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Triple-negative and HER2-overexpressing breast cancers exhibit an elevated risk and an earlier occurrence of cerebral metastases ☆

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ABSTRACT

Purpose: Evaluation of the influence of immunohistochemically defined breast cancer (BC) subtypes and other risk factors on the development of cerebral metastases (CM).
Methods: Exploratory analysis of a hospital-based prospective tumour registry including all patients with primary BC treated in our EUSOMA breast unit between 1998 and 2006.
Results: The study cohort contained 2441 patients, including 284 patients (11.6%) with triple-negative (oestrogen receptor (ER), progesterone receptor (PR) and HER2-negative) and 245 patients (10.1%) with HER2-overexpressing BC subtypes. Overall, 80 patients (3.3%) developed CM within a median follow-up period of 47 months, 19 (23.8%) of them with triple-negative and 19 (23.8%) with HER2-positive tumours. Therefore, 6.7% of all patients with triple-negative and 7.8% of patients with HER2-positive breast cancer developed CM. Multivariate analysis indicated that the highest risk for CM was triple-negative breast cancer. Further independent risk factors were: HER2-overexpression, early onset BC (age < 50 years), and large tumour size (pT3/4). Among those patients developing CM, triple-negative BC showed the shortest interval between primary diagnosis and occurrence of CM with a median of 22 months, compared to 30 and 63.5 months in HER2-positive and ER+/HER2- BC, respectively. Survival after occurrence of CM did not differ among the subtypes.
Conclusion: Patients with triple-negative or HER2-positive BC have a higher risk for CM compared with patients bearing the ER+/HER2- phenotype and develop CM earlier in the course of disease. A risk profile for CM might help adjust surveillance in high risk populations and identify patients with a need for new treatment strategies.

1. Introduction

Breast cancer (BC) is the second most common solid malignancy that spreads to the brain.[1] Historical data suggest an incidence of symptomatic cerebral metastases (CM) in BC patients between 5% and 16%[2,3]; autopsy studies revealed an even higher incidence of up to 34%.[1,2,4] Patients suffering from symptomatic CM have a poor prognosis with a median

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survival time after diagnosis ranging between 3 and 9 months.[5,6] Early onset BC, HER2-overexpression, high tumour grade and oestrogen receptor (ER) negativity have been described as independent risk factors for development of CM.[5,7–10]

In past years, carcinomas of the breast have been classified into six subgroups based on gene expression patterns, each subgroup showing a different clinical outcome. Patients with tumours bearing the basal-like subtype showed the shortest overall survival.[11] Triple-negative BC (oestrogen receptor negative (ER-), progesterone receptor negative (PR-) and HER2-negative (HER2-)) specimens exhibit the basal-like phenotype in 91% of all cases and, therefore, triple negativity can be used as a clinical surrogate for the genotypically defined basal-like phenotype.[12] As mentioned above, HER2-overexpression is associated with a more aggressive course of disease.[13] According to ER status, HER2-overexpressing BC can be classified into two subgroups: the HER2 subtype (ER-negative) and the Luminal B subtype (ER-positive).[14]

The goals of our study were (1) to determine the incidence of CM in a large cohort of patients with BC, (2) to evaluate the influence of BC subtypes (specifically triple negativity and HER2-overexpression) and other risk factors on the development of CM, and (3) to analyse the clinical course of patients with CM in BC.

2. Patients and methods

The data for this exploratory analysis were derived from the prospectively running clinical tumour registry of our EUSOMA (European Society of Breast Cancer Specialists) breast cancer unit at a city hospital (Dr. Horst Schmidt Kliniken) in Wiesbaden, Germany. The tumour registry data from BC patients included tumour characteristics, modalities and outcomes of treatments, which are updated annually by the registry physician. The follow-up information is also updated at least annually, either according to our records if patients are under continuous observation in our clinic or by contacting the referring oncologist/physician or the patient. Between 01/01/1998 and 12/31/2006, 3193 patients with primary BC were treated at our institution and further observed in a follow-up. At the time of evaluation, 99.3% of the patients had available data with > 6 months follow-up. Of the 3193 patients, 752 were excluded from this analysis because of *in situ* carcinoma only and/or incomplete pathological data (lack of TNM classification, oestrogen receptor, progesterone receptor and/or HER2 status).

