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Androgen Receptor Expression in Breast Cancer: What Differences Between Primary Tumor and Metastases?

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Abstract

Genomic studies have shown that the androgen receptor (AR) pathway plays an important role in some breast cancer subtypes. However few data are present on the concordance between AR expression in primary tumors and metastases. We investigated AR expression by using immunohistochemistry (IHC) in 164 primary tumors and 83 metastases, to explore its distribution in the different tumor subtypes and its concordance between the two sample types and according to sampling time. AR was more highly expressed in luminal A and B than HER2-positive and triple negative primary tumors. A similar distribution was found in metastases, and the concordance of AR expression between primary tumors and metastases was greater than 60%. No association between sampling time and AR expression was observed. We found a good concordance of AR expression between primary tumor and metastasis, but the variability remains high between the two types of specimens, regardless of the variation in sampling time. For this reason, if used for treatment decisions, AR evaluation should be repeated in each patient whenever a new biopsy is performed, as commonly done for the other breast cancer biomarkers.

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Introduction

Despite the heterogeneity of breast cancer (BC), global analyses of tumors using genetic profiles have identified gene expression signatures that characterize many intrinsic tumor subtypes with different biology and clinical behavior. In particular, the role of hormone status is important to define the prognosis and to predict the response to therapy for BC patients. Currently, hormone receptors are widely used as prognostic and predictive factors to manage decision-making in BC patients. Estrogen receptor (ER) expression is mostly important because it can predict about 50–70% of tumor responses under treatment with anti-estrogens, whereas response rate is less than 10% in ER-negative BCs and perhaps 0% in truly ER-absent cases [1–4]. Levels of ER affect the time-distribution of BC relapses, ER positivity being associated with more delayed recurrences compared to ER absence [5].

Androgens seem also to have importance in female BC patients. BC risk appears higher in postmenopausal women when both estrogen and androgen levels are increased [6–8]. Up to now, how androgen function can favor BC risk is not well known. Some studies showed an effect of androgens on proliferation of breast tissue [9–11]. Androgen receptor (AR) has recently been reported to have both an oncogenic and tumor suppressive role. Some studies reported that AR expression is quite elevated in most ER-positive tumors but less in

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ER-negative tumors [12–16]. These data are still controversial because the same authors described a role of AR status in predicting response rate and overall survival under hormonal therapy, and at the same time they found no association between AR expression and disease-free survival in ER-positive tumors. In the same works ER status maintained the predominant role as independent prognostic factor for disease-free survival [13–15]. For some authors, AR expression was related to a better survival when it was co-expressed with ER, [16] but not for others [17]. The availability of anti-androgen compounds (i.e., bicalutamide, enzalutamide) opened new perspectives for the treatment of advanced BC expressing AR. To select patients suitable for this kind of treatment, it is necessary to assess AR in tumor tissue. Often only primary tumor samples are available, but not metastatic samples. When we assess AR expression in the primary sample, is it important to assess its expression in metastatic tissue as well?

The concordance of AR expression between primary and metastatic samples is not well defined. Moreover the time elapsed between the biopsy of the primary tumor and the biopsy of a metastasis could affect the degree of change in AR expression between the two samples. This difference could make difficult the decision-making process for anti-androgen therapy. The purpose of this study is the analysis of AR expression in formalin-fixed, paraffin-embedded (FFPE) primary tumors and metastases, and the assessment of changes in AR expression levels over time.

Materials and Methods

Patients and Sample Selection

This retrospective study was carried out on a case series of patients enrolled from 2000 to 2008 in clinical or biological studies performed at our Institute in collaboration with the Cancer Prevention Unit and the Breast Surgery Unit of Morgagni-Pierantoni Hospital in Forlì. Patients aged ≥18 years with a histological diagnosis of invasive BC were eligible. All the patients had to be followed up for at least 5 years, unless they had relapsed earlier. The study protocol was reviewed and approved by the IRST and AVR (Area Vasta Romagna) Ethics Committee (approval no.3692) and patients provided written informed consent according to Italian privacy law. The original hematoxylin and eosin stained sections were reviewed by the pathologist in order to select the most representative inclusion of tumor tissue for each patient.

