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Original article

Biopsy of liver metastasis for women with breast cancer: Impact on survival

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ABSTRACT

Background: Biopsy of metastatic site of disease can influence treatment decisions, but its impact on survival remains uncertain.
Patients and methods: One-hundred patients with first metachronous liver metastases (LM) from breast cancer (BC) who underwent liver biopsy between 1999 and 2009 were identified. One-hundred matched control patients with LM from BC and no biopsy were selected.
Results: Liver biopsy had no statistically significant impact on survival when comparing biopsied patients to controls [HR 0.82 (95% CI 0.58–1.16)]. Patients with early metastasis (within 3 years) undergoing liver biopsy had a better survival [HR 0.60 (95% CI 0.38–0.97)] compared to those who did not. Liver biopsy had no statistically significant impact on survival in patients with late LM (after 3 years) [HR 1.09 (95% CI 0.69–1.74)]. We observed that 18 out of 100 biopsied patients (18.0%) had a conversion of predictive factors which allowed adjusting for therapy, specifically new expression of ER ($n = 5$), overexpression of HER2 ($n = 12$) or both ($n = 1$). Fourteen out of 18 (77.8%) received anti-HER2 treatment for the first time at the time of metastasis and 3 others (16.7%) received hormone therapy. Those 18 patients showed a better survival compared to the other 82 biopsied patients [HR 0.55 (95% CI 0.28–1.10)] and compared to the 13 biopsied patients with disappearance of features which predicted responsiveness to a given treatment [HR 0.19 (95% CI 0.06–0.62)].
Conclusions: Liver biopsy can impact survival of patients with early metastases from BC. Discordance between primary and distant lesions can offer the patients new treatment options.

Introduction

Liver metastases (LM) are found in 55–75% of autopsies of patients with breast cancer (BC).[1,2] Despite the multidisciplinary approach, prognosis of patients with LM from BC remains poor, with a median overall survival ranging from 20 to 60 months.[3–7]

Biopsy of metastatic tissue can improve outcome by the optimization of systemic therapies,[8,9] since discordance of biological features of the disease had been observed between the primary tumour and the metastasis.[10–16] No clear evidence on survival improvement has been reported so far.

We compared 100 patients with a LM from BC undergoing a liver biopsy to 100 matched control patients with LM from BC and no biopsy. The aims of our study were: (a) to evaluate the discordance rate in tumour biology between primary and distant tissue; (b) to assess the impact of liver biopsy on the treatment reassessment; (c) to evaluate the effect of LM biopsy on survival.

Patients and methods

We collected information through the institutional Clinical–Radiological database on all consecutive breast cancer patients who underwent ultrasound-guided liver biopsy at the European Institute of Oncology (IEO), Milan, Italy, between 1999 and 2009. We

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included only women with BC and liver as the first and only metastatic site. We excluded patients with bilateral breast cancer, synchronous metastases and patients with preceding invasive tumours. A total of 100 patients were finally analyzed in the current study. For each one of the 100 patients in the study group, we selected from the IEO Tumour Registry database one matched patient who had the same characteristics listed above but did not undergo a liver biopsy (control group). The variables used to make the randomly assigned matches were age at LM (±5 years), year of primary surgery (±5 years) and time from primary surgery to LM (±2 years).

Data on clinicopathological features, type of treatment (i.e. endocrine therapy (ET), Trastuzumab, or chemotherapy (CT)) and follow-up were retrieved and analyzed. ER and PgR were considered positive when ≥1% of tumor cells were immunoreactive. HER2 immunohistochemical (IHC) expression was evaluated using a 1:400 dilution of a polyclonal antiserum (Dako, Glostrup, Denmark). IHC expression was scored as follows: 0 (no staining or faint membrane staining), 1+ (faint membrane staining in >10% of tumour cells, incomplete membrane staining), 2+ (weak to moderate membrane staining in >10% of tumour cells), and 3+ (intense circumferential membrane staining in >10% of tumour cells). For this analysis, HER2 scores of 0 and 1+ were considered negative. HER2 IHC 3+ and FISH-amplified tumours were considered positive. All IHC 2+ tumours and tumours for which IHC was not assessable were also tested for gene amplification by FISH. As for the follow-up information, in case of unavailability of a clinical examination during the previous 6 months, survival status was ascertained by telephone or via the national registry office.

