needs to be intact. Despite preoperative evaluations, liver failure occurs in up to 5% patients in the postoperative period, particularly often in older patients [11]. Tzend et al. did not observe a significant difference in the preoperative LiMAx liver function between young and older patients. However, liver regeneration is significantly different in older population in the early period. Age was inversely correlated with liver regeneration potential during the first postoperative week, without any difference between young and older patients after one month [12]. None of the studies analyzed the biologic age.

Preoperative assessment and treatment decisions

As was mentioned in our previous publications, the population of older patients is very heterogeneous in terms of co-morbidity, physical reserve, cognitive function, and social support. Chronological age alone is a poor predictor of cancer treatment outcomes and toxicities [13]. Current routine pre-operative assessments cannot adequately identify patients at risk. Many older adults have unidentified, uncommunicated, and therefore unaddressed aging-related conditions that are associated with poorer outcomes. As a result, the Comprehensive Geriatric Assessment (CGA) was introduced to help determine the primary status of the older patient, to diagnose frailty syndrome, and to identify how to optimize the patient's condition before the start of treatment [14–16]. Therefore, more and more organisations, including the International Society of Geriatric Oncology, the National Comprehensive Network, the European Organisation for the Research and Treatment of Cancer, the American College of Surgeons National Surgical Quality Improvement Program, and the American Geriatric Society have called for the routine use of the Geriatric Assessment.

Rostoft et al. analyzed the literature regarding the role of the CGA in predicting the outcome in hepatobiliary and pancreatic surgery among older patients with cancer. It was concluded that although there are not many studies, frailty and elements from the CGA are significantly associated with negative short- and long-term treatment outcomes in older patients with hepatobiliary and pancreatic cancers [17].

Clinical characteristics of older HCC patients

Hepatocellular carcinoma (HCC) is the primary tumor of the liver with the greatest incidence worldwide. It is the fifth most common neoplasm and the third highest cause of cancer-related mortality [18]. The risk of developing HCC increases with age, reaching the highest incidence in the geriatric population during the seventh decade of life. Moreover, improvements in the treatment of chronic liver disease have caused an increase in the number of potential patients who may develop HCC [19]. In Europe, HCC older patients are more likely to be women and to be infected with HCV, less common with HBV. Moreover, in older patients, HCC develops more commonly in healthy livers [19]

Liver surgery in older patients with HCC

The Barcelona Clinic Liver Cancer (BCLC) stage system is the most used tool for treatment planning in patients with HCC. Based on the characteristics of the tumor, the degree of liver failure and physical condition, patients are stratified into five categories:

- very early (BCLC 0),
- early (BCLC A),
- intermediate (BCLC B),
- advanced (BCLC C),
- terminal (BCLC D).

For the two first stages (BCLC stage 0 and A), there is a wide range of treatment options including liver resection, liver transplantation, and local ablation. In the BCLC B stage, transarterial chemoembolization is usually proposed. In turn, in the BCLC C stage patients are qualified for treatment with Sorafenib. In the terminal stage (BCLC D), the best supportive treatment seems the optimal option [20]. There are also other staging systems. However, none of them is using the comprehensive geriatric assessment caps earlier or any other geriatric scale that allows determining frailty.

Concluding recently published studies on older populations undergoing various liver resections due to HCC, the morbidity and mortality rates ranged from 9% to 51% and from 0% to 7.5%, respectively. In high volume hospitals, there was no difference between younger and older patients in short-term morbidity, mortality, and length of hospital stay [21–23]. The 5-year overall survival rate ranged between 26% and even 75.9% in well-selected older patients. However, surgical treatment was only possible in up to 14% of older patients, compared with the younger group (12–28%) [24–27].

The introduction of parenchymal sparing surgery resulted in a decrease in mortality in older patients compared with older patients undergoing major hepatectomy [28]. In experienced hands, laparoscopic and robotic techniques further reduce surgical stress and improve the outcomes. Reported morbidity ranges were 10–15% and mortality was around 1%, respectively [29]. However, when analyzing the outcomes, various selection bias must be considered. A systematic review and meta-analysis, published in 2019, showed no significant difference in terms of blood loss, transfusions, liver failure, Clavien-Dindo III/IV complications, postoperative mortality, hospital stay, R0 resection, and operative time between younger and older patients undergoing laparoscopic hepatectomy [29]. Moreover, the minimal invasive approach in HCC cirrhotic patients has also the potential to reduce risk of post-operative liver decompensation and morbidities [30, 31]. However, most of the studied patients were evaluated based on chronological

age, comorbidities, and physical function – not on the comprehensive geriatric assessment.

