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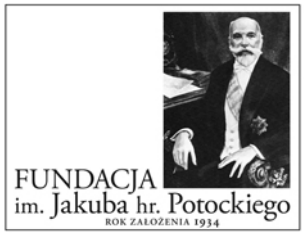

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Expression of matrix metalloproteinases (MMPs) and their inhibitor (TIMP) genes on mRNA and protein levels in oral squamous cell carcinoma

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Introduction. To investigate the mRNA and protein expression of MMP-2, MMP-9, MMP-7 and their tissue inhibitor TIMP-2 in tissue specimens with oral squamous cell cancer (OSCC) and in healthy tissues.

Material and methods. The expression genes of MMP-2, MMP-9, MMP-7 and TIMP-2 on mRNA levels were detected by the RT-PCR method in 31 samples with oral squamous cell carcinoma and in 31 healthy, control tissues. Secondly, the concentration of the analysed metalloproteinases and their inhibitor was assessed in tumor and non-tumor tissues using the enzyme-linked immunosorbent assay (ELISA) method.

Results. The mean values of gene expression of MMP-2, MMP-7, MMP-9 and TIMP in tissues with oral squamous cell cancer were significantly higher in comparison to normal ones ($p < 0.0001$). Similar observations were found for concentration levels of analysed MMPs and TIMP in tissues with and without oral cancer ($p < 0.0001$).

Conclusions. The present study demonstrated that MMP-2, MMP-7, MMP-9 and TIMP-2 gene expression on protein and mRNA levels is higher in oral squamous cell carcinoma tissues than in healthy control tissues. This may suggest that MMPs and TIMP play an important role in tumorogenesis. We did not observe any correlation between the clinicopathological characteristics of patients with OSCC and expression levels of MMPs and TIMP.

Key words: ELISA, enzyme assays, oral cancer, metalloprteinases

Introduction

According to the WHO, the incidence of only oral cancers worldwide ranges from one to ten cases per 100 000, and the number of new cases grows every year [1]. A similar situation can be observed in Poland. According to the National Cancer Registry, oral cancers account for 4% of all cancer cases in men and 1% in women. In 2010, in Poland, the frequency of oral cavity and pharynx cancer in men was 1.4 times higher than the average for men in other EU countries (data from 2009), while in women this difference was smaller (about 1.2 times) [2]. In 2012, 1725 new cases of oral mucosal cancer were recorded [3]. In 2015, there was an increase by over 4000 new cases of malignant tumours in total. It should be mentioned that in the same year the Malignant Cancer Notification Card (KZNZ) was introduced for the first time, which would help with rapid diagnosis and oncological treatment, as well as improve the quality of statistical data [4].

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Despite the fact that the knowledge on prevention and treatment of oral cancer is increasing, the number of new cases increases every year, and treatment outcomes remains poor. New prognostic factors are being searched for, which could enable more precise determination of prognosis and selection of the optimal treatment methods required. Phases of head and neck carcinogenesis are now being widely investigated.

It was observed that the initiation of metastatic process depends on the ability of the primary oral squamous cell cancer (OSCC) to destroy/digest the extracellular matrix (ECM). This enables the penetration of tumor cells to the basement membrane (BM) and the initiation of angiogenesis [5–8]. Degradation of BM, which is the first barrier inhibiting growth of the tumor, allows the invasion of the adjacent tissues and blood vessels. This process takes places in the pericellular environment and is a highly controlled cascade of events. Proteolytic enzymes are mainly responsible for these processes, among which metalloproteinases (MMPs) play a special role. MMPs calcium-dependent zinc-containing endopeptidases have various functions in the human organism. Twenty-five members of the MMP family have been identified [9, 10]. Most of them are involved in common physiological processes like tissue regeneration and angiogenesis, morphogenesis, proliferation, differentiation and cells apoptosis [9–14].

It was discovered that stromal cells take part in the up--regulation of MMPs [15, 16]. It has been assumed that the tumorogenesis of OSCC is possible due to the ability to utilise metalloproteinases produced by stromal cells of the host [16–18]. There is also a theory that cancer cells can stimulate their liberation [19].

The MMP family includes 25 different enzymes which have different functions. It appeared that MMP-2 and MMP-9 degrade collagen type IV which builds the BM, and MMP-7 degrades fibronectin, tenascin and β4 integrin [12, 17, 20–25].

A group of enzymes that are tissue inhibitors of metalloproteinases (TIMPs) have also been distinguished. Their role is to inhibit the activity of MMPs. The aim of this study was to investigate the mRNAs and protein expression of MMP-2, MMP-9, MMP-7 and their tissue inhibitor TIMP-2 in tissue specimens with oral squamous cell cancer and in healthy tissues.

Material and methods

Study group

31 patients (3 women and 28 men) aged 60.6 ± 7.3 years were included in the study. Out of the 26 examined patients, 12 declared their rural origins and this group constitutes almost half of the total assessed patients. All of them were diagnosed with oral squamous cell carcinoma and underwent surgery at the Cranio-Maxillo-Facial and Oncological Department in the years 2015–2017. Patients enrolled in this research did not obtain any induction therapy. Tumor size and cancer staging were assessed according to the guidelines of the Union for International Cancer Control (UICC) and the American Joint Committee on Cancer (AJCC) [26, 27]. We also gathered information concerning lymph node metastates (negative – N0 *versus* positive – N1, N2, N3, N4) and smoking and alcohol habits. None of the patients had distant metastases at the date of inclusion in the study. The study group characteristics were presented in table I. This study was approved by the Ethics Committee (RNN/203/13/KE). The participant's informed consent was obtained verbally.

Sample collection and preparation

We gathered tissue fragments from cancerous lesions and from normal tumor adjacent tissue from all the patients enrolled in the study. Samples were preserved and stored at –80°C. Normal tissue was taken to be a control group. Control tissues were excised from a site distant by at least 2 cm from the macroscopic tumor border and confirmed as not having precancerous or cancerous lesions in the histopathological assessment. Tumor tissues were also histopathologically examined – oral squamous cell carcinoma was confirmed in all cases.

RNA extraction and analysis

From the frozen samples, RNA was extracted with the use of TRIZOL (Invitrogen Life Technologies) liquid – a liquid extraction technique with acid guanidinium thiocyanate-phenol--chloroform. The obtained RNA fragments were separated by an agarose gel electrophoresis. Only samples with well

Table I. Characterisation of patients with oral cancer (n = 31)

Variables		No. of patients (%)
sex	female	3 (10)
	male	28 (90)
age	<65	23 (74)
	>65	8 (26)
primary tumor size (T)	T1	2 (7)
	T2	1 (3)
	T3	0 (0)
	T4	28 (90)5
nodal melostosen	N0	19 (61)
	N1	4 (13)
	N2	7 (23)
	N3	1 (3)
clinical stage	I	2 (7)
	II	1 (3)
	III	0 (0)
	IVa	27 (87)
	IVb	0 (0)
	IVc	1 (3)
histopathological grading	G1	1 (3)
	G2	25 (81)
	G3	5 (16)

preserved ribosomal 28S, 18S and 5S RNA were used in the study. Secondly, the RNA was digested with the DNAse I enzyme (GIBCO) for 15 min. at room temperature. Five RNA of prepared ribonucleic acid were used for a reverse transcription reaction at 42°C. for 60 min, according to manufacturer protocol (ImProm-IITM Reverse Transcription System kit, Promega, USA). Obtained cDNA was then used in a real-time polymerase chain reaction (PCR) (Taq Mantm, Fast Start Universal Probe Master, ROX, Roche). In the real-time PCR, we used primers that were designed with the use of the Universal Probe Library.

Substrates for real-time PCR were performed in 50 µl final volume with 0.05 µg of cDNA 25 µl of Fast Start Universal Probe Master (ROX), 250 nM probe and 1 µM of each primer. PCR was carried out in a typical manner. Initialization consisted of heating the reaction chamber to a temperature of for 10 minutes to activate the Fast Start Taq DNA polymerase. Elongation included 40 rounds of 15 sec each at 95°C. Detection of amplification was performed with the use of an ABI Prism 7000 Sequence Detection System (Applied Biosystem). Each sample was tested in triplicate in independent reactions. The obtained real-time PCR data was automatically calculated with a special module using the $2^{-\Delta\Delta Ct}$ method. Validation of PCR efficiency was performed. Serial dilution was done to prepare standard curves for each assessed gene.

Determination of MMP-2, -7, -9, TIMP-2 levels using Enzyme-Linked Immunosorbent Assay (ELISA)

The expression of the MMP-2, MMP-7, MMP-9 metalloproteinase proteins and their TIMP-2 inhibitor were assessed using a sandwich ELISA (enzyme-linked immunosorbent assay) from RayBiotech. The ELISA test was performed three times for each slice. Laboratory procedures were carried out in accordance with the manufacturer's instructions. The first step was to perform the coat stage, which was carried out by adding solid phase to the wells (where there were specific antibodies to human proteins MMP-2, MMP-7, MMP-9 and TIMP-2), tested samples (tissue homogonates) and control samples. After the incubation process, the plate is washed. Secondary antibodies labeled with horseradish peroxidase conjugated streptavidin, were then added. The wells were rinsed again. In the next step, a substrate (tetramethylbenzidine, TMB) was added for the enzyme (horseradish peroxidase) bound to the antibody; as a result of the enzymatic reaction this turns into a coloured product. Using spectrophotometry, the colour intensity was determined after a specified duration of reaction (Thermo Labsystem Multiskan Ascent 354), thanks to which the measurement of the antigen concentration in the material used for the tests was obtained.

Statistical analysis

For data distribution that differed significantly from normal distribution, non-parametric tests were applied. A Wilcoxon test for paired data and a Mann-Whitney U test was used to determine the statistical significance of differences among the various analysed independent groups. In the case of covariates of interest, Spearman rank correlation coefficients were used. The $p < 0.05$ was considered as a level of statistical significance. All the calculations were derived by means of Statistica v12.0 software.

Due to the retrospective nature of this study, it was granted an exemption in writing by the Medical University of Lodz IRB.

Results

MMPs and TIMP mRNA expression

Our study revealed that the mean values of gene expression of MMP-2, MMP-7, MMP-9 and TIMP in tissues with oral squamous cell cancer were significantly higher in comparison to normal ones ($p < 0.0001$). The detailed data was presented in table II.

We did not observe any relevant statistical correlation between the mRNA expression of analysed metaloproteinases and the clinicopathological features of patients with OSCC.

Protein levels of MMPs and TIMP

There was a significantly higher concentration of MMP-2, MMP-7, MMP-9 and TIMP in tissues with cancer than in control tissues ($p < 0.0001$), (tab. III).

No statistically significant correlation was noticed between protein levels of MMPs or TIMP analysed and the clinicopathological features of patients with OSCC like TNM advancement of tumor, clinical stage histopathological grading, smoking, patients' age and gender ($p > 0.05$).

Discussion

Our study revealed that there was a higher expression of MMP-2, MMP-7, MMP-9 and TIMP mRNA in tissues with oral cancer than in normal tissues. We also observed that the

Table II. Comparison of mRNA level of selected genes ($2^{-\Delta\Delta Ct}$ expression) in tumor samples and tumor adjacent normal tissues

	n	Tumor	Tumor adjacent normal tissues	p
		mean ± SD	mean ± SD	
MMP-2	31	0.32 ± 0.10	0.09 ± 0.03	<0.0001
MMP-7	31	0.24 ± 0.07	0.12 ± 0.04	<0.0001
MMP-9	31	0.29 ± 0.15	0.14 ± 0.04	<0.0001
TIMP-2	31	0.26 ± 0.08	0.12 ± 0.03	<0.0001

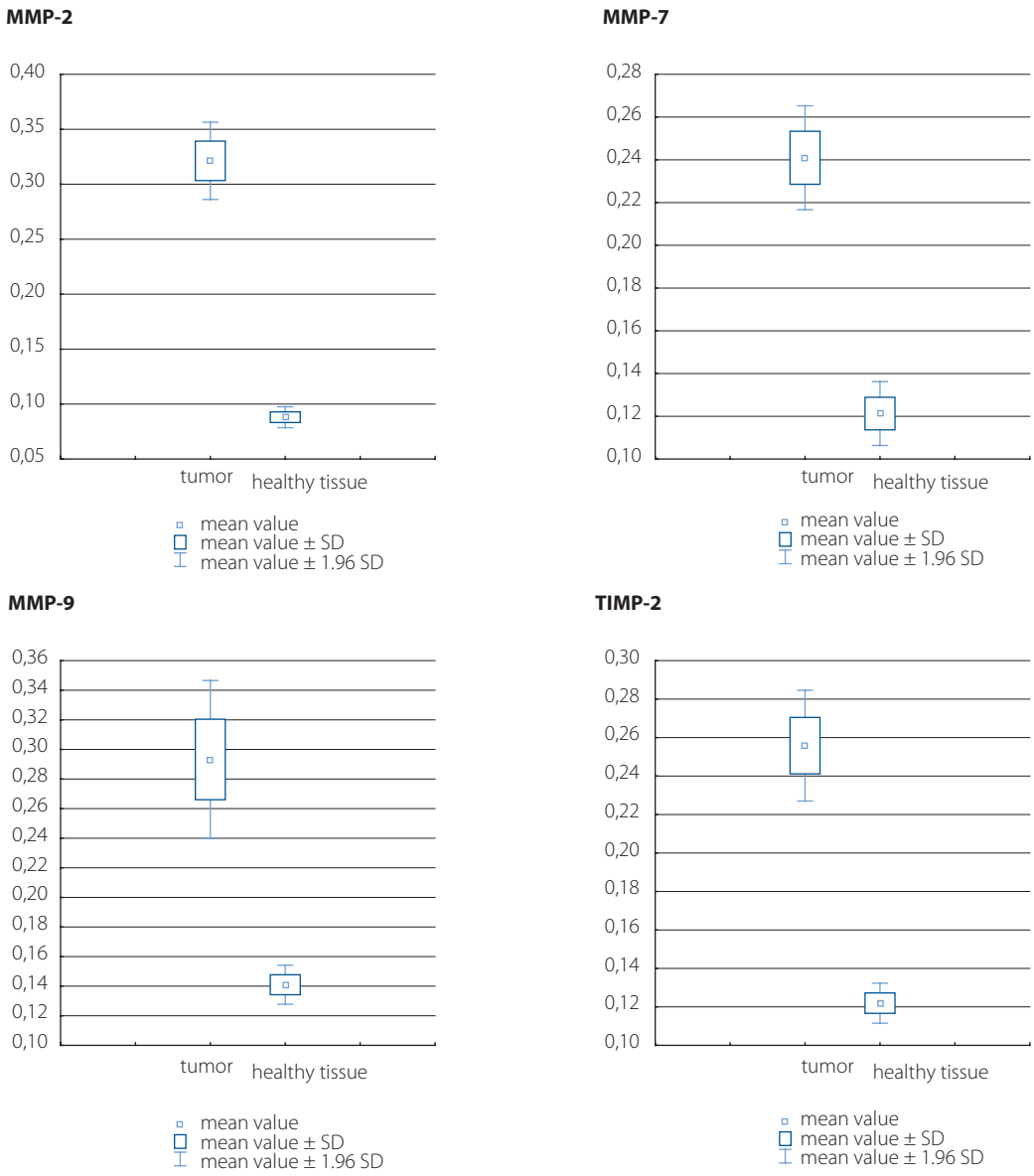

Figure 1. Comparison of mRNA level of selected genes in tumor sampels and tumor adjacent healthy tissue

Table III. Comparison of protein concentrations (ng /ml) of MMP-2, MMP-7, MMP-9, TIMP-2 in tumor samples and tumor adjacent normal tissues

	n	Tumor			Tumor adjacent normal tissues			p
		mean	±	SD	mean	±	SD	
MMP-2	31	941	±	179	498	±	102	<0.0001
MMP-7	31	237	±	45	144	±	31	<0.0001
MMP-9	31	319	±	87	172	±	33	<0.0001
TIMP-2	31	138	±	86	56	±	18	<0.0001*

affected tissues had a significantly higher concentration of the analysed MMPs and their inhibitor. Many authors noticed similar results. The MMPs expression was found to be higher in neoplastic tissues from the head and neck region [28–30]. Higher protein concentration of MMP-2, MMP-7 and MMP-9 in head and neck cancers has also been reported by other authors [29]. Numerous studies have revealed a significantly increased level of the different MMPs expression and their inhibitors in head and neck cancers in comparison to healthy tissues [16–18, 20–25]. Most frequently, researchers investigate the potential role of MMP-2, MMP-3, MMP-8 and MMP-9 in tumorogenesis of oral squamous cell carcinoma

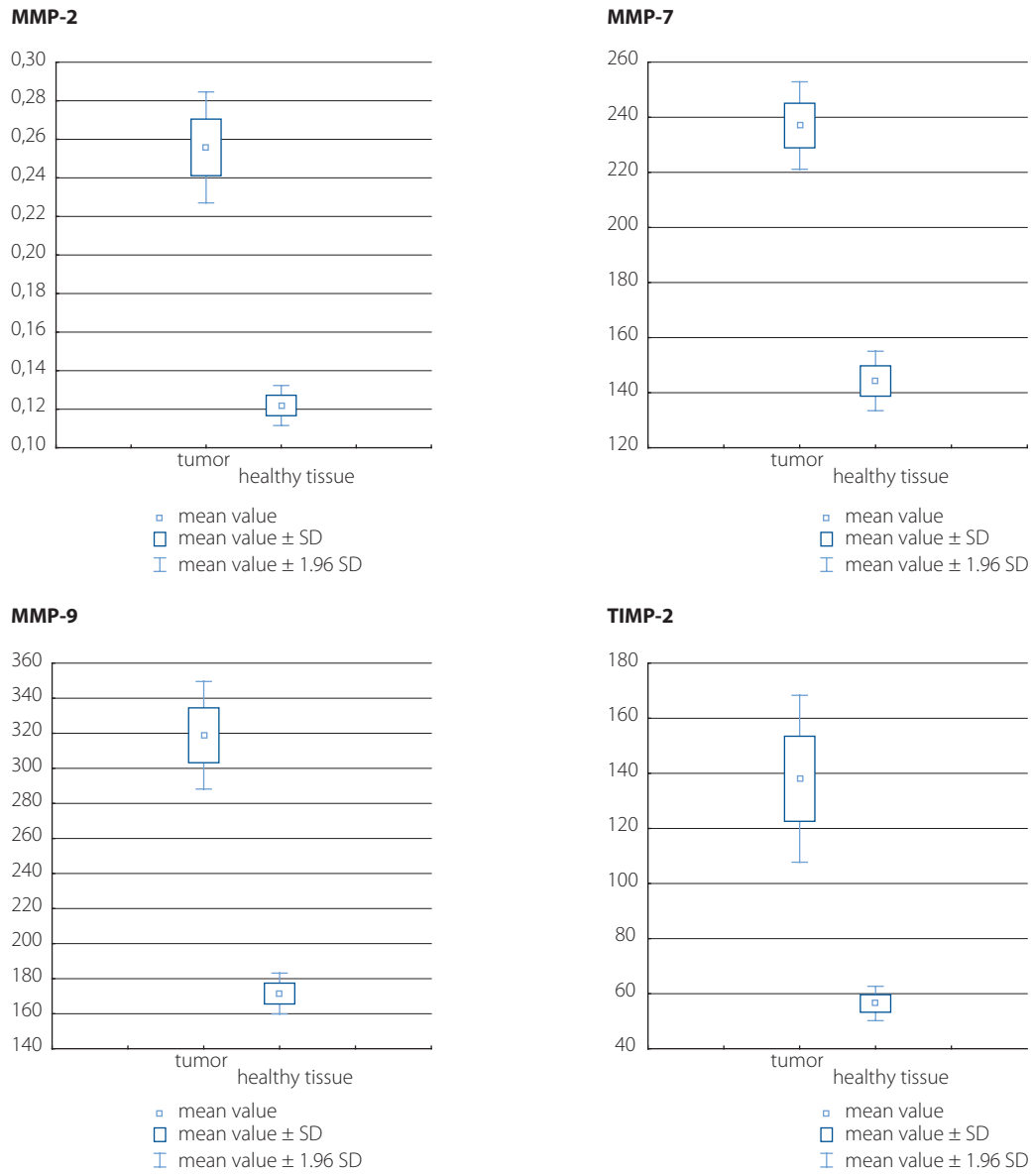

Figure 2. Comparison protein concentrations of MMP-2, MMP-7, MMP-9, TIMP-2 in tumor sampels and tumor adjacent healthy tissue

[28–31] Singh et al. investigated different combinations of MMPs and tissue inhibitors of MMPs for achieving better clinical efficacy [32].

In our study, we did not observe any significant correlation between either mRNA or the protein expression of MMP-2, MMP-7, MMP-9 and TIMP-2 and clinicopathological features like clinical stage, tumor size (T) and nodal status (N), as well as histopathological grading.

The major clinical problem in treating patients with oral cancers is local infiltration and the resulting destruction of critical structures, which is responsible for a majority of cancer related deaths, due to tumor involvement in critical organs [33]. Proteolytic degradation of ECM is an essential part of this process and different proteinases – including MMPs – have been proven to take part in it [34]. These observations prompted researchers to look for a relationship between gene expression of metalloproteinases and tumor progression. Thomas G.T. et al. and Kawamata H. et al. presented the idea that gelatinases and their tissue inhibitors are not only over-expressed in tissues with oral squamous cell carcinoma, but are also related to tumor progression and invasion [18, 35]. A statistical correlation was observed between gelatinase mRNA, immunoreactive proteins or enzyme activity and clinical advancement of the OSCC-like tumor invasion or metastases to the lymph nodes [17, 36]. Similar observations were made by Miyajima Y. et al. in hypopharyngeal squamous cell carcinoma [37]. On the other hand, there were also some reports that had results that were in accordance with ours. They proved a higher expression of MMPs in cancerous tissues but did not find any association between MMP protein expression and the stage or grade of

Table IV. Characterisation of patients with laryngeal cancer (n = 96)

Variables		No. of patients (%)
sex	female	3 (10)
	male	28 (90)
age	<65	23 (74)
	>65	8 (26)
primary tumor size (T)	T1	2 (7)
	T2	1 (3)
	T3	0 (0)
	T4	28 (90)
nodal metastases	N0	19 (61)
	N1	4 (13)
	N2	7 (23)
	N3	1 (3)
clinical stage	I	2 (7)
	II	1 (3)
	III	0 (0)
	IVa	27 (87)
	IVb	0 (0)
	IVc	1 (3)
histopathological grading	G1	1 (3)
	G2	25 (81)
	G3	5 (16)

the tumor [38]. Vicente J.C. et al. demonstrated over-expression of MMP-2 and MMP-9 and found a significant correlation between MMP-2 and MMP-9 and lymph node metastasis in patients with OSCC [39].

In the literature, there is a wide variety of results regarding MMPs, expression and their association with tumor staging in the head and neck region. The data on the correlation of the expression of metalloproteinases with invasion and nodal metastasis are inconclusive and the potential predictive role of MMPs and TIMPs in head and neck cancer progression and the influence on patients' treatment outcomes is still controversial. Several factors may contribute to this. First of all, there are different methodologies, as well as different antibodies, used in the analysed studies. Secondly, oral squamous cancer presents a heterogeneity of clinical features. Some authors failed to analyse TIMPs in association with MMPs, and it should be emphasised that the analysis of the interaction between the metalloproteinases and their inhibitors is more important than analysis of just one component [40].

Conclusions

The present study demonstrated that MMP-2, MMP-7, MMP-9 and TIMP-2 protein levels and mRNA expression is higher in oral squamous cell carcinoma tissues than in healthy control tissues. This may suggest that MMPs and TIMP play an important role in tumorogenesis. We did not observe any correlation between the clinicopathological characteristics of patients with OSCC and expression levels of MMPs and TIMP.

Conflict of interest: none declared

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Local excision *vs.* radical surgery in treating rectal nets considering the biology of neuroendocrine tumors (NETs)

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Introduction. Local excision (LE) is performed for rectal neuroendocrine tumors (NETs) <1 cm in size, whereas radical surgery (RS) is performed for larger tumors. The lack of data and limited number of studies support such approaches. Thus, we determined oncological outcomes after primary tumor resection in patients with rectal NETs and identified other factors of NETs that could influence oncological outcomes.

Material and methods. We retrospectively examined patients with I–III stage rectal NETs who underwent different surgical approaches, including LE or RS, in Severance Hospital, Korea between 2006 and 2017. The association between surgery extent, tumor size (TS), depth of invasion and biological factors of NETs was examined. Oncological outcomes were analyzed.

Results. Local excision (LE) and radical surgery (RS) were performed in 64 and 23 patients, respectively. Patients who underwent RS were more likely to have larger TS; deeper invasion; higher grade, mitotic index, Ki-67; more lymph node metastasis (LNMts); and a higher lymphovascular invasion rate ($p < 0.001$). Most patients with TS < 1.0 cm underwent LE had better DFS and OS. Primary TS > 10 mm was an independent predictor of invasion ($p = 0.001$) whereas depth of invasion was an independent predictor of LN metastases ($p = 0.003$). In the multivariate analysis, only invasion was an independent factor associated with poor DFS and OS ($p = 0.023$ and 0.015, respectively).

Conclusions. Local excision could be an effective method to use in treating rectal NETs in the early stage of the disease, and depth of invasion was an important factor influencing oncological outcomes. Our findings need to be confirmed in future prospective and randomized studies.

Key words: rectal neuroendocrine tumor, local excision, radical surgery, invasion, tumor size

Introduction

Rectal neuroendocrine tumors (NETs) are rare tumors accounting for approximately 2% of all rectal tumors. However, rectal NETs are the second most frequent tumors among all gastrointestinal tract NETs and account for 20%; they have showed the highest recent increase in incidence [1, 2]. The prognosis for rectal NETs is favorable, with a 5-year survival rate of approximately 90% [3–5]. The current National Comprehensive Cancer Network (NCCN) and European Neuroendocrine Tumor Society (ENETS) guidelines advise providing aggressive surgical treatments for rectal NETs >2 cm in size [6, 7]. However, these size-based surgical approaches are controversial because only limited studies on these approaches are available [8–10]. Common surgical methods, including low anterior resection or APR, are used to excise rectal NETs >2 cm in size [6, 11, 12].

Endoscopic approaches, including endoscopic submucosal dissection (ESD) and endoscopic mucosal resection (EMR), enable resection of rectal NETs with small sizes (<10 mm) and are recommended by the ENETS 2016 guidelines [7]. However, these endoscopic methods have a high rate of positive resec-

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tion margins (approximately 24%–46%) according to some modern studies [13, 14]. Lee at al. revealed 17% positive resection margins after EMR among patients with tumors 15 mm in diameter [15]. Studies demonstrated a significant improvement with approximately 96% complete resections when ESR was used instead of EMR [16–18]. Transanal minimally invasive surgery (TAMIS) or transanal endoscopic microsurgery (TEM) enable safe resection of rectal NETs with negative margins, although the size is >2 cm [19–20].

Some studies have suggested that the size of rectal NETs is one of the most important factors in prognosis. According to their results, positive LN metastasis significantly increase with the size of the tumor and was observed from 40 to 80% of patients with tumor size >2 cm [21–25].

In contrast, a recent assessment of the SEER database showed that LN metastasis occurred in only 11.7%, and distant metastases occurred in only 12% of tumors >2 cm [26]. According to other modern data, there are no significant differences in oncological outcomes among patients with rectal NETs of different sizes (<2 or >4 cm) who underwent local excision or standard surgical approaches with disease-free LN status [26]. Moreover, there was a paradoxical contradictory report of outcomes after local excision versus radical surgery. The 10-year overall survival (OS) rates for T2 rectal NETs after local excision or radical surgery were 79.8% and 63.2% and those for T3/4 were 82.3% and 28.3%, respectively ($p < 0.01$). In addition, Ki-67 >3% and lymphatic or venous invasion were strong predictors in multivariate analysis [27].

It is worth indicating that compared with local excision approaches, low anterior resection of the rectum or abdominoperineal resection significantly compromises the quality of life (QOL) of patients. It also can be hypothesized that the biological features of NETs appear to play a more important role than the size of NETs. For example, NET features, including high Ki-67 index, high grade and mitotic index, and lymphovascular invasion are more crucial when the surgical approach is chosen and their presence results in varying prognosis [26, 28]. However, the nature and biology of rectal NETs needs to be considered in further studies because of their increasing incidence and the promising results of local excision with no evidence of LN metastasis. The new data might prove the effectiveness of local excision with the same oncological outcomes as radical surgery but with better QOL for patients.

Thus, this study aimed to determine oncological outcomes depending on the extent of primary tumor resection in patients with I–III stage rectal NETs in addition to identifying other factors associated with NET biology and aggressiveness that can influence oncological outcomes.

Materials and methods

In this single-center, retrospective, nonrandomized study, patients with I–III stage rectal NETs who underwent different surgical treatments, including local excision or radical operation, in Severance Hospital, Yonsei University Health System, Seoul, Korea between 2006 and 2017, were examined. Data were collected from the electronic medical record database of Severance Hospital. The clinicopathological characteristics, such as patients' age at diagnosis, gender, year of diagnosis, type of operation, stage, grade, differentiation, distance from AV, mitotic index, Ki-67, CD56, synaptophysin, chromogranin status, depth of invasion, and last follow-up status (alive/dead), were obtained from the database. Type of operation was defined as radical surgery, which included low anterior resection, abdominoperineal resection and intersphincter resection performed via the open or laparoscopic/robot approach; local excision was performed by TAMIS or transanal excision. All types of operations were included for retrospective analysis based on inclusion and exclusion criteria.