ER and PR were considered positive if $\geqslant$ 10% of the tumour cells showed expression in immunohistochemistry (using the 6F-11 antibody [Novocastra, Berlin, Germany] for ER detection and the PR-312 antibody [Novocastra, Berlin, Germany] for PR detection); HER2 was considered negative if scored as 0 or 1+ by immunohistochemistry (CB11 antibody, Novocastra, Berlin, Germany) or negative by FISH in case of an IHC score of 2+. In this analysis patients were categorised into four groups according to ER, PR, and HER2 status: (1) Patients with triple-negative BC (ER, PR and HER2-negative); (2) Patients with HER2-overexpressing BC (2.1) ER-positive/HER2-positive and (2.2) ER-negative/HER2-positive; (3) All remaining patients with ER and/or PR (steroid receptor: SR) positive and HER2-negative breast cancer (referred to as SR+/HER2-). Surgery was offered to all patients without primary metastatic disease, and breast conserving therapy was applied whenever feasible. Adjuvant therapies were given according to current St. Gallen guidelines (see the Results section). Pathomorphological tumour characteristics were documented using the current diagnostic guidelines recommended by the World Health Organisation (WHO).

Diagnostic workup for CM was initiated in symptomatic patients, using cranial computer tomography (CCT) or magnetic resonance imaging (MRI). Typical indications for imaging were unexplained headache, sensory or motor peripheral or central neurologic symptoms.

Kaplan–Meier estimates were calculated with Graph Pad Prism 4.02 (La Jolla, California). All other statistical analyses were performed using SPSS 16.0 (Chicago, Illinois). Independency of continuous variables was tested with Student's *t*-test. Comparison of two or more groups of binary variables was done with either Fisher's Exact-Test or χ^2-Test. Univariate and multivariate analyses were performed using the Cox-regression model. Variables with significant results in univariate analysis were included in multivariate analysis. The Kaplan–Meier method including a log-rank test was used to determine survival differences. All *p*-values were two-sided, and $p < 0.05$ was considered significant.

3. Results

3.1. *Patients' characteristics*

The final study cohort consisted of 2441 patients with primary invasive BC. Of those, 284 (11.6%) patients had triple-negative BC, 245 (10.1%) showed HER2-positive phenotypes and 1912 (78.3%) patients expressed SR+/HER2- phenotypes.

Patient characteristics are displayed in Table 1. Statistically significant differences among the three groups were seen regarding age at BC diagnosis, size of the primary tumour, lymph-node involvement and grading. Patients with triple-negative and HER2-positive BC were more likely to have received chemotherapy than patients with SR+/HER2- disease (80.6%, 71.4%, and 38.5%, respectively), but there were no differences in frequency and extent of surgical or irradiation treatment between the three groups. Adjuvant trastuzumab was administered to 26.9% of patients with HER2-overexpressing tumours and to 76% of patients with recurrent disease. Treatment for CM did not differ between the three groups. 50 patients were treated with whole brain irradiation with 30 Gy, six patients with surgical intervention followed by radiotherapy, and eight patients with systemic therapy only; 16 patients received no treatment.

3.2. *Univariate analysis*

To identify risk factors in the three groups, univariate analysis was conducted and the results are shown in Table 2. In the univariate analysis, triple-negative phenotype, HER2-overexpression, age <50 years, larger tumour size (T 3/4) and a positive lymph-node status at primary diagnosis of BC were

Table 1 – Patient and tumour characteristics.

	Triple-negative (*N* = 284)	HER2+ (*N* = 245)	SR+/HER2- (*N* = 1912)	*p*-Value
Age				
>50	180 (63.4)	142 (60.0)	1360 (71.1)	<0.0001
⩽50	104 (36.6)	103 (40.0)	552 (28.9)	
pT				
1/2	249 (87.7)	209 (85.3)	1734 (90.7)	0.015
3/4	35 (12.3)	36 (14.7)	178 (9.3)	
pN				
–	169 (59.5)	117 (47.8)	1234 (64.5)	<0.0001
+	115 (40.5)	128 (52.2)	678 (35.5)	
M				
0	270 (95.1)	224 (91.4)	1814 (94.9)	n. s.
1	14 (4.9)	21 (8.6)	98 (5.1)	
Histology				
Invasive-ductal	237 (83.5)	215 (87.8)	1414 (74.0)	<0.0001
Others	47 (16.5)	30 (12.2)	498 (26.0)	
Grade				
1/2	129 (45.4)	140 (57.1)	1549 (81.0)	<0.0001
3	155 (54.6)	105 (42.9)	363 (19.0)	
ER/PR positive	(0)	150 (61.2)	1912 (100)	<0.000
ER/PR negative	284 (100)	95 (38.8)	(0)	

