# Relative Uptake Factor of Invasive Ductal Breast Cancer in Breast-specific Gamma Imaging as a Surrogate Parameter for Sub-typing

THOMAS MEISSNITZER[1], MATTHIAS WOLFGANG MEISSNITZER[1],
ALEXANDER SEYMER[2], KLAUS HERGAN[1] and DANIEL NEUREITER[3]

Departments of [1]Radiology and [3]Pathology, Paracelsus Medical University Salzburg, Salzburg, Austria;
[2]Department of Sociology and Cultural Science, University of Salzburg, Salzburg, Austria

**Abstract.** *Aim: The retrospective evaluation of correlations between semi-quantitative breast-specific gamma imaging (BSGI) and invasive ductal breast cancer (IDC) sub-types. Materials and Methods: The biopsy specimen of 50 histologically-proven IDCs were retrospectively evaluated concerning receptor status, human epidermal growth factor receptor 2 (HER2/Neu) status, Ki-67 level and classification into IDC sub-types. These results were compared to the relative uptake factor (RUF) in BSGI. Depending on RUF, we described four categories to outline the predictive value of RUF. Results: A total of 50 IDCs with a mean diameter of 20.36 mm were included. RUF differed reliably between luminal-A and non-luminal-A IDCs. RUF exceeding 6.5 pointed to non luminal A-carcinoma, with a specificity and positive predictive value of 100%. RUF of less than 2.6 was unlikely to be associated with a non-luminal A-carcinoma (negative predictive value: 76.5%). Comparable results were calculated for the correlation between RUF and the K-67 level. Conclusion: RUF may help classify the sub-type of an IDC in addition to pathology.*

The aim of our study was to evaluate the performance of the relative uptake factor (RUF) in breast-specific gamma imaging (BSGI) to assess immunohistochemical characteristics of invasive ductal cancer (IDC) cells. IDC is a heterogeneous disease concerning histopathological and immunohistochemical features, the risk of local recurrence, dissemination (with various patterns) and thus prognosis and outcome. This variability is based on different intrinsic sub-types, described for the first time by Perou *et al.* (1).

Current strategies to treat IDC are based on histopathological as well as immunohistochemical examinations of biopsy specimens and the evaluation of the spread of disease using imaging examinations. In fact, histopathological diagnosis and marker profile are determined by the pathologists. However, we believe that semi-quantitative BSGI may help overcome limitations in the assessment of biopsy specimen and to increase reliability of diagnosis.

In contrast to former studies, such as by Park *et al.* (2) or Tadwalker *et al.* (3), we specifically evaluated IDCs which were detected unequivocally in BSGI. We determined quantitatively the tracer accumulation of the various IDC subtypes and the correlations to certain parameters.

## Materials and Methods

This retrospective analysis is based on the data of our previous prospective study highlighting the diagnostic value of BSGI in the workup of breast lesions categorized as Breast Imaging and Data System (BI-RADS) IV or V in morphological imaging modalities (4). Fifty histologically proven IDCs with complete diagnostic work-up were included in this study, which was approved by the Local Ethics Committee [E-no: 1508, 415-EP/73/439-2014]. Written informed consent was obtained from each patient.

*Breast Specific Gamma Imaging.* BSGI particularly depicts perfusion and energy demand of breast cancer cells and so it is called a functional imaging modality, in contrast to morphological imaging modalities, such as mammography, ultrasound and magnetic resonance imaging. By calculating a semi-quantitative uptake factor we were able to evaluate scintigrams not only visually, but also quantitatively.

BSGI was performed at presentation at our Breast Cancer Center to further characterize breast lesions of categories IV and V according to BI-RADS, initially diagnosed with mammography and

*Correspondence to:* Thomas Meissnitzer, MD, Paracelsus Medical University Salzburg, Department of Radiology. Müllner Hauptstrasse 48, 5020 Salzburg, Austria. Tel: +43 662448258987, Fax: +43 66244823964, e-mail: thomas.meissnitzer@gmail.com; t.meissnitzer@salk.at
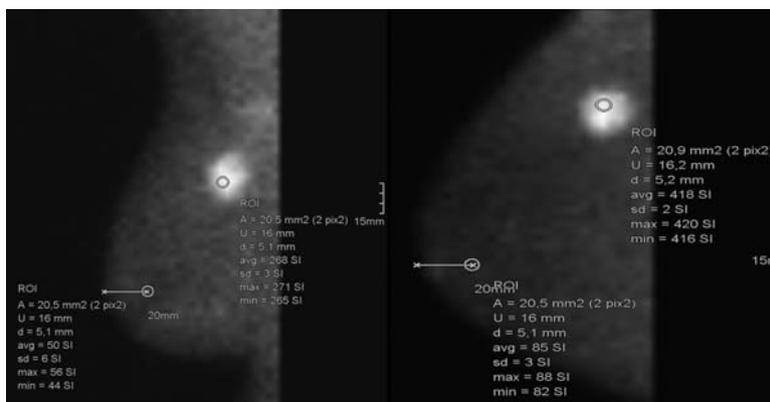
Figure 1. *Positioning of the regions of interest (ROI) to calculate the relative uptake factor (RUF). A: Circumference; U: diameter; avg: average (count=SI); sd: standard deviation; max: maximum (count, SI); min: minimum (count, SI).*

