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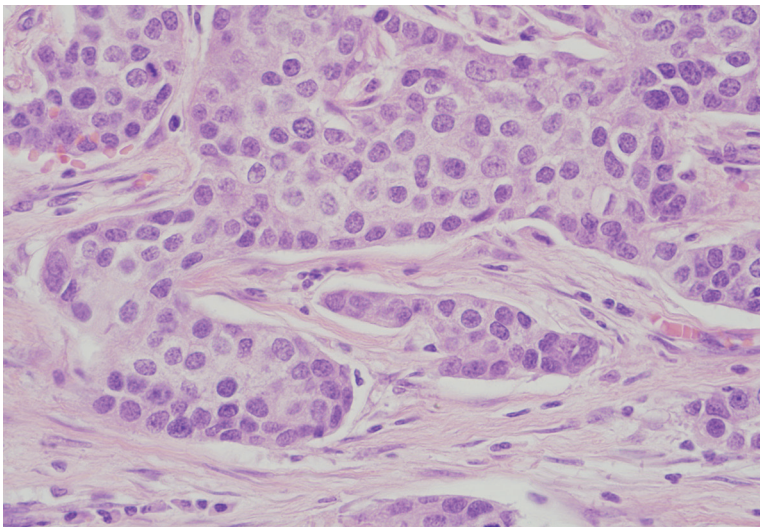

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Original article

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The impact of cervical (GTV_{CRX}) and parametrial (GTV_{LP}, GTV_{RP}) volumetric status on efficacy of radiotherapy for uterine cervix cancer in stage IIB and IIIB

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Introduction. The impact of volumetric staging of cervix and parametria on treatment outcome after combined BRT and IMRT of 135 cervix cancer patients in stage IIB and IIIB is analysed.

Material and methods. Cervical GTV_{CRX} and parametrial ($GTV_{LP, RP}$) volumes are subdivided into four subgroups. BRT with 30 Gy in three fractions was combined with IMRT 48 Gy in 24 fractions. For GTV_{CRX} ≤35 cm^3 5-year local control (LC) was 100%, which decreased to 87% for GTV_{CRX} ≥130 cm^3.

Results. Cervix and parametrial local recurrence were not higher than 3%. Major failures were periaortal nodes metastases (PNM) occurring during 5-year follow-up. Dose of ≥60 $izoGy_{2.0}$ effectively prevented the PNM. Underdosage <55 $izoGy_{2.0}$ (GTV_{RP}) resulted in an increasing PNM from 7% to 53%, strongly correlated with enlarging GTV_{CRX} from 5 cm^3 to >130 cm^3.

Conclusion. Although cervix and parametria volumetric status are highly heterogeneous, they turned out to be better prognostic predictors than traditional TNM grading.

Key words: cervix cancer, volumetric staging, radiotherapy outcomes

Introduction

Uterine cervix cancer in the stage IIB or IIIB (FIGO) develops in about 50–60% of patients and in about 25% of them periaortal lymph nodes metastases develop during 5-year follow-up [1, 2]. The EMBRACE trial [2] has shown interstage overlapping of parametrial involvement in stage IIB and IIIB, and intra-stage heterogeneity. Brachytherapy (HDR) combined with external irradiation (3D-IMRT) are used as a standard treatment modality. Traditional end-points are locoregional control, incidence of local recurrence, disease-free and overall survival, referred to as rank FIGO stages. On the contrary to head and neck cancer [3–10], volumetric status has been incidentally explored as predictive and prognostic factors in radiotherapy for cervix cancer, although Magee et al. [12], Tsang et al. [13], Dubben et al. [3] clearly documented its importance. Doubling time (Tpot) and cervix volume have been found major significant predictors for disease-free survival. These observations were strongly supported by Ito et al. [21]. These findings lead us to quantify volumes of the cervix (GTV_{CRX}), and involved left and right parametria (GTV_{LP} and GTV_{RP}) and to analyse its impact on local cervix (LTC) and parametrial control (PTC), the risk of local recurrences, and on development of the periaortal lymph nodes metastases during follow-up, as well.

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Material and methods

This retrospective study consists of 135 consecutive patients with IIB (26%) and IIIB (84%) cervix cancer treated during 2002–2008 in a single institution. The median age was 62 years (33–82 years). Using frequent serial CT scans, cervix volume (GTV_{CRX}) and volumes of both, left and right parametria (GTV_{LP} and GTV_{RP}) were contoured and counted (fig. 1).

All patients were treated with hypofractionated HDR brachytherapy (BRT) using 30 Gy in three fractions combined with 3D-IMRT 48 Gy in 24 fractions. Majority of patients also received concurrent chemotherapy (cisplatin one-a-week) during radiotherapy. Overall treatment time ranged from 46 to 51 days. Follow-up was at least 5 years.

All data was subdivided into four groups (A–D) according to the cervix (GTV_{CRX}) and parametrial volumes (GTV_{LP} and GTV_{RP}) (tab. I).

Brachytherapy of 30 Gy was hypofractionated, whereas EXRT with 48 Gy was delivered in conventional 2.0 Gy fractions. Physical doses of the HDR and EXRT should not be simply added, and therefore they were normalised to biologically izoeffective doses $EQED_{2.0}$, if given in 2.0 Gy fractions, using formula [14, 15, 16]:

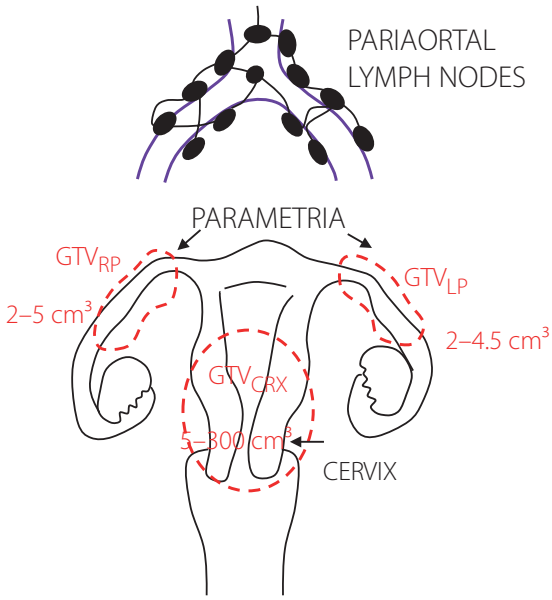

Figure 1. Topographical graph of cervix (GTV_{CRX}) and parametria (GTV_{LP}, GTV_{RP}) volumes contoured and counted on serial CTS

Table I. Characteristics of cervical (GTV_{CRX}) and parametrial (GTV_{LP}, GTV_{RP}) subgroups

Subgroup	No. cases		GTV_{CRX} cm³	GTV_{LP} cm³	GTV_{RP} cm³
A	13	10%	5–7	2–3	2–3
B	93	69%	25–35	2.2–4.2	2.2–4.1
C	14	10%	44–75	3.5–4.4	3.0–4.4
D	15	11%	130–300	4.0–4.6	4.0–4.7

$$EQED_{2.0} = TD_{EXRT}\,(d_i+\alpha/\beta)/(2.0+\alpha/\beta) + TD_{HDR}\,(1+d_i/\,\alpha/\beta)$$

where TD is a total physical dose, d_i is dose per fraction and α/β equals 10 Gy. For cervix total $EQED_{2.0}$ ranged from 108 to 115 $izoGy_{2.0}$, and 47–67 $izoGy_{2.0}$ for each parametrium. Parametrial $EQED_{2.0}$ were estimated at the midline of each parametrium. Generally, the $EQED_{2.0}$ doses, for the right parametrium were unexpectedly lower (47–55 $izoGy_{2.0}$) than those for the left one (50–67 $izoGy_{2.0}$).

The relationship between $GTV_{CRX,}$ GTV_{LP} and GTV_{RP} and treatment outcomes was estimated using the following end-points:

- local cervix and parametrial control and incidence of local recurrence;
- incidence and time of occurrence of the periaortal lymph nodes metastases (PAM);
- $EQED_{2.0}$ doses *vs.* local control (LTC) of the respective GTV targets and PAM.

Dose-effect relationships were estimated using Shapiro-Wilk, Kaplan-Meier tests and Cox regression analysis. The significance of the results was estimated by a t-Student test modified by Yates, and p = 0.05 was accepted as the significance level.

Results

Cervical and parametrial local control – distant failure

Histogram of local control recurrences and the PAM as a function of cervical and parametrial GTV and the respective $EQED_{2.0}$ are shown in details in appendix 1 (A–D). Overall 5-year LTC for the cervix cancer was 97.8% and 95.6% for involved parametria (tab. II).

For the GTV_{CRX} up to 35 cm^3 (gr. A) no cervical and parametrial failures occurred. For the GTV_{CRX} (gr. C) in the range 44–74 cm^3, local or parametrial recurrence were incidental (7%), but for GTV_{CRX} larger than 130 cm^3 (gr. D) local recurrence rate increased to 13% and in the left parametrium to 20%.

Periaortal lymph nodes metastases (PAM) occurred during follow-up (fig. 2) were the major cause of failure (24%). They occurred mainly when the GTV_{CRX} was larger than 44 cm^3 and significantly ($p < 0.001$) more frequent (≥40%), if the $EQED_{2.0}$ to the right parametrium were lower than 54 $izoGy_{2.0}$ (tab. III). The PAM never developed when the left parametrium received $EQED_{2.0}$ of ≥60 $izoGy_{2.0}$.

$EQED_{2.0}$ dose – risk of periaortal nodes metastases

Present results show that an underdosed right parametrium has likely been the main source of cancer cells that spread to the periaortal lymph nodes, although the incidence of three cervical local failures (group C and D) should not be ignored. Accumulated incidence of (PAM) as a function of follow-up time is shown in figure 2.

Table II. A – local tumour control (LTC) of the cervix and parametria depending on volumetric status (A–D) and $EQED_{2.0}$ doses; B – incidence of the PAM developing on parametrial volumes (A–D) and respective $EQED_{2.0}$ doses

Total $EQED_{2.0}$ ($izoGy_{2.0}$)	5-years local tumour control											
	Cervix				Parametrium							
					Left				Right			
	A	B	C	D	A	B	C	D	A	B	C	D
≤55									100%	100%	88%	
55.1–60					100%	100%	75%	75%	100%	100%		77%
60.1–67					100%	100%	100%	88%		100%	100%	
105–110		100%		33%								
110.1–115	100%	100%	93%	92%								
>115	100%		100%	100%								

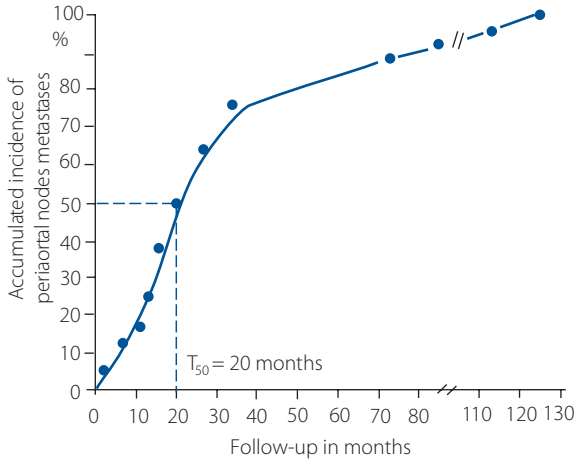

Figure 2. Accumulated incidence of periaortal nodal metastases occurring during follow-up

Table III. Risk of parametrial lymph nodes metastases depending on $EQED_{2.0}$ doses and cervical GTV_{CRX}

$EQED_{2.0}$ in right parametrium	GTV_{CRX} volumetric subgroups				
		A	B	C	D
≥51 izoGy2.0	NO RISK PAM	50%	62%	100%	100%
51–53			17%	100%	100%
53.1–56		0%		66%	50%
56.1–59		0%	0%	0%	12%
60–64		0%	0%	0%	0%
65–67		0%	0%	0%	0%
		PAM NO RISK			RISK PAM

About 80% of the PAM occurred within 40 months of follow-up. From figure 2, the T_{50} parameter (time of evidence of 50% of to be PAM) at 20 months was estimated. Assuming that 10^2–10^3 cancer cells are enough to develop a nodal metastatic lesion, the T_{50} indicated its repopulation kinetics doubling time of about 20–30 days. This may explain that 10–15% PAM occurred late, after 80 months of follow-up.

Figure 3 illustrates the significant increase in the PAM when the $EQED_{2.0}$ doses delivered to the right parametrium were lower than 55 $izoGy_{2.0}$. It has to be pointed that the $EQED_{2.0}$ doses were estimated in the midline of each parametrium. Therefore, its outer parts were even more underdosed, because of the high dose gradient using the 3D-IMRT technique.

Table III illustrates the significant increase in the risk of PAM (LP, RP) when the midline parametrial $EQED_{2.0}$ becomes lower than 53 $izoGy_{2.0}$, especially if the GTV_{CRX} volume increases to more than 44 cm^3.

$EQED_{2.0}$ – GTV_{CRX}, GTV_{LP} and GTV_{RP} control relationship

The incidences of cervix local control (LCC) and parametrial control (LPC) have been counted separately because of pronounced differences in the $EQED_{2.0}$ doses delivered to these two targets. Figure 4 shows 100% LCC for GTV_{CRX} up to

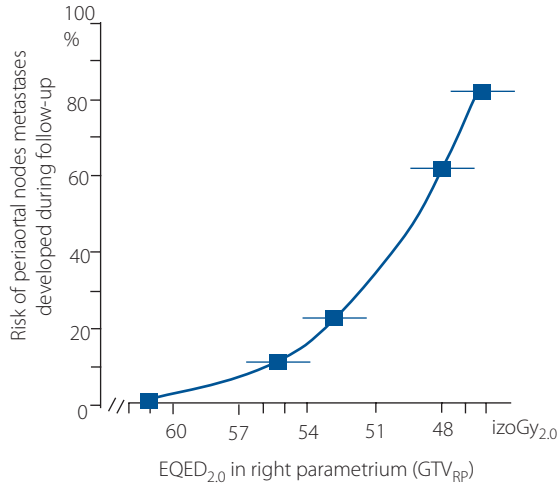

Figure 3. Risk of periaortal metastases depending on $EQED_{2.0}$ doses delivered to the right parametrium (GTV_{RP})

35-40 cm^3 (gr. A and B) for the $EQED_{2.0}$ doses higher than 110 $izoGy_{2.0}$. For GTV_{CRX} larger than 130 cm^3, $EQED_{2.0}$ lower than 110 $Gy_{2.0}$ results in only 50% LCC, which steeply increases to 100% if $EQED_{2.0}$ gets higher than 116 $izoGy_{2.0}$.

Local parametrial control (LPC) was 100% for $EQED_{2.0}$ higher than 60–65 $izoGy_{2.0}$, independently of their initial volumes, which does not differ very much (2–4.5 cm^3) within the four analysed subgroups. However, when midline $EQED_{2.0}$ was lower than 60 $izoGy_{2.0}$ the LPC (group D) sharply decreases below 60% (fig. 4). It is also important that a parametrial $EQED_{2.0}$ lower than 55 $izoGy_{2.0}$ (usually in the right parametrium) with initial GTV_{CRX} higher than 44 cm^3 led to a higher incidence of PNM occurring during follow-up.

On the contrary, too high LCC and LPC, metastases to the periaortal lymph nodes (PNM) were the major failure, which developed in 24% of cases during follow-up. The risk of the PNM increased steeply for parametrial $EQED_{2.0}$ doses lower than 54–55 $izoGy_{2.0}$. Such an underdosed parametrium can likely become a potential source of spread of the surviving cancer cells to the periaortal lymph nodes (fig. 3) to develop metastatic lesions. Uncontrolled cervix with GTV_{CRX} higher than 130 cm^3 receiving $EQED_{2.0}$ <110 $izoGy_{2.0}$ should not be ignored, because it may also contribute to increasing the risk of the PNM (tab. III, gr. C and D).

Discussion

In radiotherapy for locally advanced cervix cancer (IIB and IIIB), delivery of adequate doses to both the primary tumour and the involved parametria is a major determinant of high long-term local control. In the majority of studies, treatment outcome has been usually related to the rank of FIGO stage. Studies on radiotherapy efficacy related to initial cervix (GTV_{CRX}) and left and right parametria (GTV_{LP}, GTV_{RP}) volumes has been incidentally explored, although Dubben et al. [11] convincingly documented cervix target volume as being the only significant predictor for treatment outcome. In the EMBRACE trial [2], the importance of volumetric staging was quantified in a group of 481 patients with cervix cancer in stage IIB and IIIB. All data was divided into five volumetric subgroups with a mean GTV in the range of 12.6–79.4 cm^3. Mean total dose (D_{100}) was in the range of 88.3–103.1 Gy. However, the "dose-volume-local control relationship" was not accounted for in the analysis, and the authors have only confined themselves to the conclusion that cervical and parametrial volumes in cervix cancers stage IIB and IIIB represent a great degree of heterogeneity and radiation doses should be individually tailored to target volumes.

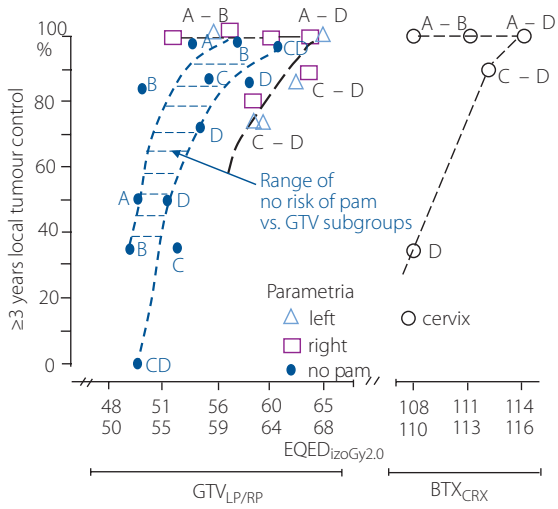

Figure 4. Local cervical and parametrial control depending on the $EQED_{2.0}$ doses

In the present study, instead of the rank FIGO stages, cervix (GTV_{CRX}) and parametria (GTV_{LP}, GTV_{RP}) volumes were estimated and subdivided into four volumetric groups. Table I shows a wide range of cervix GTV_{CRX} within 2 FIGO stages, whereas parametria volumes (GTV_{LP} and GTV_{RP}) did not differ very much. EXRT and BRT total physical doses were normalised to $EQED_{2.0}$, if given in 2.0 Gy fraction, using the L-Q model. A relatively high biological $EQED_{2.0}$ delivered to the cervix resulted in a high rate (98%) of 5-year LCC.

Local parametrial control (LPC) was also high, close to 96%. Unexpectedly, $EQED_{2.0}$ doses within the right parametrium were about 15–20% lower than within the left one. The large gradient of the HDR dose within a short distance beyond the point A may suggest its relatively small contribution to the total parametrial $EQED_{2.0}$. The 3D-IMRT also characterises heterogeneous dose distribution with a steep decrease outside of the cervix target volume [19, 20], and also in the peripheral part of the parametrium being out of its midline. Therefore, these areas can likely receive $EQED_{2.0}$ doses lower than 60 $izoGy_{2.0}$ as noted in case of right parametria. However, 5-year local parametrial control has not significantly differed from that noted for the cervix. The FIGO Cancer Report [1] and EMBRACE [2] studies pointed out that parametrial doses should not be lower than 60–65 $izoGy_{2.0}$ as noted for the left parametrium in the present study.

On the contrary, too high LCC and LPC, and metastases to periaortal lymph nodes (PAM) during follow-up occurred as a major cause of failure (24%). The risk of the PAM steeply increased when parametrial $EQED_{2.0}$ doses became lower than 54–55 $izoGy_{2.0}$. Such underdosage to the parametrium can likely be a potential source of spread of the survived cancer cells to the PNM (fig. 2) to develop metastatic lesions. The impact of uncontrolled GTV_{CRX} higher 130 cm^3 ($EQED_{2.0}$ <110 $izoGy_{2.0}$) on the risk of the PAM also cannot be ignored (tab. II).

Perez and Karanagh [17], and Girinsky, Rey and Rache [20] indicated overall treatment time (OTT) as one of the major predictors of treatment outcome, also for cervix cancer. However, in the present study OTT did not differ significantly, being in the range of 49–54 days, and therefore impact of time factor on treatment outcome was ignored.

Conclusion

The results presented clearly show a wide range of cervix cancer volumes within two FIGO stages (IIB and IIIB), and differences in the delivered biological total doses ($EQED_{2.0}$), mainly between the left and right parametria $GTV_{(LP, RP)}$. This convincingly suggests that the volumetric status of the cervix and parametria, even within the same FIGO ranks, can be a useful measurable predictor for treatment planning which should avoid "dose cold spots" (<55 $izoGy_{2.0}$) in the parametrium. A cervix volume higher than 44 cm^3 with biological total dose lower than 115 $izoGy_{2.0}$ and parametrial "dose cold spots" (<55 $izoGy_{2.0}$) may likely result in an increasing risk of development of periaortal lymph node metastases during follow-up. Therefore, such situation needs re-planning of dose distribution within the respective cervix and parametria volumes and prophylactic irradiation of the periaortic region should likely be considered.

Conflict of interest: none declared

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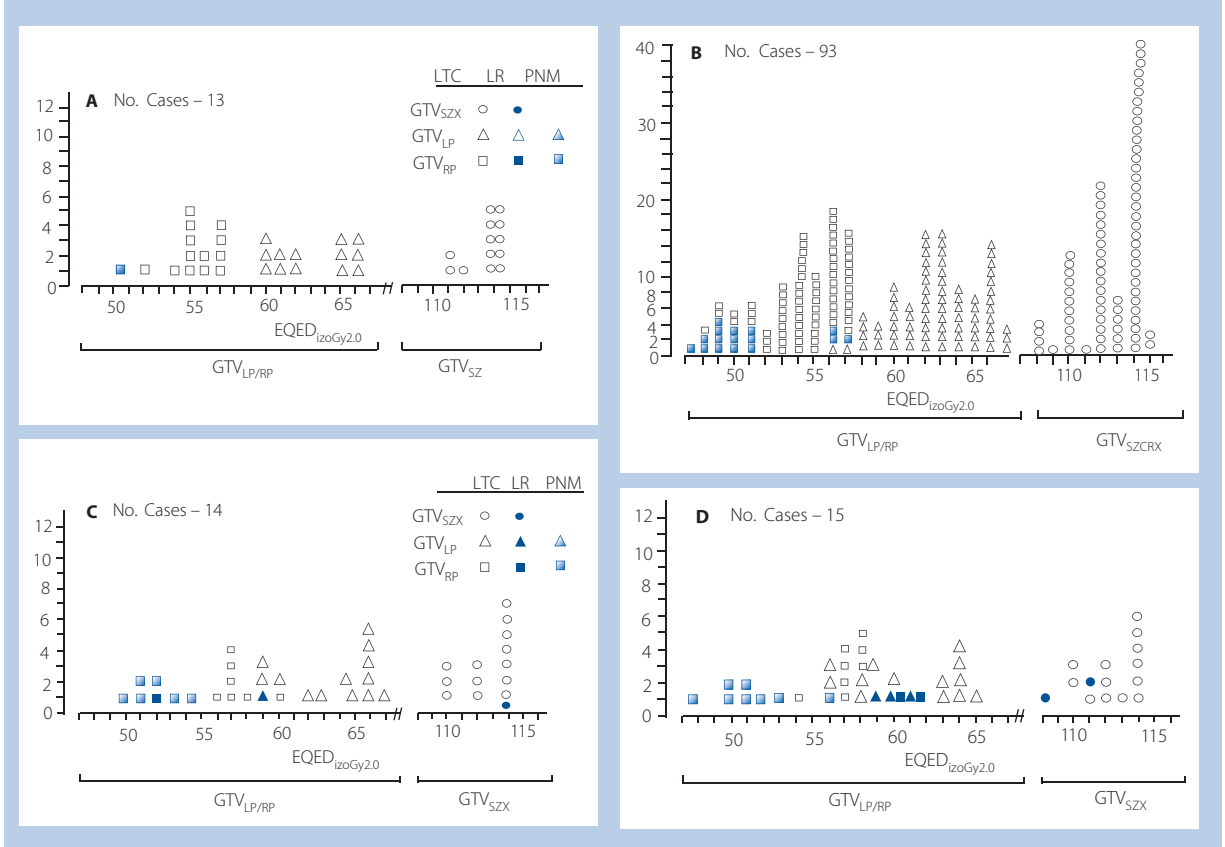

Appendix 1. Histograms of local tumour control of the cervix and parametria cancer lesion and the incidence of periaortal lymph node metastases in the group A, B, C, D (LTC – local tumour control, LR – local recurrence, PAM – periaortal lymph node metastases)

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Multiple myeloma – 2020 update on diagnosis and management

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There has been remarkable progress made in the diagnosis and treatment of multiple myeloma (MM). The median survival of the disease has doubled as a result of several new active drugs. These advances have necessitated a revision of the disease definition and staging of MM. Until recently, MM was defined by the presence of end-organ damage, specifically hypercalcemia, renal failure, anaemia, and bone lesions (CRAB features) that can be attributed to the clonal process. In 2014, the International Myeloma Working Group (IMWG) updated the diagnostic criteria for MM to add three specific biomarkers that can be used to diagnose the disease in patients who did not have CRAB features: clonal bone marrow plasma cells greater than or equal to 60%, serum free light chain (FLC) ratio greater than or equal to 100 provided involved FLC level is 100 mg/l or higher, or more than one focal lesion on MRI. In addition, the definition was revised to allow CT and PET-CT to diagnose MM bone disease.