Based on the inclusion and exclusion criteria, patients were categorized depending on the surgical approach and clinicopathologic characteristics. The inclusion criteria were as follows: localization – rectum; stage I, II and III (AJCC TNM 7th ed.); tumor size ranging from 1 to 50 mm; tumor distance within 15 cm from the anal verge; type of operation (local excision versus radical surgery); patient's age between 19 and 80 years and the availability of histopathological and radiologic data. The exclusion criteria were as follows: stage IV (AJCC TNM 7th ed.), patient's age <19 or >80 years, treatment for previous cancer besides rectal NETs; follow-up loss; or incomplete clinical/histopathological/radiologic data.

The primary endpoints of this study were long-term survival outcomes of patients with rectal NETs after surgical treatment using two different approaches, namely radical surgery or TAMIS. Different factors associated with tumor biology and aggressiveness were also investigated regardless of the surgical approaches. OS was calculated from the date of surgery to the date of death. Disease-free survival (DFS) was calculated from the date of surgery to the date of recurrence.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics, version 23 (SPSS Inc., Chicago, IL, USA). Categorical variables were expressed as frequencies (%), whereas continuous variables were presented as means, with their range or standard deviation. The means of continuous variables were compared using an independent sample t-test. Categorical variables were compared using the Pearson test. OS and DFS were estimated using the Kaplan-Meier method and were compared using a log-rank test. A multivariate Cox proportional hazards model with stepwise method was used to identify statistically significant independent prognostic factors for OS and DFS. In logistic regression analysis, p values <0.05 were used to define statistical significance of variables influencing DFS. A receiver operating characteristic (ROC) curve analysis was used to determine the optimal cutoff value of the tumor size. The variance inflation factor (VIF) method was used to detect

whether multicollinearity was presented among the independent variables (VIF >3 indicated the existent correlation among investigated variables).

Results

Overall, 1046 patients with rectal NETs underwent different treatments. An endoscopic approach was performed in 928 patients (365, cold polypectomy and 563, EMR or ESD), local excision in 72, and radical surgery in 46. Among those, based on the study aim and eligibility criteria, we further analyzed only the clinical data of 64 patients who underwent local excision and the 23 patients who underwent radical surgery. The characteristics of both groups of patients are summarized in table I.

Table I. Patients' characteristics

Variables	Radical surgery	Local excision	p
no. of patients	23	64	
age, mean ± SD (years)	52.3 ± 11.3	49.7 ± 12.1	0.420
age (years)			0.198
≤65	18 (78.3%)	57 (89.1%)	
>65	5 (21.7%)	7 (10.9%)	
gender			0.323
male	17 (73.9%)	24 (37.5%)	
female	6 (26.1%)	40 (62.5%)	
[a]primary tumor size (mm)			<0.001
≤10.2	8 (34.8%)	54 (84.4%)	
>10.2	15 (65.2%)	10 (15.6%)	
[b]primary tumor size (mm)			<0.001
≤16.5	12 (52.2%)	62 (96.9%)	
>16.5	11 (47.8%)	2 (3.1%)	
distance from AV, mean ± SD (cm)	7.7 ± 3.3	6.2 ± 2.6	0.038
differentiation			0.116
well	20 (87.0%)	62 (96.8%)	
moderate	3 (13.0%)	2 (3.1%)	
invasion			<0.001
mucosa + submucosa	8 (34.8%)	60 (93.8%)	
muscularis propria + pericolic tissue	15 (65.2%)	4 (6.3%)	
grade			<0.001
G1	12 (52.2%)	59 (92.2%)	
≥G2	11 (47.8%)	5 (7.8%)	
HPF			<0.001
≤2/10	14 (60.9%)	61 (95.3%)	
>2/10	9 (39.1%)	3 (4.7%)	
TNM stage			<0.001
I, IIA, IIB	13 (56.5%)	64 (100.0%)	
IIIA, IIIB	10 (43.5%)	0	
T status			<0.001
T1, T2	11 (47.8%)	62 (96.9%)	
T3, T4	12 (52.2%)	2 (3.1%)	
N status			<0.001
N0	13 (56.5%)	64 (100.0%)	
N1	10 (43.5%)	0	
Ki-67			0.004
≤2%	15 (65.2%)	58 (90.6%)	
>2%	8 (34.8%)	6 (9.4%)	
PNIV			0.055
positive	3 (13.0%)	1 (1.6%)	
negative	20 (87.0%)	63 (98.4%)	
LVIN			<0.001
positive	9 (39.1%)	1 (1.6%)	
negative	14 (60.9%)	63 (98.4%)	
CD56			0.044
positive	21 (91.3%)	45 (70.3%)	
negative	2 (8.7%)	19 (29.7%)	

Variables	Radical surgery	Local excision	p
synaptophysin			0.218
positive	21 (91.3%)	50 (78.1%)	
negative	2 (8.7%)	14 (21.9%)	
chromogranin			0.457
positive	4 (17.4%)	16 (25.0%)	
negative	19 (82.6%)	48 (75.0%)	

SD – standard deviation; AV – anal verge; HPF – high power field; TNM – tumor-node-metastasis; PNIV – peri-neural invasion; LVIN – lympho-vascular invasion

[a] cutoff based on mean value

[b] cutoff based on ROC curve

Patients' age, gender, differentiation of NET and some tumors markers according to immunohistochemical analysis (synaptophysin and chromogranin) were not significantly different between the two groups (all $p > 0.05$). Patients in the radical surgery group were more likely to have larger primary tumors (65.2% *vs.* 15.6%, $p < 0.001$), deeper invasion (65.2% *vs.* 7.8%, $p < 0.001$), higher grade (39.1% *vs.* 7.8%, $p < 0.001$), higher mitotic index and Ki-67 (2/10 HPFs: 39% *vs.* 4.7%; Ki-67 >2%: 30.4% *vs.* 9.4%, $p < 0.001$), T stage and LN metastasis (T3/4: 52.2% *vs.* 3.1%; N1: 43.5% *vs.* 0%, $p < 0.001$) and a higher lymphovascular invasion rate (39.1% *vs.* 1.6%, $p < 0.001$) than those in the local excision group. A vast majority of patients with tumors <1.0 cm underwent local excision (54/64, 84.4%), whereas those with tumors ≥2 cm underwent radical surgery (15/23, 65.2%). Recurrence was observed in five (21.7%) and three (4.7%) patients in radical surgery and local excision groups, respectively ($p = 0.055$).

Typically, the tumor size is one of the most important factors that predict outcomes and subsequently the surgical approach. According to the literature, tumor size >2 cm seems to be a cutoff for local excision, whereas a size between 1 and 2 cm remains controversial. We attempted to identify which primary tumor size would be applicable in our analysis. We used a ROC curve analysis to determine the optimal cutoff value of the tumor size that could influence oncological outcomes. The optimal cutoff value was 16.5 mm (sensitivity, 80%; and specificity, 90.2%) with an area under the curve of 0.877 (95% confidence interval [CI]: 0.764–0.990, $p = 0.005$). However, only two (3.1%) patients in the local excision group had a tumor size >16.5 mm compared with 11 patients (47.8%) in the radical surgery group (<0.001), resulting in inconsistency. To identify the comparable primary tumor size for analysis in both groups, we used the descriptive method to determine the mean tumor size in all 87 patients. We found that a mean size of 10.2 mm was more homogenous between the two groups. Thus, 15 and 10 patients in the radical surgery and local excision groups, respectively, had a primary tumor size >10.2 mm. A tumor size of 10 mm has been previously reported as a cutoff for local excision. Considering that, the primary tumor size of 10.2 mm was chosen for subsequent analysis of oncological outcomes in our study.

Factors associated with DFS

DFS rates were better in the local excision group than in the radical surgery. The cumulative 1-, 3-, and 5-year DFS rates of patients were 82.6%, 72.9% and 68%, respectively in the radical surgery group; 96.9%, 96.9%, and 94.4%, respectively in the local excision group ($p < 0.05$) (table III). Factors associated with an increased risk of recurrence included the type of surgical treatment (radical surgery vs. local excision), the primary tumor size >10 mm, poorer differentiation of primary tumor, invasion, grade >1, mitotic index (>1/10 HPFs), N positivity, perineural and lymphovascular invasions and Ki-67 >2% according to the univariate analysis ($p < 0.05$).

In contrast, in the multivariate analysis only deep invasion (HR, 17.385; 95% CI 3.684–82.052; $p < 0.001$) was independently associated with an increased risk of tumor recurrence and influenced DFS (HR, 8.374; 95% CI 1.342–52.248; $p = 0.023$). Table II provides details on the clinicopathologic factors associated with DFS.

Factors associated with OS

The cumulative 1-, 3-, and 5-year OS rates of patients were 95.7%, 90.6% and 84.6%, respectively, in the radical surgery group and 100% in all the years in the local excision group (tab. III). Factors associated with poor OS included primary tumor size >10 mm and tumor invasion depth ($p < 0.05$). In the multivariate analysis, depth of invasion (mucosa/submucosa *vs.* muscularis propria/pericolic tissue) was independently associated with poor OS (HR, 15.333; 95% CI, 1.710–137.447; $p = 0.015$) (tab. II).

DFS and OS were different between the two groups ($p = 0.001$). The 1-, 3-, and 5-year DFS and OS were longer in the local excision group than in the radical surgery group (tab. III).

Influence of the depth of invasion on 1-, 3-, and 5-year OS and DFS

Depth of invasion was an independent factor associated with higher recurrence rate and poor OS; thus, we analyzed the cumulative 1-, 3-, and 5-year DFS and OS with respect to this factor. We found that the cumulative 1-, 3-, and 5- DFS and OS rates were shorter in patients with deep invasion (muscular propria and pericolic tissue) than in those with superficial invasion (mucosa and submucosa) – regardless of the surgical approaches (tab. III; figs. 1 and 2). As shown in table III and

Table II. Univariate and multivariate analyses for disease-free survival (DFS) and overall survival (OS) of patients with rectal NETs after local excision of the rectum or rectum resection

Variables	Disease-free survival				Overall survival			
	Univariate analysis		[a] Multivariate analysis		Univariate analysis		[a] Multivariate analysis	
	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	P
groups	0.138 (0.036–0.534)	0.004	1.823 (0.086–3.167)	0.551	0.006 (0.001–20.571)	0.216		
age (years)	3.367 (0.867–13.077)	0.08			2.557 (0.266–24.612)	0.416		
gender	1.168 (0.302–4.519)	0.822			1.449 (0.240–8.754)	0.686		
primary tumor size (mm)	11.790 (2.496–55.686)	0.002	3.291 (0.491–22.043)	0.468	12.453 (1.387–111.771)	0.024	4.468 (0.358–55.707)	0.245
distance from AV (cm)	1.889 (0.532–6.701)	0.325			1.927 (0.321–11.562)	0.473		
differentiation	5.421 (1.145–25.675)	0.033	2.618 (0.492–13.943)	0.408	4.477 (0.464–43.247)	0.195		
invasion	17.385 (3.684–82.052)	<0.001	8.374 (1.342–52.248)	0.023	15.333 (1.710–137.447)	0.015	15.333 (1.710–137.447)	0.015
grade	5.181 (1.494–17.966)	0.01	0.156 (0.003–7.929)	0.89	2.782 (0.463–16.701)	0.263		
HPF	4.518 (1.274–16.019)	0.02	3.419 (0.147–79.551)	0.98	3.233 (0.535–19.529)	0.201		
N status	14.731 (4.131–52.529)	<0.001	4.021 (0.896–18.037)	0.069	4973.5 (0.001–7.825E)	0.518		
Ki-67	4.144 (1.165–14.747)	0.028	0.931 (0.197–5.200)	0.759	1.422 (0.159–12.727)	0.753		
CD56	1.355 (0.288–6.390)	0.701			32.824 (0.006–183676)	0.428		
synaptophysin	0.541 (0.140–2.095)	0.374			28.549 (0.002–381675)	0.489		
chromogranin	0.376 (0.048–2.966)	0.353			0.033 (0.001–327.922)	0.468		

HR – hazard ratio; CI – confidence interval; AV – anal verge; HPF – high power field

[a]Cox proportional hazards models adjusted for groups (local excision [reference], radical surgery), age (≤ 65 [reference], >65), gender (male [reference], female), primary tumor size (<10 [reference], ≥10), distance from AV (≤6 [reference], >6), differentiation (well [reference], moderate), invasion (mucosa + submucosa [reference], muscularis propria + pericolic tissue), grade (G1 [reference], G2), HPF (≤2/10 [reference], >2/10), N status (N0 [reference], N1), Ki-67 (≤2% [reference], >2%), CD56 (negative [reference], positive), synaptophysin (negative [reference], positive), chromogranin (negative [reference], positive).

Table III. Proportion of disease-free survival and overall survival of patients with rectal neuroendocrine tumors in radical surgery and local excision groups, depending on the depth of invasion of rectal neuroendocrine tumors in both groups over 1, 3, and 5 years (p = 0.001)

Time		Groups		Depth of invasion	
		Radical surgery	Local excision	Mucosa/submucosa	Muscularis/pericolic tissue
1 year	disease free survival	82.6%	96.9%	98.5%	78.9%
	overall survival	95.7%	100%	100%	94.4%
3 years	disease-free survival	72.9%	–	97.1%	72.9%
	overall survival	90.6%	–	100%	87.7%
5 years	disease-free survival	68%	94.4%	97.1%	53.1%
	overall survival	84.6%	100%	100%	87.7%

figures 1 and 2, patients with rectal NETs having invasion to the mucosa and submucosa had better 1-, 3- and 5-year DFS and OS rates than those with invasion to the muscular propria and pericolic tissue in both the groups (p = 0.001).

Factors associated with invasion and LN metastasis

Regarding survival and recurrence rates, we identified factors associated with invasion that corresponded to poor DFS and OS. We also investigated additional factors influencing DFS, such as LN positivity. We used logistic regression analysis to identify associated factors and compare potential factors. We included primary tumor size, grade, differentiation of primary tumor, T status, mitotic activity, synaptophysin, chromogranin, N status and invasion.

Factors predicting invasion

We analyzed factors that could predict the depth of invasion of the primary tumor. As shown in table IV, the mitotic index (HPF)

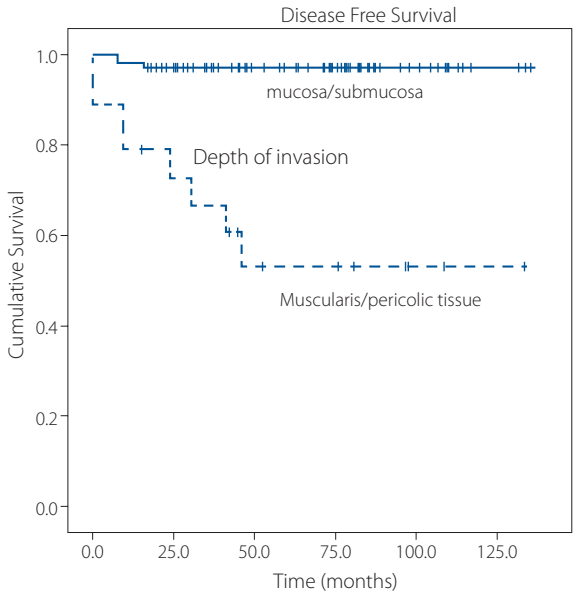

Figure 1. Disease-free survival (DFS) of patients with rectal neuroendocrine tumors (NETs) with two depths of invasion ($p < 0.001$)

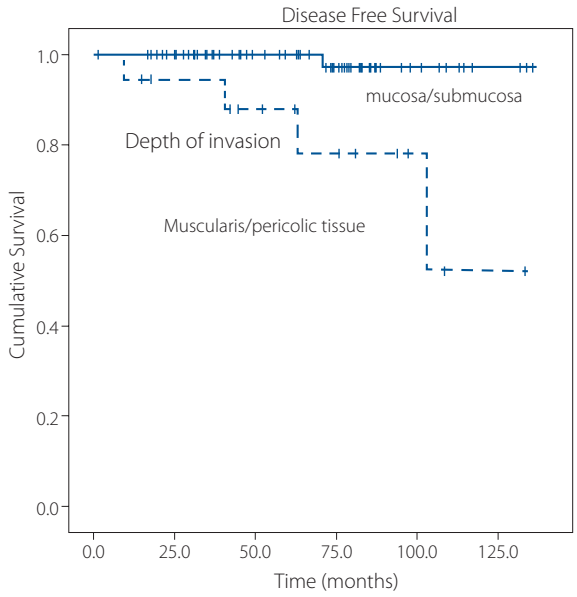

Figure 2. Overall survival (OS) of patients with rectal neuroendocrine tumors (NETs) with two depths of invasion ($p = 0.001$)

Table IV. Logistic regression analysis of factors predictive for invasion

Variables	Groups			Logistic regression analysis		
	Radical surgery	Local excision	p	Odds ratio	95% confidence interval	p
primary tumor size (>11 mm)	15 (65.2%)	10 (15.6%)	<0.001	38.515	4.343–341.594	0.001
high power field (≥2/10)	9 (39.1%)	3 (4.7%)	<0.001	55.560	4.711–655.309	0.001
grade (≥G2)	11 (47.8%)	5 (7.8%)	<0.001			[a] –
N (+)	10 (43.5%)	0	<0.001	7.570	0.871–65.766	0.066
Ki-67 (>2%)	8 (34.8%)	6 (9.4%)	0.001			[a] –
CD56 (+)	21 (91.3%)	45 (70.3%)	0.802			
synaptophysin (+)	21 (91.3%)	50 (78.1%)	0.735			
chromogranin (+)	4 (17.4%)	16 (25.0%)	0.697			
differentiation (moderate)	3 (13.0%)	2 (3.1%)	0.011			[b] –

[a]Insignificant and not presented

[b]Excluded by the variance inflation factor (VIF)

associated with NET biology was predictive of the depth of invasion in the univariate analysis and remained an independent predictor in the multivariate analysis (odds ratio [OR], 55.560; 95% CI, 4.711–655.309; $p = 0.001$). Primary tumor size >10 mm was also an independent predictor of invasion according to the univariate and multivariate analyses (OR, 38.515; 95% CI, 4.343–341.594; $p = 0.001$).

Factors predicting LN positivity

We used the same model to investigate factors that could predict LN metastasis in the multivariate analysis. We found that only invasion of the primary tumor remained an independent predictor of LN positivity (OR 14.893 CI 2.532–87.587, $p = 0.003$) in the multivariate analysis.

Discussion

According to modern data, an increasing number of patients visit hospitals with small rectal NETs because of the efficient screening program. Thus, the proportion of tumors of ≤10 mm in size with maximum invasion to the submucosa (T1) has increased over time, accounting for 45–65% of patients in recent years. This makes the local treatment of rectal NETs with good oncological outcomes feasible in many cases [1, 4, 26, 27]. In our study, we failed to include a large number of cases that were treated with local excision and radical surgery because most cases had small tumors (<10 mm) and were treated with EMR/ESD or cold polypectomy. Nonetheless, we included a small proportion of patients with tumors >10 mm in size who received bigger surgical treatments such as local excision or

radical surgery. We analyzed the effectiveness of local excision compared with radical surgery and also challenged the main idea based on NCCN and ENETS guidelines regarding the size of primary tumors that should be considered as the most important factor when treatment is planned.

We found that the groups were quite heterogeneous for analysis. Patients in the local excision group had better DFS and OS than those in the radical surgery group. However, that was because the early stage of the disease was mostly found among patients in the group with LE. The radical surgery group included patients with larger primary tumor size, LN+, higher grade and deeper invasion. However, it is worth indicating that regardless of more aggressive NETs in patients in the radical surgery group, radical surgery could not improve the oncological outcomes.

We also investigated other factors that could influence oncological outcomes. Factors such as mitotic index, Ki-67, grade, N+, PNIV and LVIV were insignificant and did not influenced DFS and OS according to the results of univariate and multivariate analysis. However, invasion appeared to be an independent factor influencing the DFS and OS of patients with rectal NETs. Patients with rectal NETs who had invasion beyond the submucosa layer had a poorer 1-, 3-, and 5-year DFS and OS compared with those having superficial invasion. Based on these results, we attempted to identify factors that influenced invasion. We found that tumor size >10 mm, mitotic index >2 and LN+ were independent predictors of the depth of invasion and thus influenced the recurrence rate and survival of patients. In contrast, we also revealed that the depth of invasion (beyond the submucosa) was an independent predictor of LN positivity, which predicted poor oncological outcomes in many previous studies. We suggest that the depth of invasion of primary NETs is the most important factor that should be considered when treatment strategies are planned. Our study also reviewed modern data found in much larger studies and discovered that where the size of the primary tumor or a more radical treatment failed to be independent factors of DFS and OS.

However, our study does have significant limitations such as its small sample size, selection bias owing to retrospective non-randomized data and group heterogeneity. We believe that more comparative prospective randomized studies with a larger number of cases are needed to corroborate our findings and investigate other prognostic factors of rectal NETs that can determine treatment strategies.

Conclusion

In summary, our findings demonstrated that local excision could be effective in treating rectal NETs in the early stage. They revealed that the depth of invasion was an important factor in influencing oncological outcomes. Nonetheless, our findings need to be confirmed in more prospective and randomized studies with a larger number of cases and more homogeneous data.

Conflict of interest: none declared

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Review article

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Neoadjuvant therapy for breast cancer patients and its impact on surgical treatment and radiotherapy (part 1.)

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Neoadjuvant therapy (NAT) is increasingly applied in patients with initially inoperable breast cancers and, frequently, in those with tumours that are initially operable, too. In most cases, the response to the applied NAT affects the scope of surgical treatment and radiotherapy, and in some situations also the complementary systemic postoperative treatment. The available studies indicate importance of response to NAT within the breast and regional lymph nodes. Assessment of response to treatment allows personalization of treatment and in some cases a change of therapy, which improves long-term outcomes.

This article summarizes the current rules of conduct in patients with early breast cancer qualified for neoadjuvant therapy, paying attention to the practical aspects and possibilities of national health insurance-covered therapies in Poland. It discusses in detail the applied regimens of systemic therapy, surgical techniques, eligibility rules and complementary radiotherapy. Systems for assessing response to neoadjuvant treatment are also presented.

Key words: breast cancer, surgery, systemic therapy, neoadjuvant therapy, adjuvant therapy

Neoadjuvant therapy (NAT) is increasingly applied in patients with initially inoperable breast cancers and, frequently, in those with tumours that are initially operable, too. In most cases, the response to the applied NAT affects the scope of surgical treatment and radiotherapy, and in some situations, also complementary systemic postoperative treatment. The available studies indicate importance of response to NAT within the breast and regional lymph nodes, allowing for treatment personalisation.

Systemic neoadjuvant therapy

The first reports of application of neoadjuvant (preoperative) chemotherapy in the treatment of patients with operable breast cancer were published by Jacquillat et al. in 1983. The authors of this study applied systemic treatment in 143 patients at the I–III stage of the disease. They observed clinical complete response (cCR) in 30% of patients. This publication triggered further clinical trials.

The first American study, NSABP B-18, which lasted from 1988 until 1993, included 1,523 patients who received (pre- or post-operatively) AC-regimen chemotherapy (adriamycin, cyclophosphamide). Among patients with preoperative treatment, 80% responded to the therapy. Clinical complete response (cCR) was observed in 36% of them, and pathological complete response (pCR) in 13%, while efficient breast-con-

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serving treatment (BCT) was achieved in 67.8% (in the group with post-operative chemotherapy – in 59.8% of the patients). In the follow-up of 5 and 9 years, no differences were found with respect to disease-free survival (DFS) and overall survival (OS) in both observed groups of patients.

Between 1995 and 2000, the NSABP B-27 study was conducted on a group of 2,411 patients. It showed that addition of docetaxel (AT regimen – adriamycin, taxotere docetaxel) to adriamycin increased the pathological complete response (pCR) rate (26% *vs.* 13%). However, no differences were found in DFS and OS between AC and AT study arms. In contrast, in the EORTC 10902 study, which assessed preoperative and postoperative treatment in FEC regimen (fluorouracil + epirubicin + cyclophosphamide) in 698 patients, a response was observed only in 49% of the patients (cCR – 7% and pCR in 1.7%). A follow-up of ten years revealed no differences in DFS and OS [1]. In a retrospective randomised clinical trials no differences in DFS and OS were found comparing preoperative and postoperative treatment [2].

Nowadays, systemic treatment allows further personalisation of therapy and better clinical and pathological response. Pathological complete response (pCR) to NAT has a positive effect on DFS and OS – especially in the case of triple-negative breast cancers (TNBC) and cancers with overexpression of human epidermal growth factor receptor 2 (HER2-positive) [2].

Indications for systemic neoadjuvant treatment

The decision to use systemic neoadjuvant therapy (NAT) at the beginning of treatment of a cancer should be taken by a multidisciplinary therapeutical team (MDT). The stage of the disease, biological subtype of the cancer, the patient's expectations and potential benefits of this treatment should be considered. Currently, NAT candidates include patients with:

1. initially inoperable breast cancer:
   - inflammatory breast cancer
   - advanced breast cancer cT4cN2/cN3;
2. initially operable breast cancer if the decision concerns performance of a breast conserving surgery in the case of limitations:
   - within the breast – a disparity between the size of the tumour and the mammary gland, if excision in the initial circumstances could lead to non-radicality or inacceptable aesthetical result.
   - within the axillary lymph nodes – metastatic regional axillary lymph nodes (cN1) when complete regression of cancerous lesions is expected;
3. initially operable breast cancer, if the performed diagnostics (especially in the case of those biological subtypes of breast cancer which are especially aggressive: HER2--positive, TNBC) would qualify the patient for post-surgical systemic treatment depending on the stage of the disease:
   - cN+/pN+,
   - at some institutions – in the case of cT ≥ 1c tumours [3].

Preoperative diagnostics

Preoperative diagnostics including interviews, physical examination, imaging, histopathological and cytological tests and other tests is described in Issue 5 of the Biblioteka Chirurga Onkologa (Oncological Surgeon's Library) titled "Chirurgiczne leczenie zmian nowotworowych piersi. II Konsensus Polskiego Towarzystwa Chirurgii Onkologicznej" ("Surgical Treatment of Breast Neoplasms. 2nd Consensus of the Polish Society for Oncological Surgery")[4].

It is particularly difficult to assess the condition of lymph nodes based on palpation and imaging tests. The primary test involves a clinical examination, although the false negative rate (FNR) in this case can be as high as 45% [5]. Metastatic cancer was found in approximately 25% of patients with cN0 stage lymph nodes, including metastases to ≥3 lymph nodes in less than 6% of cases [6].

In each case, a mammogram and ultrasound of the breast should be performed. Ultrasound with fine-needle biopsy of the suspicious lymph nodes is considered a standard diagnostic method, however, it bears a risk of inaccuracy, with its sensitivity assessed at 47–90%, specificity at 100%, and FNR at 8–24%. Sensitivity of this method is 44% for metastases <5 mm and 93% for metastases > 5 mm [5–7].

If the biopsy confirms lobular cancer, or there is a genetic background to the disease, or there are discrepancies in the spread of the disease diagnosed with mammography, US, clinical examination, and breast MRI are indicated. This technique should also be considered in cases of qualification for NAT and assessment of lesion remission during such treatment.

Management before commencement of systemic treatment

According to recommendations by many scientific oncology societies, NAT should be preceded with labelling of all diagnosed cancer foci with markers [8]. One may also consider application of similar markers at the verified (metastatic) axillary lymph node(s), if the reference oncology centre performing sentinel lymph node biopsy (SLNB) after NAT applies the TAD technique (targeted axillary dissection). TAD involves labelling of a lymph node containing a metastasis (labelling techniques are discussed below) before NAT and its targeted biopsy during SLNB afterwards. An alternative procedure involves the classical form of biopsy of sentinel lymph nodes – at least 2–3 sentinel nodes are labelled with a technique which allows their visual identification (visible staining) and with instrumental technique (probe to detect an isotope or ferromagnet).

Systemic treatment

In 2019, two important documents were published concerning rules for management of early breast cancer patients – recommendations of the European Society for Medical Oncology (ESMO) and the Consensus of Experts of the St. Gallen Conference 2019 [9, 10]. The guidelines highlight application of systemic neoadjuvant therapy in selected breast cancer patients.

According to the recommendations, the following biomarkers should always be determined for breast cancers: expression of estrogen receptors (ER), progesterone receptors (PgR) and human epidermal growth factor receptor 2 (HER2), as well as intensity of proliferation index Ki-67; and in patients with triple negative breast cancer, additionally presence of tumour infiltrating lymphocytes (TILs) [10].

Breast cancer is divided into five main subtypes, requiring a slightly different therapy:

1. ER-positive luminal A – LA cancer,
2. ER-positive luminal B – LB cancer,
3. luminal B HER2-positive cancer (HER2-LB),
4. non-luminal HER2-positive cancer (HER2-NL),
5. triple negative cancers (TNBC).

The most significant changes have been recently introduced in definition of luminal subtypes. For several years, the value of the Ki-67 proliferation index and the degree of malignancy (grade, G) have been used to distinguish them. Luminal A cancers are characterized by a low grade of malignancy (G1), high degree of expression of estrogen receptors (ER) and progesterone receptors (PgR) and a low proliferation rate (Ki-67). Meanwhile, in luminal B cancers, ER and PgR expression is lower, while the malignancy grade is higher (most frequently it's G3), and so is the Ki-67 index [11]. The proposed classification resulted in a large number of breast cancers classified as intermediate cases. This is why luminal subtypes are more easily defined by the division provided in St. Gallen recommendations of 2015, based on expression of ER, PgR, HER2 and on Ki-67:

- luminal A cancers: ER-positive, PgR ≥ 20%, HER2-negative, Ki-67 < 20–29%;
- luminal B cancers: ER-positive, HER2-negative, PgR < 20% or Ki-67 > 20–29% [12].

Systemic neoadjuvant therapy has been a standard of management in locally advanced breast cancers for years. Depending on the biological subtype, the following are applied:

- in luminal A and B cancers – hormone therapy or chemotherapy,
- in triple negative cancers – chemotherapy,
- in HER2-positive cancers – chemotherapy combined with yearly anti-HER2 therapy.