ultrasound (5). In compliance with the guidelines of the European Association of Nuclear Medicine 740-1100 MBq (20-30 mCi) [mean=843.8 MBq, 22.8 mCi] $^{99m}$Technetium SestaMIBI (CarioTOP, National Centre for Nuclear Research, Otwock, Poland) were injected in an upper extremity vein contralateral to the affected breast (6). Ten minutes after the injection, scintigrams were performed using a dedicated gamma camera for BSGI Dilon 6800 (Dilon Technologies, Newport NEWS, VA, USA) in craniocaudal and mediolateral-oblique projections, comparable to mammograms. After transmission to the electronic image archive, tracer distribution was analyzed not only visually as described by Brem *et al*. (7) but also semiquantitatively by calculating a relative uptake factor (RUF).

RUF was calculated according to the ratio RUF= $C_{max}L/C_{max}BG$. $C_{max}L$ was the highest count within the clearly definable MIBI accumulation, CmaxBG reflected the highest count within the reference region, which was always placed two cm dorsal to the nipple in an unsuspicious area (see Figure 1). The higher ratio of the two projections was used for statistics. Further technical data of BSGI and procedural instructions are described by Meissnitzer *et al*. (4). The size of the IDCs evaluated in this study was determined with morphological imaging modalities, in the majority with ultrasound due to accurate demarcation.

*Histopathological and immunohistochemical analyses.* The results of histopathological and immunohistochemical examinations of the biopsy specimen were the gold standard for this retrospective work-up, which were carried out by a pathologist with 20 years' experience in breast diagnosis. These specimen were obtained by image-guided biopsy without biopsy-related complications. Either ultrasound-guided (49 specimen) or stereotactic vacuum-assisted biopsies (1 specimen) were performed. The retrospective evaluation of the biopsy samples included verification of former diagnosis as IDC, analyses of receptor status [estrogen (ER), progesterone (PR) and human epidermal growth factor 2 (HER2/Neu) receptors], as well as Ki-67 expression and, as the final result, the assignment to the different subtypes of IDC according to the decisions of the 2011 St. Gallen International Breast Cancer Conference (8). In this classification, ER-positive IDCs are subdivided into luminal-A or -B subtypes depending on their Ki-67 expression: Luminal-A carcinomas exhibit a Ki-67 index of less than 14% and are PR-

positive or-negative and HER2/Neu-negative; luminal-B carcinomas have a Ki-67 index of 14% or higher and are PR-positive or -negative and HER2/Neu-positive or -negative; non-luminal carcinomas are either HER2/Neu-positive and negative for both endocrine receptors (ER, PR) or triple-negative (basal-like carcinomas).

*Immunohistochemistry.* Immunohistochemical stainings were performed on the whole slides for ER (Novacastra, Leica Biosystems Nussloch GmbH, Nussloch, Germany), PR (Novacastra, Leica Biosystems Nussloch GmbH, Nussloch, Germany), Ki-67 (Dako, Dako Austria GmbH, Vienna, Austria) and HER2/Neu (Ventana, Ventana Medical Systems, Inc., Tucson, AZ, USA) on an Autostainer Plus (Dako® Austria GmbH, Vienna, Austria) and Ventana®-Ultra (Roche Austria GmbH, Vienna, Austria), routinely according to the manufacturers' recommendations [heat-induced epitope retrieval (Dako® Austria GmbH, Vienna, Austria) at 95˚C for 40 min for the applied antibodies [dilutions: ER: 1:100, PR: 1:200, Ki-67: 1:500) and for HER2/Neu (ready-to use)].

*In situ hybridization.* In cases of a HER2/Neu score of 2+ an additional Her-2-SISH was performed on a Ventana® XT Stainer (Roche Austria GmbH, Vienna, Austria) routinely according to the manufacturer's recommendations to analyze the *HER2* gene amplification (9).

