

## ORIGINAL ARTICLE

# The role of interleukin 8 and matrix metalloproteinases 2 and 9 in breast cancer treated with tamoxifen

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## Summary

**Purpose:** To evaluate the role of interleukin 8 (IL8) and matrix metalloproteinase (MMP) 2 and 9 as potential parameters of response to adjuvant tamoxifen and to examine possible associations between biomarkers that might imply possible biological dependence.

**Methods:** The study included 59 postmenopausal breast cancer patients who received adjuvant tamoxifen. Biomarker levels were determined by ELISA in cytosol tumor extracts.

**Results:** Estrogen receptor (ER) proved as a reliable parameter of response to tamoxifen; patients with ER+ status had significantly longer median relapse-free survival (RFS) compared to those with ER- status ( $p=0.04$ ). Patients with IL8- status had longer median RFS compared to those with IL8+ status (77 and 53 months, respectively) but without significant difference. Patients with MMP9+ status had longer median RFS compared to those with MMP9- status

(92 and 66 months, respectively) but without significant difference. There was no significant difference in RFS between the subgroups formed according to MMP2 median value.

A significant positive correlation was found between IL8 and MMP9 levels ( $p<0.001$ ). Expression of MMP9 was significantly higher in patients with IL8 levels higher than the median ( $p=0.001$ ).

**Conclusions:** IL8 showed a tendency to act as an unfavorable parameter while MMP9 showed a tendency to act as a favorable parameter of response to tamoxifen, whereas the role of MMP2 as a potential predictive parameter is more complex. The results indicate that possible existence of positive feedback between IL8 and MMP9 might contribute to progression of breast cancer.

**Key words:** breast cancer, IL8, MMP2, MMP9, tamoxifen

## Introduction

ER and progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) are still among the most reliable prognostic and predictive parameters of breast cancer. Hormonal therapy is the most efficient adjuvant treatment of estrogen receptor-positive (ER+) breast cancer patients and 5 years of adjuvant tamoxifen reduces the recurrence rate by 50% and the mortality rate by 25% [1]. Still the efficacy of hormonal therapy is limited and about 50% of ER+ breast cancer patients will not respond well to tamoxifen [2].

IL8 is an inflammatory cytokine that belongs

to the class of CXC chemokines. Its role in tumor growth and progression is mediated by acting as mitogenic and potent angiogenic factor, as well as by chemotactic infiltration and activation of neutrophils, monocytes and other immune cells that self-secrete growth factors, angiogenic factors and proteinases [3]. It has been shown that steroid hormones (androgens, estrogens) are able to stimulate the expression of IL8 at the transcription level [4], but less is known about the role of IL8 as potential biomarker of treatment response in breast cancer patients treated with adjuvant

hormonal therapy.

MMP2 and MMP9 belong to the class of gelatinases that cleave the main structural components of basement membranes – collagen type I, III, IV, V, VII, X, XI, denatured collagen (gelatin) and elastin [5]. Besides the proinvasive role, MMP 2 and 9 act stimulatory on tumor growth by proteolytic activation of growth factors and growth factor receptors on tumor cells, endothelial and inflammatory cells [5]. Effects of MMP 2 and 9 on inflammation and angiogenesis could be both stimulatory and inhibitory, mediated by proteolytic activation/inhibition of cytokines, chemokines and angiogenic factors [5]. While most proinflammatory stimuli fail to increase the expression level of MMP2 [6], expression of MMP9 is inducible [7]. MMP9 is present constitutively only in neutrophils where it is stored in granules to be rapidly released after stimulation [8].

Available data regarding hormonal regulation of expression of these potential biomarkers in breast cancer are controversial. It is shown that several CXC chemokines including IL8 are mainly produced by ER– breast tumors [9,10], but a recent study found that estradiol increased IL8 secretion in normal human breast tissue and ER+PR+ breast cancer *in vitro* and *in vivo* [11]. Expression of both MMP2 and MMP9 is significantly increased in ER– tumors [12] and estradiol treatment significantly decreased the activity of both MMP2 and MMP9 in human breast cancer *in vitro* and *in vivo* [13,14].