Increasingly, neoadjuvant treatment is used in initially operable breast cancers – primarily TNBC and HER2-positive ones. According to the St. Gallen consensus of 2019 and ESMO guidelines of 2019, such therapy is indicated for breast cancers >2 cm and/or cytologically confirmed metastatic lymph node (cT2 and/or cN+, i.e. II stage of the disease)

This approach reflects a tendency to limit the scope of surgery in favour of conservative treatment in the area of the breast and axillary lymph node. Some publications have also shown that systemic neoadjuvant therapy may be beneficial for patients with cancers >1 cm, too [13] neoadjuvant treatment with a combination of sequential chemotherapy and HER2-targeted therapy is currently the standard of care. This is followed by breast surgery, radiotherapy (if indicated. Similar treatment opportunities for patients with HER2-positive cancers are provided by the drug prescription programme currently implemented in Poland [14]. Considering the currently binding list of reimbursed drugs in the case of anti-HER2 drugs applied in treatment of the early stage of breast cancer, it seems that inclusion of patients with cT1c stage of cancer in neoadjuvant therapy improves distant treatment results. Many European centres specialising in breast cancer treatment accept this opinion, too [15]. A document by the Department of Breast Cancer & Reconstructive Surgery of the National Research Institute of Oncology confirmed especially high rate of pathological complete remissions achieved in patients included in TCH-regimen neoadjuvant therapy (docetaxel, carboplatin, trastuzumab) – 55% in the group of patients with cancers of 10–50 mm, cN0 or cN1 (while pCR rate in the subgroup of patients with non-luminal HER2-positive cancers was 66%). Meanwhile, in a cohort of patients included in the TCH-P regimen (doctaxel, carboplatin, trastuzumab, pertuzumab), pCR rate was 76% (while in the group of patients with non-luminal, HER2-positive cancers, pCR as high as 87% was observed, especially for less advanced cancers), which will probably affect distant results of the treatment [16, 17] trastuzumab and carboplatin (TCH.

Application of systemic neoadjuvant therapy enables also verification of efficiency of the applied cytotoxic drugs in an individual patient by follow-up of changes in the size of the breast tumour and/or metastatic lymph nodes. At the Department of Breast Cancer & Reconstructive Surgery of the National Research Institute of Oncology, eligibility for neoadjuvant therapy includes also patients with diagnosed TNBC tumours assessed at up to cT1C cN0 stage of cancer.

The clinical trial results published within only the last 2–3 years led to introduction of patient selection for adjuvant therapy based on histopathological results of the operated material. Achieving pCR is an important factor which improves prognosis in patients with triple-negative and HER2-positive breast cancers [18]such as disease-free survival, event-free survival (EFS). Therefore, therapy should be focused on maximising the group of patients with pathological complete response (pCR). This can be achieved with intensive systemic treatment. In patients with diagnosis of TNBC the preferred chemotherapy regimen is the one with reduced intervals between cycles (*dose-dense chemotherapy)*. In such cases, primary prophylaxis of neutropenic fever with granulocyte growth factor is necessary. Besides, in addition to paclitaxel administered in the second step of cytostatic treatment, inclusion of carboplatin may be considered (ACdd regimen – doxorubicin, cyclophosphamide every second week, later paclitaxel +/– carboplatin every week). Meanwhile in patients diagnosed with II and III stage of HER2-positive cancer, double blockade of HER2 receptor (pertuzumab with trastuzumab) is indicated

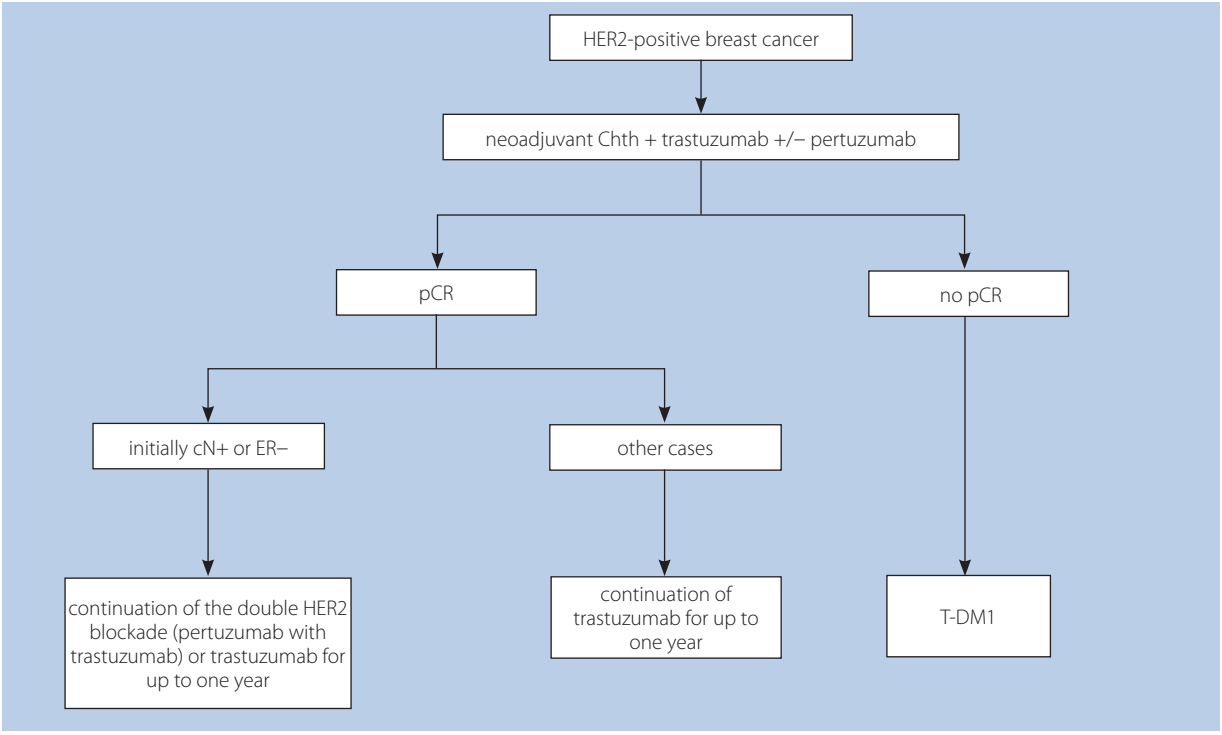

Figure 1. Perioperative treatment of patients with HER2-positive breast cancer – based on ESMO recommendations (2019) [9]

in combination with chemotherapy. Two treatment regimens are recommended: AC, then paclitaxel and PT (pertuzumab, trastuzumab) or TCHP (docetaxel, carboplatin, pertuzumab, trastuzumab). The current Polish drug prescription programme allows for pre-operative treatment with pertuzumab with trastuzumab in the case of III stage or breast tumours >2 cm with absent expression of ER and PgR or else with cytologically confirmed metastasis to an axillary lymph node [14].

The timing of application of HER2 double blockade is controversial. According to the ESMO recommendations, an annual trastuzumab with pertuzumab therapy should be considered in patients with higher risk of recurrence: initial cN+ stage or ER/PgR(–). Treatment with double anti-HER2 blockade combined with chemotherapy starts before or after the surgery. In Poland, only pre-operative treatment is covered by health insurance.

Further, in patients with HER2-LB breast cancer at high risk of recurrence (N+), prolonged complementary treatment can be considered, applying neratinib for a year after completion of trastuzumab therapy, provided that pertuzumab was not used.

An important change in the procedure concerns the choice of therapy based on pathology result of the surgical material. KATHERINE study revealed that in patients who did not achieve pCR in the surgical material, complementary T-DM1 treatment (trastuzumab, emtansine) is more effective than follow-up trastuzumab therapy after the surgery [19]. In December 2019, T-DM1 drug was registered for this application.

Similarly, results of the CREATE-X study were significant in the case of patients of TNBC [20]. They showed that if residual disease was found in the material after the surgery, additional complementary treatment with capecitabine reduced recurrence risk and improved the patients' survival.

The results of these two important clinical trials changed the management standard and have been included for the first time in ESMO and St. Gallen guidelines in 2019. The rules of management are summarised in figures 1 and 2.

Another strategy applies to patients with luminal cancers. If a breast-sparing surgery is initially possible, it should be performed, and then, based on the pathology results, a decision concerning systemic complimentary treatment should be taken. For other cases, neoadjuvant therapy is indicated. It often consists of chemotherapy. Meanwhile, in

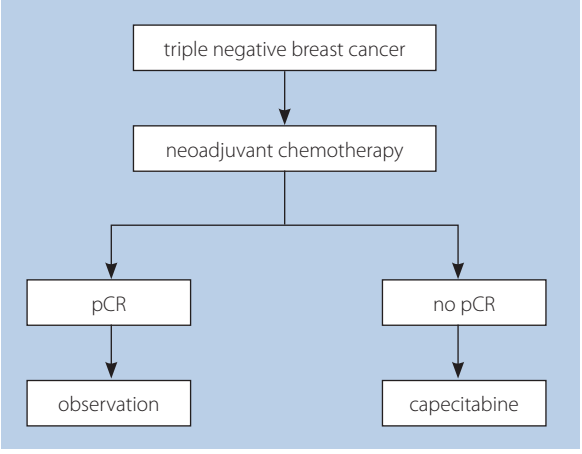

Figure 2. Perioperative treatment of patients with triple-negative breast cancer – based on ESMO recommendations (2019) [9]

post-menopausal patients with locally advanced LA cancer, neoadjuvant hormone therapy can be considered. Letrozole is the most frequently selected substance for such cases. It should be borne in mind that in LA/LB cancers, after systemic neoadjuvant therapy, the rate of pathological complete response is low, but usually these responses are not a valuable factor in prognosis. In pre-menopausal patients, neoadjuvant hormone therapy is not applied.

Neoadjuvant chemotherapy is a standard in management for all patients diagnosed with inflammatory breast cancer. After a surgery (radical mastectomy with no simultaneous reconstruction) radiotherapy is mandatory. Application of hormone therapy and anti-HER2 treatment (including within neoadjuvant therapy) depends on the condition of respective receptors. Table I presents pre- and post-operative regimens applied at the Department of Breast Cancer & Reconstructive Surgery of the National Research Institute of Oncology in Warsaw.

In recent years, important progress has been observed in treatment of patients with breast cancer. Registration of new drugs was among the factors of this progress. Most have been registered for treatment of patients with generalised breast cancer, however, application of these drugs in peri-operational treatment is currently explored, too. Results of the conducted studies will determine whether the drugs will be included as a standard in treatment of early breast cancer patients. Table II summarises the current indications for application of newly approved drugs.

It is very important to include patients in surgical treatment carefully and as quickly as possible, within 2–4 weeks

Table I. Pre-operative regimens applied at the Department of Breast Cancer & Reconstructive Surgery of the National Research Institute of Oncology

Treatment regimen	Treatment steps
HER2-positive breast cancer	
Neoadjuvant systemic treatment (cT1c-cT4, cN0-cN2)	
TCH x 6 regimen	• TCH regimen: – docetaxel 75 mg/m^2 + carboplatin AUC6 + trastuzumab *i.v.* 8 mg/kg of body weight – saturating dose, 6 mg/kg of body weight – maintenance doses; courses: 6 x every 3 weeks (+peg-GCSF) • surgery • continuation of trastuzumab up to 18 courses in total • radiation therapy, if indicated
	note: preferred regimen in patients with no history of internal disease and without indications for double anti-HER2 blockade
TCH-P x 6 regimen	• TCH-P6 regimen: – docetaxel 75 mg/m^2 + carboplatin AUC5-6 + trastuzumab *i.v.* 8 mg/kg of body weight – loading dose, 6 mg/kg of body weight – maintenance doses, pertuzumab *i.v.* 840 mg – loading dose, 420 g – maintenance doses; courses: 6 x every 3 weeks (+peg-GCSF) • surgery • continuation of trastuzumab up to 18 courses in total • radiation therapy, if indicated
	note: preferred regimen in patients with no history of internal disease and with indications for double anti-HER2 blockade (HER2 non-luminal cancers; T > 2 cm + pN+)
sequential regimen ACdd-D + T	• sequential regimen: – 4 x AC (doxorubicin 60 mg/m^2 + cyclophosphamide 600 mg/m^2) every 2 weeks (+peg-GCSF) – 4 x docetaxel 100 or 75 mg/m^2 every 3 weeks + trastuzumab *i.v.* 8 mg/kg of body weight – loading dose, 6 mg/kg of body weight – maintenance doses • surgery • continuation of trastuzumab up to 18 courses in total • radiation therapy, if indicated
PCL x 12 + T regimen	• PCL 12 regimen: – paclitaxel 60–80 mg/m^2, every 7 days x 12 + trastuzumab *i.v.* 8 mg/kg of body weight – loading dose, 6 mg/kg of body weight – maintenance doses • surgery • continuation of trastuzumab up to 18 courses in total • radiation therapy, if indicated
	note: regimen administered in patients with significant history of internal diseases and elderly
complementary systemic treatment (pT1c-pT4, pN0-pN2)	
TCH-6 regimen	• surgery • TCH regimen: – docetaxel 75 mg/m^2 + carboplatin AUC6 + trastuzumab *i.v.* 8 mg/kg of body weight – loading dose, 6 mg/kg of body weight – maintenance doses; courses: 6 x every 3 weeks (+peg-GCSF) • continuation of trastuzumab up to 18 courses in total • radiation therapy, if indicated

Treatment regimen	Treatment steps
sequential regimen ddAC-D+T	• surgery • sequential regimen: – 4 x AC (doxorubicin 60 mg/m^2 + cyclophosphamide 600 mg/m^2) every 2 weeks (+peg-GCSF); then 4 x docetaxel 100 or 75 mg/m^2 every 3 weeks + trastuzumab *i.v.* 8 mg/kg of body weight – loading dose, 6 mg/kg of body weight – maintenance doses • continuation of trastuzumab up to 18 courses in total • radiation therapy, if indicated
PCL x 12 + T regimen	• surgery • PCL 12 regimen: – paclitaxel 60–80 mg/m^2, every 7 days x 12 + trastuzumab *i.v.* 8 mg/kg of body weight every 3 weeks – loading dose, 6 mg/kg of body weight – maintenance doses • continuation of trastuzumab up to 18 courses in total • radiation therapy, if indicated
	note: regimen administered to patients diagnosed with HER2-positive luminal cancers at pT1c, pN0 stage or with significant history of internal diseases and elderly
complementary systemic treatment (pT1b, N0)	
PCL x 12 regimen	• surgery • PCL regimen: – paclitaxel 80 mg/m^2, every 7 days x 12 cycles
	note: regimen for patients with HER2-positive, non-luminal cancers
TNBC breast cancer	
neoadjuvant systemic treatment (cT1-cT4, cN0-cN2)	
ddAC PCL + Carbo regimen	• sequential regimen: – 4 x AC (doxorubicin 60 mg/m^2 + cyclophosphamide 600 mg/m^2) every 2 weeks (+peg-GCSF) – followed by 12 x paclitaxel 80 mg/m^2 + carboplatin AUC 1.5 every week • surgery • radiation therapy, if indicated
TCarbo regimen	• TC regimen: – docetaxel 75 mg/m^2 + carboplatin AUC 5–6, 6 x every 3 weeks • surgery • radiation therapy, if indicated
	note: regimen applied in patients with counterindications against anthracyclines
PCarbo regimen	• PC regimen: – paclitaxel 60–80 mg/m^2 + carboplatin AUC 1.5–2 every week x 18 • surgery • radiation therapy, if indicated
	note: regimen applied in patients with counterindications against anthracyclines
PCL x 12 regimen	• PCL 12 regimen: – (paclitaxel 60-80 mg/m^2 every 7 days) x 12 • surgery • radiation therapy in patients with indications
	note: regimen administered in patients with significant history of internal diseases and elderly
complementary systemic treatment (pT1b–pT4, pN0–pN2)	
AC-P regimen	• surgery • sequential regimen: – 4 x AC (doxorubicin 60 mg/m^2 + cyclophosphamide 600 mg/m^2) every 3 weeks; – followed by 12 x paclitaxel 80 mg/m^2 every week • radiation therapy, if indicated
TC x 4 regimen	• surgery • TC regimen: – 4 x every 3 weeks: docetaxel 75 mg/m^2 + cyclophosphamide 600 mg/m^2 • radiation therapy, if indicated
	note: regimen recommended in patients with counterindications against anthracyclines or with other history of internal diseases and with pT1c N0, G – 2
PCL x 12 regimen	• surgery • PCL 12 regimen: – Paclitaxel 60–80 mg/m^2, every 7 days x 12 • radiation therapy, if indicated
	note: regimen applied in patients at pT1b, N0 stage or significant history of internal diseases or in elderly patients

Treatment regimen	Treatment steps
complementary systemic treatment – post-neoadjuvant	
capecitabine regimen	• capecitabine x 8 every 3 weeks, at a dose of 2000–2500 mg/m^2/day for 14 days, followed by 7 days of rest period
	note: regimen for patients after a surgery with residual disease after prior neoadjuvant therapy and after completion of radiation therapy (if indicated)
luminal breast cancer	
systemic neoadjuvant therapy (cT2 – cT4, cN0-N2 – patients with luminal B, HER2-negative cancer, G3 and/or Ki-67 > 50% and cT3-cT4, cN0-N2; HER2 negative luminal A and B cancers	
AC-P regimen	• sequential regimen: – 4 x AC (doxorubicin 60 mg/m^2 + cyclophosphamide 600 mg/m^2) every 2 weeks (+ peg-GCSF); then 12 x paclitaxel 80 mg/m^2 every week • surgery • radiation therapy, if indicated
PCL regimen	• PCL regimen: – paclitaxel 60–80 mg/m^2 x 12–18 every week • surgery • radiation therapy, if indicated
	note: regimen administered in patients with counterindications against anthracyclines, in patients with significant history of internal diseases and elderly
complementary systemic treatment (luminal A and B cancers: stage IIIA-C, pT1c-pT3, pN0-pN1; luminal B cancer, G3, +/– indications from multigene tests or Magee > 31)	
AC-PCL regimen	• surgery • sequential regimen: – 4 x AC (doxorubicin 60 mg/m^2 + cyclophosphamide 600 mg/m^2) every 3 weeks; – followed by 12 x paclitaxel 80 mg/m^2 every week • radiation therapy, if indicated
AC-D regimen	• surgery • sequential regimen: – 4 x AC (doxorubicin 60 mg/m^2 + cyclophosphamide 600 mg/m^2) every 3 weeks; followed by – 4 x docetaxel 75–100 mg/m^2 • radiation therapy, if indicated
TC x 4 regimen	• surgery • TC regimen: – 4 x every 3 weeks: docetaxel 75 mg/m^2 + cyclophosphamide 600 mg/m^2 • radiation therapy, if indicated
	note: regimen recommended in patients with counterindications against anthracyclines or with other history of internal diseases
PCL x 12 regimen	• surgery • PCL 12 regimen: – paclitaxel 60–80 mg/m^2 every 7 days x 12 • radiation therapy, if indicated
	note: regimen administered in patients with significant history of internal diseases and elderly

Table II. Newly approved drugs for treatment of breast cancer patients

Biological subtype of breast cancer	Drug group	Drug	Recorded indication	Pending clinical trials in new therapeutic areas
ER + HER2–	CDK4/6 inhibitor	palbociclib ribociclib abemaciclib	generalized breast cancer: 1st or 2nd line in combination with hormone therapy (aromatase inhibitor or fulvestrant)	adjuvant treatment in patients at high risk of recurrence in combination with hormone therapy (studies by Pallas, Natalee, MonarchE)
Ttriple negative breast cancer	immunotherapy	atezolizumab	generalized breast cancer PD-L1 + in the 1st line of treatment in combination with chemotherapy	adding the drug as part of perioperative therapy
BRCA mutation carriers HER2–	PARP inhibitor	olaparib talazoparib	generalised breast cancer, in the 1st or 2nd line of treatment	adjuvant treatment in patients at high risk of recurrence (Olimpia study)
HER2+ ER+	tyrosine kinase inhibitor	neratinib	extended adjuvant treatment of breast cancer after one year of trastuzumab therapy in patients at high risk of recurrence (N+), if pertuzumab was not applied	planned as adjuvant treatment of patients previously receiving pertuzumab

after completing NAT. According to most centres, operations were performed on average 28 days after the last course of chemotherapy. NAT complications delayed surgery by approximately 8 days [21, 22].

Response assessment during systemic neoadjuvant therapy

Response to NAT, both in the breast and regional lymph nodes, should be assessed by clinical examination and imaging after the systemic treatment, analogically to tests performed before the treatment [4]. Response to NAT should be assessed on each day of chemotherapy administration and the assessment may be based on clinical evaluation [24] . Imaging complete response evidenced by magnetic resonance mammography performed after NAT does not define pCR precisely enough either in the breast or in lymph nodes. This is why guidelines by different organisations are equivocal in determining the meaning of breast MRI (magnetic resonance imaging) in decisions on the scope and type of the operation [25, 26] inadequate staging with subsequent over or under-treatment, and surgical complications. Areas covered: This review article aims to discuss these concerns and to clarify the adequate steps and procedures needed to increase safety and alleviate the possible drawbacks of NAC. The author will discuss the adequate and meticulous technical procedures needed to stage and localize the breast tumor, detect any affected axillary lymph node, improve the accuracy and safety of doing sentinel lymph node biopsy (SLNB.

In a vast majority of institutions which perform sentinel lymph node biopsy (SLNB) in patients with primary cN1 cancer with conversion to ycN0 after the systemic neoadjuvant therapy, decisions are based on clinical examination, potentially applying ultrasound evaluation of axillary lymphatic drainage. Accuracy of the clinical examination was estimated at 60% (PPV and NPV also 60%), and in the case of ultrasound – at 69% (PPV – 65%, NPV – 74%) [27, 28] there is still some degree of reluctance in applying sentinel node biopsy (SNB).

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Neuroendocrine neoplasms of the digestive system – current classification and terminology

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The system of classification and terminology of neuroendocrine neoplasms (NENs), updated in 2017 by AJCC and in 2019 by WHO, is now recommended for general use. This article is a review of this classification with respect to NENs of the digestive tract. Within the new system, two categories of neuroendocrine neoplasms of the digestive system were introduced, differing in morphology, clinical course and treatment, as based on differentiation and histological maturity grading (G). Among NENs of the digestive tract, well differentiated neuroendocrine tumours, with Ki-67 proliferation index below 20%, NET G1 and NET G2, histologically resembling normal neuroendocrine cells were distinguished. Neuroendocrine neoplasms with Ki-67 above 20% – termed neuroendocrine carcinoma NEC (poorly differentiated carcinoma G3) – were found to be heterogeneous. In every organ of the digestive tract a limited group of well differentiated tumours with Ki-67 above 20%, but typically less than 55% (well differentiated high grade NET G3) was distinguished. The remaining poorly differentiated neuroendocrine neoplasms with Ki-67 above 20%, usually over 55%, were classified as NEC (high grade neuroendocrine carcinoma). Within NEC, two groups were distinguished – large cell and small cell carcinomas. By introducing this new classification based on clinical and molecular research, any confusion between NET G3 and NEC is avoided. NEC, goblet-cell carcinoid of the appendix and MiNEN, which should be classified according to criteria applied to adenocarcinomas of their respective organs of the digestive system, are not discussed.

Key words: neuroendocrine neoplasms, NET, NEC, G grading system, TNM classification, staging

Introduction

Neuroendocrine neoplasms (NENs) are rare neoplasms found throughout the body. They originate from endocrine organs, the nervous system (peptidergic neurons) or from neuroendocrine cells of the diffuse endocrine system (DES). Here, only NENs present in the gut and pancreas will be discussed.

According to recent epidemiology studies in the US, based on The Surveillance, Epidemiology and End Results (SEER) programme [1], there is an increase in NEN incidence. Currently, the yearly incidence of these neoplasms is estimated at about 35 cases per 100 000 individuals. Of these, about 70% are gastroenteropancreatic neuroendocrine neoplasms (GEP NENs), constituting only 2% of all neoplasms of the digestive system [2, 3]. With respect to their embryonic development, NENs of the digestive system are classified as those of the foregut (i.e. from the oropharynx to the upper duodenum, liver, gallbladder, pancreas), the midgut (middle part of the duodenum to the right two-thirds of the transverse colon) and the hindgut (the left one-third of the transverse colon including the upper anal canal) [4].

Histopathological classification of NENs

In its Neuroendocrine Neoplasm/Neuroendocrine Tumour (NEN/NET) classification system, the European Neuroendocrine

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Tumour Society (ENETS) considers the type of cell, organ location and histological type including differentiation. According to ENETS, the histological maturity of the tumour (G – grading) is of main clinical significance [5]. The pTNM classification of the tumour [6] and clinical advancement staging (S) [5] need also to be considered.

The histological tumour maturity grading (G) is a microscopic feature of prognostic value in treating NENs of the digestive system. It is an independent predictive parameter of clinical outcome for patients with low (G1), intermediate (G2) or high (G3) NEN malignancy.[7–11]. The criteria for determining the histological malignancy grading of NENs based on mitotic and Ki-67 proliferation indices are presented in table I [3].

The mitotic index is the number of mitotic figures in hot spots counted in no less than ten HPFs (high power fields, 2 mm^2) at 40x magnification. The Ki-67 proliferation index is evaluated by immunocytochemistry (ICH) with MIB1 antibody as the percentage of cells presenting a positive reaction, counted in 500–2000 tumour cells. Selection of the higher value of these two indices is recommended as the G grade. NEN classification systems and therapeutic decisions rely on the G grade, as based on those two indices [3, 6, 12–14].

The grading system developed by ENETS for all NETs arising in the pancreas and gastrointestinal tract was adopted by the World Health Organization (WHO) in 2010. Within this system, two categories of neuroendocrine neoplasms of the digestive system were introduced, differing in morphology, clinical course and treatment, as based on differentiation and histological maturity grading (G) [15]. The first category consisted of well differentiated neoplasms (termed carcinoids prior to the year 2000), the Ki-67 proliferation index of which ranges between 0–20%, NET G1 and NET G2 (well differentiated neuroendocrine tumours: G1, G2). Histologically, NET G1 and NET G2 cells resemble normal neuroendocrine cells, expressing neuroendocrine markers (synaptophysin and chromogranin A [CgA]) and site-dependent hormones, low or medium nuclear atypia, and no more than 20 mitotic figures per 10 HPFs. Tumours classified as NET G1 or NET G2 should be treated according to standards pertaining to well differentiated neuroendocrine tumours [3,16–18].Within the 2010 WHO classification, the second category included neuroendocrine neoplasms with a Ki-67 proliferation index above 20%, termed neuroendocrine carcinoma NEC (poorly differentiated carcinoma G3). According to the 2017 Eighth Edition of the American Joint Committee on Cancer (AJCC) [11] and the new 2019 WHO Classification of Digestive System Tumours (presented in the 5th edition of the WHO Classification of Tumours series [13]), the group of poorly differentiated neuroendocrine neoplasms G3 was found to be heterogeneous [7,19]. In every organ of the digestive system a limited group of well differentiated tumours with Ki-67 proliferation index above 20%, typically ranging between 21 and 55% (well differentiated high grade NET G3) was distinguished [9, 10, 13, 20–24]. Unlike in the case of NET G3, the remaining poorly differentiated neuroendocrine neoplasms with Ki-67 proliferation index above 20%, usually over 55%, were classified as NECs (high grade neuroendocrine carcinoma). Within NEC, two groups were distinguished – large cell or small cell carcinomas, the course of the latter resembling that of aggressive small cell lung cancer. NEC strongly express synaptophysin and weakly express chromogranin A, demonstrate apparent nuclear atypia and over 20 mitotic figures per 10 HPFs. By introducing this new classification, based on clinical and molecular research, the confusion between NET G3 and NEC is avoided [13]. The currently proposed classification of NENs, which includes 2017 AJCC and 2019 WHO recommendations, is presented in table II.

Mixed neoplasms with exo- and endocrine components, earlier classified as mixed adenoneuroendocrine carcinoma

Table I. Grading criteria for assessing malignancy of neuroendocrine neoplasms [3, 11]

Histological malignancy grade of NEN (G)	Mitotic activity/no. of mitotic figures/10 HPF	Ki-67 proliferation index/% of cells (per 2,000 cells)
G1 – well differentiated, low grade	<2	<3
G2 – moderately differentiated, intermediate grade	2–20	3–20
G3 – poorly differentiated, high grade	>20	>20

Table II. Neuroendocrine neoplasms grading according to WHO 2019 and AJCC 2017 [11, 13–14]

Neuroendocrine neoplasm (NENs)			
NET G1	well-differentiated tumours	proliferation index <20%	well-differentiated tumours with Ki-67 proliferation index below 3%
NET G2	well-differentiated tumours		well-differentiated tumours with Ki-67 proliferation index from 3% to 20%
NET G3	well-differentiated tumours	proliferation index >20%	well-differentiated tumours with Ki-67 proliferation index usually between 21 and 55%
NEC	neuroendocrine cancers poorly-differentiated		neuroendocrine cancers with proliferation index above 21%, usually above 55% – large-cell cancers – small-cell cancers

(MANEC) [5, 15], are presently termed mixed neuroendocrine--non-neuroendocrine carcinoma (MiNEN) if both components are distinguishable, with each component to be graded individually [13, 25].

NEC, goblet-cell carcinoid of the appendix and MiNENs should be classified according to classical criteria applied to adenocarcinomas of organs of the digestive system, and are not discussed here.