*Interpretation of immunohistochemistry.* The interpretation of ER and PR expression was performed according to Remmele and Stegner (10) by evaluating the intensity (0: negative, 1: weak, 2: moderate, 3: strong) and the percentage of positive cells (0: negative, 1 <10%, 2: 10 to 50%, 3: 51 to 80% and 4: >80%). As a result, a score from 0 to 12 was calculated.

The Ki-67-based proliferation rate was assessed with the optimized particle analysis module according to the software manual ImageAccess 9 Enterprise (Imagic Bildverarbeitung AG, Glattbrugg, Switzerland) on three digitized hot-spot areas and related to the total number of cells as published earlier (11). The expression of HER2/Neu was determined according to the recent recommendations of the American Society of Clinical Oncology, ranging from negative (score 0 and 1+) to weakly positive (2+) and positive (3+) (12).
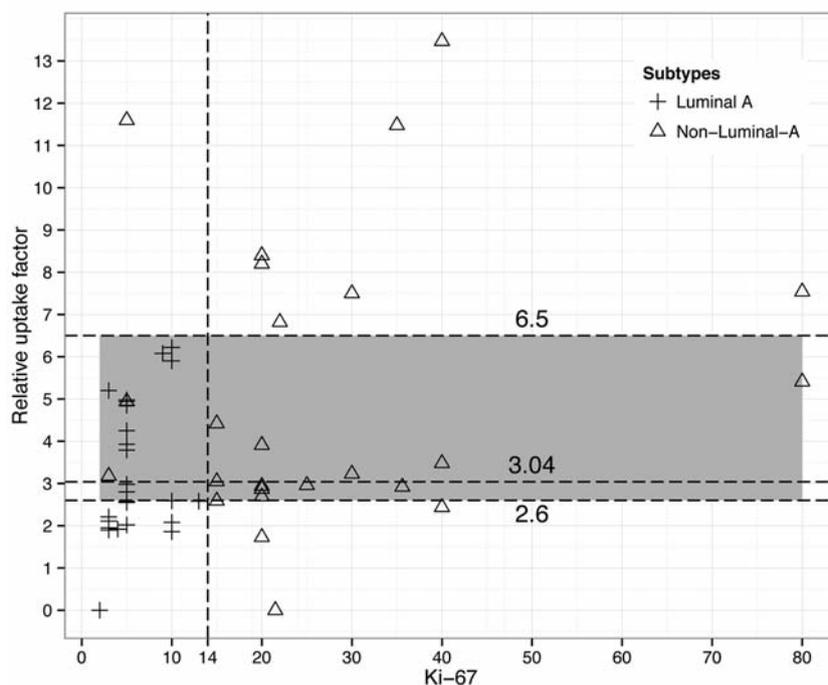
Figure 2. *Correlations of relative uptake factor (RUF), Ki-67 values and invasive ductal cancer (IDC) sub-types in the scatterplot. A relative uptake factor (RUF) of 2.6 and 6.5 can be regarded as valid thresholds.*

*Statistical analysis*. The RUFs as well as the immunohistochemical features of all included IDCs (ER, PR, Ki-67, HER2/Neu) and the resulting sub-categories were noted in an Excel spreadsheet (Microsoft Corporation, Redmond, WA, USA). Sensitivity, specificity, predictive values [positive predictive value (PPV), negative predictive value (NPV)] and accuracy statistically outlined the performance of RUF. Additionally, the area under the receiver operating characteristics curve (AUC) was calculated to summarize sensitivity and specificity of each parameter and to determine thresholds for RUF. DeLongs difference test was used to define differences in AUC values. Cohen's Kappa outlined the concordance of the different RUF levels with IDC subtypes as well as the correlation of RUF and the abovementioned markers. Similarly, the McNemar test described the level of compliance.

Calculations and graphics were created with R 3.1.2, pROC, ggplot2 and package psych (13-16). In order to determine the best performance for RUF in distinguishing non luminal-A cancer from the other subtypes of IDCs, three different thresholds were evaluated: The lowest threshold was 2.6 and was derived from the AUC curves, 3.04 was identified in our previous study to reliably subdivide malignant and benign lesions. The highest threshold of 6.5 was based on the assessment of the scatter plot (see Figure 2).

