The effect of neoadjuvant therapy and examination of prognostic factors in breast cancer patients

PhD. thesis

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1. INTRODUCTION

Epidemiological and ethiological background

Breast cancer is the most common cancer among women worldwide, accounting for 22% of all cancers in women. According to the literature, one in eight women will certainly develop some form of breast cancer during their lifetime. In Hungary, approximately 8.000 new cases are diagnosed in every year, and about 2.800 women die as a result of the disease, making breast tumors the third most common cause of cancer death. Causes of breast cancer development include genetic predisposition, hormonal imbalance, effects of certain environmental factors, familial accumulation, and gene mutations of BRCA-1 and BRCA-2 (Breast Cancer Genes 1 and 2). Any endogenous or exogenous ovarian hormone exposure, such as premature menarche, late menopause, hyperestrogenism, and the use of hormonal contraceptives for more than five years, also increases the risk of developing breast cancer.

Morphology, signs and diagnostical methods

Invasive tumors are mostly presented as painless lumps, which can be detected by the patient himself or through the required screening tests. The tumor cells may form metastasis in the axillary lymph nodes via the lymphogen route. Advanced stage breast cancer can infiltrate the subcutaneous adipose tissue, the muscles of the chest wall, the skin, and the mammilla, causing the retraction of the latter. Hematogenous metastasis occurs primarily in bones (70%) in the lungs (60%) in the liver and in adrenal glands.

Mammography and breast ultrasound (UH), as well as cytological examination from core biopsies or fine-needle aspiration biopsies (FTAB), are currently the most commonly used and accepted diagnostics. The stage of the disease is determined by the size of the tumor and the number of metastatic lymph nodes or the presence of distant metastases. Axillary lymph node status is one of the most important prognostic factors for disease-free survival and overall survival. Although it has been shown that the presence or the lack of hormone receptors (estrogen, progesterone, herceptin receptors), determined during the histological processing of tissues, are also predictive. Currently, to monitor the course of the disease besides the radiological methods (mammography, CT, MRI, PET-CT), specific tumor markers (CEA, CA 15.3), which can be detected in the blood, are also used.

Surgical treatment of the breast cancers

Until the 1970s, the mechanical approach prevailed in surgical care, according to which the breast cancer was a loco-regional disease, and the expected survival was determined by the surgical radicality. Thus, the effort for total radicalization and mutilating surgeries were preferred. By the end of the 20th century, the so-called biological approach was developed, according to which breast cancer is a systemic disease and should, therefore, be treated systematically, and the survival is not determined by the surgical radicality itself. So-called breast-conserving surgeries (BCS), with reduced radicality, have come to the front. Extensive clinical studies have shown that in terms of local recurrence or long-term survival, there is no difference between mastectomy and those breast-conserving surgeries which were completed with irradiation.

If total breast removal is required, in current practice, modified radical mastectomy is the "gold standard" technique, whereby next to the breast, only the level I and level II axillary lymph nodes are removed. In the case of early-stage invasive tumors, besides oncological radicality, aesthetics also plays an essential role in the modern surgical therapy of breast tumors. Therefore, we aim to leave as much normal breast tissues as possible by performing sectoral excision- breast conserving surgery. However, irradiation of the remaining glandular tissue is essential after BCS. In some cases, subcutaneous mastectomy is another option, which means the removal of the glandular tissue while the breast skin is spared. This surgery is only recommended if the procedure ends with immediate breast reconstruction, using free or pedicled flaps, implants, or tissue expanders.

There has also been a need to reduce radicalization in the care of axillary lymph nodes due to frequent consequences such as lymphoedema and nerve dysfunction, occurring during the total axillary lymph node dissection. The Sentinel lymph node biopsy technique has been introduced, which helps to remove selectively the most likely location of the regional metastasis. If this is tumor-free, it is not necessary to remove all the axillary lymph nodes.

In the case of breast cancer patients, the determination of surgical indication and the decision of the need for adjuvant and neoadjuvant therapy is a work of the "onco-team," which includes radiologists, pathologists, oncologists, and surgeons. Radicality of surgical care is determined by the size of the tumor and the involvement of the axillary lymph nodes.

Oncological treatment options

Adjuvant therapy

Adjuvant therapy includes post-operative irradiation, chemotherapy, hormone therapy based on receptor status, and biological treatment in HER2 (Human Epidermal Growth Factor Receptor 2) positive cases. Irradiation, by destroying viable tumor cells that are still lying in the tumor bed, reduces the risk of local recurrence by a third. In the case of breast cancers, chemotherapy is based on the use of anthracycline and taxane-containing agents.

In the case of estrogen receptor (ER) positive tumors, we aim to reduce the level of estrogen, that reaches the receptors, using GNRH analogous, aromatase inhibitors (AI), anti-estrogens, and tamoxifen (Zitazonium®). Reduced hormone levels inhibit the proliferation of tumor cells. In progesterone-sensitive tumors, therapy can be supplemented with the administration of luteinizing hormone-releasing hormone (LHRH) analogous, which may further reduce sex hormone secretion.

