


A new approach for modeling patient overall radiosensitivity and predicting multiple toxicity endpoints for breast cancer patients

Chamberlain Mbah^{a,b}, Kim De Ruyc^c, Silke De Schrijver^c, Charlotte De Sutter^a, Kimberly Schiettecatte^a, Chris Monten^{a,d} , Leen Paelinck^d, Wilfried De Neve^{a,d}, Hubert Thierens^c, Catharine West^e, Gustavo Amorim^b, Olivier Thas^{b,f} and Liv Veldeman^d

^aDepartment of Radiotherapy and Experimental Cancer Research, Ghent University, Ghent, Belgium; ^bDepartment of Mathematical Modelling, Statistics, and Bioinformatics, Faculty of Bioscience Engineering, University of Ghent, Ghent, Belgium; ^cDepartment of Basic Medical Sciences, Faculty of Health Sciences, University of Ghent, Ghent, Belgium; ^dDepartment of Radiation Oncology, Ghent University Hospital, Ghent, Belgium; ^eTranslational Radiobiology Group, Institute of Cancer Sciences Radiotherapy Related Research Christie Hospital NHS Trust, Manchester, UK; ^fNational Institute for Applied Statistics Research Australia (NIASRA), School of Mathematics and Applied Statistics, University of Wollongong, Wollongong, Australia

ABSTRACT

Introduction: Evaluation of patient characteristics inducing toxicity in breast radiotherapy, using simultaneous modeling of multiple endpoints.

Methods and Materials: In 269 early-stage breast cancer patients treated with whole-breast irradiation (WBI) after breast-conserving surgery, toxicity was scored, based on five dichotomized endpoints. Five logistic regression models were fitted, one for each endpoint and the effect sizes of all variables were estimated using maximum likelihood (MLE). The MLEs are improved with James–Stein estimates (JSEs). The method combines all the MLEs, obtained for the same variable but from different endpoints. Misclassification errors were computed using MLE- and JSE-based prediction models. For associations, *p*-values from the sum of squares of MLEs were compared with *p*-values from the Standardized Total Average Toxicity (STAT) Score.

Results: With JSEs, 19 highest ranked variables were predictive of the five different endpoints. Important variables increasing radiation-induced toxicity were chemotherapy, age, SATB2 rs2881208 SNP and nodal irradiation. Treatment position (prone position) was most protective and ranked eighth. Overall, the misclassification errors were 45% and 34% for the MLE- and JSE-based models, respectively. *p*-Values from the sum of squares of MLEs and *p*-values from STAT score led to very similar conclusions, except for the variables nodal irradiation and treatment position, for which STAT *p*-values suggested an association with radiosensitivity, whereas *p*-values from the sum of squares indicated no association. Breast volume was ranked as the most significant variable in both strategies.

Discussion: The James–Stein estimator was used for selecting variables that are predictive for multiple toxicity endpoints. With this estimator, 19 variables were predictive for all toxicities of which four were significantly associated with overall radiosensitivity. JSEs led to almost 25% reduction in the misclassification error rate compared to conventional MLEs. Finally, patient characteristics that are associated with radiosensitivity were identified without explicitly quantifying radiosensitivity.

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Introduction

One out of eight women develops breast cancer during her lifetime. Worldwide, the incidence is rising and is expected to increase in the next decade [1]. Fortunately, the number of breast cancer survivors is also increasing due to better awareness, earlier detection and more successful treatment modalities. In Belgium, the five-year relative survival rate of female breast cancer patients is now about 90% (www.kankerregister.org). More than 80% of patients with early-stage (non-metastasized) breast cancer receive radiation treatment in the adjuvant setting after surgery. Eventually, radiotherapy may be combined with systemic therapies like hormone therapy, chemotherapy or targeted therapy. Late radiotoxicity effects

have been well documented [2]. Skin fibrosis and pigmentation changes, telangiectasia and cosmetic alterations have been described [3,4]. Some of these side effects are attributed to outdated techniques and larger target volumes in older publications, but even with contemporary intensity-modulated radiotherapy (IMRT) techniques, toxicity is seen in a significant number of patients [5,6]. About 25% of patients diagnosed with breast cancer are younger than 50 years of age. Given the high incidence of breast cancer, the favorable survival rates, and the often young age at diagnosis, late side effects are important to consider when prescribing an adjuvant treatment. Inter-individual differences in the prevalence and severity of radiation-related side effects are large. It has been shown that

radiotherapy-related factors like total dose, dose per fraction, irradiated volume and dose homogeneity are predictive for late skin toxicity and cosmesis [7]. Patient and surgery-related factors like body mass index (BMI), breast volume, smoking, hypertension, baseline cosmesis and post-operative infection also play a role in developing toxicity after breast irradiation [3,8,9]. Inter-patient differences in radiation sensitivity are also assumed to be explained by underlying genetic factors [3]. Identifying predisposing factors can contribute to the prevention of severe late toxicities by tailoring treatments to the individual patient. Predictive models are also important when informing a patient about her chance of toxicity.

Inter-individual differences in toxicity outcomes are partly related to radiosensitivity, but also partly endpoint specific [10–12]. Therefore, it is vital to consider multiple endpoints in prediction models. This paper aimed to present a new approach for modeling overall radiosensitivity and predicting multiple toxicity endpoints and apply this approach to a 2-year toxicity data of 269 irradiated breast cancer patients. In our study cohort, five toxicity endpoints were measured with two goals in mind.

Firstly, prediction models were developed for all endpoints simultaneously, improving the predictions of a given endpoint by borrowing information from other endpoints.

Secondly, predisposing factors associated with radiosensitivity were identified without explicitly defining/quantifying radiosensitivity. Radiosensitivity is never directly observed, only multiple surrogate endpoints are measured. A technique currently used to model associations of patient characteristics with radiosensitivity is the Standardized Total Average Toxicity (STAT) score [10]. The STAT score summarizes several endpoints into a single value to mimic radiosensitivity. However, the STAT score is an arbitrary function (standardized average) of multiple endpoints with no clear clinical meaning. Radiosensitivity is a latent outcome, and it is only inferred through measurements of various types of radiotoxicity. Quantifying radiosensitivity from measured toxicities might be beyond the bounds of possibility. Our goal was to avoid the challenging task of quantifying radiosensitivity while identifying factors associated with it.

