Age in breast conserving therapy

# The effect of age in breast conserving therapy: A retrospective analysis on pathology and clinical outcome data

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## ARTICLE INFO

## ABSTRACT

*Background and propose:* Age is an important prognostic marker of patient outcome after breast conserving therapy; however, it is not clear how age affects the outcome. This study aimed to explore the relationship between age with the cell quantity and the radiosensitivity of microscopic disease (MSD) in relation to treatment outcome.

*Materials and methods:* We employed a treatment simulation framework which contains mathematic models for describing the load and spread of MSD based on a retrospective cohort of breast pathology specimens, a surgery simulation model for estimating the remaining MSD quantity and a tumor control probability model for predicting the risk of local recurrence following radiotherapy.

*Results:* The average MSD cell quantities around the primary tumor in younger (age $\leqslant$ 50 years) and older patients were estimated at $1.9 * 10^8$ cells and $8.4 * 10^7$ cells, respectively ($P < 0.01$). Following surgical simulation, these numbers decreased to $2.0 * 10^7$ cells and $1.3 * 10^7$ cells ($P < 0.01$). Younger patients had smaller average surgical resection volume ($118.9$ cm$^3$) than older patients ($162.9$ cm$^3$, $P < 0.01$) but larger estimated radiosensitivity of MSD cells ($0.111$ Gy$^{-1}$ versus $0.071$ Gy$^{-1}$, $P < 0.01$).

*Conclusion:* The higher local recurrence rate in younger patients could be explained by larger clonogenic microscopic disease cell quantity, even though the microscopic disease cells were found to be more radiosensitive.

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For many women with early-stage breast cancer, breast conserving therapy (BCT) has become a favorable option due to the comparable survival rates as those after mastectomy and better cosmetic outcome [1]. Despite the good results of BCT, younger patients (age $\leqslant$ 50) are still at increased risk of breast cancer recurrence compared with older patients [2,3]. Underlying this observation may be differences in tumor biology [4], adjuvant systemic therapy [5], and margin status [6]. Indeed, young patient age is associated with increased risk of positive resection margins [7]. One possible explanation may be larger disease load in younger patients [6], although this hypothesis has not been investigated in detailed pathology studies.

The correlation between tumor control and age was studied previously. In 1994, Nixon reported a higher frequency of adverse pathological factors (necrosis, pathology grade, etc.) seen in patients under 35 years of age [8]. Zhou studied 130 patients under age 40, supported the previous result and argued that the majority of the breast excisions should be performed with careful evaluation of microscopic margin status [9]. However, very few studies to date have examined the combined effect of surgical margins and age on local control rates.

In this study, we focused on the potential role of pathology and radiobiology differences to explain differences in local control between younger and older patients. For this purpose we employed a previously developed treatment simulation framework [10] to model the treatment outcome. We aimed specifically at answering three questions:

(1) Does age affect the quantity of microscopic tumor cells?
(2) Does age affect the radiosensitivity of these tumor cells?
(3) Which factor, if any, plays a more important role, quantity or radiosensitivity?

## Material and methods

### Overview

In order to study the effect of patient age on the outcome of breast conserving therapy (BCT), we deployed a retrospective analysis of patients in two different age groups: patients at age 50 years or below, and patients older than 50 years [3]. The effect

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on the treatment local control was analyzed by tracking the change in microscopic disease (MSD) quantity around the primary tumor after each treatment component through an earlier developed simulation framework [10]. In short, this framework contains three sequential stages: disease load prediction, surgery simulation and radiotherapy (RT) modeling. MSD quantity and spread distribution were first estimated using a patient model derived from a retrospective cohort of pathology data. The simulated surgery was performed and the remaining MSD quantity was quantified using typical surgical resection margins. The tumor control probability (TCP) was estimated using an extended Webb–Nahum model and compared with local control in separate datasets. In the following subsections, we first describe the pathology dataset and the outcome datasets, and then explain how the simulation framework was employed to model the effect of age on TCP.

*Pathology dataset*

We used the pathology data from the MARGINS (Multi-modality Analysis and Radiological Guidance IN breast-conServing therapy) study to model the MSD quantity and spread distribution in the breasts of patients with early breast cancer eligible for breast conserving therapy [11]. More than 1800 microscopic slides of invasive breast cancers in 60 patients (48 patients with age > 50 and 12 with age ⩽ 50) were examined by two experienced breast cancer pathologists [12,13]. MSD was observed on the microscopic slides. The disease quantity and distance from the primary tumor were recorded after slice three-dimension reconstruction. The MSD cell densities were estimated using a nuclei counting software on digitalized microscopic slides [14].

*Outcome datasets*

In the EORTC (European Organization for Research and Treatment of Cancer) 22881-10882 (boost-versus-no-boost) trial [15], a subset of 1616 patients with central pathology review showed that an increased risk of local recurrence was associated with age younger than 50 years and omission of a boost dose of 16 Gy to the tumor bed [16]. We accordingly separated our data into four groups to calculate the local control rates. These four groups were younger patients (⩽50 years) with and without a boost, and older patients (>50 years) with and without a boost.

In addition to the EORTC trial data, we used data from the EBCTCG (Early Breast Cancer Trialists' Collaborative Group), who centrally reviewed the randomized trials performed world-wide in early breast cancer every five years since 1985. The sixth cycle data in 2011 consist of 10,801 women from 17 trials [17]. The reported result of 4138 pooled patients who only received breast conserving surgery (BCS) (i.e., no RT) was selected to determine the baseline values in the two age groups at 0 Gy dose.

*Disease load prediction*

To model disease quantity as a function of age, a Zero-Inflated Model was employed [10]. To estimate disease cell density, we selected 36 pathology slides from the five youngest patients and the seven oldest patients and analyzed the difference between these two groups. The cell density data in the two age groups were fitted separately to a Gaussian model.

