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Coffee prevents early events in tamoxifen-treated breast cancer patients and modulates hormone receptor status

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Abstract

Purpose: Whether coffee modulates response to endocrine therapy in breast cancer patients is currently unknown. The CYP1A2 and CYP2C8 enzymes contribute to tamoxifen and caffeine metabolism. The purpose was to investigate the impact of coffee consumption on tumor characteristics and risk for early events in relation to breast cancer treatment and *CYP1A2* and *CYP2C8* genotypes. *Methods:* Questionnaires regarding lifestyle were completed preoperatively by 634 patients in Southern Sweden. *CYP1A2*1F* and *CYP2C8*3* were genotyped. Clinical data and tumor characteristics were obtained from patients' charts, population registries, and pathology reports. Coffee consumption was categorized as low (0-1 cups/day), moderate (2-4 cups/day), or high (5+cups/day). *Results:* The proportion of estrogen receptor negative (ER–) tumors increased with increasing coffee consumption (P_{trend}=0.042). Moderate to high consumption was associated with lower frequency of discordant receptor status (ER+PgR–) OR 0.38 (0.23-0.63) compared to low consumption. Median follow-up time was 4.92 (IQR 3.01-6.42) years. Tamoxifen-treated patients with ER+ tumors (n=310) who consumed two or more cups/day had significantly decreased risk for early events compared to patients with low consumption, adjusted HR 0.40 (0.19-0.83). Low consumption combined with at least one *CYP1A2*1F* C-allele (n=35) or *CYP2C8*3* (n=13) was associated with a high risk for early events in tamoxifen-treated patients compared to other tamoxifen-treated patients, adjusted HRs 3.49 (1.54-7.91) and 6.15 (2.46-15.36), respectively. *Conclusion:* Moderate to high coffee consumption was associated with significantly decreased risk for early events in tamoxifen-treated patients and modified hormone receptor status. If confirmed, new recommendations regarding coffee consumption during tamoxifen-treatment may be warranted.

Keywords**:** breast cancer, estrogen receptor, progesterone receptor, coffee, CYP1A2, CYP2C8

Introduction

In 2010, 7917 women were diagnosed with breast cancer in Sweden, making breast cancer the most common cancer to affect women in Sweden [1]. Here, the reported frequency of breast cancers expressing estrogen receptors (ER) ranges from 66.0% to 86.1% [2,3]. Patients with ER+ tumors are usually treated with endocrine therapy to reduce the proliferation of tumor cells. However, 40-50% of the tumors expressing estrogen and progesterone receptors (PgR) are insensitive to endocrine therapy [4,5]. Treatment effect may be influenced by host and tumor genetic factors, and medications, as well as environmental factors, including lifestyle factors such as coffee consumption. A recent study reported that coffee reduced the risk for aggressive ER– tumors in a Swedish cohort [6]. However, whether coffee consumption is associated with modulated ER/PgR status and the response to endocrine therapy is currently unknown. More knowledge about the interaction between lifestyle factors and different breast cancer treatment is needed.

According to the European Coffee Report 2009, Swedes are the third largest coffee consumers in Europe, with an average individual consumption of 3.4 cups/day. Less than 1% of the coffee consumed in Sweden is decaffeinated [7]. Coffee contains several bioactive ingredients such as the phenolics caffeic acid and chlorogenic acid [3], caffeine, and phytoestrogens [8]. The different ingredients in coffee have both carcinogenic and anti-carcinogenic effects, which may influence the prognosis of breast cancer. For example, caffeine has been shown to inhibit mitosis and induce cell differentiation [9]. Daily coffee consumption in 173,141 women who were 50 to 71 year old at baseline was strongly associated with lower total mortality, but not for death due to any type of cancer [10]. However, breast cancer specific mortality was not investigated.

In premenopausal women, selective estrogen receptor modifiers (SERMs) such as tamoxifen are mainly used as endocrine treatment [11]. Tamoxifen deactivates the ER and inhibits the proliferation of tumor cells [12]. CYP2D6 is the key enzyme metabolizing the pro-drug tamoxifen into its metabolites 4-hydroxy tamoxifen, 4-desmetyl tamoxifen and its most active metabolite, endoxifen [13,14]. The CYP1A2 and CYP2C8 enzymes also contribute to tamoxifen metabolism [15,16] as well as the metabolism of caffeine [17], while SULT1A1 has been proposed to be involved in sulfation of 4-hydroxytamoxifen and may also play a role in endoxifen clearance [13]. In a subset of the present study cohort, it has previously been shown that moderate to high coffee intake modulates ER status in women with the *CYP1A2*1F* A/A genotype, but not in those with the *CYP1A2*1F* C-allele [18]. The CYP1A2 enzyme is more inducible by coffee in *CYP1A2*1F A/A* carriers [19,20]. It is

therefore plausible that tamoxifen response is influenced by the *CYP1A2*1F* genotype and coffee intake and that any effect of coffee may be dependent on the *CYP1A2*1F* genotype. Similarly, *CYP2C8*3* genotypes have previously been shown to be associated with an increased risk for early events in a subset of tamoxifen-treated patients from the present cohort [21]. Tamoxifen response may therefore also be influenced by *CYP2C8* genotype and coffee intake.

The aims of this study were to investigate the impact of coffee consumption on tumor and patient characteristics and risk for early events in relation to breast cancer treatment and different genotypes of *CYP1A2* and *CYP2C8*.

Materials and Methods

Study Population

As of October 2002, women diagnosed with a first breast cancer at the Lund University Hospital in Sweden were invited preoperatively to participate in an ongoing cohort. The overall aims are to study factors that may be of prognostic or predictive value. Patients with a prior history of breast cancer or another cancer diagnosis within the last ten years were not invited to participate. There was no question on ethnicity, but the vast majority was ethnic Swedes. This paper is based on data collected from 634 patients between October 2002 and October 2008. The patients were asked to fill out questionnaires preoperatively, on clinical follow-up visits after three to six months, one, two and three years, and then at home at five, seven and nine years postoperatively. The follow-up rates of the patients without preoperative treatment included in the study and who were alive and breast cancer-free were as follows for the 9-year, 7-year, 5-year, and 3-year: 80.0%, 86.3%, 92.6%, and 93.5%, respectively. Patients who had received preoperative treatment (n=42, including one patient with missing information regarding interstitial laser thermotherapy and one patient who received treatment for another cancer between the initial surgery and reoperation) were excluded from analyses regarding tumor characteristics and risk for early events. A flowchart of patients included in the different analyses is presented in *fig 1*. Written informed consents were obtained from all patients. This study was approved by the ethics committee of the Lund University (Dnr 75-02 and 37-08).

