Prevalence of circulating tumor cells (CTCs) after five years of zoledronate treatment in the adjuvant SUCCESS-A study

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Aim

The prognostic value of CTCs at primary diagnosis has recently been confirmed by the SUCCESS A study (Rack et al., JNCI 2014). Key questions on the role of adjuvant bisphosphonate treatment, including patient populations deriving benefit and optimal timing/scheduling of therapy are still controversial. Aim of this study was therefore to evaluate the predictive value of zoledronic acid treatment (for 5 years vs for 2 years) on the prevalence of CTCs at 5 years after primary diagnosis additionally to other well-known predictors.

Methods

The SUCCESS A trial is a large, randomized, open-label, 2x2 factorial design Phase III study in patients with high risk breast cancer (stage N1 or T2–T4 or grade 3 or age ≤ 35 or hormone-receptor negative). Patients were first randomized to adjuvant chemotherapy treatment with 3 cycles of Epirubicin-Fluorouracil-Cyclophosphamide (FEC) followed by either 3 cycles of Docetaxel or 3 cycles of Gemcitabine-Docetaxel. In addition, patients were randomized to 2 years vs. 5 years of zoledronate treatment. CTC status before and after chemotherapy, as well as 2 and 5 years after primary diagnosis was assessed using the FDA-approved CellSearch System (Veridex, USA), and CTC positivity was defined as ≥ 1 CTC.

Statistical Methods

The primary objective was to study the predictive value of zoledronic treatment (for 2 years vs. for 5 years) on the prevalence of CTCs at 5 years after primary diagnosis, additionally to well-known predictors. A multiple logistic regression model was fitted with CTC status at 5 years as binary outcome (0 CTCs, > 0 CTCs) and the following predictors: zoledronate treatment (categorical; 2 years vs. 5 years), age at diagnosis (continuous), BMI at diagnosis (continuous), tumour stage (ordinal; pT1, pT2, pT3, pT4), lymph node status (categorical; pN0 and pN+), ER, PR, and HER2neu (each categorical; negative vs. positive), tumor type (categorical; ductal, lobular, other) , CTC status before chemotherapy (categorical; 0 CTCs, >0 CTCs), adjuvant chemotherapy (categorical; FEC-Doc vs. FEC-DocG). The Wald test was performed for zoledronate treatment. A significant p-value would indicate that zoledronate treatment influences the prevalence of CTCs at 5 years after primary diagnosis in addition to the considered predictors. Furthermore, the regression model was used to estimate adjusted odds ratios (ORs), especially for zoledronic treatment.

Patients with missing outcome and patients with missing CTC information at 5 years were excluded. Missing predictor values were imputed using single "best guesses" (median value of continuous predictors, the most common value of categorical or ordinal predictors) based on non-missing data across all subjects. Continuous predictors were used as natural cubic spline functions to describe non-linear effect (Hastie and Tibshirani, 1995). The number of knots of each predictor was determined by first fitting several simple logistic regression models which differ from each other by the number of knots (from 0 to 4) and then choosing the number of knots which optimizes the Akaike information criterion (AIC).

A sensitivity analysis was performed with a reduced logistic regression model to address the problem of over- as well as underestimation of regression coefficients due to too many variables per outcome event (Harrell et al., 1984; Peduzzi et al., 1996) and related to this, the problem of overfitting. A backward stepwise variable selection procedure with AIC as stop criteria was carried out on the condition that zoledronate treatment was kept in each selection step resulting in a reduced logistic regression model with zoledronate treatment and "the most important" predictors of the full model. As above, the Wald test was performed and adjusted ORs were estimated.

The performance of the logistic regression models was measured with the area under the receiver operator curve (AUC) and the Hosmer-Lemeshow $\chi 2$ test, where frequencies of predicted events were compared with frequencies of observed events. A large p-value indicates a satisfactory calibration.

Model building was evaluated by 10-fold cross-validation with 50 repetitions to measure the amount of over-fitting. For this purpose, the model building process (i.e., determination of degrees of freedom for continuous predictors, the estimation of regression coefficients, variable selections) was done on each training set resulting in a logistic regression model, which was used then to calculate the AUC on the corresponding validation data set. The average of all these AUCs was taken as evaluation measure.

All of the tests were two-sided, and a P value of < 0.05 was regarded as statistically significant. Calculations were carried out using the R system for statistical computing (version 3.0.1; R Development Core Team, Vienna, Austria, 2013)

Results

Patient and tumour characteristics

Data on CTC status at 5 years after primary diagnosis were available for 728 (19.4%) out of 3754 randomized patients. 65 patients (8.9%) had CTCs at five years after diagnosis and 663 (91.1%) had no CTCs. 310 patients had been randomized to 2 years of zoledronate treatment and 418 patients had been randomized to 5 years of zoledronate treatment. 93.8% of all patients had complete observations in all patients and tumour characteristics but CTCs at 2 years after diagnosis which was not considered for statistical analyses. The percentage of missing values in each predictor variable was below 0.5% except for HER2neu (1.8%) and CTC before chemo therapy (4.4%). The missing values of predictor variables were imputed as described above. 19.2% of all patients had no information about CTCs at 2 years after diagnosis. Patient and tumour characteristics according to CTC prevalence at 5 years after diagnosis are shown in Table 1.