The MSD spread model was described as disease quantity within each 1 mm distance from the edge of the macroscopic tumor relative to the total quantity recorded for each patient. The MSD spread distribution (thirty relative ratios) of each patient was modeled with a truncated Gaussian distribution independently. The mean and the standard deviation of these truncated Gaussian models were described with a negative binomial distribution and a Poisson distribution, respectively. The mean of the

Gaussian model describes the center of disease load and the standard deviation parameter of the Gaussian model indicates how wide the distribution of tumor cells was. The parameters of the spread model were compared using Student's *t*-tests between two groups.

*Surgery simulation*

The remaining MSD in the operated breast was estimated as the difference between the total MSD quantity and that within the volume of the tissue removed together with the macroscopic tumor. It was previously reported that younger patients have a larger probability of smaller excision volumes [18]. We compared the distribution of the negative surgical margins in the two age groups in the EORTC dataset using the Kolmogorov–Smirnov test and incorporated this difference in excision volumes between the two age groups within our surgery simulation module. As reported previously [10], the removed tissue around the primary tumor in the MARGINS dataset was estimated using the surgical margins in six directions. The sum of surgical margins minus minimal surgical resection margin was modeled with a Poisson distribution. The asymmetry in each direction was modeled with a Beta distribution. We compared the parameters of the Poisson model and the Beta model using Student's *t*-tests. The similarity of the remaining MSD quantities between the two age groups was evaluated using the Mann–Whitney *U* test because the estimated values did not follow a Gaussian distribution.

*Radiotherapy modeling*

The estimated remaining MSD quantity after simulated surgery was taken as the input into the extended Webb-Nahum TCP model [19] to model the local control of radiotherapy. The unknown parameters in the TCP model (i.e., MSD radiosensitivity, clonogenic cell fraction[1]) were estimated by fitting the simulation results to the clinical outcome for three different treatments: uniform irradiation with 50 Gy, 50 Gy plus 16 Gy boost and no radiation (i.e., 0 Gy). We defined the local control rate at median 10 year follow-up as the TCP of the patients. We then compared the clonogenic cell fraction (CCF) between the two groups of patients using Mann–Whitney *U* test as the estimated values of CCF across patients do not follow a Gaussian distribution. We used Student's *t*-tests for comparing the mean and standard deviation of radiosensitivity.

*Statistical tests*

We ran all statistical tests on the open-source software $R^2$. A Bayesian Inference framework was implemented with the software package 'mcmc' (Markov Chain Monte Carlo simulation). In order to quantify the statistical accuracy, we designed a framework to implement our simulations one-hundred times and ran the statistical tests one-hundred times accordingly. We reported the combined *P*-value as the average of all *P*-values times two with 1.0 as maximum [20]. The rejection of the null hypothesis for equivalence was reported at the 0.05 level of significance.

*Factors potentially associated with TCP*

To further study which patient/tumor/clinical factors underlie the inferior outcome of younger patients, we explored two scenarios in which we tested the impact of four factors on the dose-TCP

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[1] Clonogenic cell is a cell that has the potential to proliferate and give rise to a colony of cells. We assume only a proportion of microscopic disease cells is clonogenic [19].

[2] www.r-project.org.

relationship: total disease quantity before surgery, surgical effect (removed tissue volume and disease spread), clonogenic cell fraction, and radiosensitivity of tumor cells. In the first scenario, we estimated the dose-TCP relationship using the average parameter values over older and younger patients in three of the four factors while the remaining factor was set to the average of the two age groups separately. Consequently, the heterogeneity between patients was ignored and the effect of age on each of four specific factors was observed.

In the second scenario, patient heterogeneity was taken into account. To that end, the heterogeneity with the combined population of older and younger patients was modeled for three out of four parameters while modeling the remaining parameter separately for the two age groups. Similarly, we compared the variation of dose-TCP relationship by age difference on each of four factors.

For both scenarios and all four factors we estimated the dose-TCP relationships separately. The effect of age on TCP was analyzed by comparing the difference in TCP results between the younger and older groups with the varying radiation dose.

## Results

### Disease load prediction

The total microscopic disease (MSD) volume was negatively associated with age ($P = 0.03$) (Table 1). The probability of a patient having zero MSD increased with age but a significant difference between the two age groups was not observed. Therefore, we discarded the age term in the zero-inflated part of the model. As a result, younger patients had an estimated average (one standard deviation (SD)) MSD volume of 431 (632) mm$^3$ compared to MSD in older patients: 180 (241) mm$^3$.

The average cell density of MSD in younger patients was measured at $4.8 * 10^5$ cells/mm$^3$ (SD $5.9 * 10^4$ cells/mm$^3$) ($N = 5$). In older patients, the average MSD cell density was $4.3 * 10^5$ cells/mm$^3$ (SD $9.5 * 10^4$ cells/mm$^3$) ($N = 7$). No significant difference between cell densities was found between these two age groups. Therefore in the following sections we modeled the cell density using one single model. We took the average as $4.5 * 10^5$ cells/mm$^3$ and the standard deviation as $8.4 * 10^4$ cells/mm$^3$ from the twelve patients.

Regarding the distribution of disease spread, the mean and the standard deviation of the relative quantity ratios per shell are shown in Fig. 1. We observed a larger tail in the distribution for older patients while the disease is more focused around the macroscopic tumor for younger patients. The results from the Student's *t*-test showed that the parameters in the Negative Binomial distribution and the Poisson distribution of the spread model are significantly different between the two age groups (Table 2).

### Surgery simulation

Significant difference in the negative surgical margin width was not found in the two age groups in the EORTC dataset ($P = 0.27$).