Statistical methods

The Chi-square test was used to assess differences in the distribution of prognostic variables and treatment approaches between study and control groups. The Chi-square test was also used to compare the percentages of treatment changes between groups.

The main end point was overall survival (OS), defined as the length of time from the date of LM to death from any cause. The OS distribution was estimated by using the Kaplan–Meier method, and differences were evaluated by the Wilcoxon test. Multivariable Cox proportional hazards models were also applied. Adjusted hazard ratios (HR) with 95% confidence intervals (CIs) were reported. All analyses were carried out with SAS software (SAS Institute, Cary, NC). All reported *P* values were two sided.

Results

The median age at first diagnosis of patients with liver biopsy was 50 years (range 29–78). The median time to LM was 3 years (range 0.2–15.9); 78.0% of primary tumours were estrogen receptors (ER) positive and 17.0% overexpressed HER2. According to stage and tumour biology, 72% received CT at first diagnosis, 79% ET and only 1% Trastuzumab. No significant differences were observed between cases (biopsied) and controls (unbiopsied), except for the number of positive lymph nodes at primary diagnosis. Clinicopathological features of cases and controls are reported in Table 1.

Liver biopsy had no statistically significant impact on survival when comparing biopsied patients to controls [adjusted HR 0.82 (95% CI 0.58–1.16)]. Patients with early metastasis (within 3 years) undergoing liver biopsy had a better survival [adjusted HR 0.60 (95% CI 0.38–0.97)] compared to those who did not. Liver biopsy had no statistically significant impact on survival in patients with late LM (after 3 years) [adjusted HR 1.09 (95% CI 0.69–1.74)]. The interaction between the impact of biopsy and the time to metastasis was borderline statistically significant ($P = 0.065$).

Table 1
Characteristics of biopsied and unbiopsied patients.

		Biopsy	No Biopsy	*P*-value[a]
All		100	100	
Year of primary diagnosis	<1995	7 (7%)	11 (11%)	Match
	1995–1999	45 (45%)	44 (44%)	
	2000–2004	39 (39%)	37 (37%)	
	2005–2007	9 (9%)	8 (8%)	
Age at metastasis (years)	≤40	21 (21%)	14 (14%)	Match
	41–50	31 (31%)	32 (32%)	
	51–60	22 (22%)	29 (29%)	
	>60	26 (26%)	25 (25%)	
Years between primary surgery and liver metastasis	<2	24 (24%)	28 (28%)	Match
	2–3	23 (23%)	21 (21%)	
	3–5	27 (27%)	29 (29%)	
	>5	26 (26%)	22 (22%)	
Histology	Ductal	89 (89%)	86 (86%)	0.75
	Lobular	7 (7%)	10 (10%)	
	Other	4 (4%)	4 (4%)	
pT	Unknown	5 (5%)	19 (19%)	0.07
	1	45 (45%)	39 (39%)	
	2	45 (45%)	30 (30%)	
	3–4	5 (5%)	12 (12%)	
Number of positive lymph nodes	Unknown	3 (3%)	15 (15%)	<0.01
	0	5 (5%)	24 (24%)	
	1–3	39 (39%)	28 (28%)	
	4–9	44 (44%)	18 (18%)	
	10+	9 (9%)	15 (15%)	
Estrogen receptor at primary	Unknown	1 (1%)	6 (6%)	0.59
	Negative	21 (21%)	23 (23%)	
	Positive	79 (79%)	71 (71%)	
Progesterone receptor at primary	Unknown	1 (1%)	10 (10%)	0.42
	Negative	34 (34%)	36 (36%)	
	Positive	65 (65%)	54 (54%)	
HER2 status at primary	Unknown	40 (40%)	29 (29%)	0.28
	Not Overexpressed	43 (43%)	45 (45%)	
	Overexpressed	17 (17%)	26 (26%)	
Chemotherapy at primary	Unknown	(–)	2 (2%)	0.47
	No	28 (28%)	23 (23%)	
	Yes	72 (72%)	75 (75%)	
Endocrine therapy at primary	Unknown	(–)	2 (2%)	0.36
	No	21 (21%)	26 (26%)	
	Yes	79 (79%)	72 (72%)	
Trastuzumab at primary	Unknown	(–)	2 (2%)	0.30
	No	99 (99%)	95 (95%)	
	Yes	1 (1%)	3 (3%)	
Chemotherapy at metastasis	Unknown	3 (3%)	10 (10%)	0.97
	No	12 (12%)	11 (11%)	
	Yes	85 (85%)	79 (79%)	
Endocrine therapy at metastasis	Unknown	3 (3%)	10 (10%)	0.15
	No	31 (31%)	38 (38%)	
	Yes	66 (66%)	52 (52%)	
Trastuzumab at metastasis	Unknown	3 (3%)	10 (10%)	0.43
	No	66 (66%)	66 (66%)	
	Yes	31 (31%)	24 (24%)	