Genetics

Current knowledge of genetics and molecular differences between different types of NENs stimulated a meeting of experts at the International Agency for Research on Cancer (IARC) in November 2017. A consensus was proposed to distinguish between well differentiated neuroendocrine tumours (NETs) and poorly differentiated neuroendocrine carcinomas (NECs) at all sites where these neoplasms arise, as based on differences in their molecular structure [8]. Mutations in MEN1, DAXX and ATRX are found in well-differentiated NETs, while NECs usually carry *TP53* or *RB1* mutations [26]. The 2017 IARC consensus has been incorporated in the 2019 WHO 5th Edition of classification of neuroendocrine neoplasms [20].

Neuroendocrine tumours of the stomach (gastric NETs)

Within the gastric NET group, the 2017 AJCC staging system includes gastric "carcinoid" tumours (NET G1 and G2, and rare well-differentiated G3). High-grade neuroendocrine carcinoma (NEC) and mixed adenoneuroendocrine carcinoma MiNEN) are staged according to the classification of stomach adenocarcinomas.

The following changes were introduced in the 2017 AJCC staging system: stage Groups I–IV were condensed, i.e. substages A and B were excluded; gastrin was added as an additional recommended clinical care factor; pancreastatin was added as an emerging prognostic factor of clinical care [9]. Over the years 1975–2014, the yearly incidence of gastric neuroendocrine tumours has increased from 0.31 to 4.85 per 100 000 individuals [27]. This most likely results from the availability of more sophisticated methods and diagnostic tools in endoscopy, laboratory tests or nuclear medicine.

Gastric NETs may develop from different cells: histamine--producing enterochromaffin-like (ECL) cell NETs located in the corpus/fundus, somatostatin-expressing D-cell and gastrin-expressing G-cell NETs located in the antrum, or rare enterochromaffin-cell NETs producing serotonin, located in the antrum and corpus/fundus. Measurements of gastric pH, α-intrinsic factor or α-parietal cell antibody and gastrin levels are useful in differentiating between the three different types of gastric ECL NETs and in diagnosing type I, type II and type III gastric NET [11,13, 28–31].

Several prognostic factors may also be useful in diagnosing these three types of gastric NETs: gastrin is expected to be elevated in type I and type II gastric NETs [31], while gastrin is expected to remain within the normal range in type III gastric NETs.[13]. CgA is a general NET marker, however with known limitations [32]. Plasma or serum CgA is used as a marker in patients with gastric NETs. Higher CgA levels are associated with a worse prognosis [31]. Moreover, changes in the CgA level within follow up may be useful in the prognosis of recurrence after surgery or the response to therapy of metastatic disease patients [33].

Type 1 gastric NET

Type 1 gastric NETs, composed of ECL-cells are most common and typically occur as multiple small polyps in the corpus or fundus. These NETs are associated with autoimmune chronic atrophic gastritis, causing hypochlorhydria and leading to hypergastrinemia. Type 1 gastric NETs rarely metastasize. The 5-year survival rate of patients is close to 100%.

Type 2 gastric NET

Type 2 gastric NETs are rare ECL-cell tumours diagnosed in patients with multiple endocrine neoplasia type 1 (MEN1) presenting with multiple gastrinoma of the duodenum or pancreas, leading to secondary hypergastrinemia. These tumours exhibit a more aggressive phenotype with metastases in 10–30% of cases. The 5-year survival rate of patients is 60–90%. As type II gastric NETs lead to the Zollinger–Ellison syndrome, the gastric pH on endoscopy is typically very low, due to high acidity levels.

Type 3 gastric NET

Type 3 gastric NETs are sporadic tumours of no specific etiology (such as atrophic inflammation or MEN1). These solitary tumours with normogastrinemia have the worst prognosis of all three ECL cell NETs (50% metastasize). The 5-year survival rate of patients does not exceed 50% [28–30].

Gastric NENs of type 1 and type 2 are usually graded as well differentiated NETs G1 and NETs G2. Type 3 Gastric NENs are graded as NETs G3 or poorly differentiated NECs [9, 13, 21, 25]. Gastric NECs and MiNENs are usually located in the antrum or in the cardiac regions [34]. Gastric NECs usually deeply infiltrate the gastric wall. Gastric NECs and MiNENs have a poor prognosis, progress rapidly and take an aggressive course [28]. The TNM classification and staging of gastric NETs are given in tables III and IV.

Neuroendocrine tumours of the duodenum and the ampulla of Vater

The 2017 AJCC staging system applies to well-differentiated neuroendocrine tumours of the duodenum and the ampulla of Vater. Carcinomas of the ampulla of Vater, including high-grade, poorly differentiated neuroendocrine carcinomas are not staged within this system and should be classified according to classical criteria applied to adenocarcinomas of organs of the digestive system.

Table III. AJCC 2017 TNM classification for neuroendocrine tumours of the stomach [9, 11]

Definition of primary tumour (T)	
T category	**T criteria**
TX	primary tumour cannot be assessed
T0	no evidence of primary tumour
T1*	tumour invades the lamina propria or submucosa and is less than or equal to 1 cm in size
T2*	tumour invades the muscularis propria or is greater than 1 cm in size
T3*	tumour invades through the muscularis propria into subserosal tissue without penetration of overlying serosa
T4*	tumour invades visceral peritoneum (serosa) or other organs or adjacent structures
Definition of regional lymph node (N)	
N category	**N criteria**
NX	regional lymph nodes cannot be assessed
N0	no regional lymph node metastasis
N1	regional lymph node metastasis
Definition of distant metastasis (M)	
M category	**M criteria**
M0	no distant metastasis
M1	distant metastasis
M1a	metastasis confined to liver
M1b	metastases in at least one extrahepatic site (e.g., lung, ovary, nonregional lymph node, peritoneum, bone)
M1c	both hepatic and extrahepatic

*For any T, add (m) for multiple tumours [TX(#) or TX(m), where X = 1–4 and # = number of primary tumours identified; for multiple tumours with different Ts, use the highest

Table IV. AJCC 2017 prognostic stage groups for neuroendocrine tumours of the stomach [9, 11]

TNM	Stage group
T1 N0 M0	I
T2–T3 N0 M0	II
T4 N0 M0	III
any T N1 M0	III
any T, any N M1	IV

The following changes were introduced by the 2017 AJCC staging system: neuroendocrine tumours of the duodenum and ampulla, being different in tumour biology and prognosis, are now considered separately from those in the jejunum and ileum. The Tis (tumour *in situ*) distinction has now been eliminated [10, 11].

Over the years 1983–2010, the yearly incidence rate of duodenal NETs was observed to increase from 0.27 to 1.1 per 100 000 individuals [36]. The duodenal NET outcome relies on the histologic grade, depth of invasion and size of the tumour [36]. Duodenal NETs (95%) are mostly located in the first part or in the ampullary region of the duodenum. NETs arising in the ampulla of Vater are extremely rare but are often larger and of higher grade (G3), and frequently metastasize – even while being small and of low grade (G1, G2). Poorer overall survival than in the case of duodenal NETs can be expected [37]. Most duodenal NETs are below 2 cm in diameter, usually without lymph node involvement [38]. However, gastrinomas may metastasize, despite being very small in size (<1 cm) [36].

Most duodenal NETs are non-functioning. Gangliocytic paragangliomas contain NET-like elements but also show variable amounts of ganglion-like cells and spindled Schwann cells. Being indolent, they typically do not recur after resection. Gangliocytic paraganglioma and somatostatin-expressing NET occur almost exclusively in the ampullary and periampullary region [29]. Less frequent are functioning duodenal NETs – gastrinomas associated with the Zollinger-Ellison syndrome (ZES), which usually occur in the duodenum (60–80% of cases) and pancreas. However, duodenal/ampullary NETs may produce somatostatin (about 1% of gastrointestinal NETs), adrenocorticotropic hormone, VIP or serotonin, leading to the traditional carcinoid syndrome [10, 11, 38].

Not much is known about the etiology of NETs of the duodenum/ampulla of Vater. Most of these NETs are sporadic, however a small fraction (below 10%) is ascribed to hereditary cancer syndrome, such as multiple endocrine neoplasia type 1 (MEN1), neurofibromatosis type 1 (NF1) and von Hippel-Lindau (VHL) syndrome. Patients with MEN1 develop multiple duodenal gastrinomas, patients with NF1 – somatostatin expressing tumours [10, 25, 29, 39].

Most duodenal NETs are well-differentiated (G1 and G2) tumours [12]. NECs (G3 by definition) of the small bowel occur only within the ampullary region and should be staged as carcinomas in this location [37]. An adenocarcinoma component may also be present in MiNENs. The TNM classification and staging of NETs of the duodenum and ampulla of Vater are given in tables V and VI.

Neuroendocrine tumours of the jejunum and ileum

The 2017 AJCC staging system applies to neuroendocrine tumours of the jejunum and ileum. These include small bowel "carcinoid" tumours NET G1 and G2, and rare well-differentiated NET G3 arising in these locations. High-grade neuroendocrine carcinomas (NEC) and mixed adenoneuroendocrine carcinomas (MiNEN) should be classified according to classical criteria applied to the small intestine.

The following changes were introduced by the 2017 AJCC staging system: a new classification of nodal involvement, N2, is proposed; stages I–IV were condensed, i.e. substages A and B were excluded; the duodenum is considered separately; neurokinin A (NKA) was added as a possible prognostic factor for clinical care [41].

Over the years 1973–2012, the yearly incidence of well-differentiated small intestinal NETs varied between 0.32 in England and 1.2 in the US, per 100 000 inhabitants [42, 43]. The location of jejunoileal NETs is mainly in the distal part of the ileum, close to the ileocaecal valve. Therefore diagnostics of these NETs depends on their accessibility in routine endoscopy. About 33% of those NETs in the small intestine are multifocal. Jejunal or ileal NETs are usually small, growing at a slower rate than adenocarcinoma, but disseminating to the locoregional lymph node and liver [43–45]. Due to the absence of clinical symptoms, diagnosis is typically delayed until the tumour has metastasized to the liver [16, 40]. However, despite this advanced presentation, the prognosis for patients is reasonably favourable. A higher risk of long-term recurrence is suggested in patients with nodal metastases, mesenteric involvement and lymphovascular or perineural invasion [42, 43, 45, 46].

Intestinal NENs are either functioning or non-functioning NETs. Functioning NETs are mostly composed of enterochro-

Table VI. AJCC 2017 prognostic stage groups for neuroendocrine tumours of the duodenum and the ampulla of Vater [10, 11]

TNM	Stage group
T1 N0 M0	I
T2–T3 N0 M0	II
T4 N0 M0	III
any T N1 M0	III
any T, any N M1	IV

Table V. AJCC 2017 TNM classifications for neuroendocrine tumours of the duodenum and the ampulla of Vater [10, 11]

Definition of primary tumour (T)	
T category	**T criteria**
TX	primary tumour cannot be assessed
T1	tumour invades the mucosa or submucosa only and is ≤1 cm (duodenal tumours); tumour ≤1 cm and confined within the sphincter of Oddi (ampullary tumours)
T2	tumour invades the muscularis propria or is >1 cm (duodenal);
	tumour invades through the sphincter into the duodenal submucosa or muscularis propria, or is >1 cm (ampullary)
T3	tumour invades the pancreas or peripancreatic adipose tissue
T4	tumour invades the visceral peritoneum (serosa) or other organs
Definition of regional lymph node (N)	
N category	**N criteria**
NX	regional lymph nodes cannot be assessed
N0	no regional lymph node involvement
N1	regional lymph node involvement
Definition of distant metastasis (M)	
M category	**M criteria**
M0	no distant metastasis
M1	distant metastases
M1a	metastasis confined to liver
M1b	metastases in at least one extrahepatic site (e.g., lung, ovary, nonregional lymph node, peritoneum, bone)
M1c	both hepatic and extrahepatic metastases

Multiple tumours should be designated as such (and the largest tumour should be used to assign the T category): 1) If the number of tumours is known, use T(#); e.g., pT3(4)N0M0; 2) If the number of tumours is unavailable or too numerous, use the suffix m – T(m) – e.g., pT3(m)N0M0

Table VII. AJCC 2017 TNM classification for neuroendocrine tumours of the jejunum and ileum [11, 22]

Definition of primary tumour (T)	
T category	**T criteria**
TX	primary tumour cannot be assessed
T0	no evidence of primary tumour
T1*	tumour invades lamina propria or submucosa and is less than or equal to 1 cm in size
T2*	tumour invades the muscularis propria or is greater than 1 cm in size
T3*	tumour invades through the muscularis propria into subserosa tissue without penetration of overlying serosa
T4*	tumour invades the visceral peritoneum (serosal) or other organs or adjacent structures
Definition of regional lymph node (N)	
N category	**N criteria**
NX	regional lymph nodes cannot be assessed
N0	no regional lymph node metastasis has occurred
N1	regional lymph node metastasis less than 12 nodes
N2	large mesenteric masses (>2 cm) and/or extensive nodal deposits (12 or greater), especially those that encase the superior mesenteric vessels
Definition of distant metastasis (M)	
M category	**M criteria**
M0	no distant metastasis
M1	distant metastasis
M1a	metastasis confined to liver
M1b	metastases in at least one extrahepatic site (e.g., lung, ovary, nonregional lymph node, peritoneum, bone)
M1c	both hepatic and extrahepatic metastases

*For any T, add (m) for multiple tumours [TX(#) or TX(m), where X = 1–4, and # = number of primary tumours identified; for multiple tumours with different T, use the highest

maffin (EC) cells secreting serotonin or other peptides. In Europe, the term carcinoid is used to represent a midgut-originating tumour, secreting serotonin and associated with the carcinoid syndrome. The 2019 WHO classification only distinguishes between well-differentiated NETs G1, G2, G3 and poorly differentiated NECs in all sites where they occur.

In the case of functioning NETs, 30% of patients present with hormone-induced symptoms (flushing, sweating, diarrhoea, wheezing), called "carcinoid syndrome" which are difficult to control [47, 48]. Factors causing fibrosis (5-HT, tissue growth factors, tachy- and bradykinins) may also induce right-sided cardiac valve damage (carcinoid heart disease or Hedinger syndrome) [16, 47].

Hormonally non-functioning NENs of the small intestine, usually asymptomatic, are found accidentally during colonoscopy in the ileocaecal region or when looking for the primary tumour in patients with metastases. Tumours, of over 1 cm diameter, are often malignant and metastatic [16, 22, 40].

Most NENs of the small intestine are well-differentiated NET G1 and NET G2, with a reasonably good 5-year prognosis [16, 40]. Well-differentiated NET G3 are rare, presenting as well-differentiated neoplasms, unlike poorly differentiated NECs. They are located almost exclusively in the ampullary region and should be staged according to carcinomas arising in this location [37]. The TNM classification and staging of NETs of the jejunum and ileum are given in tables VII and VIII.

Neuroendocrine tumours of the appendix

The 2017 AJCC staging system applies to neuroendocrine tumours of the appendix. These include appendiceal NETs (carcinoid) tumours (NET G1 and G2, and rare well-differentiated NET G3). High-grade neuroendocrine carcinomas (NEC), goblet cell carcinoids, mixed adenocarcinomas and adenocarcinomas should be staged according to the classification for appendix carcinomas. The following changes were introduced by the 2017 AJCC staging system: stages I–IV were condensed, i.e. substages A and B were excluded.

Appendiceal NETs similarly to jejunoileal midgut NETs, used to be called appendiceal carcinoids. However, within the current 2017 AJCC staging system, they are now classified separately from jejunoileal NETs due to behavio-

Table VIII. AJCC 2017 prognostic stage groups for neuroendocrine tumours of the jejunum and ileum [11, 22]

TNM	Stage group
T1 N0 M0	I
T2–T3 N0 M0	II
T4 N0 M0	III
T1–T4 N1, N2 M0	III
any T, any N M1	IV

Table IX. AJCC 2017 TNM classification for neuroendocrine tumours of the appendix [11, 23]

Definition of primary tumour (T)	
T category	**T criteria**
TX	primary tumour cannot be assessed
T0	no evidence of primary tumour
T1	tumour is 2 cm or less in greatest dimension
T2	tumour is more than 2 cm but less than or equal to 4 cm
T3	tumour is more than 4 cm or with subserosal invasion or involvement of the mesoappendix
T4	tumour perforates the peritoneum or directly invades other adjacent organs or structures (excluding direct mural extension to adjacent subserosa of adjacent bowel), e.g., abdominal wall and skeletal muscle
Definition of regional lymph node (N)	
N category	**N criteria**
NX	regional lymph nodes cannot be assessed
N0	no regional lymph node metastasis
N1	regional lymph node metastasis
Definition of distant metastasis (M)	
M category	**M criteria**
M0	no distant metastasis
M1	distant metastasis
M1a	metastasis confined to liver
M1b	metastases in at least one extrahepatic site (e.g., lung, ovary, nonregional lymph node, peritoneum, bone)
M1c	both hepatic and extrahepatic metastases

ural differences and higher incidence [12]. Over the years 1973–2012, according to the SEER database, the yearly incidence of appendiceal NETs was 0.2 per 100 000 individuals [23, 43]. Of all appendiceal neoplasms, typically arising in the tip of the appendix and discovered accidentally at appendectomy [50], appendiceal NETs are the most frequent (up to 85%) [23].

Most NETs of the appendix are smaller than 1 cm in diameter. Major criteria of potential aggressiveness are the tumour size and infiltration of the mesoappendix. Appendiceal NETs have an excellent prognosis. With tumours smaller than 1 cm in diameter, metastases occur only in some 2% of cases [40, 49, 51]. In patients with appendiceal NETs without lymph node metastases, the 10-year survival rate ranges between 90–100% [43, 51]. Appendiceal NENs are graded as well – differentiated NETs G1 and G2. Appendiceal NECs are morphologically similar to colonic counterparts. They are rare and may occur in any part of the appendix [13].

Goblet cell carcinoids are now termed goblet cell adenocarcinomas, as neuroendocrine cells are their minor component, while mucin-secreting cells are their major element. MiNENs of the appendix are also rare and may display a combination of NEC and adenocarcinoma, as do colonic MiNENs. The term mixed adenoneuroendocrine carcinoma (MANEC) is no longer used. The TNM classification and staging of NETs of the appendix are given in tables IX and X.

Neuroendocrine tumours of the colon and rectum

The 2017 AJCC staging system applies to neuroendocrine tumours of the colon and rectum. These include colonic and rectal "carcinoid" tumours (neuroendocrine tumour G1 and G2, and rare well-differentiated NET G3). High-grade neuroendocrine carcinomas and mixed adenoneuroendocrine carcinomas should be staged according to the classification of colon and rectum carcinomas. Against the 2010 Seventh Edition, no changes were introduced in the 2017 Eight Edition of AJCC [13].

The yearly incidence of colonic and rectal NENs is rising and estimated at 0.2 and 1.2 new cases per 100 000 individuals, respectively [53]. Colorectal NENs are usually silent or associated with mass-related nonspecific symptoms, such as pain, haemorrhage or constipation.

Table X. AJCC 2017 prognostic stage groups for neuroendocrine tumours of the appendix [11, 23]

TNM	Stage group
T1 N0 M0	I
T2–T3 N0 M0	II
T4 N0 M0	III
any T N1 M0	III
any T, any N M1	IV

Colonic and rectal NENs differ significantly [2]. Colonic NETs are rare tumours, typically larger than their rectal counterparts, are more aggressive, poorly differentiated and of histologically higher grade G3 [2]. Rectal NETs tend to be smaller, over 50% being below 1 cm in diameter in younger patients and are of low or intermediate grade G1/G2 [54].

Colonic NETs have the worst prognosis among gastrointestinal (GI) NETs – about 67% of patients have a 5-year survival rate, while for rectal NETs the 5-year survival rate is about 96%[55]. Apparently, NETs originating from the midgut and the hindgut exhibit different clinicopathological features [56].

On diagnosis, colorectal NECs and MiNENs may be widely disseminated. MiNENs of the colorectum contain a poorly differentiated neuroendocrine and an adenocarcinoma component. Occasionally, in patients with idiopathic inflammatory diseases, MiNENs with a low-grade NET component may occur [57]. The TNM classification and staging of NETs of the colon and rectum are given in tables XI and XII.

Neuroendocrine tumours of the pancreas

The 2017 AJCC staging system applies to well-differentiated neuroendocrine tumours arising in the pancreas. Carcinomas of the pancreas, including high-grade (G3) and poorly differentiated neuroendocrine carcinomas, should be staged according to the classification for exocrine pancreas carcinomas.

The following changes were introduced by the 2017 AJCC staging system: pancreatic neuroendocrine tumours are now staged using a TNM staging system based on size; the criterion of peripancreatic soft tissue invasion was eliminated; the Tis distinction was eliminated; M1 is subdivided into M1a – metastases confined to the liver, M1b – metastases in at least one extrahepatic site (e.g. lung, ovaries, nonregional lymph nodes, peritoneum, bones) and M1c – both hepatic and extrahepatic metastases. In the AJCC Cancer Staging Manual, the 8th Edition staging system has been modified to be consistent with the ENETS system [58, 60].

Pancreatic neuroendocrine neoplasms (pNENs) occur in 2–5% of all pancreatic tumours [61]. Due to improvements in imaging, the yearly worldwide incidence of pancreatic neuroendocrine tumours (pNETs) has rapidly increased to 2.5–5 per 100

Table XI. AJCC 2017 TNM classification for neuroendocrine tumours of the colon and rectum [11, 24]

Definition of primary tumour (T)	
T category	**T criteria**
TX	primary tumour cannot be assessed
T0	no evidence of primary tumour
T1	tumour invades the lamina propria or submucosa and is ≤2 cm
T1a	tumour is <1 cm in greatest dimension
T1b	tumour is 1–2 cm in greatest dimension
T2	tumour invades the muscularis propria or is >2 cm with invasion of the lamina propria or submucosa
T3	tumour invades through the muscularis propria into the subserosal tissue without penetration of overlying serosa
T4	tumour invades the visceral peritoneum (serosa) or other organs or adjacent structures
Definition of regional lymph node (N)	
N category	**N criteria**
NX	regional lymph nodes cannot be assessed
N0	no regional lymph node metastasis has occurred
N1	regional lymph node metastasis
Definition of distant metastasis (M)	
M category	**M criteria**
M0	no distant metastasis
M1	distant metastasis
M1a	metastasis confined to liver
M1b	metastases in at least one extrahepatic site (e.g., lung, ovary, nonregional lymph node, peritoneum, bone)
M1c	both hepatic and extrahepatic metastases

For multiple synchronous tumours, the highest T category should be used and the multiplicity or the number of tumours should be indicated in parenthesis, e.g., T3(2) or T3(m)

Table XII. AJCC 2017 prognostic stage groups for neuroendocrine tumours of the colon and rectum [11, 24]

TNM	Stage group
T1 N0 M0	I
T2 N0 M0	IIA
T3 N0 M0	IIB
T4 N0 M0	IIIA
any T N1 M0	IIIB
any T, any N M1	IV

000 individuals [62–63]. ENETS developed the grading classification system for pancreatic NETs adopted by the WHO in 2010 [15, 64] and updated in 2017 by AJCC [8, 21, 65, 66]. A new group of well differentiated high grade G3 tumours of the pancreas with favourable prognosis, compared with poorly differentiated NEC, was introduced [67]. The new category of well differentiated G3 pNETs show intact *TP53* and *RB1* in primary G3 pNETs [26]. G3 pancreatic NETs may contain low-grade components. Grade is a significant predictor of outcome in pancreatic NETs [17, 21, 25, 58, 65, 66, 68]. While pNECs grow rapidly and have a poor prognosis, the survival rate for slow-growing pNETs is better [69].

Clinically, pNENs may be categorised as functional (F-pNET) or non-functional (NF-pNET) tumours. Up to 20% of pNETs are responsible for specific clinical syndromes due to hormone excess. These F-pNETs, located mostly in the tail of the pancreas, include gastrinomas, insulinomas, VIPomas, glucagonomas and, less common tumours secreting ACTH, PTHrP, CCK, GHRH and serotonin (tab. XIII).

In cases where expression of various hormones by immunohistochemistry does not correlate with secretion, these tumours are termed non-functional pancreatic NETs (NF-pNETs) [70]. However, NF-pNETs do secrete several substances into the serum, including chromogranin A (CgA), pancreatic polypeptide (PP), pancreastatin, and neuron-specific enolase, some of which are used as markers of NENs [70]. Most NF-pNETs, occurring at least twice as frequently as F-pNETs, are located in the head of the pancreas [8, 21].

In the 5th edition of the WHO classification, mixed neuroendocrine neoplasms of the pancreas, previously termed mixed adenoneuroendocrine carcinomas (MANEC), are now termed mixed neuroendocrine- non-neuroendocrine neoplasms (MiNEN) [13]. Following the 2017 WHO classification update, the term hyperplastic and preneoplastic lesions, described only in some hereditary cancer syndromes such as MEN1 or VHL (von Hippel-Lindau syndrome), are no longer in use [71].

The etiology of pancreatic NETs is unknown. Most pancreatic NETs are sporadic, harbouring somatic mutations (43% DAXX/ATRX mutations, 44% MEN1 mutations or mutations of mTOR pathway genes) [52]. Less than 10% of all pancreatic NETs are part of the hereditary cancer syndrome [1]. Multiple endocrine neoplasia type 1 (MEN1) is the most common. Less common are: von Hippel-Lindau disease (mutation in the *VHL* gene), Neurofibromatosis type 1 (mutation in *Nf1*). Quite rare are the Tuberous sclerosis complex (mutation in *TSC1* or *TSC2*) or Mahvash disease (pancreatic NET caused by inactivating glucagon receptor mutation) [59]. The TNM classification and staging of NETs of the pancreas is given in table XIV and table XV.

Proposal of new classification framework

In 2018 the International Agency for Research on Cancer of the World Health Organization (IARC-WHO) proposed a new framework for general classification of neuroendocrine neoplasia in all organs [8]. The currently applied NEN definitions which may complicate patient evaluation and treatment, are predominantly organ-based rather than ordered by similarity in their genetic origin, morphology or clinical behaviour, as recognised by recent advances in these disciplines. Thus, in the new proposal, the neuroendocrine phenotype is a unique cancer category, now recommended as a neuroendocrine neoplasm, NEN, for all organs. Rindi and Inzani propose [72] that in this cancer category two classes be distinguished: a well differentiated neoplasm is defined as a neuroendocrine tumour (NET) while a poorly differentiated neoplasm – as a neuroendocrine carcinoma (NEC), in all anatomical sites. NETs are further graded according to their proliferation into G1, G2 and G3, while NEC are G3 only, by definition. Within the NEC class, small cell and large cell types are distinguished.

As described above, organ-specific grading (G) cut-offs are known for the digestive system (and also for the lung), however, such cut-offs for other organs remain to be established. It is suggested that current pathology reports contain the above-discussed newly recommended classification together with the currently observed classification. To provide an example, the new classification of NENs of the gut and pancreas is given in table XVI.

Table XIII. Characteristics of functional pancreatic neuroendocrine tumours [58]

Name	Neurohormone secreted	Common symptoms
insulinoma	insulin	hypoglycemic symptoms, Whipple's triad
gastrinoma	gastrin	abdominal pain, gastroesophageal reflux, diarrhoea, duodenal and stomach ulcers
VIPoma	vasoactive intestinal peptide (VIP)	diarrhoea, hypokalemia, dehydration, muscle weakness, nausea
glucagonoma	glucagon	rash, glucose intolerance, weight loss, erythematous lesions over the distal extremities
somatostatinoma	somatostatin	diabetes mellitus, cholelithiasis, diarrhoea
ACTHoma	ACTH	Cushing's syndrome

Table XIV. A JCC 2017 TNM classification for neuroendocrine tumours of the pancreas [11, 58]

Definition of primary tumour (T)	
T category	**T criteria**
TX	tumour cannot be assessed
T1	tumour limited to the pancreas,* <2 cm
T2	tumour limited to the pancreas,* 2–4 cm
T3	tumour limited to the pancreas,* >4 cm; or tumour invading the duodenum or common bile duct
T4	tumour invading adjacent organs (stomach, spleen, colon, adrenal gland) or the wall of large vessels (celiac axis or the superior mesenteric artery)
Definition of regional lymph node (N)	
N category	**N criteria**
NX	regional lymph nodes cannot be assessed
N0	no regional lymph node involvement
N1	regional lymph node involvement
Definition of distant metastasis (M)	
M category	**M criteria**
M0	no distant metastasis
M1	distant metastases
M1a	metastasis confined to liver
M1b	metastases in at least one extrahepatic site (e.g., lung, ovary, nonregional lymph node, peritoneum, bone)
M1c	both hepatic and extrahepatic metastases

Table XV. AJCC 2017 prognostic stage groups for neuroendocrine tumours of the pancreas [11, 58]

TNM	Stage group
T1 N0 M0	I
T2–T3 N0 M0	II
T4 N0 M0	III
any T N1 M0	III
any T, any N M1	IV

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Table XVI. The new framework proposed for general classification of neuroendocrine neoplasia (NEN) in all organs [8, 72]

Site	Category	Family (class)	Type	Grade	Current terminology
gut	neuroendocrine neoplasm (NEN)	neuroendocrine tumour (NET)	GUT site[1] NET	G1 G2 G3	GUT site[1] NET G1 GUT site[1] NET G2 GUT site[1] NET G3
		neuroendocrine carcinoma (NEC)	GUT site[1] NEC small cell type GUT site[1] NEC large cell type		GUT site[1] NEC small cell type GUT site[1] NEC large cell type
pancreas	neuroendocrine neoplasm (NEN)	neuroendocrine tumour (NET)	pancreas NET (PanNET)	G1 G2 G3	PanNET G1 PanNET G2 PanNET G3
		neuroendocrine carcinoma (NEC)	pancreas NEC small cell type pancreas NEC large cell type		Pancreas NEC small cell type Pancreas NEC large cell type

[1]Site stands for the adjective connoting the different districts of the tubular gut where the NEN develops, that is, oesophageal, gastric, duodenal, small intestinal, appendiceal, colonic, rectal and anal canal NET or NEC.