However, we found significant differences between margin widths of the specimen in the pathology dataset (Table 2). The average width of the surgical margin was 23.6 mm in the older age group, and 17.6 mm in the younger age group. We found that the removed volume of tissue was significantly smaller in younger patients than in older patients ($P < 0.01$), while the average tumor diameter was slightly larger in younger patients (17.7 mm vs. 16.6 mm). Younger patients had $1.2 * 10^2$ cm$^3$ tissue removed on average (standard deviation: $1.1 * 10^2$ cm$^3$), ranging from 1.3 cm$^3$ to $1.1 * 10^3$ cm$^3$ (a normal breast size is roughly between $1.7 * 10^2$ cm$^3$ and $2.7 * 10^3$ cm$^3$ [21]). The average removed tissue volume in older patients was $1.6 * 10^2$ cm$^3$ (standard deviation: $1.5 * 10^2$ cm$^3$), ranging from 1.1 cm$^3$ to $1.5 * 10^3$ cm$^3$.

After simulated surgery, younger patients had significantly larger quantity of MSD remaining in the breast ($P < 0.01$). The average MSD quantity in younger patients was decreased from $1.9 * 10^8$ cells to $2.0 * 10^7$ cells. The percentage of the remaining MSD quantity over the original MSD quantity for younger patients ranged from 0 to 100% with a mean (1SD) of 10.4 (18.1)%. The average MSD quantity in older patients was decreased from $8.4 * 10^7$ cells to $1.3 * 10^7$ cells by surgery. The mean (1SD) percentage of reduction was 16.4 (25.1)%, range 0–100%.

### Radiotherapy modeling

The tumor control probabilities in the two age groups in the EORTC boost-versus-no-boost trial and the EBCTCG collective study are listed in Table 3. Note that the average TCP in the EORTC study is larger than the average TCP in the EBCTCG study, but falls into the 95% confidence interval of the EBCTCG study [17]. We took the TCP outcome at 10 years in the breast conserving surgery (BCS) arm only in the EBCTCG dataset and the 50 Gy and 66 Gy radiotherapy arm in the EORTC dataset. We estimated the clonogenic cell fractions for younger and older patients to be one in $5.2 * 10^4$ cells and one in $3.7 * 10^6$ cells respectively ($P < 0.001$; Table 2). The mean radiosensitivity in the younger age group was estimated at 0.111 Gy$^{-1}$, and in the older age group at 0.071 Gy$^{-1}$ ($P < 0.001$). The standard deviations were 0.037 Gy$^{-1}$ in the younger age group and 0.018 Gy$^{-1}$ in the older age group ($P < 0.001$).

With the above estimated model parameters, we obtained the relationship between radiation dose and TCP (Fig. 2). These two dose-TCP curves for the two age groups had different slopes. In the younger patient group, a higher absolute increase of TCP with the same increase of radiation dose was observed, but the starting point (TCP after breast conserving surgery only) was significantly lower.

### Factors potentially associated with TCP

Comparison of the impact of four factors on TCP for the first scenario is illustrated in Fig. 3. It was observed that total disease quantity and clonogenic cell fraction play an important role in explaining the inferior outcome of younger patients while surgery and the radiotherapy have more positive impact on younger patients. Note that by modeling that every patient had the same amount of non-zero residual clonogenic cells, the dose-respond curve starts at zero TCP at zero dose.

In the second scenario, including realistic patient heterogeneity, we obtained a similar result as for the first scenario but with smaller differences between two groups (Fig. 4). The TCP difference by age for these four factors is shown in Fig. 5. We found the difference in TCP was increasing with increasing dose for radiosensitivity to reach a maximum at 59 Gy. Differences in surgery had little impact on the TCP and were mostly compensated by large radiation dose. Total MSD quantity of younger patients had a negative impact on TCP and this impact was gradually reduced by increasing dose after
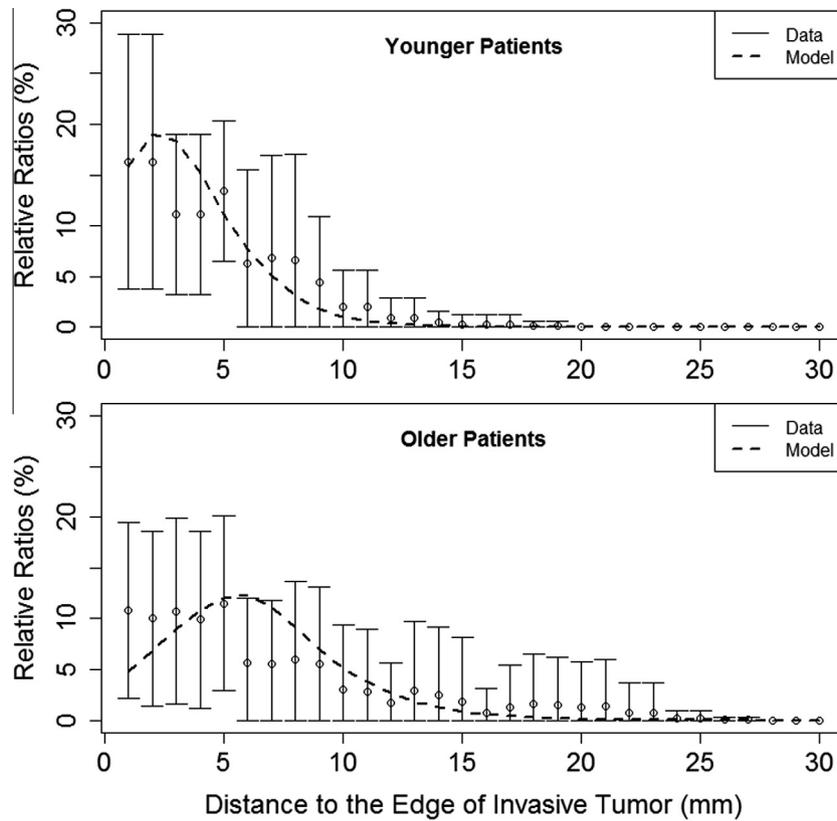
**Table 1**
Regression parameters in Zero-inflated Geometric Model. The quantity was modeled with the Geometric distribution and a larger-proportion of zeroes was corrected with the Zero-inflation part of the model.