SR+/HER2-: At least one steroid receptor positive/HER2-negative; pT: Pathological tumour size category, pN: Pathological lymph-node status, M: Metastases status, all according to TNM classification system, 6th Edition; ER/PR: Oestrogen receptor/progesterone receptor: "positive" if at least ER or PR were positive, "negative" if both ER and PR were negative.

Table 2 – Univariate analysis of risk factors in three predefined groups.

Parameter	Triple-negative (*N* = 284)		HER2+ (*N* = 245)		SR+/HER2- (*N* = 1912)	
	%	HR (95% CI)	%	HR (95% CI)	%	HR (95% CI)
Age						
>50	4.4	1	6.1	1	1.5	1
⩽50	7.7	1.8 (0.7–4.8)	8.1	1.0 (0.4–2.7)	2.6	1.3 (0.7–2.4)
pT						
1/2	5.7	1	6.8	1	2.0	1
3/4	13.2	**2.5 (1.5–8.6)**	7.9	**1.9 (1.2–7.2)**	3.3	**4.5 (3.1–22.0)**
pN						
Neg.	1.8	1	5.1	1	1.0	1
Pos.	11.3	**8.3 (2.3–29.1)**	8.6	2.6 (0.9–7.5)	3.4	**3.9 (2.1–7.4)**
M						
0	6.2	1	6.6	1	1.7	1
1	7.1	1.7 (0.8–15.2)	14.7	**3.2 (1.1–32.3)**	5.5	**4.7 (1.9–11.3)**
Grade						
1/2	7.7	1	6.7	1	1.5	1
3	3.9	0.7 (0.3–1.9)	7.1	1.1 (0.4–3.0)	1.8	0.9 (0.4–2.0)
ER/PR pos.			6.7	1		
ER-/PR-	n.a.		7.4	1.4 (0.5–3.8)	n.a.	

Percentage (%) of patients with CM; Hazard Ratio (HR) for development of CM; bold values statistically significant. SR+/HER2-: At least one steroid receptor positive/HER2-negative; pT: Pathological tumour size category, pN: Pathological lymph-node status, M: Metastases status, all according to TNM classification system, 6th Edition; ER/PR: Oestrogen receptor/progesterone receptor: "positive" if at least ER or PR were positive, "negative" if both ER and PR were negative.

significantly associated with development of CM. Adjuvant administration of chemotherapy did not alter the risk for CM significantly, but a trend towards a higher risk was observed. HER2-positive patients did not have a higher risk for CM when adjuvant trastuzumab was given, but 12 of 19 (63.2%) HER2-overexpressing patients who developed CM received trastuzumab in the metastatic setting. 33 of 80 (41.25%) patients with newly diagnosed CM had no evidence of other metastatic sites, or had stable disease considering previous known metastases.

Comparing the two ER-positive subgroups, SR+/HER2- negative versus ER+/HER2-positive, patients with ER+/HER2-positive phenotype showed an elevated hazard ratio of 6.8 (95% confidence interval [CI]: 3.1–14.6) for CM.

3.3. *Multivariate analyses*

In the multivariate analysis of all patients, triple-negative phenotype and HER2-overexpression were the strongest independent predictors for development of CM with hazard ratios of 4.2 (95% CI: 2.3–7.6) and 3.4 (95% CI: 3.1–10.9), respectively. Further significant results were seen for the following risk factors: large tumour size, nodal involvement and early onset BC (Table 3A).

Results of the multivariate analysis in the three subgroups are displayed in Table 3B. Patients with triple-negative BC had a significantly higher risk of developing CM if they had been diagnosed below the age 50 years or if they had a large primary tumour. In the group of patients with HER2-overexpressing BC, no predictors (including adjuvant trastuzumab therapy) could be identified.

Comparing the two HER2-overexpressing subgroups, HER2- phenotype (ER-negative) and Luminal B (ER-positive), the HER2- phenotype had a higher risk for developing CM.