[a] Chi-square test. Unknown category was not considered in the calculation of the *P*-value.

We observed that 18 out of 100 biopsied patients (18.0%) had a conversion of predictive factors which allowed adjusting for therapy, specifically new expression of ER ($n = 5$), overexpression of HER2 ($n = 12$) or both ($n = 1$) (Table 2). Fourteen out of 18 (77.8%) received anti-HER2 treatment for the first time at metastasis and other 3 (16.7%) received hormone therapy. Those 18 patients showed a significantly better survival compared to the other 82 biopsied patients [borderline significance, adjusted HR 0.55 (95% CI 0.28–1.10)] and even better compared to the 13 biopsied patients with disappearance of features, such as expression of ER and overexpression of HER2, which predicted responsiveness to a given treatment [adjusted HR 0.19 (95% CI 0.06–0.62)] (Figs. 1–4).

Patients with late metastasis had more frequently an ER and/or HER2 positive conversion in the metastatic tissue (12 out of 53,

Table 2
Information on biology of primary tumor and liver metastasis.

Information at primary	Information at biopsy				
	Er −/Her2 −	Er −/Her2 +	Er +/Her2 −	Er +/Her2 +	Total
ER −/Her2 −	3	0[a]	0[a]	0[a]	3
ER −/Her2 +	1	4	0[a]	2[a]	7
ER +/Her2 −	3	1[a]	34	2[a]	40
ER +/Her2 +	0	5	4	1	10
ER −/Her2 missing	5	3[a]	2[a]	1[a]	11
ER +/Her2 missing	0	1[a]	22	5[a]	28
ER missing/Her2 missing	0	0	1[a]	0	1
Total	12	14	63	11	100

[a] Patients with a conversion of predictive factors which allowed adjusting for therapy.

22.6%) than in patients with early metastases (6 out of 47, 12.8%). The difference was not statistically significant ($P = 0.200$).

We then limited the analysis to the 60 patients with complete information on ER and HER2 at both primary and metastatic lesions and we observed a change in biology in 18 patients (30.0%). Five out of 18 (27.8%) had a newly identified expression of ER ($n = 2$) or overexpression of HER2 ($n = 3$). Despite the small numbers, we could confirm the findings reported above: those 5 patients showed a better survival compared to the other 55 biopsied patients [borderline significance, adjusted HR 0.30 (95% CI 0.08−1.16)] and compared to the 13 biopsied patients with disappearance of ER expression or HER2 overexpression [adjusted HR 0.08 (95% CI 0.02−0.45)]. When considering ER and HER2 independently, we observed a change in ER in 15 patients out of 99 (15.2%), with 5 (5.1%) changing from negative to positive and 10 (10.1%) changing from positive to negative. Analysis of HER2 alone (60 patients evaluable) showed a change in 8 patients (13.1%), with 3 (5.0%) changing from not overexpressed to overexpressed and 5 (8.2%) changing from overexpressed to not overexpressed.

Among the 100 patients with liver biopsy, all liver tissues and 72 primary tumor tissues were evaluated in the pathology division of our institute, while 28 primary tumor tissues were analyzed in other institutes. In a sensitive analysis, we limited the investigation to the 72 patients that had both tissues analyzed at our institute and the results were similar to the ones above reported: patients with a conversion of predictive factors which allowed adjusting for therapy showed a better survival compared to patients with disappearance of ER expression or HER2 overexpression [adjusted HR 0.25 (95% CI 0.05−1.16)].