| | Estimate | Std. error | *P*-value |
|---|---|---|---|
| *Geometric model coefficients:* | | | |
| Intercept | 4.09 | 0.18 | <0.001 |
| Age ⩽ 50 | 0.77 | 0.35 | 0.028 |
| *Zero-inflation model coefficients:* | | | |
| Intercept | −0.98 | 0.30 | <0.001 |

**Fig. 1.** Relative ratios of disease quantity (disease quantity in a shell over total disease quantity) as a function of distance to the edge of macroscopic tumor for both patient groups derived from pathology data. The circles represent the average and the error bars represent one standard deviation for each shell.

**Table 2**
The estimated parameters from our simulation framework. The Negative Binomial model and the first Poisson model (Spread) are used in the MSD spread model in disease load prediction. The Beta model and the second Poisson model (Margin) are used in surgery simulation. CCF and the mean and the standard deviation of radiosensitivity are used in the TCP model in radiotherapy modeling. (CI = Confidence Interval).

| | Patients ⩽ 50 years | | Patients > 50 years | | Combined P-value |
|---|---|---|---|---|---|
| | Estimate | 95% CI | Estimate | 95% CI | |
| Negative Binomial *mu* | 3.92 | [2.5–5.3] | 6.87 | [5.7–9.0] | <0.001 |
| *Scale* | 15.25 | [1.5–95.2] | 3.91 | [3.0–4.9] | 0.576 |
| Poisson (Spread) | 2.67 | [2.1–3.4] | 3.32 | [2.8–4.0] | 0.013 |
| Poisson (Margin) | 12.23 | [11.3–13.9] | 17.85 | [16.9–18.9] | <0.001 |
| Beta (symmetric) | 1.97 | [1.3–2.6] | 1.34 | [1.1–1.7] | <0.001 |
| Clonogenic Cell Fraction | $1.86 * 10^{-5}$ | $[4.3 * 10^{-7}–8.5 * 10^{-5}]$ | $2.57 * 10^{-7}$ | $[0.5 * 10^{-7}–4.8 * 10^{-7}]$ | <0.001 |
| Radiosensitivity *mean* $(Gy^{-1})$ | .111 | [.091–.132] | .071 | [.050–.091] | <0.001 |
| Radiosensitivity *s.d.* $(Gy^{-1})$ | .037 | [.030–.044] | .018 | [.012–.023] | <0.001 |

**Table 3**
The TCP outcome of patients with a median 10 year follow-up in the EBCTCG dataset [17] and the EORTC dataset [15]. (BCS = Breast Conserving Therapy, RT = Radiation Therapy, CI = Confidence Interval).

| Outcome TCP | BCS only[a] | BCS + RT 50 Gy | BCS + RT 66 Gy |
|---|---|---|---|
| Age ⩽ 50 years | 52% | 78.9% | 89.9% |
| 95% CI | [47–56%] | [73.9–83.5%] | [86.2–93.1%] |
| #Patients | 932 | 273 | 296 |
| Age > 50 years | 69% | 92.5% | 95.6% |
| 95% CI | [67–72%] | [90.1–94.6%] | [93.6–97.2%] |
| #Patients | 3206 | 528 | 519 |

[a] In EBCTCG study, patients were separated into five categories by age. We took the first two categories as the younger patients and the other as the older patients.

23 Gy. The clonogenic cell fraction was the most important factor we may use to explain the difference in TCP between younger and older patients.

## Discussion

This study aimed to explain the effect of age on the tumor control probability (TCP) in patients undergoing breast conserving therapy (BCT) for early-stage breast cancer. To pursue this goal, we used a simulation framework which contains mathematic models for describing the load and spread of microscopic disease (MSD) based on a retrospective cohort of breast pathology specimens, a surgery simulation model for estimating the remaining disease quantity and a TCP model for predicting the risk of local recurrence. The results indicated that the inferior outcome in younger patients may be explained by larger total MSD quantity and larger clonogenic cell fraction (CCF), even though MSD cells in the younger patients are more radiosensitive.