Biomarker Determination

Tumor material obtained during surgery was fixed in neutral buffered formalin and embedded in paraffin. Four-micron sections were mounted on positive-charged slides for each patient (Bio Optica, Milan, Italy). Biomarker determinations were performed according to European Quality Assurance guidelines. Immunostaining for conventional biomarkers and AR expression was performed using the Ventana Benchmark^{XT} staining system (Ventana Medical Systems, Tucson, AZ, USA) with the Optiview DAB Detection Kit (Ventana Medical Systems). ER, PgR, Ki67 (Leica, Novocastra, Newcastle, UK), HER2 (Dako, Carpinteria, CA, USA) and AR (SP107 Cell Marque, Ventana Medical Systems) antibodies were used. For ER, PgR, Ki67 and HER2 detection, tissue sections were incubated for 60 minutes with antibodies diluted 1:80, 1:40, 1:100 and 1:350, respectively, in antibody diluents (Ventana Medical Systems). AR antibody, pre-diluted by the supplier, was used. Sections were incubated for 16 minutes and automatically counterstained with hematoxylin II (Ventana Medical Systems). Biomarker positivity was detected and semiquantitatively quantified as the percentage ratio between immunopositive tumor cells and the total number of tumor cells. All samples were evaluated by 2 independent observers and any disagreement (>10% of positive cells

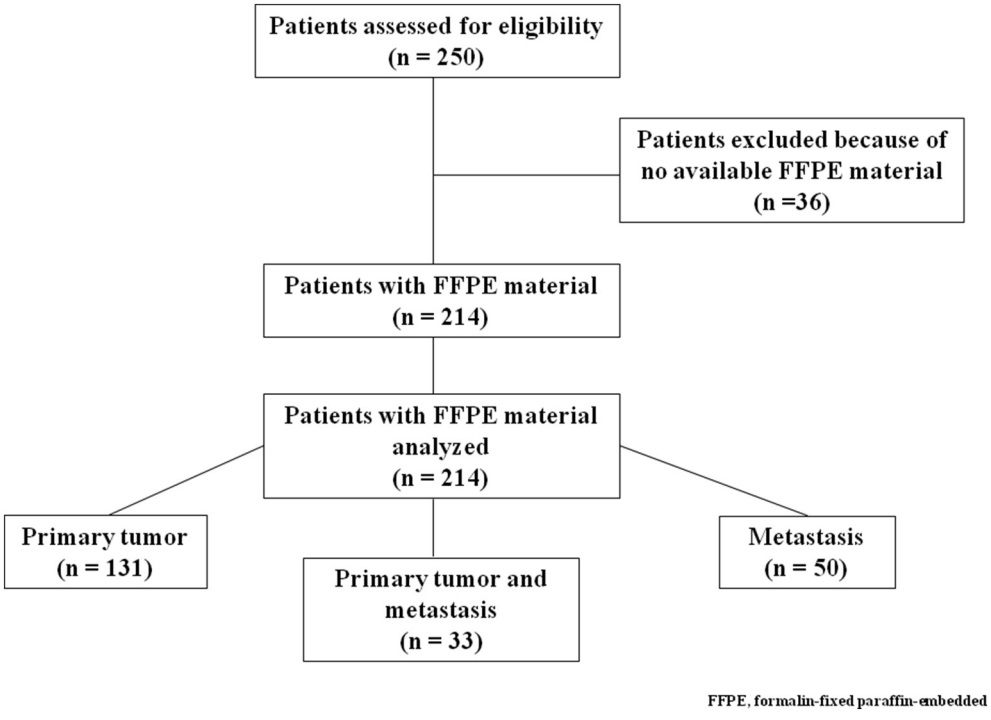

Figure 1. Consort diagram of the study.

