Chapter 4

Effects of neoadjuvant chemotherapy with or without zoledronic acid on pathological response: a meta-analysis of randomized trials

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Abstract

Background
The addition of bisphosphonates to adjuvant systemic therapy improves survival in postmenopausal patients with early breast cancer. We report a meta-analysis of four randomized trials of neoadjuvant chemotherapy (CT) +/- zoledronic acid (ZA) in stage II/III breast cancer to investigate the potential for enhancing pathological response within the breast and axilla.

Methods
Individual patient data from four prospective randomized clinical trials reporting the effect of the addition of ZA on pathological response after neoadjuvant CT were pooled. Primary outcomes were pathological complete response in the breast (pCRb) and in the breast and lymph nodes (pCR). Trial-level and individual patient data meta-analyses were done. Predefined subgroup analyses were performed for postmenopausal women and patients with triple-negative breast cancer.

Results
pCRb and pCR data were available in 735 and 552 patients respectively. In the total study population ZA addition to neoadjuvant CT did not increase pCRb or pCR rates. However, in postmenopausal patients, the addition of ZA resulted in a significant, near doubling of the pCRb rate (10.8% for CT only vs. 17.7% with CT+ ZA; OR 2.14, 95% C.I. 1.01-4.55) and a suggested benefit of the pCR rate (7.8% for CT only vs. 14.6% with CT+ ZA; OR 2.62, 95% C.I. 0.90 – 7.62). In patients with triple-negative breast cancer a trend was observed favoring CT+ZA.

Conclusion
This meta-analysis shows no overall impact from the addition of ZA to neoadjuvant CT on tumor response. However, as has been seen in the adjuvant setting, the addition of ZA to neoadjuvant CT seems to augment the effects of CT in postmenopausal patients with early breast cancer.

Introduction

The antitumor effect of bisphosphonates is still an issue of debate. Recently, the EBCTCG meta-analysis in 17,791 patients demonstrated that adjuvant bisphosphonates reduces bone metastases and improves survival in postmenopausal women with early breast cancer.(1) Several studies have also suggested that the addition of zoledronic acid to chemotherapy in the neoadjuvant setting may be beneficial and result in increased rates of pathological complete response (pCR).(2;3)(4) However, the body of evidence for this is limited due to the low number of patients and relatively discordant findings.(2;3;5)(4)In the neoadjuvant subset of the AZURE study, consisting of 205 patients with cT3 or cT4 disease or biopsy-proven lymph node involvement, the pCR rate nearly doubled in the cohort of patients who received zoledronic acid (4 mg q3-4 weeks, 6 doses) as an adjunct to neoadjuvant chemotherapy. Aft et al. reported that zoledronic acid administration resulted in a significant decrease in detectable disseminated tumor cells in patients with clinical stage II/III breast cancer treated with four cycles of neoadjuvant epirubicin plus docetaxel, in comparison to patients who were treated with chemotherapy only.(3) In contrast, the two comparative phase III trials which were prospectively designed to evaluate pCR rates following neoadjuvant chemotherapy with or without zoledronic acid 4 mg intravenously at the beginning of each cycle failed to show a beneficial effect in their stage II/III early breast cancer population.(4)(5) However, in both of these studies a numerical benefit was observed in postmenopausal women specifically, seemingly concordant with the data from the neoadjuvant AZURE subgroup analysis and the adjuvant meta-analysis.
Together, study results support the hypothesis that zoledronic acid may have an anti-tumor effect and that synergism may occur with chemotherapy.(6) We report a meta-analysis of individual patients data from all randomized studies that have compared the use of zoledronic acid (4 mg, 4-6 doses, q3-4 weeks) combined with neoadjuvant chemotherapy versus no bisphosphonate in patients with early breast cancer.