The aim of this study was to evaluate the role of IL8 and MMP 2 and 9 as potential biomarkers of treatment response in breast cancer patients treated with adjuvant tamoxifen and to examine possible associations between these potential biomarkers and clinicopathological parameters that might imply possible biological dependence.

## Methods

### Study population

The study included 59 postmenopausal breast cancer patients with known clinicopathological parameters. All patients received adjuvant hormonal therapy (tamoxifen) due to detectable levels of hormone receptors. All patients underwent surgical removal of primary tumor at the Institute of Oncology and Radiology of Serbia, Belgrade. After surgery, histological specimens were examined and classified according to the criteria of AJCC/UICC (American Joint Committee on Cancer / Union International Contre le Cancer) for TNM stage and histological type, and part of the tumor was frozen. Age and menopausal status, regional lymph node sta-

tus (N), tumor size (T), tumor grade (G) and histological type were obtained after the Institutional Review Board approval. In this retrospective study the course of disease was followed from surgical intervention till relapse to assess the relapse free survival (RFS). The median follow-up time was 61.5 months (range 4-171).

### ER, PR and HER2 estimation

Cytosol tumor extracts were prepared from frozen tumors with the following workflow: homogenization in 5 mM phosphate buffer pH 7.4-7.7 containing 20% glycerol, 1 mM monothioglycerol and 1.5 mM EDTA, centrifugation at 800-1,000 g for 30 min, followed by ultracentrifugation at 100,000 g for 60 min. The whole procedure was performed at +4°C. Tumor extract protein concentrations were assayed by the Lowry method. Hormone receptor concentrations were determined by the standard ligand-binding assay. ER levels  $\geq 10$  fmol/mg and PR levels  $\geq 20$  fmol/mg were considered as positive.

HER2 status (absence or presence of gene amplification) was determined on formalin-fixed, paraffin-embedded tumor tissue sections by chromogenic *in situ* hybridization (CISH), according to the manufacturer's instructions (Invitrogen SPOT-Light HER2 CISH Kit, USA). Hybridization results were evaluated at 40x and 100x magnification. One to 5 gene copies per nucleus were defined as no amplification, while more than 6 gene copies per nucleus or large gene copy clusters in more than 50% of tumor cells was defined as amplification.

### IL8, MMP2 and MMP9 estimation

IL8 levels were determined by ELISA in cytosol tumor extracts according to the manufacturer's instructions (RayBio Human IL8 ELISA kit, USA). MMP2 and MMP9 levels were determined by ELISA in cytosol tumor extracts according to manufacturer's instructions (Quantikine Human MMP2 and Quantikine Human MMP9 Immunoassay kits, USA). Cut-off values were selected according to observed median values.

### Statistics

*SigmaStat* software was used for data analyses. Survival curves for RFS were constructed according to the Kaplan-Meier method and compared with the log-rank test. The Mann-Whitney rank sum test was used to examine the distribution of quantitative levels of selected biomarkers (IL8, MMP2 and MMP9) between different subgroups of patients formed according to cut-off values of selected biomarkers and clinicopathological parameters. The correlations between steroid receptors and selected biomarkers were analyzed by the Spearman's rank order correlation test. A p value less than 0.05 was considered as statistically significant.

## Results

### Expression profiles

Patient's clinicopathological parameters at the time of primary diagnosis are presented in Table 1. There were no statistically significant differences (Mann-Whitney rank sum test) in the expression of IL8 between subgroups of patients formed according to patient's age, tumor size, nodal status and tumor grade. IL8 expression was significantly higher ( $p=0.03$ ) in patients with invasive ductal carcinoma (median IL8 value 11.10 pg/mg) compared to patients with invasive lobular carcinoma (median IL8 value 4.08 pg/mg). MMP2 expression was significantly higher ( $p=0.04$ ) in patients younger than 60 years (me-