During the preoperative visit, body weight, height, waist and hip circumferences, and breast volumes were measured. The measurement of the breast volume has previously been described [22]. Body mass index (BMI) was calculated as weight in kilograms divided with height in meters squared. Waist to hip ratio (WHR) was calculated as waist circumference divided with hip circumference. The questionnaires included questions regarding birth date, date of surgery, reproductive history, and use of exogenous hormones. Lifestyle factors included smoking (yes/no/occasional smoker), alcohol, and coffee consumption. Alcohol consumption was reported in terms of frequency and the number of drinks consumed during the past week. In regards to coffee intake, the questionnaire provided nine consumption levels ranging from 0 to 8+ cups of coffee a day. The definition of a cup was not specified.

Information regarding type of adjuvant treatment, sentinel node biopsy, axillary node dissection and type of surgery was collected from each patient's chart. Treatment was registered up to the last recurrence-free follow-up. Data on tumor size, histological type and grade, and involved axillary lymph nodes were obtained from each patient's pathology report. ER- and PgR-status were determined by means of immunohistochemistry using the Dako LSAB™ kit system (Dako, Glostrup, Denmark) [18]. Tumors with >10% positive nuclear staining where considered ER+ or PgR+. The tumors were analyzed at the Department of Pathology at Lund University Hospital.

Information concerning breast cancer events, including local or regional recurrence, new breast cancer, or distant metastases, was obtained from patient charts, pathology reports and the Regional Tumor Registry. Date of death was obtained from the Population Registry.

Genotyping

Genomic deoxyribonucleic acid (DNA) was extracted from the leukocyte portion of the whole blood using the Wizard Genomic DNA Purification Kit (Promega, Madison, USA). The genotyping was performed at the Region Skåne Competence Centre (RSKC Malmö), Malmö University Hospital, Malmö, Sweden. The two *CYP2C8*3* SNPs (rs11572080 and rs10509681) were analyzed with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry on a Sequenom MassARRAY® platform (Sequenom, San Diego, CA, USA), using iPLEX reagents according to the manufacturers' protocol. The Sequenom MassARRAY® software was used for multiplex SNP analysis design. The concordance was 100% between the two *CYP2C8*3* SNPs. The *CYP1A2*1F* (rs762551) SNP was genotyped using a Taqman SNP allelic discrimination assay in 384-well format on ABI PRISM 7900 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Nineteen patients were not successfully genotyped for the *CYP1A2*1F* (rs762551) SNP using TaqMan. For 16 of these patients, rs762551 genotypes were available from DNA sequencing from the previous study [18]. The concordance rate between the two methods was 99.8%.

Data Analyses

The statistical analyses were performed with IBM SPSS Statistics 19.0 (New York, USA). The patients who considered themselves smokers or party smokers were grouped as current smokers. Patient characteristics such as age at diagnosis, BMI, and WHR were analyzed in relation to daily coffee intake at the preoperative visit. As

there were few patients with 0 cups/day and 1 cup/day these groups were combined. The consumption was categorized as low (0-1 cup), moderate (2-4 cups) or high (5+ cups). Kruskal-Wallis tests were used since these variables were not normally distributed. The moderate and high coffee consumers were combined into a group called "moderate to high" and analyzed with Mann-Whitney U-test. Chi-square analyses were used to investigate the relationship between the categorical variables coffee (0-1, 2-4, 5+ or 2+ cups) and parous (yes/no), ever use of oral contraceptives (yes/no), ever use of hormone replacement therapy (yes/no), current smoking (yes/no), alcohol abstaining (yes/no), pT (0-4), histological grade (I-III), axillary node involvement (0, 1-3, 4+), and hormone receptor status (ER+/ER– and PgR+/PgR–) as well as (ER+/PgR+, ER+/PgR–, ER–/PgR–, ER–/PgR+).

For the analyses of risk for early events, patients were followed from inclusion to last follow-up, any breast cancer event or death from non-breast cancer related causes prior to January 1st 2012, whichever came first. Patients with carcinoma *in situ* (n=14) and patients with metastases detected earlier than three months after study inclusion (n=2) were excluded from the survival analyses. After exclusion, 73 patients were diagnosed with some type of breast cancer event during the nine-year follow-up, out of whom 46 patients had distant metastases.

Kaplan-Meier LogRank was used to compare risk for early events in relation to coffee consumption in different treatment groups. Patients were stratified according to whether or not they had been treated with chemotherapy, radiation therapy, or endocrine therapy prior to the first breast cancer event, last follow-up, or death. Treatment after any breast cancer event was not considered. Since few of the patients had invasive tumor sizes pT3 or pT4 (skin or muscular involvement independent of size), these patients were combined with pT2 group in the survival analyses. Cox regression was used to analyze risk for early events in relation to coffee, adjusting for age above median age at diagnosis (60+ years), invasive tumor size (>20mm or pT4), axillary lymph node involvement (yes/no), histological grade III (yes/no), and ER status.

In the survival analyses, *CYP*-genotypes and different levels of coffee consumption were first analyzed as independent variables. Secondly, two interaction variables were created to analyze whether there were any gene-environment interaction between having at least one minor allele and a low coffee consumption on the risk for early events.

A *P*-value <0.05 was considered significant. All *P*-values were two-tailed. Nominal *P*-values were presented without adjustments for multiple testing.

Results

Patient Characteristics

The preoperative patient characteristics are presented in table 1. Age ranged from 25 to 99 years. The median age at diagnosis among the 634 patients was 59.6 years. Patients with moderate coffee consumption were older than patients with low or high coffee consumption (P<0.0001). Current smoking was significantly associated with increasing coffee consumption (P_{trend}<0.0001) and the proportion of alcohol abstainers decreased with increasing coffee intake (P_{trend}=0.001).

