The Impact of Tumor Phenotype (Hormone receptor and HER-2) Discordance during Breast Cancer Progression on Patient Management and Survival

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Abstract: Background: The assessment of hormone receptors (HRs) and human epidermal growth factor receptor (HER)-2 are necessary to select patients who are candidates for hormonal and anti–HER-2 therapy. Several retrospective reviews suggest change in tumor phenotype during breast cancer progression. The aim of this prospective study is to investigate discordance in receptor status between primary and recurrence disease and to assess its impact on patient management and survival. **Patients and methods:** One hundred patients with breast cancer progressive disease were recruited and underwent histopathological sampling. All biopsies were analyzed for ER, PR, and HER2 using similar methodology in the same lab by the same pathologist for both Primary and metastases disease. **Results:** Seventy four patients (74%) maintain the same tumor phenotype [i.e. the same hormone receptors (HR) and HER2 status] at recurrence, while 26 patients (26%) had discordance in tumor phenotype during progression. Biopsy led to a reported change of management in 18% of patients (P=0.003).Rates of discordance were 20%, 32%, 16% and 10% for ER, PR, HR and HER2, respectively. Tumor phenotype discordance was associated with worse time to treatment failure (TTF) and overall survival (OS) ($P < 0.001$ and $P = 0.003$, respectively); those cases who turned into triple-negative experienced the poorest TTF and OS outcome, respect to the concordant group ($P < 0.001$ for both TTF&OS). **Conclusion:** Tumor phenotype discordance is a fact and is associated with detrimental effects on outcome and led to altered management. Tissue confirmation should be considered as a routine in breast cancer patients with suspected metastatic recurrence whenever feasible.
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1. Introduction

Breast cancers are routinely assessed for hormone receptor status [estrogen receptor (ER)and progesterone receptor (PR) a] by immunohistochemistry (IHC) and human epidermal growth factor receptor 2 (HER2) expression by IHC or amplification by FISH, in addition to pathological grade and stage, in order to guide the choice of the appropriate adjuvant therapy [1].

Hormonal therapy and anti–human epidermal growth factor receptor (HER)-2 treatments represent the most successful examples of targeted therapy for breast cancer [2]. For at least three decades, the expression of hormone receptors (HRs) has been recognized as the main determinant of the efficacy of endocrine manipulation [2]. The evaluation of these predictive parameters is generally carried out in the primary tumor, and this assessment is taken into account to select treatment even in cases of metastases that occur several years later with the increasing of availability for targeted therapies [2,3,4]. Those cases showing the same HR status and HER2 status in both primary and recurrent samples were considered concordant [5].

Several reports have shown a lack of concordance in the expression of these predictive factors between primary tumors and metastatic sites [6,7]. Nowadays, the National Comprehensive Cancer Network (NCCN) guidelines recommend a biopsy of metastatic deposits when feasible [8,9], the practice of obtaining biopsies of metastatic lesions varies considerably across centers; therefore, the clinical management of the majority of patients is still based on the initial assessment [2].

The discordance between primary tumors and metastases could be due to pre-analytical and analytical errors, or as a result of genetic drift occurring during tumor progression[10] or due to intratumoral heterogeneity where the clone with the more aggressive phenotype starts the micrometastatic process from the beginning [11,12]. The aim of this prospective study was to evaluate discordance in the expression of tumor phenotype [i.e. Hormone receptors (HR) and HER2 status] between primary breast cancers and subsequent metastases to assess the

impact of discordance on patient management and survival.

2. Patients and Methods

Study Population

This prospective study took place at a clinical oncology department –Tanta University. One hundred patients with recurrent or progressive metastatic breast cancer were included over 3 years from August 2008 to August 2011. Patients were enrolled in this study if tumor samples from both primary and corresponding metastases were available and suitable for IHC analysis (Availability of archival primary tumor was mandatory).