With the introduction of immunomodulatory agents (IMiDs) and proteasome inhibitors (PIs), major improvements have been achieved in the treatment and outcome of MM. Different treatment combinations are now in use and newer therapies are being developed. However, nearly all MM patients ultimately relapse, even those who experience a complete response to initial therapy. Management of the relapsed disease remains a critical aspect of MM care and an important area of ongoing research. The aim of this review is to summarise the current methods of diagnosis and treatment of MM.

Key words: multiple myeloma, diagnosis, treatment, novel agents, transplantation, supportive care

Incidence and epidemiology

Multiple myeloma (MM) accounts for 1% of all cancers and 10-15% of all blood cancers. The incidence in Europe is 4.5–6.0/100 000/year with a median age at diagnosis of 72 years; the mortality is 4.1/100 000/year [1]. Over 90% of MM cases refer to patients >50 years old. Only 35% of the patients are younger than 65 years at the moment of diagnosis. Individuals under 40 years of age count for up to 2% of all cases [2]. The annual incidence in Poland in 2017 was approximately 8/100 000/year [3]. The median overall survival in MM is approximately 6 years [4]. In the subset of patients eligible for autologous stem cell transplantation (ASCT), 4-year survival rates are more than 80%; the median overall survival (OS) among these patients is approximately 8 years [5]. Among elderly patients (age >75 years), median OS is lower, and is approximately 5 years [4]. Particularly poor prognosis concerns MM patients with central nervous system involvement (median OS: 7 month) [6].

Multiple myeloma arises from a terminally differentiated postgerminal centre plasma cell. The pathogenesis of MM is complex, and many steps in the pathway are not fully elucidated. Most cases of MM are preceded by the premalignant asymptomatic states of monoclonal gammopathy of undetermined significance (MGUS) and smouldering MM (SMM) [7]. The progression of MGUS to MM is approximately 1% of cases

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per year, whereas SMM has a much higher rate of progression of 10% of cases annually. Approximately 73% of SMM patients will progress to MM within 15 years [8].

Multiple myeloma is a heterogeneous disease that is based on various genetic aberrations. Many of the chromosomal abnormalities include translocations in the immunoglobulin--heavy chain of chromosome 14, aberrations in chromosomes 1, 5, 13, and 17, and trisomies [9]. Genetic abnormalities and molecular changes are thought to contribute to cell-cycle dysregulation and lead to active MM [10].

Diagnosis

In 2014, the International Myeloma Working Group (IMWG) revised the diagnostic criteria for MM [10]. The revised diagnostic criteria for MM allow the use of specific biomarkers to define the disease in addition to the established CRAB (hyperCalcaemia, Renal failure, Anaemia, or lytic Bone lesions) features. They also allow the use of modern imaging tools to diagnose MM bone disease and clarify several other diagnostic requirements.

The diagnosis of MM requires the presence of one or more MM defining events in addition to evidence of either 10% or more clonal plasma cells (PC) in bone marrow (BM) examination or a biopsy-proven plasmacytoma. Multiple myeloma defining events consists of established CRAB features as well as three specific biomarkers: clonal PC in BM ≥60%, serum free light chain (sFLC) ratio ≥100 (provided involved FLC level is ≥100 mg/l), and more than one focal lesion on magnetic resonance imaging (MRI). Diagnosis of MM should be based on the following tests [11, 12]:

1. Detection and evaluation of the monoclonal (M) component by serum and/or urine protein electrophoresis (concentrate of 24 hours urine collection); nephelometric quantification of IgG, IgA and IgM immunoglobulins; characterisation of the heavy and light chains by immunofixation; and serum FLC measurement.
2. Evaluation of BM, PC infiltration: BM aspiration and/or biopsy. Moreover, the BM sample should be used for cytogenetic/fluorescent *in situ* hybridisation (FISH) studies on immunologically recognised or sorted PC and also has the potential for immunophenotypic and molecular investigations.
3. Evaluation of lytic bone lesions: whole-body low-dose computed tomography (WBLD-CT) is the new standard for the diagnosis of lytic disease. Conventional radiography can also be used if WBLD-CT is not available. 18F-fluorodeoxyglucose positron emission tomography with CT (PET--CT) can be performed to evaluate bone lesions, according to availability and resources.
4. Complete blood cell count, with differential serum creatinine, creatinine clearance and calcium level.

The definition of MGUS has not changed. Patients need to have less than 30 g/l serum M-protein, less than 10% clonal PC in BM, and no end-organ damage for this diagnosis. Currently no data is available to support the treatment of MGUS patients.

The diagnosis of a MM demands the presence of a serum M-protein of ≥30 g/l, and/or ≥10% of clonal PC in BM. Asymptomatic patients without myeloma-defining events have a so-called SMM, which may progress to a symptomatic MM over time. The presence of end organ damage, primarily the CRAB--criteria, define an underlying MM in need of therapy. In the most recent update of the criteria for diagnosis of MM, three additional myeloma-defining events have been introduced to discriminate symptomatic MM without evidence of classical end-organ damage from SMM: clonal PC of 60% or greater in the bone marrow, a serum FLC ratio of 100 or greater, or more than one focal lesion larger than 5 mm on MRI [10]. To address these additional MM defining events, the term "SLiM-CRAB" (SLiM: S = sixty; Li = light chain; M = MRI) was coined soon after publication of the updated criteria. The new definitions of MGUS, SMM and symptomatic MM are shown in table I [10].

Staging and risk classification

The course of MM is highly variable, and the clinical behaviour is remarkably heterogeneous. Many studies have identified prognostic factors capable of predicting this heterogeneity in survival: serum ß2-microglobulin, albumin, C-reactive protein (CRP), and lactate dehydrogenase (LDH). More precise estimation of prognosis requires an assessment of multiple factors. As in other cancers, OS in MM is affected by host characteristics, tumour burden (stage), biology (cytogenetic abnormalities), and response to therapy [13].

Tumour burden in MM has traditionally been assessed using the Durie-Salmon Staging (DSS). The International Staging System (ISS) has now replaced the DSS system [14]. The R-ISS staging system is a new risk stratification algorithm with an improved prognostic power incorporating ISS, chromosomal abnormalities, and LDH levels (tab. II) [15].

Some institutions are also incorporating a risk-adapted approach to treatment decisions. The Mayo Stratification of Myeloma and Risk-Adapted Therapy (mSMART) classifies risk based on cytogenetic abnormalities [16]. Patients with deletions of the long arm of chromosome 13 and translocations of chromosomes 4 and 14 are considered to have high-risk disease. Deletion of 17p13, which results in mutations in the tumour-suppressor protein 53, is also associated with a poorer outcome [16].

Response evaluation

The definition of response established by the IMWG in 2006 has been updated in 2016 [17]. The IMWG uniform response criteria are most often used to assess response to drug therapy. Responses include stringent complete response (sCR), complete response (CR), very good partial response (VGPR), partial response (PR), and stable disease (SD) [17]. The response criteria incorporate the degree of reduction of serum, and urine M-protein by electrophoresis and immunofixation, plasmacy-

Table I. Diagnostic criteria for monoclonal gammopathy of undetermined significance, smouldering multiple myeloma, and symptomatic multiple myeloma

Definition of Monoclonal gammopathy of undetermined significance
All tree criteria must be met: • serum M-protein (non-IgM type) <30 g/l • clonal bone marrow plasma cells <10%* • absence of end-organ damage such as hyperCalcemia, renal insufficiency, anaemia, and bone lesions (CRAB) that can be attributed to the plasma cell proliferative disorder
Definition of smouldering multiple myeloma
Both criteria must be met: • serum M-protein (IgG or IgA) ≥30 g/l, or urinary monoclonal protein ≥500 mg per 24 hours and/or clonal bone marrow plasma cells 10–60% • absence of MM defining events or amyloidosis
Definition of symptomatic multiple myeloma
Both criteria must be met: • Clonal bone marrow plasma cells ≥10% or biopsy-proven bony or extramedullary plasmacytoma • Any one or more of the following MM defining events: – Evidence of end organ damage that can be attributed to the underlying plasma cell proliferative disorder, specifically: · Hypercalcemia: serum Ca >0.25 mmol/l (>1 mg/dl) higher than the upper limit of normal or >2.75 mmol/l (> 11 mg/dl), · Renal insufficiency: CrCl <40 ml per minute or serum creatinine >177 µmol/l (>2 mg/dl), · Anaemia: Hb value of >2 g/dl below the lower limit of normal, or a Hb value <10 g/dl, · Bone lesions: one or more osteolytic lesions on skeletal radiography, CT, or PET-CT – Clonal bone marrow plasma cell percentage ≥60% – Involved: uninvolved serum FLC ratio ≥100 (involved FLC level must be ≥100 mg/l) – >1 focal lesions on MRI studies (at least 5 mm in size)

Ca – calcium; CT – computed tomography; CrCl – creatinine clearance; FLC – free light chain; Hb – hemoglobin; Ig – immunoglobuline; MRI – magnetic resonance imaging; MM – multiple myeloma; PET-CT – positron emission tomography-CT

*A bone marrow can be deferred in patients with low risk MGUS (IgG type, M protein <15 g/l, normal free light chain ratio) in whom there are no clinical features concerning for myeloma

Table II. The International staging system (ISS) and revised International staging system (R-ISS) for multiple myeloma

Stage	Criteria	Frequency (%)	Median OS (months)
	ISS		
I	• β2-microglobulin <3.5 mg/l, and • Albumin (serum) ≥35 g/l	28	62
II	• Neither I or III	62	45
III	• β2-microglobulin ≥5.5 mg/l	10	29
	R-ISS		
I	• β2-microglobulin <3.5 mg/l, • Albumin (serum) ≥35 g/l, and • No high-risk cytogenetics, and • Normal LDH (defined as less than ULN)	28	82
II	• Not R-ISS stage I or III	62	62
III	• β2-microglobulin ≥5.5 mg/l regardless of albumin levels (serum), and • High-risk cytogenetics: del(17p), t (4;14) or t (14;16) or • High LDH (defined as higher than ULN)	10	40

ISS – International staging system; LDH – lactate dehydrogenase; OS – overall survival; R-ISS – revised International staging system; ULN – upper limit of normal

tomas, and PC in BM. Standard IMWG uniform response criteria for MM are presented in table III [17].

The quality and the depth of response have improved over the last 5 years in the context of novel agent-based therapies, allowing for the introduction of new response grades, namely minimal residual disease (MRD) criteria including sequencing MRD negativity, flow MRD negativity, imaging plus negativity and sustained MRD negativity. There is a statistical relationship between the achievement of CR, MRD negativity and progression free survival (PFS), or OS.

Treatment overview

The goals of MM treatment have evolved with advances in drug therapy, and more sensitive monitoring. The primary goal is to achieve a deep, long-lasting response. Additionally, therapy should control disease, minimise complications, and improve quality of life. Myeloma treatment depends on whether the patient is symptomatic. Patients with MGUS, and SMM are usually observed, and treatment is initiated upon disease progression to active MM. There is no evidence that early treatment of SMM prolongs OS. Patients with symptomatic

Table III. Standard International Myeloma Working Group uniform response criteria for multiple myeloma

Response subcategory	Response criteria
Molecular CR	CR plus negative ASO-PCR, sensitivity 10^{-5}
Immunophenotypic CR	Stringent CR plus absence of phenotypically aberrant PCs (clonal) in BM with a minimum of 1 million total BM cells analysed by multiparametric flow cytometry (with >4 colours)
Stringent CR	CR as defined below plus normal sFLC ratio and absence of clonal PCs in BM biopsy by immunohistochemistry or 2- to 4-colour flow cytometry
CR	Negative IF on the serum and urine and disappearance of any soft tissue plasmacytomas and <5% PCs in BM
VGPR	Serum and urine M-protein detectable by IF but not on electrophoresis or ≥90% reduction in serum M-protein plus urine M-protein level <100 mg per 24 hours
PR	• ≥50% reduction of serum M-protein plus reduction in 24-hour urinary M-protein by ≥90% or to <200 mg per 24 hours. If the serum and urine M-protein are unmeasurable, a ≥50% decrease in the difference between involved and uninvolved FLC levels is required. • If serum and urine M-protein are unmeasurable, and sFLC assay is also unmeasurable, ≥50% reduction in plasma cells is required in place of M-protein, provided baseline bone marrow PCs percentage was ≥30%. • In addition to these criteria, if present at baseline, a ≥ 50% reduction in the size of soft tissue plasmacytomas is also required
MR	• ≥25% but ≤49% reduction of serum M-protein and reduction in 24-hour urine M-protein by 50–89%. • In addition to the above listed criteria, if present at baseline, a ≥50% reduction in the size of soft tissue plasmacytomas is also required
PD	Any one or more of the following criteria: • Increase of 25% from lowest confirmed response value in one or more of the following criteria: – Serum M-protein (absolute increase must be ≥5 g/l), – Serum M-protein increase ≥10 g/l, if the lowest M component was ≥5 g/dl,\ – Urine M-protein (absolute increase must be ≥ 200 mg/24 hours). • In patients without measurable serum and urine M-protein levels, the difference between involved and uninvolved sFLC levels (absolute increase must be >10 mg/dl). • In patients without measurable serum and urine M-protein levels and without measurable involved sFLC levels, BM PCs percentage irrespective of baseline status (absolute increase must be ≥10%). • Appearance of (a) new lesion(s), ≥50% increase from nadir of >1 lesion, or ≥50% increase in the longest diameter of a previous lesion >1 cm in short axis, ≥50% increase in circulating PCs (minimum of 200 cells per µl) if this is the only measure of disease.

ASO-PCR – allele-specific oligonucleotide polymerase chain reaction; BM – bone marrow; CR – complete response; IF – immunofixation; M – monoclonal; MR – minimal response; PR – partial response; PCs – plasma cells; PD – progression disease; sFLC – serum free light chain; VGPR – very good partial response

MM require treatment. This treatment is patient-specific and depends on numerous factors, including cytogenetics, disease stage, age, comorbidities, and performance status.

Survival in MM has improved significantly in the last 15 year. The initial impact came from the introduction of thalidomide, bortezomib, and lenalidomide. In the last decade, carfilzomib, pomalidomide, panobinostat, ixazomib, elotuzumab, daratumumab, isatuximab, and selinexor have been approved by the European Medicines Agency (EMA) for the treatment of relapsed MM, and promise to improve outcomes further.

All patients with a diagnosis of symptomatic MM require immediate treatment. Initial choice of therapy is driven by whether a patient is eligible for an ASCT, because certain agents, such as alkylating agents, should typically be avoided in those who are transplant eligible. Initial therapy for patients with MM is also based on genetic risk stratification of the disease. Patients with high-risk disease require a CR treatment for long-term OS and thus benefit from an aggressive treatment strategy. Standard-risk patients have similar OS regardless of whether or not CR is achieved and thus can either be treated with an aggressive approach, or a sequential therapy approach.

The clinician must decide whether the patient is eligible or not for ASCT. The eligibility criteria vary from country to country. In European countries, ASCT is recommended under 65–70 years of age, but nowadays it depends upon the "physiological age" rather than the chronological age of the patient. Furthermore, serum creatinine level, the Eastern Cooperation Oncology Group (ECOG) performance status, and the New York Heart Association functional status need to be considered. The current guidance of European Society for Medical Oncology (ESMO) for MM treatment is shown in figure 1 [18].

Initial treatment in patients eligible for autologous stem cell transplantation

The current treatment paradigm for newly diagnosed MM patient eligible for ASCT consists of four phases: induction remission, transplantation, post-transplant treatment (consolidation, and maintenance therapy).

Induction remission

Induction therapy usually consists of four to six cycles of therapy with the aim of achieving rapid disease control, improving symptoms and allowing for subsequent stem cell collection. Bortezomib with dexamethasone (VD) is the standard back-

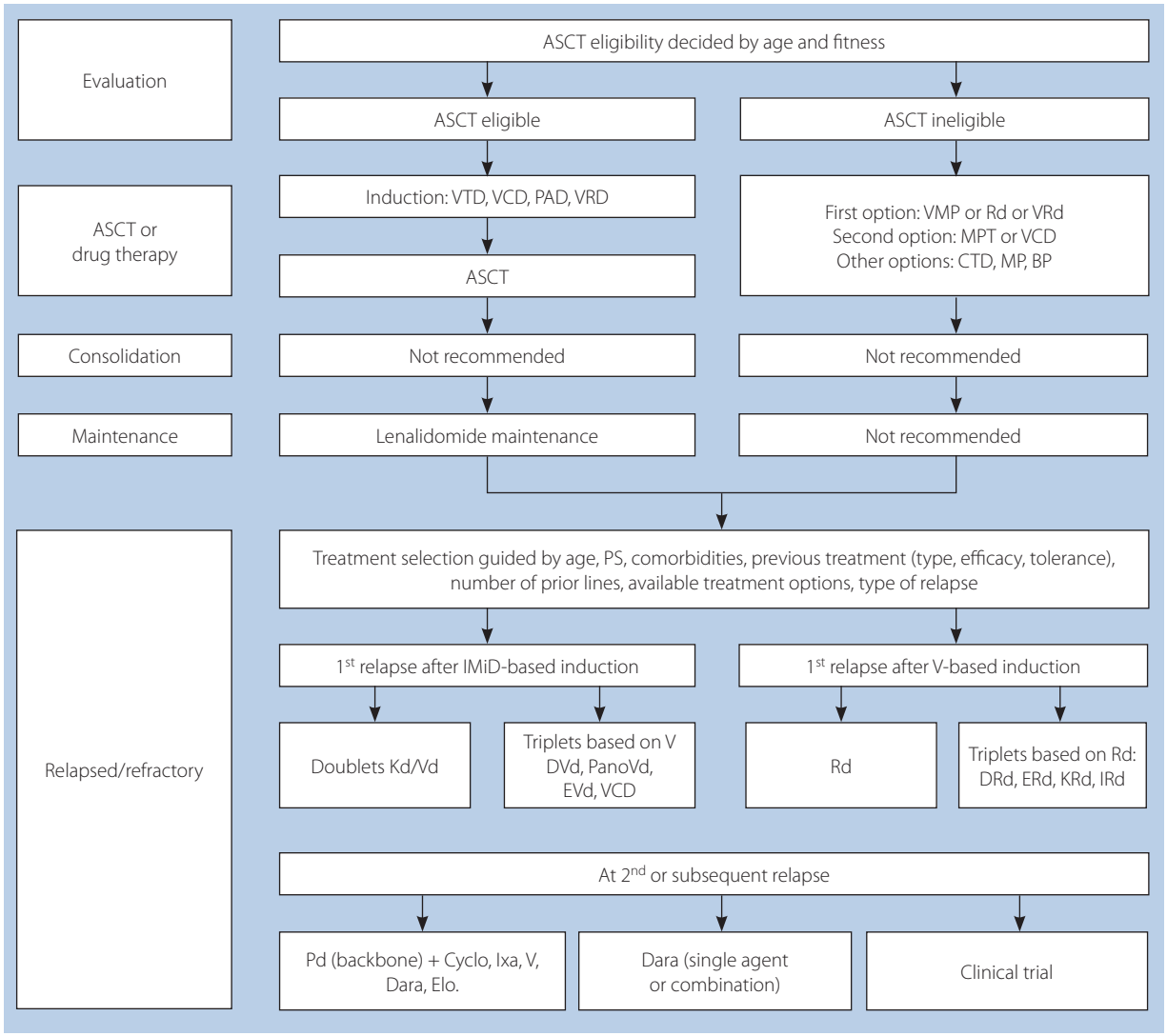

Figure 1. European Society for Medical Oncology guidance for multiple myeloma treatment; ASCT – autologous stem cell transplantation; BP – bendamustine, prednisone; Cyclo – cyclophosphamide; CTD – cyclophosphamide, thalidomide, dexamethasone; Dara – daratumumab; DVd – daratumumab, bortezomib, dexamethasone; Elo – elotuzumab; ERd – elotuzumab, lenalidomide, dexamethasone; EVd – elotuzumab, bortezomib, dexamethasone; Ixa – ixazomib; IRd – ixazomib, lenalidomide, dexamethasone; IMiD – immunomodulathory drug; Kd – karfilzomib, dexamethasone; MP – melphalan, prednisone; MPT – melphalan, prednisone, thalidomide; PAD – bortezomib, doxorubicin, dexamethasone; PanoVd – panobinostat, bortezomib, dexamethasone; Pd – pomalidomide, dexamethasone; PS – personal status; Rd – lenalidomide, low-dose dexamethasone; RVD – lenalidomide, bortezomib, dexamethasone; V – bortezomib; VCD – bortezomib, cyclophosphamide, dexamethasone; Vd – bortezomib, dexamethasone; VMP – bortezomib, melphalan, prednisone; VRd – bortezomib, lenalidomide, low-dose dexamethasone; VTD – bortezomib, thalidomide, dexamethasone

bone of induction therapy [19, 20]. The addition of a third agent, thalidomide (VTD) [21], cyclophosphamide (VCD) [22], doxorubicine (PAD) [23], or lenalidomide (VRD) [24] provides higher response rates. In prospective trials, induction with VTD is superior to VCD in terms of response rate, at the cost of a higher incidence of peripheral polyneuropathy (PN) but lower incidence of haematological toxicities [25]. To reduce the PN incidence, the French Intergroupe Francophone du Myelome (IFM) proposed the VTD regimen with reduced doses of bortezomib, and thalidomide, which is associated with a lower incidence grade ¾ PN (14% *vs.* 34%), but at the expense of lower response rates [26]. Bortezomib, cyclophosphamide, and dexamethasone was also shown to be, as effective as PAD in terms of response, but less toxic [27]. Replacement of thalidomide by lenalidomide in the VRD regimen induces higher CR rates before and after ASCT (47%, and 88% of patients with a VGPR or better, respectively) [24]. Current regimens used in the front-line are listed in table IV.

Other highly effective combinations such as carfilzomib, lenalidomide, and dexamethasone (KRd), or ixazomib, lenalidomide, and dexamethasone (IRd) are currently under evaluation in phase III trials.

However, the introduction of monoclonal antibodies will change the landscape of induction therapy in the near future. Ongoing prospective trials combining daratumumab with VTD (Cassiopeia) or VRD (Perseus), or elotuzumab with VRD are exploring the role of induction with antibody-based quadruplets.

Stem cell collection

Peripheral blood progenitor cells are usually collected for more than one ASCT (at least 2.5 x 10^6 CD34 + cells/kg per transplantation). Since the use of lenalidomide can impair stem cell collection, apheresis in this situation should be performed after 3–4 cycles, and may require the use of cyclophosphamide or plerixafor.

High dose melphalan (HDM) and ASCT

High-dose melphalan (melphalan 200 mg/m^2, MEL200) remains the standard conditioning regimen prior to ASCT. A dose reduction (100–140 mg/m^2) is recommended in case of renal impairment (estimated GFR <60 ml/min). In this group of patients, including those requiring dialysis, ASCT is feasible but exposes the patient to severe mucositis, prolonged hospitalisation and an increased risk of transplant-related mortality (4% *vs.* <1%) [28].