Tumor Characteristics

The tumor characteristics in relation to coffee intake are listed in table 2. Moderate to high consumption was associated with smaller tumors (P=0.026). The proportion of ER+ tumors decreased with increasing consumption (P_{trend}=0.042), while the proportion of PgR+ tumors was higher in patients with moderate to high consumption compared to patients with low consumption (P=0.047). The proportion of tumors with discordant receptor status was less than half in patients with moderate to high consumption compared to patients with low consumption (OR 0.38; 95% CI 0.23-0.63; P<0.0001)._Other tumor characteristics were not significantly associated with coffee consumption.

Coffee Consumption Variation between Visits

Coffee consumption at the preoperative visit correlated strongly with the consumption at the follow-up visits. Compared to the preoperative coffee consumption, the frequency of patients who still consumed 2+ cups/day ranged from 88.8% to 92.0% at the follow-up visits.

Coffee and Breast Cancer-Free Survival

Among the 574 patients with invasive tumors who had not received any preoperative treatment, who had no early distant metastases, and for whom data on preoperative coffee consumption was available, the median follow-up time was 4.92 (IQR 3.01-6.42) years. Coffee intake was not associated with median duration of either tamoxifen or aromatase inhibitor (AI) use.

Overall, moderate to high coffee consumption was non-significantly associated with lower risk for early events (LogRank; P=0.19), adjusted HR 0.65 (95% CI 0.36-1.17; P=0.15), adjusted for invasive tumor

size, axillary lymph node involvement, histological grade, age, and ER status. Moderate to high coffee consumption was also associated with a lower risk for early events in chemotherapy-treated patients (LogRank; *P*=0.070), adjusted HR 0.36 (95%CI 0.13-0.99; *P*=0.047), and a non-significantly decreased risk for early events in radiation therapy treated patients compared to patients with low coffee consumption (LogRank; *P*=0.25), adjusted HR 0.57 (95% CI 0.27-1.18; *P*=0.13).

After stratification according to ER status, moderate to high coffee consumption was non-significantly associated with a lower risk for early events compared to low coffee consumption in patients with ER– tumors (LogRank; *P*=0.21), adjusted HR 0.39 (95% CI 0.11-1.44; *P*=0.16) and in patients with ER+ tumors (LogRank; *P*=0.18), adjusted HR 0.73 (95% CI 0.38-1.39; *P*=0.34).

After stratification according to type of endocrine treatment, moderate to high coffee consumption was non-significantly associated with an increased risk for early events among patients with ER+ tumors who did not receive tamoxifen (LogRank; *P*=0.15), *fig. 2a*. Coffee consumption was not associated with risk for early events, neither in patients with ER+ tumors who did not receive any endocrine treatment (LogRank *P*=0.32) nor in patients who had been treated with AIs with or without tamoxifen (LogRank *P*=0.59). In patients who had only received AIs, all nine events appeared in the 54 patients with moderate to high coffee intake, while no events appeared in the 10 patients with low coffee consumption. However, the association between early events and coffee intake was not significant (LogRank *P*=0.28).

For the 310 patients with invasive ER+ tumors who had been treated with tamoxifen and for whom data on coffee consumption was available, moderate to high coffee consumption at the preoperative visit was significantly associated with a lower risk for early events (LogRank; *P*=0.002), *fig. 2b*. In the multivariate model including the tamoxifen-treated patients, moderate to high coffee consumption was significantly associated with less than half the risk for early events compared to patients with low consumption, adjusted HR 0.40 (95%CI 0.19-0.83; *P*=0.015). In this model, coffee consumption was the strongest factor for early events. Adjustments were also made for BMI, HRT, smoking, and alcohol abstinence. None of these variables materially changed the association between coffee consumption and early breast cancer events. Similarly, after stratification according to PgR status, the association remained significant in both groups (LogRank; *P*=0.015). After excluding patients who had been treated with both AI and tamoxifen, the association also remained significant (LogRank: *P*=0.024).

Moderate to high coffee consumption was also associated with a lower risk for early distant metastases compared to low coffee consumption in tamoxifen-patients with ER+ tumors (LogRank: *P*=0.006), adjusted HR 0.41 (95% CI 0.17-1.00; *P*=0.05).

CYP1A2 and CYP2C8 Genotypes and Coffee Consumption in Tamoxifen-Treated Patients

The *CYP1A2*1F* genotype distribution in the 631 patients where this information was available was as follows: A/A (50.9%), A/C (40.6%), and C/C (8.6%). The *CYP1A2*1F* A/A genotype was not associated with risk for early events in the tamoxifen-treated patients with ER+ tumors (LogRank; *P*=0.62). The combined effect of the *CYP1A2*1F* genotype and coffee consumption on the risk for early events was assessed in the following four groups: *CYP1A2*1F* A/A with low coffee intake, *CYP1A2*1F* any C-allele with low coffee intake, *CYP1A2*1F* A/A with moderate to high coffee intake, and *CYP1A2*1F* any C-allele with moderate to high coffee intake. There was no significant difference in risk for early events between the following three groups: *CYP1A2*1F* A/A with low coffee consumption, *CYP1A2*1F* A/A with moderate to high coffee consumption, and *CYP1A2*1F* any C-allele with moderate to high coffee consumption (LogRank; *P*=0.19). However, in the 35 patients with at least one *CYP1A2*1F* C-allele and low coffee consumption, a significantly higher risk for early events compared with the other tamoxifen-treated patients with ER+ tumors was observed (LogRank; *P*=0.002), *fig. 3*, adjusted HR 3.49 (95%CI: 1.54-7.90; *P*=0.003).

