PRECLINICAL STUDY

Three-dimensional pathological size assessment in primary breast carcinoma

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Abstract Maximal tumor diameter (MD) is traditionally an important prognostic factor in breast cancer. It must be questioned, however, how well a one-dimensional parameter alone can represent the actual morphologic condition of a three-dimensional body. Along with the pathologically assessed MD and two perpendicular diameters (PDs) of a lesion, eccentricity (EF) and the three-dimensional parameters tumor volume (TV) and surface area (TSA) of 395 ductal invasive breast carcinomas of limited size (10–40 mm) were calculated. The dependent prognostic variable was axillary lymph node involvement (ALNI). MD, TV and TSA area were highly significant predictors of ALNI; these variables had similar levels of prediction accuracy (univariate analyses: MD: $P = 0.0003$, TV: $P = 0.0009$, TSA: $P < 0.0001$; multivariate analyses: MD: $P = 0.0018$, TV: $P = 0.0109$, TSA: $P = 0.0009$; pseudo R-squared values: MD: 0.42, TV: 0.39, TSA: 0.39). Despite certain variations in tumor shape, TV and TSA with similar MD, there is no evidence that three-dimensional pathologic measurements (TV/TSA) are more precise prognostic predictors of ALNI compared to the one-dimensional measurement alone.

Keywords Breast carcinoma · Prognosis · Tumor size · Tumor volume · Tumor surface area · Three-dimensionality

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Introduction

Tumor size is traditionally one of the most sensitive and powerful indicators of prognosis in breast cancer, second only to the presence of nodal metastasis in importance [1]. Thus, it is a critical morphologic feature in staging. However, the question remains as to how well a one-dimensional assessment of the largest tumor diameter alone can represent the actual morphologic condition of a three-dimensional body. Theoretic models have shown that the propensity for metastases depends on the total number of cells of a tumor combined with the probability of each individual cell to disseminate [2]. This number of cells is probably better estimated by three-dimensional parameters such as tumor volume (TV) or surface area (TSA) (assuming that cells located at the advancing front of the tumor have a significant probability for dissemination). Depending on the shape of the tumor, there might be considerable variability in the TVs and TSAs within lesions of the same diameter. To exemplify this, a lesion

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measuring $30 \times 30 \times 30$ mm has a TV of 14.14 cm^3, a lesion measuring $30 \times 25 \times 20$ mm one of 7.85 cm^3 and a flat tumor measuring $30 \times 22 \times 15$ mm a volume of only 5.18 cm^3; the corresponding TSAs are as follows: 28.27, 19.55, and 15.48 cm^2.

Our study addresses two important issues regarding three-dimensional pathologically measured parameters in breast cancer:

1. To analyze tumor shape and its variability, and to assess factors that influence tumor shape
2. To assess the prognostic impact of three-dimensional size measures: is TV and/or TSA, a more accurate predictor of axillary lymph node involvement than the established one-dimensional measurement (maximal diameter)?

Our study answers the question of whether routine pathological three-dimensional measurement of a tumor would have clinical relevance. At this time, the reporting of these measurements in the current pathology guidelines and protocols remains optional [3–5]. This study is reported according to the "REporting recommendations for tumor MARKer prognostic studies (REMARK)" [6].

Patients and methods

Patient characteristics and data collection (REMARK requirements 2, 4, 6 [6])

The data of 395 female breast cancer patients whose surgically removed primary carcinomas were pathologically examined at the Institute for Pathology of the University of Basel, Switzerland, between 1998 and 2005 form the basis of the current analysis. We restricted our study to the analysis of histologically proven unifocal invasive ductal carcinoma of limited size, in which the largest pathologically assessed tumor diameters measured between 11 and 40 mm (T1c, T2 ≤ 4 cm). Three-dimensional measurements of each lesion, i.e. three PDs, were available for all tumors: the maximal diameter (MD) and two PD1, PD2; the data were organized as follows: MD ≥ PD1 ≥ PD2. Pathologic lymph node status was known for all patients. We excluded patients who had multifocal/multicentric breast tumors or had tumors with clinical or pathological evidence of any kind of skin or chest wall involvement (T4 category). Patients who had preoperative neoadjuvant therapy were also excluded. Invasive lobular carcinomas and rare histologic subtypes with more favorable prognosis (e.g., tubular carcinoma, papillary carcinoma, medullary carcinoma) were not considered. In all patients included in the analysis, hormonal receptor assay, assessment of the HER-2/neu status and histolopathologic grading according

to the Nottingham modification of the Scarff-Bloom-Richardson grading scheme had been performed. Furthermore, clinical data concerning age and tumor location (stratified as inner quadrant, outer quadrant and central region) were available. The study was carried out in accordance with the guidelines of the Ethics Committee of the University of Basel.