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SARS-CoV-2 infection: etiopathogenesis, clinical picture, current therapeutic options – the author's observations

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Currently, the scenario of a self-contained disappearance of the epidemic (as it was in the case of SARS) is no longer taken into consideration, whilst the SARS-CoV-2 virus will stay with us forever, similarly to other coronaviruses or flu. It is quite likely that periodical exacerbations of the epidemics – their growth and decrease – depend on many factors, which comprise, among others, the approval of the restrictions by the society or the manner in the epidemiological supervision is carried out and whether it is consistent. We must be ready for about 18–24 months of a high activity of COVID-19 with periodic active hot spots in many world regions. This requires efficient health services and the access to efficacious medication. Without an effective prophylactic vaccine, it seems that we will not be able to prevent the spread of the pandemic

Key words: COVID-19, SARS-CoV-2, interstitial pneumonia

Introduction

The first, officially confirmed case of infection with the new beta-coronavirus SARS-CoV-2 was discovered on 1st December 2019 in China, in the city of Wuhan – a large industrial centre with a population of several million, which had numerous business connections practically with the entire world. Additionally, in the city, there is a renowned, highly-specialist scientific laboratory (BSL4), which also conducts research on coronaviruses. This laboratory was a French-Chinese joint venture, yet for some reasons, the French withdrew from the collaboration.

Probably the first cases of SARS-CoV-2 infections, undoubtedly of animal origin (bats), occurred slightly earlier than was officially reported (local authorities kept it secret). Unfortunately, it was not possible to contain the epidemic focus to the place of its origin and the virus disseminated quickly to other regions of the world. Even by 11th February the WHO had declared the virus a pandemic (mainly interstitial pneumonia leading in some patients to acute respiratory distress syndrome), naming it COVID-19.

SARS-CoV-2 is one of 7 known coronaviruses pathogenic for humans – the majority of which (about 20% cases) are responsible for a mild cold-like condition. Two other coronaviruses, with very strong genetic affinity to SARS-CoV-2, were or still are highly dangerous: virus connected with SARS (the epidemic in 2002–2003 with mortality at the level of 10%) and MERS (isolated cases from 2012 mainly on the Arabian Peninsula burdened with very high mortality – 30%). SARS-CoV-2 in comparison with these other two viruses is much more infectious, yet less virulent, with significantly lower mortality rates. Initially 5 genomes of the new virus have been isolated and described :

- betaCoV/Wuhan/IVDC-HB-01/2019;
- betaCoV/Wuhan/IVDC-HB-04/2020;
- betaCoV/Wuhan/IVDC-HB-05/2019;
- betaCoV/Wuhan/WIV04/2019;
- betaCoV/Wuhan/IPBCAMS-WH-01/2019.

However, the virus, when passaging through consecutive human populations, gradually changes and the strains which

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are currently dominating are not the same as those which existed at the beginning of 2020. We see it clearly in our own clinical observations.

In spite of the large number of patients infected with SARS-CoV-2, which definitely exceeds hospitalisation and staff capacity of the health services, the rate of severe cases is lower than at the beginning of the epidemic, although definitely there are more cases in absolute numbers. Now, unfortunately, the self-contained disappearance of the epidemic (as it was in the case of SARS) is no longer taken into consideration, so the SARS-CoV-2 virus will stay with us forever, similarly to other coronaviruses or flu. It is quite likely that periodical exacerbations of epidemics – their growth and decrease – depend on many factors, which comprise, among others, the approval of the restrictions by the society or the manner in the epidemiological supervision is carried out and whether it is consistent. We must be ready for about 18–24 months of a high activity of COVID-19 with periodic active *hot spots* in many world regions [1, 2].

Etiopathogenesis

A dominating infection route is airborne and droplet transmission with conjunctival contamination being less frequent. Also alimentary transmission is possible by means of transmitting the virus particles from infected objects (e.g. from paper where the virus may survive for up to 40 hours) onto food or directly into the oral cavity. Although the presence of the virus was also detected in urine and faeces, the possibility of such a transmission, has not been confirmed.

A key role in the infection mechanism is played by the virus fusion protein (*S-spike*), which is present on its surface and manifests affinity with the ACE2 receptor protein (angiotensin-converting enzyme 2). The fusion of these two proteins allows the virus particles to penetrate into the cells of the host. ACE2 is a receptor existing on the mucosal membranes of the upper and lower respiratory tract, small intestine erythrocytes, in the kidneys, heart, testicles, cholangiocytes (but not hepatocytes) and – unfortunately – also in the vascular endothelium (that is why in severe cases it leads also to micro-thrombosis).

It was experimentally proven that other proteins: CD147, GRP78 and ADAM17, can also be receptors for SARS-CoV-2, whilst the very process of intracellular fusion requires the activation of glycoprotein S2 – via enzyme's cut with TMPRSS2, protein, cathepsin or furin. It was also proven that together with the ageing process and tobacco smoking, the ACE2 receptors count increases, which has a significant effect on the course of infection. These data form the foundation for the construction of various medications potentially helpful in the treatment of COVID-19 (numerous studies are in progress).

Finally the clinical picture and patient history influence the character and type of immunological response to the infection – which depends on many factors, including:

- individual genetic predisposition of each patient;
- specific immunological response to infections;
- viral load which the infected person received from another infected person (it has been proven that the use of personal protection measures reduces the number of severe cases of COVID-19 by 60%).

In about 5% of infections, a cytokine storm takes place. This is a general systemic response of a relatively healthy immune system which leads to a quick release of more than 150 known mediators of inflammatory reaction: among others, proinflammatory cytokines (e.g. tumour necrosis factor alpha, interleukin-1 and interleukin-6) and anti-inflammatory cytokines (e.g. interleukin-10 and ineterleukin-1 receptor blockers), numerous oxygen free radicals and coagulation factors. Cytokines signal and activate the cells of the immune system, including macrophages and T-cells to migrate in the direction of the locus of infection. The cytokines, which are located in the locus of infection, in turn, activate a mechanism within the cells which encourages them to produce even more cytokines. A correctly functioning immune system keeps this feedback loop within reasonable limits. Unfortunately, in some patients with COVID-19, especially in those with multimorbidity, the immunological response is uncontrolled – by means of activating other cells of the immune system in one location. The exact cause of the situation has not been completely clarified, yet it is definitely connected with a large number of virus particles. It may also be caused by the excessive reaction of the immune system, when it encounters a new, more aggressive pathogen and also by individual genetic predispositions of an infected person. In some way, it is imitated by the hemophagocytic syndrome.

Finally, the patient's fate is determined by the following factors:

- the stage of the pulmonary lesions, and also the lesions in other organs (heart and kidneys in particular),
- interstitial involvement,
- slowing down the blood flow within the patient's organism
- coagulopathy with the formation of micro- and macro--thrombosis [1, 3–5].

A clinical picture of the disease

According to world data, in 80% of infected persons there are no clinical symptoms or the clinical symptoms are mild. In other patients, i.e. about 20% of infected persons, the symptoms of severe interstitial pneumonia with various intensity are dominating, with the critical course concerning about 5% of this population (cytokine storm). Yet, mortality does not exceed 2–4% of all cases of infection. These statistical data are reflected in our own observations – among people who must be hospitalised.

Groups of increased risk of infection and also of a more severe course of the disease comprise the following:

- **Patients staying in nursing homes and extended care facilities**. Also other patients, in all age groups, hospitali-

sed for many reasons (nevertheless, in clinical practice this group comprises most frequently old-age persons with multi-morbidity, including advanced stages of oncological disease). Mortality for COVID-19 in the patient group >80 reaches 14–20% of all cases; yet in the cases of people very advanced in age (>95 years) the infection, for unknown reasons has a subclinical course.

- **With decreased immunity**: cancer patients (in particular with onco-haematologic conditions, patients in immuno-, chemo- or radiotherapy and 5 years after the completion of oncological treatment), HIV patients (in our practice – only untreated or with a low D4 cell count), patients with chronic unspecific colitis (e.g. Crohn's disease or ulcerative colitis), patients with some types of arthritis, systemic connective tissue diseases or dermatologic diseases, persons after cell or solid organ transplants and also persons chronically treated with glucocorticosteroids or other immunosuppressive medication.
- **Patients with cardiovascular conditions**, in particular with coronary disease and arterial hypertension.
- **Patients with respiratory diseases** – such as chronic obstructive pulmonary disease, asthma (moderate to severe form).
- **Obese persons** (with a BMI ≥ 40 or higher), also diabetics, patients with chronic renal disease (during dialysis therapy) or those suffering from chronic liver diseases.

The group at increased risk of infection and its severe course comprises also Afro-Americans and Latinos – yet in practice this does not concern Poland [4, 5].

General and local problems in the care of patients with COVID-19

The most important areas which must be stressed in this context:

1. The general failure in preparing many health systems to deal with the enormous scale of the epidemic (with regards to organisation, equipment and staff). The epidemic of SARS-CoV-2 exposed in many countries those areas which had been underinvested and with shortages of staff, especially with regard to infectious disease care.
2. The negative attitude of some (fortunately small) part of the medical staff (quitting the job, prolonged medical leave, reluctance of primary care doctors to offer individual consultations for patients with a suspicion of COVID-19). The effect of these superficial telephone consultations was the influx of patients to the ER departments in the hospitals for infectious diseases which had a negative influence on their efficiency .
3. Significant problems with medical transport.
4. Lack of consequence in capitalising on the effects on the lockdown in spring. Premature easing of the restrictions and the failure to observe those existing already (and to impose them again), as well as a reluctance to withdraw from loosening the restrictions, high risk of infections (weddings, open restaurants and clubs, church services, funerals). In spite of the almost 4-month holiday period and the number of infections being curbed, the country was not prepared for the expected typical exacerbation of the epidemic in the autumn and winter period.
5. The lack of a credible system to inform society about the causes and purposes of upholding the restrictions.
6. The lack of a definite reaction to the scandalous – in social and medical terms – activity of the "anti-COVID" movements, which deny the existence of the epidemic.

Positive attitudes:

1. The great commitment of the majority of medical staff on many levels as well as local administration in the region of Lower Silesia (also high-level one) in their battle with the epidemic (not only in a clinical sense).
2. The development of social solidarity in fighting epidemics and the organisation of support for healthcare staff.

Therapeutic procedure options

The therapeutic procedures depend on the stage of COVID-19 and the presence of comorbidities. The ordinal scale for SARS-CoV-2/COVID in its version announced by the WHO in 2020 may be useful here: this scale divides the patients infected with coronavirus into 8 functional groups:

1. Patients without hospitalisation and without any restriction of their activity;
2. Patients without hospitalisation, yet requiring restriction of their activity;
3. Patients who require hospitalisation but without oxygen therapy;
4. Patients who require unconditional hospitalisation and a low flow of oxygen through a face mask or nasal cannulas;
5. Patients who require unconditional hospitalisation and a high flow of oxygen (≥15 l/min), CPAP2, BIPAP3, non-invasive ventilation;
6. Patients who require unconditional hospitalisation and intubation and mechanical ventilation (without additional organ support);
7. Patients who require unconditional hospitalisation at the Intensive Care Unit and mechanical ventilation with additional organ support (e.g. vasopressors, RRT4, ECMO5);
8. Patients who die of SARS-CoV-2.

In clinical practice, a 4-stage scale is used whilst therapeutic procedures were defined by some scientific associations. The most complete and regularly updated recommendations in Poland seem to be the recommendations of PTEiLCH (the Polish Association of Epidemiologists and Doctors of Infectious Diseases) and those which are close to them – although expanded with experimental therapies (clinical trials) – the recommendations of local therapeutic committees. In our case, the treatment of COVID-19 in the region of Lower Silesia

is coordinated by J. Gromkowski Regional Specialist Hospital in Wrocław [6].

Stage I of COVID-19 disease – an asymptomatic patient or with a subclinical infection (applies to 80% of all infected persons) does not require hospitalisation, and therapeutic procedures are limited to the recommendations concerning isolation, rest, appropriate hydration, the use of anti-fever medication and saturation control – with respect to the possibility of a sudden progression of the disease. The use of glucocorticoids at this stage may increase viral replication and is clinically unadvisable.

Stage II with full symptoms – the patient requires hospitalisation (usually they already have interstitial pneumonia at a various stage of intensification) with regards to the necessity to apply oxygen therapy (various techniques), the prophylaxis of thromboembolic complications (low molecular weight heparin) and anti-viral treatment. At this point the recommendations from the first stage of the disease are still valid.

Currently the only known medication with a proven anti-viral action which is used in our centre (apart from the medical in therapeutic clinical trials), are remdesivir and the convalescent plasma – agents with a limited efficacy and not always available [7–10]. A significant completion of the therapy consists in adding antibiotic therapy (cephalosporins) and the administration of glucocorticosteroids – dexamethasone at the daily dose of 6–8 mg/day, started within the period between the second and the fifth day of the first administration of remdesivir or plasma.

The use of dexamethasone reduces the risk of death (evaluation within 28 days of randomisation) in patients with mechanical ventilation and in oxygen therapy – by 35% and 20% respectively. The use of remdesivir in patients who do not require oxygen therapy and more than a week after the appearance of symptoms does not make any sense and does not bring any clinical effects. So far the effectiveness of other drugs with potential anti-viral activity has not been proven – these drugs comprise lopinavir/ritonavir (used for HIV infections), baloxavir, marboxil, favipiravir (used in the treatment of flu), umifenovir (used in the treatment of flu), camostat mesylate (serine protease inhibitors), interferon, ribavirin, losartan (hydroxy) chloroquine, darunavir, nitazoxanide (antiparasitic drug), oseltamivir (used in the treatment of flu), azithromycin, sofosbuvir, daclatasvir, verdinexor and selinexor (its analogue). A number of other preparations are currently undergoing clinical trials.

Stage III – a patient with respiratory distress (the onset of a cytokine storm). The procedures are like in stage II plus additional high-flow oxygen therapy and anti-cytokine medication. In practice, the only approved preparations comprise tocilizumab (in patients with an increased concentration of IL-6) and sarilumab. There are also many other anti-cytokine medications undergoing clinical trials: IL-1 inhibitor (anakinra), human monoclonal antibody IgG1k: against IL-6 (sirukumab), against IL12/23 (ustekinumab), human monoclonal antibody p/GM-CSF (otilimab), immunoglobulins *i.v.*, inhibitors (baricitinib, ruxolitinib).

It appears that the use of remdesivir and convalescent plasma at this stage of disease is not justified with regards to aetiopathogenesis.

Stage IV – this is acute respiratory distress syndrome (ARDS) which requires mechanical ventilation and, possibly, extracorporeal membrane oxygenation (ECMO). The patient must be treated at the intensive care unit. At this stage of disease, remdesivir and convalescent plasma are not justified with regards to aetiopathogenesis (the lack of active viral replication) [6–11].

Conflict of interest: none declared

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SARS-CoV-2 as a new possible long-lasting determining factor impacting cancer death numbers. Based on the example of breast, colorectal and cervical cancer in Poland

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Introduction. So far, cancer burden has mainly been connected with the age structure of a given population and changes in risk factor exposure combined with lifestyle. Nowadays, available data indicates that the SARS-CoV-2 virus may be a new strong agent impacting the number of cancer deaths in the future.

Material and methods. In our study we analyzed changes in cancer screening as well as participation in a fast path of oncological diagnosis and treatment – before and during the SARS-CoV-2 pandemic in Poland – taking into consideration breast, colorectal and cervical cancer.

Results. We investigated substantial changes connected with the pandemic. In the case of cancer screening – despite the end of lockdown – population coverage and participation percentages are still lower than before the pandemic.

Discussion. Similar results were observed in different studies, e.g. in the United Kingdom similar declines are evident as well as a simultaneous prognosis of an increase in cancer death numbers.

Conclusions. Immediate health policy actions are needed in order to reverse unfavorable trends in cancer screening, treatment and ultimately in the number of cancer deaths in Poland.

Key words: SARS-CoV-2, COVID-19, cancer deaths, cancer prevention, Poland

Introduction

Breast cancer and colon cancer are one of the most frequent malignancies in Poland. In accordance to the latest data from the National Cancer Registry in Poland, in 2017 breast cancer was the most frequent cancer type among women causing 18 529 new cases (*European Standard Population 2013* – ESP2013: 91.3) and 6 670 deaths (ESP2013: 32.7), being also the second cause of cancer deaths among the female population. Among women, the colon is also the second leading cancer location – 5073 cases (ESP2013: 25.2). In the male population – the third most frequent – 5832 cases (ESP2013: 41.6). Looking solely at colon cancer in Poland, this tumor contributes to 3573 deaths (ESP2013: 17.6) in women and 4181 (ESP2013: 32.2) in the male population and it is the third highest cause of cancer deaths among both sexes. Additionally, rectum cancer was diagnosed among 2198 (ESP2013: 10.9) women and 3419 (ESP2013: 23.13) men causing respectively 1377 (ESP2013: 6.8) and 2161 (ESP2013: 16.4) deaths. Considering cervical cancer, in 2017 there were 2502 new cases (ESP2013: 12.3) and 1609 deaths (ESP2013: 7.9) due to this cancer type [1]. Despite its relatively low incidence, cervical cancer in Poland is characterized by a high mortality rate. In comparison with other countries

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from the World Health Organization (WHO) European Region (40 countries), Poland stands at 11th place concerning world age-standardized mortality rates for cervical cancer (ranked in descending order of mortality) – about 5 deaths per 100 000 women/year (in 2018) [2, 3].

So far, cancer burden was mainly connected with the age structure of a given population and changes in risk factor exposure combined with lifestyle. Nowadays, available data indicates that SARS-CoV-2 virus may be a new strong agent that is significantly impacting cancer deaths. Breast, colorectal and cervical cancers seems to be particularly prone to the discussed phenomenon. Despite the previous low screening coverages and participation rates for the above-mentioned cancer sites, this secondary prevention action provided constant protection against abrupt increases in deaths for those cancers. Similarly, in the case of oncology diagnosis and treatment cards (ODaTCs), a solution introduced in Poland in 2015 within the "Oncological Package" aimed at faster oncological diagnosis and treatment [4] coronavirus pandemic impaired in a large extension a path of rapid oncological diagnosis and treatment. A combination of the above mentioned epidemiological conditions and a meaningful slowdown in cancer screenings as well as issuing ODaTCs, may result in an increase in advanced breast, colorectal and cervical cancer cases and consequently in the number of deaths in Poland in the coming years. Similar scenarios may also be seen in other countries – for example in the Netherlands, where the percentage of cancer diagnoses during the pandemic decreased by about 25%, or in the United Kingdom (UK), where approximately 50% of cancer patients experienced delays in treatment [5].

Material and methods

The aim of our study was to analyze changes in two main areas that have been affected by the SARS-CoV-2 pandemic with potentially the biggest impact on future deaths from breast, colorectal and cervical cancer. These areas are: screening and participation in a fast path of oncological diagnosis and treatment, measured by the number of issued ODaTCs.

Data sourced from the National Health Fund in Poland (NHF) concerns screening coverage[1] [6] – for breast cancer (ICD-10: C50) and cervical cancer (ICD-10: C53) in the period from January to September 2019 and analogous time for 2020. We also analyzed data on colorectal cancer [7] (ICD-10: C18-21) screening participation rates sourced from the Maria Sklodowska-Curie National Research Institute of Oncology in Warsaw, Poland in the period between January and July 2019 and 2020. Moreover, we analyzed also data on ODaTCs issued from January to September 2019 and 2020 for breast, cervical and colorectal cancer (obtained from the Polish NHF [8]). However, in order to limit the effect of understated numbers of ODaTCs due to diagnostic difficulties, we used additional ICD-10 codes for breast cancer: D05 – breast cancer *in situ* (excluding breast skin cancer *in situ* – D04.5 and malignant melanoma of the breast *in situ* – D03.5) and D48.6 – tumors of uncertain or unknown characteristics in the breast. For cervical cancer: D07 – carcinoma *in situ* of other and unspecified genital organs (excluding skin cancer *in situ* – D03.5) and D39 – carcinoma *in situ* of an uncertain or unknown characteristic in the female genitalia. In colorectal cancer cases: D01 – *in situ* carcinoma of other and unspecified parts of the digestive system organs and D37 – tumors of uncertain or unknown characteristics of the mouth and digestive system organs.

The above indicated periods of time included the beginning of the SARS-CoV-2 outbreak in Poland which occurred in early March 2020. After collecting the data, we created a structured database and preliminarily analyzed the data with the use of Microsoft Excel ver. 15.22 (160506).

Limitations of the study

The main limitation of our study is the scarcity of data due to short, several-month-duration of the SARS-CoV-2 outbreak. However, based on the available data on changes in cancer screenings and issued ODaTCs numbers, we can currently indicate a possible future scenario for breast, cervical and colorectal cancer deaths in Poland that can be connected with the pandemic.

Results

In our study we focused on the data concerning three cancer sites: the breast, cervix and colorectum. In the case of breast cancer (fig. 1), we observed a sharp decrease in the number of issued ODaTCs in the period between January and April 2020 – from 4965 to 2730 cards. However, from May 2020, an increase in the number of ODaTCs is evident and it reaches an even slightly higher level that in May 2019 (4320 *vs.* 3954). As far as mammography is concerned, we can see a heavy decrease in the coverage percentages. In January 2020, 39.17% of women performed a mammography, in July it was 33.87% (until now, this was the lowest percentage this year). Before the pandemic, in 2019 during an analogous period of time, the lowest percentage in mammography coverage was at a level of 37.15% (in February) and was constantly increasing to 38.52% in September. However, the highest coverage in mammography in 2019 within the considered period was observed in January and reached a level of 39.26%.

Similarly, when examining the data on cervical cancer (fig. 2), we can also see a decrease in the number of ODaTCs during the pandemic. The lowest number of cards were issued on April 2020 – 472. For comparison, in April 2019, this number was equal to 710 ODaTCs. For all considered months in 2020 (1–9), screening coverage percentages for cytology were lower in comparison with those from comparable months in 2019, reaching a historically low level – 14.35% – in September

[1] Coverage – the % of eligible women who were screened for a mammography between the age of 50–69 years old, and for cytology – 25–59 years old.

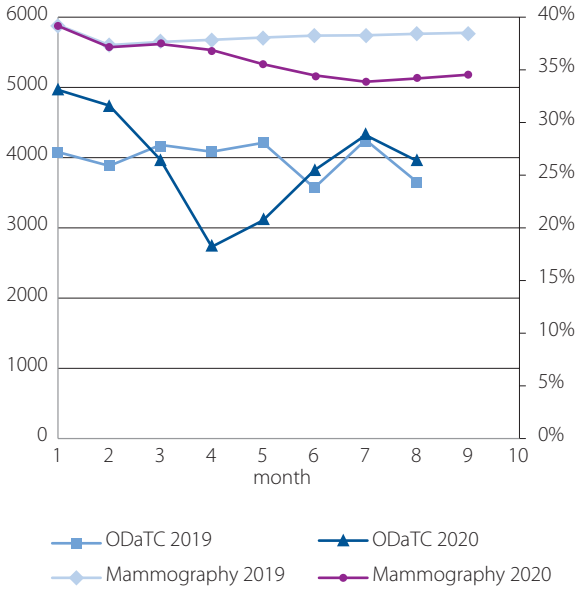

Figure 1. Absolute numbers of issued ODaTCs for breast cancer (ICD-10: C50, D05, D48) treatment and mammography coverage percentages in Poland from January to September (1–9) in the years 2019 and 2020

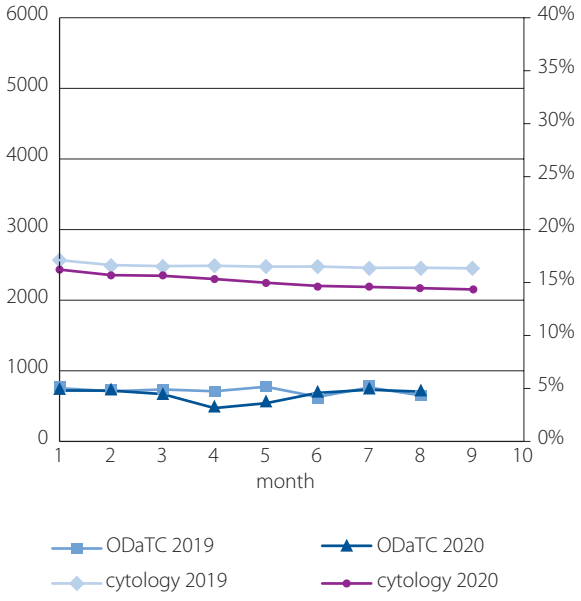

Figure 2. Absolute numbers of issued ODaTCs for cervical cancer treatment (ICD-10: C53, D07, D39) and cytology coverage percentages in Poland from January to September (1–9) in the years 2019 and 2020

2020. Moreover, collected data suggest that in 2020 cytology coverage systematically decreased – without any temporary increase or at least stabilization of the trend (unlike mammography or colonoscopy).

A notable decrease in the number of issued ODaTCs was also observed in colorectal cancer (fig. 3). In April 2020, 2063 cards were issued. In the same month in 2019, this number was equal to 3980. Despite this fact, since May an increase was visible and in July 2020 the number of issued ODaTCs is almost the same as July 2019 (3448 *vs.* 3583). Considering the data on colonoscopies, in every analyzed month of 2020 participation rates were much lower than in 2019. In April and May 2020, colonoscopy participation percentages were at the lowest level and for both months were equal to 4.93%. In 2019, percentages for these months were at the level of about 17%.

Despite the end of the lockdown in Poland, the situation regarding screenings and the number of issued ODaTCs did not fully return to its previous state. In the case of screenings, the analyzed data indicates that coverage in mammography and cytology is still lower than before the pandemic. Similarly – in the case of colonoscopy– participation percentages are currently much lower than in the analogous period in 2019. Only in the case of ODaTCs issued for breast and cervical cancer patients can we observe a return to the previous numbers. Considering solely colorectal cancer, approximately 100 less cards were issued for these patients in July 2020 in comparison with 2019 (table I).

Discussion

The epidemiological prognosis provided by the International Agency for Research on Cancer (IARC) shows that in the coming decades we can expect further increases in the cancer burden in Poland. For 2025, the absolute number of new breast cancer cases (for all ages) has been estimated at the level of 21 169 (with an overall change of +4.8% since 2018). For cervical cancer it was 3363 new cases (overall change +4.4% since 2018) and for colorectal cancer in women – 6829 (+10.5% since 2018) and in men – 8104 new cases (+14.3% since 2018).

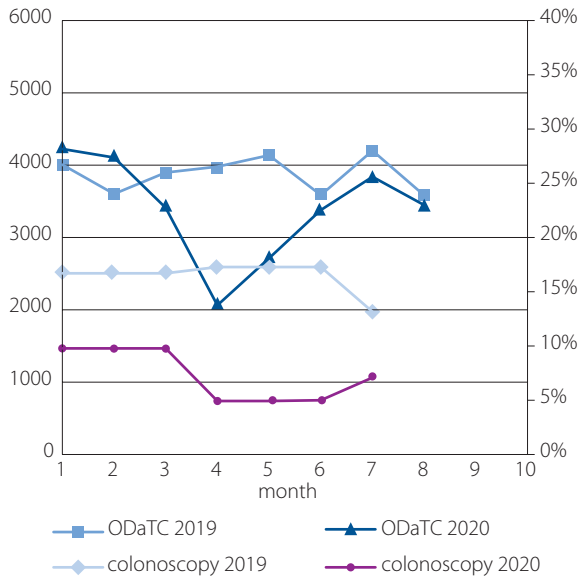

Figure 3. Absolute numbers of issued ODaTCs for colorectal cancer treatment (ICD-10: C-18-21, D01, D37) from January to August in the years 2019-2020 and colonoscopy participation percentages in Poland from January to July (1–7) in the years 2019 and 2020

Table I. Current state of screenings and the numbers of issued ODaTCs based on data from the last available month in 2020 and an analogous month in 2019

Screening	Screening coverage and participation rate* (last available month data)		Return to pre-pandemic state?	Cancer site	Absolute number of issued ODaTCs (last available month data)		Return to pre-pandemic state?
	2019	2020			2019	2020	
mammography *(September)*	38.52%	34.54%	NO	**breast** *(August)*	3654	3954	YES
cytology *(September)*	16.34%	14.35%	NO	**cervix** *(August)*	651	705	YES
colonoscopy *(July)*	13.16%	7.09%	NO	**colon, rectum** *(August)*	3583	3448	NO

*Participation rate for colonoscopy

Apart from the predicted increase in new cases for those cancers, IARC estimates also show a probable increase in the cancer death burden. In 2025 in Poland, 7494 deaths due to breast cancer are expected (with an overall change of +8.3% since 2018). For cervical cancer the number is equal to 2077 (+6.7% since 2018). For colorectal cancer in women – 4558 (+11.9% since 2018) and in men – 5603 (+17.1% since 2018) [9]. However, it must be highlighted that the IARC estimates above were prepared before the SARS-CoV-2 pandemic. Taking into consideration the results of our study, we can assume that the above epidemiological prognosis may be underestimated, particularly in light of the number of deaths for the discussed three cancer sites due to the multiplication of unfavorable effects such as: historically low participation in cancer screenings (exacerbated by the pandemic), an impaired system of fast treatment and diagnosis (the significantly lower number of issued ODaTCs, e.g. for breast cancer treatment – 4084 *vs.* 2730 in April 2019 and 2020), the constant increase in incidence, but also in mortality rates in cases of breast cancer [10] and colorectal cancer [1].