Table 1. Patient's Characteristics

	All Patients, as Per Clinical Practice* (n = 214)
	N. (%)
Available specimen	
Primary tumor	164 (76.6)
Metastasis	83 (34.4)
Both primary and metastasis	33 (15.4)
Age (years): median value (range)	58 (26–86)
Adjuvant chemotherapy	
No	64 (36.8)
Yes	110 (63.2)
Unknown	40
Adjuvant endocrine therapy	
No	67 (38.5)
Yes	107 (61.5)
Unknown	40
Histotype	
Ductal	169 (82.4)
Lobular	28 (13.7)
Other	8 (3.9)
Unknown	9
Tumor stage	
1	90 (49.2)
2	70 (38.3)
3	7 (3.8)
4	16 (8.7)
Unknown	31
Nodal involvement	
0	72 (40.0)
1	71 (39.5)
2	20 (11.1)
3	17 (9.4)
Unknown	34
Metastases at diagnosis	
Yes	40 (19.1)
No	169 (80.9)
Unknown	5
1st-line endocrine therapy for advanced BC	
Letrozole	72 (46.5)
Anastrozole	32 (20.6)
Exemestane	39 (25.2)
Tamoxifen	9 (5.8)
Fulvestrant	3 (1.9)
Unknown	59

* Clinical practice: biomarker expression measured in metastases (when a biopsy was performed on metastases) or in primary tumors (when biopsy on metastases had not been performed).

Table 2. Tumor biological Characteristics

	Primary Tumor (n = 164)	Metastases (n = 83)	As Per Clinical Practice* (n = 214)
	N. (%)	N. (%)	N. (%)
Grade			
1	6 (4.6)	0	6 (3.7)
2	49 (38.0)	11 (47.8)	67 (40.8)
3	74 (57.4)	12 (52.2)	91 (55.5)
Unknown	35	60	50
ER status			
<1%	30 (18.7)	8 (9.8)	33 (15.4)
≥1%	130 (81.3)	74 (90.2)	181 (84.6)
Unknown	4	1	0
PgR status			
<1%	49 (30.6)	30 (36.6)	75 (35.0)
≥1%	111 (69.4)	52 (63.4)	139 (65.0)
Unknown	4	1	0
<20%	81 (50.6)	41 (50.0)	113 (52.8)
≥20%	79 (49.4)	41 (50.0)	101 (47.2)
Unknown	4	1	0
Ki67 status			
<20%	75 (47.8)	48 (62.3)	113 (53.6)
≥20%	82 (52.2)	29 (37.7)	98 (46.4)
Unknown	7	6	3
HER2 status			
Negative	100 (63.7)	71 (88.7)	152 (71.4)
Positive	57 (36.3)	9 (11.3)	61 (28.6)
Unknown	7	3	1
AR status			
<1%	28 (17.1)	22 (26.5)	46 (21.5)
≥1%	136 (82.9)	61 (73.5)	168 (78.5)
Unknown	0	0	0
<10%	33 (20.1)	33 (39.8)	62 (29.0)
≥10%	131 (79.9)	50 (60.2)	152 (71.0)

* Clinical practice: biomarker expression measured in metastases (when a biopsy was performed on metastases) or in primary tumors (when biopsy on metastases had not been performed).

for the different markers) was resolved by consensus after joint review using a multihead microscope.