The availability of the following basic data was mandatory to be included in our study; diagnosis of primary, unilateral breast cancer with subsequent development of locally and or distantly recurrent disease with recorded expression status of ER, PR, and HER2 in both primary tumor and recurrence. Patient characteristics including age, menopausal status, medical history, stage at diagnosis, type of surgery, adjuvant therapies, sites of relapse and biopsy also were documented. In patients with multiple lesions, the safest and most practical location lesion was chosen for biopsy (excision biopsy or core biopsy). Patients could have any form of surgical, systemic (neoadjuvant and adjuvant) therapy as well as radiotherapy for the primary disease.

Exclusion criteria comprised bilateral breast cancer, male gender, and ductal carcinoma in situ as initial diagnosis, or if they had already started on therapy for metastatic disease. Patients were also excluded if the location of the lesion was not amenable to biopsy by the following criteria: brain metastases and lesion<1 cm in size, or lesion in a location that could not be reached by core biopsy techniques available with interventional radiology. Metastatic disease documented only by Fine-needle aspiration (FNA) only was also excluded.

Local anesthesia was routinely used *for* true cut biopsy of the suspected recurrence and the choice of CT versus sonographic guided biopsy was made in consideration of factors such as location, safe access, and comorbidity.

Trial End Points

The primary end point of this study was to define the discordance rates in tumor phenotype (HR and HER2 status) between primary and metastatic disease. The secondary end point of this study was the proportion of patients in whom results of the metastatic biopsy led to a change in management and evaluate time to treatment failure (TTF) and overall survival (OS).

Histopathological and immunohistochemistry assessment

Formalin fixed paraffin-embedded (FFPE) tissue was biopsied (as a core biopsy or excision biopsy) at the time of recurrent or metastatic disease and diagnosis was conducted by the pathologist to confirm the presence of invasive breast cancer. Reevaluation of hematoxylin and eosin (H&E) section of all specimens to confirm the presence of sufficient, suitably fixed invasive breast cancer in both primary and recurrent specimens. All patients should have a full-size archival tissue block from the original primary cancer.

The pathologist analyzing the samples was blinded as to the patients' original hormone receptor and Her2 status. FFPE from the primary cancer was subsequently reassessed to confirm the original hormone receptor status and Her2 status with the prospectively collected recurrent breast cancer FFPE block. The cases of primary breast cancer were staged according to the American Joint Committee on Cancer (AJCC) Cancer Staging Manual, sixth edition [13]. Nuclear grading of tumors was done according to the modified Black's nuclear grading system [14], and histological classification was done according to WHO criteria[15]. Hormone receptor–positive disease was defined when either ER- or PR was positive.

Immunohistochemical (IHC) staining was carried out in order to evaluate levels of ER and PR according to Harvey scoring system with antigen retrieval (using immune DAKO system) stain for ER and PR. The cut-off for ER positivity (ER+) and PR positivity (PR+) was ≥10% tumor cells with nuclear staining [16].

HER2 status was obtained from patient medical records. We defined HER2 complete membranous staining-positivity as either presence of HER2 gene amplification as evident by an immunohistochemical (IHC) analysis score of 3 or fluorescent in situ hybridization (FISH) for IHC score 2 positivity. In case of disagreement between IHC and FISH, the HER2 status was defined according to the FISH results [2,17].

Immunohistochemical (IHC) Ki67 staining was considered positive nuclear after score 14% in malignant epithelial cells [1].

Statistical Analysis

SPSS [Statistical package (version 18.0)] was used for data analysis. Mean and standard deviation were estimates of quantitative data. Chi-square/ Fischer exact were tests of proportion independence.

Overall survival was calculated from the time of study entry until death or last follow-up according to the Kaplan-Meier method [18]. Breslow test was used to compare curves. Cox-regression analysis was used to estimate odds of recurrence and 95% CI. Overall survival and progression-free survival were compared by the Kaplan–Meier method [18] with statistical significance assessed by the log-rank test. All *P* values

were two-tailed; a value of ≤ 0.05 was considered significant [19].

3. Results

Patient characteristics

Over a 3-year period (from August 2008 to August 2011), 100 women with confirmed recurrent breast cancer and fulfilling the inclusion criteria were recruited. The mean age at disease recurrence was 51.4 years (standard deviation of 12.3 years), median age was 50 years, and range was 24 to 72 years. The median time to first recurrence of breast cancer following completion of primary therapy was nearly 5 years (59.2 months) and 59 out of 100 subjects (59%) were postmenopausal. Table 1 summarizes the characteristics of the 100 patients and their primary tumors.