HER2 is found in large amounts on the surface of certain tumor cells, where it stimulates the growth of tumor cells. Trastuzumab (Herceptin®) binds selectively to these receptors, stopping the growth of tumor cells and causing the destruction of them. Another effective medicine is the bevacizumab (Avastin®), which causes reduction in the number of the blood vessels in the tumor, leading to insufficient oxygen and nutrient supply and causing its atrophy.

Neoadjuvant therapy

Pre-operative chemotherapy treatment, also known as neoadjuvant therapy or primary systemic therapy (PST), can be offered to all patients who will require postoperative adjuvant chemotherapy. The advantage of it is that the systemic treatment may begin as early as possible after diagnosis. Based on the extent of tumor regression, we are informed about the efficacy of the therapy, and in the case of inefficacy, the treatment may be modified. As a result of the neoadjuvant treatment, the size of the tumor decreases, so an unresectable tumor may become removable. Moreover, in some instances, due to the shrinkage of an originally resectable tumor, BCS can also be considered as an option. Its further advantage is that the initiation of chemotherapy treatment is not delayed by possible postoperative complications, and the efficacy of PST in vivo, also refers to a subsequent prognosis of the disease. Among the drawbacks, we should highlight the fact, that unfortunately, some patients do not respond to the treatment.

2. NEOADJUVANT (PST) TREATMENT OF BREAST CANCER PATIENTS

Introduction

Neoadjuvant chemotherapy, also known as primary systemic therapy, is currently routinely used, besides the treatment of locally advanced breast cancer (LABC) and inflammatory breast cancer (mastitis carcinomatosa), in some cases of operable breast cancer as well. The goal of PST is to reduce the viability of tumorous tissues, to prevent the vascular and lymphatic invasion of tumor cells, and to reduce the chance of local recurrence by destroying micrometastases. As a result of PST, tumor size may be reduced, resulting in the potential resection of unresectable tumors and a significant increase in the rate of breast preservation surgery.

Aim of this study

In the present study, we retrospectively processed the radiological and histological findings of our patients who underwent breast surgery in our clinic between 2007 and 2012 and got PST because of breast cancer. Our goal was:

- to evaluate the effectiveness of our PST protocol and compare results with the literature data
- investigate the impact of PST on surgical treatment
- mapping the rate at which breast conserving surgery can be performed after PST
- to study the relationship between PST response and histological features
- assess the effectiveness of preoperative examination of axillary lymph node metastases, and
- review survival data of the patients we treated

Patients and methods

In our retrospective study, we enrolled 204 patients who were undergoing surgery after preoperative chemotherapy because of breast cancer between March 2007 and September 2012 at the Department of Surgery of the Clinical Center University of Pécs (PTE KK). Patients were divided into two groups according to their stage: in one group the patients received PST because of an advanced-stage unresectable cancer or mastitis carcinoma (24.5%, n=50) and thus, because of the so-called conversion chemotherapy, became operable. The other group included patients with primary resectable cancer (75.5%, n = 154).

The 21% of patients had FEC (5-Fluorouracil 500 mg/m^2 iv + Epirubicin 100 mg m^2 iv + Cyclophosphamide 500 mg/m^2 iv) while 79% of patients got TXT + EPI (Docetaxel 75 mg/m^2 iv) + Epirubicin 75 mg/m^2 iv) combination therapy for 6 cycles. After the third cycle of chemotherapy, radiological control was performed. In the case of regression or stagnation

another three cycles of chemotherapy were applied, but in the case of progression, the patient was immediately operated on, and mastectomy was performed. In small tumors (T1c-T2) without extensive DCIS component or in the case of well responsive tumors BCS, or in other words, sectoral excision was performed. In large tumors (T3, LABC) and cases of mastitis carcinomatosa, regardless of the extent of remission, mastectomy was the chosen procedure. If the axillary lymph node metastasis was not confirmed at the patient during the early staging, then during the operation Sentinel biopsy was performed, and further axillary care was made dependent on this. If metastatic lymph node was detectable already before chemotherapy, axillary block dissection was performed regardless of the degree of radiological regression.

During our retrospective analysis, pTNM was also considered, which involves the histological type, localization, receptor status of the tumor, and it also includes the involvement of axillary lymph nodes. Specimens removed during surgery were divided into four groups based on the histological picture: complete, significant, moderate remission and those who did not respond to treatment. According to our definition, in the case of complete pathological remission, there were no detectable viable tumor cells in the glandular tissue. Significant remission was observed when tumor body size was reduced compared to imaging examinations, became hypocellular, and less than 10% of the tumor cells seemed viable. In moderate remission, tumor size was reduced compared to the original size, and the proliferative activity was abolished, but only a slight decrease in cellularity was seen. The patient did not respond to the treatment (a.k.a. non-responder) if the proliferation activity was not reduced and the tumor size stagnated or possibly increased.

Results

Based on the histological findings, regarding the stadium T, complete remission was found in 20% (n = 41) of patients and significant remission in 25.5% (n = 52). A further 33.8% (n = 69) of patients had moderate regression after neoadjuvant treatment, while 21% (n = 42) did not respond (non-responder) to the treatment.