Post-transplant treatment

The concept of consolidation and/or maintenance is a commonly adopted approach after transplantation. Consolidation after ASCT is a short-term intensive therapy aimed at improving the quality of response after transplant. Maintenance consists of the administration of a therapy for a prolonged period in order to maintain the response achieved after ASCT and prevent progression.

Consolidation with second ASCT

Before the era of novel agents, the main approach was to propose a second ASCT. However, tandem ASCT did not provide any OS, or PFS advantage, except in patients not achieving VGPR after the first transplant. [29, 30]. Currently, tandem ASCT with HDM as conditioning is recommended for transplant-eligible patients with high-risk cytogenetic features at diagnosis.

Consolidation with new drugs

Initially, bortezomib or VT(D) consolidation were shown to increase the quality of response by 30% and were considered at least in patients who failed to achieve a VGPR or CR/near CR (nCR) after ASCT [31] Nowadays, the role of consolidation remains unclear. Trials using either carfilzomib or ixazomib in this setting are currently ongoing. Overall, consolidation remains a reasonable practice in patients who failed to achieve a VGPR or nCR/CR after transplantation.

Maintenance therapy

In young patients following ASCT, phase III randomised trials have demonstrated that maintenance therapy with immunomodulatory drugs (IMiDs), either thalidomide or lenalidomide, prolongs PFS [19]. A meta-analysis demonstrated that lenalidomide maintenance following ASCT is associated with an overall OS benefit of more than two years [32]. Bortezomib maintenance was also evaluated during a two-year study and was associated with a survival benefit over thalidomide maintenance, but induction was not identical in the two arms of this prospective trial [23]. Currently, bortezomib and thalidomide are not approved in this setting.

In elderly patients following induction, several randomised trials have explored the benefit of maintenance therapy in terms of OS using either IMiDs or bortezomib: melphalan with prednisone (MP) or a reduced-dose regimen of cyclophosphamide, thalidomide, and dexamethasone (CTD) with or without thalidomide maintenance [33], MP versus melphalan, prednisone, lenalidomide (MPR) versus melphalan, prednisone, lenalidomide and followed by maintenance with lenalidomide (MPR-R) [34], bortezomib, melphalan, prednisone, thalidomide followed by maintenance with bortezomib, and thalidomide (VMPT-VT) versus bortezomib, melphalan, prednisone (VMP) [35], VMP versus bortezomib, thalidomide, prednisone (VTP) followed by either bortezomib, and prednisone (VP) or VT maintenance [36] systematic maintenance therapy currently can not be recommended in elderly patients.

Initial treatment in patients not eligible for ASCT

For patients with newly-diagnosed (ND) MM who are ineligible for ASCT due to age or other comorbidities, chemotherapy is the only option. Many patients will benefit not only in survival, but also in quality of life. Immunomodulatory agents, such as lenalidomide and thalidomide, and proteasome inhibitors (PIs), such as bortezomib, are highly effective and well tolerated. There has been a general shift to using these agents upfront in transplant-ineligible patients.

All the previously mentioned regimens can also be used in transplant-ineligible patients. Although no longer the preferred treatment, melphalan can be considered in resource--poor settings [37]. Patients who are not transplant-eligible are treated for a fixed period of 9 to 18 months, although lenalidomide, and dexamethasone (Rd) is often continued until relapse [38, 39].

The two following options are recommended based on data from randomised phase III trials: VMP (bortezomib administered subcutaneously) [39] or Rd [40]; both VMP and Rd are approved in this setting by the European Medicines Agency (EMA). Melphalan, prednisone, thalidomide (MPT) is also approved by the EMA, but is inferior to Rd in terms of PFS and OS [40, 41]. The regimen has a high toxicity rate (>50%) and a deep vein thrombosis rate of 20%, so patients undergoing treatment with this regimen require thromboprophylaxis. Bortezomib, cyclophosphamide with dexamethasone induces high response rates and prolonged PFS [19]. Lenalidomide with dexamethasone has recently been compared prospectively with Rd with bortezomib (VRd), and the addition of bortezomib resulted in significantly improved PFS and OS and had an acceptable risk-benefit profile [42]. Bendamustine, and prednisone (BP) is also approved by the EMA in patients who have clinical neuropathy at time of diagnosis, precluding the use

Table IV. Currently used first-line regimens in eligible- and ineligible-transplant newly diagnosed multiple myeloma patients

Regimen	ORR (%)	>VGPR (%)	Median PFS (months)	3-years OS rate (%)
Transplant-eligible				
VTD [21]	93	63	NR	90
VCD [22]	88	71	NA	NA
PAD [23]	90	42	35	61
VRD [24]		CR: 49	50	81% at 4 years
Transplant-ineligible				
MPT [40]	62	28	21.2	51% at 4 years
VMP [39]	71	CR: 30	22	41
Once-weekly VMP [46]	85	55	33.1	88
VCD [22]	88	71	NA	NA
Rd [38] (continuous)	75	44	25.5	59% at 4 years
VRd [42]	81.5	27.8	43	median OS: 75 months

CR – complete response; MPT – melphalan, prednisone, thalidomide; NA – not available; NR – not reached; ORR – overall response rate; OS – overall survival; PAD – bortezomib, doxorubicin, dexamethasone; PFS – progression free survival; Rd – lenalidomide, low-des dexamethasone; VCD – bortezomib, cyclophosphamide, dexamethasone; VGPR – very good partial response; VMP – bortezomib, melphalan, prednisone; VRd – bortezomib, lenalidomide, low-dose dexamethasone; VRD – bortezomib, lenalidomide, dexamethasone; VTD – bortezomib, thalidomide, dexamethasone

Table V. Definitions of relapsed and refractory multiple myeloma

Multiple myeloma	Definition
Primary refractory	Non-responsive disease, in which MR or better has never been achieved, with no significant change in M-protein level, and no evidence of clinical progression
Refractory	Non-responsive disease, while on primary or salvage therapy, or progressing within 60 days of last therapy
Relapsed	Previously responding disease that progresses and requires initiation of salvage therapy, but does not meet criteria for either primary refractory disease or relapsed and refractory disease
Relapsed and refractory	Non responsive disease, while on salvage therapy or progressing within 60 days of last therapy, in patients who have achieved at least MR at some point previously before, then progressing in their course
Double refractory	Disease refractory to both PIs and IMiDs

IMiDs – immunomodulatory inhibitors; MR – minimal response; PI – proteasome inhibitors

of thalidomide according to the MPT regimen or bortezomib according to the VMP regimen [43]. Melphalan, prednisone, and lenalidomide is not routinely used and cannot be considered as a standard of care. Cyclophosphamide, thalidomide, and dexamethasone has also been compared with MP and is superior in terms of response rates, but does not induce a clear survival advantage over MP. Current regimens used in front-line are listed in table IV.

Treatment of relapsed/refractory multiple myeloma

Table V shows definitions of relapsed and refractory (RR) MM [44]. In the relapsed setting, optimal management of MM is complex and ESMO guidelines indicate that the selection of therapy should be guided by a number of different parameters including: patient age; performance status; comorbidities; the type, efficacy and tolerance of the previous treatment; the number of prior treatment lines; the available remaining treatment options; the interval since the last therapy; and the type of relapse [18]. Relapses in MM may be clinical or biochemical, and in the case of biochemical relapse, salvage treatment can be delayed. For the youngest, fittest patients who have initially benefited from their first ASCT, a second ASCT may be considered, although, this option is still infrequently used [18, 45].

For most patients, the treatment approach will need to be based on prior exposure and toxicity. Wherever prior treatment was IMiD-based, current guidelines advise a switch to a proteasome inhibitor (PI) doublet (bortezomib or carfilzomib with dexamethasone) or bortezomib-based triplet therapy with dexamethasone and either daratumumab, panobinostat, elotuzumab or cyclophosphamide (fig. 1) [18].

In first relapse after bortezomib-based induction, treatment should be changed to an IMiD-based treatment regimen with or without a novel agent. Other options include doublet Rd therapy or triplets on an Rd backbone – for example, with the addition of daratumumab, carfilzomib, ixazomib or elotuzumab (fig. 1) [18].

If both IMiD's and PI's have been exhausted and the patient is experiencing a second or subsequent relapse, current ESMO guidelines recommend the alternative option of a clinical trial or daratumumab monotherapy if this has not been previously

tried, while combinations based on a pomalidomide backbone with ixazomib, cyclophosphamide, bortezomib, daratumumab or elotuzumab should also be evaluated (fig. 1) [18].

Compelling data from randomised, controlled phase III trials support the ability of novel agent-based triplets to achieve both superior response rates and prolonged disease control versus doublet combinations. In several phase III studies Rd with ixazomib, carfilzomib, elotuzumab and daratumumab versus Rd alone in patients with RRMM have demonstrated statistically significant improvement in the primary clinical endpoint of PFS when combined with Rd versus Rd alone in patients with RRMM. Table VI shows the results of selected phase III clinical trials assessing IMiD-based (lenalidomide, pomalidomide) chemotherapy in RRMM. Significant improvements in PFS were also obtained with daratumumab or panobinostat when added to a Vd backbone compared to Vd in the relapsed/refractory setting in phase III studies. However, the clinical benefit of triplets may

Table VI. Results of selected phase III clinical trials assessing IMiD-based (lenalidomide, pomalidomide) chemotherapy in relapsed/refractory multiple myeloma

Trial	Regimen	ORR (%)	>CR (%)	Median PFS (months)	Median OS (months)
Lenalidomide-based					
MM-010 [47]	Rd *vs.* Dex	60 *vs.* 24 p < 0.001	16 *vs.* 3.4 p < 0.001	11.3 *vs.* 4.7 p < 0.001 HR = 0.66	NR *vs.* 20.6 p = 0.03 HR = 0.66
ASPIRE [48, 49]	KRd *vs.* Rd	87 *vs.* 67 p < 0.001	32 *vs.* 9 p < 0.001	26 *vs.* 17.6 p = 0.0001 HR = 0.69	2-years: 73% *vs.* 65% p = 0.04 HR = 0.79
TOURMALINE-MM1 [50]	IRd *vs.* Rd	78 *vs.* 71.5 p = 0.004	≥ VGPR: 48 *vs.* 39 p = 0.01	20.6 *vs.* 14.7 p = 0.01 HR = 0.74	NR
POLLUX [51]	DRd *vs.* Rd	93 *vs.* 76.4 p < 0.0001	51 *vs.* 21 p < 0.0001	NR *vs.* 17.5 p < 0.0001 HR = 0.41	NR HR = 0.64
ELOQUENT-2 [52]	ERd *vs.* Rd	79 *vs.* 66 p < 0.001	≥VGPR: 35 *vs.* 29	18.5 *vs.* 14.4 p = 0.0004 HR = 0.72	43.7 *vs.* 39.6 p = 0.0257 HR = 0.77
Pomalidomide-based					
MM-003 [53]	Pd *vs.* Dex	32 *vs.* 11 p < 0.001	7 *vs.* 1	4.0 *vs.* 1.9 p < 0.001 HR = 0.5	13.1 *vs.* 8.1 p = 0.009 HR = 0.72
OPTIMISMM [54]	VPd *vs.* Vd	82 *vs.* 50 p < 0.0001	52.7 *vs.* 18.3 p < 0.0001	11.2 *vs.* 7.1 p < 0.0001 HR = 0.61	NR
ELOQUENT-3 [55]	EPd *vs.* Pd	53 *vs.* 26	20 *vs.* 9	10.3 *vs.* 4.7 p = 0.008 HR = 0.54	NR HR = 0.62

CR – complete response; DRd – daratumumab, lenalidomide, dexamethasone; Dex – dexamethasone; EPd – elotuzumab, pomalidomide, dexamethasone; ERd – elotuzumab, lenalidomide, dexamethasone; HR – hazard ratio; IRd – ixazomib, lenalidomide, dexamethasone; IMiD – immunomodulathory drug; KRd – karfilzomib, lenalidomide, dexamethasone; NR – not reached; ORR – overall response rate; OS – overall survival; Pd – pomalidomide, dexamethasone; PFS – progression free survival; Rd – lenalidomide, dexamethasone; Vd – bortezomib, dexamethasone; VPd – bortezomib, pomalidomide, dexamethasone;

Table VII. Results of selected phase III clinical trials assessing inhibitor proteasoms-based chemotherapy in relapsed/refractory multiple myeloma

Trial	Regimen	ORR (%)	>CR (%)	Median PFS (months)	Median OS (months)
APEX [56]	V *vs.* Dex	38 *vs.* 18 p < 0.001	6 *vs.* 1 p < 0.001	6.2 *vs.* 3.5 p < 0.001 HR = 0.55	12 months: 80% *vs.* 66% p = 0.001 HR = 0.57
ENDEAVOR [57, 58]	Kd *vs.* Vd	77 *vs.* 63 p < 0.0001	13 *vs.* 6 p = 0.001	18.7 *vs.* 9.4 p < 0.0001 HR = 0.53	47.6 *vs.* 40 p = 0.01 HR = 0.79
CASTOR [59]	DVd *vs.* Vd	83.8 *vs.* 63 p < 0.0001	28.8 *vs.* 9.8 p < 0.0001	16.7 *vs.* 7.1 p < 0.0001 HR = 0.31	NR
Panorama-1 [60]	PanoVd *vs.* d	61 *vs.* 57 p = 0.009	28 *vs.* 16	12.0 *vs.* 8.1 p < 0.0001 HR = 0.63	40.3 *vs.* 35.8 p = 0.54 HR = 0.94

Dex – dexamethasone; DVd – daratumumad, bortezomib, dexamethasone; HR – hazard ratio; Kd – karfilzomib, dexamethasone; ORR – overall response rate; OS – overall survival; PanoVd – panobinostat, bortezomib, dexamethasone; PFS – progression free survival; V – bortezomib; Vd – bortezomib, dexamethasone

be less evident in elderly or frail patients. The older or more unfit patients with poor performance status may benefit from less-intensive triplet regimens or dose reductions. Table VII shows the results of selected phase III clinical trials assessing IP-based chemotherapy in RRMM.

Treatment of patients with RRMM in Poland using new drugs (lenalidomide, pomalidomide, daratumumab, carfilzomib) is carried out in accordance with the Ministry of Health's drug programme "Treatment of patients with refractory or recurrent myeloma" which is available at the internet address www.gov.pl/web/zdrowie/zdrowie-onkologiczne [61].

Supportive care

Patients with RRMM are more at risk of frequent infections, bone disease or anaemia.

Infections with encapsulated germs should be managed proactively, and patients should be vaccinated against influenza, haemophilus influenza and pneumococcus. Intravenous bisphosphonates should be started or restarted at relapse, in combination with calcium and vitamin D supplementation. Local radiation therapy (20–40 Gy) may be required for local bone lesions in case of pain or imminent fracture. Anaemia should be treated with EPO (erythropoietin 40.000 UI per week, or darbepoetin 500 µg per three weeks) or transfusion [62]. Prevention of PN and thrombosis should follow the published guidelines [63].

Conclusions

Multiple myeloma can present a difficult diagnostic issue, as there are a wide variety of presenting symptoms. MM should be suspected in patients presenting signs of back pain combined with other systemic symptoms such as fatigue and weight loss, or back pain combined with abnormal blood tests. Confirmation of a MGUS and an increased (>10%) BM plasmacytosis are key determinants for the final diagnosis of MM. Despite significant advances in the management of MM, the disease remains incurable. Virtually all patients will develop relapsed disease, although strides in the field have provided opportunities for longer-term remissions.

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Oncogeriatrics (part 8.)
Frailty screening tools

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Various frailty screening tools have been developed. However, there is currently no single ideal model; some scores are better for population-level, whereas others are best suited for clinical screening and preoperative assessment. Therefore, the choice of the score might relay on specific clinical condition, the aim of the tool and department resources. The G8 and the aCGA seem to be the the most suitable in the case of preoperative frailty assessments of older patients with solid abdominal cancer who are undergoing high-risk surgery. They also may be used to identify patients at risk for adverse postoperative outcomes. They may support the decision process particularly in situations of lack of experience in full Geriatric Assessment (easy to master and implement), in acute admitted patients (time pressure or some of the domains cannot be assessed) and in case of low-/moderate-risk surgery (where extensive frailty evaluation may not influence the postoperative outcome).

Key words: older cancer patients, frailty screening, G8, aCGA, VES-13, TRST, Fried, GFI, Rockwood, Balducci

As was mentioned in the previous paper, the routine format of current preoperative requirements do not provide the information needed for optimal, tailored treatment of older patients with cancer. Therefore, Geriatric Assessment (GA) was introduced which allows for an initial assessment of the patient's condition, the identification of previously unknown health problems, a diagnosis of frailty, and an assessment of the likelihood of complications [1]. However, GA requires experience, it is time-consuming (although the additional 40 minutes during the preoperative assessment seems to be a small price to pay to decrease perioperative morbidity) and not necessary in all patients [2, 3]. Therefore, various screening tools for frailty have been developed. The Vulnerable Elderly Survey (VES-13) [4], Triage Risk Screening Tool (TRST) [5], Geriatric 8 (G8) [6], Groningen Frailty Index (GFI) [7], abbreviated Comprehensive Geriatric Assessment (aCGA) [8], Rockwood [9], Balducci [10], and Fried [11] screening scores are commonly used. Table I presents the glossary of the above-mentioned tests, including the number of questions, rang and literature cut-off scores for a patient to be considered frail.

In 2015, an update on the International Society of Geriatric Oncology (SIOG) recommendations on the use of frailty screening tools was published [12]. In the review, the most common studied tools in older patients with cancer were the VES-13, the TRST and the G8. The highest results were observed for: G8 (median sensitivity and specificity was 77–92% and 39–75%, respectively), Balducci (94% and 50%) and TRST (91% and 47%). In our recently published paper, the G8 had the highest sensitivity and negative predictive value in frailty screening among patients with cancer undergoing high-risk abdominal surgery. In turn, the aCGA had the highest discriminatory ability in terms of frailty screening in this population [13].

Most of the mentioned screening tests (VES-13, TRST, GFI, Rockwood, Balducci, Fried criteria) were developed based on older general populations. Only the G8 and the aCGA were designed specifically for older oncology patients [4–11]. One of the most

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Table I. Glossary of the different screening tests used in the study

Test	Developed for	Number of items	range	Cut-off score*
VES-13 [4]	general older population	13	0–15	≥3
TRST [5]	older patients at ED	6	0–6	≥1
G8 [6]	oncology patients	8	0–17	≤14
GFI [7]	general older population	15	0–15	≥4
aCGA [8]	oncology patients	15	ADL: 3 IADL: 4 GDS: 4 MMS: 4	≥1 dependent ≥1 dependent ≥2 ≤6
Rockwood [9]	general older population	4	0–3	≥2
Balducci [10]	general older population	4	0–4	1
Fried score [11]	general older population	5	0–5	≥3

VES-13 – Vulnerable Elders Survey, TRST – Triage Risk Screening Tool, G8 – Geriatric 8, GFI – Groningen Frailty Index, aCGA – abbreviated Comprehensive Geriatric Assessment, ED – Emergency Department, *Cut-off score for a patient to be considered frail

important characteristics of a screening tool is its ability to exclude the possibility of vulnerability, which is equivalent to a negative predictive value. Most of the papers regarding this topic present the G8 as a score meeting these conditions. In turn, its low specificity may result from the fact that it was not designed to specifically detect an abnormal GA. Thus, the aCGA, which does derive from the GA, may achieve the highest overall accuracy. The VES-13 and Fried criteria assess mainly functional status and do not identify impairments in other geriatric domains such as nutritional status, mood, or cognitive level. The TRST was designed for the screening of frailty in the emergency department but various authors have used it also in other settings [5].

To conclude, there is currently no single perfect frailty-screening tool; some scores are better for population-level, whereas others are best suited for clinical screening and preoperative assessment. Therefore, the choice of the score might relay on specific clinical conditions, the aim of the tool and department resources [14]. In the case of preoperative assessment of older patients with solid abdominal cancer, the G8 and the aCGA seems the most suitable [13].

The screening tests were not originally designed to predict the postoperative course, however, they are being increasingly studied as outcome predictors. Biganzoli et al. assessed patients with early-stage solid cancers using the Balducci, the Fried and the VES-13 score. The VES-13 score of ≥7 was a valuable discriminating tool for predicting functional decline or death. However, the authors used a higher cut-off level (≥7) in comparison to most publications (≥3) [15]. Bongue et al. evaluated the predictive performance of four frailty screening methods (aCGA, GFI, VES-13 and Fried score) and their AUC in predicting mortality ranged from 0.63 to 0.75. The tool with the greatest sensitivity for predicting the occurrence of disability, mortality and institutionalisation was the VES-13 [16]. In turn, Hall D et al. showed the clinical usefulness of the screening tool implemented in the preoperative decision process of 9153 patients undergoing various surgical procedures. On that basis, physicians decided to perform detailed evaluations of the patients and to modify their perioperative plans accordingly. As a result the mortality rate decreased significantly 30, 180 and 365 days after the surgery [17]. In turn, Huisman M.G. et al. analysing patients undergoing surgery for various solid tumours did not observe any significant predictive ability of the VES-13 and the GFI for the 30-day postoperative outcome [18].

Concluding, frailty-screening tools can be very beneficial in a variety of surgical fields. They can identify patients at risk of frailty and for adverse outcomes, particularly in situations of lack of experience in full GA (they are easy to master and implement), in acute admitted patients (when there is not enough time or some of the domains cannot be assessed) and in cases of low-/moderate-risk surgery (where extensive frailty evaluation may not influence the postoperative outcome). However, only a full Geriatric Assessment allows for: an appropriate preoperative evaluation (currently also the reference method for frailty diagnosis), identifying the age-related areas of vulnerability that can be missed in a routine clinical evaluation and enabling their preoperative modification. It also thoroughly supports the process of shared preoperative decision-making. In this age group the treatment goal is not only extension of life, but more importantly, a return to the preoperative functional and intellectual level in the postoperative period. Arguments raised about the time-consuming nature of this process are absurd, particularly when one considers the time and resources required to treat complications. Therefore, the use of the GA prior to high-risk surgery for all older patients with cancer should be recommended.

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Genetics and Oncology (part 2.)

Fundamentals of personalised medicine in the treatment of breast and ovarian cancer

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Individualisation of medical management based on prognostic and predictive markers (personalised medicine) allows customisation of prophylaxis and optimisation of treatment by increasing its efficiency and minimisation of adverse effects. In the case of breast cancer, therapy selection is still based on histopathology and immunohistochemical assessment including analysis of estrogen receptor (ER) expression, progesterone receptor (PgR) expression and over-expression or amplification of receptor tyrosine kinase erbB-2 gene (*ERBB2* aka *HER2*). An additional role, facilitating decision on application or waiver of chemotherapy in early breast cancer, may be played by panels assessing gene expression within tDNA (tumour DNA, i.e. DNA isolated from tumour cells) and evaluation of concentration of uPA (urokinase-type plasminogen activator) and PAI-1 (plasminogen activator inhibitor type 1) in tumour cells. Growing hope surrounds the new, targeted therapies, including: inhibitors of CDK 4/6 (cyclin-dependant kinases 4 and 6), mTOR inhibitors (rapamycin's mammalian target), inhibitors of poly(ADP-ribose)polymerase(PARP) or inhibitors of PI3K (phosphatidylinositol-4,5-bisphosphate 3-kinases). For ovarian cancer, treatment selection is based on assessment of the histopathologic type, malignancy degree, FIGO classification and platinum sensitivity of the tumour. However, the increasing use of PARP inhibitors and angiogenesis inhibitors is noteworthy. In the context of personalised medicine for both these cancers, an important element involves also individualisation of prophylactic and therapeutic recommendations in carriers of germline mutations associated with hereditary cancer syndromes.