Molecular subtypes were defined by the detection of ER, PgR, Ki67 and HER2. ER-positivity and PgR-positivity were considered as ≥1% tumor cells staining for ER and PgR, respectively; Ki67 was considered high when detected in ≥20% of tumor cells; HER2-positivity was defined as 3+ staining intensity by IHC or as HER2 amplification (HER2/Chromosome 17 centromere ratio ≥2.0, or mean HER2 gene copy number ≥6 per tumor cell). The expression of these biomarkers allowed to classify samples according to the St. Gallen expert consensus and the ASCO-CAP guidelines [18,19]. Luminal A-like (ER-positive, PgR ≥ 20%, low Ki67 (<20%), HER2-negative), luminal B-like (ER-positive, PgR <20%, high Ki67 (≥20%), HER2-positive or HER2-negative), HER2-positive non-luminal (ER-negative, PgR-negative, HER2-positive), and triple-negative (ER-negative, PgR-negative, HER2-negative).

As regards AR expression, we chose two different cut off values ≥1% and ≥10% of immunopositive tumor cells to assess AR positivity. Staining intensity (i.e., 0, 1+, 2+, 3+) was also analyzed in order to calculate the H-score, defined as the product of the percentage of AR-positive tumor cells and staining intensity.

Statistical Analyses

All the data were summarized using descriptive statistics. Concordance of AR expression was defined as either positive or negative in both tumor and metastasis, while discordance was defined as positivity at one site and negativity at the other or *vice versa*. The concordance rate was calculated as the proportion of concordant cases with respect to the total number of patients. The two-sided exact binomial 95% confidence interval (95% CI) was estimated. McNemar's test was performed in order to compare AR status between the primary tumor and paired metastatic sites.

Univariable linear regression was used to assess and graphically display the relationship between the time elapsed from the removal of the primary tumor to sampling of the metastasis and the difference of AR expression between the two samples.

All statistical analyses were carried out using SAS software, version 9.4 (SAS Institute, Cary, NC, USA).

Results

Two hundred fourteen patients meeting eligibility criteria were included in the study (Figure 1). Primary breast cancer archival tissue was available for 164 patients, 154 of whom had complete tumor characterization (expression of ER, PgR, Ki67, and HER2 status) and established BC subtype. Eighty-three patients had tissue samples from a metastasis, and 79 of them had the molecular subtype determined. For 33 patients both primary and metastatic tumor

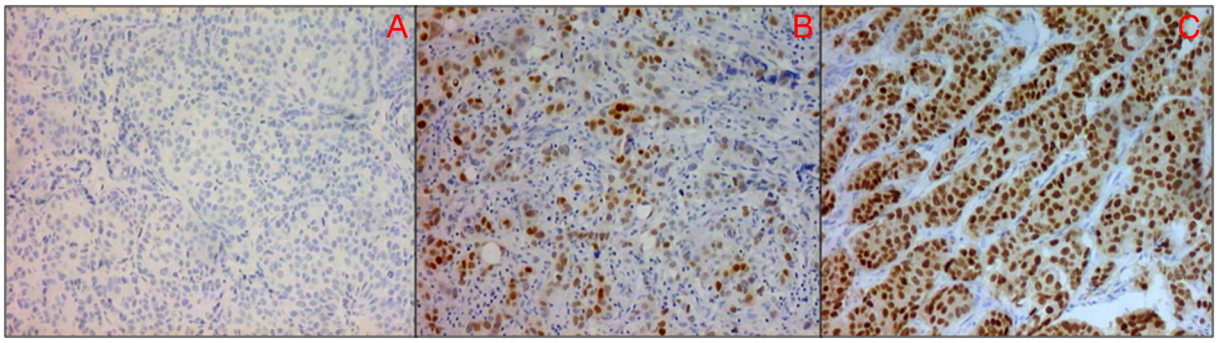

Figure 2. Ductal infiltrating carcinomas of the breast showing negativity for AR expression (A), a moderate and heterogeneous (2+) AR nuclear positivity (B), and a strong and homogeneous (3+) AR nuclear positivity (C). All 10× magnification.

tissues were available. All patients' characteristics are reported in Table 1. Forty (19.1%) had metastatic disease at diagnosis. The median age was 58 years (range: 26–86). Tumor biological characteristics are reported in Table 2.