Liver transplant in older patients

Data from the United Network for Organ Sharing and the European Liver Transplant Registry show a significant increase in the number of patients over 70 years with end-stage liver disease who qualified for a liver transplant in the last decade; it was also one of the fastest-growing patient populations [32]. In the 2019 systematic review and meta-analysis, Gavara et al. did observe acceptable short- and long-term results. They also did not find any difference in the risk of complications between young and older patients [33]. Although long-term liver transplant results are very good, older patients are rarely qualified because of their low priority on the list of available organs [32, 33].

Radiofrequency ablation for older HCC patients

The European Association for the Study of the Liver (EASL) guidelines recommends RFA as a standard of care for patients with BCLC stage 0–A, in the case of tumors not suitable for surgical resection [34, 35]. However, in the case of older patients, the results of published studies are inconsistent. Some of them report comparable outcomes between young and older population [36–38]. In turn, others reveal higher complication rates due to patients' comorbidities, use of antiplatelet or anticoagulant drugs, and preoperative low functional levels. The overall survival rates in the older group were significantly lower than those in the younger population and the recurrence-free survival rates were comparable [39].

Transarterial chemoembolization for older HCC patients

The transarterial chemoembolisation (TACE) is a procedure combing the transcatheter delivery of an anticancer drug into the hepatic artery followed by vascular obstruction with embolic agents [40]. Current guidelines recommend TACE as the standard of care for patients with multinodular, asymptomatic tumors without vascular invasion or extrahepatic spread (BCLC stage B tumors). Recent studies showed that the TACE is a safe and effective treatment in older HCC patients. The morbidity rate ranged from 4.5% to 27%, without any significant difference between older and younger patients, including also contrast medium-induced renal dysfunctions [41]. The 3-year and 5-year OS ranged between 14.9–48% and 8.4–33.8%, respectively [42–44].

Immunotherapy for older HCC patients

Sorafenib has shown efficacy in two randomized trials, resulting in a significant 30% improvement in survival of HCC patients [45]. The European Association for the Study of the Liver recommends sorafenib as the preferred treatment for patients with HCC who cannot tolerate potentially more effective therapies, particularly in the case of preserved liver function (Child–Pugh grade A) and advanced tumor stages (BCLC stage C) [46]. In the case of the older population, it turned out to be equally safe among older and younger patients with similar toxicity-related discontinuation rates between these groups [47]. There was also no difference between these groups regarding overall survival and time to treatment failure [48]. After 10 years, another multikinase inhibitor, Lenvatinib, was approved in first-line treatment [49]. Studies have proven its non-inferiority compared with sorafenib in cases of overall survival. Moreover, lenvatinib may have some potential benefits over sorafenib for patients with HBV chronic infection [49]. Atezolizumab plus bevacizumab as first-line treatment is the next treatment possibility in the treatment of advanced HCC [50]. However, we have to wait for further studies including those on the geriatric population.

Concluding, we need well-designed studies on a larger group of older patients using various advances of geriatric oncology. The Comprehensive Geriatric Assessment, evaluation of life expectancy, and analysis of patients' goals should become routine preoperative instruments allowing for better selection of older patients for a tailored treatment. They are proven to correlate much more with the short- and long-term outcomes in comparison to the currently evaluated factors. Therefore, Suda et al. proposed the percent life expectancy (%LE). It is the survival time for each patient divided by the life expectancy. This parameter may evaluate the benefits of a given treatment for older HCC patients. The authors showed that patients aged 80 years or older had the best survival benefit according to the %LE [52].

Moreover, there are currently many unintentional selections bias in most of the studies. The physicians tend to qualify older patients for surgical treatment with a good performance status and preserved liver function. This might favor similar outcomes to those of younger patients. In turn, the chronologically oldest patients are often qualified for non-curative treatment, which might favor poorer prognosis compared with younger patients [51].

Colorectal liver metastasis in older patients

Recent studies have shown that patients aged 70 and more who undergo liver resection for colorectal liver metastases have the possibility to achieve a 5-year survival of 21–44%, with postoperative morbidity and mortality rates of approximately 20–40% and 0–7%, respectively. This is despite patients undergoing more complex treatment for more extensive disease [53–62]. The main reason for the outcome improvement is the introduction of parenchymal sparing liver surgery. It has been shown to be associated with less surgical stress, fewer postoperative complications, non-inferior cancer-related outcomes, and higher feasibility of future resections [63]. There is also a higher rate of R1 resection. However, it is not associated with poorer disease free survival [64, 65]. Therefore, major

resection should be limited only to patients where it is the only curative option.

More and more older patients are getting neoadjuvant chemotherapy with intend to downstage the disease and to converse the disease into resectable. It has been proven that chemotherapy in combination with surgical techniques was not associated with poorer postoperative outcomes in older patients in comparison to younger groups [66]. So, excellent perioperative outcomes can be achieved with morbidity and mortality of 38.2% and 0.3%, respectively, using parenchymal sparing liver surgery, chemotherapy, and ablation. That combination should be used to avoid unnecessary major liver resection [67–71]. Implementation of the ERAS program in the postoperative period may further improve outcomes. A 2015 meta-analysis of randomized controlled trials on the efficacy of the ERAS program in liver surgery showed that this approach significantly reduces post-operative morbidity, length of stay, and accelerates functional recovery [72].