Regarding the receptor status, HER2 + patients responded best to PST, 36% of the patients achieved complete pathological remission (cPR) and a further 17% achieved significant remission. Complete remission rates were also very high in triple-negative breast cancer patients (24%), but non-responder rates were also the highest in this patient group (38%), and further 38% of these cases were showing only moderate remission. Tumors with ER+ and PR + receptor status had the lowest incidence of cPR, but significant remission was also seen in 32% of these patients.

After PST and surgery, a significant shift towards the lower T stages (down-staging) was observed with respect to the pathologist-determined stage. The distribution of surgical types is shown in Table 1.

Table 1. Surgical solutions

	n	%
Sectoral excision	71	35
Mastectomy	129	63
Subcutan mastectomy	4	2
Re-excision		
rate	33	16
cause		
- involvement by the tumor or by DCIS	15	45
- narrow resection margin	18	55

Compared the baseline axillary status with the pathological findings, the number of the patients who had lymph node metastases, from the initial 69% (n=141) decreased to 52,5 % (n=107) by the effect of PST.

The median survival for patients with advanced-stage breast cancer (T3-T4) was 78.3 months (~6,5 year) (St. Dev: 42.5) and 96.2 months (~8 year) (St. Dev: 37.1) in patients with primary resectable breast cancer (T1-T2) who underwent PST, which was a significant difference. Five-year survival was 65,1% in our advanced-stage breast cancer patients and 83,7% in primary resectable breast cancer patients, which was also significant (p=0.01171).

Discussion

In our study, the effectiveness of PST was monitored by comparing the results of preoperative staging examinations with those pathological staging results, which were determined from the surgical specimen. Histological examination revealed complete remission in 20% of patients, and this rate is in the range reported in the literature (5-30%). Significant remission was observed in 25.5% of patients, and moderate regression was achieved in a further 33.8%. The 21% of patients did not respond to the treatment, proliferation activity did not decrease, and the size of the tumor stagnated, or in some cases, it increased.

The therapeutic response to the neoadjuvant treatment was the best in the HER2 + patients, which means similar result to those data which was found in the literature. Although

cPR was found more rarely in tumors with ER and PR positive receptor status, PST proved to be also very effective in these patients due to many significant remissions, so altogether, 42% of patients responded well. The rate of cPR was also very high in triple-negative patients (24%); however, the highest rate of non-responder patients was also found in this group (38%), and only a modest remission was observed in another 38% of patients. Thus, PST was the least effective in this group of patients, but in those patients, who responded to the treatment, complete remission was seen. The results of our retrospective study are consistent with data from Starver et al. who demonstrated, by multivariate analysis, that the only significant predictor factor of cPR is the receptor status of the tumor.

There is no consensus or a well-established criteria system for surgical care regarding the indication of BCS after neoadjuvant treatment. Given that, the neoadjuvant treatment has virtually no effect on the in situ component, despite cPR or pronounced down-staging, BCS can not be safely recommended in those patients where the tumor is surrounded by radiologically demonstrable extensive DCIS, as subsequent tumor recurrence are based on these cells.

Patients who have already had lymph node metastases during preoperative staging, independently of the effects of PST, we performed level I-II. axillary block dissection. Sentinel lymph node biopsy and low axillary dissection (removal of 1-4 lymph nodes) were performed in the initially axillary negative patients. If metastasis was confirmed at the histological examination, a further operation was performed to remove level I-II. axillary lymph nodes.

Examining the response to PST, depending on the localization of the tumors, we found that 70% of central tumors, 38% of lower-inner quadrant tumors, 34% of upper-outer quadrant tumors, and 23% of both lower-outer and upper-inner quadrant tumors responded extremely well to the treatment (achieving complete and significant regression). Thus, centrally located tumors responded best to treatment. It is believed that this is due to the breast blood supply, but its explanation requires more studies with a higher number of cases, like international studies.

In conclusion, we can say that the neoadjuvant treatment we applied in breast cancer patients was significantly effective in reducing tumor size, whereas we noticed less effect on lymph node status. Often, after neoadjuvant therapy, because of the residual DCIS, we are not able to reduce the volume of resection as much as the tumor size reduction would allow. During the processed 5-year period, thanks to the PST, 26 patients became operable from the primary unresectable stage (conversion chemotherapy). Our results strongly support the multimodal treatment of breast tumors and the need for neoadjuvant therapy.

3. DSC ANALYSIS OF THE BLOOD PLASMA OF BREAST CANCER PATIENTS

Introduction

Differential scanning calorimetry (DSC) is a thermoanalytical method can detect and tracking small temperature changes between the test sample and the reference material, thus, it is suitable to examine biological structures as well. By changing the temperature, we can reach the point where the chemical form or crystal structure of the given substance changes. By detecting the temperature of the conversion and the thermal energy absorbed/released during this process, we can identify the chemical structure of the material. The DSC instrument measures that electrical power which is needed to maintain the sample and reference material at the same temperature when they are heated or cooled. Evaluation of the DSC thermogram answers how the heat flux, which is the amount of heat passing through the material per unit of time, has changed on the curve by increasing the temperature.