Material and methods

Patients

Two hundred and sixty-nine early-stage breast cancer patients treated between 2009 and 2014 at Ghent University Hospital with whole-breast irradiation (WBI) after breast-conserving surgery were included in this analysis. All patients gave informed consent and agreed to prospective data collection including digital photographs and a blood sample for genotyping. Patients participated in one of the clinical trials approved by the local Ethics Board and registered on www.clinicaltrials.gov (NCT00887523, NCT01973634). Only patients with two-year follow-up data were included.

Radiation treatment

All patients received WBI of 40.05Gy in 15 fractions; also, 32 patients were treated to the periclavicular and axillary lymph

node regions following the same fractionation scheme. The latter patients were treated in supine position, while patients receiving WBI without lymph node irradiation (LNI), were either treated supine or prone. A sequential boost of four times 2.5Gy was added, for 206 patients.

Assessment of toxicity

The grade of fibrosis and edema was scored by the responsible radiation oncologist at baseline (before the start of radiotherapy) and at every follow-up visit using the LENT–SOMA (late effect of normal tissue–subjective, objective, medical management, and analytical evaluation) toxicity scale [13]. Pigmentation change was scored at the same time points on a three-point scale: none, transient or permanent. Since most patients experience fibrosis of the surgical scar, only fibrosis outside the tumorbed was considered. Before and two years after treatment, digital photographs of the thorax were taken in standard conditions as described earlier [14]. Retraction was scored on the photographs, also per the LENT–SOMA scale. The BCCT.core (breast cancer conservation treatment.cosmetic results) software of the INESC Porto Breast Research Group [15], was used to assess retraction. Photographs were taken with the arms at the level of the hips and above the head. Based on measurements of asymmetry, the software provides a score from 1 (excellent cosmetic outcome) to 4 (poor cosmetic outcome) in comparison with the non-treated breast. To assess toxicity related to radiotherapy, the difference Δ , between the baseline score and the score at two years was used. Patients with a Δ_0 experienced no radiation-related toxicity. Patients with Δ_1 , Δ_2 or Δ_3 , experienced an increase in toxicity of 1, 2 or 3 grades, respectively. Telangiectasia was not included for endpoint analysis, because of too few events.

Genotyping

Genotyping was performed for seven single nucleotide polymorphisms (SNPs), located in six genes. The focus was on validating previously reported relationships with late skin toxicity. The relation between rs2682585 polymorphism in the XRCC1 gene [16] and the rs1800629 polymorphism in the TNF gene [17], and radiation-induced toxicity was analysed. In the genome-wide association study [18], four more polymorphisms were identified (SATB2: rs2881208, CCDC129: rs882460, SLFN14: rs2840044 and CCRN4L: rs13116075). These were also included. In addition, the rs321601 polymorphism near the variation mentioned above in SLFN14 was genotyped. Genomic DNA was obtained from blood using the Puregene genomic DNA purification kit (Gentra Systems, Minneapolis, MN, USA). Genotyping was performed using restriction fragment length polymorphism (RFLP) analyses, high-resolution melting (HRM) curve analyses and direct sequencing. Primer sequences and restriction enzymes are available in the [Supplementary Tables S1 and S2](#). For reproducibility control, 15% of all samples were duplicated. The concordance rate between duplicate samples was 100%. All genotype distributions were in Hardy–Weinberg equilibrium

($p < .05$). Only one data point for one polymorphism was missing. Only the dominant model coding was looked at for all SNPs. The recessive and the additive model coding (other possible SNPs model coding) led to categories with too few patients and unreliable statistical conclusions.

Data

The endpoints were dichotomized as follows: patients with Δ_0 (experiencing no radiation-related toxicity) were classified as 0 (controls) and patients with any grade of toxicity (Δ_1 , Δ_2 or Δ_3) as 1 (cases). Dichotomizing between mild (grouping Δ_0 and Δ_1) versus severe (grouping Δ_2 and Δ_3) would have resulted in a disproportionately small group of severe toxicity (<5% of cases). For that reason, we classified Δ_0 as control versus Δ_1 , Δ_2 or Δ_3 as cases.

Variables investigated are listed in Table 1, for the categorical and continuous variables. Missing variable values were imputed by multiple imputations, before estimating the effect sizes of interest.

Statistical analysis

In this section, we discuss the models, how the effect sizes are estimated and describe an alternative approach that leads to better predictions of individual endpoints. Moreover, a statistical test is introduced for the identification of risk factors that are significantly associated to radiosensitivity, without quantifying radiosensitivity.

With five binary endpoints, we fitted five multivariate logistic regression models one for each endpoint, to assess the impact of the variables on the endpoints. The standard procedure for estimating parameters of a logistic regression model is based on the maximum likelihood estimators (MLEs). For prediction, individual MLEs of a given variable can be further improved by using a combination of other MLEs for the same variable but from different endpoints. This seems controversial at first, and it is known as Stein's paradox [19]. When there are at least three parameters analysed simultaneously, this approach results in a lower mean squared error (MSE), as has been demonstrated by James and Stein [20]. This estimator will be called the James–Stein estimator (JSE). JSE compared to MLE, may also lead to better predictions when used in a prediction rule [21]. Five effect sizes (MLEs) were estimated per variable, one for each of the five endpoints. Therefore, the James–Stein estimator is directly applicable and can be constructed from these MLEs (see Supplementary Appendix for details). In constructing the JSEs, the sum of squares of the MLEs for the i th variable is computed. In particular, $S_i = \sum_j \hat{\beta}_{ij}^2$ where $\hat{\beta}_{ij}$ is the MLE of the effect size of variable i , for the j th endpoint and J is the total number of endpoints ($J=5$). The JSE of the i th variable, for the j th endpoint, is constructed using S_i , as $\left(1 - \frac{J-2}{S_i}\right) \hat{\beta}_{ij}$. The parameter $\lambda_i = \left(1 - \frac{J-2}{S_i}\right)$, which is called the shrinkage parameter, drives the procedure. It is assumed that the average effect size across endpoints is zero for all variables, since

it is likely that most variables have no effect on the endpoint. Therefore, the JSE shrinks the MLE towards zero, when S_i is approximately equal to $J-2$. When S_i is much larger than $J-2$, there is almost no shrinkage, meaning the MLEs cannot benefit from the other endpoints. Finally, when S_i is smaller than $J-2$, the shrinkage parameter λ_i becomes negative. To avoid negativity, the parameter is transformed to λ_i^+ (the positive part of λ_i) which takes the value zero when λ_i is negative. With λ_i^+ equal to zero, the effect size of variable i is zero across all endpoints. This is a form of variable selection (only variables with non-zero effect sizes stay in the model) and may improve predictions.