The *CYP2C8*3* genotype distribution in the 634 patients where this information was available was as follows: *1/*1 (82.5%), *1/*3 (16.6%), and *3/*3 (0.9%). Increasing number of *CYP2C8*3* alleles was associated with increased risk of early events in the 310 tamoxifen-treated patients with ER+ tumors (LogRank; *P*=0.019), adjusted HR 2.24 (95%CI 1.12-4.47; *P*=0.022) per allele. Again, the combined effect of *CYP2C8* genotypes and coffee consumption was assessed in four groups. There were no differences in the risk for early events between the following three groups: *CYP2C8**1/*1 and low coffee intake, *CYP2C8**1/*1 and moderate to high coffee intake, and patients with any *CYP2C8**3 allele and moderate to high coffee intake (LogRank; *P*=0.39). However, in the 13 patients with any *CYP2C8*3* allele and low coffee consumption, the risk for an early event was significantly higher compared to the other tamoxifen-treated patients (LogRank; *P*<0.0001), *fig. 4*, adjusted HR 6.15 (95%CI: 2.46-15.36; *P*=0.0001).

Discussion

The main finding of this study was that coffee consumption was associated with a significantly decreased risk for early breast cancer events in tamoxifen-treated patients with ER+ tumors. However, the long-term effects of coffee consumption are unknown since patients with ER+ breast cancer tend to experience later events than patients with ER– tumors [23]. Moreover, coffee consumption was significantly associated with modulated hormone receptor status including a higher frequency of ER– and a lower frequency of ER+PgR– tumors. ER+PgR– breast cancers respond less well to tamoxifen than ER+PgR+ tumors [24,25]. However, coffee consumption was associated with a lower frequency of early events in tamoxifen-treated patients with ER+PgR– as well as ER+PgR+ tumors.

Caffeine has been shown to inhibit Akt phosphorylation [26]. Phosphorylated Akt (pAkt) down-regulates PgR levels and activity and, in addition, induces ER ligand independent activity [27]. These results are consistent with the lower frequency of tumors with discordant ER and PgR status we found among patients with a moderate to high coffee consumption. The reduced clinical response to tamoxifen among patients with lower coffee consumption may result from a higher level of pAkt and thus a higher level of ligand independent ER activity. However, a recent study showed that MCF-7 cells that were treated with caffeine showed a 4-fold increase in the PgR mRNA expression [28]. Further mechanistic studies are needed to investigate how coffee influences hormone receptor expression and activity in breast cancer.

A higher frequency of ER– tumors was associated with increasing coffee consumption in the present study. In contrast, a recent Swedish case-control study by Li *et al*. [6] found that postmenopausal patients with a consumption of five or more cups/day had a lower frequency of ER– tumors compared to patients with a lower coffee consumption. One reason for the difference between the results of the present study and the Li *et al*. study may be that the present study also included premenopausal and postmenopausal patients. ER– tumors are more common among premenopausal women [29]. However, the association between a moderate to high coffee consumption and a higher frequency of ER– tumors in the present study became even stronger after adjustment for age (data not shown). Hormone receptor status was only available for 65.4% of the patients in the study by Li *et al*., versus 97.9% in the present study. Coffee may be associated with smaller tumor size [18] and lack of

tumor material for receptor analyses may have biased the association between coffee intake and ER status in the study by Li *et al*. [6].

Another mechanism for the decreased risk of early events among coffee drinkers may be that coffee induces an increase in the plasma ratio of 2OHE/16αOHE [30,31], which was also found among tamoxifen-treated patients in a pilot study of the present cohort [32]. Tamoxifen metabolites deactivate the ER, but it is possible that tamoxifen is unable to deactivate all tumor ERs. A higher plasma ratio of 2OHE/16αOHE may thus be favorable since 2OHE is a weak estrogen or may even act as an anti-estrogenic substance [33], while 16αOHE is a potent estrogen with procarcinogenic effects and high affinity for the ER [34]. A decreased level of 16αOHE, mediated by coffee, may thus lead to a better effect of tamoxifen. Thibodeau found in 1998 that methotrexate resistance in MCF-7 breast cancer cells was inducted 88-fold by 16αOHE and 33-fold by 2OHE [35]. The effect of coffee on the response to chemotherapy may similarly be mediated through alternation in the 2OHE/16αOHE ratio.

Moreover, CYP1A2 is a key enzyme in the 2-hydroxylation of estrone and estradiol [36], in the metabolism of caffeine [37], and it contributes to tamoxifen metabolism [15]. CYP2C8/9 also contributes to the metabolism of caffeine [17] and tamoxifen [16]. *CYP2C8*3* genotypes, which may influence CYP2C8 enzyme metabolic activity [38], have previously been associated with a higher risk for early breast cancer events in tamoxifen-treated patients, in a subset of the present cohort [21]. Intake of caffeinated coffee may thus result in increased activation of tamoxifen via CYP1A2 or CYP2C8/9, a hypothesis consistent with our finding of fewer early events in tamoxifen-treated patients with ER+ tumors and moderate to high coffee consumption. Moreover, others have reported that carriers of the *CYP1A2*1F A/A* genotype but not the *CYP1A2*1F* C-allele who consume 3+ cups/day have a higher inducibility of CYP1A2 compared to non-consumers [20]. This may explain the finding of an association between low coffee consumption in combination with at least one *CYP1A2*IF* C-allele or *CYP2C8*3* allele with an over 3-fold risk for early breast cancer events in tamoxifen-treated patients with ER+ tumors.

Coffee consumption was associated with several potential confounders. Coffee intake and smoking have been shown to be associated [10,39]. In the present study, current smoking was significantly associated with increasing coffee consumption, but not associated with early events. Increasing coffee consumption in the study population was also significantly associated with a decreasing frequency of alcohol abstainers. After adjustments

for alcohol and smoking in the multivariate models, the association between coffee and risk for early events remained essentially the same. Use of HRT prior to diagnosis in this population was not significantly associated with either coffee consumption or risk for early events. Some studies have previously reported that HRT increases disease-free survival [40-42], while others have reported a higher mortality rate [42-44]. None of these potential confounders could explain the results of this study. A recent publication based on this cohort reported that poor adherence was associated with early events [45]. However, coffee intake was not associated with duration of endocrine therapy and could thus not explain the results.