## Results

In our previous prospective study, 92 lesions were detected in 67 patients and histopathological analyses of biopsy specimen revealed 67 malignant lesions. Among the latter, immunohistochemical work-up was fully-available or

Table I. *Descriptive statistics.*

| Parameter (units) | Mean | SE |
|---|---|---|
| Ki-67 (%) | 16.502 | 2.430 |
| ER (*) | 9.700 | 0.584 |
| PR (*) | 5.800 | 0.586 |
| Relative uptake factor [RUF] (**) | 4.200 | 0.396 |
| Size (mm) | 20.360 | 1.300 |

*Score calculated according to [10] ranging from a minimum of 0 to maximum of 12. **RUF calculated according to the ratio CmaxL/CmaxBG. CmaxL=highest count within the clearly definable MIBI accumulation, $C_{max}BG$=highest count within the reference region placed two cm dorsal to the nipple. ER: Estrogen receptor; PR: progesterone receptor; SE: standard error.

retrospectively feasible in 50 IDCs with a mean size of 20.36 mm [standard error (SE)=1.3 mm] because the other patients were treated elsewhere. There were a total of 25 luminal-A and 18 luminal-B IDCs, 7 were non-luminal IDCs; among the latter, 2 were classified as HER2/Neu-positive and negative for endocrine receptors, and 5 were basal-like carcinomas (triple-negative). The calculated mean values for Ki-67, ER, PR, RUF and size including SEs are listed in Table I.

Table II. *Statistics of the different relative uptake factor (RUF) categories.*

|  | RUF | Sensitivity (SE) | Specificity | (SE) | PPV | (SE) | NPV | (SE) | AUC | *p*-Value | Kappa | McNemar |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Non-luminal-A | >2.6 | 0.840 (0.052) | 0.520 | (0.071) | 0.636 | (0.068) | 0.765 | (0.060) | 0.680 | (0.008) | 0.360 | 0.080 |
|  | >3.04 | 0.640 (0.068) | 0.600 | (0.069) | 0.615 | (0.069) | 0.625 | (0.068) | 0.620 | (0.059) | 0.240 | 1.000 |
|  | >6.5 | 0.320 (0.066) | 1.000 | (0.000) | 1.000 | (0.000) | 0.595 | (0.069) | 0.660 | (0.016) | 0.320 | 0.000 |
| Ki-67 >14% | >2.6 | 0.818 (0.055) | 0.464 | (0.071) | 0.545 | (0.070) | 0.765 | (0.060) | 0.620 | (0.239) | 0.268 | 0.022 |
|  | >3.04 | 0.591 (0.070) | 0.536 | (0.070) | 0.500 | (0.071) | 0.625 | (0.068) | 0.560 | (0.559) | 0.124 | 0.522 |
|  | >6.5 | 0.318 (0.066) | 0.964 | (0.026) | 0.875 | (0.047) | 0.643 | (0.068) | 0.680 | (0.057) | 0.303 | 0.001 |

SE: Standard error, PPV: positive predictive value, NPV: negative predictive value, AUC: area under the curve.

Although our data showed no statistically valid correlation between RUF and ER or PR status, we found a relationship between RUF and the IDC sub-type, that is to say to the differentiation between luminal-A *versus* the others. RUF exceeding 6.5 excludes luminal-A IDC with a maximum specificity and PPV of 100% and an acceptable AUC ($p<0.05$). For RUF between 2.6 and 6.5, no reliable conclusion about the sub-type could be drawn according to the low PPVs and NPVs. A RUF of below 2.6 points to a luminal-A IDC, as the NPV for a non-luminal-A IDC reaches at least 77% (Figure 2 and Table II).

As a consequence, we suggest the application of RUF comparable to a traffic light: RUF exceeding 6.5 excludes a luminal-A IDC (red light), a value of between 2.6 and 6.5 is inconclusive (yellow light) and RUF of below 2.6 identifies a luminal-A IDC (green light).

Comparable results were calculated for the abovementioned categories of RUF and the Ki-67 level of IDCs as the most important proliferation marker (Figure 3). Table II lists statistical parameters for distinguishing the different types of IDCs correlated with RUF.

## Discussion

Considering the pharmacokinetics of SestaMIBI, correlations between SestaMIBI uptake in IDCs and their biologic behavior can be expected. We postulate that if the RUF could be linked to a certain histological subtype of IDC, the accuracy of diagnosis based on imaging examinations and the analysis of biopsy specimen could be increased: For radiologists, not only the assessment of a breast lesion as suspicious or unsuspicious, but also the selection of biopsy site (according to the region with the highest uptake) could be facilitated. Pathologists could focus on the analysis of samples from areas with the highest uptake. Moreover, therapeutic decisions could also be based on semi-quantitative BSGI, especially if there exist uncertainties in the histopathological classification.