To evaluate the predictive value of MLEs versus JSEs, given a specific medical history, we ran one-hundred repeats of five-folds cross-validated misclassification error rates, based on MLEs and JSEs in a prediction rule, separately.

The sum of squares, S_i , can also be used as a test statistic (when endpoints are independent, which is the case). The statistic, S_i , has a chi-squared distribution with $J=5$, degrees of freedom. With five endpoints, and at a 5% significance level, the critical value is 11.07. Sum of squares greater than 11.07 are significantly different from zero and their corresponding p -values can be calculated. Based on these p -values, variables associated with radiosensitivity can be found without explicitly quantifying radiosensitivity. The entire modeling process is summarized in a flow diagram in Figure 1.

In calculating the STAT score, on the other hand, the sum of the five endpoints for each patient, its mean and variance across patients are computed. The sum of toxicity scores for each patient is then subtracted from the mean and divided by the standard deviation (standardization). The STAT score computed this way is the quantity that represents radiosensitivity. A multiple linear regression model with the STAT score as the response variable is fitted, from which p -values are used to represent the association of radiosensitivity and patient characteristics.

Results

Table 2 shows the MLEs and their corresponding JSEs for the five endpoints. Ranking of the variables is based on the shrinkage parameter λ_i (and its positive counterpart λ_i^+), which measures how far the MLEs of the i th variable are from zero. Breast volume is the highest ranked variable. Figure 2 gives a visual comparison between the MLEs and JSEs. Here, the dashed line represents the distance of MLEs from zero, i.e., the sum of squares (scaled to fit on the plot) of effect sizes across all five endpoints. The variables were ranked by their sum of squares, which measures how close the effect sizes are to zero. The MLEs and their respective JSEs are also displayed for every endpoint. Based on the JSEs, only 19 highest ranked variables stay in the prediction model. In descending order, most important variables increasing radiation-induced toxicity were breast volume, chemotherapy, age, SATB2 rs2881208 SNP and nodal irradiation. The most protective variable was treatment position (prone position), which came at the eighth rank. Sums of

Table 1. Variables and endpoints investigated.