For older patients with unresectable CRLM who meet the eligibility criteria for radioembolization, 90Y-radioembolisation microspheres appear to be effective and well-tolerated, regardless of age. Therefore, the selection of patients for radioembolization should not include chronological age as an exclusion factor [73].

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Phacomatoses, genetic testing for personalisation of clinical management (part 2)

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Von Hippel-Lindau disease and tuberous sclerosis are rare genetic disorders, which belong to the group of phacomatoses. They involve an increased risk of development of multiple cancers, mostly benign ones, which may undergo malignant transformation. Genetic diagnostic including identification of the pathogenic variant of the *VHL* and *TSC1* and *TSC2* genes enables optimisation of patient care and identification of relatives who carry the mutation.

Key words: von Hippel-Lindau disease, VHL, tuberous sclerosis, sclerosis tuberosa complex, *TSC*, phacomatosis

Von Hippel-Lindau disease

Von Hippel-Lindau disease (VHL, OMIM 193300) owes its name to the German ophthalmologist Eugen von Hippel and the Swedish pathologist Arvid Lindau, who, working independently from each other, described in 1904 and 1926 clinical syndromes characterised by the presence of tumours of the retina and the central nervous system [1]. VHL is a genetically determined syndrome, predisposing to development of neoplasms, which is inherited in an autosomal dominant manner with almost full penetration. In about 20% of patients, the mutation occurs *de novo* , but it is passed on by the carrier to their offspring (50% risk of passing the mutation on), and in subsequent generations the course of the disease is more severe, and the symptoms occur earlier, in a process referred to as genetic anticipation [2]. The disease is diagnosed in 1 person per 38–91,000 and the incidence is 1 in 36–45,000 births [1]. The first symptoms appear as early as in the second decade of life, the diagnosis criteria are met in all patients before the age of 70 [1]. If VHL disease is diagnosed, constant patient surveillance is necessary, as it allows early detection of neoplasms and implementation of the optimal therapy. Nevertheless, life expectancy for people with VHL is the shortest among those with other hereditary cancer syndromes [3]. The course of the disease involves development of multiple benign and malignant tumours within the central nervous system (CNS), eye, internal organs, especially kidneys, pancreas, adrenal glands [4].

Hemangioblastomas of the central nervous system are often the first symptom of the disease and occur in 72–75% of patients [1]. They can be located in the cerebellum (*hemangioblastoma cerebelli*), in the medulla oblongata (*hemangioblastoma medullae oblongatae*), and in the spinal cord (*hematoblastoma medullae spinalis*). Depending on their location and size, they lead to a variety of clinical symptoms. The mass effect of intracranial tumours may lead to an increase in intracranial pressure manifested by nausea, vomiting, displacement of brain structures with impaction leading to death. In the case of smaller tumours, there may be focal symptoms, headaches or they may be asymptomatic. Cerebellar location causes balance disturbances, which are also present in the case of the endolymphatic sac tumours (ELST), observed in about 15% of patients with VHL. This tumour is characterised by local

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malignancy, and as it increases in size, it destroys the structures of the inner ear and the temporal bone pyramid. It may also infiltrate cranial nerves (facial and vestibulocochlear nerves). As it grows towards the cerebellum, it causes the pontocerebellar angle syndrome. Typical symptoms include complete or partial hearing loss which occurs in 95–100% of patients, tinnitus in 77% of patients, vestibular balance disorders - 62% of patients and facial nerve palsy – 8% of patients [1, 5]. Treatment of CNS hemangioblastomas, as well as ELSTs is mainly surgical and depends on the location, tumour size, as well as possible infiltration of adjacent anatomical structures.

Retinal hemangioblastoma (retinal capillary hemangioma, hemangioblastoma) is observed in 50–60% of patients with VHL [6]. Ophthalmological examination reveals a sharply delineated orange-red lesion, richly vascularized, with intra- and subretinal exudate. The lesions are located in the peridural part or on the periphery of the retina (upper or lower temporal area). In about 25% of patients, permanent loss of vision occurs, and presence of multiple lesions predisposes to formation of further foci [7].

Patients diagnosed with VHL require constant ophthalmic supervision, including fluorescein angiography (AF – distinguishing nutrient arterioles from drainage veins), ultrasound examination (determination of the tumour diameter, visualization of fluid), optical coherence tomography (determination of the subretinal fluid accumulation site). The treatment includes laser photocoagulation, cryotherapy, photodynamic therapy, and techniques of vitroretinal surgery. Pharmacotherapy involves attempts to administer antagonists of vascular endothelial growth factor (VEGF) in the case of posterior pole hemangiomas [1, 7].