Methods

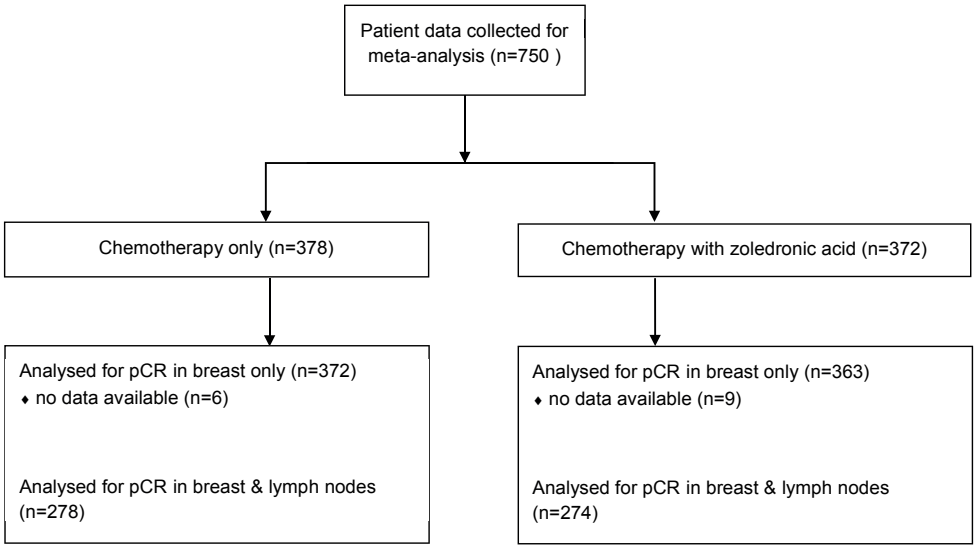
Included studies
Patients from four prospective randomized studies were included in this meta-analysis. The NEOZOTAC trial randomized 250 patients to receive 6 three-weekly cycles of TAC chemotherapy (docetaxel, doxorubicin, cyclophosphamide with pegylated G-CSF within 24 hours) with or without zoledronic acid (4 mg i.v.). The JONIE1 trial randomized 180 patients to receive neoadjuvant chemotherapy (4 three-weekly cycles FEC [5-fluorouracil, epirubicine, cyclophosphamide] followed by 12 weekly cycles paclitaxel) with or without 7 infusions of zoledronic acid 4 mg. In the neoadjuvant subset of the AZURE study, 205 patients received neoadjuvant chemotherapy following local guidelines, with or without zoledronic acid 4 mg, every 3-4 weeks, for 6 dosis. In the study by Aft et al. 120 patients received four cycles of intravenous neoadjuvant epirubicin plus docetaxel every 3 weeks, with granulocyte-stimulating factor support, with or without zoledronic acid (4 mg i.v.).

Variables collected from each study
Participating study groups were asked to provide patient data on the following variables: estrogen receptor (ER)-status, progesterone receptor (PR)-status, cT-status, cN-status, menopausal status, age, pathological complete response in the breast (pCRb)-status, pathological complete response in the breast and lymph nodes(pCR)-status and allocated treatment. pCRb was defined as absence of invasive tumor cells in the breast.

Statistical analysis
Patient characteristics in treatment arms were compared with the Pearsons's Chi-Square Test, or if applicable Fisher's Exact Test, in case of categorical variables or the Mann-Whitney-U test in case of continuous variables. Homogeneity of the treatment effect among studies was tested using the Q statistic and by calculating the I2-value. Data were analyzed on a trial-level as well as on individual patient level. Due to the homogeneity of study effect sizes fixed-effects models were used. For the trial-level approach, Odds Ratios (OR) of each separate study were calculated, correcting for the classical predictors ER-status and cT-status (cN-status was not completely collected in some studies). Pooling of odds ratios was performed using inverse variance weighting.
As for individual patient data analyses multivariate logistic regression correcting for ER-status and cT-status was used to calculate the OR. Subgroup analyses based on menopausal status (pre/perimenopausal vs. postmenopausal as defined per trial and by age) and hormone-receptor status (triple-negative tumors which are more likely to achieve pCR vs. all other tumors with known receptor status) were pre-specified.(7) The null hypothesis that the effect sizes of the intervention (zoledronic acid) did not differ significantly between subgroups was tested by adding an interaction between subgroup characteristics and treatment(neoadjuvant CT+ZA vs. neoadjuvant CT alone) in the logistic regression analyses. Statistical analyses were done using SPSS (version 20.0 for Windows, IBM SPSS Statistics) and R (package 'meta', version 2.15.0, The R Foundation for Statistical Computing).

Results

Patients
Demographics and tumor characteristics of the pooled population are summarized in Table 1. 735 and 552 patients were included for respectively the pCRb and pCR analysis respectively. pCRb data were available from 735 patients (CONSORT diagram). The median age of our pooled population was 48 (range 25-75). 37% of the included women were postmenopausal (Supplementary file: definitions of postmenopausal status in included studies). 73% of the tumors were ER-positive and 8% were HER2-positive. 19% of the tumors were triple-negative.