Variables	Control (223)	Edema case (41)	Missing (5)	Control (194)	Retraction case (73)	Missing (2)	Control (213)	Fibrosis case (51)	Missing (5)	Control (190)	Pigmentation case (74)	Missing (5)	Control (173)	BCCT_score case (90)	Missing (6)
<i>Clinical variables</i>															
Menopause															
No	35 (0.95)	1 (0.03)	1 (0.03)	31 (0.84)	6 (0.16)	0 (0)	31 (0.84)	5 (0.14)	1 (0.03)	31 (0.84)	5 (0.14)	1 (0.03)	30 (0.81)	6 (0.16)	1 (0.03)
Yes	188 (0.81)	39 (0.17)	4 (0.02)	162 (0.7)	67 (0.29)	2 (0.01)	181 (0.78)	46 (0.2)	4 (0.02)	159 (0.69)	68 (0.29)	4 (0.02)	142 (0.61)	84 (0.36)	5 (0.02)
Diabetes															
No	208 (0.83)	38 (0.15)	4 (0.02)	181 (0.72)	68 (0.27)	1 (0)	199 (0.8)	47 (0.19)	4 (0.02)	177 (0.71)	69 (0.28)	4 (0.02)	163 (0.65)	81 (0.32)	6 (0.02)
Yes	15 (0.79)	3 (0.16)	1 (0.05)	13 (0.68)	5 (0.26)	1 (0.05)	14 (0.74)	4 (0.21)	1 (0.05)	13 (0.68)	5 (0.26)	1 (0.05)	10 (0.53)	9 (0.47)	0 (0)
Hypertension															
No	167 (0.86)	24 (0.12)	3 (0.02)	142 (0.73)	51 (0.26)	1 (0.01)	158 (0.81)	33 (0.17)	3 (0.02)	137 (0.71)	54 (0.28)	3 (0.02)	125 (0.64)	64 (0.33)	5 (0.03)
Yes	56 (0.75)	17 (0.23)	2 (0.03)	52 (0.69)	22 (0.29)	1 (0.01)	55 (0.73)	18 (0.24)	2 (0.03)	53 (0.71)	20 (0.27)	2 (0.03)	48 (0.64)	26 (0.35)	1 (0.01)
Smoking status															
No	125 (0.81)	28 (0.18)	2 (0.01)	109 (0.7)	44 (0.28)	2 (0.01)	112 (0.72)	41 (0.26)	2 (0.01)	108 (0.7)	45 (0.29)	2 (0.01)	97 (0.63)	54 (0.35)	4 (0.03)
Yes	98 (0.86)	13 (0.11)	3 (0.03)	85 (0.75)	29 (0.25)	0 (0)	101 (0.89)	10 (0.09)	3 (0.03)	82 (0.72)	29 (0.25)	3 (0.03)	76 (0.67)	36 (0.32)	2 (0.02)
Alcohol															
No	60 (0.77)	16 (0.21)	2 (0.03)	54 (0.69)	24 (0.31)	0 (0)	56 (0.72)	20 (0.26)	2 (0.03)	53 (0.68)	23 (0.29)	2 (0.03)	48 (0.62)	29 (0.37)	1 (0.01)
Yes	163 (0.85)	25 (0.13)	3 (0.02)	140 (0.73)	49 (0.26)	2 (0.01)	157 (0.82)	31 (0.16)	3 (0.02)	137 (0.72)	51 (0.27)	3 (0.02)	125 (0.65)	61 (0.32)	5 (0.03)
<i>Treatment variables</i>															
Hormone therapy															
No	37 (0.95)	0 (0)	2 (0.05)	24 (0.62)	14 (0.36)	1 (0.03)	34 (0.87)	3 (0.08)	2 (0.05)	26 (0.67)	11 (0.28)	2 (0.05)	30 (0.77)	8 (0.21)	1 (0.03)
Yes	186 (0.81)	41 (0.18)	3 (0.01)	170 (0.74)	59 (0.26)	1 (0)	179 (0.78)	48 (0.21)	3 (0.01)	164 (0.71)	63 (0.27)	3 (0.01)	143 (0.62)	82 (0.36)	5 (0.02)
Chemotherapy															
No	157 (0.84)	27 (0.14)	3 (0.02)	143 (0.76)	42 (0.22)	2 (0.01)	149 (0.8)	35 (0.19)	3 (0.02)	139 (0.74)	45 (0.24)	3 (0.02)	122 (0.65)	60 (0.32)	5 (0.03)
Yes	66 (0.8)	14 (0.17)	2 (0.02)	51 (0.62)	31 (0.38)	0 (0)	64 (0.78)	16 (0.2)	2 (0.02)	51 (0.62)	29 (0.35)	2 (0.02)	51 (0.62)	30 (0.37)	1 (0.01)
Target therapy															
No	208 (0.83)	37 (0.15)	5 (0.02)	177 (0.71)	71 (0.28)	2 (0.01)	196 (0.78)	49 (0.2)	5 (0.02)	174 (0.7)	71 (0.28)	5 (0.02)	163 (0.65)	81 (0.32)	6 (0.02)
Yes	15 (0.79)	4 (0.21)	0 (0)	17 (0.89)	2 (0.11)	0 (0)	17 (0.89)	2 (0.11)	0 (0)	16 (0.84)	3 (0.16)	0 (0)	10 (0.53)	9 (0.47)	0 (0)
Treatment position															
Supine	140 (0.81)	31 (0.18)	1 (0.01)	120 (0.7)	51 (0.3)	1 (0.01)	136 (0.79)	35 (0.2)	1 (0.01)	115 (0.67)	56 (0.33)	1 (0.01)	109 (0.63)	61 (0.35)	2 (0.01)
Prone	83 (0.86)	9 (0.09)	4 (0.04)	74 (0.77)	21 (0.22)	1 (0.01)	76 (0.79)	16 (0.17)	4 (0.04)	74 (0.77)	18 (0.19)	4 (0.04)	64 (0.67)	29 (0.3)	3 (0.03)
Nodal irradiation															
No	202 (0.85)	30 (0.13)	5 (0.02)	179 (0.76)	56 (0.24)	2 (0.01)	193 (0.81)	39 (0.16)	5 (0.02)	175 (0.74)	57 (0.24)	5 (0.02)	158 (0.67)	74 (0.31)	5 (0.02)
Yes	21 (0.66)	11 (0.34)	0 (0)	15 (0.47)	17 (0.53)	0 (0)	20 (0.62)	12 (0.38)	0 (0)	15 (0.47)	17 (0.53)	0 (0)	15 (0.47)	16 (0.5)	1 (0.03)
Boost															
No	55 (0.87)	5 (0.08)	3 (0.05)	50 (0.79)	12 (0.19)	1 (0.02)	53 (0.84)	7 (0.11)	3 (0.05)	45 (0.71)	15 (0.24)	3 (0.05)	41 (0.65)	20 (0.32)	2 (0.03)
Yes	168 (0.82)	36 (0.17)	2 (0.01)	144 (0.7)	61 (0.3)	1 (0)	160 (0.78)	44 (0.21)	2 (0.01)	145 (0.7)	59 (0.29)	2 (0.01)	132 (0.64)	70 (0.34)	4 (0.02)
<i>Genetic variables</i>															
TNF rs1800629															
GG	164 (0.84)	27 (0.14)	4 (0.02)	137 (0.7)	56 (0.29)	2 (0.01)	157 (0.81)	34 (0.17)	4 (0.02)	142 (0.73)	49 (0.25)	4 (0.02)	127 (0.65)	65 (0.33)	3 (0.02)
GA + AA	58 (0.79)	14 (0.19)	1 (0.01)	56 (0.77)	17 (0.23)	0 (0)	55 (0.75)	17 (0.23)	1 (0.01)	47 (0.64)	25 (0.34)	1 (0.01)	46 (0.63)	24 (0.33)	3 (0.04)
XRC1 rs2682585															
GG	138 (0.83)	26 (0.16)	2 (0.01)	127 (0.77)	38 (0.23)	1 (0.01)	135 (0.81)	29 (0.17)	2 (0.01)	118 (0.71)	46 (0.28)	2 (0.01)	118 (0.71)	45 (0.27)	3 (0.02)
GA + AA	85 (0.83)	15 (0.15)	3 (0.03)	67 (0.65)	35 (0.34)	1 (0.01)	78 (0.76)	22 (0.21)	3 (0.03)	72 (0.7)	28 (0.27)	3 (0.03)	55 (0.53)	45 (0.44)	3 (0.03)
SATB2 rs2881208															
CC	93 (0.84)	16 (0.14)	2 (0.02)	81 (0.73)	29 (0.26)	1 (0.01)	90 (0.81)	19 (0.17)	2 (0.02)	83 (0.75)	26 (0.23)	2 (0.02)	73 (0.66)	37 (0.33)	1 (0.01)
TT + CT	130 (0.82)	25 (0.16)	3 (0.02)	113 (0.72)	44 (0.28)	1 (0.01)	123 (0.78)	32 (0.2)	3 (0.02)	107 (0.68)	48 (0.3)	3 (0.02)	100 (0.63)	53 (0.34)	5 (0.03)
CCDC129 rs882460															
TT	127 (0.84)	22 (0.14)	3 (0.02)	111 (0.73)	39 (0.26)	2 (0.01)	124 (0.82)	25 (0.16)	3 (0.02)	109 (0.72)	40 (0.26)	3 (0.02)	96 (0.63)	54 (0.36)	2 (0.01)
TC + CC	96 (0.82)	19 (0.16)	2 (0.02)	83 (0.71)	34 (0.29)	0 (0)	89 (0.76)	26 (0.22)	2 (0.02)	81 (0.69)	34 (0.29)	2 (0.02)	77 (0.66)	36 (0.31)	4 (0.03)
SLFN14 rs2840044															
AA	79 (0.83)	14 (0.15)	2 (0.02)	63 (0.66)	31 (0.33)	1 (0.01)	77 (0.81)	16 (0.17)	2 (0.02)	62 (0.65)	31 (0.33)	2 (0.02)	61 (0.64)	30 (0.32)	4 (0.04)
AG + GG	144 (0.83)	27 (0.16)	3 (0.02)	131 (0.75)	42 (0.24)	1 (0.01)	136 (0.78)	35 (0.2)	3 (0.02)	128 (0.74)	43 (0.25)	3 (0.02)	112 (0.64)	60 (0.34)	2 (0.01)
SLFN14 rs321601															
AA	59 (0.86)	9 (0.13)	1 (0.01)	49 (0.71)	20 (0.29)	0 (0)	54 (0.78)	14 (0.2)	1 (0.01)	53 (0.77)	15 (0.22)	1 (0.01)	47 (0.68)	21 (0.3)	1 (0.01)
AG + GG	164 (0.82)	32 (0.16)	4 (0.02)	145 (0.72)	53 (0.26)	2 (0.01)	159 (0.8)	37 (0.18)	4 (0.02)	137 (0.68)	59 (0.3)	4 (0.02)	126 (0.63)	69 (0.34)	5 (0.02)
CCRN4L rs13116075															
AA	145 (0.83)	28 (0.16)	1 (0.01)	126 (0.72)	46 (0.26)	2 (0.01)	140 (0.8)	33 (0.19)	1 (0.01)	124 (0.71)	49 (0.28)	1 (0.01)	115 (0.66)	55 (0.32)	4 (0.02)
AG + GG	78 (0.82)	13 (0.14)	4 (0.04)	68 (0.72)	27 (0.28)	0 (0)	73 (0.77)	18 (0.19)	4 (0.04)	66 (0.69)	25 (0.26)	4 (0.04)	58 (0.61)	35 (0.37)	2 (0.02)
<i>Continuous variables</i>															
Median age (Years)	59	63	0	58.5	61	0	58	64	0	59	61	0	57	61	0
Median body mass index (kg/m ²)	25.5	27	1	25	27.5	1	25	27.5	1	25	27	1	25	26.5	1
Median breast volume (cc)	459.6	708.096	2	485.875	573.534	2	479.3	580.932	2	463.05	595.208	2	452.629	612.568	2
Median hot spots (cc)	4.7786	8.3714	11	2.894	21.7337	11	4.75	12.2214	11	4.4504	8.625	11	4.9251	9.2048	11
Median tumor size (cc)	1.4	1.5	0	1.4	1.6	0	1.4	1.5	0	1.4	1.5	0	1.5	1.4	0