The coffee variable and other potential risk factors were self-reported and may thus constitute an error in measurement. The definition of cup size was not further described, but the lack of precision regarding the actual coffee intake cannot explain the findings, since imprecision would bias the results towards the null hypothesis. However, the median coffee consumption in the study population was similar to the average coffee consumption in Sweden [7]. The questionnaire did not include a question as to whether the coffee was caffeinated or decaffeinated. However, only a minority (1%) of the coffee consumed in Sweden is decaffeinated [7]. When reported coffee intake at the follow-up visits was used as the exposure variable, the association between coffee intake and risk for early events in tamoxifen-treated patients remained significant (data not shown). Since the correlation between coffee consumption during the preoperative and follow-up visits was high, preoperative coffee intake reliably predicted postoperative coffee consumption. As there were few patients with no coffee consumption, zero cups/day group would have been a suboptimal reference group, especially for subgroup analyses. Instead, a 0-1 cup/day coffee consumption group was used as reference. The results for the groups with no coffee consumption and 1 cup/day were overlapping. Similarly, the survival rates for the 2-4 cups/day and 5+ cups/day were completely overlapping in the survival analyses (data not shown).

The Skåne University Hospital in Lund serves almost 300 000 inhabitants. Since patients are not referred to other hospitals for surgery, the study is population-based. During the time the cohort was compiled, 1090 patients went through breast cancer surgery. Approximately 58% of these patients were included in the study. Patients were mostly missed due to lack of available research nurses. However, included patients were similar to non-included patients regarding age and hormone receptor status. The follow-up rates were high. The overall response rate among patients who were alive and event-free was somewhat lower in patients with moderate to high coffee intake compared to low coffee intake (data not shown). Since data on recurrences, new cancers and

deaths were retrieved from clinical charts and Swedish Registries (which are virtually 100% complete), this means that there was a proportionally larger loss of patients that were still in remission in the group with moderate to high coffee intake. The difference in follow-up rates may therefore have biased the results towards the null. Clinical data and date of death were obtained from patients´ charts and the population registry and thus reliable. Therefore, the findings of this study are considered generalizable to breast cancer patients in Sweden. However, the follow-up time was approximately five years, and long-term effects of coffee consumption in breast cancer patients could not be evaluated.

Conclusion

The main finding of this study was that tamoxifen-treatment combined with moderate to high coffee consumption was associated with less than half the risk of an early breast cancer event compared with low consumption. Furthermore, low coffee consumption combined with any *CYP1A2*1F* C-allele or *CYP2C8*3* was associated with an over 3-fold risk for an early breast cancer event in tamoxifen-treated patients. These patients may benefit from more personalized treatment. We also found a strong association between moderate to high coffee consumption and hormone receptor status, which suggests that coffee may influence the expression of both ER and PgR and modulate the relationship between the two. If the main finding of this study is confirmed in an independent cohort, patients should be informed of the potential benefits of combining coffee consumption with tamoxifen-treatment.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Figure Legends

Fig. 1 Flow chart of patients included in the different analyses.

Fig. 2a Coffee consumption was not significantly associated with risk for early events among patients who did not receive tamoxifen treatment (LogRank 1 df; P=0.15).

Fig. 2b Tamoxifen-treated patients with ER+ tumors and moderate to high coffee consumption had a significantly lower risk for early events, compared to patients with low coffee consumption (LogRank: 1 df; P=0.002), adjusted HR 0.40 (95% CI 0.19-0.83; P=0.015). As this is an ongoing cohort there are fewer patients with longer follow-up times.

Fig. 3. In the 35 patients with at least one *CYP1A2*1F* C-allele and low coffee consumption, a significantly higher risk for early events compared with the other tamoxifen-treated patients with ER+ tumors was observed (LogRank; *P*=0.002), adjusted HR 3.49 (95%CI: 1.54-7.90; *P*=0.003). As this is an ongoing cohort there are fewer patients with longer follow-up times.

Fig. 4. In the 13 patients with any *CYP2C8*3* allele and low coffee consumption, the risk for an early event was significantly higher compared to the other tamoxifen-treated patients (LogRank; *P*<0.0001), adjusted HR 6.15 (95%CI: 2.46-15.36; *P*=0.0001). As this is an ongoing cohort there are fewer patients with longer follow-up times.

