Prognosis, stage and oestrogen receptor status of contralateral breast cancer in relation to characteristics of the first tumour, prior endocrine treatment and radiotherapy.

Alkner, Sara; Ehinger, Anna; Bendahl, Pär-Ola; Rydén, Lisa; Fernö, Mårten

Published in:
European Journal of Cancer

DOI:
10.1016/j.ejca.2015.07.016

2015

Prognosis, stage and estrogen receptor status of contralateral breast cancer in relation to characteristics of the first tumor, prior endocrine treatment and radiotherapy

Sara Alkner[a, b], Anna Ehinger[a, c], Pär-Ola Bendahl[a], Lisa Rydén[a, d], Mårten Fernö[a].

[a] Division of Oncology and Pathology, Clinical Sciences Lund, Lund University Medicon Village, 223 63 Lund, Sweden.

[b] Skåne Clinic of Oncology, Skåne University Hospital Lund, 222 41Lund, Sweden.

[c] Department of Pathology and Cytology, Blekinge County Hospital, 371 85 Karlskrona, Sweden.

[d] Clinic of Surgery, Skåne University Hospital Lund, 222 41 Lund, Sweden.

E-mail addresses:

Sara Alkner: Sara.Alkner@med.lu.se

Pär-Ola Bendahl: Par-Ola.Bendahl@med.lu.se

Anna Ehinger: anna.ehinger@med.lu.se

Mårten Fernö: Marten.Ferno@med.lu.se

Lisa Rydén: Lisa.Ryden@med.lu.se

Corresponding author: Sara Alkner, MD, PhD, Skåne Clinic of Oncology, Skåne University Hospital Lund, 22241, Lund, Sweden. Telephone: +46736002927 or +4646177617.

Fax: +4646147327.

Acknowledgements

We want to thank Kristina Lövgren for construction of the TMA and for retrieving tumor paraffin blocks. We also want to thank Gunilla Chebil for locating and collecting paraffin blocks, and Sara Baker for data base construction and collection of paraffin blocks.
In addition we are indebted to the South-Swedish Oncological Centre providing us with data from the Cancer Register, and to the skilled and helpful staff at the surgical, oncological and pathological clinics, and at the medical archives in Lund, Malmö, Helsingborg, Ängelholm, Landskrona, Ystad, Trelleborg, Kristianstad, Växjö, Ljungby, Halmstad, Karlshamn and Karlskrona. Without their help this study could never have been accomplished. We are also indebted to Per Hall and Kamila Czene, Karolinska Institutet, Stockholm for providing us with the protocol used for data abstraction from patients' charts.

Financial support: This work was supported by the Swedish Breast Cancer Association (BRO) (2011, 2014); the Skåne University Hospital foundation (2014-94413); the Percy Falk Foundation (2011); the Swedish Cancer Society (13 0499); the Swedish Research Council (B0262601, 521-2011-3021); the Gunnar Nilsson Cancer Foundation (2013); the Mrs. Berta Kamprad Foundation (13/2013); the Anna and Edwin Bergers Foundation (2014); Skane County Council's Research and Development Foundation (REGSKANE-434091); and Governmental Funding of Clinical Research within the National Health Service (2011-2014).

Role of the Funding Sources: None of the funding sources had any impact on study design; the collection, analysis and interpretation of data; the writing of the report; or in the decision to submit the article for publication.

Abstract

Aim: A contralateral breast cancer (CBC) is today treated as an independent primary tumor, although recent data suggest risk and prognosis of CBC to be influenced by characteristics of and treatment given for the first tumor (BC1). We hereby investigate phenotypical and prognostic features of the second tumor (BC2) in relation to prior endocrine treatment and radiotherapy.

Methods: From a well-defined population-based cohort of CBC-patients, we have constructed a unique tissue-microarray including 600 pairs of primary tumors and CBCs. Breast cancer mortality was primary end-point for prognosis.