Table 1. Summarizes the characteristics of the 100 patients and their primary tumors.

Age	No
Median age 50 years	
Range 24–72	
Clinical stage	
I	5
IIA/IIB	21
IIIA/IIIB	46
IIIC/IV	28
Histologic type	
Ductal	83
Lobular	11
Other	6
Histological grade	
1	0
2	38
3	62
Menopausal status	
Premenopausal	44
Post menopausal	56
Hormonal status	
ER	
Positive	76
Negative	24
PR	
Positive	64
Negative	36
HR	
Positive	80
Negative	20
HER2 status	
Positive	18
Negative	82
Tumor phenotype	
HR+/HER2−	70
HR+/HER2+	10
HR−/HER2+	8
HR−/HER2−	12
Mean Ki67	27%, range 4%–87%
Previous therapy	
Neoadjuvant treatment	26
Adjuvant chemotherapy	75
Adjuvant hormone therapy	78
Adjuvant trastuzumab	4

Twenty six patients (26%) had received neoadjuvat treatment, 75 (75%) had received adjuvant chemotherapy, 78 (78%) had received adjuvant endocrine therapy, and 4 had received adjuvant trastuzumab; 46 were still receiving endocrine therapy when metastases were diagnosed.

Sites of biopsied were locoregional recurrences in 38 cases and distant metastases in 62 cases; in 11 of these 62 patients synchronous locoregional recurrences were also present.

Sites of local recurrences biopsy were: chest wall 12, breast 10, ipsilateral axilla nodes 9 and homolateralclavicular nodes 7. Sites of distant metastases biopsy were liver 30; lung and pleura 12, bone 10 and distant lymph node /skin 6. Seven patients with DM were surgical sampled, whereas the reminder 55 patients were biotical sampled. Table 2 lists the sites of biopsy from the recurrent tumors.

Table 2:- Site of biopsy from recurrent tumors

Locoregional recurrences biopsy :- (38/ 100 patients)		
Site	No/100 Patients	(%)
Chest wall	12	(12)
Breast	10	(10)
Axillary lymph node	9	(9)
Supraclavicular lymph node	5	(5)
Infraclavicular lymph node	2	(2)
Distant metastases (metastasis biopsy) :-(62 / 100 patients)		
Site	No /100 Patients	(%)
Liver	30	(30)
Lung/pleura	12	(12)
Bone	10	(10)
Distant lymph node /skin	6	(6)
Other	4	(4)

Based on the primary tumor, 76 of 100 patients (76%) were ER+, 64 of patients (64%) were PR+, 80 of 100 patients(80%) were HR +,and 18 of 100 patients(18%) were Her2+.

Discordance: - Figure 1 illustrates the changes in tumor phenotype, ER, PR, HER2 and HR between the primary tumor and the recurrence.

Discordance in single-receptor measurement

A discordance in ER status was observed in 20 cases (20%), with the loss and gain of ER positivity in 16 out of 76 patients (21.05%) and 4 out of 24 patients (16.7%) respectively. The highest rate of discordance was observed for PR (32%), with PR loss rate of 37.5% (24/64 patients) countered as the main change. Total discordance rate for HR was 16% with the loss and gain of HR positivity in 14 out of 80 patients (17.5%) and 2 out of 20 patients (10%), respectively. For HER2-statusthe discordance was observed in 10 cases (10%), with4 cases out of 18 patients (22.2%) changed to HER2 negative and 6 patients (7.3%) out of 82 patients gain of HER2 positivity. The rates of discordance in single-receptor measurements are summarized in figure 1.

Discordance in tumor phenotype

According to the expression of HR and HER2, patients were classified into four subtypes: HR positive/HER2 negative, HR positive/HER2 positive, HR negative/HER2 positive and HR negative/HER2 negative (triple negative; TN). Seventy four patients (74%) maintain the same tumor phenotype at recurrence, while 26 patients (26%) had discordance in tumor phenotype during progression, 8 patients (8%) of them shifted to TNBC whereas 18 discordant patients showed either HR or HER2 positivity at recurrence.