The number inside parenthesis denote the total number of individuals for each category.

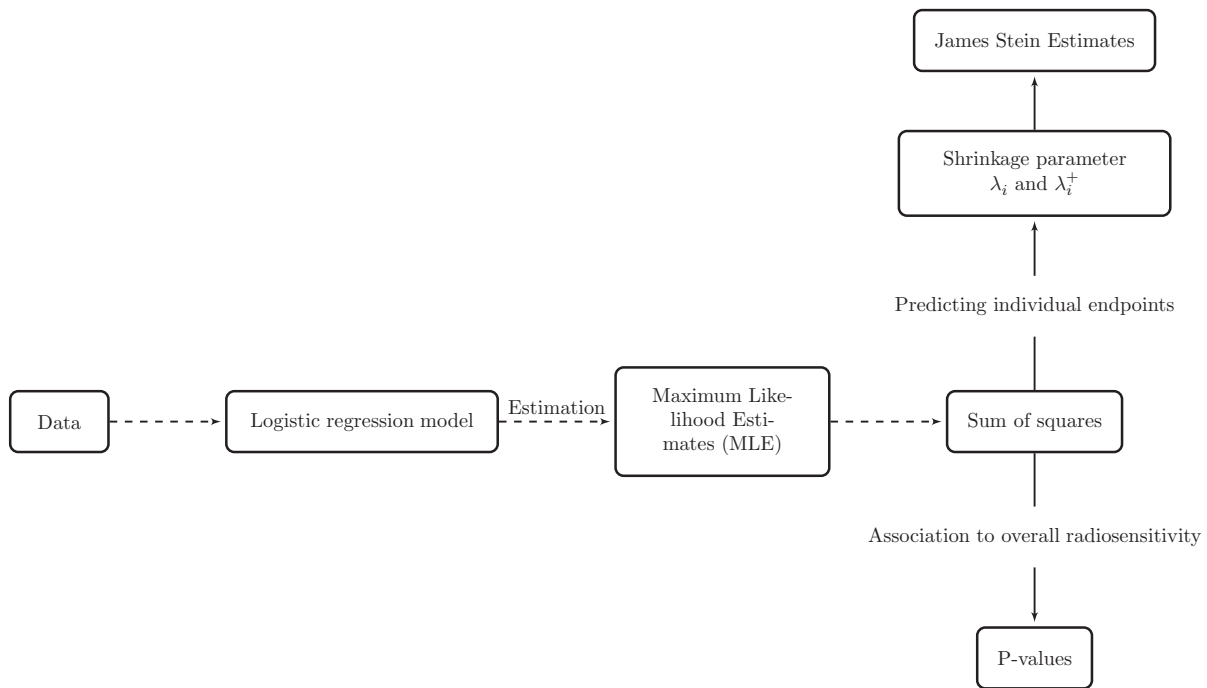


Figure 1. Flow diagram showing the modeling steps. From the data, the model is fitted, estimation of model parameters is by maximizing the likelihood for each endpoint. Sum of squares are computed from which association of variables to radiosensitivity can be investigated with p -values. On the other hand, for predicting individual endpoints, the sum of squares can be transformed to shrinkage parameters, and James–Stein estimates (JSE) are computed. JSE led to better predictions when used in the prediction rule compared to maximum likelihood estimates.

squares smaller than $J - 2$ were observed for the clinical variables, diabetes, boost and tumor size (Table 2). Which means the effect sizes of these variables are close to zero; consequently, their shrinkage parameters (λ_i^+) were zero and their MLEs were shrunken to zero for all endpoints (Table 2 and Figure 2). This indicates that these variables were not predictive of any of the endpoints and therefore omitted from the prediction model. Boxplots (Figure 3) show the misclassification error rates of the MLE and the JSE when used in the prediction rule. For all the different endpoints, misclassification error rates with JSEs are lower than those obtained via MLEs. Overall, the misclassification error of the MLE was 45% and that of the JSE was 34%, which is nearly 25% lower, suggesting that JSE is better.