Renal cell carcinoma (RRC) occurs in approximately 30% of patients with VHL. It originates from the renal tubular epithelium, histologically most often it is a clear cell carcinoma, differing from the sporadic form by multifocal manifestation and association with renal cysts. It may be bilateral [1, 8]. Clinical symptoms in the form of a palpable tumour mass, pain in the lumbar region and haematuria occur in patients with large tumours (Virchow's triad). Smaller tumours remain asymptomatic and are detected incidentally in screening imaging studies. Occasionally, the so-called paraneoplastic symptoms occur, with hypercalcemia related to the secretion of PTH-like peptide (PThrP), arterial hypertension caused by production of renin by tumour cells or polyglobulia – resulting from release of erythropoietin [9].

The basic diagnostic tools are computed tomography and magnetic resonance imaging. Histological diagnosis is made after harvesting a tumour fragment by kidney biopsy or during nephrectomy, which is one of the basic therapeutic methods in RRC. Surgical treatment for small tumours (smaller than 3 cm) consists in removing the tumour mass with a healthy kidney margin. In advanced stages, pharmacotherapeutic attempts are made to treat the cancer with tyrosine kinase inhibitors that block angiogenesis and mTOR kinases. Interferon alpha immunotherapy is also used.

Pheochromocytoma occurs in approximately 16% of VHL patients [1]. It is a catecholamine-secreting tumour occurring in VHL, usually benign, affecting mainly adrenal glands, often bilateral. It may also be multifocal. As in the sporadic form, the main clinical symptom is arterial hypertension (paroxysmal or permanent), which may be associated with headaches and increased sweating. Other observed symptoms include paroxysmal paling of the skin, a feeling of anxiety, tremors, cardiac arrhythmias in the form of tachycardia, ventricular accessory contractions, atrial fibrillation or additional ventricular beats, which may cause sudden cardiac death or chronic heart disease – cardiomyopathy with development of pulmonary congestion.

Diagnostic includes testing of free catecholamines or their metabolites (vanillylmandelic acid-VMA, methoxycatecholamines) in the 24-hour urine collection. Methoxycatecholamine can also be measured in the serum. Tumour location is determined with computed tomography or magnetic resonance imaging, or occasionally iodine-labelled metaiodobenzylguanidine (MIBG) scintigraphy, which is especially useful for diagnosing small lesions and metastases [1]. Abdominal ultrasound is also used as a screening method to detect *pheochromocytoma*-type tumours.

Treatment of pheochromocytomas involves surgical resection (total or sparing adrenalectomy) after pharmacological pre-treatment, in which blood pressure and heart rate should be normalised. For this purpose, alpha blockers are employed, as the basic drug (phenoxybenzamine) is used for 2 weeks before the planned surgery. Alpha-blocker therapy can be supplemented with beta-blocking drugs, especially in people with concomitant tachycardia. Beta blockers cannot be used as monotherapy [1]. Patients after surgical resection of a pheochromocytoma require constant supervision to enable early detection of the potential tumour recurrence.

Changes in the pancreas are cysts or benign cystic neoplasms (cystadenomas). They occur in a large group of patients with VHL disease – 72% [1]. They may remain asymptomatic or affect the pancreatic and exocrine capacity due to effected pressure. Pancreatic neuroendocrine tumours (PNET) may also occur in the course of VHL.

The multi-organ manifestation of VHL disease and the multitude of possible clinical symptoms associated with it require multidisciplinary supervision and the selection of treatment according to the type of lesions affecting the individual patient. In general, two basic types of the disease can be distinguished: 1, 2, with the latter including a, b, c subtypes [10]. The diagnostic criteria include a clinical analysis of the patient with a diagnosed coexistence of multiple neoplastic lesions [1]. VHL can be diagnosed in the case of detection of:

- at least two haemangioma-type tumours of the central nervous system (central nervous system hemangioblastomas),

- at least one hemangioblastoma of the central nervous system and one of the neoplastic tumours described below,
- at least one of the tumours described below and a mutation typical of VHL or having a first-degree relative diagnosed with VHL.

Typical symptoms of VHL included in the diagnostic criteria refer to occurrence of:

- a CNS neuroblastoma (including a diagnosed retinal hemangioblastoma),
- endolymphatic sac tumours,
- renal-cell carcinoma,
- pheochromocytoma, paraganglioma (glomus tumour),
- neuroendocrine tumours and / or multiple pancreatic cysts.