Among the 164 primary tumor samples, 136 (82.9%) were AR positive (Figure 2) according to a cut off value of ≥1%, and 131 (79.9%) with the cut-off value of ≥10% (Table 2). Similar data were obtained for metastases: on a total of 83 metastases analyzed, 61 (73.5%) were AR positive according to a cut off value ≥1%, and 50 (60.2%) by using ≥10% cut off value (Table 2). AR H-score median value was 240 (range 0–300) in primary tumors and 210 in metastases (range 0–300). AR expression was higher in luminal A and luminal B tumors than in HER2-positive and triple negative tumors, both on primary tumors (Table 3A) and metastases (Table 3B).

The characteristics of patients for whom samples for both primary tumor and metastasis were evaluable are reported in Table 4. Tumor biological characteristics for the same group are reported in Table 5. The concordance between AR-positivity in primary tumor and metastasis was 66.7% (95% CI 50.6–82.8; P = .035) by using 1% as cut off value, and it dropped to 60.6% (95% CI 43.9–77.3; P = .002) by using the cut off value of 10% (Table 6).

We used univariable linear regression to study the association between the time elapsed from the removal of the primary tumor to the metastasis biopsy (months; x-axis) and the changes in AR expression

Table 3A. Distribution of AR Expression in the Different Primary Tumor Subtypes

	Primary Tumor Subtypes (N. = 154)				
	LA*	LB#	LB-HER2+**	TN^	HER2+ (HR-)@
	N. (%)	N. (%)	N. (%)	N. (%)	N. (%)
Primary tumor					
AR negative (<1%)	3 (9.4)	8 (14.5)	7 (17.9)	5 (50.0)	5 (27.8)
AR positive (≥1%)	29 (90.6)	47 (85.5)	32 (82.1)	5 (50.0)	13 (72.2)
AR negative (<10%)	4 (12.5)	8 (14.5)	9 (23.1)	6 (60.0)	7 (38.9)
AR positive (≥10%)	28 (87.5)	47 (85.5)	30 (76.9)	4 (40.0)	11 (61.1)

* LA, luminal A-like: ER+, PgR ≥20%, Ki67< 20%, HER2–;
\# LB, luminal B-like: ER+, PgR <20% or Ki67 ≥ 20%, HER2–;
** LB-HER2+, luminal B-like HER2-positive: ER+, PgR <20% or Ki67 ≥ 20%, HER2+;
^ TN, triple-negative: ER–, PgR–, HER2–;
@ HER2+ (HR–), HER2-positive, hormone receptor-negative: ER–, PgR–, HER2+.

Table 3B. Distribution of AR Expression in the Different tumor Subtypes on Metastases

	Tumor Subtype on Metastases (N. = 79)				
	LA*	LB#	LB-HER2+**	TN^	HER2+ (HR-)@
	N. (%)	N. (%)	N. (%)	N. (%)	N. (%)
Metastasis					
AR negative (<1%)	2 (12.5)	12 (25.5)	2 (25.0)	4 (57.1)	1 (100)
AR positive (≥1%)	14 (87.5)	35 (74.5)	6 (75.0)	3 (42.9)	0
AR negative (<10%)	4 (25.0)	17 (36.2)	4 (50.0)	6 (85.7)	1 (100)
AR positive (≥10%)	12 (75.0)	30 (63.8)	4 (50.0)	1 (14.3)	0

* LA, luminal A-like: ER+, PgR ≥20%, Ki67< 20%, HER2–;
\# LB, luminal B-like: ER+, PgR <20% or Ki67 ≥ 20%, HER2–;
** LB-HER2+, luminal B-like HER2-positive: ER+, PgR <20% or Ki67 ≥ 20%, HER2+;
^ TN, triple-negative: ER–, PgR–, HER2–;
@ HER2+ (HR–), HER2-positive, hormone receptor-negative: ER–, PgR–, HER2 + .