Regarding association, p -values are presented for the sum of squares and from the model with the STAT score as endpoint (Table 2). Results of p -values from the sum of squares versus STAT p -values show discrepancies for the variables nodal irradiation and treatment position. For both variables, STAT p -value suggests an association, whereas none can be concluded based on the sum of squares. For all other variables, results were quite similar. Breast volume was ranked as the most important variable in both techniques.

Fifteen variables with a p -value greater than 5% and a non-zero λ_i^+ , are nevertheless retained, because inclusion of these variables may lead to better prediction. It must be noted that two of these 15 are only significant for STAT score analysis.

Discussion

Overall, radiosensitivity can be defined as an unknown combination of several toxicity endpoints. We aimed at: (1)

searching variables associated with overall radiosensitivity without explicitly quantifying it and (2) predictive for the individual endpoints. We exploited James and Stein's approach [20], which maximizes the use of the data. That is, it uses information from other endpoints for estimating the effect size of a single endpoint. The sums of squares of the MLEs was used as a test statistic to test associations of the unknown radiosensitivity and patient characteristics.

The variables diabetes, boost irradiation and tumor size were shrunken to zero and considered non-predictive, hence not included in the prediction model. Modeling with the JSE has the advantage of maintaining the information on individual endpoints, from which patients' risk profiles can be computed. Clinicians can rank patients by their risk of toxicities, and their treatment is then tailored (e.g., adapting the doses). Partial breast irradiation has shown to reduce toxicity, but some studies show a higher recurrence risk. In the clinic, patient toxicity risk profiles computed from JSEs can be used to open discussions with patients, e.g., do you prefer whole-breast irradiation with a high risk of toxicity or partial breast irradiation, which is probably safe in low-risk tumors, but the long-term outcome is unknown?

The misclassification error rate quantifies the percentage of wrong predictions, i.e., if the model predicts a patient with 'toxicity' as 'no toxicity' and vice versa. The JSE led to smaller misclassification errors, as presented in Figure 3. There is always a bias-variance trade-off when parameters of a model are estimated. The MLE is unbiased asymptotically, but may end up with a large variance, and hence a higher rate of misclassification. Unlike the MLE, the JSE introduces bias which may reduce its variance and may lead to a low misclassification error. With the JSE, only 19 variables (the variables with

Table 2. Maximum likelihood estimates (MLEs) of each variable and their James Stein estimates (JSEs).

Rank	Variables	Edema	Retraction	Fibrosis	Pigmentation	BCCT.core	Edema	Retraction	Fibrosis	Pigmentation	BCCT.core	λ_j	λ_j^+	P-value (sum of squares)	P-value (STAT)
1	Breast volume (cc)	3.482	2.168	1.662	1.468	1.703	3.058	1.904	1.460	1.289	1.496	0.878	0.878	<.001	<.001
2	Chemotherapy (Yes, No)	1.788	1.308	0.448	2.528	1.687	1.414	1.034	0.354	2.000	1.334	0.791	0.791	.014	.006
3	Age (Years)	1.225	0.006	1.265	2.697	1.848	0.958	0.005	0.990	2.110	1.446	0.782	0.782	.017	.008
4	SATB2 rs2881208	0.850	1.382	1.144	2.268	1.409	0.620	1.008	0.834	1.654	1.027	0.729	0.729	.050	.009
5	Nodal irradiation (Yes, No)	1.264	0.792	2.067	1.585	0.512	0.855	0.536	1.398	1.072	0.346	0.676	0.676	.099	.006
6	Smoking (Yes, No)	1.383	2.078	-0.780	0.059	-1.453	0.920	1.382	-0.519	0.039	-0.966	0.665	0.665	.111	.740
7	XRCC1 rs2682585 (GG, AA + GA)	-1.096	1.420	1.082	-0.045	2.125	-0.727	0.942	0.718	-0.030	1.410	0.663	0.663	.113	.134
8	Treatment position (Prone, Supine)	-1.539	-1.489	-0.923	-1.718	-1.119	-1.016	-0.983	-1.133	-1.134	-0.738	0.660	0.660	.116	.016
9	Trastuzumab (Yes, No)	0.270	-1.615	-0.202	-1.722	1.449	0.176	-1.051	-0.601	-1.121	0.944	0.651	0.651	.126	.373
10	Hot spots (counts)	-1.526	1.844	-0.972	-0.077	0.648	-0.881	1.065	-0.561	-0.045	0.374	0.577	0.577	.213	.817
11	Hormone therapy (Yes, No)	0.017	-1.467	1.297	0.381	1.764	0.010	-0.846	0.748	0.220	1.018	0.577	0.577	.214	.091
12	Hypertension (Yes, No)	0.876	-1.040	-0.010	-2.007	-0.569	0.452	-0.537	-0.005	-1.036	-0.294	0.516	0.516	.287	.270
13	Alcohol (Yes, No)	-0.712	-0.562	0.354	2.042	0.551	-0.318	-0.251	0.158	0.912	0.246	0.447	0.447	.367	.520
14	TNF rs1800629 (GG, GA + AA)	0.869	0.474	0.883	1.827	-0.043	0.358	0.195	0.364	0.753	-0.018	0.412	0.412	.404	.165
15	SLFN14 rs2840044 (AA, AG + GG)	0.372	-1.471	0.348	-1.027	0.580	0.080	-0.315	0.074	-0.220	0.124	0.214	0.214	.576	.541
16	CCRN4L rs13116075 (AA, AG + GG)	-0.394	1.069	0.190	0.835	1.267	-0.069	0.187	0.033	0.146	0.221	0.175	0.175	.603	.256
17	SLFN14 rs321601 (GG,GT + TT)	0.063	-1.561	-0.659	0.585	0.646	0.011	-0.272	-0.115	0.102	0.113	0.175	0.175	.603	.847
18	CCDC129 rs882460 (TT, TC + CC)	0.401	0.696	1.139	1.152	-0.373	0.048	0.083	0.136	0.138	-0.045	0.120	0.120	.637	.236
19	Menopause (Yes, No)	-1.096	-0.764	0.776	-0.054	-0.963	-0.105	-0.073	0.074	-0.005	-0.092	0.096	0.096	.651	.635
20	Diabetes (Yes, No)	-0.734	-1.038	-0.091	-1.057	-0.068	0.000	0.000	0.000	0.000	0.000	-0.092	0.000	.739	.201
21	Boost (Yes, No)	0.539	-0.062	0.336	-1.093	-0.819	0.000	0.000	0.000	0.000	0.000	-0.320	0.000	.810	.530
22	Tumor size (cm)	-0.731	0.572	0.096	-1.139	-0.853	0.000	0.000	0.000	0.000	0.000	-0.855	0.000	.899	.813