Discussion

In our retrospective case−control series of patients with BC and liver metastasis, liver biopsy had no statistically significant effect on overall survival. We observed a positive effect of liver biopsy on survival in patients with early metastases. We also identified a subgroup of patients with a conversion of predictive factors (i.e. positive conversion of ER and/or HER2) which allowed adjusting for therapy.

We already documented that biopsies of metastasis are useful for confirmation/exclusion of advanced disease and for the reassessment of the biology of the metastatic disease, contributing to define a more effective treatment strategy, either by proposing the patients new treatment options or avoiding ineffective therapies.[11] There is emerging evidence that tumour biology may change dynamically during the natural history of the disease, with possible impacts on treatment decisions and, possibly, on survival.[10–16] Changes in receptor expression may occur as a consequence of transcriptional or post-transcriptional modifications of gene expression, which may occur spontaneously or as a consequence of clonal selection in response to therapy. In our study population, discordance in the biology profile between the primary lesion and metastasis was observed in 15 cases out of 99 evaluable cases (15.2%) for ER status, and in 8 out of 60 evaluable cases (13.1%) for HER2. Comparable results are reported in the literature.[17–22] In a similar study,[17] 258 patients underwent biopsy and discordance rates in ER and HER2 between the primary and recurrent diseases were 13% and 5%, respectively. The biopsy results altered management in 15.9% of patients (95% CI 11.7−20.9%, $P < 0.0001$). A

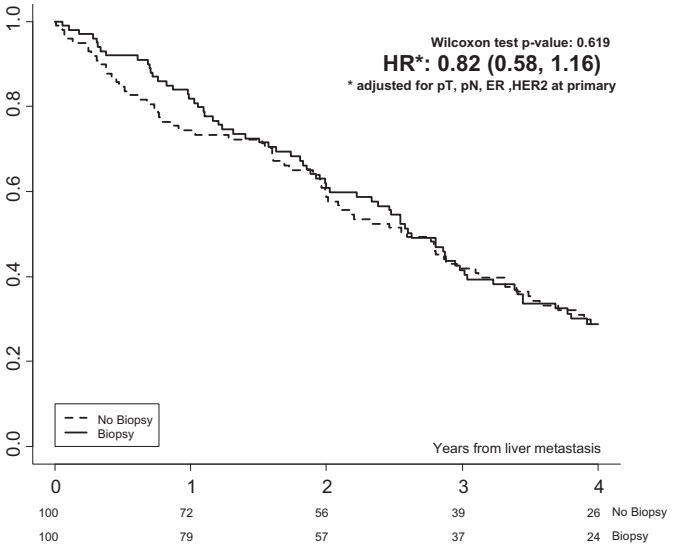

Fig. 1. Overall survival.

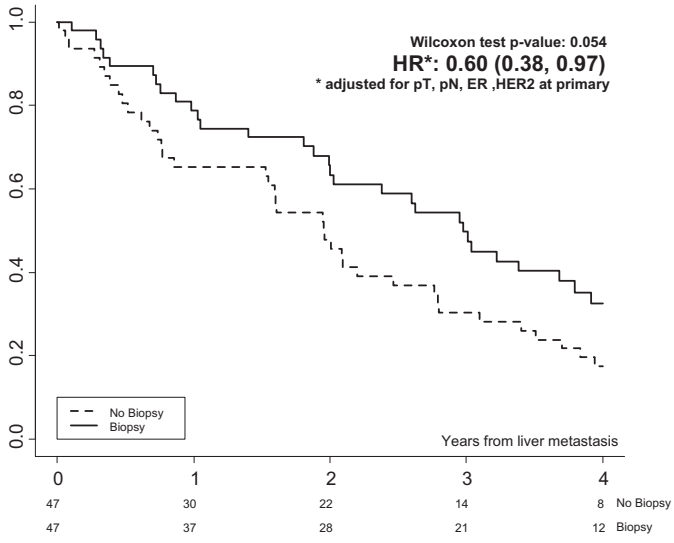

Fig. 2. Overall survival in patients with early metastasis (within 3 years).