An overview of clinical trial data has shown that breast conserving surgery (BCS) should be followed by radiotherapy to achieve results comparable with mastectomy in terms of recurrence and
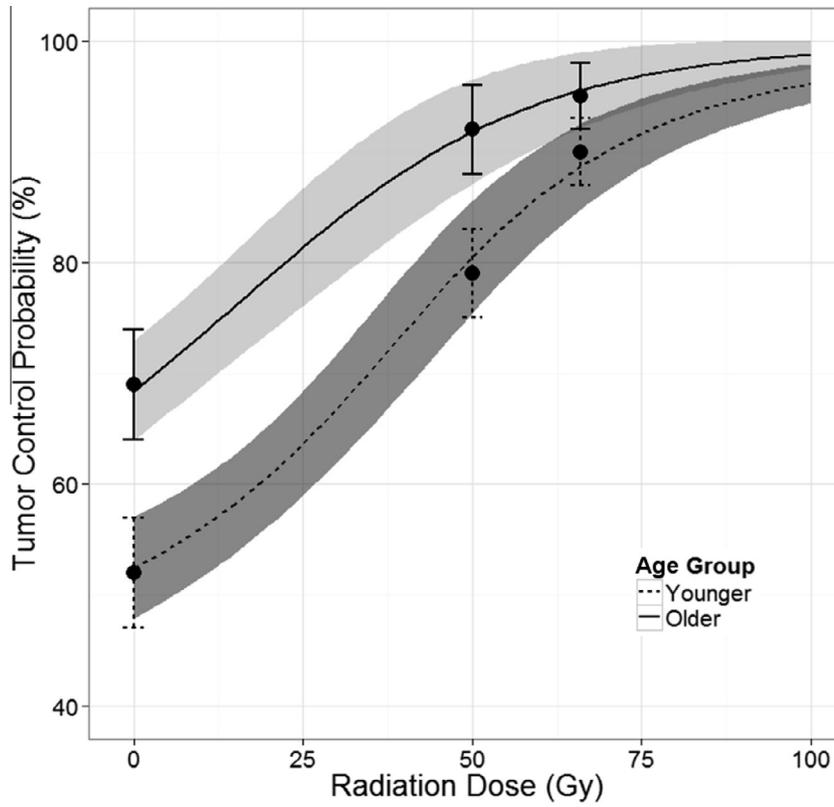
**Fig. 2.** The relationship between radiation dose and TCP in the different age groups. The shaded areas indicate the 95% confidence interval of the estimates. The error bars represent the reported clinical outcome from the EORTC and the EBCTCG studies.
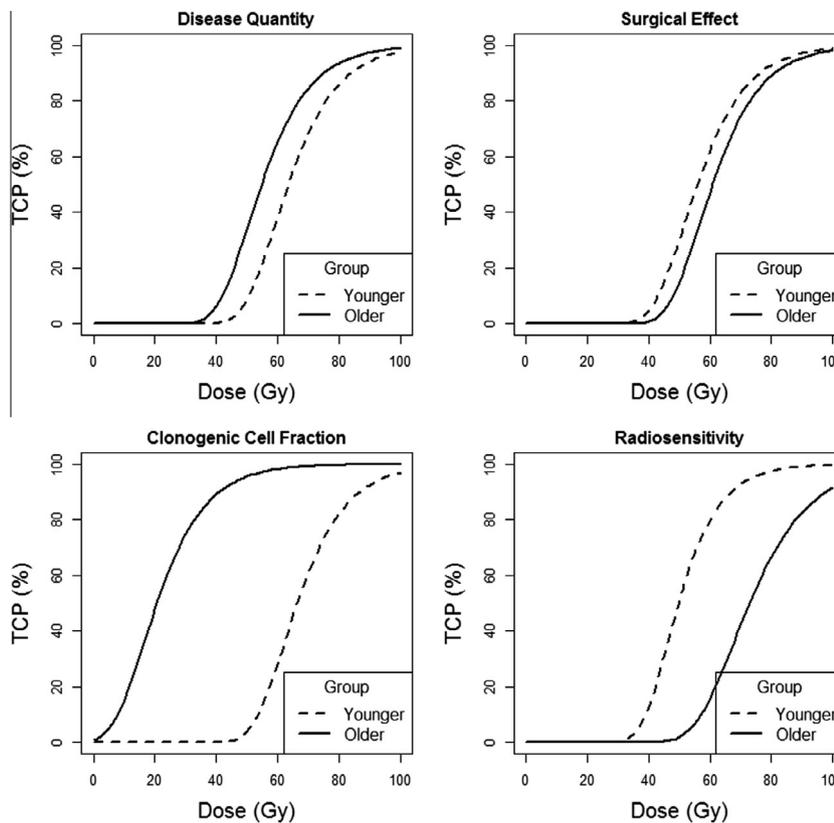


**Fig. 3.** The dose-TCP relationship curves for younger and older patients stratified for different factors that affect TCP in the first scenario. The dash curve represents the result for younger patients; the solid curve represents the result for older patients.
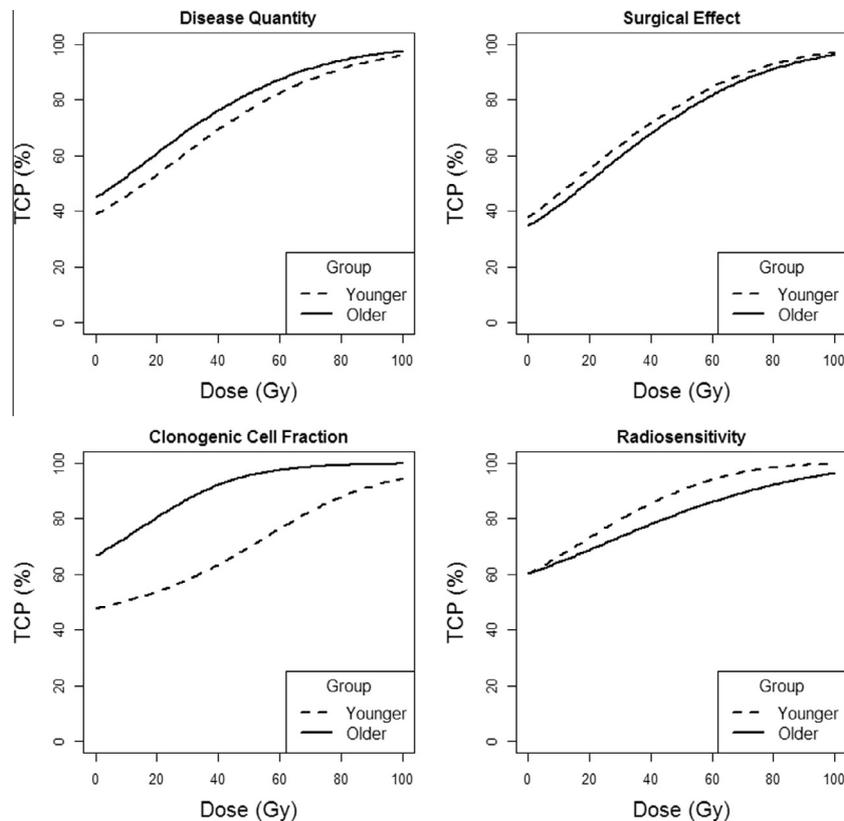
**Fig. 4.** The dose-TCP relationship curves for younger and older patients stratified for different factors that affect TCP in the second scenario. The dash curve represents the result of younger patients; the solid curve represents the result of older patients.