Results: Both estrogen receptor (ER) status and stage was strongly correlated between BC1 and BC2 within CBC-pairs. Although BC2 had the highest prognostic impact, BC1 continued to influence prognosis after diagnosis of CBC. Patients diagnosed with two high stage tumors within a short time-interval had a particularly bad prognosis. Prior endocrine therapy and radiotherapy both correlated to ER-negativity of BC2. An ER-negative BC2 was associated with an inferior prognosis compared to an ER-positive BC2 regardless of ER-status of BC1 or prior endocrine therapy.

Conclusions: Our results suggest that both the residual prognostic impact of BC1, the possibility of contralateral metastasis, as well as prior treatment given, need to be considered when determining appropriate diagnostic work-up and treatment of CBC. In addition, radiation to the contralateral breast and risk of inducing CBC with an aggressive ER-negative phenotype should be considered when establishing new radiation treatment techniques. This study indicates loss of ER-expression as an important "endocrine treatment escape mechanism", although further studies are warranted.

Highlights

We investigate contralateral breast cancer (CBC) in relation to prior treatment

Two high stage tumors within a short time-interval indicate a bad prognosis

CBC developed after prior endocrine therapy is to a higher percentage ER-negative

Loss of ER-dependence seems to be an important “endocrine treatment escape mechanism”

CBC after prior radiotherapy is often of an ER-negative more aggressive phenotype

Keywords

Breast Neoplasms, Humans, Neoplasm Staging, Prognosis, Estrogen Receptor, Progesterone Receptor, Tissue Microarray Analysis, Radiotherapy, Hormonal Antineoplastic Agents, Tamoxifen.

Introduction

Prior breast cancer patients have a life-time risk of 2-20% of developing a contralateral breast cancer (CBC) (1-3). A CBC is today treated as a new independent primary tumor, although recent data suggest that the second tumor (BC2) may in some cases be a metastasis of the first (BC1) (4, 5). In addition, CBC diagnosed in close connection to prior adjuvant treatment is presumably resistant to the treatment given. Indeed, prior endocrine therapy, chemotherapy and radiotherapy have all been associated with a worse prognosis once diagnosed with CBC (4, 6, 7). CBC may hence be used as an *in vivo* model for studies of adjuvant treatment resistance. In addition, with new radiotherapy techniques becoming clinically available, importance of scattered dose to the contralateral breast and risk of radiation induced CBC need to be further evaluated.

We have hereby studied TNM-stage, estrogen (ER) and progesterone receptor (PR) status of BC2 in relation to characteristics of BC1 and prior treatment, using a unique tissue-microarray (TMA) including >700 CBC-patients. This is to our knowledge the largest cohort of CBC-patients with access to detailed patient, tumor and treatment information as well as tumor tissue ever studied. We hereby wish to clarify the biological relationship between CBC-pairs, and find indications as to when contralateral metastasis should be suspected and clonal relationship further investigated. We also want to investigate phenotypical and prognostic features of BC2 in relation to prior treatment. This could not only give us important information on how to optimize treatment for patients with CBC, but also increase our knowledge on treatment escape mechanisms *in vivo*.

Patients and Methods

Tissue microarray and immunohistochemistry

Inclusion criteria and data abstraction have been described before (4). Briefly all patients within the Southern Swedish Healthcare Region with two breast cancers reported in the Swedish Cancer Registry, and BC2 diagnosed between 1977 and 2007 were included. Clinical data was abstracted from individual charts and paraffin-embedded tissue collected. We focused on patients with metachronous CBC (≥3 months between tumors), excluding patients with synchronous CBC, patients with distant metastasis or another malignancy diagnosed before BC2, and patients with BC2 found only in the axilla. For the remaining 764 patients, paraffin blocks were available for 643 BC1 and 685 BC2, giving a total of 728 patients included in the TMA (Figure 1). After exclusion according to predefined criteria 688 patients were considered in the main statistical analysis. From representative areas of the invasive breast cancers, tissue-core-biopsies (diameter 1.0 mm) were punched out and mounted into the recipient block using a tissue-array-machine (Beecher Instruments, USA).