Key words: personalised medicine, breast cancer, ovarian cancer, predictive tests, prognostic tests, germline mutations

Introduction to personalised medicine

Personalised therapies are currently among the most notable trends in medicine, especially in management of cancer patients. Progress in genetics and molecular pathology allowed selection of a number of biomarkers of patient-specific status. Their analysis enables selection of an optimal, individually tailored procedure. The said biomarkers can be diagnostic (helpful in precise diagnosis), prognostic (allowing estimation of the probable course of the disease in terms of recurrence risk), and predictive (allowing prediction of the likely response to particular therapies, and therefore helpful in selecting personalised therapy). Normally, biological material from the tumour is used for marker evaluation which may be carried out at the level of genetic changes (using appropriately se-

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lected cytogenetic and/or molecular tests) as well as protein changes (usually using immunohistochemical methods). The concept of personalised medicine in oncology is very broadly defined and it includes choosing patient-specific treatment, taking into account both individual prophylaxis and the type, time and sequence of therapy, as well as doses of drugs used. Management adapted to the needs of an individual patient is aimed at increasing the effectiveness of prophylaxis and therapy and at reducing the frequency and intensity of side effects [1].

The following review presents basics of personalised medicine as applied in breast cancer and ovarian cancer patients, considering especially guidelines of the European Society for Medical Oncology (ESMO).

Genetic profile of breast and ovarian cancer

The basis of the neoplastic transformation process lies in mutations. Their accumulation leads to genetic instability within neoplastic cells.

Most cancers, including most breast cancers (70–75%) and most ovarian cancers (75–90%) are sporadic in nature and develop as a consequence of accumulated somatic mutations, which are non-hereditary changes acquired during the individual's life and limited to the genome of neoplasm's cells. Presence of somatic mutations is thus limited to tDNA – DNA isolated from tumour cells. Characteristically, sporadic neoplasms are usually diagnosed in older age patients with no family history of cancer.

Some cancers, including 15–20% of breast cancers, are familial. In these cases, their origin is characterised by aggregation of neoplasms of a specific type among members of a family. In patients with familial neoplasms, multi-gene variations are observed in constitutive genome, which increase susceptibility to environmental cancerogenic factors. Therefore, familial neoplasms develop as a consequence of combined effect of constitutive genetic susceptibility and adverse environmental factors, which together lead to occurrence of mutations related to neoplastic transformation. However, the complexity and limited penetrance of constitutional variants do not allow their application as markers that would unequivocally define the individual risk of developing a cancer.

Hereditary neoplasms (developing on the basis of inherited mutation) are relatively rare e.g. hereditary breast cancer accounts for 5–10% of all breast cancer cases, and hereditary ovarian cancer for 10–25% of all ovarian cancer cases. They are characterised by unique clinical features. A suspicion of a hereditary cancer should be raised with such clinical features as: atypical cancer, young age of diagnosis (e.g. pre-menopausal breast cancer), multifocal and/or bilateral lesions, occurrence of two or more primary neoplasms in an individual or occurrence of neoplasms of the same spectrum in several members of a family. In patients with hereditary breast and/or ovarian cancer, observations confirmed an increased lifetime risk of development of not only those cancers, but also other neoplasms of the spectrum characteristic of the syndrome in question. A hereditary cancer is associated with carrying a specific germline mutation, i.e. a hereditary, congenital mutation of a single gene which is present in all cells of the body, and therefore identified both in tDNA tests and on DNA isolated from cells outside the tumour (e.g. peripheral blood lymphocytes, saliva cells, oral mucosa cells, fibroblasts). Identifying people with hereditary breast and/or ovarian cancer is important not only because of the individualisation of prophylactic and therapeutic recommendations for such patients, but also considering the necessity to provide genetic counselling to other family members [1].

Breast cancer – individualisation of therapy based on histological and immunohistochemical classification

Selection of treatment for patients with breast cancer is still based on histopathology and immunochemistry of the tumour.

Decisions concerning targeted therapy are mostly based on the tumour's biological profile. In the case of breast cancer, this profile refers to the immunohistochemical (IHC) analysis of expression of the estrogen receptor (ER), expression of the progesterone receptor (PgR) and overexpression of the human epidermal growth factor type 2 receptor (HER2) or (if this assessment is equivocal) analysis of amplification of receptor tyrosine kinase erbB-2 aka HER2 receptor gene (*ERBB2* aka *HER2*). The above biomarkers are diagnostic, prognostic and predictive, too (tab. I).

According to ESMO recommendations, examination of HER2 status should conform to standards of the American Society of Clinical Oncology – College of American Pathologists (ASCO-CAP). Additionally, amplification of *HER2* gene may be analysed by *in situ* hybridisation (ISH) or fluorescence *in situ* hybridisation (FISH), usually applied as an additional test in cases of equivocal immunochemistry results (+2) [2].

Each patient with an invasive breast cancer should have ER, PgR and HER2 status assessed, optimally in the biopsy specimen [2, 3]. In cases of equivocal or triple negative receptor status of the biopsy specimen, additionally post-operative material should be immunohistochemically tested. Furthermore, HER2 status should be re-evaluated in post-operative material in cases in which a test of the biopsy specimen revealed G1 ER+, PgR+, HER2+ NST breast cancer, as well as for selected cases of specific-type breast cancer. In all of the above situations, the postoperative results should be considered final [2]. ESMO also recommends that in cases of advanced breast cancer in the metastatic stage, at least one IHC assessment on biological material from a metastatic focus should be performed to assess the biological profile, which may be different from that of the primary tumour [4].

Table I. Individualisation of systemic treatment of breast cancer depending on the tumour's biological profile

Classification	ER	PgR	HER-2	Prognosis	Systemic treatment [2–6]
luminal A	+	+	–	good	1. Hormone therapy 5–10 years: • estrogen receptor blockers like tamoxifen, toremifene, fulvestrant and/or • aromatase inhibitors like anastrazole, letrozole, exemestane. 2. Chemotherapy in cases of T3 and/or involvement of 4 lymph nodes. 3. CDK 4/6 inhibitors (palbociclib, ribociclib, abemaciclib) or mTOR inhibitors (everolimus) in cases of advanced cancer. 4. PI3K inhibitors (alpalisib) with fulvestrant in the next line of treatment (after hormone therapy) in patients with advanced breast cancer and *PIK3CA* mutation in tDNA. 5. PARP inhibitors (olaparib or talazaparib) in the next line of treatment (after anthracycline and taxanes) in patients with a germline *BRCA1* or *BRCA2* mutation in cases of advanced cancer.
luminal B HER2-negative	+	–	–	moderately good	1. Hormone therapy 5–10 years: • estrogen receptor blockers like tamoxifen, toremifene, fulvestrant and/or • aromatase inhibitors like anastrazole, letrozole, exemestane 2. Chemotherapy. 3. Anti-HER2 therapy in HER2-positive cases: • monoclonal antibodies such as trastuzumab, pertuzumab, T-DM1 and/or • kinase inhibitors such as lapatinib, neratinib, tucatinib. 4. CDK 4/6 inhibitors (palbociclib, ribociclib, abemaciclib) or mTOR inhibitors (everolimus) in cases of advanced HER2-negative luminal B cancer. 5. PI3K inhibitors (alpalisib) with fulvestrant in the next line of treatment (after hormone therapy) in patients with advanced HER2-negative luminal B breast cancer and *PIK3CA* mutation in tDNA. 6. PARP inhibitors (olaparib or talazaparib) in the next line of treatment (after anthracycline and taxanes) in patients with a germline *BRCA1* or *BRCA2* mutation in cases of HER2-negative advanced cancer.
luminal B HER2-positive	+	+/–	+		
non-luminal HER2-positive	–	–	+	moderately severe	1. Anti-HER2 therapy in HER2 positive cases: • monoclonal antibodies such as trastuzumab, pertuzumab, T-DM1 and/or • kinase inhibitors such as lapatinib, neratinib, tucatinib. 2. Chemotherapy.
triple negative (TNBC)	–	–	–	severe	1. Chemotherapy (considering platinum derivatives, among others). 2. PARP inhibitors (olaparib or talazaparib) in the next line of treatment (after anthracycline and taxanes) in patients with a germline *BRCA1* or *BRCA2* mutation in cases of advanced cancer.

Breast cancer – therapy individualisation based on molecular changes and ancillary markers

PI3K inhibitors

A lot of hope is now associated with PI3K inhibitors (inhibitors of phosphatidylinositol-4,5-bisphosphate 3-kinases), such as alpelisib, which are new targeted therapeutical substances applied in the next-line treatment (after hormonal anti-estrogen therapy) in patients with advanced ER-positive, HER2-negative breast cancer displaying presence of *PIK3CA* gene mutation (catalytic subunit of alpha-phosphatidylinositol-4,5-bisphosphate 3-kinase) in tDNA. In these cases, it is recommended to use the PI3K inhibitor in combination with fulvestrant [5]. Importantly, in tDNA of luminal breast cancers, mutation of *PIK3CA* gene is the most frequent molecular lesion, so a large group of women with luminal breast cancer would benefit from application of the targeted therapy using PI3K inhibitors [5, 6].

PARP inhibitors

The latest ESMO recommendations suggest also possibility to apply PARP inhibitors (poly (ADP-ribose) polymerase – PARP) – olaparib or talazoparib – in the next-line treatment (after anthracycline and taxanes) in patients with germline mutation of *BRCA1* or *BRCA2* gene (mutation responsible for hereditary breast cancer and ovarian cancer syndrome – table II) and diagnosed triple negative advanced breast cancer or advanced luminal HER2-negative breast cancer [4].

Gene expression panels

In the targeted therapy of breast cancer, gene expression panels on tDNA can be applied, too: MammaPrint (Agendia, Amsterdam, The Netherlands), Oncotype DX (Genomic Health, Redwood City, CA, USA). Prosigna (PAM 50, NanoString Technologies, Seattle, WA, USA), Endopredict (Myriad Genetics SaltLake City, UT, USA), Breast Cancer Index (Biotheranostics, Inc., San Diego, CA, USA). The above tests assess expression of selected – usually several dozen – genes related to processes of proliferation, angiogenesis, metastasis and others, allowing determination of the specific expression profile of the tumour. According to the ESMO recommendations, gene expression panels are used as a supplementary prognostic marker (enabling estimation of the course of the neoplastic disease and the risk of metastasis) and a predictive marker mainly in cases of early ER-positive and HER2-negative breast cancer, without

nodal involvement or with involvement of up to 3 lymph nodes. In such cases, results of the discussed panels play an auxiliary role in equivocal situations, where application or withdrawal of chemotherapy is considered [2, 3].

Assessment of uPA and PAI-1 concentrations in tumour cells

Tests to assess uPA (urokinase-type plasminogen activator) and PAI-1 (plasminogen activator inhibitor-1) concentrations in tumour cells have similar application in individualisation of breast cancer therapy as gene expression panels. The test is based on the ELISA technique, and it requires a fresh and unfixed or freshly frozen tumour specimen. According to ESMO recommendations, the test should be considered primarily in cases of early breast cancer without lymph node involvement. High concentrations of uPA and /or PAI-1 are considered unfavourable prognostic markers suggesting high risk of recurrence and indicating that adjuvant chemotherapy is advisable [2, 3].

Broad-panel tDNA molecular testing

Next generation sequencing (NGS) allows analysis of the whole exome (whole-exome sequencing – WES) or even the whole genome (whole genome sequencing – WGS) of the tumour, raising hopes for application of new targeted therapies. Broad-panel molecular tests showed that the most frequent molecular changes in cancer cells include gene mutations in *PIK3CA, TP53, GATA3, PTEN, AKT1, CDH1, ARID1B, CASP8, BRCA1, RB1, MLL3, MAP3K1, MAP3K13, NCOR1, SMARCD1, CDKN1B, TBX3, RUNX1, CBFB, AFF2, PIK3R1, PTPN22, PTPRD, NF1, SF3B1* and *CCND3*, as well as copy number variants (CNV) in *PIK3CA, ERBB2, TP53, MAP2K4, MLL3, CDKN2A, PTEN* and *RB1* [6]. Comprehensive genomic profiling (CGG) enables establishment of a molecular classification of breast cancers based on changes in individual signalling pathways, such as PI3K / AKT / mTOR pathway (molecular target for such therapeutical substances as everolimus, temsirolimus, alpelisib), double-strand DNA break repair in genes *BRCA1 / BRCA2 / PALB2* (their mutations are a good predictive factor for PARP inhibitors), estrogen receptor ER pathway, cell cycle regulatory pathway CCND1 / CDK4 / RB1 (molecular target for palbociclib, ribociclib, abemaciclib), growth factors ERBB2 / EGFR / FGFR1 (molecular target for such therapeutic substances as trastuzumab, pertuzumab, afatinib, lapatinib, neratinib, pazopanib, ponatinib) [7]. Thus, detection of changes in the cellular pathways identified in breast cancer allows assumptions concerning potential efficacy of therapies targeted at them. There are commercial broad-panel genetic tests available on the market, offering sequencing of many genes within tDNA, both in the form of WES and WGS tests of the tumour genome, as well as gene panels selected for a given tumour. Their analysis is potentially important in the context of developing targeted therapies. However, ESMO recommendations do not provide for routine application of broad-panel molecular tests of tDNA, due to their currently limited use in clinical practice in patients with breast cancer.

Hereditary breast cancer

There is a specific dimension of personalised medicine applied in breast cancer which concerns carriers of mutations typical for hereditary cancer syndromes including breast cancer. Breast cancer appears in the spectrum of many hereditary cancer syndromes caused by germline mutations of such genes as: *BRCA1* and *BRCA2* (HBOC hereditary breast and ovarian cancer), *CHEK2, PALB2, TP53* (Li-Fraumeni syndrome), *ATM*, *PTEN* (Cowden syndrome), *CDH1* (hereditary diffuse gastric cancer) or *STK11* (Peutz-Jeghers syndrome). The role of mutations of individual genes in aetiopathogenesis of the hereditary breast cancer varies depending on the studied ethnic group, but most hereditary breast cancers are associated with germline mutation of *BRCA1* or *BRCA2* gene responsible for the hereditary breast and ovarian cancer syndrome [8, 9].

Currently in Poland, module I of the National Cancer Control Programme of the Ministry of Health (Narodowy Program Zwalczania Chorób Nowotworowych Ministerstwa Zdrowia – NPZChN MZ) recommends for all breast cancer patients genetic counselling. Genetic testing to assess critical hereditary mutation is recommended for selected group of patients, in whom the hereditary form of disease has been suspected on the basis of pedigree analysis. The recommendations concern the presence of five germline mutations of *BRCA1* gene which are the most common in the Polish population (c.5266dupC, c.181T>G, c.4035del, c.66_67AG, c.3695_3699GTAAA), two selected germline mutations of *PALB2* gene (c.509_510del, c.168_171TTGT) and three selected germline mutations of *CHEK2* gene (c.1100del, del5395, c.444+1G>A) . This range of molecular diagnostics has been developed based on the specificity of the Polish population, with dominating carriers of one of the five founder mutations of *BRCA1* gene, which account for aetiology of approximately 64% of BRCA-dependent hereditary breast cancers [10].

In cases of hereditary history (tab. II) module I of the NPZChN MZ Programme recommends expanded molecular diagnostics, including sequencing of *BRCA1* and *BRCA2* genes, currently by the next generation sequencing (NGS) technique. However, this test, too, has some limitations. It is not recommended for analysing large rearrangements (deletions and duplications), which account for up to 10% of mutations identified in *BRCA1* and *BRCA2* genes [11]. Their occurrence should be verified by another method such as MLPA technique (multiplex ligation-dependent probe amplification). Furthermore, sequencing entire gene-coding sequences yields a lot of information requiring diligent bio-IT analysis for verification of clinical significance of the identified variations. Identifying variations is a complex process, requiring advanced *in silico* analysis, assessment of the variation's frequency in the general population, access to available databases, such as: ClinVar, dbSNP, Breast

Cancer Information Core, Varsome, 1000GP, Consensus PathDB, Gene Ontology, GWAS, OMIM, UniProt or HGMD, etc. The currently applied classification recommended by the American College of Medical Genetics and Genomics (ACMG) provides for five classes of pathogenic effect of variations:

- non-pathogenic variant (class 1),
- possibly non-pathogenic variant (class 2),
- variant of unknown clinical significance (VUS, class 3),
- possibly pathogenic variant (class 4),
- pathogenic variant (class 5) [12].

Class 4 and Class 5 variations are considered to be mutations, i.e. changes of clinical significance. The analysis of germline VUS variants remains a major challenge in genetic counselling. Therefore, it is recommended that prophylactic and therapeutic recommendations should be based on history and clinical analysis. Further – considering progress of knowledge on molecular changes and constant updating of databases – it is stressed that the identified variation should be consulted again after 2–3 years.

The variability of clinical significance of individual variations within a studied gene can be observed in the case of *CHEK2* gene: its shortened protein or frameshift variants have a far more significant impact on neoplasm risk than missens variations. Therefore, individual medical recommendations should be based not only on the gene where the mutation is found, but also on the type of change identified.

Identification of a germline mutation is a molecular confirmation of a specific hereditary cancer syndrome. However, due to the limitations of genetic testing presented above, non-detection of mutations in the tested range does not allow for clear exclusion of the suspected hereditary cancer syndrome. The result of a genetic test should therefore be supported by specialised genetic counselling, and individual medical recommendations should take into account not only results of molecular tests, but also clinical and history evaluation.

Patients with family and clinical history suggestive of hereditary form of disease who do not have mutations revealed in sequencing of *BRCA1* and *BRCA2* remain a challenge. Hereditary breast and ovarian cancer (HBOC), while dominant, is not the only syndrome of hereditary predisposition to cancers with breast cancer in the spectrum. In some of the other, rarer syndromes, there are associated characteristic signs and symptoms, such as specific family history, macrocephaly (Cowden syndrome) or typical changes in skin and mucosa (Peutz-Jeghers syndrome), facilitating identification of a specific suspicion and referring to targeted genetic testing. In non-specific cases, the only option is to consider broad-panel genetic NGS testing, which allows sequencing of many genes associated with many hereditary cancer syndromes within a single test. There are many commercial wide-panel tests available on the market, differing in the scope of the studied genes. They may take into account both genes of high penetrance (in the case of occurrence of a germline mutation, they increase the risk of developing neoplasms from a given spectrum very strongly, even fivefold) and of moderate penetrance (increasing the risk of developing neoplasms from a given spectrum about 2–5 times in the case of occurrence of a germline mutation).

Carriers of germline mutations should be informed about the risk of occurrence of the mutation in their relatives. Genetic counselling should cover not only people with hereditary breast cancer, but also selected members of their families. Individualisation of medical procedures applies to all mutation carriers (including those diagnosed with another cancer from the spectrum of a given cancer syndrome, as well as those without cancer diagnosis), and also families with cancer in which no causative mutation was detected.

Detailed characteristics of the most frequent hereditary cancer syndromes of spectrum including breast cancer, and therapeutic and prophylactic recommendations for mutation carriers considering ESMO guidelines and module I of the NPZChN Programme are shown in table II.

Ovarian cancer – classification

In patients with diagnosed ovarian cancer prognosis of the course of disease and potential response to applied therapy depends on the neoplasm's histopathology type, tumour grading, four-stage FIGO classification and tumour's platinum sensitivity.

According to the classification of the World Health Organisation (WHO), epithelial ovarian tumours include:

- serous type (about 80% of cases),
- endometrioid type (about 10% of cases),
- clear-cell type (about 5% of cases),
- mucous type,
- transitional epithelial tumours (Brenner tumour),
- mixed type,
- undifferentiated type,
- unclassified type [20].

Additionally, there is a separate group of borderline epithelial tumours of the ovary, accounting for 10–15% of ovarian tumours and characterised by equivocal histopathology, which doesn't allow their identification as either malignant or non-malignant ovarian neoplasms. Serous borderline tumours are the most frequent, followed by mucous and endometrioid types [20].

Apart from the standard classification, there is also another division of epithelial ovarian tumours, considering jointly: etiopathogenetic factors, histopathological type, histologic malignancy stage, molecular changes, response to chemotherapy and prognosis. This division distinguishes the following types:

- type 1 ovarian cancer, characterised by low histological malignancy, a more stable course and frequent mutations of *KRAS, BRAF, ERBB2, PTEN, PIK3CA* and *ARID1A* genes in the genetic material of the tumour (*ARID1A* mutations are particularly frequently identified in cases of endometrioid and clear cell carcinoma). Type 1 ovarian cancer includes low-grade serous, endometrioid, mucous and clear-cell

Table II. Selected hereditary cancer syndromes with breast cancer in the spectrum - characteristics and therapeutic and prophylactic management

Hereditary cancer syndrome	Genes in which germline mutations are present	Indications for genetic testing	Risk of breast cancer in carriers of the mutation	Other neoplasms in the spectrum of increased risk and associated symptoms	Recommendations
hereditary breast and ovarian cancer (HBOC) [8, 11]	*BRCA1*	According to ESMO[1]: a) genetic testing for HBOC should be considered in families with: – breast cancer ≤50 years of age – triple negative TNBC breast cancer – ipsilateral and/or contralateral breast cancer – male breast cancer – breast cancer in an Ashkenazi woman – two breast cancers among first / second / third degree relatives – three breast cancers among first / second / third degree relatives, regardless of age – ovarian cancer – pancreatic cancer and/or prostate cancer of Gleason score ≥7 and breast cancer and/or ovarian cancer b) optimal scope of the test includes sequencing and evaluation of large rearrangements (deletions/duplications) of *BRCA*1 and *BRCA2* genes c) in families with identified specific *BRCA1/2* mutation, an occurrence of hereditary mutation should be verified in the carrier's family, above all first-degree relatives. According to module I of the NPZChN MZ Programme [2]: a) analysis of occurrence of five mutation of *BRCA1* gene which are the most common in the Polish population (c.5266dupC, c.181T > G, c.4035del, c.66_67AG, c.3695_3699GTAAA) in: – any person with diagnosed with breast cancer (including DCIS, male breast cancer) – any person with ovarian cancer (including peritoneal cancer and fallopian tube cancer) – in families with breast and/or ovarian cancer, in cases where the person with cancer is unavailable for examination, the analysis should be performed in the closest relatives (optimally first or second degree) b) sequencing of *BRCA1* and *BRCA2* genes: – only in people diagnosed with breast and/or ovarian cancer – only in cases where 5 most common mutations in the Polish population have been excluded – only if: • the patient has both breast cancer and ovarian cancer, including the first diagnosis before the age of 50 • the patient has had bilateral breast cancer, including the first diagnosis before the age of 50	– risk of developing breast cancer in carriers up to approx. 87% – risk of developing contralateral breast cancer in carriers up to approx. 83% – risk of developing breast cancer in male carriers up to approx. 1%	– risk of developing ovarian cancer up to approx. 63% – risk of developing prostate cancer up to approx. 8,5% – risk of developing pancreatic cancer up to approx. 3%	– each carrier should perform monthly self-palpation of the breasts – each carrier is recommended to breastfeed for a long time and to abandon/limit hormone replacement therapy (HRT) – each carrier is recommended to have a medical palpation of the breasts and breast imaging every 6 months (the age of beginning the tests depends on the family and clinical assessment, but should not be later that 25 years of age): MRI alternating with ultrasound (up to 30 years of age) or mammography (after 30 years of age) – chemoprevention with tamoxifen may be considered in any carrier – each carrier may consider a prophylactic bilateral mastectomy, optimally with simultaneous reconstruction – in carriers who developed breast cancer, breast conserving surgery should be abandoned in favour of mastectomy, possibly with prophylactic contralateral mastectomy, ideally with simultaneous reconstruction – in carriers who have developed triple-negative or luminal type breast cancer that progresses despite anti-estrogen therapy, PARP inhibitors (olaparib or talazaparib) should be considered as the next-line treatment (after anthracycline and taxanes) – each carrier from 30 years of age is recommended to have a TV-USG of the small pelvis and assessment of serum CA125 concentration every 6–12 months – in each carrier, prophylactic, bilateral adnexectomy is recommended, optimally at the age of 35–40 years, after completion of procreation plans – carriers diagnosed with ovarian cancer are expected to respond well to platinum derivatives and PARP inhibitors – each male carrier should conduct regular breast self-examination, and from 30–35 years of age annual medical palpation of the breast is recommended (especially in carriers of the *BRCA2*mutation) – each male carrier (especially with *BRCA2* mutuation) may consider annual screening for prostate cancer starting from 40–45 years of age
	BRCA2		– risk of developing breast cancer in carriers up to approx. 84% – risk of developing contralateral breast cancer in carriers up to approx. 62% – risk of developing breast cancer in male carriers up to approx. 9%	– risk of developing ovarian cancer up to approx. 27% – risk of developing prostate cancer up to approx. 20% – risk of developing pancreatic cancer up to approx. 7% – a discreetly increased risk of developing melanoma (of the skin and/or eyeball)	