*BSGI and pharmacokinetics of SestaMIBI.* [99m]Technetium SestaMIBI [Hexakis (2-methoxy-2-methylpropylisonitrile)]

is a lipophilic cationic compound and an isonitrile. Being lipid-soluble, it diffuses passively from the blood into the cytoplasm and is retained in the region of the mitochondria because of the electrostatic attraction between the positive charge of the [99m]Technetium SestaMIBI and the negative charge of the mitochondria (17, 18). Uptake and retention of [99m]Technetium SestaMIBI depend on angiogenesis and regional perfusion, plasma and mitochondrial membrane potentials and thus the level of tissue metabolism (19, 20).

*Quantification of SestaMIBI uptake.* As opposed to the study of Park *et al.* (21), influencing factors on the calculation of the relative tracer uptake, such as differences in positioning of the breast on the detector or a varying background activity due to asymmetry of glandular tissue are less significant using our method. By the use of the absolute count within an exactly defined region of interest (ROI), significant fluctuations of the standard deviations in counts within the applied small ROIs can be neglected. The size of a lesion, its distance from the detector and the breast thickness were not taken into account, as we used a single-head detector to calculate an approximate RUF. Hence there exist significant differences compared to the results acquired with dedicated dual-head cameras, as described by Hruska *et al.* (22).

*Correlations between RUF and immunohistochemical characteristics of IDC.* Our former prospective study described the outstanding diagnostic potential of BSGI compared to morphological imaging modalities, especially concerning specificity and PPV for malignancy of a lesion. A poor sensitivity of 60% for lesions smaller than 1 cm was the only relevant limitation of BSGI.

In the current study, we demonstrated that more aggressive, often locally advanced IDCs, such as luminal-B, non-luminal or triple-negative sub-types exhibit higher energy demand because of up-regulated angiogenesis, increased perfusion, higher proliferative activity and surrounding inflammation and clear correlations demonstrated between SestaMIBI uptake and immunohistochemical characteristics, also shown by Mankoff *et al.* (23) and Scopinaro *et al.* (24). The exact
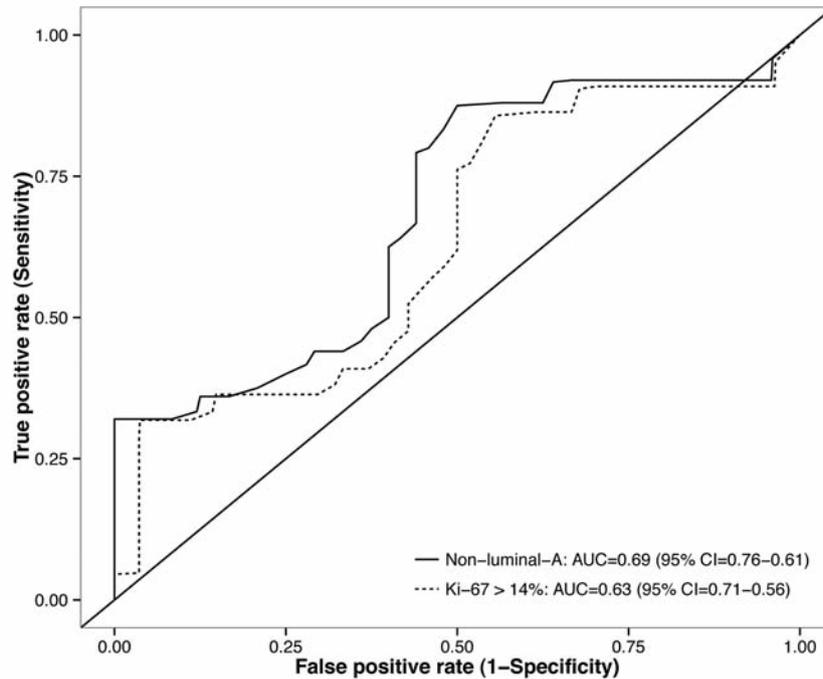
Figure 3. *Correlations between relative uptake factor (RUF) and Ki-67 levels outlined in a Receiver Operating Characteristic plot.*

characterization of IDCs is decisive for the choice of therapy and prognosis because nowadays we understand IDCs to be a heterogeneous group of different biological sub-types (1). The genetic fundamentals of these cancer sub-types are well-known (25). The proliferative signature, which is crucial for the distinction between luminal-A and non luminal-A IDCs, is routinely determined by immunohistochemical investigation of the Ki-67 level encoded by MKI67 and 14% is a generally accepted cut-off level (26). Trihia *et al*. described the Ki-67-index as a surrogate parameter for prognosis and grading, which can be assessed in biopsy specimen (27). Recent studies have demonstrated the increased and premature incidence of local recurrence, as well as metastasis, of IDCs with certain marker profiles, for example if Ki-67 exceeds 14% and cancer cells are HER2/Neu-positive (28, 29). Nishimura *et al*. underlined the baseline Ki-67 value as a significant predictor for time and site of breast cancer recurrence with lower index values in patients with bone metastases and higher levels in patients with liver and brain metastases. Additionally, higher nuclear grade and ER[−], PR[−] and HER2/Neu-negative IDCs with shorter disease-free interval were correlated with higher Ki-67 values (30).