ER and PR were reevaluated by a pathologist (AE), using immunohistochemistry (Ventana Benchmark system, 790-4324 clone SP1 and 790-2223 clone 1E2) (8). In line with Swedish clinical standard during this period, tumors with ≥10% stained nuclei were considered positive. The project was approved by the Regional Ethical Review Board of Lund University (LU240-01) and carried out in accordance with the code of ethics of the World Medical Association. All data was handled confidentially according to Sweden's Personal-Data-Act.

Statistical analysis

Survival-data and cause of death was retrieved from the Swedish National Board of Health and Welfare (March 2014), and breast cancer mortality (BCM) chosen as primary end-point.

BCM includes breast cancer death or death after metastasis as a primary event. Event-free survival was measured from diagnosis of CBC.

For statistical calculations, the software package Stata 11.2 (StataCorp. 2009. TX, USA) was used. General comparisons between groups of BC1 and BC2 were done with McNemar's test, Wilcoxon matched-pairs signed-ranks test or McNemar-Bowker's test of symmetry (Table 1). Associations between tumor-pairs or treatment groups were evaluated with χ^2-test or χ^2-test for trend (Table 2). Prognosis after BC2 was summarized graphically as cumulative BCM and cause-specific Cox-regression, treating competing events (death not related to breast cancer) as censoring, was used to estimate hazard ratios (HR). Plots were curtailed when ≤5 individuals remained at risk. When relating stage of BC1 and BC2 to prognosis after BC2 (Table 3), a full factorial Cox-model with 8 parameters was fitted.

Assumptions of proportional hazards were checked graphically. Risk factors for ER-negativity of BC2 were determined with logistic-regression. The Wilcoxon rank-sum test evaluated the relationship between time-interval and ER-status. Age at BC1 was categorized as <50 *vs.* ≥50 years. To adjust for calendar period, the material was divided in thirds by diagnosis-date of BC1. P-values correspond to two-sided tests and values <0.05 were considered significant.

Approximately 90% of patients with endocrine therapy for BC1 received tamoxifen (Table 1) (remaining 16 patients: 14 oophorectomy, 1 oophorectomy+tamoxifen, 1 tamoxifen-aromatase inhibitor). Initially, patients with endocrine treatment for BC1 were compared with those without. Other prior adjuvant treatment did not significantly differ between groups (Among patients with endocrine therapy for BC1, 64% received radiotherapy and 6% chemotherapy for BC1. Among patients without endocrine therapy for BC1, 61% received radiotherapy and 11% chemotherapy for BC1). Analyses were repeated comparing prior

tamoxifen *vs*. no prior endocrine therapy, and only prior tamoxifen *vs*. no prior adjuvant treatment (data not shown).

Patients with only prior radiotherapy were compared with patients without any prior adjuvant treatment, and analyses repeated for all patients with prior radiotherapy *vs*. all other patients (data not shown).

Multivariable analyses were adjusted for characteristics and when appropriate also for treatment of BC1 (calendar period, age, time-interval to BC2, size, lymph-node status, ER, PR, endocrine treatment, chemotherapy and radiotherapy) (Table 4). No interaction terms were considered and the main effects were assumed to be independent. Since effect of biology and treatment of BC1 on BC2 was to be investigated, adjusting for characteristics of BC2 would risk concealing such effects.

Results

Tumor characteristics

Patient and tumor characteristics are described in Table 1. Median follow-up time was 11.4 years for patients without breast cancer related death, and 9.1 years in the whole patient cohort. BC2 was generally smaller than BC1 and more often PR-negative. In addition, BC2 was less often treated with radiotherapy, but more often with endocrine therapy. No general difference was seen between BC1 and BC2 in type of surgery used, lymph-node status, TNM-stage, or ER-status. When comparing tumors within CBC-pairs, both TNM-stage, ER- and PR-status were significantly correlated (Table 2).