3.4. *Incidence of CM and survival analyses*

Within the median follow-up period of 47 months, 80 of 2441 patients (3.3%) developed CM. The brain was the first metastatic site in 10–12% of the patients with recurrence regardless of subgroup. Patients with triple-negative and HER2-positive BC had a higher frequency of CM (6.7% and 7.8%, respectively; $p = 0.3$) compared to the SR+/HER2- group (2.2%) ($p < 0.05$ for the comparison) (Fig. 1). The highest incidence of CM (20%) was seen in younger patients (age < 50 years) with node-positive, triple-negative BC.

The median time to development of brain metastases was shortest in patients with triple-negative BC (22 months), followed by patients with HER2-overexpressing BC (30 months) and SR+/HER2- tumours (63.5 months) (Fig. 1). Similar patterns were observed in patients with other metastases: the median time of distant recurrence free survival was 21 months in triple-negative BC patients, 24 months in patients with HER2-overexpressing BC and 28 months in patients with SR+/HER2- tumours ($p < 0.05$).

During the observation period, 334 deaths were reported. Median 5-year survival was significantly lower in patients with triple-negative (68%) or HER2-positive (73%) BC compared to patients with SR+/HER2- tumours (87%).

After the first diagnosis of CM, the prognosis of all patients was poor with the median survival ranging between 3 months in triple-negative and 11 months in HER2-positive BC patients with no statistical differences (Fig. 2).

4. Discussion

This retrospective, single-institution study analysing the risk factors for development of brain metastases was based on a relatively large population with 2441 patients with invasive BC. Although some related studies were conducted on a larger cohort,[15,16] other comparable analyses were done on a smaller scale.[17–19]

The frequency of symptomatic CM has been reported to be between 5 and 16%,[2,3] and in asymptomatic patients even up to 34%.[1,10,20] A higher incidence of symptomatic brain metastasis is often reported in studies focusing explicitly on high risk collectives, like HER2-positive patients treated with trastuzumab[7,22] or patients treated with chemotherapy.[20,23] The present analysis, however, was based on a non-selected cohort including a large number of low risk patients who had received neither chemotherapy nor trastuzumab. Thus, the analysis showed a lower frequency of symptomatic brain metastases (3.3%), which agrees with some population-based reports.[16,21]

In this study, 11.6% of the patients had triple-negative BC, which is consistent with other studies showing a rate of 10–14%.[24,25] Triple-negative BC, a clinical surrogate for the basal-like phenotype, was the strongest independent risk factor for development of CM. Recently, it was shown that patients with the basal-like phenotype had a higher rate of brain metastases than other patients.[27,28] Others reported that patients suffering from ER-negative disease have a higher risk

Table 3 – (A) Multivariate risk factors for the development of CM in all patients. (B) Multivariate risk factors for the development of CM in subgroups; bold values statistically significant. HR: Hazard ratio; ER+/HER2-: At least one steroid receptor positive/HER2-negative; pT: Pathological tumour size category, pN: Pathological lymph-node status, according to TNM classification system, 6th Edition.

	HR	95% CI	*p*-Value
A parameter			
Triple-negative	**4.2**	**2.3–7.6**	**<0.0001**
Her-2neu+	**3.4**	**3.1–10.9**	**0.005**
Age < 50 years	**2.0**	**1.2–3.5**	**0.012**
T	**1.9**	**1.2–3.6**	**0.02**
N +	**2.4**	**1.1–5.1**	**0.028**
B parameter	Triple-negative (N = 84) HR (95%CI)	HER2+ (N = 245) HR (95%CI)	SR+/HER2- (N = 1912) HR (95%CI)
Age ⩽ 50 years	**3.8 (1.3–11.2)**	1.0 (0.4–2.8)	**2.0 (1.2–3.5)**
T	**2.2 (1.1–9.7)**	1.5 (0.9–6.7)	**2.1 (1.2–5.1)**
N +	4.0 (0.8–19.7)	2.2 (0.6–8.8)	**2.4 (1.1–5.1)**