Zoledronic acid treatment and prevalence of CTCs at 5 years

We could not show that the duration of zoledronate treatment (2 or 5 years) influenced the prevalence of CTCs at 5 years after diagnosis (p = 0.13, Wald test). The adjusted OR for 2 years vs 5 years of treatment was 0.65 (95% CI: 0.37 to 1.13). The ORs for the predictors of the full logistic regression analysis is shown in Table 2.

The reduced logistic regression model confirmed the main analysis. The adjusted OR and the p-value of the Wald test were exactly the same as above. ORs are shown in Table 3

Model performance and evaluation

The full logistic regression model seemed to be bad calibrated, whereas the calibration of the reduced model seemed to be rather good. Both models seemed to be over-fitted to a certain degree, because the cross-validated AUCs are lower than the apparent AUCs on the original data.

Conclusions

We could not show an influence of zoledronate treatment duration on the prevalence of CTCs five years after primary diagnosis. Other trials in this setting might provide additional information on the predictive role of CTCs in the context of bisphosphonate treatment.

References

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Characteristic		No CTC Mean or N	No CTC SD or %	At least one CTC Mean or N	At least one CTC SD or %
Age		52.9	10.2	54.4	10.4
BMI		26.1	4.8	26.1	4.4
Tumor Stage	pT1	310	46.8	23	35.4
	pT2	322	48.6	41	63.1
	pT3	22	3.3	1	1.5
	pT4	9	1.4	0	0.0
Grading	G1	33	5.0	2	3.1
	G2	341	51.4	27	41.5
	G3	289	43.6	36	55.4
Nodal status	pN+	450	67.9	47	72.3
	pN0	213	32.1	18	27.7
Tumor type	Ductal	546	82.4	54	83.1
	Lobular	72	10.9	6	9.2
	Other	45	6.8	5	7.7
ER	Negative	198	29.9	23	35.4
	Positive	465	70.1	42	64.6
PR	Negative	256	38.6	21	32.3
	Positive	407	61.4	44	67.7
HER2	Negative	484	73.0	53	81.5
	Positve	179	27.0	12	18.5
Adjuvant chemotherapy	FEC-DocG	323	48.7	33	50.8
	FEC-Doc	340	51.3	32	49.2
CTCs before chemoth.	0	543	81.9	52	80.0
	> 0	120	18.1	13	20.0
CTCs at 2 years	0	450	84.0	41	75.9
	> 0	86	16.0	13	24.1
Zoledronic treatment	2 years	778	56.6	43	66.2
	5 years	288	43.4	22	33.8

Table 1: Patient characteristics at primary diagnosis according to the CTC status at 5 years after diagnosis.

Predictor		OR
Age (years)	per unit increase	1.02 (0.99, 1.05)
BMI (kg / m²)	per unit increase	0.98 (0.92, 1.04)
Lymph node status	pN0 vs pN+	0.68 (0.36, 1.27)
Tumor type	lobular vs ductal	0.76 (0.30, 1.91)
	other epithelial vs ductal	1.07 (0.40, 2.89)
ER	ER+ vs ER-	0.46 (0.21, 1.01)
PR	PR+ vs PR-	2.39 (1.09, 5.23)
HER2neu	positive vs negative	0.56 (0.28, 1.09)
Adjuvant chemotherapy	AB vs AA	0.95 (0.56, 1.60)
Tumor stage	per unit increase	1.19 (0.78, 1.81)
Grading	per unit increase	1.88 (1.10, 3.18)
CTCs before chemo therapy	CTC > 0 vs CTC = 0	1.12 (0.58, 2.16)
Zoledronic acid treatment	2 years vs 5 years	0.65 (0.38, 1.12)

Table 2: Predictors of CTC positivity at 5 years. Full logistic regression model showing odds ratios (ORs) with 95% confidence intervals.

Predictor		OR
Age (years)	per unit increase	1.02 (1.00, 1.05)
ER	ER+ vs ER-	0.47 (0.22, 1.02)
PR	PR+ vs PR-	2.60 (1.20, 5.64)
HER2neu	positive vs negative	0.58 (0.30, 1.13)
Grading	per unit increase	1.75 (1.05, 2.90)
Zoledronic acid treatment	2 years vs 5 years	0.65 (0.38, 1.12)

Table 3: Predictors of CTC positivity at 5 years. Reduced logistic regression model, showing odds ratios (ORs) with 95% confidence intervals.

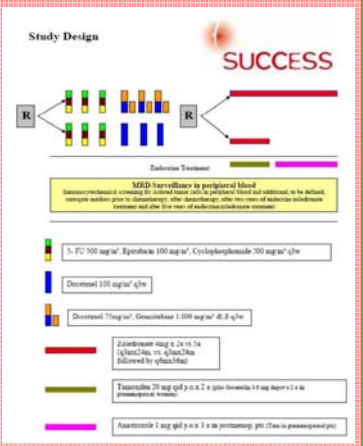

Figure 1: SUCCESS study design

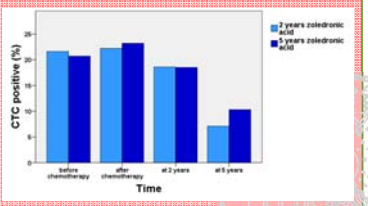

Figure 2: CTC positivity rate before and after chemotherapy as well as 2 and 5 years after primary diagnosis

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