Prognostic significance of BC1 *vs.* BC2.

As expected, BC2 had the highest impact on prognosis after diagnosis of CBC (Figure 2, Table 3). However, within the individual stages of BC2, stage of BC1 contributed with prognostic information. The additional prognostic effect of BC1 diminished with increasing time between tumors. Patients diagnosed with two stage III tumors within five years had a twelve times higher HR for breast cancer mortality than if both tumors were of stage I (17 of 17 patients with two stage III tumors diagnosed within 5 years died from breast cancer). This effect seemed to be higher than what would be expected if the two tumors represented two independent events (HR 1.8, p=0.3). However, patients with two stage III tumors were few, making results more unreliable and statistical significance hard to achieve.

An ER-positive BC2 was associated with a better prognosis compared to an ER-negative BC2 regardless of ER-status of BC1 (Figure 2c) (multivariable Cox-regression HR 0.5, 95%CI 0.3-0.7, p<0.001). Patients with an ER-negative BC2 had a slightly, although not significantly, better prognosis if BC1 was ER-positive. Similar results as above were seen for PR (data not shown).

Characteristics of BC2 in relation to prior endocrine therapy

Prior endocrine therapy was significantly correlated to ER- and PR-negativity of BC2 (Supplemental Table 1). Without prior endocrine therapy 92% of patients with an ER-positive BC1 developed an ER-positive BC2 and 41% of patients with an ER-negative BC1 an ER-negative BC2. The corresponding numbers after endocrine therapy was 77% *vs.* 59%. Without prior endocrine therapy 14% of CBCs were ER-negative compared to 27% when prior endocrine treatment had been given (p<0.001). This effect was more pronounced in older women (ER-negative BC2: <50 years at BC1 23% *vs.* 30%, ≥50 years 9% *vs.* 27%. Term of

interaction p=0.04), and non-significantly so also with a short time-interval to BC2 (<5 years to BC2 15% *vs.* 37%; ≥5 years to BC2 12% *vs.* 19%. Interaction p=0.1). Similar results were seen in regard to PR (Supplemental Table 1).

A multivariable logistic-regression model with tumor and treatment characteristics of BC1 as covariates showed young age, short time-interval to BC2, ER-negativity of BC1, and endocrine treatment given for BC1 to be significant risk factors predicting development of an ER-negative BC2 (Table 4). There was also a trend for ER-negativity after prior radiotherapy. A similar analysis showed prior endocrine treatment (OR 3.6, 95%CI 1.7-7.7) and prior radiotherapy (OR 2.5, 95%CI 1.1-5.6) to be predictive of a change in receptor status from an ER-positive BC1 to an ER-negative BC2.

There was no significant difference in time to development of an ER-positive or an ER-negative BC2 in patients without any prior adjuvant therapy (ER-positive 6.6 years, ER-negative 6.3 years), or in patients without prior endocrine treatment (ER-positive 8.6 years, ER-negative 9.1 years). With prior endocrine therapy, on the other hand, there was a shorter mean time-interval to development of an ER-negative (6.1 years) than an ER-positive BC2 (7.9 years) (p=0.05). However, this difference in time-interval to BC2 in relation to ER-status and endocrine treatment was not significant (p=0.2 for the interaction term in a linear-regression model).

With or without prior endocrine treatment an ER-positive BC2 was associated with a better prognosis than an ER-negative BC2 (Figure 2d). Patients developing ER-positive CBC within 5 years of BC1 had a higher BCM if they had received endocrine treatment for BC1 (Prior endocrine treatment *vs.* no prior endocrine treatment: HR 1.5, 95% CI 0.94-2.6, p=0.09. Only prior endocrine treatment *vs.* no prior adjuvant treatment: HR 2.9, 95% CI 1.5-5.5, p=0.001). However, significance did not remain in multivariable analysis.