Data analysis (REMARK requirements 7–9 [6])

1. Tumor shape (eccentricity). To assess the "roundness" of a tumor, the "Eccentricity Factor" (EF), as previously described by Schwartz et al. [7], was employed as a measure of tumor shape. The EF describes the overall change in tumor shape based on the following formula (where MD is the maximal diameter and PD2 is the smaller of the two measured PDs:

$$EF = \sqrt{1 - (PD2/MD)^2}$$

According to this formula, the EF values fall between zero and one: the higher the EF, the flatter the shape of the tumor. For example, a lesion measuring $30 \times 30 \times 30$ mm would have an EF of 0, whereas a lesion measuring $30 \times 25 \times 20$ mm would have an EF of 0.75 (Fig. 1); the highest EF value in our study population was 0.98 with corresponding tumor measurements of $29 \times 14 \times 6$ mm.
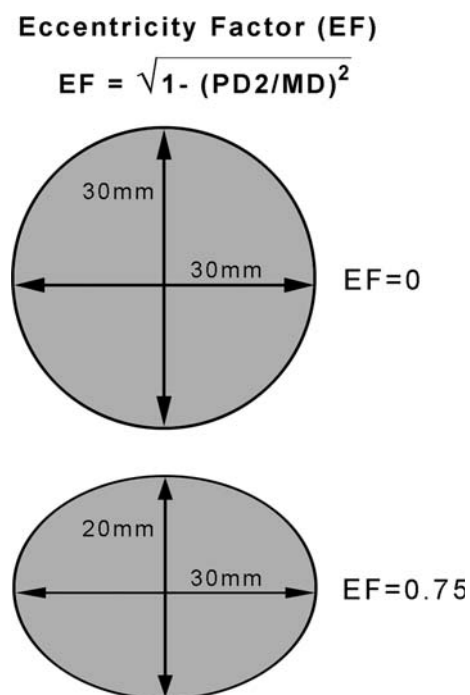

Fig. 1 Mathematical formula for the eccentricity factor and diagram illustrating two different eccentricity values. PD2: the smaller of both PDs; MD: maximal diameter

2. Tumor volume (TV). For volume computation, the shape of the tumor was approximated to an ellipsoid. The volume was computed using the following equation (where MD is the maximal diameter and PD1/PD2 are diameters that are perpendicular to the MD and to each other in the three-dimensional space of the tumor body):

$$V = 1/6 \cdot \pi \cdot MD \cdot PD1 \cdot PD2$$

3. Tumor surface area. The surface area was approximated by the following formula (where $p = 1.6075$):

$$S = 4\pi \left(\frac{\left(\frac{MD}{2}\right)^P \left(\frac{PD1}{2}\right)^P + \left(\frac{MD}{2}\right)^P \left(\frac{PD2}{2}\right)^P + \left(\frac{PD1}{2}\right)^P \left(\frac{PD2}{2}\right)^P}{3} \right)^{1/p}$$

4. Dependent variable. The dependent variable of our study was ALNI, the single most powerful prognostic factor for primary breast cancer. In our analyses, ALNI refers not to the nodal status (expressed as either the presence of negative or positive nodes), but rather the number of lymph nodes involved.

For every tumor analyzed in this study, we determined six specific morphologic values: largest diameter (MD), two PDs (PD1, PD2), TV, TSA and the EF.

Statistical analysis (REMARK requirements 10, 11 [6])

Ordinal linear regression was used to model the relation between tumor shape (EF) and selected morphologic features; to achieve approximately normal distribution for MD/PD2, this variable was logarithmically transformed, and regression parameters were expressed as geometric mean ratios. To evaluate the influence of prognostic factors on ALNI, particularly MD, TV, TSA and EF, we used univariate and multivariate Poisson regression method [8]. This model was performed allowing for overdispersion. TV and TSA values were logarithmically transformed. Regression parameters are reported as risk ratios with 95% confidence intervals. In all tests, a P-value <0.05 was considered significant. To decide which of the three multivariate models including one of the highly confounded variables MD, TV and TSA has the best impact on ALNI, pseudo R-squared values according to Cox and Snell were calculated [9]. Statistical analyses were performed with R Development Core Team software, version 2.5.0 (Vienna, Austria).