Hereditary cancer syndrome	Genes in which germline mutations are present	Indications for genetic testing	Risk of breast cancer in carriers of the mutation	Other neoplasms in the spectrum of increased risk and associated symptoms	Recommendations
		• the patient has been diagnosed with breast cancer or ovarian cancer and has a first and / or second degree relative who was diagnosed with breast and/or ovarian cancer, at least one of these cases before the age of 50 • the patient was diagnosed with breast cancer before the age of 50 or ovarian cancer at any age, and in addition first and/ or second grade relatives were diagnosed with male breast cancer and/or ovarian cancer c) in families with identified specific *BRCA1/2* mutation, an occurrence of hereditary mutation should be verified in the carrier's family, above all first-degree relatives d) if a specific *BRCA1/2* mutation is identified in DNA from tumour cells (tDNA), its presence should be analysed on DNA isolated from outside the tumour cells (blood, saliva, oral swab, skin biopsy specimen) to assess the nature of the mutation (somatic, i.e. non-hereditary or germline, i.e. hereditary).			– in all carriers of the *BRCA2* mutation, annual dermatological and ophthalmological testing for melanoma may be considered, especially in the presence of this tumour in relatives – in any carrier of the *BRCA2* mutation, especially in cases with a family history of pancreatic cancer, annual pancreatic cancer screening (EUS or MRI) may be considered, starting from the age of 50 or 10 years earlier than the youngest family history of pancreatic cancer
germline mutations of *PALB2* [8, 11, 13]	*PALB2*	According to module I of the NPZChN MZ Programme[2]: two selected mutations of *PALB2* (c.509_510del, c.168_171TTGT) should be analysed: a) in any person with breast cancer b) in the case when the person diagnosed with breast cancer is unavailable for examination and the family has been diagnosed with: – bilateral breast cancer – breast cancer before 40 years of age – male breast cancer – 2 cases of breast cancer and/or ovarian cancer in people who are first/second degree relatives.	– risk of developing breast cancer in carriers up to approx. 58%	– increased risk of developing pancreatic cancer – increased risk of developing breast cancer in male carriers	– each carrier should perform monthly self-palpation of the breasts – each carrier is recommended to have a medical palpation of the breasts and breast imaging every 6 months (the age of beginning the tests depends on the family and clinical assessment, but should not be later that 20–25 years of age): MRI alternating with ultrasound (up to 30 years of age) or mammography (after 30 years of age) – each carrier may consider bilateral prophylactic mastectomy, optimally with simultaneous reconstruction, but no such recommendations are included in module I of NPZChN MZ Programme – the person with identified mutation should be notified that if she gets pregnant by a carrier of *PALB2* mutation, the risk of giving birth to a child with Fanconi anaemia type N is 25%
germline mutations of *CHEK2* [8, 11]	*CHEK2*	According to module I of the NPZChN MZ Programme [2]: three selected mutations of*CHEK2* (c.1100del, del5395, c.444+1G > A) should be analysed: a) in any person with breast cancer b) in the case when the person diagnosed with breast cancer is unavailable for examination and the family has been diagnosed with: – bilateral breast cancer – breast cancer before 40 years of age – male breast cancer – 2 cases of breast cancer and/or ovarian cancer in people who are first/second degree relatives.	– risk of developing breast cancer up to approx. 39% *The spectrum and risk of cancer development largely depend on the type of mutation found. Literature data are ambiguous for many CHEK2 variants.*	– risk of developing prostate cancer – risk of developing papillary thyroid cancer *The spectrum and risk of cancer development largely depend on the type of mutation found. Literature data are ambiguous for many CHEK2 variants.*	– each carrier should perform monthly self-palpation of the breasts – each carrier is recommended to have a medical palpation of the breasts and breast imaging every 6 months (the age of beginning the tests depends on the family and clinical assessment, but should not be later that 20–25 years of age): MRI alternately with ultrasound (up to 30 years of age) or mammography (after 30 years of age), but no recommendation for breast MRI in module I of NPZChN MZ Programme [2] – module I NPZChN MZ[2] additionally recommends annual ultrasound of the thyroid gland

Hereditary cancer syndrome	Genes in which germline mutations are present	Indications for genetic testing	Risk of breast cancer in carriers of the mutation	Other neoplasms in the spectrum of increased risk and associated symptoms	Recommendations
Li-Fraumeni Syndrome (LFS) [8, 14]	*TP53*	Sequencing of *TP53* gene is recommended if: 1. Chompret criteria are met: – diagnosed breast cancer in a patient aged ≤30 – diagnosed malignant neoplasm in LFS spectrum in a patient aged ≤45 and at least one relative in first/second degree with diagnosed malignant neoplasm in LFS spectrum (excluding breast cancer if in the original patient) at the age of ≤55 or in a multifocal form – diagnosed multiple primary malignancies (except multiple primary breast cancer lesions), of which at least 2 belong to the LFS spectrum and the first diagnosis occurred at the age ≤45 – diagnosed rare malignant neoplasm typical of LFS, such as: adrenal cortex cancer, choroid plexus cancer, anaplastic embryonal rhabdomyosarcoma 2. The patient was diagnosed with hypodiploid acute lymphoblastic leukaemia (ALL) before 21 years of age. 3. Findings in tumour cell studies: – presence of *TP53* mutation of allele frequency approaching 50% or higher in tDNA – absent or decreased expression of TP53 in IHC tests	– risk of breast cancer development in carriers up to approx. 54% [14] – 79% [8] (usually premenopausal breast cancers)	– notably young age of oncological diagnoses (including <18 years of age) and risk of multifocal primary cancers in a single patient – neoplastic disease develops in at least 90% of female carriers and 70% of male carriers of the *TP53* mutation – risk of developing a soft tissue sarcoma up to approx. 27% – risk of developing osteosarcoma up to approx. 16% – risk of developing a malignant tumour of the CNS up to approx. 14% (especially *glioblastoma*, *astrocytoma*) – risk of developing adrenal cortex cancer (ACC) up to approx. 13% – risk of developing leukaemia (especially ALL, AML, MDS) up to approx. 4% – risk of developing lymphoma up to approx. 2% – risk of developing colorectal cancer (up to approx. 3–8%) and gastric cancer – risk of developing melanoma	– each carrier should perform monthly self-palpation of the breasts – in each carrier from 20 years of age medical palpation of the breasts is recommended every 6–12 months – in each carrier from 20 years of age annual breast MRI is recommended – in carriers who developed breast cancer, breast conserving surgery should be abandoned in favour of mastectomy, possibly with prophylactic contralateral mastectomy, ideally with simultaneous reconstruction – each carrier may consider a prophylactic bilateral mastectomy, optimally with simultaneous reconstruction – in all carriers from 25 years of age gastroduodenoscopy and colonoscopy is recommended (at least every 5 years, the endoscopic image determines the frequency of the examination) – annual neurological examination is recommended for all carriers and consideration of annual whole-body MRI and six-monthly blood counts – in all carriers, ultrasound of the abdominal cavity and small pelvis may be considered: every 3–4 months until the age of 18 and every year after the age of 18 – annual dermatological examination is recommended for all carriers – X-ray and ionizing tests and therapies should be abandoned (or limited) in all carriers
Cowden syndrome [8, 15]	*PTEN*	performance of *PTEN* genetic testing is recommended in patients with a Cleveland Clinic score (CC score) of at least 10; the scale considers assessment of occurrence of malignant cancers (including breast, endometrium, thyroid, renal cancer) and non-malignant tumours and non-neoplastic lesions typical for Cowden syndrome spectrum. https://www.lerner.ccf.org/gmi/ccscore/	– risk of developing breast cancer in female carriers up to approx. 50%, according to some sources up to approx. 85%	– risk of developing thyroid cancer (especially follicular cancer) up to approx. 35% – risk of developing renal cancer (especially papillary cancer) up to approx. 35% – risk of developing endometrial cancer up to approx. 28% – risk of developing colorectal cancer up to approx. 9% – risk of developing melanoma up to approx. 5% – very frequent occurrence on non-malignant tumours:	– each carrier should perform monthly self-palpation of the breasts – in each carrier from 20–25 years of age medical palpation of the breasts is recommended every 6–12 months – in each carrier from 30 years of age annual breast MRI and/or mammography is recommended – in each carrier from 30–35 years of age annual TV-USG with endometrial biopsy is recommended (unless a hysterectomy has been previously performed) – each carrier may consider a prophylactic bilateral mastectomy, optimally with simultaneous reconstruction – any carrier may consider prophylactic hysterectomy – in all carriers, annual dermatological control and annual ultrasound of the thyroid gland are recommended

Hereditary cancer syndrome	Genes in which germline mutations are present	Indications for genetic testing	Risk of breast cancer in carriers of the mutation	Other neoplasms in the spectrum of increased risk and associated symptoms	Recommendations
				– gastrointestinal polyps (hamartomatous, juvenile adenomas), fibrocystic breast dysplasia, nodular goitre and/or thyroid adenomas, uterine fibroids, vascular malformations – frequent coexistence of dermal and mucosal lesions: trichilemoma lesions of the face, skin papillomatosis, oral papillomas, acral keratosis, freckle lesions on the skin of the penis, lipomas and skin fibromas – macrocephaly – possible signs in the autism spectrum and psychomotor retardation in childhood – possible coexistence of Lhermitte-Ducros disease (LDD), i.e. dysplastic cerebellar ganglion	– in all carriers, colonoscopy is recommended from the age of 35, frequency of the examination depends on the endoscopic image – in all carriers from 40 years of age annual renal CT or MRI is recommended
germline *ATM* mutations [8, 16]	*ATM*		– risk of developing breast cancer in carriers up to approx. 52%	– carriers are also likely to have a moderately increased risk of developing gastric and colorectal cancer	– each carrier should perform monthly self-palpation of the breasts – each carrier is recommended to have a medical palpation of the breasts every 6–12 months and annual MRI of the breasts (no clear recommendations as to the age to start the tests, probably no later than 25) – X-ray and ionizing tests and therapies should be abandoned (or limited) in all carriers – the person with identified mutation should be notified that if she gets pregnant by a carrier of ATM mutation, the risk of giving birth to a child with ataxia-telangiectasia is 25%
hereditary diffuse gastric cancer [8, 17, 18]	*CDH1*	sequencing with the analysis of rearrangement (deletion / duplication) of *CDH1* gene is recommended in the following cases: – diagnosed diffuse gastric cancer at any age and at least one relative in the first/second degree with diagnosed any gastric cancer – diffuse gastric cancer diagnosed in the patient or a relative in the first/second degree before the age of 40 – the patient or family member has had both diffuse gastric cancer and lobular breast cancer, and at least one diagnosis was made before the age of 50.	– risk of developing lobular breast cancer in carriers up to approx. 42%	– risk of developing diffuse gastric cancer up to approx. 70% in men and up to approx. 56% in women	– each carrier should perform monthly self-palpation of the breasts – in each carrier from 20 years of age medical palpation of the breasts every 6 months is recommended and also regular breast imaging: annual MRI (from 20 years of age) alternating with annual mammography (additionally from 30 years of age) – each female carrier may consider a prophylactic bilateral mastectomy, optimally with simultaneous reconstruction – in each adult male carrier, it is recommended to consider prophylactic gastrectomy, performed optimally between 20 and 30 years of age

Hereditary cancer syndrome	Genes in which germline mutations are present	Indications for genetic testing	Risk of breast cancer in carriers of the mutation	Other neoplasms in the spectrum of increased risk and associated symptoms	Recommendations
					– in carriers who do not decide to undergo prophylactic gastrectomy, it is optimal to perform high-resolution chromoendoscopy using indigo carmine and the Cambridge protocol (starting 5–10 years younger than the youngest diagnosis in the family, examination repeated every 6–12 months) – in every carrier from the age of 40 it is recommended to perform a colonoscopy examination at least every 5 years (frequency of the examination is determined by the endoscopic image)
Peutz-Jeghers Syndrome (PJS) [8, 18, 19]	*STK11*	Sequencing of *STK11* gene is recommended for patients with: – identified presence of ≥2 hamartomatous polyps in the GI tract, confirmed by histopathology – identified presence of at least 1 hamartomatous polyp of the gastrointestinal tract and the family history indicative of PJS – identified presence of at least 1 hamartomatous polyp and presence of dermal and mucosal discoloration spots typical of PJS – identified presence of dermal and mucosal discolorations typical do PJS and family history suggesting	– risk of developing breast cancer in carriers up to approx. 54%	– presence of multiple polyps (usually hamartomatous) of the GI tract – risk of developing colorectal cancer up to approx. 39% – risk of developing pancreatic cancer up to 36% – risk of developing gastric cancer up to approx. 29% – risk of developing small intestine cancer up to approx. 13% – risk of development of ovarian tumour –SCTAT (sex cord tumours with annular tubules) up to approx. 21% – risk of developing a malignant cervical adenoma up to approx. 10% – risk of developing endometrial cancer up to approx. 9% – risk of developing Sertoli cell tumour of the testicle up to approx. 9% – risk of developing lung cancer up to approx. 17% – frequently associated dermal and mucosal discoloration: around lips, eyes, nose and on the oral mucosa, around the perineum and on fingers	– each carrier should perform monthly self-palpation of the breasts – in each carrier from 20–25 years of age medical palpation of the breasts every 6 months is recommended and also regular breast imaging: annual MRI (from 20–25 years of age) alternating with annual mammography (additionally from 30 years of age) – each female carrier may consider a prophylactic bilateral mastectomy, optimally with simultaneous reconstruction – in each carrier imaging of the upper GI tract and small intestine (gastroscopy/MR endoscopy/capsule endoscopy) and colonoscopy are recommended at the of 8 and then: • every 2–3 years in the case of polypoid lesions identified at the age of 8 • at 18 years of age in the case of no polypoid lesions identified at the age of 8 and then from 18 years of age every 2–3 years – in girls, ultrasound of the pelvis minor with ovary evaluation is recommended from childhood until the age of 12 – in each adult carrier, annual TV-USG examination and annual evaluation of serum CA125 concentration are recommended – in each carrier from 30 years of age annual screening for pancreatic cancer (EUS or MRI-MRCP) is recommended – in boys, annual examination of the testicles (palpation and possibly ultrasound) is recommended, from childhood until approx. 12 years of age

[1]ESMO – European Society for Medical Oncology
[2]National Cancer Control Programme of the Ministry of Health

ovarian cancers, malignant Brenner tumours, and borderline epithelial tumours;

- type 2 ovarian cancer, characterised by high histological malignancy, aggressive course and metastatic tendency, poor prognosis and frequent *TP53* mutations (very common in high-grade serous ovarian cancer), *BRCA1* and *BRCA2* mutations (any of the above identified in approximately 20% of type 2 ovarian cancers) in genetic material of the tumour. Type 2 ovarian cancer includes serous or endometrioid high-grade ovarian cancers, mixed-type tumours and undifferentiated tumours. Characteristically, in the most common cases of type 2 ovarian cancers, i.e. serous tumours and those of high histological malignancy, a specific etiopathogenetic mechanism is suggested, with the neoplastic process starting within the hyphae of the fallopian tube [20].

Ovarian cancer – individualisation of therapy

Histopathological classification vs. response to chemotherapy

The mainstay of treatment in patients with ovarian cancer is radical surgery and adjuvant chemotherapy, usually applying platinum derivatives (carboplatin, cisplatin) in combination with paclitaxel. In further treatment lines, depending on platinum sensitivity of the tumour, it is possible to apply platinum preparations and praclitaxel, traditional or pegylated liposomal doxorubicin (PLD), topotecan, gemcitabine and trabectedin [20].

Predictive markers of response to the classic chemotherapy regimen include histopathological type and degree of malignancy of ovarian cancer. Low effectiveness of standard chemotherapy protocols based on platinum compounds is observed in cases of serous ovarian tumours of low histological malignancy and clear-cell ovarian cancer [20].

PARP inhibitors

In systemic treatment of patients with ovarian cancer, poly-(ADP-ribose) polymerase (PARP) inhibitors such as olaparib, niraparib and rucaparib are becoming increasingly important. The effect of these substances relies on inducing double-strand DNA breaks in neoplasm cells, leading to interruption of the cell cycle and death of the cancer cells. Therefore, the best effects of treatment with PARP inhibitors are achieved in the presence of the *BRCA1* or *BRCA2* mutation in the tDNA, because in such tumours DNA break repair is impaired by homologous recombination deficiency (HRD) and dependence of the repair process on the mechanisms related to PARP polymerases. Consequently, routine sequencing of both genes on tDNA isolated from post-operative material, cell block or possibly biopsy specimen has been introduced to the diagnostic process. Until recently, inclusion of PARP inhibitors in ovarian cancer therapy depended on presence of the pathogenic variants (class 5) or probably pathogenic variants (class 4) of *BRCA1* or *BRCA2* in tDNA.

DNA break repair failure due to homologous recombination deficiency may occur also because of other molecular changes than *BRCA1* or *BRCA2* mutation and further clinical trials showed that therapeutical effect of application of PARP inhibitors was observed in general in cases of ovarian cancer with evidence of homologous recombination deficiency. There are commercial tests on the market that enable assessment of the homologous gene recombination deficiency and the resulting genomic instability in tumour cells. These tests are based on the measurement of loss of heterozygosity (LOH), telomeric allelic imbalance (TAI) and damage to chromosomal structure (large scale state transitions – LST), among other parameters. However, the tests are notably expensive. Furthermore, the latest clinical trial results show that PARP inhibitor exhibit efficacy which is also significant, although lower, in the group of patients with ovarian cancer without *BRCA1* and *BRCA2* mutations in tDNA, and even without evidence of HRD [20].

Current recommendations provide for administration of any PARP inhibitor in patients with recurrent platinum-sensitive ovarian cancer of high grade of malignancy, regardless of mutational status of *BRCA1* and *BRCA2* in tDNA in the case of supportive treatment after administration of chemotherapy based on platinum compounds, as well as in patients with advanced (FIGO grades III and IV), platinum-sensitive ovarian cancer of high grade of malignancy with known *BRCA1* or *BRCA2* mutation in tDNA in supportive treatment after administration of chemotherapy based on platinum compounds.

There have also been recommendations concerning consideration of rucaparib monotherapy as next-line treatment in patients with ovarian cancer and known *BRCA1/2* mutation in tDNA, who have counterindications to chemotherapy with platinum derivatives [20].

Inhibitors of angiogenesis

Enhanced angiogenesis is one of the pathomechanisms leading to increasing mass of ovarian cancer. Therefore, angiogenesis inhibitors such as bevacizumab, a monoclonal antibody directed against vascular endothelial growth factor (VEGF) are considered in the treatment of ovarian cancer. Current ESMO recommendations provide for application of bevacizumab in first-line treatment along with paclitaxel and carboplatin in patients with advanced ovarian cancer (FIGO stage IV and FIGO stage III after suboptimal cytoreduction with residual lesions exceeding 1 cm) in an adjuvant and supportive scheme for one year and in cases of recurrence in patients with platinum-sensitive ovarian cancer, who have not received bevacizumab as first-line treatment [20].

Hereditary ovarian cancer

Personalised medicine in cases of ovarian cancer, similarly to breast cancer, also extends to an individual approach in

Table III. Selected hereditary cancer syndromes with ovarian cancer in the spectrum – characteristics and therapeutic and prophylactic management

Hereditary cancer syndrome	Genes in which germline mutations are present	Indications for genetic testing	Risk of ovarian cancer in carriers of the mutation	Other neoplasms in the spectrum of increased risk and associated symptoms	Recommendations
breast and ovarian cancer (HBOC) [8, 11]	*BRCA1*	according to ESMO[1]: a) genetic testing for HBOC should be considered in families with: – ovarian cancer – breast cancer ≤50 years of age – triple negative TNBC breast cancer – ipsilateral and/or contralateral breast cancer – male breast cancer – breast cancer in an Ashkenazi woman – two breast cancers among first/second/third degree relatives – three breast cancers among first/second/third degree relatives, regardless of age – pancreatic cancer and/or prostate cancer of Gleason score ≥7 and diagnosed breast cancer and/or ovarian cancer b) optimal scope of the test includes sequencing and evaluation of large rearrangements (deletions/duplications) of *BRCA*1 and *BRCA2* genes c) in families with identified specific *BRCA1/2* mutation, an occurrence of hereditary mutation should be verified in the carrier's family, above all first-degree relatives according to module I of the NPZChN MZ Programme[2]: a) analysis of occurrence of five mutations of *BRCA1* gene which are the most common in the Polish population (c.5266dupC, c.181T > G, c.4035del, c.66_67AG, c.3695_3699GTAAA) in: – any person with breast cancer (including DCIS, male breast cancer) – any person with ovarian cancer (including peritoneal cancer and fallopian tube cancer) – in families with breast and/or ovarian cancer, in cases where the person with cancer is unavailable for examination, the analysis should be performed in the closest relatives (optimally first or second degree) b) sequencing of *BRCA1* and *BRCA2* genes: – only in people diagnosed with breast and/or ovarian cancer – only in cases where 5 most common mutations in the Polish population have been excluded – only if: • the patient has both breast and ovarian cancer, with the first diagnosis before the age of 50 • the patient has had bilateral breast cancer, with the first diagnosis before the age of 50 • the patient has been diagnosed with breast cancer or ovarian cancer and has a first and/or second degree relative who was diagnosed with breast and/or ovarian cancer, at least one of these cases before the age of 50	– risk of developing ovarian cancer up to approx. 63%	– risk of developing breast cancer in carriers up to approx. 87% – risk of developing breast cancer in male carriers up to approx. 1% – risk of developing prostate cancer in carriers up to approx. 8,5% – risk of developing pancreatic cancer up to approx. 3%	– each carrier from 30 years of age is recommended to have a TV-USG of the small pelvis and assessment of serum CA125 concentration every 6–12 months – in each carrier, prophylactic, bilateral adnexectomy is recommended, optimally at the age of 35–40 years, after completion of procreation plans – carriers diagnosed with ovarian cancer are expected to respond well to platinum derivatives and PARP inhibitors – each carrier should perform monthly self-palpation of the breasts – each carrier is recommended to breastfeed for a long time and to abandon/limit hormone replacement therapy (HRT) – each carrier is recommended to have a medical palpation of the breasts and breast imaging every 6 months (the age of beginning the tests depends on the family and clinical assessment, but should not be later that 25 years of age): MRI alternating with ultrasound (up to 30 years of age) or mammography (after 30 years of age) – in carriers who developed breast cancer, breast conserving surgery should be abandoned in favor of mastectomy, possibly with prophylactic contralateral mastectomy, ideally with simultaneous reconstruction – each female carrier may consider a prophylactic bilateral mastectomy, optimally with simultaneous reconstruction – chemoprevention with tamoxifen may be considered in any carrier – in carriers who have developed triple-negative or luminal type breast cancer that progresses despite anti-estrogen therapy, PARP inhibitors (olaparib or talazaparib) should be considered as the next-line treatment (after anthracycline and taxanes)
	BRCA2		– risk of developing ovarian cancer up to approx. 27%	– risk of developing breast cancer in carriers up to approx. 84% – risk of developing breast cancer in male carriers up to approx. 9% – risk of developing prostate cancer in carriers up to approx. 20% – risk of developing pancreatic cancer up to approx. 7% – a discreetly increased risk of developing melanoma (of the skin and/or eyeball)	