Discordance in Ki67

A non-significant increase in mean Ki67 from primary tumor (27%, range 4–87%) to recurrence (36%, range 4–95%) was observed (P =<0.076).

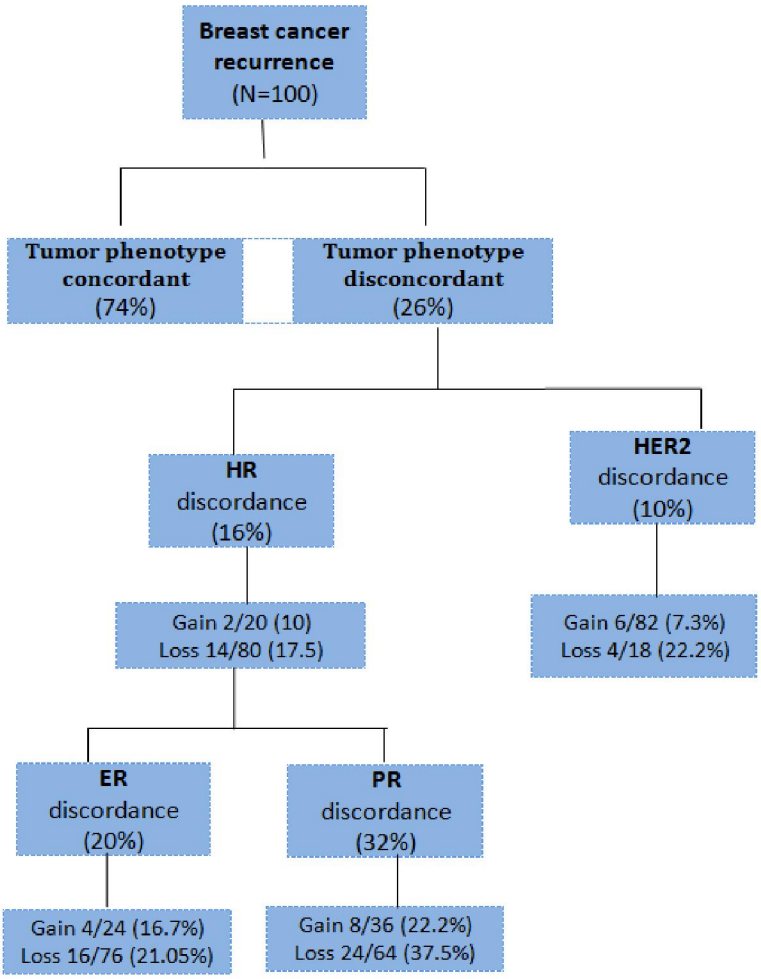


Figure 1. Changes in tumor phenotype, ER, PR and HER2 between the primary tumor and the recurrence

Prognostic impact of tumor discordance

Time to treatment failure (TTF) and overall survival (OS) of the ER- concordant cases were higher when compared with the ER-discordant cases (median 6.27 versus 4 months, P = 0.003 and median 36.47 versus 19.2 months, P = 0.001, for TTF and OS respectively).

Median TTF for the patients who changed from ER positive to ER negative and for the concordant ER-positive group was 3 months versus 7.3 months respectively, P<0.001. Median OS was 17.13 months versus 42.5 months, respectively for the same group of patients, P<0.001.

Time to treatment failure (TTF) and overall survival (OS) of the PR- discordant cases were 5 and 26.3 months respectively, whereas they were 6.1 and 32.3 monthsrespectivelyfor PR concordant and the differences were not statistically significant for TTF and OS in both groups (P = 0.8 and P =0.23 for TTF and OS, respectively).

So discordance in HR status resulted in a worse TTF (median 3 months versus 6.27 months, P < 0.001) and OS (median 17.13 versus 36.47 months, P < 0.0001).Those patients with HR loss experienced both worse TTF (median 3 versus7.3 months, P <0.001) and OS (median 17.13 versus 42.5 months, P <0.001) when compared with those who maintained HR positivity.