CONSORT diagram

Trial-level analysis

Study effects among trials were homogeneous (I2=0%, p=0.44), and for this reason a fixed-effects model was used for analysis. In the total pooled population of patients with early breast cancer, the addition of zoledronic acid to neoadjuvant chemotherapy did not increase pCRb (OR=1.28, 95% C.I. 0.84-1.97) or pCR (OR=1.39, 95% C.I. 0.79-2.48) (Figure 1A,B). pCR and pCRb were next investigated separately in pre/perimenopausal and postmenopausal women. A suggested benefit of the addition of zoledronic acid with regards to pCR and pCRb was observed in postmenopausal patients (pCR: OR=2.69, 95% C.I. 0.87-8.33; pCRb: OR 1.99, 95% C.I. 0.90-4.39), but not in pre/perimenopausal patients (pCR: OR=0.93, 95% C.I. 0.46-1.90; pCRb: OR=1.07, 95% C.I. 0.62-1.86)(Figure 1 C,D).

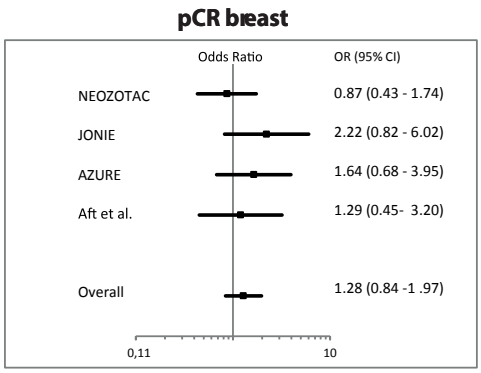
Individual patient-data analysis

In the individual patient-data analysis, no difference in pCRb or pCR rates with the addition of zoledronic acid were observed in the total study population (Table 2). However, a significantly greater proportion of postmenopausal patients attained pCRb if treated with the addition of zoledronic acid (10.8% vs. 17%, OR 2.14, 95% C.I. 1.01-4.55, p=0.048). For pCR, a tendency towards better response after zoledronic administration (7.8% vs. 14.6%, OR-2.62, 95% C.I. 0.90-7.62, p=0.076) was observed. However, the data were not sufficient to show a significant interaction between the intervention (zoledronic acid) and postmenopausal status as regards treatment effect. (p-value for interaction=0.17). A post-hoc exploratory analysis based on age as a surrogate for menopausal status suggested that the benefit of

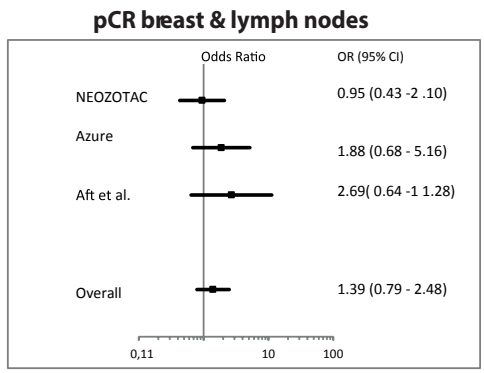
zoledronic acid addition increases with age (Figure 2). A pre-specified exploratory analysis on patients that were postmenopausal and had triple-negative breast cancer (and were therefore more likely to achieve pCR) suggested an even stronger treatment effect of zoledronic acid (pCR: OR=5.83, 95% C.I. 1.21-28.0; pCRb: OR=7.52, 95% C.I. 1.93 – 29.20).

Characteristic		Pooled population
N		750
Median age(range)		48 (25-75)
T-status	T1/T2	374 (49.9)
	T3/T4	375 (50.0)
	Unknown	1 (0.1)
N status*	N-	223 (29.7)
	N+	333 (44.4)
	Unknown	194 (25.9)
ER status	ER-	201 (26.8)
	ER+	548 (73.1)
	Unknown	1 (0.1)
PR status	PR-	239 (39.1)
	PR+	397 (52.2)
	Unknown	60 (8.0)
HER2-status	HER2-	623 (83.1)
	HER2+	58 (7.7)
	Unknown	69 (9.2)
Triple-negative tumor	Yes	141 (18.8)
	No	514 (68.5)
	Unknown	95 (17.7)
Postmenopausal	Yes	277 (36.9)
	No	455 (60.7)
	Unknown	18 (2.4)