Hereditary cancer syndrome	Genes in which germline mutations are present	Indications for genetic testing	Risk of ovarian cancer in carriers of the mutation	Other neoplasms in the spectrum of increased risk and associated symptoms	Recommendations
		• the patient was diagnosed with breast cancer before the age of 50 or ovarian cancer at any age, and in addition first and/or second grade relatives were diagnosed with male breast cancer and/or ovarian cancer c) in families with identified specific *BRCA1/2* mutation, hereditary mutation should be verified in the carrier's family, above all first-degree relatives if a specific *BRCA1/2* mutation is identified in DNA from tumor cells (tDNA), its presence should be analysed on DNA isolated from outside the tumor cells (blood, saliva, oral swab, skin biopsy specimen) to assess the nature of the mutation (somatic, i.e. non-hereditary or germline, i.e. hereditary)			– each male carrier should conduct regular breast self-examination, and from 30–35 years of age annual medical palpation of the breast is recommended (especially in carriers of the *BRCA2* mutation) – each male carrier (especially with *BRCA2* mutation) may consider annual screening for prostate cancer starting from 40–45 years of age – in all carriers of the *BRCA2* mutation, annual dermatological and ophthalmological testing for melanoma may be considered, especially in cases of presence of this tumor in relatives
hereditary non-polyposis colorectal cancer (HNPCC, Lynch syndrome) [8, 18, 22]	*MLH1, MSH2, MSH6, EPCAM, PMS2*	according to ESMO recommendations [1]: a) in each case of colorectal cancer, the following tests should be performed: IHC studies to analyse expression of MLH1, MSH2, MSH6 and PMS2 proteins and/or microsatellate instability (MSI) on tumour cells; and a genetic test (sequencing and evaluation of large gene rearrangement associated with HNPCC) only in cases of abnormal IHC and/or MSI results b) in cases where tumour cells and/or the person diagnosed with colorectal cancer are unavailable for testing, a genetic test (sequencing and evaluation of large gene rearrangements associated with HNPCC) should be performed, if at least Bethesda criteria are met: – colorectal cancer diagnosed before the age of 50 – the patient has at least 2 malignant neoplasms from the HNPCC spectrum – colorectal cancer diagnosed before 60 years of age and displaying the histological features typical of high microsatellite instability – a patient with colorectal cancer, who has at least 1 first-degree relative with malignant neoplasm of the HNPCC spectrum, and at least one of these diagnoses was made before the age of 50 – a patient with colorectal cancer, who has at least 2 first/second-grade relatives with malignant neoplasm from the HNPCC spectrum, regardless of the age of diagnosis according to module II of the NPZChN MZ Programme [2]: a) in cases with Bethesda criteria met, IHC test should be performed analysing MLH1, MSH2, MSH6 and PMS2 proteins expresion on cells of the intestine cancer and if results are out of normal range, diagnostics should include a genetic test verifying HNPCC-related genes, too b) in cases where tumour cells and/or the person diagnosed with colorectal cancer are unavailable for testing, a genetic test analysing HNPCC--related genes should be performed, if at least Bethesda criteria are met	– risk of developing ovarian cancer up to approx. 20% The risk of developing particular tumours depends on the gene in which the germline mutation occurred	– risk of developing colorectal cancer up to approx. 74% – risk of developing endometrial cancer up to approx. 54% – risk of developing gastric cancer up to approx. 18% – risk of developing small intestine cancer up to approx. 12% – risk of developing bile duct cancer up to approx. 6% – risk of developing urinary tract (urothelial) cancer up to approx. 25% – risk of developing a CNS tumor (so-called Turcot's syndrome) up to approx. 6% – additionally, in carriers of the *MLH1* or *MSH2* mutation: • increased risk of developing prostate cancer to approx. 30% • increased risk of developing breast cancer to approx. 18% – increased risk of developing pancreatic cancer to approx. 9% • increased risk of developing sebaceous gland tumor (called Muir-Torre syndrome) to approx. 9%	– annual colonoscopy is recommended for each carrier: • starting from 20–25 years of age in carriers of the *MLH1* and *MSH2* mutations • starting from 30–35 years of age in carriers of the *MSH6, PMS2 and EPCAM* mutations – in the case of diagnosed colorectal cancer, subtotal colectomy is recommended followed by endoscopic examinations of the preserved part of the large intestine at least every 2 years – in each carrier from 30–35 years of age gastroduodenoscopy every 1–3 years should be considered – in each female carrier from 30–35 years of age annual TV-USG with endometrial biopsy is recommended (unless a hysterectomy has been previously performed) and further an annual evaluation of serum CA-125 concentration – each female carrier may consider prophylactic bilateral adnexectomy with hysterctomy after the completion of procreation plans (optimally around 35–40 years of age) – annual neurological examination is recommended for each carrier

Hereditary cancer syndrome	Genes in which germline mutations are present	Indications for genetic testing	Risk of ovarian cancer in carriers of the mutation	Other neoplasms in the spectrum of increased risk and associated symptoms	Recommendations
				The risk of developing particular tumours depends on the gene in which the germline mutation occurred.	
***RAD51* germline mutations** [23]	*RAD51C* *RAD51D*		– moderately increased risk of developing ovarian cancer: • for RAD*51C* OR mutation approx. 5% • for mutation *RAD51D* OR approx. 7%	– currently, there are no sufficient data concerning increased risk of development of other cancers	– each carrier may consider prophylactic bilateral adnexectomy after the age of 45
***BRIP1* germline mutations** [23]	*BRIP1*		– moderately increased risk of developing ovarian cancer: OR approx. 4%	– currently, there are no sufficient data concerning increased risk of development of other cancers	– each carrier may consider prophylactic bilateral adnexectomy after the age of 45

[1]ESMO – European Society for Medical Oncology
[2]National Cancer Control Programme of the Ministry of Health

prophylaxis and therapy for carriers of germline mutations associated with hereditary cancer syndromes with ovarian cancer in their spectrum. As in the case of hereditary breast cancers, most hereditary ovarian cancers are associated with carrying germline *BRCA1* or *BRCA2* gene mutation, which account for the hereditary breast and ovarian cancer syndrome. However, there is also high risk of development of the ovarian cancer associated with mutations of *MLH1, MSH2, MSH6, EPCAM, PMS2* genes, responsible for the *hereditary non-polyposis colorectal cancer* (HNPCC) or germline mutations in *BRIP1, RAD51C* or *RAD51D* genes (tab. III) [8, 21].

Currently, in patients with ovarian cancer, due to the growing importance of PARP inhibitors in therapy, it is recommended to start molecular diagnostics with sequencing of the *BRCA1* and *BRCA2* genes on tDNA, which can be isolated from both postoperative and biopsy material as well as from a tissue block. The results of the sequencing are then subjected to bioinformatic processing and thorough analysis in order to assess the clinical significance of the identified variants. Presence of *BRCA1* or *BRCA2* mutation (variations of class 5 and 4) in tDNA is a good predictive marker, which indicates probably high effectiveness of PARP inhibitors in therapy. Additionally, identification of *BRCA1* or *BRCA2* mutation in tDNA requires verification of the nature of the detected change (germline mutation which is hereditary or somatic mutation which is non-hereditary) through analysis of its occurrence in DNA isolated from cells outside the neoplasm. Interpretation of VUS variants (class 3, variants of unknown clinical significance) remains a challenge, both in terms of doubtful predictive value with respect to PARP inhibitors, and unknown effect of germline VUS on neoplasia risk.

Nevertheless, apart from patients with identified mutation in tDNA, genetic counselling should cover patients with no identified mutation in tDNA and those who did not have molecular testing on tDNA performed. Each patient with ovarian cancer referred for genetic counselling has their history and clinical analysis examined and differential diagnostics is performed considering hereditary breast-ovarian cancer *(*HBOC) and hereditary nonpolyposis colorectal cancer (HNPCC aka Lynch syndrome). On the other hand, patients who did not have *BRCA1* and *BRCA2* genes sequenced in tDNA, are referred to genetic testing of constitutive genome for 5 founder mutations of *BRCA1* gene. In the case of patients with family history, the testing is then expanded to include *BRCA1* and *BRCA2* gene sequencing. And only this is a basis for individual prophylactic and therapeutical recommendations (tab. III). In ovarian cancer patients, especially with family history of cancer who did not have mutations identified in *BRCA1* and *BRCA2* gene sequencing, broad-panel, commercial NGS testing may be considered, as it allows for sequencing many genes associated with many hereditary cancer syndromes within a single test. However, each genetic test has its limitations and if no mutation is found within the tested range, it does not unequivocally exclude the suspected hereditary cancer syndrome. Therefore, the final recommendations should be formulated based on a comprehensive analysis considering molecular testing results, as well as family and clinical assessment.

After diagnosis of a germline mutation, the patient should be informed of the risk of the mutation in the family. It is also necessary to provide genetic counselling not only to patients with inherited ovarian cancer, but to selected members of their families, too. Individualisation of medical recommendations applies to all mutation carriers (including those diagnosed with another cancer from the spectrum of a given cancer syndrome, as well as those without cancer diagnosis), and also families with cancer in which no causative mutation was detected.

Detailed characteristics of the most frequent hereditary cancer syndromes of spectrum including ovarian cancer, and therapeutic and prophylactic recommendations for mutation carriers considering ESMO guidelines and modules I and II of the NPZChN Programme are shown in table III.

Conclusion

Personalised medicine is increasingly applied in prophylaxis and treatment of breast and ovarian cancers. Application of individually tailored therapies based on immunochemical and molecular markers increases the patients' chances to avoid adverse effects, to prolong survival and progression-free survival.

Increasing access to molecular broad-panel and whole-genome testing allows identification of individual pathomechanisms that lead to neoplastic transformation within the tumour as well as to metastases, and this knowledge gives hope for application of potentially effective molecularly targeted therapies. Also, identification of patients with hereditary breast cancer and/or hereditary ovarian cancer enables development of individualised prophylactic and therapeutical recommendations for the patients, and for members of their families, too. However, this approach to diagnosing breast or ovarian cancer requires comprehensive assessment of the patient and multi-specialist, coordinated care by oncologist, surgeon, gynaecologist, geneticist, pathologist and laboratory diagnostician.

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Breast cancer – extracapsular extension in the sentinel lymph node

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Invasive breast cancer is the most common malignancy in women. At present, in the majority of cases it is recognized at an early stage. Its most common site of metastasis is the axilla region, and for women without clinically suspected lymph nodes a sentinel lymph node biopsy (SLNB) is the method of choice in diagnostics and treatment process. It allows, in many cases, axillary lymphadenectomy to be avoided and the risk of complications after a surgical treatment to be diminished. Extracapsular extension (ECE) of nodal metastasis, defined as extension of cancer cells through the nodal capsule, is an important prognostic factor. The aim of this paper is to review the literature on ECE in the sentinel lymph node (SLN).

Key words: breast cancer, sentinel lymph node, extracapsular extension

Introduction

For the last decade, axillary lymphadenectomy (ALND) has not been mandatory for patients with 1-2 sentinel nodes with macrometastases who were undergoing lumpectomy and adjuvant radiotherapy as part of their treatment, according to an ACOSOG Z0011 trial or an AMAROS trial. The outcomes of these trials showed no differences in recurrence and survival between patients who had undergone ALND and those who had not undergone ALND, but the presence or absence of extracapsular extension was not analyzed in these trials [1–3].

ECE can be connected with poor prognosis and its diameter should be determined, because in many cases this factor determines the necessity of performing an ALND or regional lymph node radiotherapy.

Biological subtype of breast cancer and positive SLN

Luminal tumours are the most common breast cancer and they represent about 70% of all cases of breast cancer [4–7]. In most patients with Luminal A cancer, surgery is used up-front. In women with clinically negative lymph nodes, SLNB is the method of choice instead of an ALND. Some authors point to different factors influencing the presence of metastases in the sentinel lymph nodes, such as: age, the diameter of the tumour, grade, and the lobular type of the cancer. Luminal A breast cancers are usually of low histological grade with slow growth and a good prognosis, however quite frequently, the illness is more advanced at the moment of diagnosis [8–9]. For women with Luminal B HER2 negative cancer, an additionally high Ki-67 factor is connected with the possibility of a positive sentinel lymph node [10–11].

For triple negative or HER2 positive cancer patients, the strategy of treatment has changed lately and therapy usually starts with chemotherapy [12]. For patients with an overexpression of the HER2 receptor, the probability of metastasis to SLN and ECE is much higher [11]. Systemic treatment leads, in more than 40% of patients, to a complete pathological response (ypT0N0) and very often SLNB is advised. However, for women with clinically suspected or with metastasis diagnosed before treatment an ALND is mandatory. On the other hand, for triple

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negative or HER2 positive T1 patients, with tumours less than 1cm in diameter, surgery is an up-front strategy [12–16].

ECE in a sentinel lymph node and correlation with non-sentinel lymph node (NSLN) metastases

In most of the papers presented, ECE in sentinel lymph nodes is connected with metastases to NSLNs. More metastatic SLNs are connected with a higher probability of positive NSLNs. The ratio between metastatic sentinel lymph nodes and removed sentinel lymph nodes is very important. The higher the ratio, the greater the probability of metastases to NSLNs [17–21]. In his paper, Palamba showed that for patients with ECE the probability of the occupation of additional lymph nodes by cancer cells is much higher – 84.6% for massive, 58.5% for minimal, and only 14.5% for sentinel lymph nodes without extracapsular extension [22]. Similar conclusions were presented by Gooch et al. The risk of metastases to NSLNs was connected with the diameter of ECE. For infiltration greater than 2 mm, or for lesser than 2 mm, or for no extracapsular infiltration, the probability of the occupation of more than four axillary lymph nodes was 33%, 8.5% and 2.5%, respectively [10]. In yet another paper, this feature was also presented, but it was not an independent factor for disease free survival (DFS) and overall survival (OS) [23]. Schwentner, analyzing the outcomes of 324 women showed that the probability of increasing pN status (pN1 to pN2-3) was much higher in patients with ECE after performing an axillary dissection [24].

The diameter of ECE in the sentinel lymph node

In the literature, we do not meet a correct definition of extracapsular extension in connection with its clinical meaning. In pathological reports, however, very often, we find only the sentence that ECE is present and in some that ECE is absent which can be understood as the true absence of ECE or as the situation that this feature was not assessed by the pathologist. In the analysis presented by Vane et al., in a group of 3502 patients, information on ECE was available for about 60% of them [10]. Nottegar et al. have performed an analysis of proper papers concerning the issue of ECE, and five articles were included by them in their meta-analysis. In four out of the five articles, the analysis was connected only with a short piece of information that ECE was present or absent without estimation of its diameter [25]. The lack of information on the diameter of the ECE can be accepted in a situation where an axillary dissection was performed and there is a huge number of metastatic lymph nodes, which is connected with poorer prognosis and the necessity of systemic treatment, not only for cancers with worse prognosis (triple negative or non-luminal) but also for patients with luminal ones. After SLNB and the presence of ECE, it is mandatory to estimate the diameter of the ECE, because not only is it connected with prognosis, but it also influences the planning of further therapy. The relevance for prognosis of extracapsular extension was proven in patients with other cancers [26–28].

In the 5th edition of The American Joint Committee of Cancer (AJCC) Cancer Staging Manual, the presence of ECE was recognised and named as subcategory pN1biii, but was removed from following editions [29]. This factor has also not been assessed in clinical trials. In the ACOSOG Z0011 trial, patients with ECE were excluded from the analysis and in the AMAROS trial this factor has not been evaluated. The authors pointed to the fact that the diameter of the ECE can influence both DFS and OS. When this diameter exceeded 2 mm, the risk of locoregional failure was greater than 20% and influenced DFS [30–32]. Nevertheless, this fact was not confirmed by others. In their paper Choi et al. presented the fact that in cases of ECE less than 2 mm the risk of recurrence was the same as for patients without ECE [33].

Similar conclusions were shown by Barrio et al. However, the mean time of observation was only 21 months, there were no nodal failures in patients with ECE in sentinel lymph nodes and they were not treated with an axillary dissection, but rather biologically oriented systemic treatment and locoregional radiotherapy were used. The risk of nodal failure in this group was only 1.5% [34]. The research of Kanyilmaz et al. has shown that the extent of the ECE is a prognostic factor for survival in pT1-2N1 breast cancer patients with a diameter of extracapsular extension greater than 1mm. This factor, according to the authors, was connected with shorter OS and DFS [35].

Conclusions

In an era of diminishing surgical treatment in the breast area and axilla region, it seems to be very important to precisely estimate the diameter of any extracapsular extension in the sentinel lymph nodes. In an era of biologically directed systemic treatment and conformal radiotherapy, it is probable that we can avoid the harmful consequences of surgical procedures in many patients [12].

Pathologists should include the diameter of the ECE in their reports to help, much more so than presently, clinicians take decisions about the best oncological treatment for women with breast cancer. As the data mentioned shows, a diameter of 1–2 mm for an extracapsular extension in SLN is crucial (pivotal). The prognostic importance of ECE must also be confirmed by future clinical trials.

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Case report

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A rare complex variant translocation t(9;22;6;17;1) in chronic myeloid leukemia: case report

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The presence of the Philadelphia chromosome (Ph) in chronic myelogenous leukaemia (CML) is a specific cytogenetic change resulting from a reciprocal translocation between chromosomes 9 and 22. In 5–10% of newly diagnosed cases there are variant translocations (vPh) involving more chromosomes. This paper presents the case of a CML patient with a complex variant translocation involving chromosomes 1, 6, 9, 17 and 22. A molecular analysis did not reveal any mutation in the kinase domain of *BCR-ABL1* gene or the mutation of *TP53* gene. After the first-line treatment with imatinib no cytogenetic or molecular response was obtained. The change of treatment to dasatinib resulted in a minimal cytogenetic response (minCyR) followed by a minor cytogenetic response (mCyR). The application of nilotinib in the third-line treatment resulted in a complete molecular response (CMolR) and therapy success. The likely reason for the failure of the first- and second-line treatment was the loss of a fragment of the 17p13 region as a result of a variant translocation. The change can be a functional equivalent of the loss of one copy of *TP53*. The analysis of presented case confirms the significance of the detailed evaluation of the composition of vPh complex variant translocations as well as importance of combination cytogenetic and molecular diagnostics in CML treatment monitoring. It makes possible to adequate diagnose higher-risk patients and apply effective treatment strategies if an aberration is identified.

Key words: chronic myelogenous leukaemia, complex variant translocation of the Philadelphia chromosome, treatment of chronic myelogenous leukaemia

Introduction

Chronic myelogenous leukaemia (CML) belongs to the group of myeloproliferative disorders. The CML development is associated with the presence of the Philadelphia chromosome (Ph) and *BCR-ABL1*, a fusion gene with oncogenic properties. As a result of reciprocal translocation t(9;22), the *ABL1* protooncogene from the long arm of chromosome 9 (q34) is transferred to the *BCR* gene on the long arm of chromosome 22 (q11). The shorter chromosome 22 (Ph) carries a new oncogene, *BCR-ABL1*, which encodes tyrosine kinase with constitutive activity. The autophosphorylation of tyrosine in BCR-ABL1 kinase and the activation of the Ras/MAPK signalling pathway in a pluripotent bone marrow stem cell leads to the increased proliferation of a leukemic clone and contributes to the neoplasm growth. The product of chromosomes 9 and 22 translocation is the BCR-ABL1: p210 protein (in 99% of cases) or, less frequently, p190 or p230, which differ in terms of their mass as well as biological properties [1].

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The presence of the Ph chromosome is a specific cytogenetic abnormality which occurs in over 90% of CML cases, regardless of the disease progression stage. But in 5–10% of newly diagnosed patients there may occur complex translocations involving, except chromosomes 9 and 22, one or more other chromosomes. These are variant translocations (vPh). The mechanism of vPh formation is not entirely clear, but two alternative possibilities are taken into consideration. In a one-step mechanism, it is assumed that breaks occur simultaneously in several different chromosomes involved in the translocation, while in a two- step mechanism there are two subsequent translocations, a classical one followed by the one involving additional chromosomes.

The second mechanism of vPh formation could result in a worse prognosis because it is analogous to the mechanism of clonal evolution. Currently, it is believed that patients with Ph variants have a good response to treatment and their prognosis is similar to the one for patients with a typical translocation involving t(9;22) [2]. A cytogenetic analysis enables the detection of secondary chromosomal aberrations that accompany the primary translocation involving t(9;22) and indicate clonal evolution. Most often, there occur additional copies of chromosome 8 and 19, chromosome Ph and isochromosome 17q. The trisomy of chromosomes 21 and 17, the monosomy of chromosomes 7, 17 and Y as well as the translocation involving t(3;21)(q26;q22) are less frequent. The frequency of these changes increases during disease progression , respectively up to 5–10% in the chronic phase, up to 30% in the acceleration phase and up to 80% in the blast crisis phase [3, 4]. Among the described aberrations only some abnormalities, such as i(17)(q10), –7/del(7q) and 3q26.2, are related to prognosis deterioration [5]. According to the current European LeukemiaNet (ELN) recommendations, the detection of an additional aberration in a primary test should be a warning, but if a secondary aberration in Ph+ clone arises during the therapy, it indicates its failure [6].

The molecular diagnostics of CML using PCR (polymerase chain reaction) techniques identifies the type of *BCR-ABL1* transcript which level may be adequately monitored during the response to the tyrosine kinase inhibitors (TKI) treatment. Most translocations involving t(9;22) are characterised by the fusion between exons e13 or e14 of the *BCR* gene with exon a2 of the *ABL1* gene. This combination of gene fusions is described, respectively, as transcripts b2a2 (e13a2) and b3a2 (e14a2). About 2–5% of patients carries rare transcript variants [2].

The current CML treatment is based on tyrosine kinase inhibitors that block the binding site of ATP kinase, inhibits the phosphorylation of tyrosine residues, and prevent the activation of a cell signal. Targeted therapy include: imatinib (first-generation drug), dasatinib, nilotinib, bosutinib (second--generation drugs) and third-generation inhibitors (e.g. ponatinib). Multi-centre studies confirmed that imatinib treated patients achived a high response rate of a complete cytogenetic response and survival free from progression (over 80%) [7]. If a patient has not responded to the first-line treatment, next-generation drugs are used. Indications for the use of other drugs involve resistance to or intolerance of the first-line treatment. Mutations of the *BCR-ABL1* kinase domain are the major cause of resistance to the TKI treatment. [8]. The most unfavourable is a point mutation T315I, which is the main cause of resistance to imatinib, dasatinib, nilotinib and bosutinib.

Chromosome analysis is necessary to determine the effectiveness of the TKI treatment, which evaluates the cytogenetic response level. A total cytogenetic response (CCyR) is defined by no metaphases with Ph+ clone in tested bone marrow, a partial response (PCyR) means the presence of Ph+ in 1–35% of the metaphases , a minor response (mCyR) –in 36-65%, a minimal response (minCyR) – 66–95% metaphases with Ph+, and there is no cytogenetic response (noCyR) when Ph+ is present in over 95% of analysed metaphases. The level of the molecular response to the TKI treatment is evaluated by *BCR--ABL1* transcript type and its quantity. Therefore, without the identification of transcript variant , the monitoring of patients with rare variant transcript would give a false negative result suggesting that patient achived complete molecular response (CMolR). CMolR means that there is no mRNA *BCR-ABL1* in RQ--PCR (real-time quantitative – PCR) test or in RT-PCR (reverse transcriptase – PCR) in two consecutive blood samples. In major molecular response (MMR), the quantitative ratio of *BCR-ABL1* to *ABL1* or another reference gene is ≤0.1% on the international scale (IS) [9]. If the resistance to the first-line treatment occurs, the mutation of the *BCR-ABL1* oncogene kinase domain should be evaluated.

Case study

A 52-year old man was diagnosed with chronic myelogenous leukaemia in October 2015, which was confirmed by cytogenetic and molecular tests. The CBC results were as follows: WBC (white blood cells) – 176 thousand, Hb (haemoglobin) – 7.4g%, Ht (haematocrit) – 23.4, PLT (thrombocytes) – 327 thousand. Additionally, there was a shift towards myelocytes and MBL (monoclonal B lymphocytosis) of 6% in the peripheral blood smear.

There were no deviations from the norm in biochemical tests, except for the high activity of LDH (lactate dehydrogenase). A baseline molecular test confirmed the presence of *BCR-ABL1* transcript p210 of the b2a2 type, while the kinase domain analysis performed after the failure of the first-line treatment excluded the presence of any mutation in the kinase domain of this gene.

The cytogenetic examination performed at the diagnosis revealed the presence of a complex translocation involving t(9;22;6;17;1)(q34;q11;p11.2;p11.2;q21) (fig. 1).