Patients with a loss in HER2 expression experienced a trend to a worse TTF (median 4.50 versus6.17 months, P = 0.061 and OS (median 28.27 versus 36.47 months, P = 0.067) when compared with patients who maintained the HER2 positivity, however the differences were statistically insignificant.

Patients who maintained their tumor phenotype unchanged showed a significant better outcome when compared with discordant cases, both in terms of TTF (median 6.3 versus 4.0 months, P <0.001) (Figure 2-A) and OS (median 36.47 versus21.17 months, P = 0.003)(Figure 3-B).

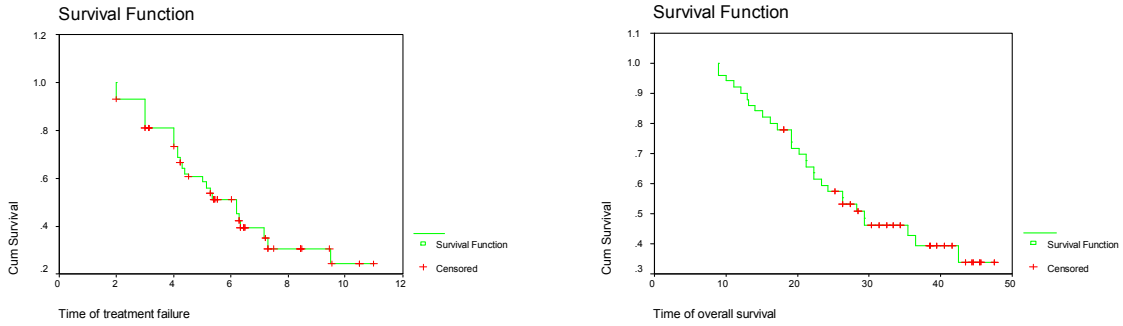
The patients whose tumor phenotype turned into TN had the worst TTF (median 3.0 versus 6.3 months, P < 0.001) and OS (median 10.07 versus 36.47

months, $P < 0.001$), when compared with the concordant group. Figure (4 A&B)

Change in Therapy

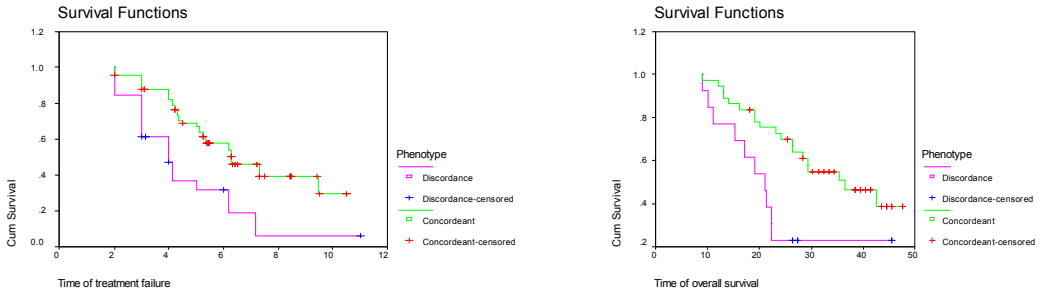
Eighteen women (18%) had a change in treatment as compared with the prebiopsy therapeutic plan ($P = 0.003$). Trastuzumab was subtracted from treatment regimen in 4 patients with Her 2 loss and in contrary it was added to the treatment regimen in those 6 patients with Her2 gain. Six patients shifted from hormonal treatment to chemotherapy as their receptor became triple negative. Two patients with single visceral metastasis from those previously TN received aromatase is inhibitor after shifting to ER +.

There were no significant associations between histological type (ductal vs. lobular)($P = <0.471$), tumor staging (1,2 vs. 3,4)($P = <0.0871$), grading (G1–G2 vs. G3)($P = <0.0912$), menopausal status (premenopausal vs. postmenopausal)($P = <0.176$), Ki-67(< 14% vs. ≥ 14%)($P = <0.076$),the time interval between evaluation of primary breast cancer and metastasis($P = <0.57$), type of previous treatments ($P = <0.0738$)and tumor phenotype discordance. Also there was no statistically significant difference between LR & DM ($P = <0.0671$) and tumor phenotype discordance.