In patients with VHL diagnosis confirmed by a genetic test result, the periodic examinations should include:

- at the age of 0–2 years – annual physical and ophthalmological examination,
- from 2 years of age – MRI of the brain and spinal cord twice a year,
- abdominal ultrasound annually, if cysts or tumours are found – computed tomography (CT) examination every 6 months,
- from 20 years of age – annual CT instead of annual ultrasound,
- from the age of 60 – computed tomography in any year in MRI was not performed; if there are no symptoms, MRI every 3–5 years [11].

Genetic background, diagnosis and genetic counselling

Mutations in the *VHL* suppressor gene constitute the molecular background of von Hippel-Lindau syndrome. The *VHL* gene is located on the short arm of chromosome 3 (locus p25.3, MIM * 608537), it consists of three exons (642 nucleotides) and encodes a highly conserved protein. Gene transcript is present in various cell types in many tissues (both in foetal and postnatal life) [12]. Depending on the point of translation initiation, determined by the presence of two methionine (start) codons, two protein isoforms (pVHL) are formed, one consisting of 213 amino acids ($VHLp_{30}$, cytoplasmic expression) and the other consisting of 160 amino acid residues ($VHLp_{19}$, nuclear expression) [13].

VHL protein acts in complexes with various proteins. First of all, it forms the VBC complex with elongin C and the complex of elongin B with kullin-2 and Rbx (binding through the α domain) [14]. Under physiological conditions (normal oxygen concentration), the VBC complex, of the activity of ubiquitin ligase E3, is responsible for ubiquitination of the alpha subunit of hypoxia-inducible factor 1 (HIF1-α), leading to its proteolysis in the proteasome and consequently inhibiting transcription of hypoxia-induced genes [15]. The domain responsible for binding the substrate to the VBC complex is the β domain of pVHL, which binds HIF1-α via hydroxylated proline residues. Under hypoxic conditions, there is no hydroxylation of HIF1-α proline residues and no binding to pVHL [16]. This results in the accumulation of HIF1-α, and consequently, transcription of genes regulated by the HIF1 protein (HIF1-α and HIF1-β heterodimer) is induced, including genes encoding growth factors such as: vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) and transforming growth factor alpha (TGF-α), as well as *EPO* gene encoding erythropoietin. Moreover, the VBC complex also regulates HIF2-α, HIF3-α and atypical protein kinase λ [10, 17, 18].

VHL protein dysfunction results in deregulation in the control of HIF1-α degradation and is associated with a permanently high level of HIF (independent of the oxygen level) which leads to overproduction of VEGF, PDGF and TGF-α. This is the most likely molecular mechanism to explain the excessive abnormal proliferation and angiogenesis in richly vascularized tumours of the VHL spectrum. It has also been shown that the dysfunction of the VBC complex contributes to development of pheochromocytomas as a result of the accumulation of atypical λ protein kinase, which leads to overexpression of the transcription factor B-jun which inhibits apoptosis in nerve crest cells in the adrenal medulla [10, 19].

VHL syndrome is inherited in an autosomal dominant manner and in about 80% of cases the mutation is inherited from one of the parents (Mendelian inheritance, 50% risk of passing the mutation). In the remaining 20% of cases, the mutation occurs *de novo* and in the family there are no people with diagnosed or suspected VHL [13]. Genetic testing is extremely important in terms of developing a preventive care program for the mutation carrier (taking into account the risk of neoplasms from the spectrum of this syndrome) and providing genetic counselling to the entire family. Genetic testing may be targeted to analyse a single gene or a panel of genes associated with phacomatoses, and in the case of an ambiguous phenotype, total or whole genome testing may be considered. The most common types of mutation in the *VHL* gene are missense (approximately 30–60%), intra-gene insertions / deletions, frameshift mutations and splice site mutations, all leading to protein truncation of approximately 20–30%. About 20–40% of the mutations are large deletions, sometimes involving the entire gene [20].

So far, more than 300 pathogenic variants in the *VHL* gene have been identified [17]. Pathogenic variants were found in all three exons. The 167 codon encoding arginine is considered a "mutational hot spot" [21]. The disease is characterised by age-dependent full penetration and variable expression (penetration is assumed to exceed 90% around the age of 65) [13]. Knudson's two-hit hypothesis explains development of VHL. One defective allele is present in all cells (constitutional mutation), and the loss of the second copy (deletion, point mutation, hypermethylation of the promoter sequence) of the gene is a factor that initiates the process of neoplastic transformation [22].

Genotype-phenotype correlations are well studied. Major changes such as whole exon deletion and mutations leading to protein truncation are most often associated with the VHL type 1 phenotype, with retinal and central nervous system haemangiomas, kidney cancer and pancreatic cysts, but no pheochromocytoma. Missense pathogenic changes are associated with type 2 VHL, in which pheochromocytoma is observed. Depending on coexistence of other organ manifestations, type 2 is divided into type 2A (pheochromocytoma, haemangioma, no kidney cancer), 2B (pheochromocytoma, haemangioma, kidney cancer), 2C (pheochromocytoma) [13]. Some researchers suggest also inclusion of 1B type, characterised, like type 1, by no pheochromocytoma and additionally low risk of renal cancer. Type 1B is characteristic of patients who, in addition to the deletion of the *VHL* gene, have a deletion in the *BRK1* gene [13].