The fluorescence in situ hybridisation (FISH) confirmed the presence of fusion *BCR-ABL1* on changed chromosome 22 and

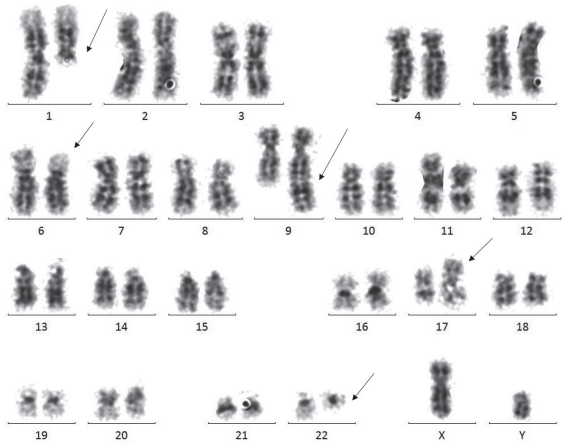

Figure 1. Karyotype: 46, XY, t(9;22;6;17;1)(q34;q11;p11.2;p11.2;q21)

the absence of the second fusion signal (visible on changed chromosome 9 in typical cases). It was confirmed that the *BCR* gene signal (from chromosome 22) had been reduced (dim) and transferred to the short arm (p) of chromosome 6 (fig. 2). The presence of a complex variant translocation between chromosomes 1, 6, 9, 17 and 22 was confirmed by additional tests with painting probes. They demonstrated the translocation of the short arm of chromosome 6 to the short arm (p) of chromosome 17 and the transfer of a part of chromosome 1 to chromosome 9q. This change was accompanied by the deletion of the fragment including *ABL1* gene from translocation chromosome 9.

At the same time, it was confirmed that the breakpoint on the short arm of chromosome 17 involved in the complex translocation resulted in the alternation of the structure of one copy of the *TP53*. In the FISH image, this change was visible

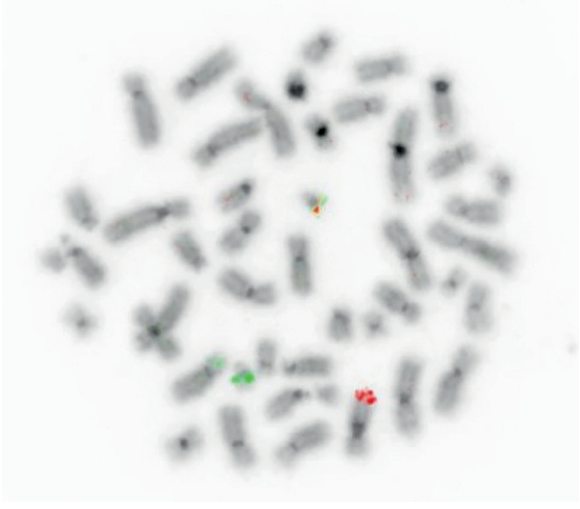

Figure 2. 46,XY,t(9;22;6;17;1)(q34;q11;p11.2;p11.2;q21).ish der(6) t(9;22;6;17;1)(BCR dim+) der(9)t(9;22;6;17;1)del(9)(q34q34)(ABL1-, BCR-),der(22)t(9;22;6;17;1)(BCR+,ABL1+)

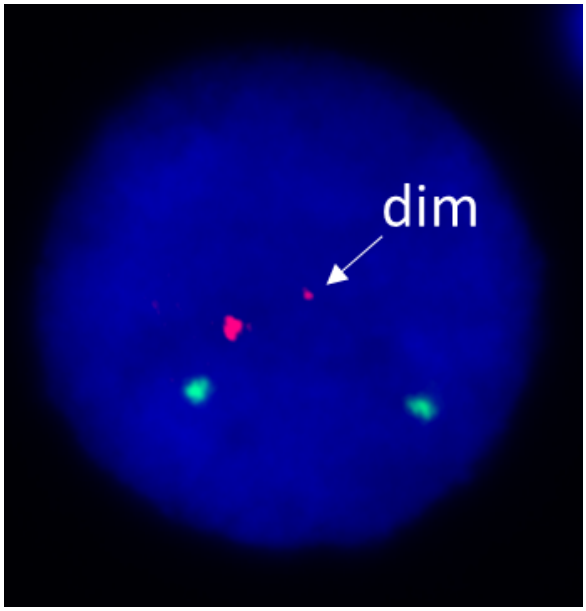

Figure 3. Difference in TP53 signal size on interphase. FISH with TP53/CEP 17 Probe Kit (Abbott): TP53 – red signal, chromosome 17 centromere – green signal. Apparent reduction of one TP53 signal (dim)

as a reduced signal of the probe specific for *TP53* gene (dim) (fig. 3).

NGS was employed to search for the mutations of oncogenes and suppressor genes typical for neoplasms. The sequencing of 50 genes, including *TP53*, using Ion AmpliSeq Cancer Hotspot Panel v2 on the Ion SS sequencer (Thermo Fisher Sci.) did not reveal any mutations of the 207 amplicons tested. The sequencing results (depth x1881) did not reveal any mutations of the 207 amplicons tested. The results of sequencing (depth x1881) for *TP53* gene were analysed for the presence of pathogenic mutations according to the COSMIC database and the additional sequences obtained were analysed using the Integrative Genomics Viewer.

Course of treatment

The first-line treatment involved cytoreduction with hydroxycarbamide. The treatment with imatinib at a dose of 400 mg/d was launched in November 2015. According to the cytogenetic analysis performed after the imatinib therapy, the aberration involving t(9;22;6;17;1)(q34;q11;p11.2;p11.2;q21) was still present in all cells (noCyR). The molecular evaluation of quantity revealed a high amount of *BCR-ABL1* transcript at the level of 1.4467%. During monitoring, a new aberrations, such as marker chromosome in Ph+ clone and additional chromosome 8 in Ph- clone, appeared. These additional aberrations indicated the progression of cytogenetic changes.

Due to no cytogenetic or molecular response as of July 2016, the second line treatment with dasatinib was introduced. After change of treatment minCyR at the level of 85% and 5 months later – mCyR at the level of 61% were achieved.

In May 2017, nilotinib at a dose of 800 mg/d was used as the third-line treatment. After 6 months there was a reduction of the number of cells with t(9;22;6;17;1) to 4% and a PCyR was observed (fig. 4). As of January 2018, the level of *BCR-ABL1* transcript also dropped to 0.0871%, which confirmed a MMolR. Subsequent molecular tests (April 2018, April and July 2019) showed a reduction in the level of *BCR-ABL1* transcript (respectively, 0.0527%, 0.0285% and 0.0019%) until a CMolR was achieved in August 2019, which was also maintained in the tests of January 2020.

Conclusions

The prognostic value of cytogenetic aberrations in CML has been changing along with the development of modern therapy methods. The prognostic value of deletion in the region of *ABL1-BCR* fusion on chromosome 9 ceased to be important along with the marginalisation of the treatment using interferon alpha, in which it was an independent negative prognostic factor [10]. In the era of advanced TKI therapies, the significance of other prognostic factors has also been evolving. The detection of additional aberrations in a baseline test should be treated as a warning, while the appearance of a secondary aberration in the Ph+ clone during the therapy indicates its failure. It should be remembered that both variant and secondary translocations may be a result of sub-microscopic changes, which are invisible in classical karyotype analysis. In this situation, it is important to use cytogenetic molecular techniques, such as fluorescence in situ hybridisation (FISH) [15, 16]. At present, ELN guidelines promote more detailed tests for patients with an increased cytogenetic risk, but they do not clearly indicate that physicians should diversify initial therapies in everyday practice [6].

In the presented case, complex translocation involving chromosomes 1, 6, 9, 17 and 22 was described. The formation of a *BCR-ABL1* fusion gene on Ph chromosome was accompanied by the loss of *ABL1* on changed chromosome 9. The complex translocation also resulted in the disruption of the structure of the short arm of chromosome 17. The *TP53* gene, located in this region, is one of the most important tumor suppressors. The loss of *TP53* function may be the main factor causing resistance to the treatment with tyrosine kinase inhibitors and may influence the disease progression [12–14]. The involvement of chromosome 17 in the variant translocation as confirmed by the authors caused the loss of a fragment of 17p13 with an atypical *TP53* aberration, which may be functionally equivalent to the loss of one copy. At the same time, the absence of the *TP53* mutation was confirmed. The loss of *TP53*, which usually occurs as a result of the formation of i(17q), is a warning indicating the possibility of the TKI treatment failure [5]. The loss of *TP53* as a result of vPh has also been described by other authors [13]. In presented case, no atypical transcript or mutation in the *BCR-ABL1* fusion gene after the failure of the first-line treatment in molecular analysis was confirmed.

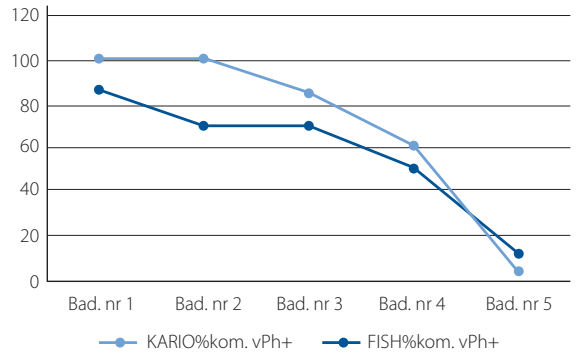

Figure 4. Changes in the proportion of Ph+ cells in karyotype and FISH tests over the course of treatment

It is generally believed that the imatinib treatment failure in patients with chronic CML is most often caused by the presence of the *BCR-ABL1* oncogene mutation [18]. Attempts to demonstrate the impact of *BCR-ABL1* transcript on the treatment results did not show any significant differences in this respect, although it was demonstrated that patients with b2a2 transcript had a higher event-free survival (EFS) [18, 19].

During the first-line treatment, the patient did not respond to imatinib and then revealed a weak response to the second-line treatment (dasatinib). Patients for whom two consecutive lines of treatment were proven unsuccessful are known to cause the most therapeutic problems, but in this case the third-line treatment with nilotinib turned out to be effective [20].

The nilotinib therapy has already been confirmed successful in patients with *TP53* deletion who were resistant to imatinib [21]. Recent tests have demonstrated that nilotinib is more effective than imatinib in increasing the level of p53 in serum in patients with chronic myelogenous leukaemia [22]. Thus, the failure of the first- and second-line treatment in this case is most likely caused by the loss of one functional copy of the *TP53* gene, which was confirmed in the FISH test. In the absence of a mutation in the *BCR-ABL1* kinase domain and no mutation in the *TP53* gene, therapy failure may have been related to loss of TP53 function as a result of complex translocation. The analysis of this case confirms the legitimacy of an expanded cytogenetic examination in the presence of vPh or atypical secondary changes during CML diagnostics as they make it possible to detect patients with an increased risk of the disease.

In conclusion, presented case confirms the importance of common analysis diagnostic and monitoring results using cytogenetic and molecular analysis methods, including the expanded possibilities offered by NGS. It should be emphasized that the limitation of a genetic aberration analysis to one innovative technique, regardless of its advancement level, causes a risk that some important data which influence the therapy and its results might be ignored. To date, there has been no standard NGS diagnostic method. Hence, close collaboration between diagnostic and clinical centres is essential to develop the most effective treatment strategies for higher-risk patients.

Conflict of interest: none declared

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The surgical anatomy of the mammary gland (part 1.)

General structure, embryogenesis, histology, the nipple-areolar complex, the fascia of the glandular tissue and the chest wall

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The rapid development of surgical techniques used in breast surgery requires an excellent knowledge of mammary gland anatomy. This article presents the most up-to-date information on embryogenesis as well as the histology and general anatomy of the breast. Particular attention has been given to the structure of the nipple-areolar complex and the anatomy of the chest wall and mammary gland fascia.

Key words: mammary gland, anatomy, embryogenesis

The breasts are paired glands located on the front wall of the chest within the outer integument. They can be found both in females and in males, although they are an anatomical attribute of women in a cultural, mental and, above all, functional sense. Anatomical descriptions of female breasts refer to some kind of a 'model'. In the majority of them, this model is purely theoretical and most often refers to the breasts of young women with a healthy weight and body structure, soon after achieving sexual maturity. Thus, it reflects the perfect breasts of an ideal woman. This ideal has been changing significantly over the centuries. Moreover, it differs depending on race, geographic or regional location, or subculture. Therefore, it is difficult to define the right size of universal breasts. It is more appropriate to describe the size and shape of breasts individually with reference to the harmony with the woman's build as well as the structure and shape of her chest. It may be assumed that the average capacity of a breast remains within the range of 200 to 500 ml.

Female breasts are subject to continuous changes dependent on a number of endogenous factors (primarily hormones), general health condition or disease, as well as exogenous factors related to the nature of physical activity, work, care, reproduction or breast feeding. Natural changes in the anatomy of the mammary gland arise with the age and successive stages of maturity reached by each individual.

The breast of a sexually mature woman is primarily made of glandular tissue which is subject to dynamic cyclical hormonal changes caused by alternate stimulation with oestrogens (the first phase of the cycle) and progesterone (the second phase of the cycle). Pregnancy is the period when glandular tissue is stimulated to grow and prepare for feeding under the influence of prolactin. The breast of a woman after menopause is filled to a large extent with adipose tissue, which is less susceptible to hormonal changes [1, 2].

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Because of the location on the front wall of the chest within the outer integument, there is a significant variety as regards the position and shape of mammary glands depending on the body position, the size and weight of the breasts as well as the woman's physical activity. Differences in the number and location of hormone receptors within the breast and the influence of external factors during each individual's development may often cause asymmetry between both breasts [1, 3, 4]. The aforementioned factors justify the special need for knowledge of breast anatomy, both static and dynamic. Regardless of the type of intervention planned (treatment or cosmetic surgery), a surgeon who embarks on surgery within the mammary gland must examine in detail the abovementioned anatomical aspects and predict the impact of the procedures performed on the changes occurring in the operated breast with the passage of time.

General structure

A breast has a conical shape and is located between the pectoralis major muscle, subcutaneous tissue and the skin. The back wall of the breast has contact with the fascia of the pectoralis major, the serratus anterior, the abdominal external oblique muscle and the rectus abdominis. The breast foundation is an oval with the diameter of 10–12 cm stretched towards the axillary fossa. The location on the chest wall is relatively stable and involves the area stretching between the anterior arch of the 2nd and 3rd rib from the top and the anterior arch of the 5th and 6th rib from the bottom as well as between the sternal line medially to the ancillary midline laterally [5, 6].

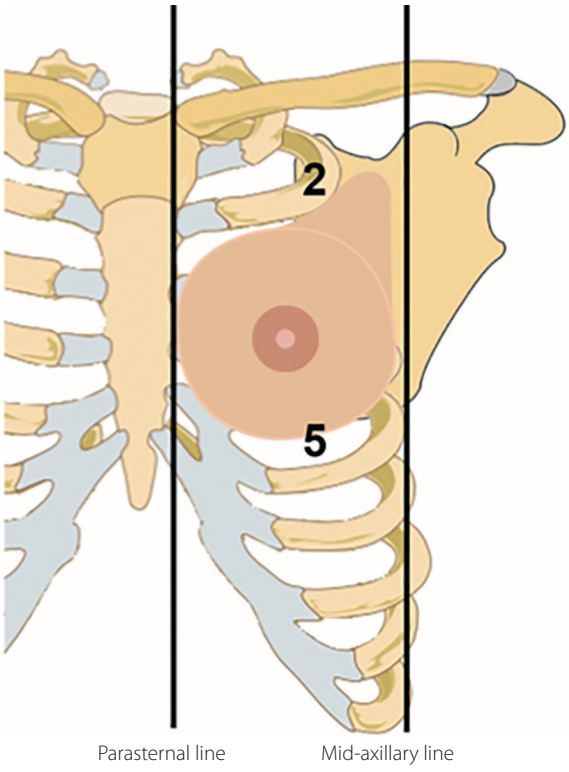


Figure 1. Projection of a breast on the chest front wall (footprint)

There are three main components of a breast: glandular tissue (*glandula mammaria*) covered with the skin, the nipple (*papilla mamariae*) and the areola (*areola mammae*) [7]. Glandular tissue is made of several lobes (*lobi glandulae mammariae*) arranged radially and divided by septa made of connective tissue and adipose tissue. The densest glandular tissue is in the top external quadrant and it extends towards the ancillary fossa creating Spence's tail. The main exosecreting ducts (*ductus lactiferi*) run from glandular lobes to their separate ostia at the top of the nipple. Except for the areola and the nipple, subcutaneous tissue (*tela subcutanea*) completely surrounds glandular tissue. For a long time, part of the subcutaneous tissue was considered superficial fascia, which, in the form of two laminas, surrounded the breast. In fact, the superficial fascia of the chest is located between the breast and the deep fascia of the pectoralis major [5, 6, 8]. In subcutaneous tissue, in particular in the top part of the breast, there are numerous fibres and hypocellular septa made of connective tissue running from the skin inward, between lobes and lobules of glandular tissue. They are attached to the fascia of the pectoralis major. These structures, responsible for maintaining the breast in the appropriate position on the chest wall, are called the suspensory ligaments of Cooper [9]. The development of a tumour within the mammary gland may cause the infiltration of these ligaments and the retraction of the skin above the tumour [10, 11].

Mammary gland development

The embryonic period

Embryonic breast development, joint and identical in female and male foetuses at the initial stage, involves a number of successive interactions between specialised groups of cells. The human skin coat is made of two layers, the epidermis and the dermis, which create diverse structures to ensure appropriate interactions of the body with the external environment.

Initially, the epidermis is created out of the superficial layers of the ectoderm which are colonised by three structures:

1. the pigment containing melanocytes from the primordial neural tube,
2. antigen-presenting Langerhans cells from the bone marrow,
3. Merkel cells of unknown origin which receive sensations related to pressure from the surface.

The dermis, which is originally created from the mesoderm, contains numerous blood vessels and sensory nerve endings.

In 4th week of foetal life individual groups of superficial ectoderm cells become thicker and, at the same time, their proliferation towards mesoderm cells located at a greater depth begins [6, 5, 12, 13].

They undergo intense differentiation to create the multi--layer skin of a mature human being. The highly specialised structures created from different skin layers include: teeth, hair, hair appendages, nails, sebaceous glands, apocrine glands, sweat glands and mammary glands. The latter three are formed from the processes of the epidermis penetrating the dermis in the form of diverticula.

Apocrine and sweat glands are located primarily in the area of axillae, the anus and genitals. Mammary glands are a special type of highly specialised apocrine glands. Their development begins in week 4th of foetal life with the double thickening of the ectoderm in the ventral part of the embryo, which forms the mammary line (milk line) running along an arch from the axilla to the groin area on both sides. In the human foetal development these structures disappear quickly, except for the primordia of mammary glands formed on both sides at the level of the 4th intercostal space on the front wall of the chest. In week 5th, the remaining part of the ectoderm line begins to proliferate into deeper levels of the skin. In this way, a primordial breast bud is created [14].

In weeks 6–7th the ectoderm diverticulum continues to actively grow into the dermis until in week 10th the primordial breast bud divides itself into branches which, in week 12th, create the secondary breast bud – a form of a lobule in a mature breast. The abovementioned process is induced by the extracellular mesoderm matrix. Growth factors and hormones produced by this and by adipose tissue, which is a lipid deposit, stimulate the development of the primordial breast bud.

In the next stage of the foetal development, which lasts until week 20th, the secondary buds of the glandular tissue primordia become extended and they branch out into narrow channels that will eventually form lactiferous ducts (*ducti lactiferi*). This stage of breast development is induced by placental hormones that pass through to the foetus: progesterone, the growth hormone, the insulin-like growth factor, oestrogens, prolactin, corticoids of the adrenal cortex and triiodothyronine. The result of this stimulation is the formation of the glandular tissue lobules that contain their own exosecreting lactiferous ducts [1, 12, 13]. Support for the forming breasts is provided by the process of skin raising and the creation of the suspensory ligaments of Cooper that anchor the breasts in the fascia of the pectoralis major. Exosecreting lactiferous ducts converge radially in the retro-areolar area creating a kind of a collective bubble, a recess and an opening in the skin. In this place, as a result of the growth of the ectoderm into internal layers of the mesoderm, the latter becomes stimulated to create an evident protrusion on the skin – a breast nipple bud. It contains numerous smooth muscle fibres arranged longitudinally and circularly. The areola surrounding the nipple with its specialised apocrine epidermal Montgomery glands is formed from the ectoderm in the 5th month of foetal life. A pigmentation process begins in the nipple in week 32th and lasts until the end of the pregnancy [3, 6, 14].

Four stages of extra-foetal breast development

The neonatal period

The breast of a neonate is built of radially arranged lobules. Their lactiferous ducts converge into a bubble and discharge in the nipple. At this stage, the nipple is a protrusion with a little recess in the central part of the areola. Soon after the delivery, the nipple, as a result of the proliferation of the neighbouring tissues, becomes raised permanently and the areola becomes slightly pigmented. The early stages of breast development to a small extent depend on steroid sex hormones. However, strong testosterone stimulation in males through the binding of mesoderm receptors causes a rapid involution of glandular tissue and hampers breast development during the neonatal period. At the same time, oestrogens stimulate further breast development and maturation in females [1, 13].

Puberty

Puberty is characterised by intense breast growth. Initially, it is the growth of deposit adipose tissue and periductal connective tissue, which is followed by the growth of glandular tissue and the extension and elongation of breast ducts. This happens under the stimulation of oestrogens, the growth hormone and prolactin, without the participation of progesterone. Usually, breast development in women ends about the age of 20 [1, 14].

Pregnancy

During pregnancy, breasts achieve functional maturity by the preparation of follicles to active lactation. Under the influence of the continuous growth of the blood levels of such hormones as progesterone, oestrogens, prolactin and placental lactogen, cytoplasmatic organelles accumulate in the epithelial cells of the follicles. After the delivery, environmental factors and intensive hormonal changes (prolactin produced in the pituitary and somatomammotropin produced by the placenta) stimulate lactocytes to produce milk containing proteins, casein and alpha-lactalbumin as well as lipids. Under the influence of the neonate's crying and sucking as well as oxytocin, which is produced in the hypothalamus and released from the posterior lobe of the pituitary, the muscle cells surrounding the follicles begin to contract. Consequently, milk is released from the nipple [15, 16]. When feeding ends, the production of milk also disappears as a result of the lesser mechanical stimulation of breasts and the reduction of prolactin levels. Follicles go back to rest.

Menopause

Decreasing hormonal stimulation during menopause (after 40 years of age) leads to the permanent rest of breasts and the disappearance of glandular tissue, which is replaced by connective and adipose tissue.

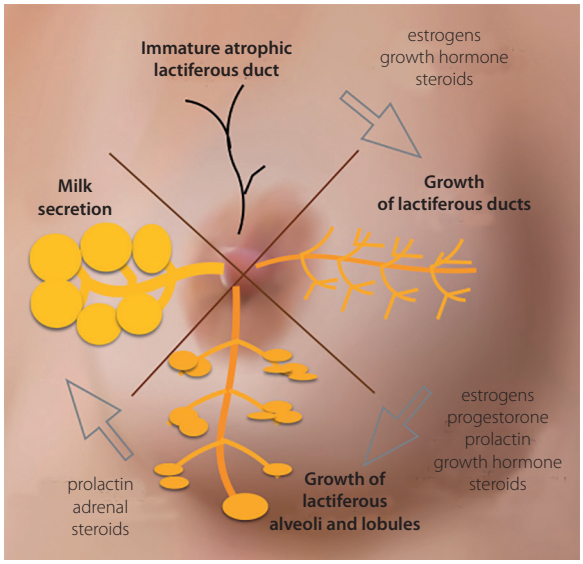


Figure 2. The influence of hormonal stimulation on the maturation of the breast gland

Anatomical disturbances of the breast development

There are many kinds of breast structure disturbances related to developmental defects. One of the most common (affecting 1–5% of the population) is the incomplete involution of the embryonic mammary line in the form of hyperthelia, i.e. the remnants of accessory elements of the areola and/or nipple along the mammary line. Polymastia, i.e. the presence of additional mammary glands, is a much rarer condition. They may appear along the entire course of the mammary line, from the axillary fossa to the groin. Because of the colour of the areola and the nipple, they may be mistaken for dermal papillae or naevi. Hypoplasia, i.e. the underdevelopment of breasts, or amastia, i.e. the absence of glandular tissue within the nipple-areolar complex, occur very seldom. Amastia, most often one-sided, is accompanied by the total absence of the pectoralis major. Another structure disturbance is one-sided amastia as an iatrogenic effect of surgery or radiotherapy. When the process of embryonic development is stopped or disturbed at the stage of the second breast bud creation (weeks 12–16th), the areola takes on the shape characteristic for a tubular breast. It is accompanied by a significant reduction in the connective tissue volume (parenchyma) and a kind of hernia – the prolapse of glandular tissue with the frontward expulsion of the nipple-areolar complex [1, 3–5, 13].