**Table 1.** Patient clinicopathological parameters at the time of primary diagnosis

Clinicopathological parameters	n	%
Age (years)		
< 60	17	28.8
≥ 60	42	71.2
NA	0	0
Menopausal status		
premenopausal	0	0
postmenopausal	59	100
NA	0	0
Tumor size (cm)		
T < 2	31	52.5
T ≥ 2	25	42.4
NA	3	5.1
Nodal status		
N0	11	18.7
N+	36	61.0
NA	12	20.3
Histological type		
IDC	27	45.8
ILC	21	35.6
Other histologies	11	18.6
NA	0	0
Tumor grade		
G1	8	13.6
G2	39	66.1
G3	12	20.3
NA	0	0
Estrogen receptor status		
ER-	16	27.1
ER+	43	72.9
NA	0	0
Progesterone receptor status		
PR-	30	50.8
PR+	29	49.2
NA	0	0
HER2 status		
HER2-	35	59.3
HER2+	14	23.7
NA	10	17.0

n: number of patients, IDC: invasive ductal carcinoma, ILC: invasive lobular carcinoma, ER-: ER<10 fmol/mg, ER+: ER≥10 fmol/mg, PR-: PR<20 fmol/mg, PR+: PR≥20 fmol/mg, HER2-: HER2 gene not amplified, HER2+: HER2 gene amplified, NA: data not available

dian MMP2 value 13.83 ng/mg) compared to older patients (median MMP2 value 10.61 ng/mg). MMP2 expression was significantly higher ( $p=0.02$ ) in tumors less than 2 cm (median MMP2 value 14.18 ng/mg) compared to larger tumors (median MMP2 value 9.82 ng/mg). There were no significant differences in the expression of MMP2 between the subgroups formed according to nodal status, histological type and tumor grade. There were no statistically significant differences in the expression of MMP9 between subgroups of patients formed according to available clinicopathological parameters.

In Table 2 is presented the distribution of quantitative values of biomarkers (IL8, MMP2 and MMP9) between subgroups of patients formed according to cut-off (median) values of biomarkers (Mann-Whitney rank sum test). There was a significant difference ( $p=0.002$ ) in the expression of IL8 between subgroups of patients formed according to median MMP9 value ( $M=2.51$  ng/mg). IL8 expression was significantly higher in patients with higher MMP9 levels (median IL8 value 11.95 pg/mg) compared to patients with lower MMP9 levels (median IL8 value 4.08 pg/mg). There was a significant difference ( $p=0.001$ ) in the expression of MMP9 between the subgroups formed according to median IL8 value ( $M=9.15$  pg/mg). MMP9 expression was significantly higher in patients with higher IL8 levels (median MMP9 value 4.77 ng/mg) compared to patients with lower IL8 levels (median MMP9 value 1.34 ng/mg). Furthermore, a significant positive correlation was found between IL8 and MMP9 levels (Spearman rank order correlation test,  $p<0.001$ ).

### Expression profiles in association with ER/PR/HER2 status

There were no significant differences in the expression of IL8 between subgroups of patients formed according to ER/PR/HER2 status (Mann-Whitney rank sum test). There were no significant differences in the expression of MMP2/MMP9 between subgroups of patients formed according to ER/PR/HER2 status. A significant negative correlation was found between ER and IL8 levels (Spearman rank order correlation test,  $p=0.03$ ). There were no significant correlations between levels of hormone receptors and MMP 2 and 9.

### Survival analyses

There were no significant differences in RFS

**Table 2.** Distribution of quantitative values of biomarkers between subgroups of patients formed according to cut-off (median) values of biomarkers (Mann-Whitney rank sum test). Bold numbers indicate statistical significance