As mentioned before, available data on the impact of the SARS-CoV-2 pandemic on cancer deaths is still scarce and preliminary. However, some research and reports are available and indicate the possible direction of future changes. Maringe C. et al. in a population-based modeling study showed that in the United Kingdom (UK), due to delays in cancer diagnoses and treatment connected with the pandemic, a notable increase in the number of cancer deaths is expected in the future. Authors estimated that only for four cancer types – breast, colorectal, oesophageal and lung – approximate an increase in the number of additional cancer deaths in the next 5 years at the level of 3291–3621 [11]. In another study, Sud A. et al. indicates that in the UK during the lockdown, the number of patients who received referrals for urgent cancer treatment pathways decreased by about 84% in comparison with the pre-pandemic period [12]. In our study we also investigated substantial decreases in the number of patients with referrals to the fast path of oncological diagnosis and treatment, measured by the number of issued ODaTCs. Comparing January and April 2020, we noticed the following declines: breast cancer – 45% (2235), cervical cancer – 72% (1249), colorectal cancer – 51% (2161). Despite the obvious differences between Polish and UK healthcare systems, results of our study suggest that we can expect a similar scenario on cancer deaths in Poland as in the UK.

Conclusions

1. Collected data suggests possible additional numbers of deaths from breast, colorectal and cervical cancer in Poland in the future.
2. There is a need to conduct urgent health policy evaluations aimed at reversing the unfavorable trend in cancer screenings, and treatment in order to stop or at least slow down expected increases in cancer deaths.
3. Special attention should be drawn to screening; despite the end of lockdown, population coverage and participation percentages are still lower than before the pandemic.
4. Combining the data from the National Cancer Registry and the National COVID-19 Registry in Poland is an unique opportunity to conduct high quality research on the consequences which the pandemic could have for Polish oncology.

Conflict of interest: none declared

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COVID-19

Invited editorial

Cancer and COVID-19

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In this issue, we would like to draw readers' attention to two articles relating to the current situation connected with the COVID-19 pandemic.

From the turn of the 19th and 20th centuries, infectious diseases became less and less common among the human population, with a few very turbulent exceptions, such as the Spanish flu pandemic from around 1918 [1], or the Asian flu in 1957 [2]. The attention of medicine and health care managers was focused rather on chronic diseases, and one of the most important challenges described by Abdel Omran in *The Epidemiologic Transition: A Theory of the Epidemiology of Population Change* (1971) [3] was the struggle for a healthy and decent old age. In his model, A. Omran also took into account another factor that significantly determines the health of the population – infectious diseases. The current epidemiological situation confirms this theory, however, due to many decades of stable situation, this factor seemed to be less and less relevant. In 2020, for the first time in the post-war history of Europe, a virus with a very high virulence – SARS-CoV-2 – appeared. The articles which we would like to recommend to read are devoted to the disease of COVID-19 which is caused by this virus. The effects it has had in the area of cancer prevention are already noticeable.

Professor Krzysztof Simon and colleagues in the article *SARS-CoV-2 infection: etiopathogenesis, clinical picture, current therapeutic options – the author's observations* (*Zakażenie SARS-CoV-2: etiopatogeneza, obraz kliniczny, aktualne możliwości postępowania terapeutycznego – doświadczenia własne*) introduce the history of the COVID-19 pandemic and organize knowledge about pathogenic coronaviruses for humans, recalling the SARS epidemic (2002–2003) and MERS (since 2012 mainly found on the Arabian Peninsula). The authors indicate that at present a sudden end to the pandemic is impossible, but rather it is expected that SARS-CoV-2 will become part of the virus landscape of humanity like the influenza virus, constantly creating new strains. In the description of the etiopathogenesis, the authors describe the ways the infection spreads, as well as the mechanism and routes of virus entry, emphasizing that the use of personal protective equipment reduces the number of severe COVID-19 cases by about 60%. Particularly interesting is the presentation of those groups at increased risk of infection and who endure a severe course of the disease. The authors point to cancer patients, especially those with hematological cancers, as a particularly vulnerable group; this should encourage both the increased protection of these patients, but also very careful monitoring of their health status, taking into account four stages of COVID-19 described in this paper.

The second article (Koczkodaj et al. *SARS-CoV-2 as a new possible long-lasting determining factor impacting cancer death numbers. Based on the example of breast, colorectal and cervical cancer in Poland*), which we would like to also recommend, concerns the impact of the pandemic on secondary cancer prevention. We investigated changes in screening percentages as well as the numbers of patients referred to fast-track cancer treatment programs (number of issued *oncology diagnosis and treatment cards* – ODaTCs). Mentioned percentages and numbers decreased rapidly during the pandemic, especially in the lockdown period. It can be assumed that the long-term consequences of this occurrence will result in a higher number

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of patients presenting more advanced stages of the disease, and may also be associated with an increased number of cancer deaths.

It is also worth highlighting that the situation of people undergoing cancer treatment is all the more difficult since these patients are at a much higher risk of having a severe course of COVID-19. According to the latest recommendations of the European Society for Clinical Oncology (ESMO) cancer patients should be vaccinated against COVID-19 (before starting treatment, if possible). Moreover, ESMO experts also point to the priority of vaccinations of staff taking care of cancer patients, which is of course related to their own health and safety, but also to the safety of the patients, who remain at very high risk of infection and severe disease [4].

A long-lasting pandemic can also have disastrous economic consequences. Forecasts for Great Britain allow for a double-digit decline in Gross Domestic Product (GDP). As shown by the history of the economic crises of 1709 (The Great Frost in Europe), 1920 (The Great Depression), or the banking crisis (2008–2013), has always translated into unfavorable changes for public health (for example by a reduction of available funds for health care). The coming decade will be difficult, and decisions taken now in order to deal with the unprecedented events of 2020 will have long-term consequences – both economic and social. An excessive number of deaths due to cancer is probably inevitable, therefore current efforts should be focused on more innovative and efficient solutions in order to strengthen primary and secondary prevention, as well as to improve access to fast treatment paths. Ferrara and Albano, in their work entitled *COVID-19 and health care systems: What should we do next*? which was published in the journal *Public Health* [5] even indicate the need for a complete redefinition of the functioning of the health care systems, in particular in terms of services, personnel, and therefore also in terms of budget. On the other hand, the work of Iyengar K. et al. [6] states that the pandemic may also be an opportunity for the sector to rationalize the efficiency of available resource use and to revise health strategies. Moreover, the authors point to the sharp development in the field of telemedicine, as well as the growing popularity of scientific publications.

The area of oncology seems particularly vulnerable to the effects of the pandemic, the sudden appearance of which has highlighted the many deficiencies and imperfections of the health care systems in Poland and around the world. Currently, we need decisive actions that should be taken, regardless of economic and political pressure, because only decisions based on empathy and compassion will be a measure of our humanity and will ultimately decide on the fate of health care in the future. Taking this fact into consideration, the ESMO statement on vaccination against COVID-19 is extremely important, indicating, as it does, that the vaccination of cancer patients should be one of the current priorities with regards to the care of these patients.

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Wound healing in older oncologic patients

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The wound itself, along with lymphatic oedema, nausea and vomiting, fatigue and psychological stress, is listed as one of the five factors most negatively influencing cancer patients. Current oncologic treatment strategies are based on multimodal protocols including surgery, radiation and chemotherapeutic regimens. Thus, in oncology we can have a patient with a surgical wound, a wound that is a complication from radio- or chemotherapy, or a wound due to cancer progression. With increasing age, skin functions deteriorate due to the quantitative and qualitative changes of skin cells. Despite this, it seems that wound healing in healthy older patients is only delayed, but not completely defective. This effect is clinically apparent by the age of 60 and becomes significant at the age of 70. In turn, scar maturation improves in comparison with young individuals. However, the skin alone is more susceptible to injury in older patients. As older patients are qualified for complex oncologic treatment more and more often, wound healing in older cancer patients has become a matter of critical importance.

Key words: older oncologic patients, elderly, wound healing

About one-half of cancer cases and two-thirds of cancer deaths occur in patients aged 65 years or older [1]. As was mentioned in the previous papers, the incidence of cancer increases with age, so the number of older patients with cancer is expected to rise further in the coming years. The fastest growing segment of this population, those over 85 years of age, is also the cohort with the highest incidence of chronic wounds. As they are undergoing complex oncologic treatment more and more often, there is also a significant increased risk of impaired wound healing among this population [2, 3].

Annually it is estimated that worldwide prevalence of surgical wounds is 114,271 thousand (with average healing time app. 10–14 days and annual growth 3.6%), of chronic wounds 40,400 thousand (with indefinite healing time and annual growth 7.6%) and of complicated skin cancer 103 thousand (with average healing time 28 days and annual growth 3.1%) [4]. In this context, wound healing in older cancer patients becomes a matter of critical importance. Moreover, the wound, along with lymphatic oedema, nausea and vomiting, fatigue and psychological stress, is listed as one of the five factors most negatively influencing cancer patients. The pain, smell, infection, exudate, bleeding, cosmetic appearance with the wound have a direct effect on feelings of fear, shame, uncertainty, inconvenience, isolation, loss of function and low self-esteem in cancer patients [5].

Current oncologic treatment strategies are based on multimodal protocols including surgery, radiation and chemotherapeutic regimens. Thus, in oncology we can have the patient with a surgically created wound, a wound that is a complication of radio- or chemotherapy, or a wound due to cancer progression. The last type, a malignant wound, affects app. 5% of oncologic patients and app. 10% of patients with metastasis [6].

Normal wound healing is an innate immune response to injury with the aim of restoring anatomic and functional tissue integrity. It is a tightly regulated series of processes involving

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numerous cell types, mediators and proteolytic enzymes. The first phase – haemostasis – starts immediately after injury, followed by distinct, but overlapping, phases of inflammation (usually lasting for 2–4 days), proliferation (begins within 72 hours of injury and lasts for about 14 days) and tissue remodelling (starting at around day 8, which can persist for 1 year or longer). During haemostasis, injured micro-vessels constrict, a coagulation cascade becomes activated, platelets aggregate, and a fibrin clot serving as a provisional matrix, is formed [7]. The inflammatory phase is characterised by the sequential infiltration of neutrophils, monocytes and lymphocytes. Within 24–48 hours, monocytes migrate into the wound and differentiate into mature macrophages – the key regulator of this phase. Next is the proliferative phase, which is characterised by the replacement of the provisional fibrin matrix with granulation tissue, angiogenesis and transformation of fibroblasts into myofibroblasts; this, in turn, will cause contraction of the wound and matrix remodeling [8].

With increasing age, skin functions deteriorate due to quantitative and qualitative changes in the skin cells. The most important changes are as follows:

- decreased proliferation of keratinocytes,
- increased keratinocyte migration time,
- decrease in the number of macrophages and in fibroblasts,
- atrophy of the epidermis,
- flattening of the dermal-epidermal junction,
- reduced, disorganised microcirculation,
- reduced vascular response,
- decrease in Langerhans cells and melanocytes.

Despite this, it seems that wound healing in healthy older patients is only delayed, but not defective. This effect is clinically apparent by the age of 60 and becomes significant at the age of 70. In turn, scar maturation improves in comparison with young individuals [9]. The lack of consensus on this matter is mainly due to the fact that most of the studies use the chronological age and not the biological. As was discussed in the previous papers, the older population is a very heterogenic cohort and its heterogeneity increases with age. However, the skin alone is more susceptible to injury in older patients. An *ex-vivo* model demonstrated that the application of a compressive load to aged skin resulted in sub-epidermal separation and altered orientation of the collagen fibres, similar to that seen in patients with pressure ulcers [10].

Multiple general processes have been associated with age that influence wound healing. Among others, malnutrition, decline of sex and steroid hormones, immobilisation, medication, obesity and comorbidities such as diabetes, renal failure, peripheral arterial disease and chronic venous insufficiency are the most often studied factors [11–15]. Between the ages of 68 and 78, a healthy person loses approximately 1% of fat-free mass per year. A reduced perception of hunger, early satiety and changes in the hormonal mediators associated with energy balance may additionally influence the process. This loss translates to a 3-fold loss of strength. The combination of sarcopenia, functional decline, malnutrition and the inability of aged skin to distribute a pressure load substantially increases the risk of impaired wound healing, formation of chronic wound and pressure ulcers [16].

The timing of surgical intervention in relation to radio- or chemotherapy is fundamentally important as regards surgical wound healing. Radiotherapy has a significant role in the local control of cancer; however, because it non-specifically damages adjacent tissue, it can further complicate wound healing. This depends mainly on the total amount of radiation exposure as well as the timing and overall duration of treatment. In cases of doses larger than 50 Gy, or treatments given less than 3 weeks before surgery, a significant increase in wound complications may be observed. In turn, chemotherapeutic agents interfere with many pathways that are essential to wound healing: they can delay cell migration, impair cell proliferation and reduce angiogenesis and matrix formation. Furthermore, they weaken the immune system and thereby increase the risk of infection [17]. As far as medication is concerned, we cannot forget systemic glucocorticoids, which may inhibit wound repair by anti-inflammatory effects suppression of fibroblast proliferation and collagen synthesis [18].

The research interest in wound healing in cancer patients will continue to grow; not only in the context of an ageing population but also because tumours appear to behave similar to wounds that fail to heal. Many cellular and molecular similarities have been studied in recent years that indicate multiple shared mechanisms between wounds and tumours; they differ only in that one is well regulated during wound healing and the other dysregulated during cancer growth/metastasis. Normal wound repair has a resolution phase, in turn, cancer cells behave more similar to a chronic wound, which has no such phase [19].

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Fundamentals of personalised medicine in colorectal cancer

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Personalised treatment which is a dynamically developing branch of medicine, is based on individualisation of diagnostic and therapeutic procedures. Its aim is to optimise treatment by increasing therapy effectiveness, while minimising side effects. It is designed both for patients with a diagnosed hereditary cancer syndrome, as well as those with sporadic cancers. In the case of a diagnosed colorectal cancer, personalised treatment requires patient selection based on predictive factors. This involves determination of the genetic status within the epidermal growth factor receptor (EGFR) signalling pathway, including assessment of the cancer tissue genotype with respect to *RAS* gene mutations (*KRAS*, *NRAS*) and *BRAF* gene mutations. In patients who do not respond to anti-EGFR targeted therapy, chemotherapy aimed at vascular endothelial growth factor (VEGF) is introduced. In personalised medicine it is also essential to introduce prophylactic and therapeutic measures, both in carriers of germline mutations, and members of their families who have not been diagnosed with this mutation, but who meet family history and clinical criteria of hereditary cancer syndrome.

Key words: personalised medicine, colorectal cancer, hereditary cancer syndrome, germline mutation, somatic mutation, epidermal growth factor EGFR, *RAS, BRAF*

Introduction

According to the National Cancer Register, the colorectal cancer is the third most common neoplasm diagnosed in Poland in men (after prostate cancer and lung cancer) and second in women (after breast cancer). The incidence is increasing gradually and since 1980 it has increased 4 times in men and 3 times in women [1].

Risk factors affecting development of the colorectal cancer include above all age, low-fibre diet, inflammation of the colon (e.g., ulcerative colitis and Crohn's disease), metabolic disorders (including mainly obesity, hypercholesterolemia, hypertension and diabetes), as well as smoking, polyps within the colon or diagnosis of the same neoplasm in members of the patient's family [2].

Genetic background of the colorectal cancer

The aetiology of colorectal neoplasms is complex. A vast majority, about 65–75% of them, are sporadic (non-hereditary) and in such cases the major risk factor is age. Further 10–15% are familial colorectal cancers. Both in the case of sporadic, and familial colorectal cancers, the basis for their development is complex: genetic ("genetic background" constituted by medium and low penetrance gene variants, which increase susceptibility to environmental carcinogens) along with the environmental exposure to carcinogens (usually shared for families). Variants in medium-penetrance genes confer increased cancer risk as compared to the general population, while variants in low-penetrance genes may modulate individual susceptibility to carcinogens [3].

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The remaining 5–10% of colorectal cancers are associated with hereditary predisposition. Such syndromes are suspected in families where the family history and clinical criteria for diagnosis/suspicion of a hereditary cancer syndrome are met (number of cases, relationship between patients, age of onset, histopathological diagnosis) [4].

Colorectal neoplasms, which develop as a result of hereditary cancer syndromes, may arise both on the basis of polyposis and without the increased number of polyps in the intestine [4, 5].

Hereditary cancer syndromes with polyposis-related colorectal cancer cases in their spectrum include [3]:

1. Adenomatous polyposis:
- *familial adenomatous polyposis* (FAP) – caused by *APC gene mutation,* characterised by autosomal dominant (AD) inheritance, including classic and benign forms of FAP, Turcot syndrome and Gardner syndrome,
- MAP syndrome (*MUTYH-associated polyposis*) – caused by mutations in the *MUTYH gene,* characterised by autosomal recessive (AR) inheritance.
2. Hamartomatous polyposis – dominantly autosomally inherited (AD):
- Peutz-Jeghers syndrome – caused by mutations in the *STK11* gene,
- Cowden syndrome – caused by mutations in the *PTEN* gene,
- hereditary mixed polyposis syndrome – caused by mutations in the *CRAC1* gene,
- juvenile polyposis of the colon – caused by mutations in the *BMPR1A* and *SMAD4* genes.

The only hereditary colorectal cancer syndrome without polyposis is Lynch syndrome (*hereditary non-polyposis colorectal cancer* – HNPCC) – caused mainly by mutations of *MLH1*, *MSH2*, *MSH6*, *PMS2* and *EPCAM* genes [3–5].

Hereditary non-polyposis colorectal cancer (HNPCC, Lynch syndrome)

HNPCC is diagnosed in approximately 3–4% of colorectal cancer patients. The risk of cancer development in carriers of the syndrome's germline mutation (hereditary mutation present in all cells of the body) increases with age, reaching the lifetime level of 80% in men and 40% in women (average age of onset is 40 years, in contrast to sporadic cancers for which average age of onset is 60–70) [6, 7].

The people diagnosed with Lynch syndrome, apart from colorectal cancer, are also at an increased risk of developing other malignant neoplasms outside the large intestine. These neoplasms belong to the so-called spectrum of Lynch syndrome and include malignant neoplasms of the following organs [8, 9]:

- endometrium (risk of developing the disease 30–51%) and ovary (4–15%) in women,
- stomach (up to 18%) and small intestine (3–5%),
- collecting system of the kidney/ureter/bladder (2–20%),
- bile ducts / gallbladder,
- pancreas (4%),
- central nervous system (typically glioblastoma and astrocytoma),
- prostate (in carriers of the mutation in the *MSH2* gene),
- breast (in carriers of the mutation in the *MLH1* gene).

Carriers of pathogenic variants in the *MLH1* and *MSH2* genes have a significantly higher risk of developing colorectal cancer at an earlier age, as compared to the carriers of pathogenic changes in the *MSH6* and *PMS2* genes. The incidence of endometrial and urinary tract cancer is higher in carriers of the *MSH2* gene mutation [10].

Clinically, the following forms of Lynch syndrome are distinguished [8, 11]:

1. colorectal cancer only,
2. colorectal cancer and other cancers within the spectrum,
3. Torre Muir syndrome – malignant tumours of the colon and other diseases within the spectrum are associated with skin cancers (e.g., spinous cell carcinoma, squamous cell carcinoma, as well as sebaceous cysts and adenomas),
4. Turcot syndrome – coexistence of malignant tumours of the large intestine with primary brain tumours.

Genetic background of Lynch syndrome

The genetic background of Lynch syndrome, inherited autosomally dominantly, involves mutations in mutator genes (*DNA mismatch repair genes* – MMR genes), mainly *MLH1*, *MSH2*, *MSH6* and *PMS2*, as well as changes in the *EPCAM* gene (approximately 1–3% of HNPCC cases). Deletion of the *EPCAM* gene causes hypermethylation of the adjacent *MSH2* gene, which results in its inactivation [6, 10, 11, 12].

The role of mutator genes concerns coding proteins involved in the process of removing mismatched bases within the DNA chain; loss of their function leads to impairment of the process of repair of mismatched bases, thus causing accumulation of mutations within a cell. Loss of MMR gene/protein function is expressed in development of the "mutator phenotype", characterised by microsatellite instability (*MSI*), i.e., increased number of errors occurring at replication of the DNA chain – mainly in repeated sequences called microsatellites. More than 70% of mutations in tumours with high microsatellite instability are identified in the *MLH1*, *MSH2* and *EPCAM* genes [6, 13].

Mutations inactivating the MMR genes lead to the lack of expression of the corresponding MMR protein evidenced by immunohistochemistry test (*immunohistochemistry staining* – IHC). MMR and IHC tests performed in colorectal tumours allow for identification of the microsatellite instability status and are characterised by high sensitivity (approx. 94%) and specificity (approx. 88%) [6].

Approximately 15–20% of sporadic colorectal carcinomas show microsatellite instability and loss of expression of *MLH1*

in tumour tissue, most commonly due to somatic hypermethylation of the *MLH1* gene promoter associated with a BRAF *V600* gene mutation. Therefore, when loss of expression of *MLH1* is present (alone or with loss of expression of *PMS2*), it is necessary to first exclude hypermethylation of the *MLH1* promoter in the tumour or assess the presence of the somatic mutation V600 in the *BRAF* gene. In the case when loss of *MLH1* expression coexists with loss of expression of *MSH2*, *MSH6*, or isolated expression of *PMS2* gene, genetic analysis should be performed for presence of germline mutations in the above genes. The MMR IHC and/or MSI test, followed by the analysis of hypermethylation of the promoter of the *MLH1* gene (in the case of loss of expression of *MLH1* gene), should also be performed in women diagnosed with endometrial cancer, due to the fact that 2–3% endometrial carcinomas belong to the spectrum of tumours in Lynch syndrome [6, 11].

Presence of somatic mutations of the *BRAF* protooncogene in colorectal tumours allows for excluding with high probability the Lynch syndrome, indicating sporadic disease. However, absence of V600 mutation within the *BRAF* does not unequivocally signify diagnosis of Lynch syndrome-related colorectal cancer [5, 6].

In patients who cannot have molecular testing of tumour tissue performed, predictive models are applied which allow estimation of probability of finding a pathogenic variant of a mutator gene (PREMM 5 MODEL). The clinical criteria used to identify people with suspicion of Lynch syndrome are the Amsterdam II criteria and the modified Bethesda criteria [3, 6, 8].

The detailed algorithm of management in patients with diagnosed colorectal cancer, depending on availability of tumour tissue, is described in ESMO recommendations [6].

Amsterdam criteria II (fulfilment of all criteria allows for clinical diagnosis of Lynch syndrome, is an indication for genetic diagnosis of this disorder and an indication for the implementation of preventive recommendations, even in the absence of molecular confirmation of the syndrome):

- at least 3 family members with histopathologically confirmed LS spectrum malignancy,
- cases of colorectal cancer or LS spectrum neoplasms in at least 2 consecutive generations,
- at least one of those suffering from colorectal cancer or LS spectrum cancer is a first degree relative to the others,
- at least one case of colorectal cancer or LS spectrum cancer occurred before the age of 50,
- in the case of colorectal cancers, familial polyposis (FAP) should be excluded,
- verified histopathological diagnosis.

Modified Bethesda criteria (meeting at least one of them is an indication for molecular diagnostics for Lynch syndrome):

- colorectal cancer diagnosed before the age of 50,
- multifocal colorectal cancer regardless of the age of diagnosis (applies to both synchronic and metachronic foci),
- colorectal cancer with high microsatellite instability, diagnosed before the age of 60,
- colorectal cancer in the patient and at least one neoplasm from the LS spectrum in 1st/2nd degree relatives, including at least one onset before 50 years of age,
- colorectal cancer in the patient and at least 2 malignant neoplasms from the LS spectrum among 1st/2nd degree relatives, regardless of age.

Genetic diagnostics in Lynch syndrome

Testing for hereditary mutations is performed on DNA isolated from the patient's somatic cells (lymphocytes, mucosa cells). Due to the complex molecular background (diversity of genes involved in the aetiology of the syndrome) and the multitude of pathogenic changes occurring within them, (nonsense mutations, missense changes) reading frame shift, splicing mutations, as well as large rearrangements, i.e. deletions/duplications or inversions), the genetic diagnostics of Lynch syndrome should include, first of all (due to the significant predominance of point mutations) sequencing (using the method *next generation sequencing* – NGS) of the gene panel of *MLH1, MSH2*, *MSH6*, *PMS2*. If no mutations are detected in the sequencing of the above genes, as well as *EPCAM*, the MLPA method (*multiplex ligation-dependent probe amplification*) should be used to analyse the presence of large rearrangements within the studied genes [3, 14].

Prophylaxis for Lynch Syndrome

Prophylactic care should be applied to people in predisposed families, as diagnosed based on the analysis of the family history and clinical criteria, and to people with diagnosed critical mutation (even if the family history and clinical criteria are not met). It is aimed at early detection of cancer through active supervision of people at increased risk, thus extending their survival time and improving the quality of life. Thanks to the advances in oncogenetics, such supervision may be adapted to the identified genetic change and family history of disease [12].

Further, at-risk patients are advised to avoid carcinogens, including especially smoking, and to observe healthy lifestyle, including maintaining the normal body weight. Detailed rules of preventive treatment are presented in table I.

Familial adenomatous polyposis (FAP)

Hereditary familial adenomatous polyposis syndrome accounts for less than 1% of all cases of malignant colorectal neoplasms, while being the most common cause of polyposis with a known genetic basis. FAP is characterised by autosomal dominant inheritance and it is caused by germline mutations in the APC suppressor gene [6, 7, 11].

Table I. Principles of prophylactic management for patients at risk of HNPCC based on the NCCN, ESMO and NMHN guidelines. [4, 6, 8, 10, 15, 16]

Organ	Study type	Age	Frequency
large intestine	• colonoscopy*1 • in patients with diagnosed cancer – colectomy*2	• MSH1/MSH2 25 years of age • MSH6/PMS2 35 years of age • or 5 years earlier than the earliest disease in the family, if the diagnosis <25 years of age	every 12–24 months
uterine body	• transvaginal ultrasound • biopsy of the uterine body*3 • prophylactic hysterectomy and/or bilateral adnexectomy*4	30–35 years of age	• every 12 months • in any case of atypical vaginal bleeding (beyond the expected menstruation or after the end of menstruation)
ovary	• transvaginal ultrasound • CA-12 marking • prophylactic hysterectomy and/or bilateral adnexectomy*4	30–35 years of age	every 12 months
stomach	• upper GI endoscopy • *Helicobacter pylori* testing should be considered in all mutation carriers	30–35 years of age	every 24–36 months
pancreas	• MRI and/or ultrasound to be considered*5	50 years of age or 5 years earlier than the earliest disease in the family	
urinary tract	no confirmation of the effectiveness of the test due to a too high percentage of false-positive results		
CNS	neurological examination		every 12 months

*1 Indigo carmine chromoendoscopy has been shown to be significantly more effective in people with LS compared to standard colonoscopy. It is recommended to perform the test in reference centres.

*2 It has been shown that there is an increased risk of metachronic colorectal cancer after partial colectomy and that patients' quality of life was similar after partial and total colectomy. Therefore, extended colectomy should be an option for patients with Lynch syndrome undergoing primary surgery for colorectal cancer, especially if the disease occurs at a young age.

*3 Recommended for identification of patients with precancerous endometrial lesions or asymptomatic endometrial cancer.

*4 In the case of mutation carriers who have completed their procreation plans (optimally at 35–40 years of age); after surgery, HRT at the lowest effective dose should be considered.

*5 Recommended for patients with pancreatic cancer who have a 1st degree relative with the same cancer.

The clinical diagnosis of familial adenomatous polyposis is based on the following phenotypes [3, 4, 17]:

1. Classic FAP:
- presence of over 100 adenomatous polyps in the large intestine (polyps may appear as early as in childhood, and from 40 to 50 years of age the risk of cancer development is up to 98%),
- fewer than 100 polyps in the large intestine and at least 1 relative diagnosed with FAP.
2. Attenuated FAP (AFAP):
- fewer than 100 colon polyps before the age of 30 and/or
- a relative with confirmed AFAP, and/or
- more than 100 polyps in the colon over the age of 40.
3. Gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS):
- presence of polyps confined to the stomach body and fundus,
- more than 100 (sometimes thousands) polyps in proximal stomach or more than 30 polyps in a 1st degree relative of a person with GAPPS,
- polyps most commonly derived from the fundic glands of the stomach (*fundic gland polyps* – FGPs), some of which may have regions of dysplasia, or a family member of FGPs with dysplasia or gastric adenocarcinoma,
- no polyps in the large intestine and duodenum.

Extraintestinal symptoms of FAP

Hereditary familial adenomatous polyposis in a disease associated with extraintestinal symptoms, and the risk of their occurrence increases with age [3, 18, 17]. They include:

- congenital hypertrophy of the retinal pigment epithelium (CHRPE) – the risk of developing it reaches 70–80%,
- epidermoid tumours – 50%,
- osteomas of the mandible – 50–90%,
- desmoid tumours – 10–15%,
- changes in dentition, including extra teeth – 11–27%,
- polyps located in the higher parts of the digestive tract (the bottom of the stomach and duodenum),
- increased risk of neoplasms, including thyroid gland (papillary carcinoma, risk of 2–3%), stomach, duodenum, brain (usually medulloblastoma, risk <1%), and hepatoma (approximately 1%).

The number of polyps is closely correlated to the risk of developing colorectal cancer and to the location of the

mutation in the *APC* gene. Nonsense mutations (appearance of a stop codon) located between codon 169 and 1600 co-exist with the phenotype of the classical FAP syndrome with hundreds of polyps. Pathogenic changes around codon 1300 cause the appearance of thousands of polyps and correlate with the highest risk of developing colorectal cancer. Mutations present in 5′ direction from codon 160 and 3′ from codon 1600 are associated with a benign form of inherited familial adenomatous polyposis.