Figure 1

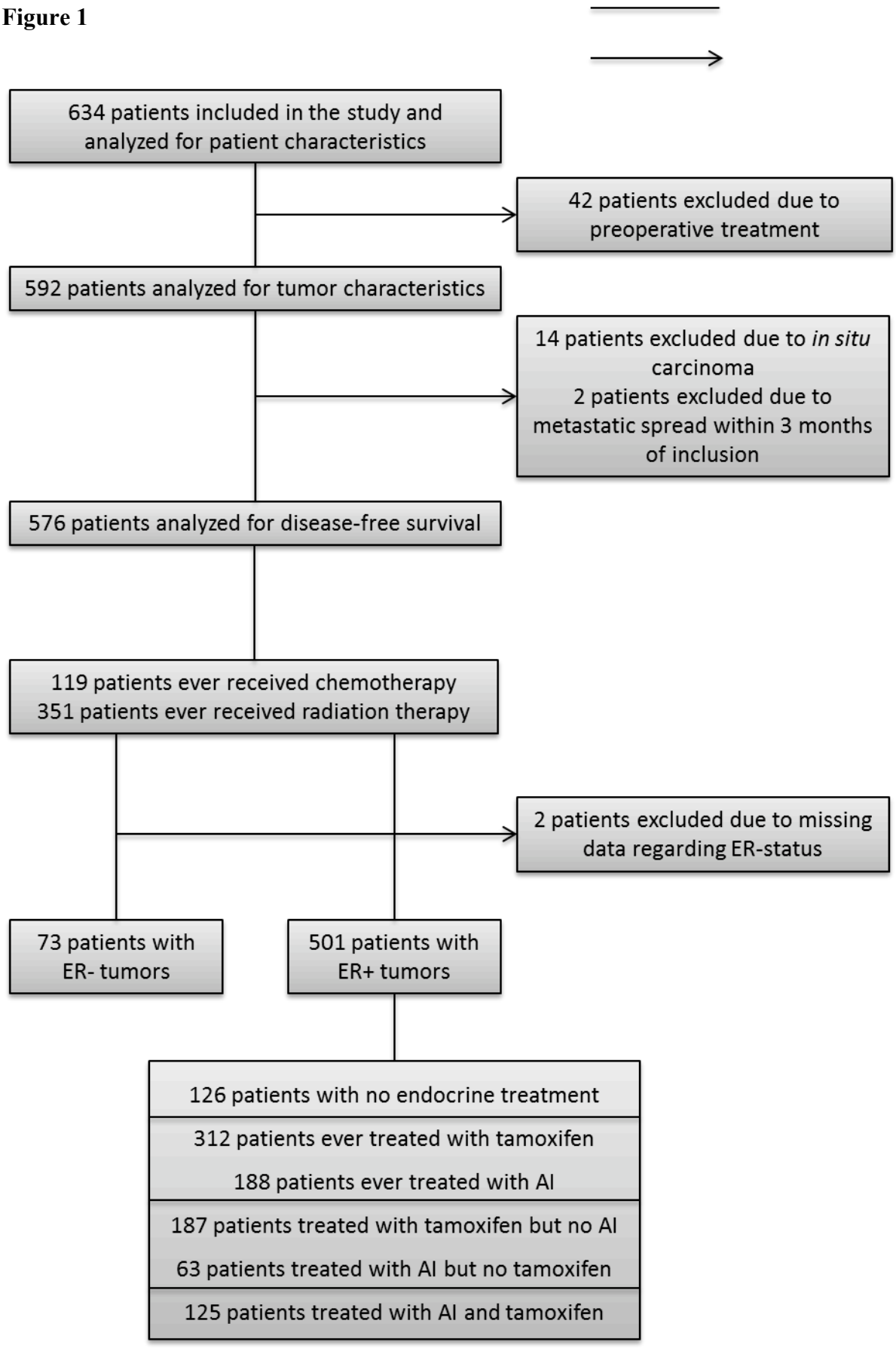
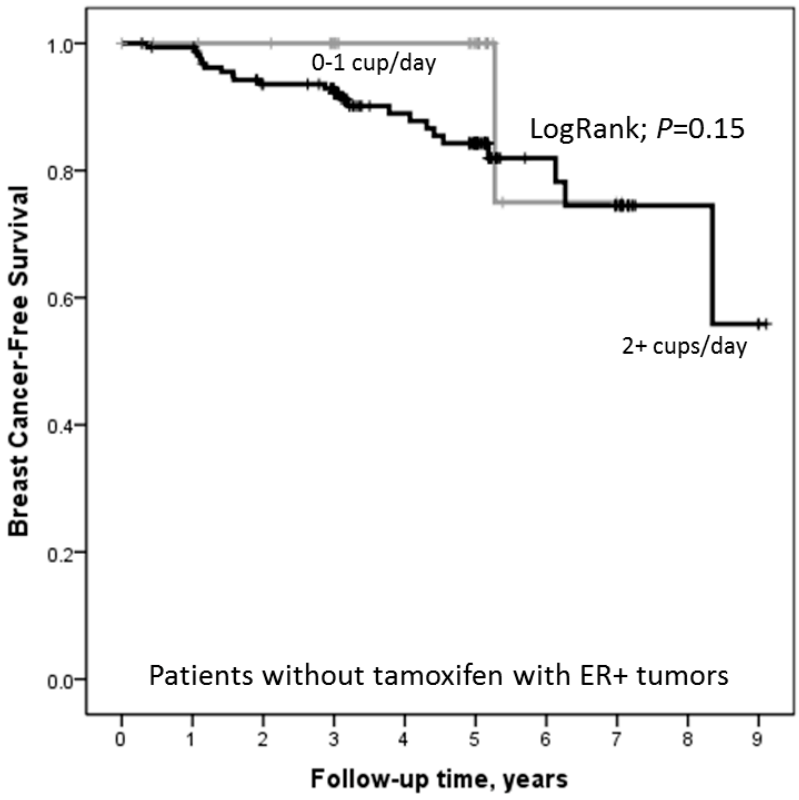
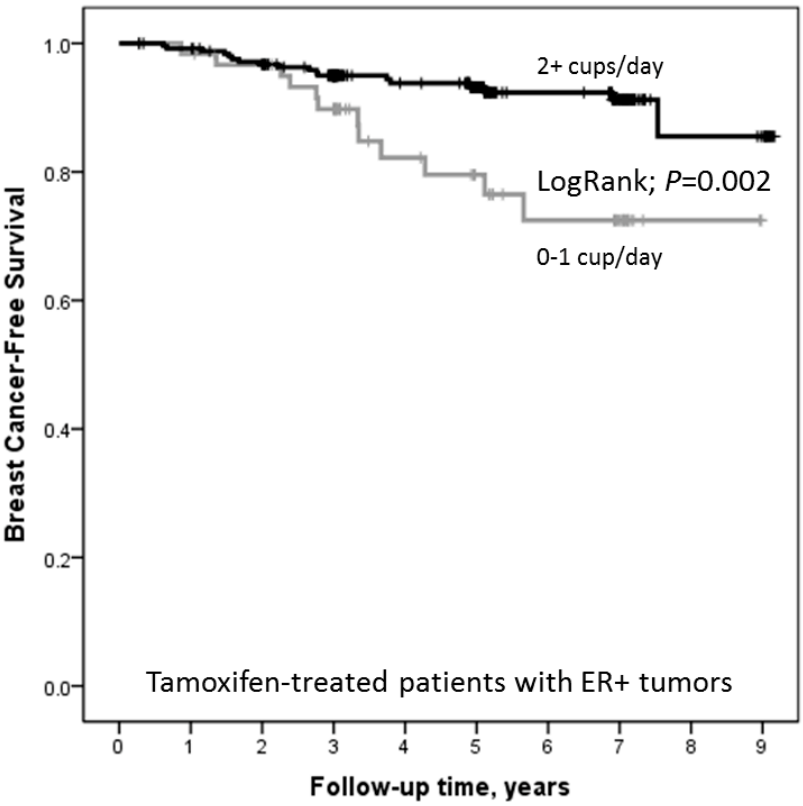