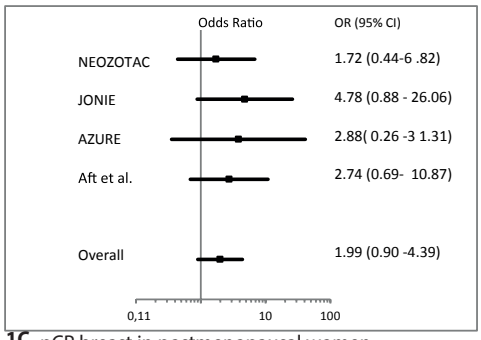
Table 1. Patient and tumor characteristics of the pooled population. *High number of missings as nodal status not prospectively collected in each study



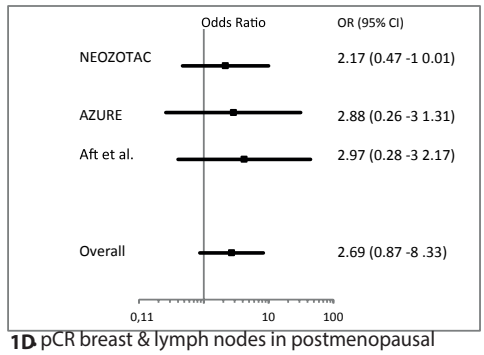
1A. pCR breast in total population



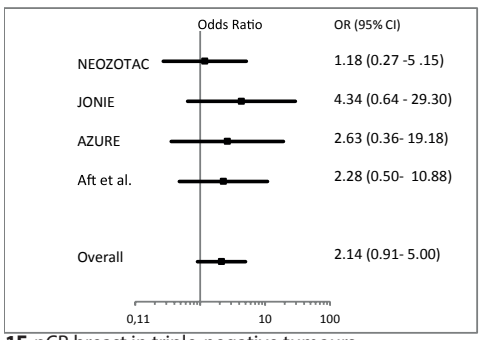
1B. pCR breast & lymph nodes in total population



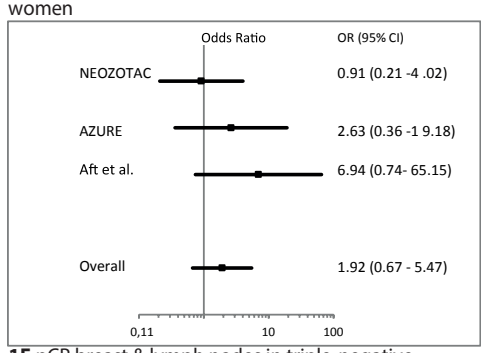
1C. pCR breast in postmenopausal women



1D. pCR breast & lymph nodes in postmenopausal women



1E. pCR breast in triple-negative tumours



1F. pCR breast & lymph nodes in triple-negative tumours

Figure 1. Forest plots of the effects of zoledronic acid on pCR and pCRb. Odds ratios are adjusted for T-status and ER-status.

Discussion

In our meta-analysis we did not observe a benefit in the pCR or pCRb rate in the overall patient population when zoledronic acid was added to neoadjuvant chemotherapy in women with clinical stage II/III breast cancer. Our study provides the first data indicating a statistically significant benefit of the addition of zoledronic acid to neoadjuvant chemotherapy on pathological complete response in postmenopausal patients with early breast cancer. Our findings are in concordance with observations in the adjuvant setting, where the addition of zoledronic acid to systemic therapy has shown survival benefit in postmenopausal patients with low levels of reproductive hormones. (1)(8;9)