Results (REMARK requirements 12–17 [6])

Clinicopathologic and morphologic characteristics of the 395 patients in the study are summarized in Table 1.

Eccentricity, an expression of tumor shape in invasive ductal breast carcinoma, is depicted graphically by a box plot (Fig. 2). The most frequent tumor shape was scalene ellipsoid, i.e. an ellipsoid in which the lengths of all three semi-principal axes are different ($n = 286$, 72.4%). 44 patients (11.1%) had tumors in which the largest diameter was repeated twice and the third dimension was a smaller number (oblate spheroid, e.g. $20 \times 20 \times 15$ mm). 56 patients (14.2%) had dimensions in which there was one larger diameter and the smaller diameter was repeated twice (prolate spheroid, e.g. $20 \times 15 \times 15$ mm). Only nine carcinomas (2.3%) had a spherical shape (all three diameters were identical; EF = 0). 50% of all lesions ranged between an EF of 0.61 and 0.83. The median eccentricity value was 0.75. This value means that the ratio between the MD and the smallest perpendicular diameter (PD2) is 1:0.67, or that PD2 is approximately 30% less than the MD. Table 2 shows that established prognostic factors (histologic grade, estrogen receptor and HER-2/neu status) had no effect on tumor shape. Only the MD had a significant influence on the shape of the tumor: the larger the tumor, the flatter the shape ($P < 0.001$).

Using univariate analysis, maximal tumor diameter (MD), TV and TSA were highly significant predictors of ALNI; these variables had similar levels of prediction accuracy (MD: $P = 0.0003$; TV: $P = 0.0009$; TSA: $P < 0.0001$; Table 3). Additionally, both perpendicular tumor diameters had significant association with ALNI (PD1: $P = 0.0086$; PD2: $P = 0.0106$).

Multivariate analyses showed that MD, TV and TSA remained significant independent predictors of ALNI (MD: $P = 0.0018$; TV: $P = 0.0109$; TSA: $P = 0.0009$; Table 4). Since these variables are highly confounded by the common value MD, they could not be calculated in a combined model [8]. Therefore, separate calculations were made for each variable. Table 4 shows the seven-variable model which includes MD in the calculation (in addition to MD/TV/TSA we chose to take six co-variables into consideration in the model: age, EF, tumor location, grading, estrogen receptor and HER-2/neu status). At the bottom of this table, the TV and TSA values are indicated. The values of the six co-variables in the calculations including TV and TSA are quite similar to the calculation including MD, therefore they were omitted from the table. The pseudo R-squared values evaluating the three confounded variables showed the following ranking: MD: 0.42; TV: 0.39; TSA: 0.39.

Discussion (REMARK requirements 19, 20 [6])

In the upcoming era of molecular and genetic characterization of breast cancer, the significance of traditional

Table 1 Clinicopathologic and morphologic characteristics of the study group

Variable	Entire study group	Tumor size: 11–20 mm	Tumor size: 21–30 mm	Tumor size: 31–40 mm
Total number of cases (%)	395 (100)	167 (41.8)	177 (45.6)	51 (12.6)
Age (yrs)				
Mean	61.7	59.8	63.1	63.2
Range	31–88	31–85	31–88	37–86
Tumor diameter[a], mean (mm)				
MD: maximal diameter	22.7	16.3	25.0	36.0
PD1: perpendicular diameter 1	18.8	13.8	20.8	27.9
PD2: perpendicular diameter 2	14.9	11.4	16.5	20.7
Tumor volume (cm^3)				
Mean	4.2	1.5	4.8	11.4
Range	0.14–25.66	0.14–4.19	0.96–14.14	3.14–25.66
Tumor surface area (cm^2)				
Mean	12.08	6.20	13.82	25.30
Range	1.46–42.20	1.46–12.57	6.02–28.27	12.98–42.20
Eccentricity factor				
Median	0.75	0.70	0.75	0.82
Range	0–0.98	0–0.97	0–0.98	0.25–0.97
Tumor location (%)				
Outer quadrants/central region	295 (74.7)	120 (71.9)	135 (76.3)	40 (78.4)
Inner quadrants	100 (25.3)	47 (28.1)	42 (23.7)	11 (21.6)
Grading (%)				
G1	52 (13.2)	35 (21.0)	17 (9.6)	–
G2	210 (53.2)	88 (52.7)	94 (53.1)	28 (54.9)
G3	133 (33.6)	44 (26.3)	66 (37.3)	23 (45.1)
Estrogen receptor positive (%)	295 (74.7)	130 (77.8)	130 (73.4)	40 (78.4)
HER-2 neu positive (%)	75 (19.0)	24 (14.4)	38 (21.5)	13 (25.5)
Axillary lymph node involvement (%)				
Negative	191 (48.4)	93 (55.7)	83 (46.9)	15 (29.4)
1–3 lymph nodes	148 (37.4)	59 (35.3)	68 (38.4)	21 (41.2)
4–10 lymph nodes	38 (9.6)	9 (5.4)	19 (10.7)	10 (19.6)
>10 lymph nodes	18 (4.6)	6 (3.6)	7 (4.0)	5 (9.8)