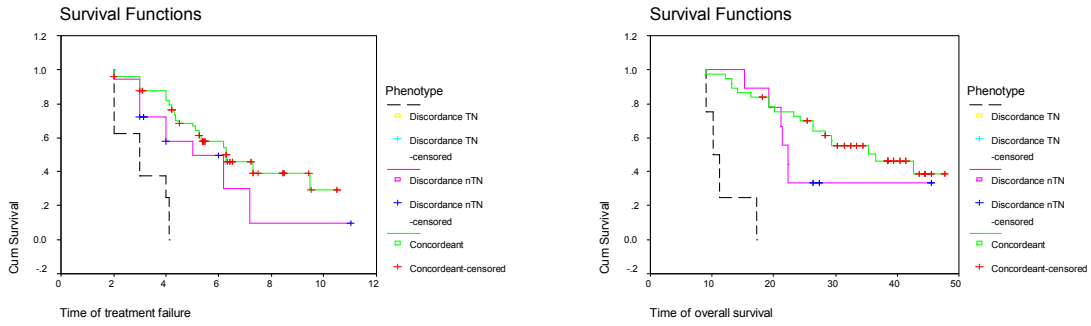
(A) (B)

Fig 2. Survival for all patients :-(A) Time to treatment failure. (B) Overall survival



(A) (B)

Fig3.Survival by discordance.(A) Time to treatment failure. (B) Overall survival.



(A) (B)

Figure (4) Survival curves for the concordant group, the discordant group with non-triple-negative phenotype at recurrence (nTN) and the discordant group with triple-negative phenotype at recurrence (TN): (A) Time to treatment failure; (B) Overall survival.

4. Discussion

Historically, original ER, PR, and HER2 status from the primary cancer have been used to direct subsequent therapy, assuming no change in the biological features of the recurrent disease compared with the original primary; this approach is no longer considered tenable [20-22], and the use of metastatic biopsy become contentious if possible [23,24]. Current opinion supports routine reassessment of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor type 2 (HER2) receptor in tumor tissue which is considered a pragmatic solution at the time of diagnosis of relapse to guide and tailor further appropriate therapy for each patient [21,22,25]. NCCN guidelines (2014) recommend that first recurrence breast cancer disease should be biopsied.

This is based largely on retrospective evidence that discordance of HR status in recurrent breast cancer [26] is an established predictor for poor response to therapy [27]. Studies of paired samples of the primary tumor and locally/regionally recurrent or distant metastases suggest that tumor receptor status may be discordant in a significant proportion of patients: 18% to 54% for ER, 36% to 54% for PR, and 3% to 22% for HER2 in both retrospective series [27-32] and small prospective series [25,33,34]. These results are comparable with our results in this current study.

With the debate of receptor discordance, two alternative explanations raised as the main cause by many authors [5,35-37]: first technical issues, such as poor reproducibility of the immunohistochemical technique and it is likely that this technical issue alone does not explain thoroughly the variation on of ER, PR and HER2 status between primary tumors and relapses observed and this is denied by:- **A)** the rate of discordance is not the same for the three receptor with the PR discordance being the highest incidence followed by ER, while HER2 recorded the least incidence and also the rate of discordance loss and gain is not equal as in our study, we found that the prevalence of negative conversion outnumbered that of positive conversion (21.05% versus 16.7 %, 37.5 % versus 22.2 %, 22.2% versus7.3%, for ER,PR and HER2, respectively), **B)** All the specimens from both primaries and recurrences were evaluated by same pathologist and at one lab and by adopting the same assays, handling procedures and with the same staining techniques to reduce pre-analytical and analytical variability. Second due to tumor heterogeneity either from the start due to the presence of small sub-clones routinely undetected within the primary lesion, or true biological drift and switch from therapy or due to progression of tumor cells to a more aggressive phenotype and this progression is arising forward and become more popular for many investigator nowadays [2, 37].