Previous studies have shown that thermodynamic changes in biological structures can be related to the development of various diseases. In addition, these thermoanalytical changes have been shown to be [transition temperature: T_m (° C); calorimetric enthalpy: ΔH (J / g)] appear specifically in different diseases and within each stage they differ from each other. In a summary published in 2009, Garbett et al. investigated thermal changes in blood plasma using the DSC technique and concluded that the examined tumors and diseases might have an unique, fingerprint-like thermal curve. Recent researches, therefore, suggest that DSC, besides the conventional diagnostic procedures, may provide useful information for oncological diseases, but there is no literature on previous DSC testing in breast cancer patients.

Aim of this study

In our study, we sought to determine whether

-there is a difference in DSC curves of blood samples from healthy and breast cancer patients, or not?

-the thermoanalysis correlate with disease stage and progression, or not?

Patients and methods

In our prospective study, we included 19 women with operable breast cancer who had undergone surgery in the Surgery Clinic of University of Pécs in Hungary. Patients were categorized by the maximum tumor diameter (5-75 mm) and by the number of metastatic axillary lymph nodes (0-10 pcs).

DSC measuring

Peripheral blood samples were taken in Vacutainer tubes containing EDTA (1.5 mg/ml sample), from healthy adults (control group, n = 3) and from patients immediately prior to surgery (n = 19). Subsequently, the plasma fraction was separated from the blood cells at 4 ° C. Plasma thermal denaturation was determined by using a SETARAM micro DSC-II calorimeter. Calorimetric enthalpy was calculated from the area under the heat absorption curve by using two-point setting SETARAM peak integration. Data were graphically processed, after the ASCII conversion, by using the Origin (ver. 6.0) software (Microcal Software Inc, Northampton, USA).

The mean and standard error (SE) were calculated to evaluate the results. Data were processed in the case of $n \geq 5$ by T-probe. The significance was $p < 0.05$. MicroCal Origin 6.0 program (Microcal Software, USA) was used for evaluation.

Results

Comparing the DSC curves of healthy controls with the results of the breast cancer patients, measurements showed different thermal domains (based on T_m and the run of curves) during the denaturation, depending on tumor size and number of metastatic lymph nodes. In terms of the tumor size, the tendency of the results was like the disease progression. On the thermograms, these changes were most noticeable in the group 11-20 mm (Group 2), and in Group 4 (where comparing to this, the tumor diameter was double). According to our measurements, T_{m3} and T_{m4} may be good indicators of the disease progression. Compared to the control group a new peak, T_{m4}, appeared in those patients where the tumor diameter was greater than 10 mm but less than 50 mm. All this is confirmed by the decreasing characteristic of calorimetric enthalpy of the plasma. It would be expedient to extend the examinations to a higher number of cases, but the characteristic (trend) of the differences among the groups is still clearly visible. Thus, according to the DSC data, in the tumor samples, the alteration of the structural unit belonging to the second and third melting temperatures can be considered as a good indicator of the severity of the disease, in terms of increasing the number of metastatic lymph nodes. The T_{m4} and T_{m5} thermal parameters were detectable only in cancer patients, and they did not appear in the healthy control group.

Discussion

Our study investigated the thermal changes in the plasma of breast cancer patients by the DSC method, as a function of tumor size and number of metastatic regional lymph nodes.

Overview the thermograms of the 19 patients, we observed a unique characteristic compared to the curves of healthy controls. Comparing the DSC curves of healthy controls to the breast cancer patients, the measurements indicated a different frequency of the T_{m2} and T_{m3} thermal domains, depending on the size of the tumor and hence, the severity of the disease. The new denaturation transition (T_{m4}) was detected only in the plasma of cancer patients.

Examining those patient groups, which were formed according to the number of metastatic lymph nodes, we found that in control samples, only two major transformation temperatures (T_{m1}, T_{m3}) were more likely to be detected and T_{m2} appeared mainly in-patient samples. The T_{m3} conversion peak showed a significantly different temperature compared to the control group. In addition, T_{m4} and T_{m5} thermal parameters were detectable only in cancer patients and did not appear in the healthy group. Calorimetric enthalpy also correlates with disease severity, but due to the low number of cases, this result was only significant in patients with 1-3 metastatic lymph nodes.

Our results are important for several reasons. On the one hand, the DSC examination can not only clarify the presence of breast cancer but can also be used to distinguish different stages and to monitor the course of the disease. On the other hand, over the past ten years, numerous oncological studies have confirmed the possible role and potential use of thermoanalytical measurements of blood plasma in the course and monitoring of the disease.