634 patients included in the study and analyzed for patient characteristics
42 patients excluded due to preoperative treatment
592 patients analyzed for tumor characteristics
14 patients excluded due to *in situ* carcinoma
2 patients excluded due to metastatic spread within 3 months of inclusion
576 patients analyzed for disease-free survival
119 patients ever received chemotherapy
351 patients ever received radiation therapy
2 patients excluded due to missing data regarding ER-status
73 patients with ER- tumors
501 patients with ER+ tumors
126 patients with no endocrine treatment
312 patients ever treated with tamoxifen
188 patients ever treated with AI
187 patients treated with tamoxifen but no AI
63 patients treated with AI but no tamoxifen
125 patients treated with AI and tamoxifen

Fig. 2a



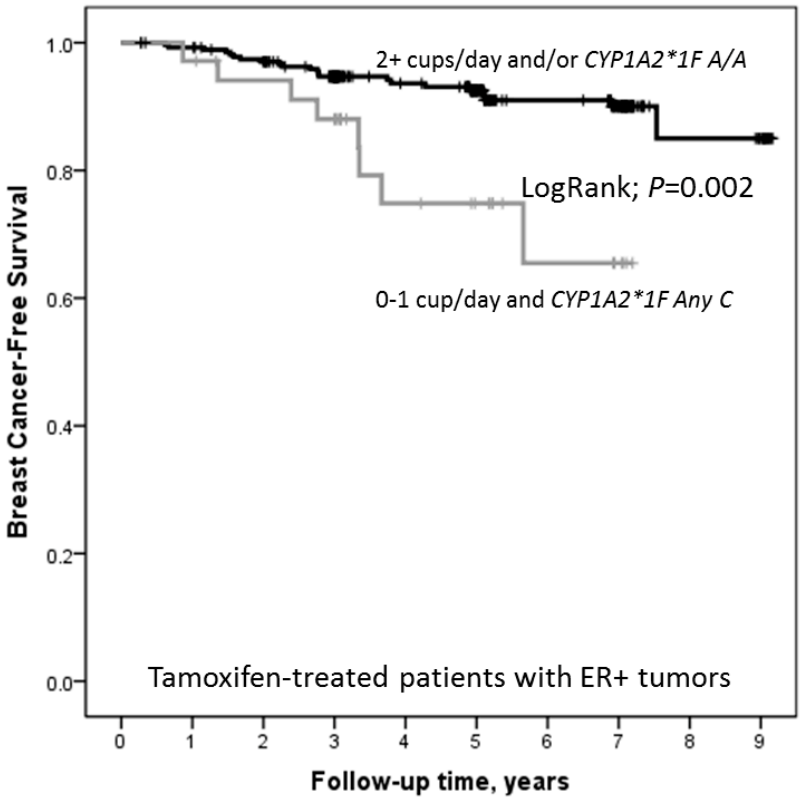
0-1 cup/day	**n=**	29	26	25	22	18	15	2	1	0	0
2+ cups/day	**n=**	160	157	142	129	76	61	22	18	4	2

Fig. 2b



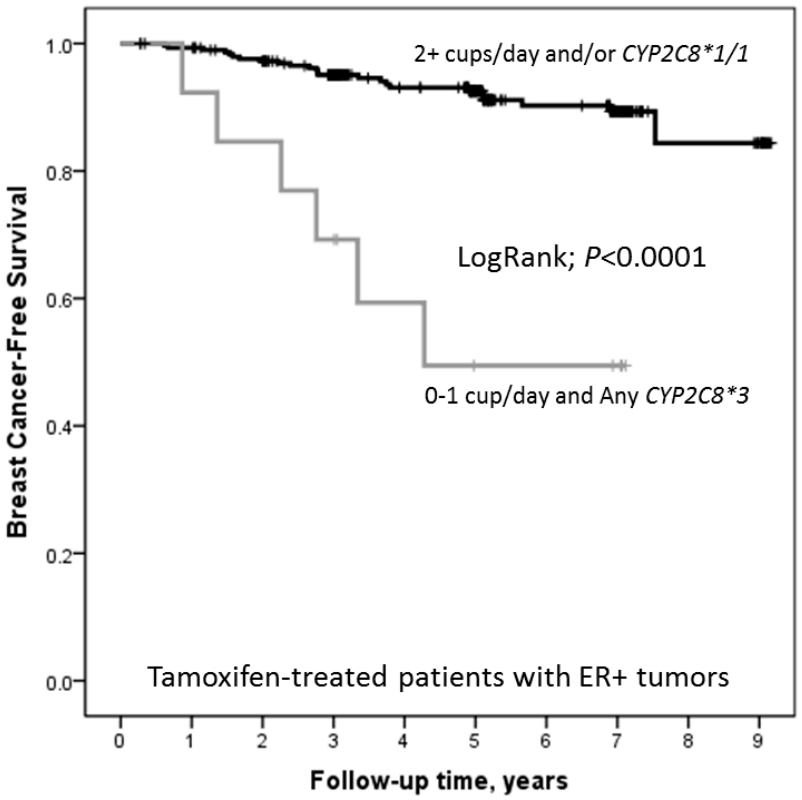
2+ cups/day	**n=**	249	244	233	199	159	128	89	70	15	13
0-1 cup/day	**n=**	61	60	57	50	32	26	18	13	2	0

Fig. 3



2+ cups and/or										
*CYP1A2*1F A/A*	**n=**	275	270	259	221	174	140	100	79	17	13
0-1 cup/day &										
*CYP1A2*1F Any C*	**n=**	35	34	31	28	17	14	7	4	0	0

Fig. 4



2+ cups/day and/or										
*CYP2C8*1*/*1*	**n=**	297	292	279	240	185	150	103	80	17	13
0-1 cup/day &										
*Any CYP2C8*3*	**n=**	13	12	11	9	6	4	4	3	0	0

Table I. Patient characteristics of the 634 patients in relation to daily coffee consumption, (cups per day)[a]