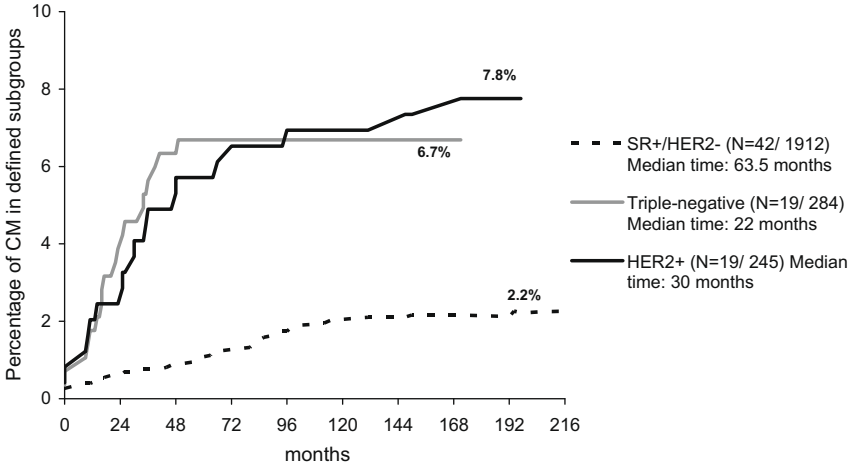

Fig. 1 – Time course and frequency of cerebral metastases (CM) in the three subgroups; in brackets: Patients with CM/all patients; Triple negative versus HER2+; Log-rank test: $p = 0.3$; Triple-negative and HER2+ versus SR+/HER2-; Log-rank test: $p < 0.05$.

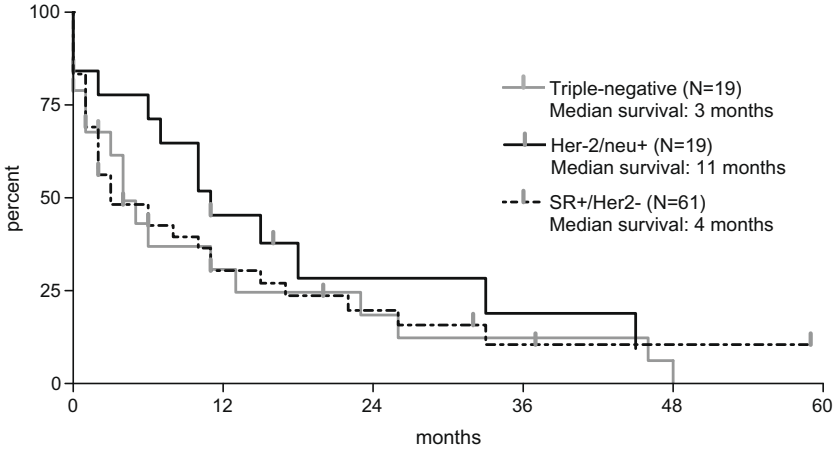

Fig. 2 – Survival of patients with CM after the first diagnosis of cerebral involvement; in brackets: Number of patients with CM in each group/all patients with CM; Censored data indicated by grey symbols; Log-rank test: $p = 0.51$.

for CM.[8,16,26] Furthermore, analyses of cerebral metastasis specimen revealed the basal-like and HER2-phenotype to be the most frequent subtypes.[12] Consistent with these earlier findings, this analysis showed HER2-overexpression (mainly with ER-negative receptor status) to be a strong independent risk factor for CM. In addition, the comparison between the two ER-positive phenotypes (SR+/HER2- and SR+/HER2+) revealed an elevated risk for patients with HER2-overexpressing tumours comparable to previous reported results.[29]

Early onset BC, large tumour size and lymph-node involvement at first diagnosis of BC were confirmed as independent risk factors for development of brain metastasis.[8] In fact, a combination of early onset, triple-negative and node-positive BC was associated with a 20% risk of developing brain metastases.

Our analysis also found that the aggressive nature of triple-negative[11,24,30,31] and HER2-overexpressing[13,30,31] disease resulted in a shortened CM free survival and overall survival. Furthermore, the median interval between first diagnosis of BC and first diagnosis of CM in triple-negative patients was shorter than that in patients with HER2-overexpressing and SR+/HER2- phenotypes, as observed earlier.[27,32]