4. THE IMPORTANCE OF THE OXIDATIVE STRESS IN BREAST CANCER PATIENTS

Introduction

The etiology of breast cancer is multifactorial, and besides the genetic predisposition, the most known risk factors (age, obesity, drugs, hormones, etc.) are associated with cellular oxidative damage of the mammary gland. Oxygen-Free Radicals (OFRs) play a key role in carcinogenesis, by triggering, and promoting tumor progression and systemic tumor invasion. One of the main sources of OFRs are polymorphonuclear leukocytes (PMNs), which directly release OFRs and indirectly enhance free radical production by a lysosomal myeloperoxidase enzyme (MPO). Tumor cells produce far more OFRs than normal cells. The deleterious effects of oxidative stress can be counteracted by non-enzymatic (e.g., reduced glutathione: GSH; sulfhydryl groups: -SH groups) and enzymatic (e.g., superoxide dismutase: SOD; catalase: CAT) antioxidants.

Aim of this study

The purpose of this study was to detect oxidative stress and antioxidant parameters in blood plasma in breast cancer patients with different tumor sizes, lymph node involvement, receptor status, mitotic activity, and chemotherapy.

Patients and methods

Our prospective study included 40 newly diagnosed women with operable breast cancer. Patients were categorized according to tumor diameter (T), number of metastatic regional lymph nodes (N), proliferation activity (MIB-1), receptor status, and the presence or absence of neoadjuvant preoperative chemotherapy. According the tumor diameter patients were classified into T1 (n = 12), T2 (n = 11), T3 (n = 10) and T4 (n = 7) groups. Based on the number of metastatic axillary lymph nodes, patients were divided into three groups: N0 (n = 12), N1 (n = 20), N2 (n = 8). Four groups were formed based on proliferation activity: MIB-1 (n = 15), MIB-2 (n = 8), MIB-3 (n = 10) and MIB-4 (n = 7). Considering the receptor status of the tumor, three groups were distinguished: Her2 + (n = 12), ER + and PR + (n = 15), and Triple- (n = 13). Regarding preoperative chemotherapy (neoadjuvant treatment), patients were divided into two groups, who did not receive (-Chemo, n = 23) and those who received (+Chemo, n = 17) chemotherapy.

Peripheral blood samples were collected preoperatively from patients (n = 40) and from

healthy women aged 26 to 60 years (control, n = 20). Free radical production of PMN leukocytes was determined, and the peak value of free radical formation was compared with the white blood cell count of the patients. Plasma MPO concentration, MDA concentration, which is known as an indirect marker of lipid peroxidation, endogenous antioxidant scavenger GSH concentration, plasma sulfhydryl (-SH) groups, SOD activity, and catalase enzyme activity were also investigated.

Results

The total production of OFRs was significantly increased in breast cancer patients compared to healthy controls. This increased proportionally with tumor size and with the number of metastatic lymph nodes (5.5 ± 1.5 AU) ($p < 0.05$; $p < 0.01$). Proliferation activity showed a parallel and significant increase compared to healthy controls ($p < 0.05$; $p < 0.01$; $p < 0.001$). Furthermore, its production was significantly higher in the Mib4 (17.69 ± 1.73 AU) group compared to the Mib1 group (10.14 ± 0.92 AU) and to the Mib2 group (9.48 ± 1.23 AU). Regarding the receptor status, the OFR release by PMN leukocytes were significantly higher in all groups compared with controls ($p < 0.05$). OFRs production was slightly elevated in that group where neoadjuvant chemotherapy was absent (-Chemo) compared to the control group, but a significant increase was also seen in that group where the patients received chemotherapy (+Chemo).

A similar tendency was observed in the change of MPO activity. In peripheral blood samples, both plasma MDA and hemolysate MDA values were significantly increased in most cases, compared to the healthy controls ($p < 0.05$; $p < 0.01$; $p < 0.001$). Values changed significantly in proportion to the severity of the disease, related to tumor size, number of metastatic lymph nodes, and whether the patients received neoadjuvant chemotherapy or not.

From among the antioxidant enzymes, activity of SOD and CAT showed significantly reduced levels in all groups compared to the control group (610 ± 34 vs. 960 ± 40 IU / ml; 1578 ± 67 vs. 2400 ± 82 BU/ml, $p < 0.001$). Considering the proliferation activity (Mib1 and Mib2 vs. Mib4), only SOD values, in the case of chemotherapy groups, both SOD and CAT activities have also changed significantly with the severity of the disease.

Discussion

In recent years, more and more studies have shown that oxidative stress produced by OFRs can play an important role in the development of breast cancer. In the present study, the examination of peripheral blood samples showed that lipid peroxidation, total OFRs release,

and MPO activity of PMN leukocytes were significantly higher in breast cancer patients than in the healthy control group. These parameters increased parallel with the severity of the disease, so with the tumor size, the number of metastatic lymph nodes, and with the proliferative activity. This can be caused by the increased production of OFRs, which can damage all cellular molecules (e.g., lipids, proteins, and DNA molecules) and, on the other hand, which can reduce or deplete the cell's antioxidant capacity. Our results agree with other authors' findings on breast tumor cell cultures and with published data of a few clinical trials.

Numerous studies have confirmed a correlation between pro-oxidant status and the extent of breast tumor dissemination. In our study, significant increases in pro-oxidant parameters were measured in the lymph node positive patient population. Literature data also point to the fact that increased levels of pro-oxidant caused by OFRs overproduction are not only a cause and a consequence but also an important maintenance factor for the growth and dissemination of breast cancer.