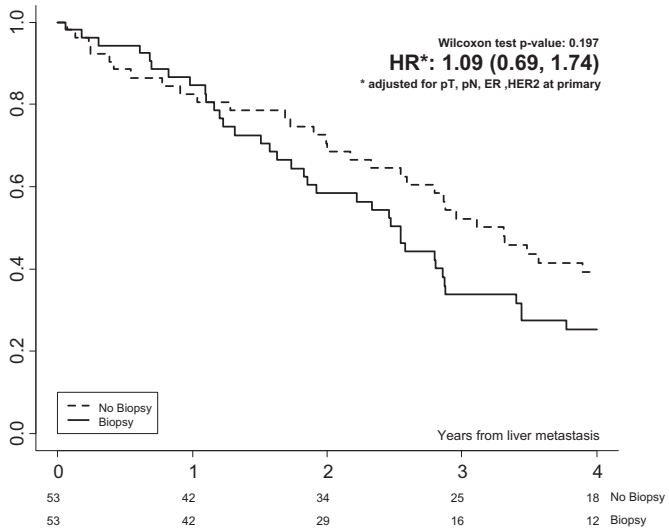

Fig. 3. Overall survival in patients with late metastasis (after 3 years).

recent review of studies comparing primary and metastatic lesions reported a discordance rate of estrogen receptor status of 30% (range 18–54%) and of HER2 status of 16% (range 0–33%).[18]

Changes in biology should be interpreted with caution since inadequate sampling of heterogeneous lesions and limited accuracy and reproducibility of receptor assays can lead to confusing results.[23–27] However, the proportion of falsely discordant results remains unclear.[27] Some authors suggest repeating the test simultaneously on both primary and recurrent tumour specimens, since repeating biopsy on the metastatic tissue alone does not necessarily improve the diagnostic accuracy and has the potential to produce a false-negative result.[27]

The original information we provided in our study is that we identified a subgroup of patient with favorable outcome that had benefit from the biopsy on the metastatic site. A statistically significant better overall survival was observed in the group of 18 patients with a positive conversion of ER and/or HER2 (from negative to positive). All these patients, as a consequence of liver biopsy, had the opportunity to receive targeted treatment as Trastuzumab or endocrine therapy.

Another interesting information we reported is related to the positive effect of liver biopsy on survival in patients with early metastases, but not in patients with late metastases. What is not clear is why patients with early metastatization should have a greater benefit in terms of overall survival from liver biopsy respect to patients with late metastatization. Our hypothesis is that an appropriate treatment reassessment could play a more significant role in rapid, hence more severe, reappearances, whose prognosis still has large margin of improvement, rather than in late, hence more indolent, reappearances, whose prognosis is more favorable by itself.

The present study has some strengths: (i) all biopsies have been carried out in a single institution; (ii) all IHC was carried out within the same pathology division; (iii) this is the first study, to our knowledge, that focused the question on the effect of LM biopsy on survival; (iv) all patients had liver only disease. Several limitations are present though, apart from its retrospective design. First, 40 tumours had an undetermined HER2 status at primary diagnosis, because they were diagnosed in the pre-Trastuzumab era. Ten of them (25%) had overexpression at progression and were counted as cases that benefited from the biopsy. Since approximately 20% of breast cancer tumours show an overexpression of HER2,[28] we might have slightly overestimated the actual number of patients that nowadays would benefit from the biopsy (approximately two patients out of 10 might have already had the HER2 overexpressed at the primary diagnosis). Anyhow, when we performed the