Histology of the mammary gland

The glandular tissue is made of follicles (*alveolus lactiferi*) grouped in grape-like bunches (*acinum lactiferi*) which together form the basic functional unit of the breast – the lobule (*lobulus*). Lobules are joined in bigger structures forming lobes (*lobus*). Both lobules and lobes are surrounded by a kind of septa made of connective tissue, which becomes thicker around lobes. It is difficult to identify precisely the boundaries of lobes for the needs of segmental mastectomy.

Follicles are made of two layers of cells: the internal layer on the side of the follicle lumen – lactocytes secreting milk during lactation, and the external layer made of muscle-epithelial cells.

Lactiferous ducts which start in lactiferous follicles come together creating interlobular ducts, which form interlobar ducts and finally main ducts beginning at a distance of about 8 mm from the areolar surface. They run almost parallel to the ostia in the form of lactiferous orifices at the top of the nipple. The walls of the lactiferous ducts are made of flexible connective tissue containing a large number of elastic fibres arranged circularly or longitudinally and smooth muscle cells. The subcutaneous tissue of the areola contains a large number of radially arranged smooth muscle fibres. In the nipple, smooth muscles have a spiralling course and create a net at the top. The main lactiferous ducts pass through the openings in this net.

From the histological perspective, there are two components building the breast: epithelium and stroma. The duct-lobule system is based on two layers of epithelial cells lying on the basal lamina and surrounded with a stroma. Epithelial cells (luminar) create a layer of cubic, pseudo-striated cells located internally – on the side of the lumen of exosecreting ducts. The other, more external layer, is created by myoepithelial cells lying directly on the basal lamina.

The basal lamina surrounding lactiferous ducts, ductules and grape-like structures of the mammary gland made of type IV collagen and laminin separates the system of ducts from the surrounding stromal tissue. The infiltration of the basal lamina and myoepithelial cells by the cells of an invasive tumour is what distinguishes it from the non-invasive ductal carcinoma *in situ* (DCIS) [6, 13].

There are two kinds of stromal tissues:

1. Intralobular tissue – contains loosely placed fibroblasts, dispersed lymphocytes, macrophages, plasmatic cells and blood vessels. This tissue does not contain elastic fibres, it is rich in mucus and responds to hormone stimulation.
2. Interlobular tissue – forms the connective tissue surrounding bigger lactiferous ducts containing more collagen fibres which are more densely arranged. Because of its greater density, this tissue causes difficulty in interpretation during mammography. This part of the mammary gland is replaced by adipose tissue during menopause and bears the main responsibility for the increase of the breast volume.

Within the stroma, one can find multi-nucleus giant cells that may raise concern for oncological reasons, but their clinical significance has not been proven [17, 18]. Lymph nodes may also be found inside nipples – most often they are incidentally diagnosed during a histological examination of a removed breast. If any carcinogenic changes are confirmed, they are classified as axillary lymph nodes according to the TNM Classification.

The skin of the nipple-areolar complex contains melanocytes with a different level of pigment saturation. Usually, pigmentation reaches its peak in the period of sexual maturity. A precisely marked pigmentation border between the areola and the rest of the skin on the breast is a convenient place for a surgical incision. Scars in this place are usually linear and do not leave any visible traces. The areola skin contains numerous sebaceous glands, independent of hair follicles, which take the form of Montgomery's tubercles, especially visible during pregnancy and lactation. Apocrine glands are also present. Moreover, the fibrous stroma contains numerous strands of smooth muscles.

In 10% of women, there are clear cell (Toker) structures within the epidermis of the nipple, which in 27% of cases exhibit the qualities of metaplasia and in 12% – atypia [52]. They may be the reason for the mistakes made when distinguishing it from the clear cell DCIS variant.

Breast skin and the nipple-areolar complex

The skin covering the mammary gland has a diverse structure. It is thicker at its perimeter, while near the areola it is thinner and more delicate. In the peri-areolar area there is no subcutaneous fat and the skin is attached to the glandular tissue by suspensory ligaments, subcutaneous muscles and nipple muscles distributed circularly around the ostia of lactiferous ducts at the top of the nipple. The skin is particularly thick and strong at the bottom part of the breast near the inframammary fold. The quality of the skin covering the breast may differ depending on the individual. In some women it is thin, flaccid and has little elasticity. This is always accompanied by the loosening and extension of the suspensory apparatus and its susceptibility to increasing ptosis [1, 11, 19]. In such a situation, during breast reduction or breast suspension surgery it is necessary to ensure that the distance between the rim of the areola and the inframammary fold be adequate. Special caution is advised when the epidermis near the areola is removed as subcutaneous blood vessel plexuses may easily be damaged because of the skin thinness in this area [19].

The areola of a medium-size breast has a wheel-like shape with the diameter of 3.5–5 cm. In the projection upon the chest wall, it is located at the level of the 4th intercostal space. What makes it different from the surrounding skin is a greater concentration of the pigment and the presence of minor protrusions (1–2 mm of the diameter of Morgagni tubercles) in the form of ostia of apocrine sebaceous glands moisturising the skin surface, also known as Montgomery glands [1, 20].

In the central part of the areola there is a nipple, i.e. a protrusion which differs individually and most often has a conical or cylindrical shape with a diameter of 10–12 mm and a height of 9–10 mm. The recesses on its surface are the ostia of exosecreting lactiferous ducts in the number equal to the number of mammary gland lobes [1, 3].

It was universally considered that each part has 15–25 lobes and the same number of lactiferous duct ostia. Recently, authors have reported that there are 9 lobes and ducts on average discharging to the nipple of each breast. The existence of milk containers in the form of lactiferous sinuses located behind the nipples is also challenged at present [11]. The skin of the nipple-areolar complex does not lie on the subcutaneous tissue but on the thin layer of smooth muscles forming two layers:

- The layer of spirally circulating exosecreting lactiferous ducts (Sappey muscle fibres)
- The radial layer forming a net (muscle fibres of Meyerholz).

The above muscle fibres run from the areola to the nipple and accompany exosecreting lactiferous ducts along their entire length [20].

The layer under the muscles below the areola is made of adipose tissue, which disappears towards the nipple. The sub-areolar vessel plexus is located in this tissue. Lactiferous ducts that start in lactiferous follicles converge to create, in turn, interlobular, interlobar and finally main ducts that begin at a distance of about 8 mm from the areola surface and run almost parallel to the ostia at the top of the nipple. The epithelium of exosecreting ducts extends to reach directly the skin of the nipple [3, 10, 11]. This creates a possibility for the carcinoma to proliferate using this path. The nipple-areolar complex includes a developed network of blood vessels, sensory nerve endings, smooth muscles containing the sympathetic system fibres and a developed network of lymphatic vessels also known as the Sappey plexus [21, 22].

Breast and chest wall fascia

The notion of fascia, which was considered a passive structure surrounding muscles, has undergone a radical change. Today, on the basis of the studies of anatomists and clinicians, it is known that the fascia is a dynamic tissue, highly innervated and well-vascularised. The *fasciae* surrounding organs are also

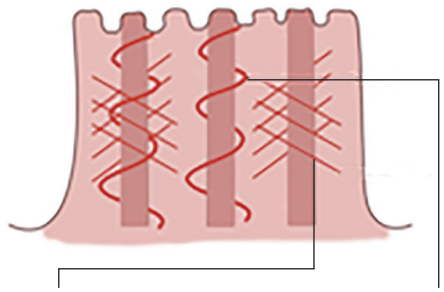


Figure 3. Types of smooth muscular coat surrounding exosecreting laciferous ducts within the nipple

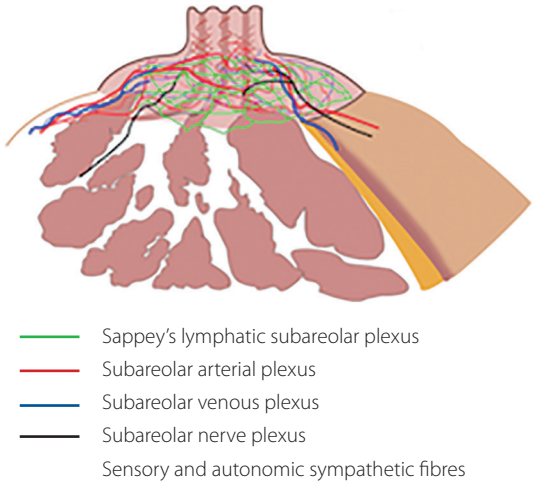


Figure 4. Cross section of the nipple-areolar complex

a source of multi-potential stem cells supporting the process of tissue repair and healing. Thorough understanding of the structure of superficial and deep fascia and its clinical significance is of key importance in breast reconstruction and aesthetic surgery.

There are significant differences between the superficial fascia (*fascia superfitialis*) and the deep fascia (*fascia profunda*) in terms of their morphology and function.

The superficial fascia is a membranous layer of connective tissue made of loosely braided collagen fibres mixed with elastic fibres, abundant in this structure. It is connected to the skin by vertical fibrous threads called retinacula (ligaments, *retinacula cutis*). Below, between the superficial and deep fascias, there is another layer of reticula arranged obliquely to the fascia. In this space, there are numerous adipose tissue lobules.

The subcutaneous layer performs the following functions:

1. enabling the skin to move over the structures located underneath it,
2. thermoregulation,
3. exchange of metabolites,
4. passage of blood vessels, lymphatic vessels and nerves.

The superficial fascia is an equivalent of the muscular layer of the skin (*panniculus carnosus*) present in mammals. Also in some areas of the human body there is a muscle coat within the superficial fascia: on the neck (platysma), on the face (superficial muscular aponeurosis system – SMAS), near the anus (external sphincter, *fascia perinealis superfitialis – Colles fascia*) and in the scrotal sac (*fascia dartos – tunica dartos*) [5, 23].

The deep fascia is located under subcutaneous tissue and covers muscles as a multilaminar layer of collagen interwoven with relatively insignificant numbers of elastic fibres. Extensions of deep fascia penetrate muscular tissue in the following forms:

1. intermuscular septa *(septi intermusculari)*,
2. epimysium,
3. perimysium,
4. endomysium, and in some regions
5. periosteum.

Muscular fascia is inherently integrated with muscular tissue. It participates in the transfer of the contraction force between related muscle groups.

Because of the accumulation of nerve endings in the form of mechanoreceptors, the deep fascia plays an important role in deep sensibility (proprioceptive).

There are differences in the structure of the deep fascia depending on its location. The deep fascia of the upper limbs, lower limbs and the lumbar-sacral area is thick, strong and able to transfer significant forces during muscle contraction. The fascia covering the superficial muscles of the chest wall (pectoralis major, muscle latissimus dorsi, trapezius muscle) is thinner, more delicate and makes a uniform layer, difficult to separate from the chest muscles it covers.

The fascia adheres to muscles thanks to numerous fibrous intermuscular septa to which individual muscular fibres are attached, which additionally improves the efficiency of muscle contraction.

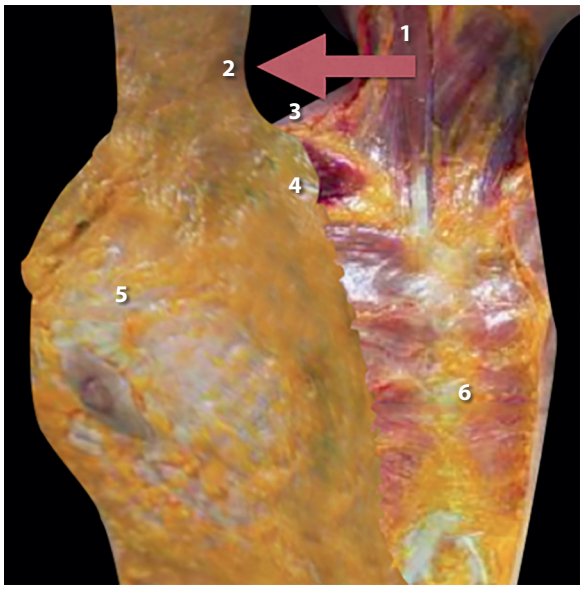
In the course of embryonic development, the superficial muscles of the chest transfer from the upper limb to extend over the chest muscles that lie deeper. In this way, they create an additional fascial-muscular layer. This explains the functional identity of the superficial muscles of the chest and the upper limb and the existence of muscular-fascial connections between limbs [23].

The mammary gland is located on the front wall of the chest and lies on the fascia of superficial muscles (greater pectoral muscle, serratus anterior). The superficial layer separating glandular tissue from adipose tissue is an important anatomical element in breast surgery. Surgical preparation of the surface of glandular tissue between the subcutaneous and pre-glandular vessel plexuses ensures radical removal of glandular tissue and, at the same time, enables surgery in the area devoid of blood vessels [24].

The skin and superficial fascia are connected with the deep fascia by a system of fibrous retinacula called the ligaments of Cooper. They are largely responsible for the fixation of the mammary gland to the skin. This is why there is no mobility of the skin with respect to the mammary gland [25, 26].

So far, it has been thought that the first person who described the existence of suspensory ligaments of the breast in 1840 was Sir Astley Paston Cooper (1768–1841) [9].

It turns out, however, that the detailed anatomical description of the breast with the entire ligament apparatus was provided 300 years earlier in the first edition of *De Humani Corporis Fabrica Libri Septem* published by Andreas Vasalius in 1543. He described the existence of three structures: a thin fascial membrane, dense adipose tissue between the breast and the chest wall muscle and numerous little fibres running vertically from the abovementioned structures to the skin [27–29].



1. Neck muscles
2. Platysma
3. Superficial fascia of the chest
4. Subcutaneous adipose tissue
5. Nipple-areolar complex
6. Intersection of the collagen fibres of superficial fascia over the sternum

Figure 5. Chest wall with dissected superficial fascia, subcutaneous tissue, adipose tissue and a mammary gland

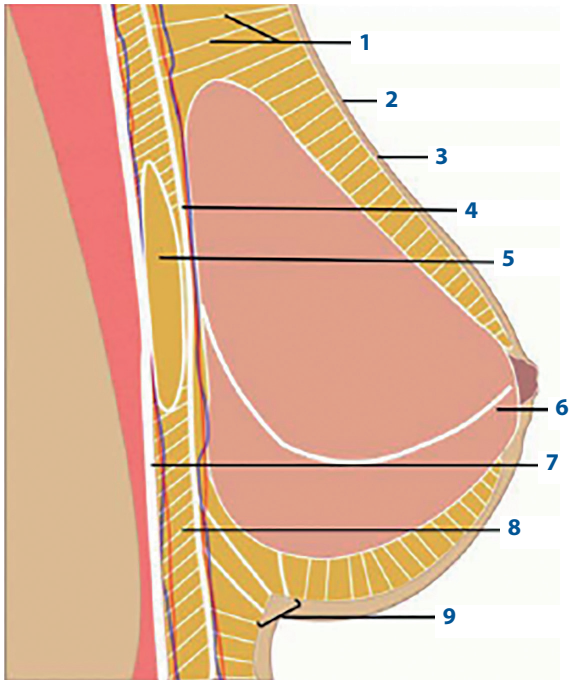
Between the lamellas of the superficial and deep fascias, directly behind the mammary gland tissue, there is a space filled with bursa which is a fatty-serous pillow (*bursa retromammaris,* Chassaignac's bursa). Numerous connections with the ligaments of Cooper make it possible for the fascial lamellas to move freely in this place, which enables the transfer of breasts on the chest wall [23, 30, 31].

The most important fascial component is the inframammary fold located near the lower edge of the greater pectoral muscle. Its detailed anatomical description was provided by Riggio (2000) on the basis of his own studies and earlier experiments by Lockwood (1991) and Navy (1998). The key element of the inframammary fold is a system of numerous subcutaneous connections in the form of strong retinacula (ligaments) between the superficial and deep fascias, which are evidently thicker in this place and create a dense adherence zone [8, 31].

Surgical preparation of tissues during skin sparing mastectomy (SSM or ASM – areola sparing mastectomy) in order to create a pocket under the pectoral muscle in immediate breast reconstruction (IBR) requires that the entire length of the inframammary fold structures and deep fascia along the lower edge of the greater pectoral muscle be preserved. Any damage to these structures may preclude immediate breast reconstruction with the application of a final implant. Preserving the entire fascia of the greater pectoral muscle during mastectomy due to carcinoma is oncologically safe. Preserving undamaged fascia in the lower-medial (parasternal) part prevents the loosening of the muscle attachment, which ensures even covering of the implant and makes it possible to place sutures in the lateral part at the border of the greater pectoral muscle and the serratus anterior muscle to prevent the implant from slipping to the side [8, 31, 32].

Anatomical studies indicate there is a close relationship between the course of main vessels and nerves inside glandular tissue and the fascial apparatus inside the breast preserving its shape and form and fixing the breast on the surrounding structures of the chest wall. This kind of an internal suspensory ligament of the breast is made of a horizontal dense fibrous septum which starts on the breast fascia at the height of the fifth rib and runs transversely to the nipple-areolar complex. The septum divides the mammary gland into two levels, upper and lower. At both ends of the septum, there is a fibrous tissue reinforcement in the form of a medial and lateral ligament whose fibres are folded at the edges and run vertically to the chest wall. These are recurring structures with consistent and defined morphology.

The vertical medial ligament extends from the strong fascia of the sternum at the level of the attachment to the 2nd and 5th rib, while the lateral vertical ligament is attached to the lateral edge of the smaller pectoral muscle. Cranially vertical ligaments attach to the breast fascia at the height



1. Superficial fascial-cutaneous ligaments
2. Epidermis
3. Dermis
4. Superficial fascia
5. Chassaignac's bursa
6. Vertical fascia of the breast – mesentery
7. Deep fascia
8. Deep interfascial ligaments
9. Inframammary fold

Figure 6. Draft of the breast gland fascia

of the second rib and in this way create an oval attachment. The lines of this attachment extend along the edges of the greater pectoral muscle. In the ventral direction, both lateral and medial ligaments enter glandular tissue creating a fibrous sac surrounding the breast [12, 31]. Most authors think that the superficial lamella of the chest fascia lies entirely on the pectoral muscle outside glandular tissue. Some of them believe that the superficial fascia is divided at the top edge of the breast into a deep lamella – extra-glandular, and superficial lamella – pre-glandular, which encapsulates the entire breast. Even the members of the latter group admit that only a part of the superficial lamella can visible at the edge of the mammary gland, which is where it connects to the deep fascia forming the crests of Duret as a direct extension of the ligaments of Cooper running from the chest wall directly toward the skin. These minor ligaments determine the real range and shape of the breast. In the medial part, the ligaments are more delicate and support the skin directly above the sternum. In the lateral part, they are strong, more developed and form a strong band between the lateral edge of the smaller pectoral muscle, the skin and the axillary fascia. In this way, employing the suspensory ligament of the axilla they create the vault of the axillary fossa [23, 32].

The inframammary fold is made of the superficial part of fascia, close to the skin, which extends transversely at the level of the 5th rib thanks to the ligaments of Cooper accumulating in the greatest numbers there. Apart from suspending the mammary gland on the chest wall and shaping the breast, the suspensory ligament system creates a pathway for vessels and nerves entering directly from the chest wall to the mammary gland and following it to reach the nipple-areolar complex. The duplicated lamella of the transverse fascia of the breast, which divides it in the upper and lower level and has an opening for vessels and nerves coming in from the chest wall, creates a similar mesentery to the small intestine mesentery in terms of its structure and build.

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The copyright fair use in scientific and publication activities

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Introduction. The use of research results, scientific statements, illustrations that come from other authors' publications is a common research practice. It is part of the scientific discourse supporting the reuse and dissemination of knowledge and scientific findings. It should, however, respect the copyrights of other authors' publications and the rules of permitted use within the framework of the so-called permitted use.

Material and methods. The analysis of the principles and limits of the use of material from other people's publications in publishing and scientific activities includes national and international standards in the field of copyright, protection of personal rights and ethics in science.

Results and discussion. In order for individual authors and research centers to legally use previous publications and their elements in their own scientific activities, they should apply the rules on permitted use. These include the so-called right of quotation, the statutory license for scientific and research institutions and the so-called reprinting right. The enrichment of one's own publications with excerpts from previous scientific studies and their graphical elements is permitted as long as it serves the purpose of research, clarification, scientific and critical analysis and respects the authorship of the source material. The marking of the source and the author is a required practice also with regard to content that is not protected by copyright but is the result of scientific creation.

Summary. The use of other people's fragments of publications and illustrations within the framework of permitted use does not require the consent of the copyright owner and payment of remuneration for the use, but must take place under the terms and within the limits set by copyright law and take into account the standards of reliable recognition of other authors research and scientific findings. Failure to comply or misapplication of this obligation may result in an allegation of infringement of copyright or personal interest in the form of scientific creation and may interfere with recognized standards of integrity in science.

Key words: fair use, permitted use of others' works, right of quotation, Creative Commons licenses

Introduction

The use of someone else's work is an essential part of scientific research and publication activities of individual scientists and scientific centres. The freedom of research, access to information and expression should be exercised with the respect of the author's economic and personal rights of other authors, standards of reliability in science and publication standards. Copyright law, ensuring exclusivity in the use of works and respect for authorship, provides for a special mechanism that takes into account the indicated interests and limits the copyright monopoly in the form of the institution of so-called fair use. At the same time, regulations on the protection of personal rights, codes of scientific ethics and guidelines of publishers limit the free multiplication of results, data and scientific findings in their own scientific and publishing activities, which are not covered by copyright protection, but are valuable results of research of other scientists.

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Material and methods

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Medical journals having registered press titles and carrying out publishing activities under the Press Law of 26 January 1984 [4], are beneficiaries of "the reprint right" (Article. 25 the Copyright Act).

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Results and discussion

For the proper determination of the limits of fair use of others' publications of scientific and publishing activities, it is of particular importance to clarify more precisely the statutory conditions for the use of the right of quotation, since it constitutes the basic legal tool for the use of works in one's publishing activities. The subject of a quotation may include not only text excerpts from other author's scientific papers, but also plastic, graphic and photographic works, which may include illustrations (drawings, tables, photographs), provided that they meet the requirements of the copyright law.

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As should be noted, diagnostic images (X-ray, ultrasound, images showing symptoms of diseases, intraoperative image, etc.), simple charts made with the use of standard IT tools, standard tables showing statistical data, test results, etc., will usually not meet this condition. Although they may constitute a key element of publications of scientific value, they are not subject to copyright protection, which means that there are no formal restrictions on their reproduction and no obligation to mark authorship. However, this does not amount to freedom to copy such elements, including not only in the finished graphic version, but also the results and data themselves. The obligation to mark the authorship and source in such a case may result from the protection of the personal rights of the original author in the form of scientific creation. Failure to do so may expose the author to a claim of unreliability in science due to appropriation of someone else's research results and scientific achievements.

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Fourthly, as long as authors usually respect the three conditions indicated above in the form of the use of the source and the author, they are not aware of another important restriction in the exercise of the right of quotation. It concerns the statutory purposes of using someone else's work, which are: explaining, polemic, critical or scientific analysis, teaching. These objectives are achieved in particular when the reference to a fragment or protected illustration is part of a scientific discussion, review, supports one's own research or views serve to illustrate the text as a reference point for discussion, polemic or criticism, etc. The need to use tables, diagrams, diagnostic and documentary photographs in their original form may be justified, among others, by the need to indicate errors in them,

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Summary

When preparing your own publications, which use excerpts and illustrations from other authors publications, should remember the following rules:

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3. The basic form of permitted use commonly used in publishing activities is the right of quotation. On the basis of this right, excerpts from domestic and foreign scientific articles and studies may be quoted in one's own publication without permission and without remuneration, regardless of whether such publication will be made available on free or commercial terms. The conditions for permitted quotation are:
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Conflict of interest: none declared

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