The present study confirmed that among antioxidant enzymes, SOD, and CAT activity was reduced in breast cancer patients compared to healthy controls. Low molecular weight antioxidants such as GSH and -SH did not change significantly in the present study. In our study, we observed that preoperative chemotherapy increased pro-oxidant levels and resulted in decreased antioxidant capacity in treated patients compared to untreated patients.

5. THE EXAMINATION OF MICRO-RNA (miRNA) EXPRESSION IN BREAST CANCERS

Introduction

The miRNAs are small, ca. 19-22 nucleotides in length, which forms the family of non-coding RNAs and they are involved in the regulation of various biological processes, including the evolution (through the fine-tuning of gene expression), the cell division and differentiation, the proliferation, the apoptosis, the metabolism, and overall the maintenance of cellular homeostasis. Their regulatory function is performed by post-transcriptional gene silencing in conjunction with messenger RNA (mRNA). They thus regulate or completely block the manifestation of the original information at the protein synthesis (translation) level, coming from the DNA. Numerous studies have confirmed the altered expression of miRNAs in different tumors. Sharp differences of miRNA expression patterns, between normal and tumorous cells, were also demonstrated in cell cultures of normal and tumorous mammary cells.

Aim of this study

The aim of this study was to determine the expression patterns of miR-21, miR-34a, miR-221, and miR-383 micro-RNAs in tumor samples of patients who were operated with advanced-stage breast carcinoma. Then we aimed to investigate these, depending on the tumor size, the number of lymph node metastasis, the tumor receptor status and the stage of the tumor.

Patients and methods

Twenty-four women with breast carcinoma were included in our study. The sampling was performed directly from the tumor tissue during the operation between January and November 2014. All the selected patients had well-palpable, advanced-stage breast cancer. Patients, based on the final histological findings, were divided into different groups according to their age (below 50 and above), tumor size (below 40 mm and above), lymph node involvement (less than 3 metastatic lymph nodes), receptor status (ER +, Her2 +, and Triple-negative), and tumor stage (stage II v. III) and analyzed for micro-RNA differences based on these variables. The examination of micro-RNA expression of tumor tissue samples was performed by quantitative real-time PCR with the assistance of the Department of Public Health of University of Pécs.

Results

There was a close correlation among the size of the breast tumor and the expression of miR-21, miR-34a, miR-221 microRNAs. The expression of miR-21 in tumors above 40 mm (n = 18) was fifteen times higher than that measured in tumors below 40 mm (n = 6). While the expression of miR-34a and miR-221 was detectable in tumors above 40 mm, they were not detectable in the tumor group below 40 mm. The expression of miR-383 was not measurable, neither in tumors below 40 mm nor in larger.

In advanced breast tumors, micro-RNA expression, measured in tumor tissue, also showed a strong correlation with the number of metastatic lymph nodes. Patients with histological examination of more than three axillary lymph node-confirmed metastases (n = 9) showed a six-fold increase in tumor-expressed miR-21 and miR-34a compared with patients with less positive lymph node (n = 14).

In terms of receptor status, miR-21, miR-34a, and miR-221 expression were increased in ER positivity (n = 17), whereas miR-21 expression was most pronounced in HER positive cases (n = 6).

Discussion

Today, we know already that miRNA patterns are different in healthy and cancerous tissues. Whenever changes occur in any area of normal cellular life, the ratio and the number of miRNAs will change. At the cellular level, miRNAs can also act as oncogenes (oncomir) or tumor suppressor genes, depending on which gene is regulated.

One of the first micro-RNA, identified as an oncogene, was miR-21. Increased expression of this both in serum and in the tumor tissue itself, has been demonstrated in many tumor types. In our study, we found that its value correlated closely with the size of the breast tumor and the number of metastatic axillary lymph nodes. Regarding the receptor status, a dramatic increase of expression was observed in HER + patients. Our results in several points agree with the data of other authors, who showed increased miR-21 expression in patients with Ki-67 +, HER2 +, ER +, and PR + receptor status. Studies performed in cell cultures and on various types of human malignant tissue (glioblastoma, lymphoma, melanoma, etc.) have confirmed that miR-21 functions as an oncogene and its inhibition may be a therapeutic target in the future.

In our study, the expression of miR-34a and miR-221increased in tumors larger than 40 mm and in the case of 3 or more involved lymph nodes, and they also appeared in the tumorous tissue in the case of stage II and III. Members of the miR-34 family have suppressor effects, which inhibit the important tumor suppressor inhibitor protein Sirtuin-1 (SIRT1). Earlier

studies have shown that increased expression of miR-34 can eliminate tumor stem cells in some malignant tumors. MiR-221 is a tumor invasion-specific, oncomyric miRNA that inhibits apoptosis. Of the micro-RNAs, previous studies on miR-221 and miR-222 have shown, that they are involved in the pathology of breast cancer through their target gene.

The presence of miR-383, both in human breast cancer cells in vitro and in mouse embryonic stem cells, has been shown to increase the susceptibility to DNA damage, but during our study, its expression did not correlate with tumor size, metastasis, receptor status or disease status.