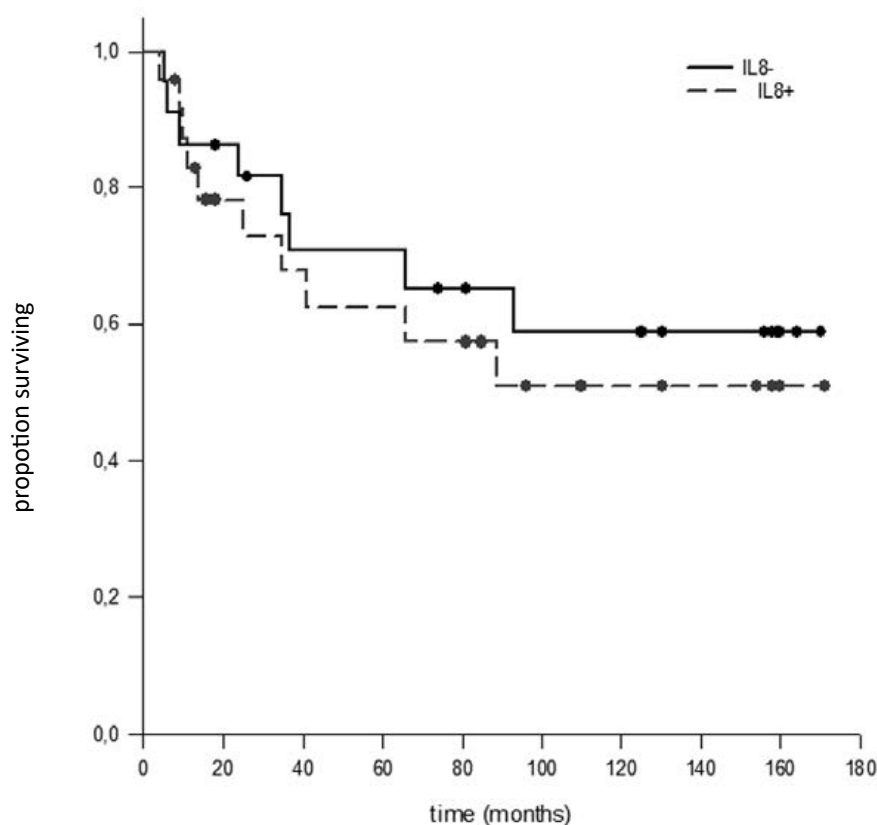
Biomarkers	M	n	Median IL8 values (pg/mg)	p	n	Median MMP9 values (ng/mg)	p	n	Median MMP2 values (ng/mg)	p
IL8										
IL8 < M	9.15	/	/	/	22	1.34	<b>0.001</b>	21	11.00	0.2
IL8 ≥ M					20	4.77		20	13.45	
MMP9										
MMP9 < M	2.51	21	4.08	<b>0.002</b>	/	/	/	19	10.79	0.2
MMP9 ≥ M		21	11.95					19	13.60	
MMP2										
MMP2 < M	11.55	20	3.97	0.2	18	1.22	0.08	/	/	/
MMP2 ≥ M		21	10.63		20	3.36				

M: median value, in pg/mg for IL8, in ng/mg for MMP9 and MMP2, n: number of patients, p: p value. Bold numbers indicate statistical significance

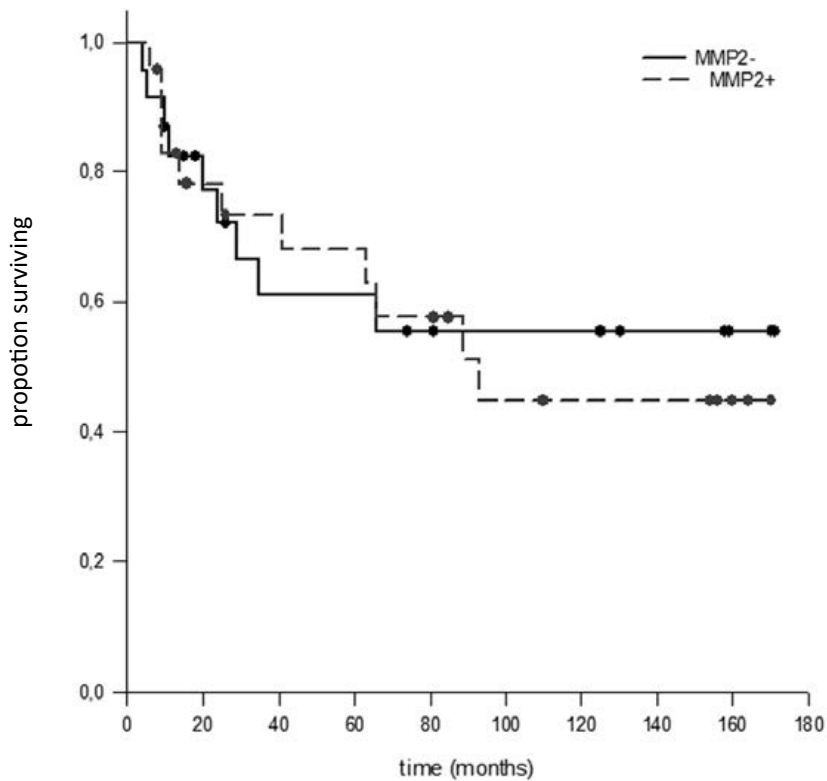
**Table 3.** Survival analyses for subgroups of patients formed according to median values of biomarkers