The accuracy and reproducibility of proliferative markers and hormone receptor status examined in biopsy specimen are limited due to small sample volume, heterogeneous expression, different lab methods and subjective reading

(31). For IDCs exceeding a diameter of 1 cm, these limitations are irrelevant for BSGI. Moreover, reliability of diagnosis also depends on the biopsy site within a tumor chosen by the radiologist. Increased MIBI uptake within a certain part of a tumor points to the most dedifferentiated and thus most aggressive part, which should be biopsied. Thereby, sample error may be obviated. According to our data, areas with a RUF exceeding 2.6 require biopsy.

Our findings are in accordance with those of Tilling *et al*., Maini *et al*. and Tofani *et al*., who reported no statistically significant correlations between SestaMIBI uptake and receptor status (32-34).

*Potential role of RUF in therapeutic decisions.* Biomarkers decisively determine timing and type of adjuvant therapy (28). In this context, not only improved response rates of non luminal-A IDCs to chemotherapy, but also the lack of response of ER[+] IDCs (luminal-A and luminal-B) to tamoxifen can be highly relevant. In particular if ER[+] IDCs lack PRs, tamoxifen may act as an estrogen agonist *via* ERs by activating growth factors (35). Nevertheless, first results on the additional use of signal transduction inhibitors, such as inhibitors of mechanistic targets of rapamycin (everolismus, temsirolismus), phosphoinositide 3-kinase, tyrosine kinases, insulin-like growth factor-receptors and epidermal growth factor receptors to overcome tamoxifen resistance are promising (36).

A RUF higher than 6.5, indicating a highly aggressive IDC sub-type, may support the decision for additional chemotherapy, especially if the results of histopathological and immunohistochemical examinations of the biopsy specimen are equivocal. Despite shorter disease-free survival, triple-negative (basal-like) and HER2/Neu-enriched IDCs have the highest response rate to chemotherapy, a fact known as the 'triple-negative paradox' (37).

In contrast, a RUF below 2.6 indicates a less aggressive sub-type, such as luminal-A IDC, with a significantly better prognosis. Voduc *et al.* for example, described a local recurrence rate of 8% within 10 years for luminal-A cancer, whereas HER2/Neu-positive and triple-negative sub-groups exhibited local recurrence rates of 21% and 14% respectively (38). These patients would expect little benefit from chemotherapy, even though they would be exposed to its side-effects (39).

Another possible reason for the low chemosensitivity of IDCs with low RUF may be an increased expression of the multidrug-resistance P-glycoprotein because as a substrate of this transport protein, SestaMIBI is more rapidly eliminated from cancer cells rich in P-glycoprotein (40).

*Limitations of our study.* Our results were obtained by retrospective work-up of a relatively small sample of 50 biopsy specimen. This may be the reason why the correlations between RUF and the receptor status did not have a higher statistical validity. However, there exist clear indications for an association between RUF and the Ki-67 level, as well as for the highly relevant distinction between luminal-A and the other IDC-subtypes.

Regarding the thresholds for RUF, it should be mentioned that a RUF exceeding 6.5 excludes luminal-A IDC with great reliability. Conversely, below a RUF of 6.5 there is a wide gray zone concerning the classification of IDCs.

## Conclusion

The RUF may be used as a surrogate parameter for the classification of IDCs into different sub-types. Due to simple and rapid calculation at presentation, the RUF can be regarded as a basis for treatment decisions and prognostic evaluations, in addition to the examinations of biopsy specimen by the pathologists and by staging with imaging modalities.

## References

1 Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lønning PE, Børresen-Dale AL, Brown PO and Botstein D: Molecular portraits of human breast tumors. Nature *406*: 747-752, 2000.

2 Park JY, Yi SY, Park HJ, Kim MS, Kwon HJ, Park NH and Moon SY: Breast-specific gamma imaging: correlation with mammographic and clinicopathologic characteristics of breast cancer. Am J Roentgenol *203*: 223-228, 2014.