[a] The data were organized as follows: MD ≥ PD1 ≥ PD2

anatomic-morphologic prognostic determinants may very well take a back seat to these new indicators. Efforts should, however, be made to enhance the accuracy of these parameters. As before, the size of the tumor remains an outstanding prognostic factor. Characterization of a three-dimensional body by a one-dimensional measurement only seems to be a rather inadequate method.

Studies concerning the three-dimensionality of breast lesions are mainly a domain of radiologists, where the tumors assessed are still in vivo [10, 11]. Only few studies have assessed the three-dimensional extent of surgically removed carcinomas themselves through pathological examination of the tumor specimen [12–14]; none of them, however, have addressed the prognostic significance of these measurements.

In our study, we analyzed the shape of pathologically examined breast carcinomas and described its variabilities. Furthermore, we assessed the prognostic impact of the three-dimensional size measurement of a lesion, namely TV and TSA and compared its predictive capacity with the established one-dimensional measurement, the MD. For improved uniformity and comparability, we analyzed only invasive ductal carcinoma, which comprises up to 80–90% of all invasive breast carcinomas, thereby representing the single largest group of invasive breast carcinomas. These carcinomas typically form well-defined solid tumors and three-dimensional measurement of their size is relatively precise. Lobular carcinomas were excluded from analysis because in many cases, there is no grossly well-demarked mass, and therefore three dimensional assessment of size is
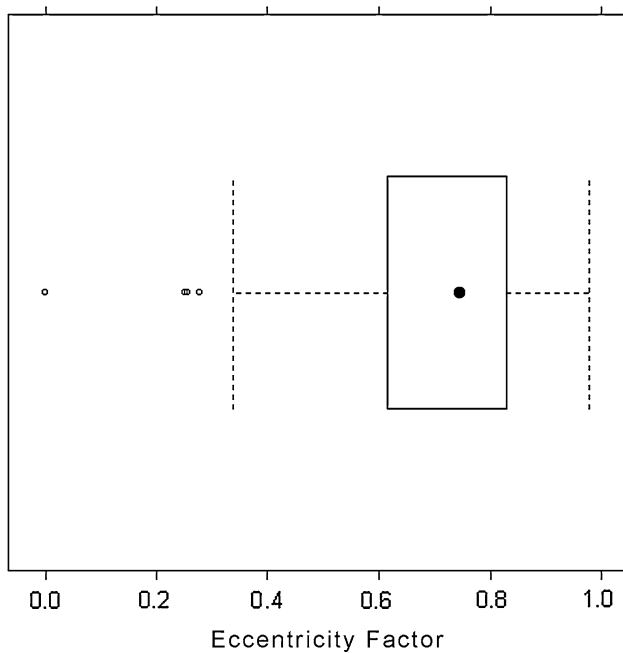
Fig. 2 Box plot for eccentricity of invasive ductal breast carcinoma. The box plot shows the median value (closed circle) and the 25% and 75% percentiles. The dashed lines label the 10% and 90% percentile, while the open circles represent outlying values

imprecise. More uncommon histologic subtypes were also excluded because they usually have a more favorable outcome (e.g., tubular, mucinous, medullary, papillary

Table 2 Associations between clinicopathologic/morphologic characteristics and tumor shape (eccentricity), univariate analysis

Variable	GMR (95% CI)	P-value
Maximal tumor diameter	1.05 (1.03–1.06)	<0.001
Poor histologic grading (G3)	0.98 (0.91–1.06)	0.640
ER-negative status	1.02 (0.96–1.08)	0.617
c-erbB2 positive status	1.01 (0.95–1.08)	0.703

GMR: Geometric Mean Ratio; CI: Confidence Interval

carcinomas) compared to invasive ductal and lobular carcinomas and the number of cases was so small that they cannot even be reasonably analyzed as independent subgroups.