Biomarker	Number of patients	Number of relapses	Relapses (%)	RFS (months)	Median RFS (months)	p value (Log Rank)
IL8						
IL8-	22	8	36.4	5 - 170	77	0.6
IL8+	24	10	41.7	4 - 171	53	
MMP2						
MMP2-	23	9	39.1	4 - 171	35	0.8
MMP2+	24	11	45.8	6 - 170	64	
MMP9						
MMP9-	21	8	38.1	5 - 170	66	0.5
MMP9+	22	7	31.8	8 - 171	92	

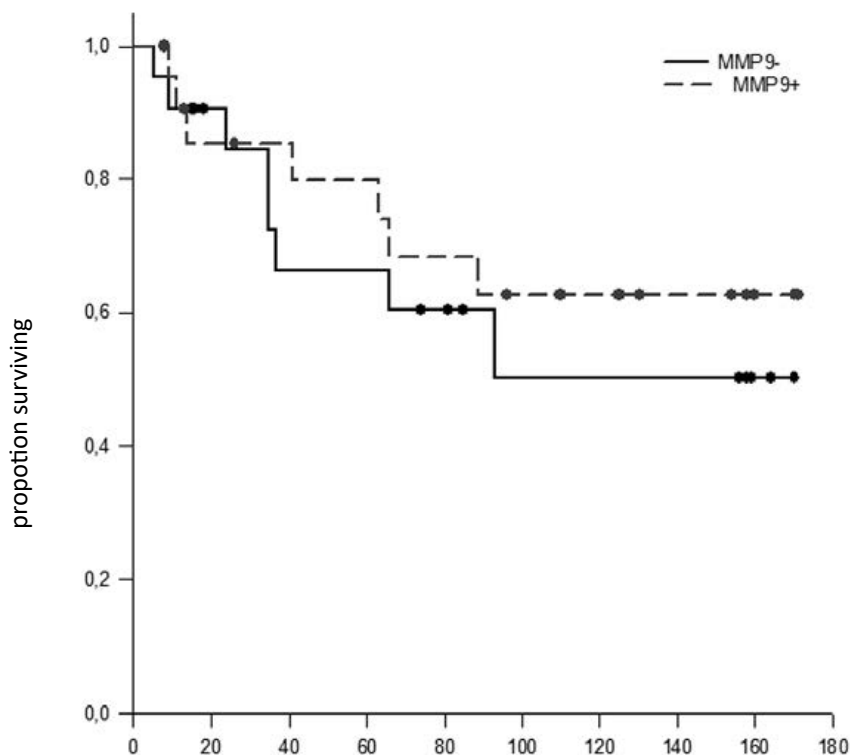
IL8-: IL8 < 9.15 pg/mg, IL8+: IL8 ≥ 9.15 pg/mg, MMP2-: MMP2 < 11.55 ng/mg, MMP2+: MMP2 ≥ 11.55 ng/mg, MMP9-: MMP9 < 2.51 ng/mg, MMP9+: MMP9 ≥ 2.51 ng/mg



**Figure 1.** Survival analysis for subgroups of patients formed according to median IL8 value (M=9.15 pg/mg) (p=0.6).



**Figure 2.** Survival analysis for subgroups of patients formed according to median MMP2 value (M=11.55 ng/mg) ( $p=0.8$ ).