3 Tadwalkar RV, Rapelyea JA, Torrente J, Rechtman LR, Teal CB, McSwain AP, Donnelly C, Kidwell AB, Coffey CM and Brem RF: Breast-specific gamma imaging as an adjunct modality for the diagnosis of invasive breast cancer with correlation to tumor size and grade. Br J Radiol *85*: 212-216, 2012.

4 Meissnitzer T, Seymer A, Keinrath P, Holzmannhofer J, Pirich C, Hergan K and Meissnitzer MW: The added value of semiquantitative breast-specific gamma imaging in the work-up of suspicious breast lesions compared to mammography, ultrasound and 3T MR Imaging. Br J Radiol *88(1051)*: 20150147, 2015.

5 D´Orsi CJ, Sickles EA, Mendelson EB and Morris EA: ACR BI-RADS® Atlas, Breast Imaging Reporting and Data System. Reston, VA, USA: American College of Radiology, 2013.

6 Bombardieri E, Aktolun C, Baum RP, Bishof-Delaloye A, Buscombe J, Chatal JF, Maffioli L, Moncayo R, Mortelmans L and Reske SN: Breast scintigraphy: procedure guidelines for tumor imaging. Eur J Nucl Med Mol Imaging *30*: 107-114, 2003.

7 Brem RF, Floerke AC, Rapelyea JA, Teal C, Kelly T and Mathur V: Breast-specific Gamma Imaging as an adjunct imaging modality for the diagnosis of breast cancer. Radiology *247*: 651-657, 2008.

8 Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thürlimann B and Senn HJ: Strategies for subtypes: dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. Ann Oncol *22*: 1736-1747, 2011.

9 Dietel M, Ellis IO, Kreipe H, Moch H, Dankof A, Kölble K and Kristiansen G: Comparison of automated silver enhanced in situ hybridization (SISH) and fluorescence ISH (FISH) for the validation of HER2 gene status in breast carcinoma according to the guidelines of the American Society of Clinical Oncology and the College of American Pathologists. Virchows Arch *451(1)*: 19-25, 2007.

10 Remmele W and Stegner HE: Recommendations for uniform definition of an immunoreactive score (IRC) for immunohistochemical estrogen receptor detection (EA-ICA) in breast cancer tissue. Pathologe *8(3)*: 138-140, 1987.

11 Kemmerling R, Weyland D, Kiesslich T, Illig R, Klieser E, Jäger T, Dietze O and Neureiter D: Robust linear regression model of Ki-67 for mitotic rate in gastrointestinal stromal tumors. Oncol Lett *7(3)*: 745-749, 2014.

12 Nechaev IN, Knyazev EN, Krainova NA and Shkurnikov MY: Express analysis of Her-2/neu status in breast cancer biopsy specimens. Bull Exp Biol Med *155(4)*: 522-526, 2013.

13 R-project.org. A language and environment for statistical computing. R Foundation for Statistical Computing [latest R version 3.2.0, released on 2015-04-16]. Vienna Austria: R Core Team. Available from: http://www.R-project.org

14 Robin X, Turck, N, Hainard, A, Tiberti N, Lisacek F, Sanchez JC and Müller M: pROC: an open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinformatics: 12,77. DOI:10.1186/1471-2105-12-77, 2011.

15 Wickham H: ggplot2: elegant graphics for data analysis. Springer New York, 2009.

16 R-project.org; Revelle W: Psych: procedures for personality and psychological research Northwestern University [latest version 1.5.2, released on 2015-02-19]. Available from: http://CRAN.R-project.org/package=psych

17 Chiu ML, Kronauge JF and Piwnica-Worms D: Effect of mitochondrial and plasma membrane potentials on accumulation of hexacis (2-methoxyisobutylisonitrile) technetium(I) in cultured mouse fibroblasts. J Nucl Med *31*: 1646-1653, 1990.

18 Pauwels EKJ, McCready VR, Stoot JHMB and van Deurzen DFP. The mechanism of accumulation of tumor-localizing radiopharmaceuticals. Eur J Nucl Med *25*: 277-305, 1998.

19 Sharma S, Sharma MC and Sakar C: Morphology of angiogenesis in human cancer: a conceptual overview, histoprognostic perspective and significance of neoangiogenesis. Histopathology *46*: 481-489, 2005.

20 Buscombe JR, Cwickla JB, Thakrar DS and Hilson AJ: Uptake of Tc-99m MIBI related to tumor size and type. Anticancer Res *17*: 1693-1694, 1997.