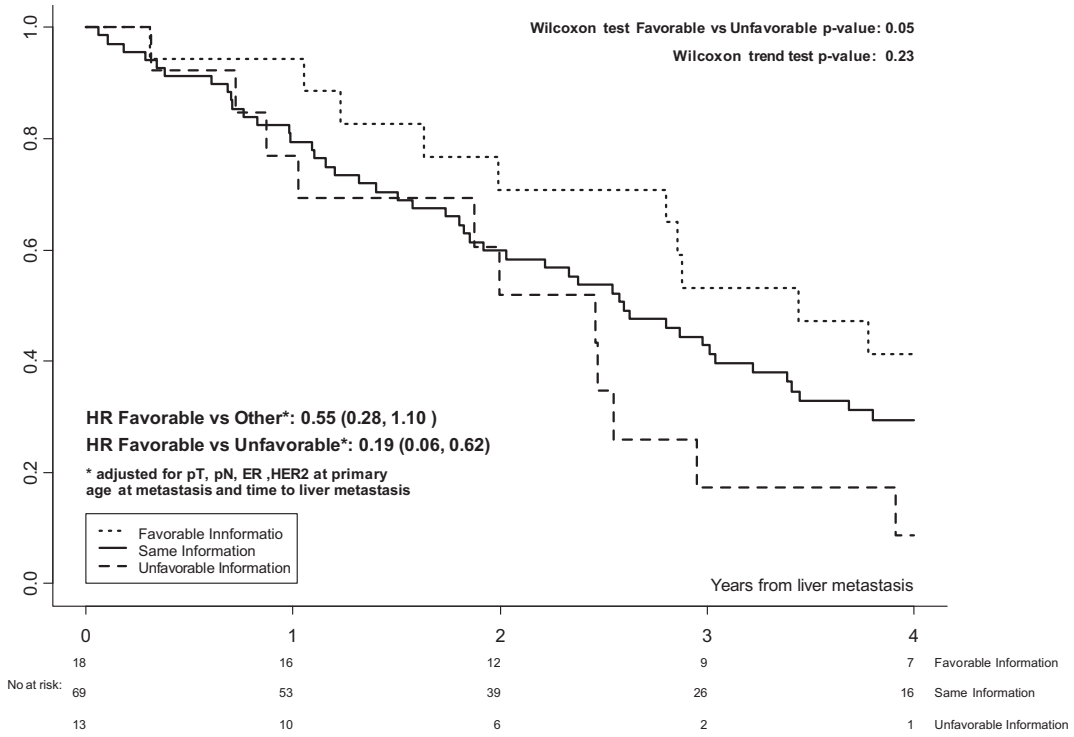

Fig. 4. Survival according to changes in biology between the primary and the metastatic lesion. *Favorable information*: conversion of predictive factors which allowed adjusting for therapy, specifically new expression of ER and/or overexpression of Her2/Neu. *Same information*: no change in expression of ER and/or overexpression of Her2/Neu. *Unfavorable information*: disappearance of features (expression of ER and/or overexpression of Her2/Neu) which predicted responsiveness to a given treatment.

analysis excluding all the 40 cases, we obtained comparable results on both discordance rates and survival. A second limitation is that discordance rate between biological profiles can be simply related to the limited accuracy and reproducibility of receptor assays, as stated above, and to the intraobserver and interobserver variability, and we do not have any tool to determine how reliable our results are. Anyhow, in order to possibly increase homogeneity of data sources, we re-performed the analysis selecting the 72 patients out of 100 that had both primary and secondary lesions analyzed in the pathology division of our institute and the results were similar to the ones obtained from the whole population.

Despite the promising results from this and other studies, some issues that may prevent clinicians from proposing a biopsy in the metastatic setting include lack of resources, technical difficulties, or reluctance to undertake an invasive procedure in a patient who has advanced disease. We should also consider that many rebiopsy procedures cannot be easily carried out due to potential complications. Since the final decision must be the result of a joint decision between the patient and the physician, another possible concern is the patient's preference to undertake another invasive procedure. Simmons and colleagues reported that up to 82% of patients with suspected metastatic lesions agreed to undergo a confirmatory biopsy.[10]

So far, whether the changes in receptor expression are due to biological evolution or to inconsistent measurements remains unclear. However, according to our results, when safe and easy to carry out, a liver biopsy of the metastatic lesion should be considered in all patients, particularly in case of early metastasis and when new treatment options are potentially available. Even if the improvement in survival that we observed should preferably be confirmed by a randomized trial, our findings on the role of biopsy in the advanced disease in the liver could be extended to other metastatic sites.

Conflict of interest statement

None declared.

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