**Figure 3.** Survival analysis for subgroups of patients formed according to median MMP9 value (M=2.51 ng/mg) ( $p=0.5$ ).

between subgroups of patients formed according to patient's age, tumor size, nodal status, histological type, tumor grade, PR status and HER2 status (Log rank test). There was a significant difference

in RFS between the subgroups formed according to ER status ( $p=0.04$ ). Patients with ER+ status had significantly longer median RFS compared to those with ER- status (63 and 50 months, respectively).

Survival analyses for subgroups of patients formed according to median values of biomarkers are presented in Table 3 and Figures 1, 2 and 3 (Log rank test,  $M=9.15$  pg/mg for IL8, range 0.19–1147.54 pg/mg;  $M=11.55$  ng/mg for MMP2, range 0.08–31.69 ng/mg;  $M=2.51$  ng/mg for MMP9, range 0.14–32.24 ng/mg). Patients with IL8– status had longer median RFS compared to those with IL8+ status (77 and 53 months, respectively) but the difference did not reach statistical significance. Patients with MMP9+ status had longer median RFS compared to those with MMP9– status (92 and 66 months, respectively), but the difference did not reach statistical significance. Furthermore, there was no significant difference in RFS between the subgroups formed according to MMP2 median value.

When comparing RFS of patients stratified in different phenotypes according to ER status and the median IL8 value ( $M=9.15$  pg/mg), there was a significant difference in RFS between patients with ER+IL8– and ER–IL8+ phenotypes, as well as between patients with ER+IL8+ and ER–IL8+ phenotypes (Log rank test,  $p=0.04$  and  $p=0.02$ , respectively). Patients with ER+IL8– phenotype had significantly longer median RFS compared to those with ER–IL8+ phenotype (77 and 19 months, respectively), and patients with ER+IL8+ phenotype had significantly longer median RFS compared to those with ER–IL8+ phenotype (83 and 19 months, respectively). When comparing RFS of patients stratified in different phenotypes according to PR/HER2 status and the median IL8 value, there were no statistically significant differences in RFS. When comparing RFS of patients stratified in different phenotypes according to ER/PR/HER2 status and the median MMP2/MMP9 value ( $M=11.55$  ng/mg for MMP2 and  $M=2.51$  ng/mg for MMP9), there were no statistically significant differences in RFS.

## Discussion

Literature has indicated that IL8 could be a biomarker of unfavorable prognosis and expression of IL8 in primary tumor tissue is expected to be in direct relation to unfavorable clinicopathological parameters. We found significantly higher expression of IL8 in patients with invasive ductal carcinoma (median IL8 value 11.10 pg/mg) compared to those with invasive lobular carcinoma (median IL8 value 4.08 pg/mg). This is consistent with the available literature according to which patients with invasive ductal carcinoma generally

had worse prognosis compared to those with invasive lobular carcinoma [15,16]. We also found significantly higher expression of MMP2 in patients younger than 60 years (median MMP2 value 13.83 ng/mg) compared to older patients (median MMP2 value 10.61 ng/mg) and MMP2 expression was significantly higher in tumors less than 2 cm (median MMP2 value 14.18 ng/mg) compared to larger tumors (median MMP2 value 9.82 ng/mg). These results support the hypothesis that MMP2 could be a biomarker of favorable prognosis. On the other hand, according to a widely accepted hypothesis, tumor growth and tumor progression/invasiveness are two independent pathways in the process of carcinogenesis [17].

The expression of IL8 was significantly higher in patients with higher MMP9 levels (median IL8 value 11.95 pg/mg) compared to patients with lower MMP9 levels (median IL8 value 4.08 pg/mg) (Table 2). Also, the expression of MMP9 was significantly higher in patients with higher IL8 levels (median MMP9 value 4.77 ng/mg) compared to patients with lower IL8 levels (median MMP9 value 1.34 ng/mg). Furthermore, a significant positive correlation was found between IL8 and MMP9 levels. Several studies have recently confirmed a significant positive relation between IL8 and MMP9 in different types of human cancers. Liu et al. found a significant positive correlation between IL8 and MMP9 levels in tumor tissue of non-small cell lung cancer patients [18]. They also found serum and tissue expression of IL8 and MMP9 remarkably higher in patients with lymph node metastasis compared to those without lymph node metastasis, and postulated that IL8 could facilitate the metastatic spread by up-regulating MMP9 [18]. Biasi et al. showed that serum levels of IL8 were increased from stage II in colorectal cancer patients, when also the enzymatic activity of MMP9 increased, and the increasing trend of the two serum markers was found significantly correlated [19]. There are only few studies related to this issue in breast cancer patients. We have recently found a significant positive relation between IL8 and MMP9 in adjuvantly untreated early breast cancer patients [20,21].