All analyses were repeated for prior tamoxifen *vs.* no prior endocrine treatment and only prior tamoxifen *vs.* no prior adjuvant treatment, with similar results (data not shown).

Characteristics of BC2 in relation to prior radiotherapy

Interestingly, also prior radiotherapy was significantly correlated to ER- and PR-negativity of BC2 (Supplemental Table 1). In patients without any prior adjuvant therapy 9% of CBCs were ER-negative compared to 16% in patients having received radiotherapy as only adjuvant treatment for BC1 (p=0.04). This effect was non-significantly more pronounced with a short time-interval to BC2 (<5 years 8% *vs.* 19%, ≥5 years 10% *vs.* 14%). No difference in time-interval to development of an ER-positive *vs*. ER-negative CBC in relation to prior radiotherapy was found (ER-positive 9.7 years, ER-negative 8.9 years). No relation was seen between ER-status of BC1 and if radiotherapy was given. Hence, these results are not explained by a selection bias of patients with an ER-negative BC1 more often receiving radiotherapy.

Previously published results of a worse prognosis for CBC-patients after prior radiotherapy (4) was confirmed in this subgroup with more detailed information on TNM-stage and hormone-receptor status (multivariable Cox-regression of only prior radiotherapy *vs.* no prior adjuvant treatment adjusted for characteristics of BC1 as described above. HR 1.9, 95%CI 1.2-3.0, p=0.006).

Analyses were repeated comparing all patients with prior radiotherapy *vs*. all other patients with similar results.

Discussion

To our knowledge, this is the largest cohort of CBC-patients with access to detailed patient information, a long follow-up period, as well as tumor-tissue, ever studied. As previously suggested, we found hormone-receptor status within tumor-pairs to be highly correlated (9, 10). In addition, TNM-stage was correlated between tumors. This may in part be explained by individual patient's delay in reporting symptoms and compliance to follow-up. Other explanations are genetic and environmental factors inducing a specific tumor-type. However, recently the possibility of BC2 representing a metastasis of BC1 has also been suggested. Supporting this are studies showing lymph-node-status of BC1 to influence risk of CBC and the time-interval between tumors to affect prognosis (4, 5). In addition, genetic comparisons show some CBC to have features similar enough to BC1 that a metastatic spread is possible (11-15).

We found that although prognosis after CBC is mainly determined by BC2, BC1 continues to have an impact. Patients developing two high stage tumors within a short time-interval seemed to have a worse prognosis than expected if these tumors represented two independent events. This may hence be a patient-group where contralateral metastasis could be more common and further investigation of clonal relationship warranted.

Prior endocrine therapy was significantly correlated to ER-negativity of BC2. This is in line with previous tamoxifen prevention-trials and CBC-studies, where tamoxifen reduced incidence of ER-positive breast cancers by half, while having less, if any, effect on development of ER-negative tumors (16-18). In fact, there have even been suggestions of an increased risk of ER-negative CBC after tamoxifen use (17, 19). We cannot from this study determine whether this is due to a selective eradication of ER-positive tumors or whether some developing tumors in fact change ER-status due to treatment given.

Hence, prior endocrine therapy correlates to an increased percentage of ER-negative CBC, which in turn has a worse prognosis. However, an inferior prognosis has also been suggested for patients developing an ER-positive CBC despite prior endocrine therapy (6). This is not instantly confirmed by our study where patients with an ER-positive BC2 had a significantly better prognosis, regardless of having received prior endocrine treatment or not. A possible explanation is differences in study design and details of statistical analysis. However, another possible explanation may be that development of endocrine treatment resistance most often includes loss of ER-expression, while a remaining ER-expression indicates a still functional ER-signaling system and a less aggressive phenotype. Nevertheless, ER-positive CBC developed in close connection to BC1 had a slightly worse prognosis if endocrine treatment had been given for the first tumor. Since these results were not significant in multivariate analysis, they may in part be due to bias by indication (i.e. more aggressive tumors receiving more treatment). However, further studies of these tumors are warranted.