The precise biological mechanism that enables a specific anti-tumor effect of zoledronic acid in patients with low reproductive hormone levels is still unknown. Postmenopausal women are known to have an increased receptor activator of nuclear factor-kappa β ligand (RANKL) to osteoprotegerin (OPG) ratio, thereby promoting osteoclastogenesis and accelerating bone turnover.(10) During bone resorption, growth factors and cytokines, such as insulin-like growth factors and transforming growth factor beta, are released from the bone which may stimulate proliferation and attract tumor cells.(11) Since, the main effect of zoledronic acid is inhibition of bone resorption, this might explain why postmenopausal women, with an increased bone turnover, benefit from zoledronic acid therapy. Another explanation might be related to an immunomodulatory effect of zoledronic acid. Low estrogen levels induce an inflammatory response with an increase in immune cells such as macrophages and T cells.(12) Tumor associated macrophages (TAM) or M2 macrophages assist tumor progression.(13;14) Bisphosphonates reverse the TAM phenotype from pro-tumoral M2 to tumoricidal M1 and help deplete these M2 macrophages.(15) In addition to this, in a preclinical model it was observed that zoledronic acid was more toxic to human macrophages rather than to breast cancer cells.(16) A study by Junankal et al. showed, using two-photon microscopy, that outside of the skeleton bisphosphonates are likely to be taken up by TAms. They found that bisphosphonates initially binds to areas of micro-calcifications and can be engulfed by TAMs.(17) This might be a mechanism through which zoledronic acid could affect primary breast tumor growth. Furthermore, stimulated T-cells may interact with antigen presenting cells, attack tumour cells and express and secrete RANKL, which can contribute to the anti-tumour effect of zoledronic acid. Consequently, the combination of a tumor microenvironment with increased immune cells, RANKL and bone turnover, caused by estrogen deprivation, might explain why zoledronic acid has an antitumor effect when administered as an adjunct to neoadjuvant chemotherapy that appears restricted to postmenopausal patients.

In an exploratory sub-analysis in patients that were both postmenopausal and had triple negative disease, we found the largest effect of zoledronic acid on pCR (Supplemental File). This might be explained by the higher immune cell infiltrated found in those patients with triple negative breast cancer in combination with the putative immunomodulatory effect of zoledronic acid.(18)

There are some limitations to our study. Our meta-analysis relied on slightly differing definitions of menopausal status as defined by each of the included studies. As confirmation of our findings, we performed an analysis based on individual patient age as a surrogate for

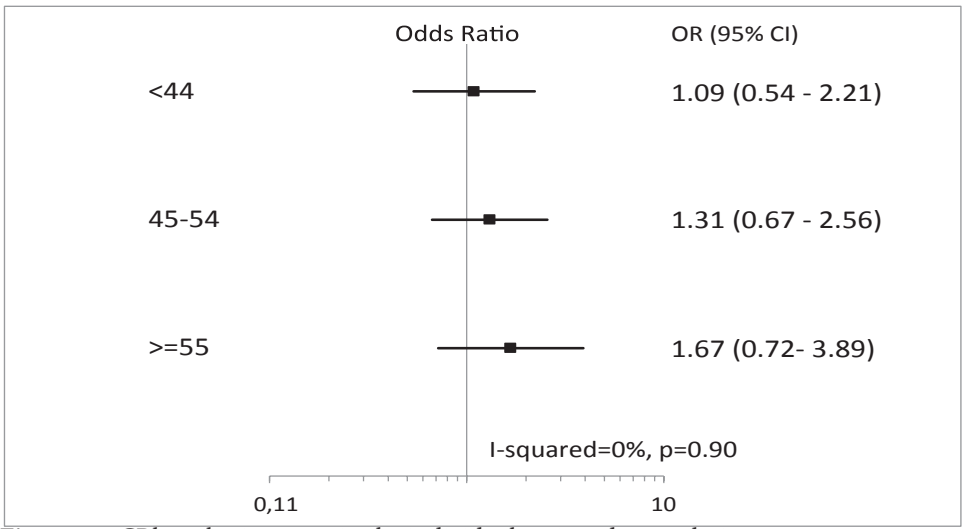
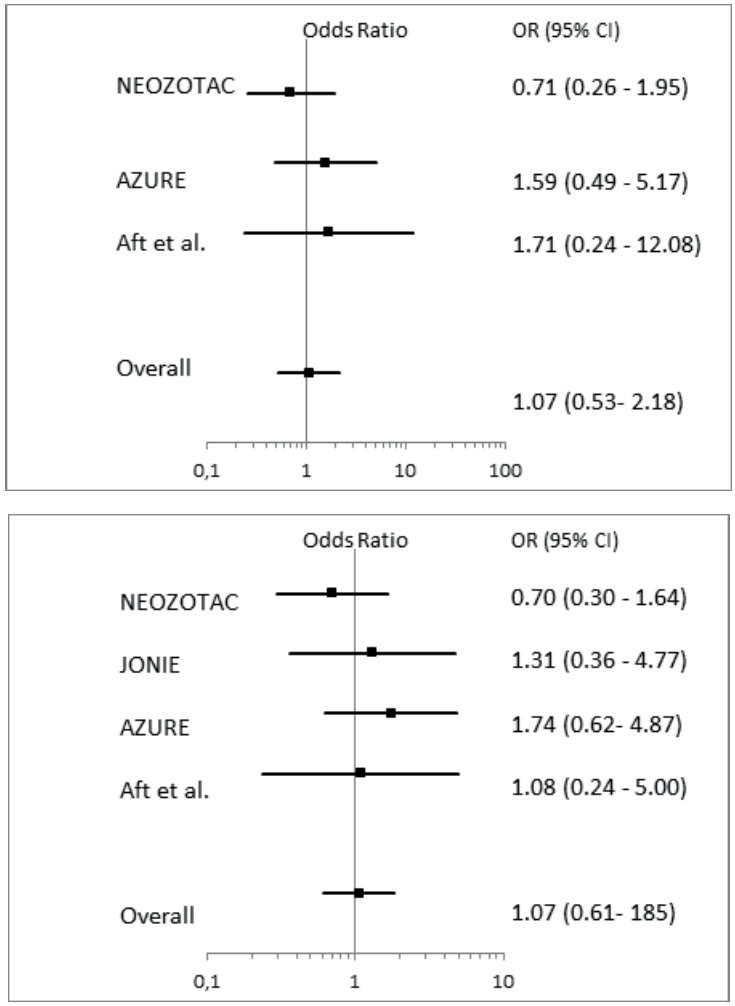