Several *in vitro* studies demonstrated that IL8 promoted the invasiveness by up-regulating MMP9 expression and activity. Inoue et al. showed that sense-transfected PC-3P prostate cancer cells overexpressed IL8-specific mRNA and protein, which resulted in up-regulation of MMP9 mRNA and collagenase activity and increased invasion [22]. Mian et al. showed that IL8 blockade by anti-

body significantly inhibited the expression and activity of both MMP2 and MMP9 in bladder cancer cells, which resulted in decreased invasion [23]. Wang et al. demonstrated that IL8 secretion by ovarian cancer cells increased anchorage-independent growth, angiogenic potential and invasion, and IL8-enhanced cell invasiveness correlated with increased expression and activity of MMP2 and MMP9 [24]. Shiau et al. showed that HPV E6 antigens significantly up-regulated IL8 expression, and increased the expression and activity of MMP2 and MMP9 in lung adenocarcinoma cells [25]. They further revealed that increased expression of MMP2 and MMP9 was mediated by the HPV E6-induced IL8 [25]. Mulayim et al. showed that IL8 promoted the invasiveness of endometrial stromal cells by up-regulating the expression and activity of MMP2 and MMP9 [26]. Van den Steen et al. demonstrated that MMP9 processed the most potent human neutrophil chemokine, IL8, into a 10–30-fold more active chemokine, which resulted in an important positive feedback loop, as IL8 induced the rapid release of MMP9 from the granules [27]. Chakrabarti and Patel demonstrated that IL8-induced MMP9 release from neutrophils was mediated through CXCR2 and involved two distinct pathways, one involving PKC and ERK1/2 and the other involving Src-family kinases [28]. A significant positive relation as well as correlation that we found in this study between IL8 and MMP9 levels indicate that possible existence of positive feedback between IL8 and MMP9 might contribute to progression of hormone-dependent breast cancer.

A significant negative correlation between ER and IL8 levels that we found indicates that expression of IL8 could be hormonally regulated in breast cancer. This is consistent with the available literature that found IL8 expression inversely related to ER status in breast tumor tissue [9,10,29] and breast cancer cells [30–32]. We previously postulated that considering the negative correlation between ER and IL8 and the effect of tamoxifen that blocks the effects of ER, there is a possibility that during the treatment period, consequently, tamoxifen could increase IL8 levels and therefore induce worse outcome of tamoxifen-treated patients [33].

No significant relations between levels of hormone receptors and MMP2 and MMP9 were noticed. This is consistent with a study by Rahko et al. who showed that MMP9 expression in breast tumor tissue was not significantly related to clinical stage, histology or hormone receptor status

[34]. On the other hand, La Rocca et al. investigated the activity levels of MMP2 and MMP9 in the sera of breast cancer patients and found an inverse correlation with regard to ER expression [35]. They also found that elevated activity levels of both MMP2 and MMP9 correlated with HER2 overexpression and postulated that HER2 could enhance signaling pathways that may lead to increased production of MMP2 and MMP9 [35]. Decock et al. found no relationship between plasma levels of MMP2 and MMP9, total or active, and clinicopathological parameters including hormone receptor status [36]. They only found active plasma MMP2 significantly increased in patients with HER2+ breast tumors [36]. Sullu et al. showed that MMP9 expression was significantly increased in high-grade, triple negative and ER-breast tumors, while MMP2 expression was significantly increased in ER- and high-grade breast tumors in the lymph node-negative group [12].