In our study, seventy four (74%) patients maintained the same tumor phenotype [i.e. the same hormone receptors (HR) and HER2 status], whereas 26% changed during progression and this is matched to the 77% and 23% that reported by Dieci *et al.* Respectively [5].We also evaluated the rates of changes in single-receptor expression, with results of 20 %, 32% and 10% for ER, PR and HER2, respectively and this is in agreement with that reported in the large meta-analysis published by Aurilio, *et al.* [37] which indicated that the rates of discordance for ER, PR and HER2 were 20%, 33% and 8%, respectively. The frequencies of ER, PR and HER2 loss or gain that we observed in our study were also consistent with that reported figures by Aurilio, *et al.* [37] and others [38-41].

We confirmed findings from other retrospective and prospective series by identifying PR as the most discordant receptor, with PR loss as the main change [38-41]. Among HER2-discordant cases, more patients gained HER2expression than those who became HER2 negative and this is comparable with the results of a recent meta-analysis [42]. No statistical significant association between ER gain, PR gain, HR gain or HER2 gain and prognosis was observed when compared with the respective concordant negative cases and this is equivocal with that reported by Dieci *et al.* [5].

Tumor phenotype discordance was associated with worse time to treatment failure (TTF,P <0.001) and overall survival(OS,P = 0.003). Within the discordant group, a loss of a receptor expression rather than gain resulted as the main determinant of poor prognosis; this is in line with that reported in previous large retrospective reports [38, 41, 43- 45]. Those cases who turned into triple-negative experienced the poorest TTF and OS outcome, with respect to the concordant group (both P = <0.001), this is also consistent with that reported by Liedtke, et al and Dieci *et al.* [5, 43].

In this study, ER+ loss was the cornerstone in single receptor discordance that significantly associated with the worse TTF and OS after alteration the management of recurrent disease and this is in consistent with many retrospective studies [2, 5, 43]. The trend toward a worse TTF and OS with HER2+ discordance observed in our study may need large prospective studies because not only that the discordance rate in HER2 is low but also the incidence of HER2 + disease is already less than that with ER+ disease.

In our study, there were no significant associations between histological type (ductal vs lobular), tumor staging (1,2 vs 3,4), grading(G1–G2

vs G3), menopausal status (premenopausal vs. postmenopausal), Ki-67(< 14% vs.≥ 14%), the time interval between evaluation of primary breast cancer and metastasis, type of previous treatments and tumor phenotype discordance. Also there was no statistically significant difference between LR &DM and tumor phenotype discordance. These data are in line with that reported by Thompson *et al.* [20] and Bogonia *et al.* [17].

Biopsy results led to a significant change of management in 18% of patients (P = 0.003) in our study, which lies in between the 14% and 20% reported by Amir *et al.* [35] and Simmons *et al.* [36]. This influence and alter the planned treatment in about 1 in 5.5 patients, consistently with that reported in literatures where the treatment plane changed from one in five to one in seven patients [20,35,36].

In our opinion, when a biopsy of the metastatic lesion is easy to be performed and safe; it should be considered as a routine procedure in all patients, particularly with the improvements in interventional radiology and where clinicians should consider carefully the method and site of biopsy to maximize analyzable data yield, since the characterization of relapsing breast cancer can play a major role and is likely to impact treatment choice [5,35].

Our results show that tissue confirmation by biopsy of metastatic sites is technically feasible and should be considered standard of care in patients with clinical and/or radiological suspicion of metastatic recurrence especially when lesions are amenable to biopsy safely. Receptor change was more common with hormone receptors than with HER2. The discordance in the tumor phenotype was associated with both significantly shorter TTF and OS and this alter the management in 18% of our cases. Expression of discordance should be expressed in tumor phenotype because at the end we treat the patient as one unit. Patients who changed their tumor phenotype to TN by losing HR and/or HER2 positivity experienced the shortest TTF and OS when compared with concordant cases, and hence our data together with those of many others investigators should lead to practice change in the management of recurrent and/or metastatic breast cancer lesions.

The aim of personalized or precision medicine is to customize treatments on the basis of the molecular and genomic features of each individual tumor [46,47]. The final decision should be based on patient and tumor-related factors and as a result of a joint decision between them.

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