Identification of the pathogenic variant of the *VHL* gene allows molecular diagnostics for family members to identify pre-symptomatic carriers of mutation, and then to introduce an appropriate diagnostic and prophylactic surveillance, reducing the need for screening in those who have not inherited the pathogenic variants [23]. Genetic counselling should also take into account the risk of germ cell mosaicism of the parent whose child has a confirmed mutation, if the same change was not found in the parents' examination. Additionally, somatic mosaicism is also possible, signifying that the pathogenic variant is present only in some of the cells of the body. The course of the disease in this case will be milder and if the pathogenic variant is absent in the germ cells, the risk of disease in the offspring is at the level of the population risk of [24].

In Poland, patients with VHL are included in the care program for families of high, hereditary risk of developing malignant neoplasms – Module III – "Prophylaxis and early detection of malignant neoplasms in families with rare hereditary cancer syndromes – retinoblastoma, Von Hippel-Lindau disease (VHL)". The programme guarantees medical procedures concerning identification of patients with VHL based on clinical diagnostic, molecular analysis – genetic testing, and regular patient care including:

- annual medical consultation,
- MRI of the head and spinal cord from the age of 11 (every 1 to 3 years depending on changes present within the CNS),
- abdominal ultrasound (annually)
- abdominal CT (or MRI) (every 2–3 years),
- ophthalmological consultation from the age of 1; fundus examination in the Goldman mirror from the age of 6.

Therefore, it is crucial to identify pre-symptomatic carriers and to introduce screening tests as early as possible. Consequently, it is also justified to perform genetic tests in children from families with the critical mutation to identify the mutation in the *VHL* gene observed in the family. Further, in families with the identified mutation, prenatal and preimplantation tests can be performed, too [21].

Tuberous sclerosis

Tuberous sclerosis (sclerosis tuberosa complex – TSC), also known as Bourneville-Pringle disease, is an autosomal dominant disease with full penetration and variable expression. In about 75% of the cases a TSC mutation arises de novo. The disease is diagnosed in 1 in 10,000 children born, and its frequency in the general population is 1: 6,800–1: 17,300 [25, 26].

Tuberous sclerosis is characterised by formation of hamartomata-type tumours of the skin, central nervous system, kidneys, lungs and heart. The characteristic triad of symptoms includes mental retardation, epilepsy, and Pringle angiofibromas, which appear in early childhood as yellowish-pink papules covering the seborrheic surfaces of the face (nose, medial cheeks, forehead). They occur in almost 90% of patients and their number increases in adolescence. They have an undesirable cosmetic effect, and may spontaneously bleed [26].

Other skin lesions seen in TSC are leaf-shaped discolorations (leaf-shaped leukoderma), often located on the scalp, showing a characteristic discoloured strand of hair growing out of the lesion. "Confetti" stains are observed, too, showing as colourless marks on the extensor surfaces of limbs, shagreen patches in the sacral region of the body or squamous fibromas in the forehead region which occur in about 25% of the patients [27]. Gingival fibromas, similarly to fibromas of the nail folds called Koenen's nodules, appear later, mainly in adults [26].

Kidney symptoms

Angiomyolipoma is a hamartoma- type tumour which occurs in 80% of the patients. It is a benign neoplasm, however, as it enlarges, it may cause spontaneous haemorrhage into the kidney capsule (Wunderlich's syndrome) or its failure, resulting in increased mortality among patients [26]. Negative prognosis is also associated with presence of the renal clear cell carcinoma. Its incidence is higher in TSC patients as compared to the general population. Mutations in the *TSC2* gene increase the risk of polycystic kidney disease in people with TSC [28].

Neurological symptoms

Epilepsy diagnosed in early childhood, often infancy, is a characteristic symptom of TSC which occurs in 79–90% of patients [26]. Behavioural disorders from the autism spectrum, ADHD, and mental retardation are also observed – in about 40% of patients [29]. Some of these disorders are related to structural changes in the brain resulting from formation of hamartoma-type cortical-subcortical tumours subependymal heterotopic / periventricular nodules. Subependymal periventricular nodules may become a starting point for a malignant tumour referred to as a subependymal giant cell astrocytoma which grows in lateral ventricles of the brain and may lead to Monro foramen obstruction, ventricular enlargement, hydrocephalus and death. Diagnosis is based on brain imaging including computed tomography and magnetic resonance imaging,

which also shows white matter heterotypes (white matter linear migration lines) occurring in 20–30% of the patients [30].