Among clinicopathological parameters, only ER proved to be a reliable parameter of response to tamoxifen. Patients with ER+ status had significantly longer median RFS compared to those with ER- status (63 and 50 months, respectively). This is consistent with the available literature according to which hormone receptors are still among the most reliable prognostic and predictive parameters of breast cancer, although the clinical outcome of patients and the response to tamoxifen are variable [37–40]. There are many possible causes of tamoxifen resistance, one of which could be hidden in the main mechanism of its action. By blocking the ER function, tamoxifen could influence the expression of ER-regulated growth factors and cytokines (ER+ cancer expression profile).

According to the available literature, IL8 has been identified as a biomarker of unfavorable prognosis in breast cancer [21,30,41,42] as well as a potential parameter of response to tamoxifen [9]. In our study IL8 showed a tendency to act as an unfavorable parameter of response to tamoxifen (Figure 1). Patients with IL8- status had longer median RFS compared to those with IL8+ status (77 and 53 months, respectively) but the difference did not reach statistical significance.

Literature related to prognostic and predictive value of MMP2 and 9 in breast cancer are controversial. Many studies identified MMP2 and MMP9 as biomarkers of unfavorable prognosis, and patients with elevated expression of MMP2 and/or MMP9 in breast tumor tissue had significantly shorter overall survival (OS) and RFS

[12,43-45]. A meta-analysis of published studies that included 2,400 breast cancer patients showed that MMP9 overexpression had an unfavorable impact on both OS and RFS [46]. Several studies indicated that tumor tissue MMP2 and MMP9 could be parameters of response to hormonal therapy in locally advanced breast cancer [34,47]. On the other hand, there are studies that indicate that MMP2 and MMP9 could be biomarkers of favorable prognosis. Kuvaja et al. showed that serum levels of the total proMMP2 correlated inversely with tumor burden, and found an association between lower levels of free active MMP2 and tumor recurrence [48]. Scorilas et al. showed that patients with elevated expression of MMP9 in breast tumor tissue had significantly longer OS and RFS [49]. Pellikainen et al. found that independent predictors of shorter RFS were HER2 overexpression, advanced-stage disease and reduced MMP9 expression in breast carcinoma cells [50]. In our study MMP9 showed a tendency to act as a favorable parameter of response to tamoxifen (Figure 3). Patients with MMP9+ status had longer median RFS compared to those with MMP9- status (92 and 66 months, respectively) but the difference did not reach statistical significance. The role of MMP2 as a potential predictive parameter is more complex. MMP2 seems to act as a favorable parameter of response to tamoxifen in the time interval of 90 months after operation, but after that time interval the trend of MMP2 changed (Figure 2).

We found that patients with ER+IL8- phenotype had significantly longer median RFS compared to patients with ER-IL8+ phenotype (77 and 19 months, respectively) and the number of relapses in ER+IL8- subgroup was 31.2 vs 75% in the ER-IL8+ subgroup. This indicates that ER as a favorable parameter and IL8 as an unfavorable parameter (which is probably downregulated by ER), could influence the response to hormonal therapy

in an additive manner. Furthermore, patients with ER+IL8+ phenotype had significantly longer RFS compared to patients with ER-IL8+ phenotype (83 and 19 months, respectively) and the number of relapses in ER+IL8+ subgroup was 25 vs 75% in the ER-IL8+ subgroup. This indicates that ER status could help to stratify breast cancer patients with higher IL8 levels into low-risk and high-risk subgroups.

## Conclusion

According to our results, IL8 showed a tendency to act as an unfavorable parameter while MMP9 showed a tendency to act as a favorable parameter of response to tamoxifen, whereas the role of MMP2 as a potential predictive parameter is more complex. Furthermore, ER as a favorable parameter of response to tamoxifen and IL8 as an unfavorable parameter, could influence the treatment response in an additive manner. Our results indicate that expression of IL8 could be hormonally regulated, and that possible existence of positive feedback between IL8 and MMP9 might contribute to progression of hormone-dependent breast cancer. However, we must refrain and point that these results deserve further investigation, since this study had small and unequal numbers of observations in the subgroups.

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## Conflict of interests

The authors declare no conflict of interests.

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