Our data show that most breast carcinomas share a similar scalene ellipsoid shape and that the variability of eccentricity is relatively low. Despite certain variations in tumor shape and at times considerable variabilities in TV and TSA with similar MD (or within subgroups of diameters with a range of 10 mm, see Table 1), there is no evidence that the three-dimensional pathologic measurements (TV/TSA) are more precise prognostic predictors of ALNI compared to MD alone. Of note, even the usually smaller PDs still have prognostic significance. This implies that imprecision of pathologic measurement of the largest dimension of the tumor might not be so critical.

Even in subgroups with tumors at extremes of shape (namely spherical or excessively flattened), three-dimensional measurement is not a more powerful predictor than MD. We expected this to be the case with flattened tumors, which have correspondingly lower TV and TSA. A possible explanation could have been that these tumors had more often characteristics that are associated with tumor aggressiveness (e.g., poor grading, hormonal receptor negative and HER-2 neu positive status). However, this was not the case; only MD had a significant influence on the shape of the tumor. The fact that tumors become flatter with increasing size probably has to do with their blood supply. Wapnir et al. demonstrated an inverse relationship between microvessel counts and TV [13].

The finding that three-dimensional measurements are not more powerful predictors of prognosis than tumor size alone might rather support the notion that only a small subpopulation of tumor cells in a whole tumor population has metastatic potential, and that this might not be dependent on total TV or total amount of tumor cells, but possibly on the individual quantity of metastases initiating

Table 3 Associations between clinicopathologic/morphologic characteristics and axillary lymph node involvement: univariate analysis

OR: Odds Ratio; CI: Confidence Interval

[a] The data were organized as follows: MD ≥ PD1 ≥ PD2

Variable	OR (95% CI)	P-value
Age	1.02 (0.88–1.19)	0.788
Tumor diameter[a]		
MD: maximal diameter	1.28 (1.12–1.45)	0.0003
PD1: perpendicular diameter 1	1.22 (1.05–1.41)	0.0086
PD2: perpendicular diameter 2	1.26 (1.05–1.49)	0.0106
Tumor volume	1.46 (1.17–1.82)	0.0009
Tumor surface area	1.79 (1.28–2.50)	<0.0001
Eccentricity factor	0.94 (0.33–3.02)	0.920
Tumor localization: outer quadrants/ central region	1.43 (0.87–2.47)	0.177
Poor histologic grading (G3)	2.73 (1.26–7.47)	0.0249
ER-negative status	1.59 (1.05–2.37)	0.0240
HER-2 neu positive status	2.04 (1.34–3.04)	<0.0001

Table 4 Multivariate Poisson regression analyses for factors that influence axillary lymph node involvement: the model was calculated separately for each of the variables maximal tumor diameter, tumor volume and surface area

Variable	OR (95% CI)	*P*-value
Maximal tumor diameter	1.23 (1.08–1.40)	0.0018
Age	1.02 (0.88–1.17)	0.831
Eccentricity factor	0.59 (0.22–1.72)	0.311
Tumor localization: outer quadrants/central region	1.39 (0.89–2.28)	0.165
Poor histologic grading (G3)	1.80 (0.84–4.68)	0.174
ER-negative status	1.28 (0.85–1.90)	0.234
c-erbB2 positive status	1.66 (1.09–2.48)	0.0164
Tumor volume	1.33 (1.07–1.66)	0.0109
Tumor surface area	1.55 (1.12–2.16)	0.0009

OR: Odds ratio; CI: Confidence interval

tumor stem cells or the gain of metastatic possibility of these cells over time [15]. Furthermore, there is a mathematical explanation for the statistical lack of higher impact of three-dimensionality: the multiplication of three metric variables results in more variability of the predictor, and this leads to higher variability of the data and to blurred statistical trends.

According to the current pathology guidelines and protocols, a three-dimensional measurement of breast carcinomas remains optional [3–5], with the comment in the College of American Pathologists protocol that this has not yet been validated [4]. Our data do not support the assumption that three-dimensional measurement has an improved prognostic capability in comparison with traditional one-dimensional measurement. Nevertheless, three-dimensional pathologic measurement of a tumor provides useful information. It allows a more exact morphologic description of the lesion compared to the assessment of MD alone. Furthermore, a routinely performed three-dimensional measurement helps the pathologist in identifying the plane of the longest tumor axis and to assess the truly largest dimension.

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