Pulmonary symptoms

Lymphangioleiomyomatosis is one of the symptoms of pulmonary TSC, and is caused by the proliferation of smooth muscle cells around the bronchi and small vessels resulting in pulmonary remodelling and cyst formation. The patients' symptoms include cough, dyspnoea and haemoptysis. Lymphangioleiomyomatosis occurs mainly in adult women [25]. In patients with TSC, multifocal micronodular hyperplasia of pneumocytes may also occur, visible in imaging as small nodules [31].

Cardiac symptoms

Rhabdomyomata are lesions which may undergo spontaneous involution. They occur in the youngest children and mostly disappear in the preschool period. In some patients, however, they may lead to cardiac arrhythmias and sometimes to heart failure [32].

Ocular symptoms

Ocular changes occurring in the course of TSC are hamartomatous nodules of the retina, which, despite their multifocal manifestation, do not deteriorate vision in most cases. They are divided into flat lesions, mulberry lesions and mixed type (transitional lesions) [33].

The multiplicity of clinical symptoms and the diverse expression may pose diagnostic difficulties. The currently applicable criteria, which were proposed in 2021 at the Washington conference [34], are helpful in establishing the diagnosis as well as the further treatment of the patient.

The criteria listed below (two major or one major and two minor ones) are required for the diagnosis of the disease.

Major criteria:

- discoloration patches (>3 patches >5 mm in diameter),
- facial angiofibromas (>3) or frontal squamous fibroids (angiofibromas) (>3) or fibrous cephalic plaque,
- periungual fibromas, non-traumatic (ungula fibromas) (>2),
- shagreen patches,
- multiple retinal hamartomas,
- cortical brain tumours (cortical dysplasia),
- subependymal nodules of the brain,
- subependymal giant cell astrocytoma,
- rhabdomyomata of the heart,
- lymphangioleiomyomatosis,
- angiomyolipoma [2].

Minor criteria:

- confetti-type skin lesions,
- multiple dental enamel pits (>3),
- intraoral fibromas (>2),
- retinal achromic patches,
- multiple renal cysts,
- nonrenal hamartomas.

Genetic background, diagnosis and genetic counselling

The genetic background of tuberous sclerosis is constituted by pathogenic variants in the tumour suppressor genes *TSC1* or *TSC2*. The *TSC1* gene is located on the long arm of chromosome 9 (locus q34.13), the longest transcript of the gene consists of 23 exons (the first two are non-coding, and exons 5 and 12 are alternatively spliced), it encodes the hamartin protein. The *TSC2* gene is located on the short arm of chromosome 16 (locus p13.3), the longest transcript consists of 42 exons (non-coding exon 1 and alternatively spliced exons 25 and 31), it encodes tuberin [35]. Hamartin and tuberin form a complex in which hamartin is responsible for stabilisation through the super-helical domain "coiled-coil", additionally interacting with other proteins, while tuberin performs, among others, the function of a GTPase activating protein (GAP) for the small G Rheb protein which regulates / inhibits mTORC1 (mTOR kinase complex 1, mammalian target of rapamycin kinase), controlling protein translation, cell growth and proliferation. The activity of the hamartin-tuberin complex is inhibited by the protein kinases Akt and p38 MAPK [36].

Dysfunction of the hamartin-tuberin complex contributes to the lack of control over many signalling pathways, including the mTOR pathway, leading to its constant activity, thus leading to uncontrolled cell division and proliferation, and further to development of benign hamartoma-type tumours in many organs [37].

So far, about 650 pathogenic variants present in *TSC1* have been identified, the most common changes leading to protein truncation. The changes are scattered throughout the gene and no "hot spots" were found with the exception of exon 15, where several repetitive mutations were noted. Missense variants are rare and occur mainly at the N-terminus coding of the protein, thus contributing to its destabilisation [38]. About 1,900 pathogenic variants are known in the *TSC2* gene. They are distributed throughout the gene and over 30% of them are located in exons 32 to 41, encoding the carboxylic domain containing important functional domains including GAP [39].

No correlation was found between the type of mutation in *TSC1* and the phenotype, moreover, those patients have a less severe disease course compared to patients with mutations in *TSC2*. Women with found mutations in the carboxyl domain of the *TSC2* gene (exons 40 and 41) are more likely to develop lymphangioleiomyomatosis [40]. In addition, TSC patients with polycystic kidney disease have a higher risk of a more severe course of the disease if pathogenic variants of the *TSC2* gene are present. If the *TSC2* gene is deleted, the *PKD1* (polycystin 1) gene is also deleted (3' ends of these genes overlap) causing a contiguous gene syndrome [41]. Interestingly, there are also reports of people / families with mutations in *TSC2*, who had a milder course of the disease, either mildly symptomatic or asymptomatic [42, 43].