Recent studies have shown adjuvant radiotherapy to increased risk of CBC (20, 21). Interestingly, we found not only endocrine therapy but also prior radiotherapy to correlate to ER-negativity of BC2. This is supported by preclinical studies showing radiotherapy to reduce ER-expression in breast cancer cell-lines and rat mammary-tumors (22-25), as well as to induce ER-negative breast cancer in premenopausal mice (26). Potential explanations could be: Activation of DNA-repair pathways influencing ER-expression (23); Disturbance of ER-autoregulation involving micoRNA (27, 28); Microenvironmental changes inducing an ER-negative stem-cell-enriched phenotype (29); and promoter-hypermethylation reducing estrogen binding-affinity and signaling (25, 28).

Clinical studies are few, but data from the SEER-database suggest an increased risk of ER-negative CBC after adjuvant radiotherapy (30), and preoperative radiotherapy reduce ER-concentration in human breast cancers (31). Induced breast carcinomas after radiation of

Hodgkin's lymphoma are also often of an aggressive ER-negative subtype (32, 33). Risk of inducing CBC with an aggressive phenotype should hence be considered when choosing an optimal radiation technique. For example, the modern techniques IMRT (intense modulated radiotherapy) and VMAT (volumetric modulated arc therapy) have been shown to increase dose and second cancer risk in the contralateral breast compared to conventional 3D-conformal radiotherapy (34).

Although radiation induced second malignancies generally develop first after several years (35), we found prior radiation to affect ER-status of BC2 already within five years of treatment. One possible explanation could be not only an induction of new tumors, but also an effect on preexisting lesions. Indeed, preclinical and clinical studies cited above suggest radiotherapy to induce both intratumoral and microenvironmental changes promoting a shift towards a more ER-negative phenotype. Since ER-negative breast cancer cells are more radio-resistant (36), another possibility may also be low doses to the contralateral breast to eradicate mainly ER-positive cancer cells present, while ER-negative cells are left unaffected.

In conclusion, our results suggest that both the residual prognostic impact of BC1, the possibility of contralateral metastasis, as well as prior treatment given need to be considered when determining appropriate diagnostic work-up and treatment of CBC. Diagnosis of two tumors of a high TNM-stage within a short time-interval is associated with a bad prognosis and may indicate risk of contralateral metastasis. In addition, both prior endocrine treatment and radiotherapy correlate to an increased percentage of CBC with an aggressive ER/PR-negative phenotype.

Conflict of Interest Statement

None of the authors have any conflict of interest to declare.

References

1. Adami HO, Bergstrom R, Hansen J. Age at first primary as a determinant of the incidence of bilateral breast cancer. Cumulative and relative risks in a population-based case-control study. Cancer 1985;55(3):643-7.

2. Rutqvist LE, Cedermark B, Glas U, Mattsson A, Skoog L, Somell A, et al. Contralateral primary tumors in breast cancer patients in a randomized trial of adjuvant tamoxifen therapy. J Natl Cancer Inst 1991;83(18):1299-306.

3. Chen Y, Thompson W, Semenciw R, Mao Y. Epidemiology of contralateral breast cancer. Cancer Epidemiol Biomarkers Prev 1999;8(10):855-61.

4. Alkner S, Bendahl PO, Ferno M, Manjer J, Ryden L. Prediction of outcome after diagnosis of metachronous contralateral breast cancer. BMC Cancer 2011;11:114.

5. Vichapat V, Garmo H, Holmqvist M, Liljegren G, Warnberg F, Lambe M, et al. Tumor stage affects risk and prognosis of contralateral breast cancer: results from a large Swedish-population-based study. J Clin Oncol 2012;30(28):3478-85.