The number of polyps has a significant impact on the risk and the age at which a polyp will become malignant. In patients with a molecular confirmation of mutated APC gene and with presence of thousands of polyps, without introducing prophylactic measures, colorectal cancer is diagnosed on average at 28 years of age, while the average age of onset in patients with hundreds of polyps is 44 years, and in people with mild polyposis – about 55 years. Further, mutations in the 3′ region from codon 1400 cause an increased risk of desmoid tumours, while pathogenic changes located 5′ from exon 9 (codons 312–438) do not cause CHRPE (except for single changes in exon 6) [19].

Genetic diagnostics of FAP

In the case of hereditary familial adenomatous polyposis, even 20–25% of the mutations are *de novo*, which means that there is no family burden (the family history and clinical criteria for suspicion/diagnosis of the syndrome are not met). Germline mutations in the *APC* gene are responsible for approximately 90% of classic FAP cases. Molecular diagnostics should include sequencing of this gene (tests can be started with the analysis of the presence of the 4 most common mutations in exon 11, i.e., c.1500T > A (p.Tyr500X), c.3183_3187delACAAA, c.3202_3205delTCAA, c.3927_3931delAAAGA). If no mutation is identified in the patient, large rearrangements within the *APC* gene or the region in which it resides should be analysed. With availability of multi-gene panels, it is also possible to analyse genes related to colon polyposis at the same time, including *MUTYH*, *POLE*, *POLD1*, *NTHL1*, *STK11*, *SMAD4*, *BMPR1A* [3, 19].

If polyposis of the colon is found or a familial mutation is identified, genetic diagnostics should be provided to all members of the family selected based on the phenotype, even before they turn 18 (considering the risk of FAP syndromes onset in early childhood) [17].

Prophylactic management of FAP

Increased surveillance should be provided for all mutation carriers, as well as members of the given family in whom no germline mutation can be identified. The rules of prophylactic treatment for patients at risk of FAP are presented in table II.

Diagnostic options available in the funding programme of the Ministry of Health

In Poland, in accordance with Module II of the National Cancer Control Program the Ministry of Health for 2018–2021,

Table II. Rules of prophylactic management for patients at risk of FAP based on the NCCN, ESMO and NMHN guidelines [4, 6, 10, 15, 16, 17]

Organ	Study type	Age	Frequency
large intestine	• flexible sigmoidoscopy and colonoscopy (in the case of adenomas and depending on age) • preventive colectomy/proctocolectomy at the age of 16–20	from 10–15 years of age	• every 12–24 months, gradually extending the period between tests to 36 months • in patients after colectomy – colonoscopy every 6–12 months (depending on the presence of polyps)
duodenum	• endoscopy of the upper digestive tract (front and side view)	• from 25–30 years of age (according to ESMO) • from 20–25 years of age (according to NCCN) • depending on the family burden	every 1–5 years[*1]
stomach	• endoscopy of the upper digestive tract (front and side view)	from 25–30 years of age	
thyroid	• thyroid ultrasound • palpation	from 25–30 years of age	every 12 months
liver	• marking of blood serum alpha-fetoprotein • abdominal ultrasound • liver palpation	up to 7 years of age	every 3–6 months
desmoid tumours	• CT • MRI		
pancreas	• abdominal ultrasound	depending on family history	
CNS tumours	• physical examination (due to limited data, no indications for imaging tests)		every 12 months

[*1] testing frequency should be based on Spiegelman's guidelines

genetic and preventive diagnostics is available for families with suspected hereditary cancer syndromes with dominant predisposition to development of colorectal cancer, including:

- familial adenomatous polyposis syndrome (FAP),
- Lynch syndrome (HNPCC),
- Peutz-Jeghers syndrome (PJS),
- juvenile polyposis (JPS),
- recessive polyposis syndrome, which is conditioned by mutations in the *MUTYH* gene.

The diagnostics is aimed at identifying the mutation (in the first place) in the sick person, or in the absence of such a possibility (e. g. death, no consent to perform a genetic test) in a 1st degree relative. This allows for the introduction of an optimal scheme of care for the mutation carrier and their family, which (in the long term) increases the survival time of the carrier of the *APC* gene mutation by about 10–12 years and helps to extend the survival time of the carriers of mutation in *MLH1*, *MSH2*, *MSH6*, *PMS2, STK11*, *SMAD4*, *BMPR1A*, *EPCAM* and *MUTYH* genes.

Patients are qualified for the program by a clinical genetics specialist on the basis of family history and clinical data, which take into account the type/location of the neoplasm and the age of disease onset in both the probate and their first- and second-degree relatives, possibly other family members. Module II provides for:

- detection of mutations in genes: *APC*, *MLH1*, *MSH2*, *MSH6*, *PMS2*, *STK11*, *SMAD4*, *BMPR1A*, *EPCAM* and *MUTYH* in the carrier (including molecular testing, immunohistochemistry and microsatellite instability assessment),
- evaluation of the expression of mutator genes in colorectal cancers diagnosed before the age of 60,
- regular colonoscopy, gastroscopy, gynaecological ultrasound and serum Ca-125 marking [15].

Personalised treatment for colorectal cancer

Personalised medicine, which is currently used in oncology, is intended both for patients with diagnosed hereditary cancer syndrome and patients with sporadic neoplasms. The concept of "individual" (personalised) treatment requires selection of patients based on molecular predictors (necessary to assess the response to treatment) in order to increase the effectiveness of therapy and minimise exposure to adverse effects [20].

Personalised treatment of colorectal cancer based on molecular characteristics of the tumour tissue, started from confirmation of the fact that downstream abnormalities in genes of the EGFR signalling pathways (epidermal growth factor receptor) contribute to development of this neoplasm. Normal cells divide in response to growth factor signals that interact with cell surface receptors. In the case of an increase in the number of growth factor receptors or their excessive sensitivity, as in the case of colorectal cancer, signals are sent to the inside of the cell leading to its excessive and uncontrolled proliferation. Therefore, for a dozen years now, cetuximab and panitumumab (monoclonal antibodies to bloc EGFR receptor) have been applied in patients with metastatic colorectal cancer. The mechanism of both antibodies is the same as they bind to the extracellular ligand-binding domain, thereby inhibiting the activity of EGFR signalling pathways. These are mainly two intracellular pathways: RAS-RAF-MEK-ERK (responsible for the proliferation of tumour cells) and PI3K-PTEN-AKT (responsible for the survival, growth and invasion capacity of the tumour) [21].

The results of many studies over the past decades have shown that both cetuximab and panitumumab become ineffective in the presence of mutations in genes regulating subsequent steps in intracellular signalling. Therefore, an indispensable part of the targeted treatment in patients with metastases of colorectal cancer is to assess the genotype of the neoplastic tissue in terms of mutations in the *RAS* genes (especially *KRAS* and *NRAS* – mutations present in 30–50% of CRC patients), as well as the *BRAF* gene (mutations occurring in approximately 8–12% of patients). Pioneering observations allowed a conclusion that benefits of treatment with anti-EGFR antibodies concern patients with the correct form of the *KRAS* gene (wild type – WT), while further analyses proved that the presence of mutations in this gene is a negative predictor of response to anti-EGFR therapy. Many analyses carried out so far also indicate that the *KRAS* gene should be assessed along with other biomarkers of the RAS-RAF-MEK-ERK and PI3K-PTEN-AKT pathways, as the expected response to treatment is also determined by the condition of the remaining components of signalling pathways [21, 22].

The success of therapy in patients with metastatic colorectal cancer is also threatened by the risk of mutations in *RAS* genes occurring during the treatment and leading to developing resistance to anti-EGFR drugs. To avoid another tumour tissue biopsy, it is possible to detect development of these mutations by analysing the DNA of the tumour tissue circulating in the patient's blood, but this technique is not routinely applied.

In patients with tumours not responsive to anti-EGFR components-based chemotherapy, another class of drugs can be applied that target vascular endothelial growth factor (VEGF) instead of epidermal growth factor. This factor promotes the growth of blood vessels, including those that supply blood to the neoplastic tissue, and when it's blocked, sufficient blood supply to the tumour is prevented, thus causing its contraction. The most commonly used anti-VEGF drug is the monoclonal antibody bevacizumab. Aflibercept and ramucirumab have also been used [22, 23].

Selection of tissue for biomarker testing is important, and so is enrichment of samples by macrosection to maximise the tumour cell content (>50%) prior to DNA isolation. Primary tumour tissue or liver metastases are recommended for the *RAS*

mutation test. Other sites of metastasis (lymph nodes, lungs) are only considered if no primary tumour specimen or liver metastases are available. In parallel, in each case, the status of the *BRAF* mutation should be assessed for prognostic and, to a lesser extent, predictive assessment [22].

RAS mutations

The presence of mutations within the proto-oncogene family of *RAS* genes (including *KRAS* – about 40%, *NRAS* – 3–8% and *HRAS* – 3–4%) is a negative predictive biomarker for anti-EGFR antibody therapy in patients with metastatic colorectal cancer. This is due to the fact that, despite the inhibition of EGFR activity, EGFR-independent signal transmission takes place via the RAS-RAF-MEK-ERK pathway to the cell nucleus. This leads to increased, uncontrolled proliferation. Therefore (in accordance with the applicable standards) testing for this mutation should be performed in all patients at the time of diagnosis; however, it is mandatory prior to treatment with anti-EGFR monoclonal antibodies (cetuximab and panitumumab). The current standards, approved by ESMO and NCCN, require confirmation of *KRAS* wild-type (no mutation) before initiating cetuximab and panitumumab treatment. Moreover, patients with a confirmed mutation in the *KRAS* gene are not treated with anti-EGFR monoclonal antibodies, because such therapy does not bring any benefits, and additionally it may lead to a shortened survival while causing exposure to numerous side effects [21, 24].

BRAF mutations

Mutations of the *BRAF* proto-oncogene are present in tumour tissue in approximately 8–12% of patients with metastatic colorectal cancer and are excluded from the *KRAS* mutations. More than 90% of these mutations concern codon 600 (V600), where valine is replaced with another amino acid, most often glutamic acid (V600E). Therefore, in accordance with the guidelines of NCCN and ESMO, it is recommended to analyse the *BRAF* mutation in cancers with wild-type *KRAS* before administration of anti-EGFR therapy (currently *BRAF* mutation status is assessed in parallel with the *RAS* mutation status).

A study of patients with metastatic colorectal cancer with identified *BRAF* mutation found that two-thirds of them had the primary tumour on the right side of the colon and associated with an increased risk of metastases to the peritoneum and distant lymph nodes, with a concomitant reduced frequency of lung metastases. Presence of the *BRAF* gene mutation was also shown to be a negative prognostic marker, with a 10.4-month survival in patients with the current mutation, compared to 34.7-month survival in patients with wild-type BRAF tumours. Nearly one-third of tumours with the *BRAF* mutation also showed microsatellite instability (MSI), and the same percentage of MSI tumours contained the *BRAF* mutation [22, 25, 27].

Contrary to the predictive status of *KRAS*, the value of the mutation *BRAF* is still under investigation. It seems that the predictive value of *BRAF* depends on whether patients receive anti-EGFR preparations in first-line treatment (most often chemosensitive tumours) or in the 2nd- or 3rd-line treatment (chemoresistant tumours) [25].

Genetic/dagnostic tests used to assess mutation status of *RAS* and *BRAF* genes

Assessment of the mutational status of the *RAS* and *BRAF* genes in treatment of metastatic colorectal cancer has become a standard of diagnostic procedure in recent years, which supports the selection of the best therapy for a given patient.

Most often, DNA is isolated from previously prepared paraffin blocks. A qualified pathologist, who assesses the percentage of neoplastic cells in such a preparation, plays an important role in choosing the right block. Therefore, close collaboration between pathologists and molecular biologists is essential. Moreover, the technique of producing the block, including the buffers used, are of great importance for the quality of the nucleic acids isolated from them.

A laboratory where genetic tests are performed requires not only appropriate equipment and highly qualified personnel, but it should also participate in international quality control tests to confirm quality of tests performed. In the case of *RAS* and *BRAF* genes, tests are organised, among others, by the European Society of Pathology: Colon External Quality Assessment Scheme.

Currently, commercially available kits are used for routine assessment of the status of the *RAS/BRAF* genes. They allow assessment of the most frequent mutations and the analysis of pathogenic changes in *RAS* should cover at least exons 2, 3 and 4 of the *KRAS* genes (codons 12, 13, 59, 61, 117 and 146), as well as exons 2, 3 and 4 of the *NRAS* gene (codons 12, 13, 59, 61 and 117) [22]. The advantage of ready-made tests over tests created independently by a laboratory involves validation as well as approval/certification for *in vitro* diagnostics (CE-IVD). This, in turn, is related to the high reliability of the obtained results. Currently, there are also several commercial kits approved by the US Food and Drug Administration (*FDA)*. The assays for the *RAS/BRAF* mutations are mainly based on the Real-Time PCR method, assessing more than ten mutations in the *KRAS/NRAS* and mutations in the *BRAF* V600 gene at the same time. There is also commercially available, FDA-approved kit for next generation sequencing, which allows assessment of 56 mutations in *KRAS/NRAS*. The scope of testing variants is constantly updated according to the latest knowledge and recommendations.

PI3K/PTEN/AKT axis

The correct form of the *RAS* gene (in particular *KRAS*) does not guarantee a positive response to anti-EGFR treatment. This means that the therapy in patients with colorectal cancer depends also on other mechanisms, hence the need to analyse other markers. The PI3K/PTEN/AKT pathway is also associated with the KRAS/

BRAF signalling pathway. Mutations in the *PIK3CA* gene (which codes the catalytic subunit p110α of the PI3K enzyme) occur in about 10–20% of colorectal cancers and are associate both with *KRAS* mutations, and tumour microsatellite instability [26]. The mutated form of *PIK3CA* leads to constant signal transmission to AKT pathway, inducing increase and proliferation of tumour cells. In turn, the protein product of the suppressor gene *PTEN* (phosphatase and tensin homolog; a component of the pathway) is responsible for inhibiting the AKT kinase pathway. Loss of PTEN activity (most often caused by gene mutations, its deletion or promoter methylation) leads to hyperactivation of the PIK3/AKT pathway. There were individual cases of coexistence of *PIK3CA* mutation and *PTEN* inactivation. Data on the influence of these disorders on the response to treatment with anti-EGFR preparations are contradictory, which makes it difficult to assess their value as predictors. This is most likely due to the variety of mutations that can appear within *PIK3CA*. The most frequently found and analysed mutations (*hotspots*) are variants present in exons 9 and 20 of the *PIK3CA* gene. Based on the results of experimental and epidemiological studies, it seems that mutations present in exon 20 play a significant role in the treatment, as opposed to mutations occurring in exon 9. At present, the predictive value of the *PIK3CA* gene mutation for anti-EGFR therapy in patients with normal (wild) type *RAS* genes is low and further research is required [21].

Defining therapeutic strategy

The optimal therapeutic strategy for each patient is determined on the basis of a clinical examination, blood count, determination of the parameters of kidney and liver function, measurement of the level of tumour markers, imaging tests (including CT and MRI of the abdominal cavity and chest) and assessment the patient's general clinical condition. The general condition and fitness of the patient are important both prognostic and predictive factors for the introduced chemotherapy (tab. III) [22, 23].

1. First-line treatment

- FOLFIRI (leucovorin + fluorouracil + irinotecan) + cetuximab (in cases of no mutations in *RAS* and *BRAF*),
- FOLFOX (leucovorin + fluorouracil + oxaliplatin) + panitumumab (in cases of no mutations in *RAS* and *BRAF*),
- FOLFIRI + panitumumab (in cases of no mutations in *RAS* and *BRAF*),
- FOLFIRI + bevacizumab (in cases of *RAS* mutation), combined with prior adjuvant chemotherapy including oxaliplatin, and resection of the primary lesion,
- FOLFOXIRI (leucovorin + fluorouracil + oxaliplatin + irinotecan) + bevacizumab (in cases with *BRAF* mutation) and removal of the primary lesion [29],
- fluoropyridine monotherapy in patients who do not tolerate aggressive treatment [22, 23, 27, 28].

2. Second-line treatment

- FOLFOX + bevacizumab (provided that no adjuvant chemotherapy containing oxaliplatin and resection of the primary lesion were applied),
- FOLFIRI + aflibercept (with no irinotecan chemotherapy applied, in cases of no effect of oxaliplatin and fluoropyrimidine chemotherapy and resection of the primary lesion) [22, 23, 27].

Second-line therapy begins with the change of the first-line therapy strategy, primarily because of the failure of the original assumptions. It is usually offered to patients in good general condition, with normal internal organ function and depends on the choice of first-line therapy.

3. Third-line treatment

- cetuximab or panitumumab (in cases of no *RAS* and *BRAF* mutations and no prior anti-EGFR treatment),
- regorafenib (recommended in patients previously treated with fluoropyrimidine, oxaliplatin, irinotecan, for whom treatment with anti-VEGF or anti-EGFR is not considered),
- trifluridine with tipiracil (Lonsurf) with insensitivity to previous systemic therapy based on fluoropyridine, oxaliplatin and irinotecan,
- if microsatellite instability (6–8% of tumours) and resistance to chemotherapy are diagnosed, anti-PD-1 immunotherapy should be considered [22, 23, 27].

Table III. Selection of systemic therapy in accordance with the treatment algorithm for patients with metastatic colorectal cancer (excluding patients with oligometastases) – based on the ESMO recommendations

Treatment objective	Cytoreduction (tumour atrophy)			Disease control (progression control)		
molecular profile	*RAS* wt	*RAS* mt	*BRAF* mt	*RAS* wt	*RAS* mt	*BRAF* mt
first line						
preferred choice	double chemotherapy + EGFR antibody	double chemotherapy + bevacizumab	FOLFOXIRI + bevacizumab	double chemotherapy + bevacizumab or dual chemotherapy + EGFR antibody	double chemotherapy + bevacizumab	FOLFOXIRI +/– bevacizumab
second choice	FOLFOXIRI +/– bevacizumab	FOLFOXIRI + bevacizumab	double chemotherapy + bevacizumab	FP + bevacizumab		double chemotherapy + bevacizumab
third choice	double chemotherapy + bevacizumab	FOLFOXIRI	FOLFOXIRI			

Treatment objective	Cytoreduction (tumour atrophy)			Disease control (progression control)		
observation						
preferred choice	FP + bevacizumab	FP + bevacizumab	FP + bevacizumab	FP + bevacizumab	FP + bevacizumab	FP + bevacizumab
second choice	intermission	intermission	intermission	intermission	intermission	intermission
second line						
preferred choice	double chemotherapy + bevacizumab	double chemotherapy + bevacizumab	double chemotherapy + bevacizumab	dual chemotherapy + bevacizumab or dual chemotherapy + EGFR antibody	double chemotherapy + bevacizumab	double chemotherapy + bevacizumab
second choice	dual chemotherapy + EGFR or FOFIRI antibody + aflibercept / ramucirumab	FOLFIRI + aflibercept / ramucirumab	FOLFIRI + aflibercept / ramucirumab	FOLFIRI + aflibercept / ramucirumab	FOLFIRI + aflibercept / ramucirumab	FOLFIRI + aflibercept / ramucirumab
third line						
preferred choice	double chemotherapy + EGFR antibody or irinotecan + cetuximab	regorafenib or trifluridine/ tipiracil	regorafenib or trifluridine/ tipiracil	double chemotherapy + EGFR antibody or irinotecan + cetuximab	regorafenib or trifluridine/ tipiracil	regorafenib or trifluridine/ tipiracil
second choice	monotherapy with EGFR antibodies			monotherapy with EGFR antibodies		
third choice	regorafenib or trifluridine/ tipiracil			regorafenib or trifluridine/ tipiracil		

FP – fluoropyrimidine; mt – mutation; wt – wild type; EGFR antibodies – cetuximab and panitumumab [22]

Conclusion

Year by year, personalised medicine is ever more broadly applied in management of cancer, especially colorectal cancer. Application of targeted therapy based on molecular predictors is aimed at administering treatment which would increase survival time and improve life comfort by minimising adverse effects of the therapy applied. Further, identification of patients with familial cancer predisposition allows introduction of prophylaxis and diagnostic-prophylactic process for all relatives of the patient selected based on the family history. Implementation of such procedures in the case of colorectal cancer and other cancers in its spectrum requires cooperation of a team of specialists, including a clinical geneticist, surgeon, oncologist, pathologist and lab diagnostician.

Conflict of interest: none declared

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The surgical anatomy of the mammary gland. Vascularisation, innervation, lymphatic drainage, the structure of the axillary fossa (part 2.)

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Dynamically developing oncoplasty, i.e. the application of plastic surgery methods in oncological breast surgeries, requires excellent knowledge of mammary gland anatomy. This article presents the details of arterial blood supply and venous blood outflow as well as breast innervation with a special focus on the nipple-areolar complex, and the lymphatic system with lymphatic outflow routes. Additionally, it provides an extensive description of the axillary fossa anatomy.

Key words: anatomy of the mammary gland

The large-scale introduction of oncoplasty to everyday oncological surgery practice of partial mammary gland resections, partial or total breast reconstructions with the use of the patient's own tissue as well as an artificial material such as implants has significantly changed the paradigm of surgical procedures. A thorough knowledge of mammary gland anatomy has taken on a new meaning. Correct arterial blood supply to tissues is a key element in plastic surgery and breast reconstruction surgery.

Vascularisation of the mammary gland

Arterial vessels

The vascularisation of breasts is characterised by rather significant individual diversity. Its relatively stable elements are the internal thoracic artery (*arteria mammaria interna*) running through the system of perforators, the lateral thoracic artery (*arteria thoracica lateralis*), the thoracoacromial artery (*arteria thoracoacromialis*), end branches of the perforators of 3th–8th intercostal arteries (*aa. intercostales*) and little vessels supplying blood to the serratus anterior [1, 2].

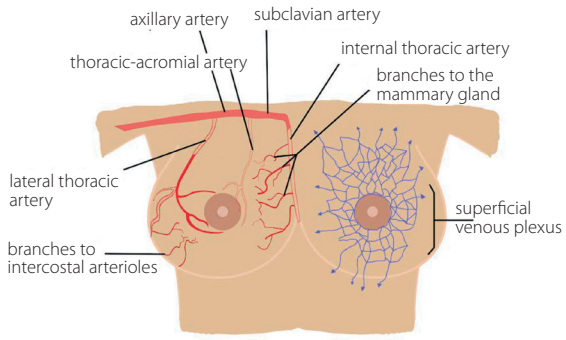

Figure 1. Arterial and venous vessels of the mammary gland

The internal thoracic artery (*arteria mammaria interna*) is a branch of the subclavian artery (*arteria subclavia*) which goes off it near the scalenus posterior (*musculus scalenus*) and enters the chest passing the subclavian vein (*vena subclavia*). Within the chest, it crosses with the phrenic nerve (*nervus*

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phrenicus) and continues its route on the internal surface of the front wall along the attachments of ribs to the sternum, 1–2 cm laterally to its edge, between the endothoracic fascia (*fascia endothoracica*) and the parietal lamina of the pleura. In each intercostal space, it divides itself into two branches:

1. the anterior cutaneous branch (*ramus cutaneus ventralis*),
2. the intercostal branch (*ramus intercostalis*) which connects directly to the appropriate intercostal artery (*arteria intercostalis*) through a direct branch of the aorta.

The arterial blood supply of the mammary gland is primarily ensured by medial thoracic branches (*rr. mammari mediales*) supplying medial and lower lateral quadrants. At the level of the 6th intercostal space, the internal thoracic artery is divided into two end branches: the musculophrenic artery and the superior epigastric artery.

The musculophrenic artery (*arteria musculophrenica*) is the final lateral branch of the internal thoracic artery with little branches going off to the 7^{th,} 8th and 9th intercostal spaces. Eventually, it divides itself and ends at the diaphragm and muscles of the lateral part of the abdomen [3, 4].

The superior epigastric artery (*arteria epigastrica superior*) is the final medial branch of the internal thoracic artery and its prolongation towards the rectus abdominis. Along its route, it branches off to muscles, the skin and the diaphragm. Its anonymous branches at the level of the xiphoid process of the sternum connect to the branches of the opposite side. The anonymous branches going off the right superior epigastric artery enter the falciform ligament of the liver and connect to the branches of the common hepatic artery. The superior epigastric artery within the rectus abdominis, at the level of the navel, connects to the inferior epigastric artery, a branch of the external iliac artery. The superior epigastric artery is the main artery supplying blood to the cutaneous--adipose flap of the hypogastrium used in TRAM flap breast reconstruction surgery.

The inferior epigastric artery (*arteria epigastrica inferior*) is a branch of the external iliac artery. It is used in breast reconstruction employing a DIEP flap with a vascular microfusion with the internal thoracic artery at the level of the 3rd rib [5, 6].

The axillary artery branches (*arateria axillaris*) supply the bones and muscles of the upper limb, pectoral muscles, the serratus anterior and the latissimus dorsi muscles, the shoulder joint and the mammary gland. The arteries forming the arterial network of the chest branch off from the axillary artery. These branches arise at different locations and because of their significant variety, they are hard to find during surgery.

The following arteries branch off from the upper section of the axillary artery:

- the superior thoracic artery (the highest) (*arteria thoracica superioris*),

the following arteries branch off from the middle section:

- **the thoracoacromial artery** (*arteria thoracoacromialis*),
- **the lateral thoracic artery** (*arateria thoracica lateralis*),

and the following arteries branch off from the lower section:

- the subscapular artery (*arteria subscapularis*),
- the anterior circumflex humeral vein (*arteria circumflexa humeri anterior*),
- the posterior circumflex humeral vein (*arteria circumflexa humeri posterior*).

The thoracoacromial artery (*arteria thoracoacromialis*) is a short stem going off on the anterior surface of the axillary artery over the upper edge of the smaller pectoral muscle. After crossing through the coracoclavicular fascia, it divides itself into four branches: thoracic, coracoid, clavian and branchial. The thoracic branch forms numerous connections with the internal thoracic artery, the lateral thoracic artery and intercostal arteries. In this way, it participates in supplying blood to the mammary gland, primarily the tail of Spence [7–9].

The lateral thoracic artery (*areteria thoracica lateralis*) goes off below the edge of the smaller pectoral muscle, runs downward and medially crossing with the ulnar nerve (*nervus ulnaris*) and the axillary vein (*vena axillaris*) at the front. Then, on the serratus anterior, it divides itself into 2th–5th intercostal spaces. Here, it gives off its lateral thoracic branches (*rami mammari laterales*), which cross through the greater pectoral muscle to supply blood to the mammary gland and the skin near it and then connect with the thoracic branches going off (as piercing branches) from the internal thoracic artery, which is the main breast-supplying artery [1, 3, 8].

Numerous connections of arterial vessels supplying blood to glandular tissue and covering the skin make three plexuses, which are the most important elements of arterial blood supply:

1. **The subdermal plexus** (*plexus subdermalis*) – very extended, formed by numerous anastomoses between the branches of the thoracobrachial artery and the neighbouring arteries: subclavian, subscapular and anterior branches of the perforators which come from the internal thoracic artery [10, 11].
2. **The preglandular plexus** (*plexus preglandularis*) – supplied by anterior and glandular branches of the lateral thoracic artery, the third perforator of the internal thoracic artery and other anterior thoracic perforators. Two major arteries, lateral and medial, form connections that circumvent the areola. Additionally, the preglandular plexus has numerous connections to the subcutaneous plexus. Together, they form an extensive network of arterial vessels covering the anterior surface of the gland and branching off, in large numbers, inside the gland, perpendicularly to the breast surface. These arterial branches penetrate the glandular tissue along the connective tissue septa surrounding lobules, lactiferous follicles and exosecreting ducts [10, 12].
3. **The retroglandular plexus** (*plexus retroglandularis*) – made by deep muscular perforators which are branches

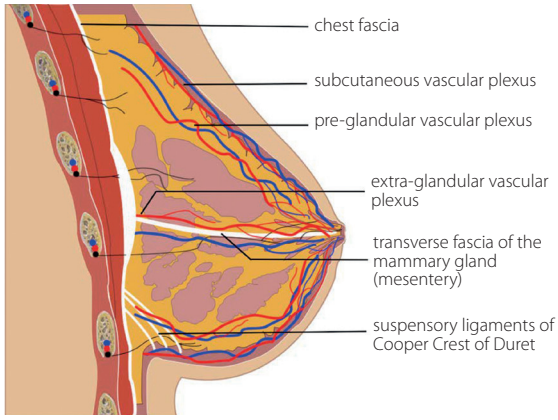

Figure 2. Sagittal cross section of the breast

of the thoracoacromial (coracoid) artery, deep branches of the medial (from the 2nd, 3rd, 4th and 5th intercostal artery) and lateral (from the 7th, 8th and 9th intercostal arteries) intercostal perforators. This plexus is of lesser functional importance, although it is closely related to the previous system of interglandular connections running along interlobar and interlobular connective tissue septa [13].

An important element of breast surgery is a thorough knowledge of the nipple-areolar complex vascularisation. Seitz et al. proposed the sources of blood supply to the areola and the nipple be divided into five anatomical spheres called *NACsomam* by the authors:

- I – medial,
- II – lateral,
- III – central,
- IV – inferior,
- V – superior.

In the studies performed, the majority of vascularisation was provided by inferior-medial sphere I. Moreover, it was confirmed that arterial blood supply to the nipple-areolar complex is symmetrical for both breasts in 96% of cases [14].

Venous outflow from the mammary gland

The main pathway for venous blood outflow is the axillary vein (*vena axillaris*). It is a short stem with a large lumen exceeding the diameter of the axillary artery, which is formed by the combination of two deep brachial veins and runs deep into the axillary fossa medially from its foundation to the lower edge of the clavicle. When it passes the clavicle, it transforms into the subclavian vein. The tributaries of the axillary vein are: the cephalic vein (*vena cephalica*), the lateral thoracic vein, the areolar venous plexus of the nipple (*plexus venosus alveolaris*), the thoracoepigastric vein and the intercostobrachial veins (*vv. intercostobrachiales*). An important anatomical element is the subareolar venous plexus, which forms a dense network of connections circumventing the areola (venous corona). From here, venous blood outflow may take two routes:

- superficial, which begins below the areola (Haller's plexus) and drains the blood to the internal thoracic vein and superficial veins of the lower part of the neck,
- deep, located at a greater depth under the superficial fascia, which transports blood to the internal thoracic vein, posterior intercostal veins and directly to the axillary vein [13, 15].