Table 4. Characteristics of Patients for Whom Samples of Both Primary Tumor and Metastasis were Available

	No. (%)
Median age, years (range)	55 (33–76)
Adjuvant chemotherapy	
No	10 (33.3)
Yes	20 (66.7)
Unknown	3
Adjuvant endocrine therapy	
No	8 (26.7)
Yes	22 (73.3)
Unknown	3
Histotype	
Ductal	23 (71.9)
Lobular	6 (18.7)
Other	3 (9.4)
Unknown	1
Tumor stage	
1	14 (48.3)
2	14 (48.3)
3	0
4	1 (3.4)
Unknown	4
Nodal involvement	
0	13 (44.8)
1	11 (37.9)
2	5 (17.3)
3	0
Unknown	4
Metastases at diagnosis	
Yes	3 (9.4)
No	29 (90.6)
Unknown	1
First-line endocrine therapy for advanced breast cancer	
Letrozole	8 (27.6)
Anastrozole	10 (34.5)
Exemestane	8 (27.6)
Tamoxifen	3 (10.3)
Fulvestrant	0
Unknown	4

Table 5. Tumor Biological Characteristics of Patients for Whom Samples of Both Primary Tumor and Metastasis were Available

	Primary Tumor (n = 33)	Metastasis (n = 33)
	No. (%)	No. (%)
Grade		
1	1 (4.5)	0
2	8 (36.4)	5 (50.0)
3	13 (59.1)	5 (50.0)
Unknown	11	23
ER status		
<1%	5 (17.2)	4 (12.5)
≥1%	24 (82.8)	28 (87.5)
Unknown	4	1
PgR status		
<1%	7 (24.1)	18 (56.2)
≥1%	22 (75.9)	14 (43.8)
Unknown	4	1
<20%	13 (44.8)	18 (58.1)
≥20%	16 (55.2)	13 (41.9)
Unknown	4	2
Ki67 status		
<20%	12 (41.4)	18 (58.1)
≥20%	17 (58.6)	13 (41.9)
Unknown	4	2
HER2 status		
Negative	23 (85.2)	28 (90.3)
Positive	4 (14.8)	3 (9.7)
Unknown	6	2
AR status		
<1%	4 (12.1)	11 (33.3)
≥1%	29 (87.9)	22 (66.7)
Unknown	0	0
<10	4 (12.1)	15 (45.5)
≥10	29 (87.9)	18 (54.5)

ER, estrogen receptor; PgR, progesterone receptor; HER2, human epidermal growth factor receptor 2; AR, androgen receptor.

between the two samples (absolute variation in the percentage of AR-positive cells between the two samples, y-axis). No association between time and AR expression was observed (R-squared = 0.04 and adjusted R-squared = 0.0091, P = .264) (Figure 3).

Discussion

Collins and colleagues reported that AR is most commonly expressed in luminal A and B invasive BC and it is present in approximately one-third of basal-like cancers [20].

Our results are in agreement with these findings because in our case series AR is more frequently expressed in luminal than the other subtypes, both in primary tumors and metastases. Nonetheless, the low number of HER2-positive and triple negative BC in our study precludes firm conclusions about the distribution of AR expression in different molecular subtypes. Despite the increasing use of gene expression profiles, such as Oncotype Dx or PAM50, we classified tumors according to a conventional immunohistochemistry marker panel (hormone receptors, HER2 and Ki67 expression). We opted for the latter because it has been seen that molecular assays do not furnish superior prognostic information to that of tumor morphology and immunohistochemistry [21,22].

Only a few articles have been published on the comparison of AR expression evaluated on primary tumor and metastasis [23,24]. We found a statistically significant concordance between AR expression in primary tumor and metastasis using two cut off values (1% and 10%). Due to the retrospective nature and potential selection bias of our study, we did not evaluate the prognostic or predictive role of AR expression in these two types of specimens.