survival [3]. However, the value of radiotherapy (RT) after BCS in older breast cancer patients is still a subject of debate [3,22]. In the current clinical setting, over 60% of breast cancer patients are diagnosed at the age above 50 years. The treatments of these older patients vary and the outcome differ [23]. For some older women, breast conserving surgery without radiation may be used to minimize potential treatment-related complications [24]. Gruenberger et al. suggested that radiation may be safely omitted for low-risk tumors in women over age 60 years [22]. Unfortunately, different results were reported by the Harvard 2006 pilot study [25] and the Italian randomized trial [26]. Consequently, these lead to the conclusion that the omission of RT should be considered carefully in subgroups of patients. However, this goal was not straightforward due to the lack of knowledge on the correlation between radiation dose and TCP. This study pursued to fill in this research blank and provided a quantified relationship between radiation dose, MSD and TCP for the older patients.

On the same note, several studies reported that younger breast cancer patients had inferior outcome than older patients [2,27,28]. The EORTC boost-versus-no-boost trial showed that local recurrence was strongly correlated with age. Although there was a statistically significant benefit to the boost in all age groups, the absolute benefit was higher for younger women. A randomized phase III trial [29] was initiated in 2005 to investigate the potential benefit of an additional 10 Gy dose to the tumor bed in younger patients in addition to a uniform 50 Gy dose and a standard boost 16 Gy. The 10 year local recurrence results have not been reported yet. Using our simulation framework for younger patients, we predict that the TCP at 10 years will increase from 81.8% to 89.8% by a standard boost of 16 Gy and further to 91.9% (95% CI: 89.2 ∼ 94.6%) using a standard boost plus the additional 10 Gy with the same eligibility criteria for adjuvant chemotherapy/hormone therapy as

the EORTC boost-verse-no-boost trial. Note that the local recurrence rate of the total cohort in the young boost trial [29] shall be better than our prediction on this patient-matched case, because more patients in the young boost trial received the adjuvant systemic therapy and benefited from many improvements in the treatment technology. These issues are discussed in the following sections.

In previous work [10], the total MSD quantity was considered as a function of age at diagnosis, tumor grade and tumor diameter, and the function parameters were estimated using the pathology dataset. In the current study, the effect of age on treatment outcome was the major focus; therefore, we only chose age to establish a link with the patient's disease quantity. The younger patients had significantly larger quantity of disease than older patients both before and after surgery. This finding supports the necessity of an additional radiation dose in younger patients.

Different from the previous research paper [33], we treated the age parameter as a discrete variable instead of a continuous variable. The advantage of separating patients into two groups is that we can deploy a structural modeling approach for analyzing the correlation between the histopathology characteristics of tumors and the outcome of the trials. Clearly, our study approach has the predictive ability on the local recurrence rate in a new trial with varying radiation doses. A similar prediction is impossible to obtain in a regression-form analysis. So far, to the best of our knowledge, no similar study exists that provides a predictive modeling platform.

The radiosensitivity in younger patients was estimated to be larger on average than that in the older patients, which may explain the larger absolute TCP benefit of the boost dose in the younger age group [15]. Moreover, the analyses of factors that may affect TCP suggest that clonogenic MSD cell quantity plays a
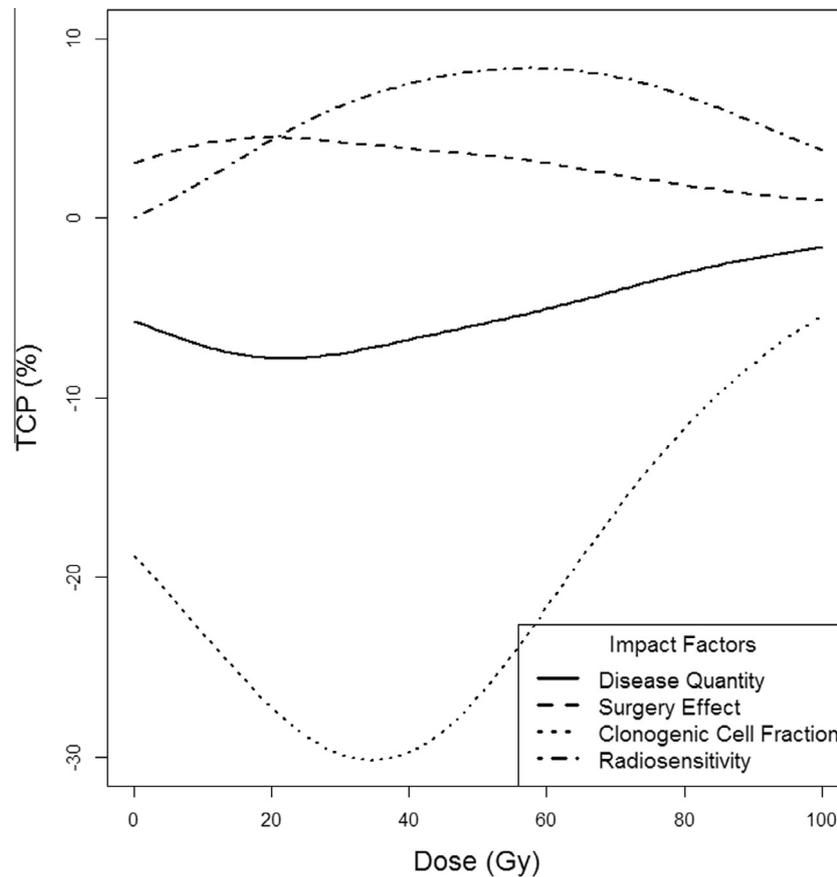
**Fig. 5.** The TCP difference curves as a function of radiation doses for the four factors impacting treatment outcome between the two age groups. Total MSD quantity (solid), surgery effect (dash), clonogenic cell fraction (dot) and radiosensitivity (dash-dot).

more important role on the TCP than radiosensitivity. A large research focus exists world-wide on genomic profiling and experimental study to understand the radiobiology of breast cancer [4,30,31], but very few studies address the prediction of tumor cell quantity [32]. We recommend that additional effort should be spent on predicting tumor cell quantity (e.g., building mathematic models to predict microscopic disease extension using multiple imaging modalities [13]) in order to design effective treatment plans for high-risk subgroup patients.