Breast innervation

Nerves providing innervation to the mammary gland come from the somatic peripheral nervous system and the autonomic sympathetic system. Within the breast, there are no parasympathetic system nerve endings. The innervation of the mammary gland tissue and the anterior-lateral area of the chest together with the covering skin is closely related. This is confirmed by the common ectodermal origin of both structures [16].

Breast innervation comes from three sources:

1. Ventral branches of spinal nerves, from Th2 to Th6 (intercostal nerves from 2nd to 6th). Cutaneous-glandular nerves are end branches of both lateral and medial perforators of intercostal nerves (*nn. intercostales*). After crossing through the pectoral muscles, the anterior medial branches of intercostal nerves 2nd–6th run on the breast surface providing innervation both to the mammary gland and the skin covering it. The branch running from the 4th intercostal nerve goes directly to the nipple [17, 18]. The group of lateral nerves is created by external branches of lateral perforators, from the 3rd to the 6th intercostal nerve, which enter the glandular tissue from the back, near the external borders. After branching off from the cutaneous branches, the main stems run upward along the posterior breast surface and regular glandular branches go off from them in the posterior-anterior direction. They run along the ligaments of Cooper and form an extensive network of connections innervating the areola skin. Numerous sensory endings, sensory bodies as well as pressure and temperature receptors make the nipple-areolar complex one of the best innervated areas of the female body [17, 19, 20].
2. Branches of the supra- and subclavian parts of the brachial plexus:
   - the medial thoracic nerve (C8–Th1, *nervus thoracicus medialis*) and the lateral thoracic nerve (C5–C7, *nervus thoracicus lateralis*) provide innervation to the greater and smaller pectoral muscles,
   - the long thoracic nerve (C5–C6, *nervus thoracicus longus*) provides innervation to the serratus anterior muscle,
   - the thoracodorsal nerve (C6–C8, *nervus thoracodorsalis*) provides innervation to the latissimus dorsi muscle [19, 21].
3. Supraclavicular nerves (C3–C4, *nn. supraclaviculares*) from the jugular plexus provide innervation to the upper part of the chest (near the clavicle).

PECS I and PECS II (*pectoral nerve blocks I & II*) are septum blocks within the chest wall commonly used as one of the elements of multimodal analgesia in breast surgery [13].

The branches of the autonomic system from the paravertebral sympathetic chain of superior thoracic ganglions form an important element of breast innervation. The motor fibres of the sympathetic system provide innervation to the smooth muscles of the areola and the nipple as well as the smooth muscles of the arterial vessels of the nipple-areolar complex.

The innervation of the nipple-areolar complex is extremely complex, because of frequent differences in the pathways of the nerves providing it. The most stable source of innervation comes from the lateral branch of the intercostal nerve. The innervation provided by the anterior branches of the 3rd, 4th and 5th intercostal nerves is characterised by greater diversity. The cutaneous branches of the 2nd and 6th intercostal nerves do not participate in the innervation of the nipple or the areola as they exclusively innervate the peripheral segments of the breast skin [13, 22].

Lateral cutaneous branches, of a greater diameter than the anterior branches, cross through the deep fascia along the medial axillary line and run in the medial direction on the pectoral muscle. At the level of the medial-clavicular line, they suddenly bend under the straight angle and run along the connective tissue septa of the breast towards the nipple, which they innervate in the form of numerous little branches. Only in rare cases do lateral branches run on the surface of the subcutaneous tissue directly to the nipple.

Anterior cutaneous branches innervate the medial part of the nipple-areolar complex. They cross through the fascia along the parasternal line (*linea parasternalis*) and divide into medial branches running to the tissue covering the sternum while the lateral branches in the subcutaneous tissue go on the surface to the direction of the nipple. These branches reach the edge of the areola of the left breast within the area between 8 and 11 o'clock and the right breast – between 1 and 4 o'clock. Therefore, peri-areolar incisions should be avoided in these areas because of the risk of damage to the main branches innervating the nipple. This may be the cause of a partial or complete loss of sensation [8, 17, 21].

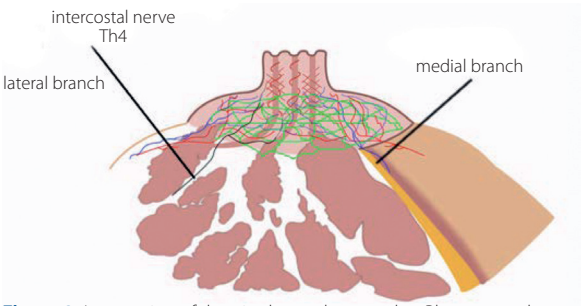

Figure 3. Innervation of the nipple-areolar complex. Please note that the lateral and medial branches of the intercostal nerve 4 run along a different route

There is an interesting relationship between the diameter and number of nerve branches providing innervation to the areola and the nipple – the smaller the diameter, the more numerous the branches [17].

Lymphatic drainage

The anatomical foundations of lymphatic drainage were presented in 1874 by Sappey, who suggested that the lymph from the mammary gland is drained separately from other parts of the trunk. Until today, knowledge with regard to this subject is derived from the works by Sappey, Poirier and Cuneo [23].

The understanding of the interstitial fluid circulation in the breast makes it possible to better realise the significance of correct surgical incisions and the rules for soft tissue movement during oncoplasty. The routes of lymph drainage from the mammary gland allow better planning and performance of such surgeries in patients with breast cancer.

Routes of lymphatic outflow

Lymph vessels make a network of open vessels draining the interstitial fluid from all areas of the human body. Having a small diameter initially, they connect to form increasingly greater vessels, in a similar way to the venous system. On the way, they pass through lymph node stations, which serve as filters and a form of the body's defence against microorganisms and tumour cells. Eventually, the lymph flowing through the thoracic duct (*ductus thoracicus*) and the right thoracic duct reaches, respectively, the left and right venous angle at the junction of the subclavian vein and the internal jugular vein, where it flows into the venous system.

The lymphatic drainage of the breast starts in the intercellular spaces of glandular tissue lobules through a network of non-valvular lymph capillaries (20–70 µm in diameter). Over the network of pre-collectors (70–150 µm in diameter), which already have valves and are located in the dermis, the lymph flows to deep lymph collecting vessels located in deep tissues underneath the deep fascia. A network of many lymph vessels located just under the breast areola is created by the superficial and deep sub-areolar plexus called, after its discoverer, the Sappey plexus [23, 24].

Due to the ectodermal origin of breasts, the lymphatic draining of the mammary gland is closely related to the skin drainage. Lymph from the skin is drained through an extended network of lymph vessels running to the subcutaneous plexus located between the skin and the superficial fascia. In a similar way, lymph from the mammary gland is drained through extended lymph plexuses around each lobe flowing to the superficial main collector and creating the sub-areolar Sappey plexus, which, in turn, connects to the deep fascia plexus through numerous vessels crossing the glandular tissue.

Thus, the network of breast lymph vessels is made of four connected plexuses:

- the cutaneous plexus (*plexus cutaneus*),
- the subcutaneous plexus (*plexus subcutaneus*),
- the fascial plexus of the greater pectoral muscle (*plexus fascialis*),
- the glandular plexus (*plexus glandularis*), which includes lobules, lobes and lactiferous ducts [24, 25].

The glandular plexus drains lymph directly to the subcutaneous plexus located under the areola called the Sappey plexus. The fascial plexus is also connected to the subcutaneous plexus through the vessels running in interlobular septa made of connective tissue. Subareolar plexuses drain lymph in two directions: to axillary lymph nodes and to the lymph nodes located along the internal thoracic artery. Moreover, there are lymphatic connections between both breasts, which may be the cause of rare metastases to the lymph nodes of the opposite side. The drainage from the fascial plexus does not have a significant share in the lymphatic drainage of the breast, but it may be an alternative route if the main drainage pathway is closed. Lymph in the fascial plexus comes from the drainage of the greater and smaller pectoral muscles and, from there it flows to the apical axillary nodes. The intermuscular lymphatic route along the thoracoacromial artery, also known as Groszman's route, goes through 1–4 Rotter's nodes located between the greater pectoral muscle and the smaller pectoral muscle.

Because of the very extensive network of lymph vessels and numerous connections between lymph nodes, each breast may be drained both to the lateral axillary nodes and medial retrosternal nodes. However, most of the lymph from the breasts is drained to axillary lymph nodes [3, 24].

Lymphatic drainage may also be achieved through vessels accompanying lateral branches of intercostal arteries to nodes located just behind the ribs and, from there, directly to the thoracic duct (*ductus thoracicus*). Another possible direction of lymphatic drainage is to the antephrenic node, the liver and then to the ventral nodes (Gerota's pathway) [13, 24].

From the superficial (subareolar) and deep plexuses, the lymph is further drained along three main pathways:

1. **the axillary or lateral pathway** which drains the lymph directly from the subareolar plexus, satellite lymph nodes and most parenchymal lymph vessels. Lymphatic drainage occurs along the lower edge of the greater pectoral muscle going to the group of axillary lymph nodes,
2. **along the internal thoracic artery**, where drainage starts both in the medial and lateral part of the breast and lymph vessels go through the greater pectoral muscle inside the chest wall. Along the medial edge of the breast there are pathways combining the areas of lymphatic drainage from both breasts and going to parasternal lymph nodes [3],
3. the **retromammary pathway** (*retromammary pathway*) – lymphatic drainage from the posterior part of glandular tissue [13].

Lymph nodes

Axillary lymph nodes are the main station filtering the lymph from the mammary gland, although they are located outside the gland. Additionally, they are also a part of the lymph flow pathway from the upper limb and the chest wall.

Axillary lymph nodes can be divided into 5 groups:

1. Lateral thoracic nodes (or thoracic-axillary) usually make a group of 5–10 lymph nodes located along the lateral thoracic vessels directly behind the greater pectoral muscle, below the smaller pectoral muscle.
2. Acromial nodes (lateral axillary) are a group of 1–6 nodes located along the posterior surface, outside the axillary vein and the lower edge of the smaller pectoral muscle. They drain the upper limb. They should be preserved during surgical lymphadenectomy because their removal causes lymphatic oedema of the upper limb. The boundary of correct lymphadenectomy is the lower edge of the axillary vain.
3. Subscapular nodes (lower scapular) are a group of about 5 lymph nodes located along the nerves and vessels running to the latissimus dorsi muscle. They drain the lateral part of the back but should be removed during lymphadenectomy because of numerous connections with lymph pathways draining the lower-lateral parts of the mammary gland.
4. Central axillary nodes are 2–6 nodes occupying the central part of the axillary fossa which are located below the smaller pectoral muscle and partially behind it. They drain lymph from

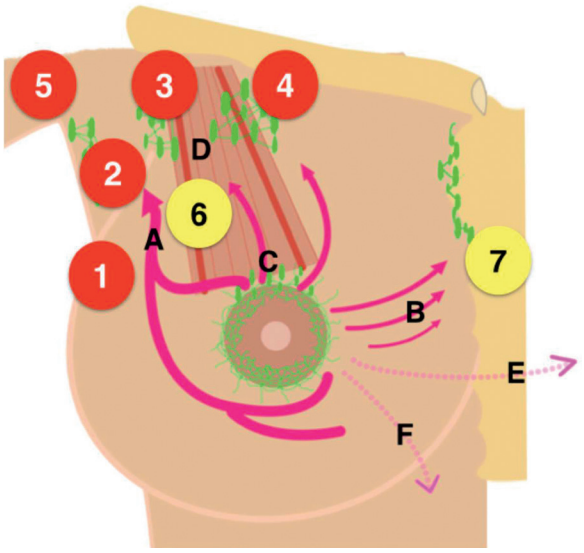

Figure 4. Lymphatic drainage of the mammary gland
Lymph nodes: 1 – lateral thoracic nodes (5–10), 2 – subscapular nodes (5), 3 – central nodes (2–6), 4 – subclavian nodes – apical (10), acromial nodes (1–6), 6 –Rotter's nodes (1–4), 7 – retrosternal nodes
Routes of lymphatic outflow from the mammary gland: A – axillary route (lateral), B – peristernal route (medial), C – extra-glandular route, D – Groszmann's route, E – route to the opposite breast, F – route along epigastric and subhepatic plexuses of the sac of the rectus muscle of the abdomen

the three previous lymph node groups. In the original method of mastectomy including lymphadenectomy described by Patey, the cutting of the attachment of the smaller pectoral muscle enabled easier access to this group of lymph nodes.

5. Subclavian nodes (apical axillary) are 10–11 lymph nodes located at the top boundary of the smaller pectoral muscle.

Rotter's interpectoral lymph nodes (1–4) are located between the greater and smaller pectoral muscle. They collect the lymph from the upper quadrants and the central part of the breasts. From these nodes, the lymph flows directly to the lymph nodes located outside or above the smaller pectoral muscle [3, 13, 24–26].

The parasternal lymph nodes (internal pectoral) are located along internal thoracic vessels within the chest. They can be found at the level of sternal attachments, from the 1st to the 6th rib. Lymph flows to them from the medial quadrants of the breasts, in particular at the level of the 2nd, 3rd and 4th intercostal spaces. These nodes, because of their location inside the chest, cannot be examined as part of a routine clinical examination and scintigraphy is necessary.

For the needs of surgical anatomy, the axillary lymph nodes are divided into 3 levels as proposed by Berg in 1955:

- The first level of lymph nodes contains 9–24 nodes located laterally from the mammary gland and medially from the lateral edge of the *latissimus dorsi*. The boundary is the lateral edge of the smaller pectoral muscle. This group contains lateral thoracic nodes, subscapular nodes, acromial nodes and central axillary nodes.
- The second level of lymph nodes contains 2–7 nodes located behind the greater pectoral muscle between its lateral and medial edge. This group contains superior axillary nodes and intermuscular lymph nodes.
- The third level of lymph nodes contains 1–12 lymph nodes located above the medial edge of the greater pectoral muscle. This group contains subclavian nodes.

Most (80–90%) lymphatic drainage from the breasts is achieved through the first level of axillary lymph nodes. In 4–20% of cases, the route of the lymphatic flow may pass over the first level and go directly to superior axillary nodes and intermuscular nodes, i.e. to the second level. Only in 3–5% of cases may lymph flow directly to the 3rd level of axillary lymph nodes with the passing over of the two lower levels. This is why the correct location of the sentinel node during oncological breast surgeries is so important in practice [26, 27].

About 75% of lymph flows along collective lymph vessels from the mammary glands through the peri-areolar plexus to the side in the direction of axillary lymph nodes. The remaining part goes directly to the lymph nodes located within the chest along the internal thoracic artery, to the opposite breast and to the superficial plexus of the rectus abdominis. Some of the lymph from the upper quadrants of the breasts may go directly to the lymph nodes located between the pectoral muscles (Rotter's route) [27].

Axillary fossa

The axillary fossa (*fossa axillaris*) is an important element in breast surgery. It is located below the acromial joint, which is the main connection between the chest wall and the upper limb, between two axillary folds, the anterior and the posterior. In its anatomical position, the axillary fossa is a narrow space, which, when an arm is abducted, forms a three-dimensional area looking like a pyramid with a cut-off peak in the cranial direction. In this place, under the clavicle, such important anatomical structures as arterial vessels, veins, nerves and lymph vessels enter the axillary fossa [18, 28].

Boundaries of the axillary fossa

The boundaries of the axillary fossa are made of 4 walls:

1. The narrow lateral boundary created by the intertubercular sulcus (*sulcus intertubercularis*) located between the major tuberculum (*tuberculum majus*) and the minor tuberculum (*tuberculum minus*) of the humerus.
2. The medial boundary made of the serratus anterior muscle (*musculus serratus anterior*), ribs and intercostal muscles (*mm. intercostales*).
3. The anterior boundary limited by the pectoral major (*musculus pectoralis major*), the pectoralis minor (*musculus pectoralis minor*) and the subclavian muscle (*musculus subclavicularis*).

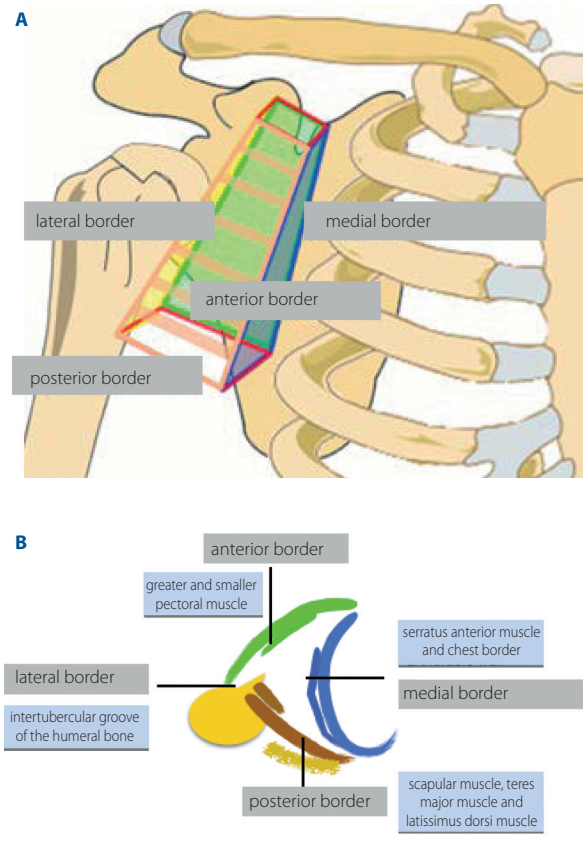

Figure 5. Anatomical borders of the axillary fossa: A. frontal view, B. transverse view

4. The posterior boundary between the subscapular muscle (*musculus subscapularis*), the latissimus dorsi muscle (*musculus latissimus dorsi*) and the teres major muscle (*musculus teres major*) [28, 29].

The apex, which is an inlet for clinically important structures running through the axillary fossa, is limited by the lateral edge of the 1st rib, the top edge of the scapula and the posterior edge of the clavicle.

The foundation of the axillary fossa is the superficial axillary fascia (*fascia axillaris superficialis*), which becomes the superficial pectoral fascia (*fascia pectoralis superficialis*) near the inframammary fold, the thoracic fascia (*fascia thoracica anterolateralis*) on the lateral side of the trunk and the superficial dorsal fascia (*fascia superfitialis dorsi*) near the posterior axillary fold [30–32].

The axillary fossa can be divided into three separate spaces: the subpectoral space (*spatium subpectorale*), the subfascial axillary space (*spatium axillare subfasciale*) and the space for the neurovascular bundle. The very narrow subpectoral space is located between the perimysium and the deep fascia of the pectoral muscle, from the clavicle to the anterior axillary fold. It is especially visible when lymph collects there after the removal of the axillary fossa lymph nodes. The interpectoral space limited by the superficial and deep axillary fascia is much more important in practice. This is where intercostobrachial nerves (*nn. intercostobrachiales*), the basilic vein (*vena basilica*) and deep axillary lymph nodes (*nodi lymphatici axillares profundi*) are located. The precise preparation of lymph nodes in this space enables their removal together with the surrounding adipose tissue without jeopardising the main neurovascular bundle and risking damage to it. [31].

Contents of the axillary fossa

The axillary fossa is built of the following structures:

1. the axillary artery (*arteria axillaris*) – the main artery supplying blood to the upper limb. Its medial and posterior parts cross the axillary fossa,
2. the axillary vein (*vena axillaris*) – the basic vein draining blood from the upper limb. Its tributaries within the axilla are the cephalic vein (*vena cephallica*) and the basilic vein (the royal vein, *vena basilica*),
3. the brachial plexus (*plexus brachialis*) made of spinal nerves C5–Th1. The main nerves going off from this plexus supply the upper limb, the chest wall and the breast,
4. axillary lymph nodes (*nodi lymphatici axillares*) – draining nodes for the lymph flowing from the upper limb, the chest wall and the mammary gland,
5. the biceps branchii muscle (*musculus biceps brachii*) and the coracobrachialis muscle (*musculus coraco-brachialis*). The tendons of these muscles run through the axillary fossa and attach to the coracoid process (*processus coracoideus*) of the scapula [29].

Conflict of interest: none declared

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Plagiarism and self-plagiarism – facts and myths

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The problem of plagiarism and self-plagiarism is becoming more and more important in the context of the discussion on pathologies in science. Although in many cases these are reprehensible phenomena, the assessment of situations associated with these pejorative terms is not always justified. While using someone else's work (including, in particular, scientific publications) without the affiliation of authorship is an infringement of copyright moral rights, from a legal point of view, self-plagiarism is a neutral practice. The duplication of one's work or a significant part of it without a proper and clear reference to an earlier publication may be, however, considered an infringement of the principles of reliability and ethics in science. It may also have negative consequences for procedures for obtaining scientific degrees and titles. It also exposes the author of the self-plagiarism to a loss of scientific credibility and reputation.

Key words: plagiarism, self-plagiarism, unreliability in science, authorship

Introduction

Recently, a topic which is sometimes raises more attention than scientifically important research results, is cases of plagiarism and anti-plagiarism in the academic community, including in the medical field. Both phenomena, consisting of the duplication and/or multiplication of someone else's or one's own scientific work, is incentivised by a the system for evaluating scientific achievements, easy access to others publications in electronic form and wide publication opportunities in the growing number of journals in Poland and abroad. The verification of publications during procedures for obtaining scientific titles, and in some cases rather personal conflicts as opposed to those of a purely scientific basis, help to identify and publicise plagiarism and self-plagiarism cases.

The qualification and evaluation of such forms of "creativity" are not always conclusive and correct. The problem of unclarity in a decisive assessment is influenced by different circumstances relevant to assessments of particular case and by different approaches on plagiarism and self-plagiarism under copyright law, scientific reliability and publication standards. To avoid misunderstandings, it is necessary to systematise the situations in which plagiarism or self-plagiarism is concerned. It is also important to indicate the consequences of such practices based on existing legislation and codes of ethics in science.

Is any reproduction of someone else's work a form of plagiarism?

Although commonly the term "plagiarism" refers to various forms of appropriation of someone else's creativity, it is not defined in legal texts. However, in copyright law, as an area appropriate for the protection of scientific works, it is understood as an infringement of the personal copyright to be recognised as an author of a work (Article 16 of the Act on Copyright and Related Rights of 14 February 1994. – hereinafter referred to as "author") [1]. Such action is threatened by civil and criminal liability (Articles 78 and 115 of the Copyright Act). The crime of plagiarism is prosecuted ex officio, which means that proceedings against the person who committed may be initiated even without the knowledge and will of the author whose copyright have been infringed.

To consider plagiarism under copyright law, the following conditions must be met:

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2. Unauthorised misappropriation of someone else's work takes place where there is an attribution of the authorship of another person's work or parts of it. This takes place when such copying does not allow the reader to recognise who the actual author is, and therefore suggests that the author is the person whose name is attributed to the work. This situation can be avoided if copying takes place under provisions of copyright fair use, namely the so-called right of quotation, which under certain conditions allows the multiplication of other author's fragments of works, but with the clear indication of source and authorship [2].
3. A work containing plagiarised content is disseminated (made available to the public).

In practice, not all publishing and scientific activities that reproduce the work of others, even if ethically questionable, constitute plagiarism under copyright law. Often, situations in which the research results, static data, research ideas, discoveries, etc. are taken from someone else's publication are wrongly qualified as plagiarism. As such, they are not subject to copyright protection and do not involve protection of authorship. It does not mean, however, that the misappropriation of someone else's scientific results in one's own publications without indicating the authorship of the original source is acceptable and allowed. Such a practice may constitute the basis for an allegation of infringement of personal rights in the form of the right to scientific creation under the provisions on the protection of personal rights (Articles 23 and 24 of the Civil Code). In the context of scientific activity, the consequence of finding such abuse may be disciplinary proceedings based on the provisions of the Act of 20 July 2018 – the Law on Higher Education and Science [3]. Also, recommended, but not legally binding, the Code of Ethics for Researchers developed by the Commission on Ethics in Science treats all forms of unreliable use of someone else's creativity as a gross violation of the principles of ethics in scientific activity [4]. According to the explanation contained in the Code of Ethics in Science, "committing plagiarism consists of appropriating someone else's ideas, research results or words without correctly mentioning the source, which constitutes an infringement of intellectual property rights". This statement may be misleading since such creations are explicitly excluded from protection under intellectual property law, including copyright (Article 2 of the Copyright Act). There is, therefore, an apparent inconsistency in the classification of the plagiarism in the light of copyright law and standards of scientific reliability. This may lead to confusion for both scientists and the bodies responsible for the proper assessment of the use of various forms of re-using someone else's work from a legal and ethical point of view.

Is self-plagiarism not the plagiarism?

Misunderstandings about legal qualification also apply to self-plagiarism, that is to say, the re-use, or even repeated publication, of the same work or part of it – including the results and scientific findings of previous publications.

Although the term itself refers to plagiarism and suggests that it is an activity that should be judged on the same basis and consequently considered prohibited as plagiarism, from a copyright perspective, "self-plagiarism" of ownworks is neutral. An author may (by executing hiscopyright moral right to authorship) indicate his name at all (also subsequent, even similar works . In such a case, there is no misrepresentation of authorship – which is the essence of plagiarism as a form of infringement of copyright moral rights.

However, various practices known as self-plagiarism may create the wrong image of scientific achievements and the originality of all the publications. Thus, self-plagiarism, like plagiarism, is treated as an act that violates the principles of scientific integrity and ethics. Such a critical assessment is based on the unjustified benefits of artificially duplicated scientific output as the basis for obtaining a title and degree in procedures where the number of publications is one of the important criteria for its evaluation.

Such "recycling of scientific publications" is also ethically questionable from the point of view of misleading readers as to the validity, relevance and credibility of scientific studies. Such an author's action is treated as a breach of readers' confidence in the reliability of scientific findings, research and publications [5]. It is of particular importance in medical science, where the results of milestone studies for treatment methods, diagnosis of diseases, risks associated with treatment, etc. are described. The double publication of original studies is particularly problematic. It can falsify data and distort test results (result in double-counting of data or incorrect weighting of individual test results).

In any case, the qualification of self-plagiarism as a reprehensible action should be judged carefully [6]. Expertise in narrow fields of science inevitably leads to dealing with specific problems in one's research, the description of which in different contexts or the publication in an updated or extended form should not in itself be questioned. Only situations in which the reproduction of the same scientific work or parts of it in different languages, under different titles, in different journals, should be considered problematic and unreliable when it is made without clearly indicating that the text in question has already been disseminated and/or published in the same or modified form. Repeated publication of the same or similar article or its parts is acceptable from copyright point of view. It may be considered unreliable and be questioned as a scientific misconduct, if the subsequent publication of an earlier

article does not contain a reference to the earlier publication (suggests that it is the first and original publication).

The absence of such an explicit reference may result in the same text being counted as several separate publications or involve scientifically unreliable suggestions of novelty. It may also harm the rights and interests of the publishers of earlier publications, including the infringement of their economic rights to the work, acquired from the author in the case of an earlier publication. Such conduct may result in the simultaneous publication of an identical text by competing publishers, which deprives it of its originality.

For the above reasons, European and national guidelines for the scientific community [7] and publication policies for scientific journals, including the current recommendations of The International Committee of Medical Journal Editors [8], introduce requirements for authors to reduce the duplication of publications. According to them, it is the responsibility of the author:

- to inform the publisher (editor) of other identical published works or manuscripts that have been prepared for and/or published in other journals, or
- to make a declaration of the originality of the article and the absence of any previous publication.

Medical journals do not consider the prior publication of clinical trial results in relevant databases or registers (however, a reference to this fact is recommended).

Earlier publication of preliminary or partial research results should not limit later publication based on such results. In this case, the consent of the publisher of the original publication could be required. (e.g., publisher of preliminary study report, preprint, abstract or poster presented at a scientific conference where research results were presented).

How to publish in accordance with the law, the principles of integrity and ethics in science?

Given the increasing number of scientific studies and the more common monitoring of manifestations of unreliability in science, it is important to observe the following principles of lawful publication and the principles of integrity and ethics in science.

1. The use of even small fragments of someone else's publication without respecting conditions of the right to quote and attribute authorship is a violation of copyright moral rights (plagiarism). Also, by the multiplication of fragments of work, the economic rights are often infringed. It may have legal consequences in the form of civil and criminal liability under copyright law.
2. As a rule, an infringement is not self-plagiarism, i.e., the re-use of one's earlier publications in a different way, in a parallel publication or another language version. In the case of previous transfer of economic copyrights to the original publication to the publishing house, such an action may result in copyright infringement and civil liability.
3. Repeatable publication of the content published in other journals may be justified in some situations, and even beneficial for a given field (e.g., in the case of secondary analysis of data from clinical trials). To ensure that the publication of the same work or an essential part of it does not lead to an accusation of unreliability in science or a violation of publication ethics and publishing standards, the work should:
   - include a clear and visible reference to the previous study,
   - provide information that there are secondary analyses or test results,
   - take place with the consent of the publishing house (editors) responsible for the original publication.

   The studies and publications which are similar to a significant extent should be included on the list of scientific achievements only once [9].
4. Accepting and not disclosing cases of plagiarism is an example of pathology in science. However, the public dissemination of allegations of plagiarism or self-plagiarism against other authors should be preceded by a careful verification of all circumstances. As shown, they do not always meet the criteria of copyright infringement and/or unlawful or reprehensible coping, whereas hasty judgments and harsh assessments may result in the loss of the author's credibility and scientific reputation. Also, integrity and caution in the formulation of decisions regarding the discussed offences should be regarded as good practice and an element of ethics in science.

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