Some authors observed that hormone receptor status (ER and PgR) may change several times over the course of the disease. These changes could be associated with prognostic worsening. Hence, they suggest repeating the hormone receptor determination in metastatic BC patients [25]. For this reason we assessed the association between the time-interval from primary tumor removal to biopsy of the metastatic site, and the change in AR expression between the two samples. We found that the variation in the sampling time of the two types of specimens does not explain the difference of AR expression between

Table 6. Concordance Between AR Evaluated in Primary Tumor and in Metastasis

	Metastasis			
	Negative	Positive	Total	McNemar Test
	No. (%)	No. (%)	No. (%)	p
Primary tumor				
AR negative (<1%)	2 (50.0)	2 (50.0)	4 (12.1)	
AR positive (≥1%)	9 (31.0)	20 (69.0)	29 (87.9)	
			Concordance: 66.7%	
			(95% CI 50.6–82.8)	0.035
AR negative (<10%)	3 (75.0)	1 (25.0)	4 (12.1)	
AR positive (≥10%)	12 (41.4)	17 (58.6)	29 (87.9)	
			Concordance: 60.6%	
			(95% CI 43.9–77.3)	0.002

CI, confidence interval.

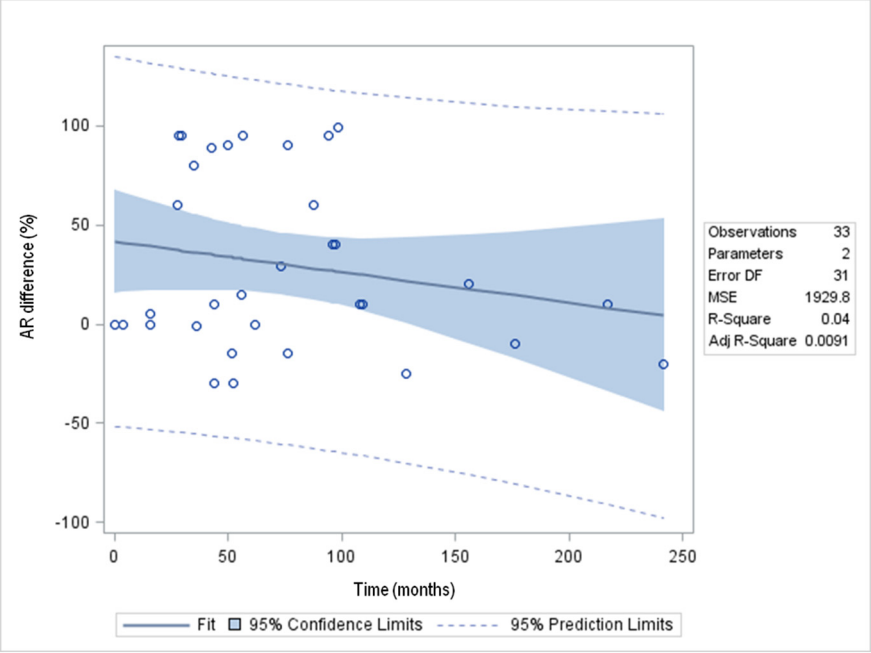

Figure 3. Univariable linear regression to assess the association between the time elapsed from the removal of the primary tumor to the metastatic biopsy (months; x-axis) and the changes in AR expression between the two samples (absolute variation in the percentage of AR-positive cells between the two samples, y-axis).

primary and metastatic lesions, because R-squared value of a linear regression of time to AR change is close to 0. This finding might reflect the high spatiotemporal variability of AR expression, with intratumor spatial heterogeneity exceeding temporal heterogeneity.

Conclusions

Although our results must be interpreted cautiously due to the low number of paired primary tumor and metastasis samples analyzed, they nevertheless suggest that the evaluation of AR by IHC should be performed in all biological material available for each patient, regardless of the time interval between samplings, to plan an anti-AR therapeutic approach.

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