Due to the nature of a retrospective study, our analysis is only based on the simulations using the cohorts, which consist of the treatments of surgery and radiotherapy. Adjuvant therapy is frequently used to treat patients while our framework does not include the effect of chemotherapy or hormone therapy. As has been well demonstrated, both Tamoxifen and chemotherapy reduce the risk of local recurrence by half, seen for example in the complete data of the boost-versus-no-boost trial of 5318 patients [33]. To incorporate systemic treatment in our framework, one may thus approximate the local recurrence risk with a correction factor of 0.5 when adjuvant therapy is given, however, should be also always cautious on the interaction between different types of treatments.

Several limitations of our study should be mentioned. First, we split our cohort data by age into only two groups due to the limitation of a small number of younger patients ($N = 12$) in our completely embedding pathology dataset. Menopausal status or other age related factors (e.g., ER receptor) may also play an important role on the local recurrence rate which unfortunately could not be investigated using the current datasets of this study. However, the analysis can be easily empowered with a larger pathology dataset for the desired factors. Second, the higher recurrence risk of

breast cancer patients was observed in our study than those in other recent studies. Possible explanations for the lower rate in new trials are better preoperative staging imaging procedures, use of image-guided surgery with pathological assessment of the margins, optimized radiotherapy with 3D treatment planning, and more widespread use of effective adjuvant systemic treatment [34]. Third, under-sampling may have occurred in the estimated microscopic disease quantity data. The resection specimens were first sliced macroscopically in 4 mm slabs, and then further trimmed and examined on microscopic slices of 4 μm thickness. To increase the robustness of the estimation, we proposed to use statistical models and a simulation framework to account for the uncertainties in data. Fourth, we generalized the disease spread from the pathology data in our own institute ($N = 60$) to the patients enrolled in the multi-institutional clinical trials based on the assumption that the patient groups have comparable characteristics. We believed this was a valid assumption because all patients in the pathology study received BCT. Fifth, the microscopic cell density was estimated from only 12 patients. No further data were analyzed because the likelihood to find a significant difference between younger and older patients is small due to the close average values and the large variation. Sixth, we assumed that all MSD cells received the prescribed dose, ignoring dose heterogeneity and setup errors.

**Conclusion**

The microscopic disease quantity, impact of surgery, radiosensitivity and clonogenic cell fraction of breast cancer patients were studied and compared between two age groups. Inferior outcome of treatment in younger patients could be explained by the larger

microscopic disease quantity and larger clonogenic cell fraction, even though the microscopic disease cells in younger patients were estimated to be more radiosensitive.

## Conflict of interest

None.

## Acknowledgements

## References

[1] Early Breast Cancer Trialists' Collaborative Group. Effects of radiotherapy and surgery in early breast cancer – an overview of the randomized trials. N Engl J Med 1995;333:1444–56.

[2] Antonini N, Jones H, Horiot JC, Poortmans P, Struikmans H, Van den Bogaert W, et al. Effect of age and radiation dose on local control after breast conserving treatment: EORTC trial 22881-10882. Radiother Oncol 2007;82:265–71.

[3] Beadle BM, Woodward WA, Buchholz TA. The impact of age on outcome in early-stage breast cancer. Semin Radiat Oncol 2011;21:26–34.

[4] Nuyten DS, Kreike B, Hart AM, Chi JT, Sneddon JB, Wessels LF. Predicting a local recurrence after breast-conserving therapy by gene expression profiling. Breast Cancer Res 2006;8:R62.

[5] Elkhuizen PH, van Slooten HJ, Clahsen PC, Hermans J, van de Velde CJ, van den Broek LC, et al. High local recurrence risk after breast-conserving therapy in node-negative premenopausal breast cancer patients is greatly reduced by one course of perioperative chemotherapy. J Clin Oncol 2000;18:1075–83.

[6] Dillon MF, Hill ADK, Quinn CM, McDermott EW, O'Higgins N. A pathologic assessment of adequate margin status in breast-conserving therapy. Ann Surg Oncol 2006;13:333–9.

[7] Tartter P, Kaplan J, Bleiweiss I. Lumpectomy margins, reexcision, and local recurrence of breast cancer. Am J Surg 2000;179:81–5.

[8] Nixon AJ, Neuberg D, Hayes DF, Gelman R, Connolly JL, Schnitt S. Relationship of patient age to pathologic features of the tumor and prognosis for patients with stage I or II breast cancer. J Clin Oncol 1994;12:888–94.

[9] Zhou P, Gautam S, Recht A. Factors affecting outcome for young women with early stage invasive breast cancer treated with breast-conserving therapy. Breast Cancer Res Treat 2007;101:51–7.

[10] Chen W, Gilhuijs K, Stroom J, Bartelink H, Sonke J. A simulation framework for modeling tumor control probability in breast conserving therapy. Radiother Oncol 2014;111:289–95.