Statistically, we did not find significant differences in our results due to the low number of samples, but there is a tendency for the micro-RNA expression and clinicopathological features in advanced-stage breast cancer cases. This, besides the diagnostic use of miRNAs as early biomarkers, raises the possibility of their subsequent therapeutic use in oncological patient care. Mapping and understanding of the signaling pathways can greatly contribute to the development of more effective molecular target therapies, in addition to those which are currently in use.

6. NOVEL FINDINGS

1. During our retrospective study, which included 204 patients, diagnosed with advanced-stage unresectable breast cancer, mastitis carcinomatosa, and primary resectable tumor showed that following primary systemic therapy and surgery the neoadjuvant therapy in breast cancer patients was extremely efficient to decrease the size of cancer, while in the aspect of the lymph nodes status smaller effect was noticed. Furthermore, breast cancers with central localization had a better reaction to the treatment than the others.

2. Differential scanning calorimetry (DCS) analysis of the blood plasma of 19 patients ,with various stages of breast cancer, showed that the change of the transient temperature of the thermograms and the change in the calorimetric enthalpy are related to the size of the tumor and the number of metastatic axillary lymph nodes.

3. Examination of peripheral blood samples from 40 patients treated with operable breast carcinoma, showed a significant increase in pro-oxidant status (lipid peroxidation, total OFRs release, increase in MPO activity) and a decrease in antioxidant capacity of cells (SOD, CAT) in proportion to the severity of the disease. Neoadjuvant chemotherapy as a "double-edged weapon" significantly increased the oxidative stress.

4. During the determination of micro-RNA expression of tumor tissue samples from 24 patients, who were operated with advanced breast cancer, we showed that miR-21, miR-34a, and miR-221 expression correlated closely with the tumor size, the number of metastatic lymph nodes and with the tumor receptor status.

7. LIST OF PUBLICATION

Topic related journal articles

1. **Zapf I**, Tizedes G, Pavlovics G, Kovács G, Kálmán E, Szalai G, Kövér E, Farkas R, Horváth ÖP. Emlődaganatos betegek primer szisztémás terápiája során elért eredményeink (2007-2010). Magyar Sebészet 2011;64:223-8.

2. **Zapf I**, Fekecs T, Ferencz A, Tizedes G, Pavlovics G, Kálmán E, Lőrinczy D. DSC analysis of human plasma in breast cancer patients. Thermochimica Acta 2011;524:88-91.
   IF: 1,805 Independent citations: 22, Dependent citations: 11, Total: 33

3. **Zapf I**, Fekecs T, Moezzi M, Tizedes Gy, Pavlovics G, Kálmán E, Horváth PÖ, Ferencz A. Emlődaganatos betegek vérplazmájának differenciál pásztázó kalorimetriás vizsgálata. Magyar Onkológia 2012;56:274-9.

4. **Zapf I**, Moezzi M, Fekecs T, Nedvig K, Lőrinczy D, Ferencz A. Influence of oxidative injury and monitoring of blood plasma by DSC on breast cancer patients. Journal of Thermal Analysis and Calorimetry 2016;123:2029-35.
   IF: 1,953 Independent citations: 8, Dependent citations: 6, Total: 14

5. Ferencz A, **Zapf I**, Lőrinczy D. Harmful effect of neoadjuvant chemotherapy monitoring by DSC on breast cancer patients' blood plasma. Journal of Thermal Analysis and Calorimetry 2016;126:55-59.
   IF: 1,953 Independent citations: 5, Dependent citations: 1, Total: 6

IF of topic related first author publications: 3,758
IF of all topic related publictaions: 5,711
Independent citations: 35, Dependent citations: 18, Total: 53

The list of the topic related, cited abstracts

1. **Zapf I**, Ferencz A, Fekecs T, Tizedes Gy, Pavlovics G, Kálmán E, Lőrinczy D. Humán vérplazma differenciál scanning kalorimetriás vizsgálata emlődaganatos betegekben. Magyar Sebészet 2011;64:158.
2. **Zapf I**, Gombos K, Juhász K, Ferencz A, Tizedes Gy, Pavlovics G, Kovács Gy, Horváth ÖP, Vereczkei A. Emlőcarcinomás betegek mikro-RNS-expressziójának meghatározása. Magyar

Sebészet 2015;68:117-118.