Reasons for a higher propensity for CM in triple-negative phenotypes are most likely to be different from those for HER2- phenotypes. There is increasing evidence that HER2-positive tumours show an organotropism, driven by chemokine-mediated movement of malignant cells to specific organs. The chemokine receptor CXCR4 is expressed in most BC cells, but HER2-overexpression was associated with up regulation of CXCR4 receptor expression and protection from protein degradation.[33] SDF-1α (stromal cell-derived factor-1α) is the exclusive ligand for CXCR4, which is expressed in the brain.[34] BC cells that express CXCR4 are attracted by tissues expressing high levels of SDF-1α, which causes BC cells to leave the circulation and to proliferate and induce angiogenesis and metastasis.[33] This phenomena of 'soil and seed' in HER2-overexpressing BC may explain the higher frequency of CM: 1.9% in HER2-negative compared to 9% in HER2-positive patients.[29] Trastuzumab therapy might not overcome this inherited risk for CM in HER2-positive patients, because trast-

uzumab is a large molecule which is unable to penetrate the intact blood-brain barrier.[2] CM are found in up to 48% of HER2-positive, trastuzumab treated patients.[7] In our population, 63.2% of all HER2-overexpressing patients developing CM had received trastuzumab in the metastatic setting, which confirmed other reports suggesting trastuzumab therapy as a risk factor for CM[2,22] in terms of stabilising systemic disease and allowing brain metastases to develop.

Triple-negative BC is the clinical surrogate for the basal-like phenotype and is routinely assessed during histo-pathological work-up.[35] There is no evidence that the CXCR4-SDF-1α axis in basal-like BC is as important as in HER2-positive disease. The aggressive nature of basal-like BCs, mainly leading to haematogenous metastatic spread, is poorly understood. However, several histological findings, such as high nuclear grade, accumulation of p53 and high expression of the proliferation marker KI-67,[36] as well as invasive ductal carcinomas with large, central acellular zones,[37] correlate with worse clinical outcomes. A molecular explanation for increased aggressiveness and invasive and metastatic potential includes Epithelial-Mesenchymal Transition (EMT), defined by the loss of epithelial characteristics (E-cadherin, occludins and luminal cytokeratins) and the gain of mesenchymal features (vimentin N-cadherin, ß-catenin), mediated by specific transcription factors: 'EMT inducers' (Snail, Slug, Twist and ZEB).[38,39] Sarrio and colleagues reported a focal expression of these mesenchymal markers preferentially in basal-like BC and concluded that the proclivity of basal-like cells to mesenchymal transition may be related to the high aggressiveness and characteristic metastatic spreading of these tumours.[39]

After the development of CM the prognosis was poor in all patients within the expected range.[5,6,32,40] In our analysis, BC subtype had no significant impact on survival once brain metastases had been diagnosed, although others have reported significant shorter survival in patients with triple-negative disease[27,32] and prolonged survival for patients with HER2-overexpressing tumours, especially when patients were treated with trastuzumab in advanced stage disease.[7,27,32] This contradiction might be the result of a limited number of HER2-positive patients with CM or the restricted use of trastuzumab beyond disease progression in our study.

There are some limitations regarding this study. The retrospective character might lead to a selection bias and the restriction to a single institution may cause a referral bias. Although the HER2 detection was accomplished in a national reference centre, we found a rather low proportion of HER2-overexpressing patients compared to Slamon and colleagues,[13] which might lead to an underestimation of the impact of HER2-overexpression as a risk factor for CM.

Patients with triple-negative and HER2-positive BC have a high risk for CM and develop CM earlier in the course of disease. An approach to use BC phenotype for risk evaluation together with other risk factors (e.g. age at diagnosis, tumour size, nodal involvement) may therefore help to identify patients who could benefit from early detection and treatment of CM.

Currently, there is no evidence for a benefit of early detection of CM in BC patients.[41] However, this may be due to the lack of adequate selection criteria for cohorts at high risk. In small cell lung cancer, Sanchez and colleagues demonstrated a survival benefit of approximately 3.5 months when asymptomatic brain metastases were treated as soon as they had been detected.[42] Since patients with triple-negative disease usually develop brain metastases within the first few years after BC diagnosis, CNS-screening would not have to be extended over a longer period of time. Also, the expected number of patients who would have to be screened is small.

The risk factors described here for the development of brain metastases (triple-negative, premenopausal, nodal involvement) will be evaluated prospectively in one large adjuvant triple-negative BC trial, BEATRICE (Bevacizumab Adjuvant Therapy in Triple Negative Breast Cancer protocol).

Conflict of interest statement

None declared.

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