[11] Stroom J, Schlief A, Alderliesten T, Peterse H, Bartelink H, Gilhuijs K. Using histopathology breast cancer data to reduce clinical target volume margins at radiotherapy. Int J Radiat Oncol Biol Phys 2009;74:898–905.

[12] Schmitz AC, van den Bosch MA, Loo CE, Mali WP, Bartelink H, Gertenbach M. Precise correlation between MRI and histopathology – exploring treatment margins for MRI-guided localized breast cancer therapy. Radiother Oncol 2010;97:225–32.

[13] Schmitz AC, Pengel KE, Loo CE, van den Bosch MAA, Wesseling J, Gertenbach M, et al. Pre-treatment imaging and pathology characteristics of invasive breast cancers of limited extent: potential relevance for MRI-guided localized therapy. Radiother Oncol 2012;104:11–8.

[14] Chen W, Stroom J, Sonke J-J, Bartelink H, Schmitz AC, Gilhuijs KG. Impact of negative margin width on local recurrence in breast conserving therapy. Radiother Oncol 2012;104:148–54.

[15] Bartelink H, Horiot J-C, Poortmans PM, Struikmans H, Van den Bogaert W, Fourquet A, et al. Impact of a higher radiation dose on local control and survival in breast-conserving therapy of early breast cancer: 10-year results of the randomized boost versus no boost EORTC 22881-10882 trial. J Clin Oncol 2007;25:3259–65.

[16] Jones HA, Antonini N, Hart AAM, Peterse JL, Horiot J-C, Collin F, et al. Impact of pathological characteristics on local relapse after breast-conserving therapy: a subgroup analysis of the EORTC boost versus no boost trial. J Clin Oncol 2009;27:4939–47.

[17] Darby S, McGale P, Correa C, Taylor C, Arriagada R, Clarke M, et al. Effect of radiotherapy after breast-conserving surgery on 10-year recurrence and 15-year breast cancer death: meta-analysis of individual patient data for 10,801 women in 17 randomised trials. Lancet 2011;378:1707–16.

[18] Vrieling C, Collette L, Fourquet A, Hoogenraad W, Horiot J-C, Jager J. Can patient-, treatment- and pathology-related characteristics explain the high local recurrence rate following breast-conserving therapy in young patients? Eur J Cancer 2003;39:932–44.

[19] Webb S, Nahum A. A model for calculating tumour control probability in radiotherapy including the effects of inhomogeneous distributions of dose and clonogenic cell density. Phys Med Biol 1999;38:653–66.

[20] Rüschendorf L. Random variables with maximum sums. JSTOR. Adv Appl Prob 1982;14:623–32.

[21] Hoe AL, Mullee MA, Royle GT, Guyer PB, Taylor I. Breast size and prognosis in early breast cancer. Ann R Coll Surg Engl 1993;75:18–22.

[22] Gruenberger T, Gorlitzer M, Soliman T, Rudas M, Mittlboeck M, Gnant M, et al. It is possible to omit postoperative irradiation in a highly selected group of elderly breast cancer patients. Breast Cancer Res Treat 1998;50:37–46.

[23] Mandelblatt JS. Predictors of long-term outcomes in older breast cancer survivors: perceptions versus patterns of care. J Clin Oncol 2003;21:855–63.

[24] Cirrincione C, Mccormick B, Shank B, Ph D, Wheeler J, Champion LA, et al. Lumpectomy plus Tamoxifen with or without Irradiation in women 70 years of age or older with early. Breast Cancer 2013;971–7.

[25] Lim M, Bellon JR, Gelman R, Silver B, Recht A, Schnitt SJ, et al. A prospective study of conservative surgery without radiation therapy in select patients with Stage I breast cancer. Int J Radiat Oncol Biol Phys 2006;65:1149–54.

[26] Tinterri C, Gatzemeier W, Costa A, Gentilini MA, Zanini V, Regolo L, et al. Breast-conservative surgery with and without radiotherapy in patients aged 55–75 years with early-stage breast cancer: a prospective, randomized, multicenter trial analysis after 108 months of median follow-up. Ann Surg Oncol 2014;21:408–15.

[27] Van der Leij F, Elkhuizen PHM, Bartelink H, van de Vijver MJ. Predictive factors for local recurrence in breast cancer. Semin Radiat Oncol 2012;22:100–7.

[28] Miles RC, Gullerud RE, Lohse CM, Jakub JW, Degnim AC, Boughey JC. Local recurrence after breast-conserving surgery: multivariable analysis of risk factors and the impact of young age. Ann Surg Oncol 2012;19:1153–9.

[29] The Netherlands Cancer Institute. Radiation dose intensity study in breast cancer in young women. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2000–[April 18, 2014]. Available from: <http://clinicaltrials.gov/show/NCT00212121 NLM Identifier: NCT00212121>.

[30] van't Veer L, Dai H, Vijver Van De M. Gene expression profiling predicts clinical outcome of breast cancer. Nature 2002;415:530–6.

[31] Van de Vijver MJ, He YD, van't Veer LJ, Dai H, Hart AAM, Voskuil DW. A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med 2002;347:1999–2009.

[32] Margenthaler J, Gao F, Klimberg VS. Margin index: a new method for prediction of residual disease after breast-conserving surgery. Ann Surg Oncol 2010;17:2696–701.

[33] van Werkhoven E, Hart G, Van Tinteren H, Elkhuizen P, Collette L, Poortmans P, et al. Nomogram to predict ipsilateral breast relapse based on pathology review from the EORTC 22881-10882 boost versus no boost trial. Radiother Oncol 2011;100:101–7.

[34] Bartelink H, Maingon P, Poortmans P, Weltens C, Fourquet A, Jager J, et al. Whole-breast irradiation with or without a boost for patients treated with breast-conserving surgery for early breast cancer: 20-year follow-up of a randomised phase 3 trial. Lancet Oncol 2014;16:47–56.