3. Ferencz A, **Zapf I**, Lőrinczy D. Chemotherapy is a double-edged sword. Blood plasma monitoring in patients by DSC. Maria Curie-Skłodowska University Press, Lublin, 2015.p. 484. (ISBN:978-83-7784-684-1)

Topic related scientific presentations

1. **Zapf I**, Ferencz A, Fekecs T, Tizedes Gy, Pavlovics G, Kálmán E, Horváth ŐP, Lőrinczy D. Humán vérplazma differenciál pásztázó kalorimetriás vizsgálata emlődaganatos betegekben. Magyar Sebész Társaság, Kísérletes Sebészeti Szekció, 2011. évi XXIII. Kísérletes Sebész Kongresszus, 2011. június 2-4, Budapest.
2. Ferencz A, Fekecs T, **Zapf I**, Moezzi M, Lőrinczy D. DSC analysis of blood plasma on patients with skin and breast cancer, and psoriasis. In: 4th Joint Czech-Hungarian-Polish-Slovak Thermoanalytical Conference, Czech Republic, Prague 2013.06.24-27.
3. Ferencz A, Fekecs T, Mehdi M, **Zapf I**, Lőrinczy D. DSC as a diagnostic tool in the medical applications. Thermal Analysis and Calorimetry in Industry and Research - 40 Years of GEFTA Annual congress with participation of GEFTA's European partner associations, Germany, Berlin, 2014.09.16-19.
4. **Zapf I**, Gombos K, Juhász K, Ferencz A, Tizedes Gy, Pavlovics G, Kovács Gy, Horváth ÖP, Vereczkei A. Emlőcarcinomás betegek mikro-RNS-expressziójának meghatározása. A Magyar Sebész Társaság Kísérletes Sebészeti Szekciójának XXV. Kongresszusa. Pécs, 2015.05.14-15.
5. Ferencz A, **Zapf I**, Lőrinczy D. Chemotherapy is a double-edged sword. Blood plasma monitoring in patients by DSC. 12th Conference on Calorimetry and Thermal Analysis: Poland, Zakopane 2015.09.06-10.

The list of non-topic related scientific publications and abstracts

1. Molnár FT, Horváth ÖP, Varga G, **Zapf I.** Intratrachealis pH viszonyok és a GERD: lehetséges kapcsolat a tüdőrák carcinogenesisével? Medicina Thoracalis 2004;57:56.
2. **Zapf I**, Benkő I, Szántó Z, Molnár FT. A kolorektális eredetű tüdőmetasztázisok sebészi kezelésének retrospektiv vizsgálata. Magyar Sebészet 2006;59:323.
3. **Zapf I**, Molnár FT, Benkő I, Kalmár NK, Szántó Z, Pótó L, Horváth ÖP. A colorectalis tumorok tüdő metastasisainak sebészi kezelése. Magyar Sebészet 2007;60:130-5. Independent citations: 2, Total: 2
4. Wolf M, Benkő R, Undi S, Dékány A, Illényi L, Papp A, Varga C, **Zapf I**, Barthó L. In vitro

pharmacology of inosine, with special reference to possible interactions with capsaicin-sensitive mechanisms and inflammatory mediators. Methods and Findings in Experimental and Clinical Pharmacology 2009;31:359-66. IF: 1,136 Independent citations: 1

5. Nedvig K, **Zapf I**, Fekecs T. A vékonybél meleg és hideg ischaemiás károsodásának kimutatása differenciál pásztázó kalorimetriás vizsgálattal. Magyar Sebészet 2011;64:207-12.
6. Fekecs T, Kalmár-Nagy K, Szakály P, Németh K, Moezzi M, **Zapf I**, Horváth ÖP, Barthó-Szekeres J, Ferencz A. Changes of progesterone-induced blocking factor in patients after kidney transplantation. Transplantation Proceedings 2011;43:3694-6. IF: 1,005 Independent citations: 2
7. Fekecs T, **Zapf I**, Ferencz A, Lőrinczy D. Differential scanning calorimetry (DSC) analysis of human plasma in melanoma patients with or without regional lymph node metastases. Journal of Thermal Analysis and Calorimetry 2012;108:149-52. IF: 1,982 Independent citations: 31 Dependent citations: 7, Total: 38
8. Csanaky K, Bánki E, Szabadfi K, Reglődi D, Tarcai I, Czeglédi L, Helyes Zs, Ertl T, Gyarmati J, Szántó Z, **Zapf I**, Sipos E, Shioda S, Tamás A. Changes in PACAP immunoreactivity in human milk and presence of PAC1 receptor in mammary gland during lactation. Journal of Molecular Neuroscience 2012;48:631-7. IF: 2,891, Dependent citations: 7, Total: 7
9. Fekecs T, Moezzi M, **Zapf I**, Ferencz A. Humán plazma differenciál pásztázó kalorimetriás vizsgálata melanoma malignumos betegekben = Analysis of human plasma with differential scanning calorimetry in melanoma patients. Egészség-Akadémia 2012;3:34-40.
10. Mehdi M, Fekecs T, **Zapf I**, Ferencz A, Lőrinczy D. Differential scanning calorimetry (DSC) analysis of human plasma in different psoriasis stages. Journal of Thermal Analysis and Calorimetry 2013;111:1801-4. IF: 2,206 Independent citations: 19 Dependent citations: 7, Total: 26
11. Moezzi M, **Zapf I**, Fekecs T, Nedvig K, Lőrinczy D, Ferencz A. Influence of oxidative injury and monitoring of blood plasma by DSC on patients with psoriasis. Journal of Thermal Analysis and Calorimetry 2016;123:2037-43. IF: 1,953, Independent citations: 7 Dependent citations: 4, Total: 11

Author's cumulative impact factor: 20,642

Independent citations: 97, Dependent citations: 43, Total: 140

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