6. Sandberg ME, Hartman M, Klevebring D, Eloranta S, Ploner A, Hall P, et al. Prognostic implications of estrogen receptor pattern of both tumors in contralateral breast cancer. Breast Cancer Res Treat 2012.

7. Hartman M, Czene K, Reilly M, Adolfsson J, Bergh J, Adami HO, et al. Incidence and prognosis of synchronous and metachronous bilateral breast cancer. J Clin Oncol 2007;25(27):4210-6.

8. Alkner S, Bendahl PO, Ferno M, Nordenskjold B, Ryden L. Tamoxifen reduces the risk of contralateral breast cancer in premenopausal women: Results from a controlled randomised trial. Eur J Cancer 2009;45(14):2496-502.

9. Swain SM, Wilson JW, Mamounas EP, Bryant J, Wickerham DL, Fisher B, et al. Estrogen receptor status of primary breast cancer is predictive of estrogen receptor status of contralateral breast cancer. J Natl Cancer Inst 2004;96(7):516-23.

10. Coradini D, Oriana S, Mariani L, Miceli R, Bresciani G, Marubini E, et al. Is steroid receptor profile in contralateral breast cancer a marker of independence of the corresponding primary tumour? Eur J Cancer 1998;34(6):825-30.

11. Imyanitov EN, Hanson KP. Molecular pathogenesis of bilateral breast cancer. Cancer Lett 2003;191(1):1-7.

12. Janschek E, Kandioler-Eckersberger D, Ludwig C, Kappel S, Wolf B, Taucher S, et al. Contralateral breast cancer: molecular differentiation between metastasis and second primary cancer. Breast Cancer Res Treat 2001;67(1):1-8.

13. Tse GM, Kung FY, Chan AB, Law BK, Chang AR, Lo KW. Clonal analysis of bilateral mammary carcinomas by clinical evaluation and partial allelotyping. Am J Clin Pathol 2003;120(2):168-74.

14. Brommesson S, Jonsson G, Strand C, Grabau D, Malmstrom P, Ringner M, et al. Tiling array-CGH for the assessment of genomic similarities among synchronous unilateral and bilateral invasive breast cancer tumor pairs. BMC Clin Pathol 2008;8:6.

15. Teixeira MR, Ribeiro FR, Torres L, Pandis N, Andersen JA, Lothe RA, et al. Assessment of clonal relationships in ipsilateral and bilateral multiple breast carcinomas by comparative genomic hybridisation and hierarchical clustering analysis. Br J Cancer 2004;91(4):775-82.

16. Cuzick J, Powles T, Veronesi U, Forbes J, Edwards R, Ashley S, et al. Overview of the main outcomes in breast-cancer prevention trials. Lancet 2003;361(9354):296-300.

17. Li CI, Daling JR, Porter PL, Tang MT, Malone KE. Adjuvant hormonal therapy for breast cancer and risk of hormone receptor-specific subtypes of contralateral breast cancer. Cancer Res 2009;69(17):6865-70.

18. Swain SM. Tamoxifen and contralateral breast cancer: the other side. J Natl Cancer Inst 2001;93(13):963-5.

19. Li CI, Malone KE, Weiss NS, Daling JR. Tamoxifen therapy for primary breast cancer and risk of contralateral breast cancer. J Natl Cancer Inst 2001;93(13):1008-13.

20. Clarke M, Collins R, Darby S, Davies C, Elphinstone P, Evans E, et al. Effects of radiotherapy and of differences in the extent of surgery for early breast cancer on local recurrence and 15-year survival: an overview of the randomised trials. Lancet 2005;366(9503):2087-106.

21. Wickberg A, Holmberg L, Adami HO, Magnuson A, Villman K, Liljegren G. Sector resection with or without postoperative radiotherapy for stage I breast cancer: 20-year results of a randomized trial. J Clin Oncol 2014;32(8):791-7.