Tuberous sclerosis is inherited in an autosomal dominant manner with a significant predominance of disease cases with *de novo* mutations. It is estimated that about 70% of patients have no family history of an affected person, while the remaining 30% are family cases [35]. Mutations in the *TSC1* gene are almost twice as frequent in hereditary cases as compared to the sporadic form. Penetration of the *TSC1* and *TSC2* mutations is complete, while expression of disease is variable [42]. Symptoms of the disease occur in people in whom the second copy of the gene is silenced due to changes in the DNA sequence (mutations) or epigenetic changes – in line with Knudson's two-hit theory [35].

Identification of the pathogenic change is necessary for prophylactic treatment that is optimal for the patient and genetic counselling for the patient's family. The risk of a carrier passing a critical change over to their offspring is 50%. Currently, genetic testing involves analyses of the sequences of both key genes and searching of deletion / duplication. The method that allows quick sequence analysis is next generation sequencing (NGS), while for deletion / duplication analysis, recommended methods include those based on e.g. MLPA (multiplex ligation-depend probe amplification) and FISH (fluorescent in situ hybridization) as well as aCGH (array comparative genomic hybridization) [35, 39]. In cases of uncertain clinical diagnosis, application of a test based on a selected panel of genes (differential diagnosis) may be considered. About 70% of the mutations are found in the *TSC2* gene and further 25% in the *TSC1* gene. Finding no pathogenic variant in a patient with a clinical diagnosis is often associated with presence of mosaicism. Therefore, examination of the patient's other tissues should be considered. Moreover, germline mosaicism is also possible in healthy parents (without the mutation) who have an affected child [35]. In the case of identification of a germinal mutation, it is also possible to perform prenatal and preimplantation tests [39].

Conclusions

Von Hippel-Lindau disease and tuberous sclerosis belong to the group of phacomatoses, genetically determined diseases predisposing to development of multiple neoplasms. Due to the similarity of skin lesions associated with the discussed disorders, it is necessary to differentiate them from neurofibromatosis 1 and 2 and schwannomatosis. Early detection and, consequently, placing patients under multidisciplinary supervision improves the prognosis, enabling implementation of cancer treatment in the early stages of the disease. The constantly expanding genetic knowledge makes it possible to better understand the molecular aspect of both diseases, which will probably allow introduction of personalised treatment in the future, which will significantly increase the patients' quality of life.

Conflict of interest: none declared

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Letter to the Editor

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Is the elimination of cervical cancer now 3 times easier? One-dose vaccine efficacy has far-reaching implications

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More and more studies have proven that low trust in vaccines has become a universal phenomenon, regardless of the region of the world and type of vaccine [1–3]. Particularly intense public debate on vaccines efficacy and safety has been (and still is) visible during the COVID-19 pandemic. A plethora of misconceptions, myths and fake news on vaccines has come to the fore recently, causing a lack of trust, not only in COVID-19 vaccines. Searching for new paths to cope with this public health challenge should be one of the key points of current international and national health policy agendas. In this context, recent research results look very promising, i.e. a single dose of vaccination against HPV has a similar efficacy to two and three doses [4]. These results may have far-reaching implications – primarily in a public health context, as it may enable a much faster eradication of HPV regionally and worldwide, moreover, those reluctant to get the HPV vaccine will be more likely to take just one dose, rather than three.

Europe's Beating Cancer Plan launched by the European Commission at the beginning of 2021 assumes vaccinations of target population of girls will be at the level of 90% by 2030 as well as acceleration of vaccinations among boys. However, the document describes a "fully vaccinated" target population, implying having completed a 3-dose scheme which can be much more difficult taking into consideration high social HPV vaccine hesitancy. Moreover, in the coming years (at least 4-5 years), due to rapidly growing demand, there are predictions of further HPV-vaccine shortages on the world market. Furthermore, despite the plans of new manufacturers to enter the market, it will take some time to begin efficient production (even 4 years are needed for manufacturing the final product). Moreover, legal difficulties impede shifting HPV vaccinations supplies from one country to another [5]. Discussing all of these obstacles, it is crucial to significantly increase social trust in HPV vaccinations. Implementation of the one-dose vaccination scheme seems to be one of the easiest and most beneficial ways to achieve this goal. Additionally, faster HPV eradication can be obtained by combining vaccination and screening in organised programs [6, 7] and this strategy would be greatly facilitated if both screening and vaccination could be completed in a single visit.

The WHO has specifically called for further research on innovative ways to achieve the elimination goal faster. The fact that it has also now been shown for the quadrivalent vaccine (that a single-dose HPV vaccination is as effective as a 3-doses full scheme), means the results could help to overcome one of the most important barriers to broad vaccine coverage – low social trust.

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