The variables are ranked with the best variable ranked 1. The shrinkage parameter λ_j and its positive part λ_j^+ are shown. p -values based on the sum of squares (p -value (sum of squares)) and the Standardized Total Average Toxicity, STAT, (p -value (STAT)) score are shown.

ranks 1–19 in Table 2) were needed for prediction, thus included in the model. In predicting different endpoints, the JSE guaranteed 66% correct classification overall. The MLE is making use of twenty-two variables resulted in only 55% correct classifications, which comes very close to coin flipping. Referring to each endpoint, JSE again outperformed MLE, with lower misclassification errors for every endpoint.

The p -values from the sum of squares are informative for hypothesis testing/association studies. Based on these p -values, variables associated to radiosensitivity can be found without explicitly quantifying radiosensitivity. However, the STAT score quantifies radiosensitivity (an unknown entity) and goes on to investigate variables associated with it. Using a significance level of 5%, only the first four variables from Table 2 were significant. Larger breast volume, chemotherapy, older age and the SATB2 rs2881208 SNP were associated with a higher chance of developing any toxicity. These associations have been reported before. The correlation between breast volume and late toxicity has been seen in different publications [3,22–25]. Since no correlation was found between breast volume and hot spots in this dataset, the effect cannot only be attributed to dose inhomogeneity, as already reported by Goldsmith et al. [22]. A possible explanation may be the increased presence of more adipose tissue in large breasts. Adipocytes are known to secrete adipokines involved in inflammation like leptin, IL-6, IL-8 or TNF α [26]. Large breast size is often seen in combination with obesity. An increased number of macrophages and an inflammatory signature has been shown in breast adipose tissue of obese women compared to normal-weight women [27,28]. There is also an association between hypertrophic adipocytes, as often seen in obesity, and the production of pro-inflammatory adipokines [26]. This chronic inflammatory state could predispose to increased toxicity after breast irradiation. In this dataset, there was a very strong correlation between BMI and breast volume. If BMI was included in the model, it was shrunk to zero, and the overall prediction performance of the model dropped. Without breast volume in the model, BMI became a predictive variable, but weaker than breast volume. For this reason, BMI was left out of the model. There seems to be a strong association between chemotherapy and toxicity, even if not given concomitantly with radiation as was the case in our study. This confirms literature data [3,29,30]. There is emerging evidence that the synergistic effect of radiotherapy and chemotherapy is explained by an effect on the immune system. Recent investigations show that the innate immune system is involved in the tumor response after radiation treatment [31].

Some chemotherapeutic agents typically used in breast cancer, like anthracyclines, taxanes and cyclophosphamides are also capable of influencing the immune response [32]. An increased risk of late toxicity with older age has been reported in the boost-no boost trial [33], and in other trials [3,29,34,35]. With increasing age, the glandular tissue is replaced by adipose and stromal tissue, which might lead to a higher susceptibility to radiation-related side effects as described above. Another explanation could be the reduced DNA repair capacity at an older age. The last significant variable in the model, based on the sum of squares p -value,

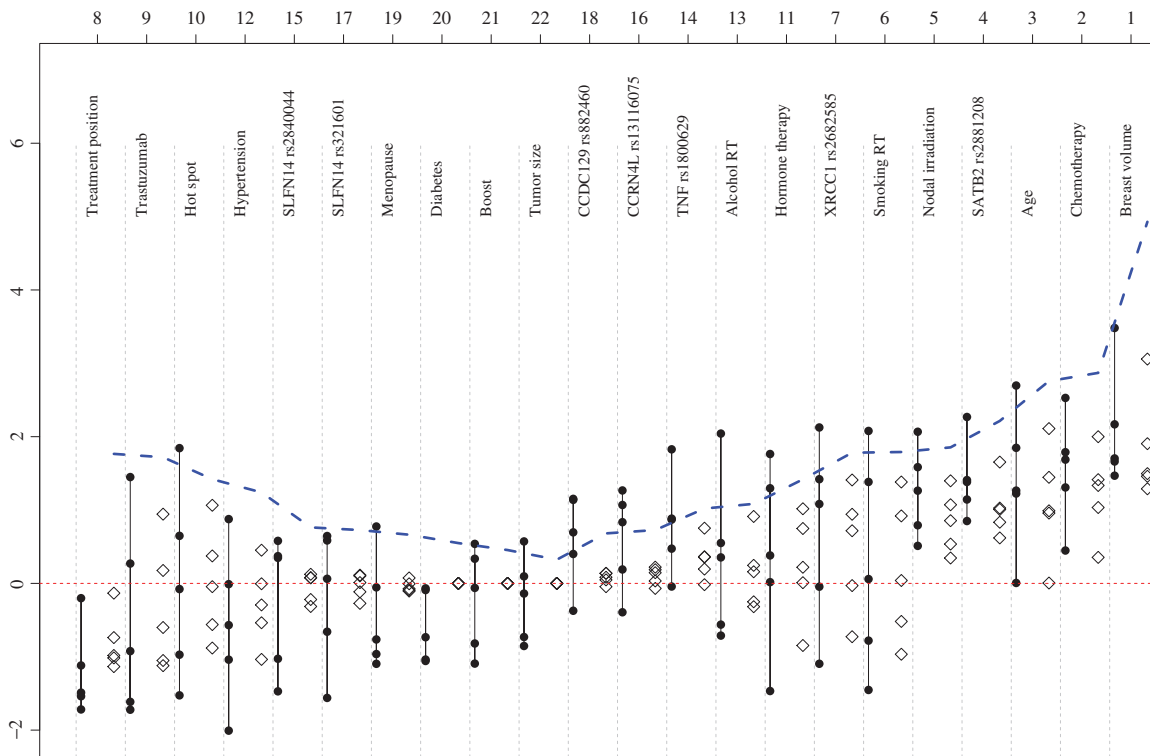


Figure 2. Maximum likelihood estimates, MLEs, (●) and their corresponding James Stein estimates JSE (◇). Each set of MLEs and JSEs are labeled with the variable name. The blue dash line is a scaled version of the variance around zero, of each variable. On top of the plot are the ranks of the variables. Line segments are used to connect the MLEs to illustrate their scatter.