22. Janssens JP, Wittevrongel C, Van Dam J, Goddeeris P, Lauwerijns JM, De Loecker W. Effects of ionizing irradiation on the estradiol and progesterone receptors in rat mammary tumors. Cancer Res 1981;41(2):703-7.

23. Toillon RA, Magne N, Laios I, Lacroix M, Duvillier H, Lagneaux L, et al. Interaction between estrogen receptor alpha, ionizing radiation and (anti-) estrogens in breast cancer cells. Breast Cancer Res Treat 2005;93(3):207-15.

24. Paulsen GH, Strickert T, Marthinsen AB, Lundgren S. Changes in radiation sensitivity and steroid receptor content induced by hormonal agents and ionizing radiation in breast cancer cells in vitro. Acta Oncol 1996;35(8):1011-9.

25. Devriendt D, Ma Y, Kinnaert E, Journe F, Seo HS, Van Houtte P, et al. Effect of low dose irradiation on estrogen receptor level in MCF-7 breast cancer cells. Int J Cancer 2001;96(1):32-40.

26. Imaoka T, Nishimura M, Iizuka D, Nishimura Y, Ohmachi Y, Shimada Y. Pre- and postpubertal irradiation induces mammary cancers with distinct expression of hormone receptors, ErbB ligands, and developmental genes in rats. Mol Carcinog 2011;50(7):539-52.

27. Di Leva G, Gasparini P, Piovan C, Ngankeu A, Garofalo M, Taccioli C, et al. MicroRNA cluster 221-222 and estrogen receptor alpha interactions in breast cancer. J Natl Cancer Inst 2010;102(10):706-21.

28. Fucic A, Gamulin M. Interaction between ionizing radiation and estrogen: what we are missing? Med Hypotheses 2011;77(6):966-9.

29. Nguyen DH, Oketch-Rabah HA, Illa-Bochaca I, Geyer FC, Reis-Filho JS, Mao JH, et al. Radiation acts on the microenvironment to affect breast carcinogenesis by distinct mechanisms that decrease cancer latency and affect tumor type. Cancer Cell 2011;19(5):640-51.

30. Neta G, Anderson WF, Gilbert E, Berrington A. Variation in the risk of radiation-related contralateral breast cancer by histology and estrogen receptor expression in SEER. Breast Cancer Res Treat 2012;131(3):1021-7.

31. Janssens B, Drochmans, Mulier, Rutten, Wittevrongel, De Loecker. Effect of Presurgical Radiotherapy on the Steroid Receptor Concentration in Primary Breast Carcinoma. Eur J Cancer 1981;17(6):659-64.

32. Castiglioni F, Terenziani M, Carcangiu ML, Miliano R, Aiello P, Bertola L, et al. Radiation effects on development of HER2-positive breast carcinomas. Clin Cancer Res 2007;13(1):46-51.

33. Broeks A, Braaf LM, Wessels LF, van de Vijver M, De Bruin ML, Stovall M, et al. Radiation-associated breast tumors display a distinct gene expression profile. Int J Radiat Oncol Biol Phys 2010;76(2):540-7.

34. Abo-Madyan Y, Aziz MH, Aly MM, Schneider F, Sperk E, Clausen S, et al. Second cancer risk after 3D-CRT, IMRT and VMAT for breast cancer. Radiother Oncol 2014;110(3):471-6.

35. van Leeuwen FE, Klokman WJ, Veer MB, Hagenbeek A, Krol AD, Vetter UA, et al. Long-term risk of second malignancy in survivors of Hodgkin's disease treated during adolescence or young adulthood. J Clin Oncol 2000;18(3):487-97.

36. Debeb BG, Xu W, Woodward WA. Radiation resistance of breast cancer stem cells: understanding the clinical framework. J Mammary Gland Biol Neoplasia 2009;14(1):11-7.