Figure 2. pCRb on basis on age in the individual patient data analysis.

menopausal status which showed similar results to the analyses using trial-defined menopausal status. Although our study represents the largest population to date, the sample size of our meta-analysis was not sufficient to prove a significant effect of zoledronic acid on pCR in breast and lymph nodes, as this endpoint was not collected in one of the included studies. In addition, our data were not sufficient to show statistical interaction between postmenopausal status and zoledronic acid intervention as regards pCR, although clear differences, consistent with findings in the adjuvant setting, were found in favor of the postmenopausal subset of patients.

Further translational research is necessary and ongoing in order to elucidate the specific anti-tumor mechanism of zoledronic acid, especially concerning the alleged immunomodulatory role, in order to select those patients that would benefit most from including zoledronic acid in their treatment regimen. The NEOZOL study for example, aims to evaluate changes in Vascular Endothelial Growth Factor and gamma-delta T-cell activity (NCT01367288) (19).

Another important remaining question is whether the beneficial effect in postmenopausal women during neoadjuvant treatment will translate into improved survival, especially in cases with triple-negative breast cancer. Reduction of dissemination in the bone microenvironment may provide survival benefit. As the data matures over the next few years, an update of this meta-analysis with long term follow up results will hopefully provide a conclusive answer to this.



Supplementary 1. Forest plots of the effects of zoledronic acid on pCR and pCRb in pre/perimenopausal women. Above for pCR, under for pCRb. Odds ratios are adjusted for T-status and ER-status.

Study	Definition Postmenopausal status
JONIE-1	- Women >= 60 years
	- Bilater oophorectomy (prior to diagnosis)
	- Twelve consecutive months of amenorrhea
Aft et al.	- 1 year with no menstrual activitity, previous bilateral oophorectomy, age>56
AZURE	- > 5 years since menopause
NEOZOTAC	- Assessed by local physician
	- If revision possible: FSH >20 U/L & estradiol <110 pmol/l

Supplementary 2. Definitions of postmenopausal status used in included studies.

	Chemotherapy only		**Chemotherapy + ZA**		**Odd's Ratio**	**95% C.I.**
	pCRb/total	%	pCRb/total	%		
pCR in breast						
Triple-negative breast cancer	3/20	15.0	10/21	47.6	7.98	2.01-30.72
Non-triple-negative disease	3/65	4.6	5/78	6.4	1.08	0.42-2.76
	pCR/total	%	pCR/total	%		
pCR						
Triple-negative disease	4/30	13.3	14/29	48.3	6.10	1.27-29.27
Non-triple-negative disease	9/95	9.5	11/108	10.2	1.33	0.30-5.91

Supplementary 3. Effect of zoledronic acid on pCR in postmenopausal women only stratified on triple-negative receptor status (yes/no).

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