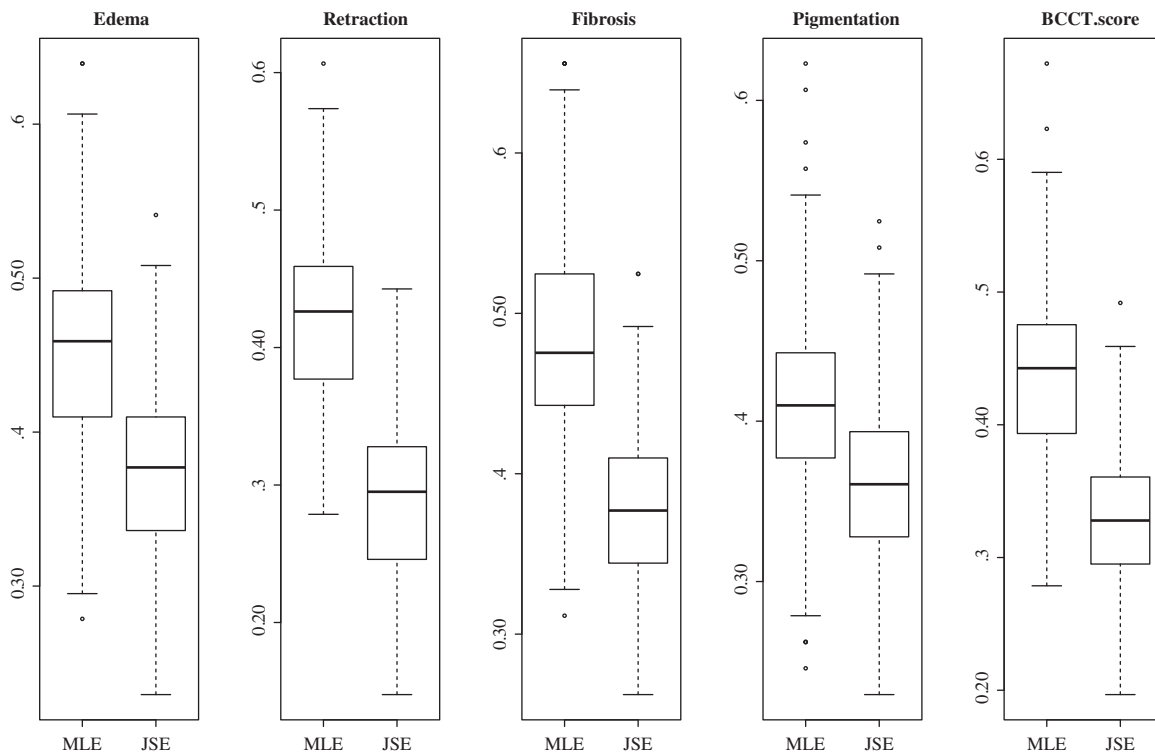


Figure 3. One-hundred repetition of five-folds cross validated misclassification error rates of maximum likelihood estimates (MLEs) and James–Stein estimates (JSEs). Smaller misclassification errors are observed for the JSE compared to the MLE. Overall, the misclassification error of the MLE is 45% and that for the JSE 34%.

was the rs2881208 polymorphism near SATB2. In the genome-wide association study [18], this SNP was significantly associated with increased toxicity for all endpoints and the STAT score. Besides these four variables, the STAT score also

detects a significant association with toxicity for nodal irradiation and treatment position, even though the *p*-values of these variables from the sums of squares were insignificant. Effect sizes with consistent signs across endpoints end up

having a significant STAT score p -value, no matter how small these effect sizes are. When the nodes are irradiated, the treated volume increases and dose inhomogeneity is more likely to occur. These confounders may lead to significant STAT scores. Therefore, the increase in late toxicity, as seen in other studies [30,35], is logical. Patients treated in a prone position are less likely to develop any of the reported toxicities, which agrees with a randomized trial comparing prone and supine breast radiotherapy [14]. Due to the shape of the breast in prone position, it is much easier to obtain homogeneous dose distributions than in supine position. Thirteen other variables were predictive, while their p -values were insignificant. Most of these variables were selected based on previously described associations with late toxicity after breast irradiation. The effect sizes in our cohort were often small, and the direction of the association was not always consistent with literature data. Several reasons can be found. A major limitation of our study is the small sample size. A second explanation might be the way the endpoints were dichotomized. Due to the very low number of serious adverse events, i.e., grade 2 or 3 toxicity, the groups were allocated based on no toxicity at all (controls) and any grade of toxicity (cases). Since grade 1 side effects are not unusual at two years, this dichotomization may have biased the results. We only looked at cosmetic outcomes. Given the limited follow-up of two years, we could not evaluate data on other, more relevant, endpoints like heart toxicity, lung toxicity and secondary malignancies. However, we aimed at pioneering research in the direction of modeling overall radiosensitivity from multiple endpoints. The association of variables with overall radiosensitivity we have modeled will improve if data on other endpoints become available.

Conclusion

JSE is used for selecting variables that are predictive for multiple toxicity endpoints. With this estimator, 19 variables were predictive for all toxicities, of which only four were significantly associated with overall radiosensitivity. JSEs led to almost 25% reduction in the misclassification error compared to MLEs. With the sum of squares of MLEs, patient characteristics that are associated with radiosensitivity were identified without explicitly quantifying radiosensitivity.

Disclosure statement

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ORCID

Chris Monten  <http://orcid.org/0000-0003-2599-7313>

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