	All	Missing	Low (0-1)	Moderate (2-4)	High (5+)	P-value[c]	(2+)	*P*-value[d]
	Median (IQR) or %		Median (IQR) or %	Median (IQR) or %	Median (IQR) or %	(0-1) vs (2-4) vs (5+)	Median (IQR) or %	(2+) vs (0-1)
n=	634		111	397	124		521	
Age at diagnosis. yrs	59.6 (51.1-66.1)	0	56.0 (46.6-65.2)	61.1 (53.3-67.7)	57.2 (48.3-63.0)	**<0.0001**	60.1 (52.2-66.5)	**0.002**
Weight. kgs	68.0 (61.0-76.2)	2	65.5 (58.0-74.5)	68.0 (61.0-76.0)	70.0 (62.0-80.0)	0.062	68.6 (61.1-77.0)	0.060
Height. m	1.66 (1.62-1.70)	1	1.65 (1.60-1.69)	1.65 (1.61-1.70)	1.67 (1.63-1.72)	0.115	1.66 (1.62-1.70)	n.s
BMI, kgs/m²	24.6 (22.3-27.8)	3	24.4 (21.8-27.0)	24.6 (22.5-27.7)	24.7 (22.4-29.0)	n.s	24.6 (22.5-27.9)	n.s
Waist-Hip Ratio	0.84 (0.78-0.89)	4	0.84 (0.78-0.90)	0.83 (0.78-0.89)	0.85 (0.80-0.89)	n.s	0.84 (0.78-0.89)	n.s
Total breast volume, mL[b]	1000 (625-1450)	88	975 (500-1450)	1000 (675-1600)	850 (556-1288)	0.090	1000 (650-1450)	n.s
Age at menarche, yrs	13.0 (12.0-14.0)	5	13.0 (12.0-14.0)	13.0 (12.0-14.0)	13.0 (12.0-14.0)	n.s	13.0 (12.0-14.0)	n.s
Parous, %	84.7%	0	82.0 %	85.4 %	88.7 %	n.s	85.4 %	n.s
Age at first full term pregnancy, yrs	25.0 (22.0-28.0)	100	25.0 (23.0-28.0)	24.0 (22.0-28.0)	24.5 (21.0-29.0)	0.17	24.0 (22.0-28.0)	0.061
Ever use of OC, %	70.3 %	0	72.1 %	68.3 %	75.8 %	n.s	70.1 %	n.s
Ever use of HRT, %	45.3 %	1	37.8 %	49.1 %	39.5 %	n.s	46.9 %	0.081
Current smoker, %	21.3 %	0	9.0 %	18.4 %	41.1 %	**<0.0001**	23.8 %	**0.001**
Alcohol abstainer, %	11.1 %	1	19.8 %	10.1 %	6.5 %	**0.001**	9.2 %	**0.001**

[a] Information on coffee consumption was missing for two patients

[b] Patients with prior breast surgery were excluded (n=81) Breast volume was missing for seven patients

[c] Kruskall-Wallis test/Chi square

[d] Mann-Whitney U-test/Chi square

Table II. Tumor characteristics of the patients in relation to daily coffee consumption, cups/day[a]

	All	**Low (0-1)**	**Moderate (2-4)**	**High (5+)**	***P-value*[b]**	**(2+)**	**(0-1) vs (2+)**
n=	634	111	397	124		521	***P*-value (df)**
Neoadjuvant treatment	30	11	15	4		19	
Interstitial laser thermotherapy	11	1	6	4		10	
Missing	1	0	0	1		1	
No pretretment	592	99	376	115		491	
pT					0.068		**0.026 (4)**
In Situ	14 (2.4 %)	2 (2.0 %)	8 (2.1 %)	4 (3.5 %)		12 (2.4%)	
1	424 (71.6 %)	65 (65.7 %)	275 (73.1 %)	83 (72.2 %)		358 (72.9%)	
2	144 (24.3 %)	27 (27.3 %)	89 (23.7 %)	27 (23.5 %)		116 (23.6%)	
3	9 (1.5 %)	5 (5.1 %)	3(0.8 %)	1 (0.9 %)		4 (0.8%)	
4	1 (0.2 %)	0	1 (0.3 %)	0		1 (0.2%)	
Missing	0	0	0	0		0	
Axillary node involvement					n.s		n.s
0	368 (62.4 %)	58 (59.2 %)	233 (62.1 %)	76 (66.1 %)		309 (63.1%)	
1-3	167 (28.3 %)	29 (29.6 %)	109 (29.6 %)	28 (24.3 %)		137 (28.0%)	
4+	55 (9.3 %)	11 (11.2 %)	33 (8.8 %)	11 (9.6 %)		44 (9.0 %)	
Missing	2	1	1	0		1	
Histological grade					n.s		0.112 (2)
I	157 (26.6 %)	22 (22.2 %)	103 (27.5 %)	32 (27.8 %)		135 (27.6%)	
II	308 (52.1 %)	61 (61.6 %)	190 (50.7 %)	56 (48.7 %)		246 (50.2%)	
III	126 (21.3 %)	16 (16.2 %)	82 (21.9 %)	27 (23.5 %)		109 (22.2%)	
Missing	1	0	1	0		7	
Hormone receptor status							
ER+PgR+	398 (68.7 %)	59 (60.8 %)	263 (71.5 %)	74 (66.1 %)	n.s	337 (70.2%)	0.069 (1)
ER+PgR-	104 (18.0 %)	31 (32.0 %)	54 (14.7 %)	19 (17.0 %)	**0.008**	73 (15.2%)	**<0.001 (1)**
ER-PgR-	73 (12.6 %)	7 (7.2 %)	49 (13.3 %)	17 (15.2 %)	0.092	66 (13.8%)	0.077 (1)
ER-PgR+	4 (0.7 %)	0	2 (0.5 %)	2 (1.8 %)	n.s	4 (0.8%)	n.s
ER+	502 (86.7 %)	90 (92.8 %)	317 (86.1 %)	93 (83.0 %)	**0.042**	410 (85.4 %)	0.052
PgR+	402 (69.4 %)	59 (60.8 %)	265 (72.0 %)	76 (67.9 %)	0.114	341 (71.0 %)	**0.047**
Missing	13	2	8	3		11	

[a] Information on coffee consumption was missing for two patients in the following groups: pT1 and pT2, axillary lymph node 0 and 1-3, histolgical grades II and III, ER+PgR+, ER+ and PgR+

[b] Chi square/Linear-by-linear association test