21 Park KS, Chung HW and Yoo YB *et al*: Complementary role of semiquantitative analysis of breast-specific gamma imaging in the diagnosis of breast cancer. Am J Roentgenol *202*: 690-695, 2014.

22 Hruska CB and O'Connor MK: Quantification of lesion size, depth, and uptake using a dual-head molecular breast imaging system. Med Phys *35(4)*: 1365-1376, 2008.

23 Mankoff DA, Dunnwald LK, Gralow JR, Ellis GK, Schubert EK, Charlop AW, Tseng J, Rinn KJ and Livingston RB: [Tc-99m]-sestamibi uptake and washout in locally advanced breast cancer are correlated with tumor blood flow. Nucl Med Biol *29*: 719-727, 2002.

24 Scopinaro F, Schillaci O, Scarpini M, Mingazzini PL, Di Macio L, Banci M, Danieli R, Zerilli M, Limiti MR and Centi Colella A: Technetium-99m sestamibi: an indicator of breast cancer invasiveness. Eur J Nuc Med *21*: 984-987, 1994.

25 Sorlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, Deng S, Johnsen H, Pesich R, Geisler S, Demeter J, Perou CM, Lønning PE, Brown PO, Børresen-Dale AL and Botstein D: Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci USA *100(14)*: 8418-8423, 2003.

26 Cheang MC, Chia SK, Voduc D, Gao D, Leung S, Snider J, Watson M, Davies S, Bernard PS, Parker PS, Perou CM, Ellis MJ and Nielson TO: Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. J Nat Cancer Inst *101*: 736-750, 2009.

27 Trihia H, Murray S, Price K, Gelber RD, Golouh R, Goldhirsch A, Coates AS, Collins J, Castiglione-Gertsch M and Gusterson BA. Ki-67 expression in breast carcinoma: its association with grading system, clinical parameters, and other prognostic factors-a surrogate marker? Cancer *97(5)*: 1321-1331, 2003.

28 Ribelles N, Perez-Villa L, Jerez MS, Pajares B, Vicioso L, Jimenez B, de Luque V, Franco L, Gallego E, Marquez A, Alvarez M, Sanchez-Mūnoz A, Perez-Rivas L and Alba E: Pattern of recurrence of early breast cancer is different according to intrinsic subtype and proliferation index. Breast Cancer Res *15*: R98, 2013.

29 Kennecke H, Yerushalmi R, Woods R, Cheang MC, Voduc D, Speers CH, Nielsen TO and Gelmon K: Metastatic behavior of breast cancer subtypes. J Clin Oncol *28*: 3271-3277, 2010.

30 Nishimura R, Osako T, Nishiyama Y, Tashima R, Nakano M, Fujisue M, Toyozumi Y and Arima N: Prognostic significance of Ki-67 index value at the primary breast tumor in recurrent breast cancer. Mol Clin Oncol *2(6)*: 1062-1068, 2014.

31 Yerushalmi R, Woods R, Ravdin PM, Hyes MM and Gelmon KA: Ki67 in breast cancer: prognosis and predictive potential. Lancet Oncol *11*: 174-183, 2010.

32 Tiling R, Stephan K, Sommer H, Shabani N, Linke R and Hahn K: Tissue-specific effects on uptake of $^{99m}$Tc-Sestamibi by breast lesions: a targeted analysis of false scintigraphic diagnoses. J Nucl Med *45*: 1822-1828, 2004.

33 Maini CL, Tofani A, Sciuto R, Semprebene A, Cavaliere R, Mottolese M, Benevolo M, Ferranti F, Grandinetti ML, Vici P, Lopez M and Botti C: Technetium-99m-MIBI scintigraphy in the assessment of neoadjuvant chemotherapy in breast carcinoma. J Nucl Med *38*: 1546-1551, 1997.

34 Tofani A, Sciuto R, Semprebene A, Festa A, Pasqualoni R, Giunta S, Mottolese M, Benevolo M, Botti C and Maini CL: 99Tcm-MIBI scintimammography in 300 consecutive patients: factors that may affect accuracy. Nucl Med Commun *20*: 1113-1121, 1999.

35 Thakkar JP and Mehta DG: A review of an unfavorable subset of breast cancer: estrogen receptor positive, progesteron receptor negative. Oncologist *16*: 276-285, 2011.

36 Baselga J, Semiglazov V, van Dam P, Manikhas A, Bellet M, Mayordomo J, Campone M, Kubista E, Greil R, Bianchi G, Steinseifer J, Molloy B, Tokaji E, Gardner H, Phillips P, Stumm M, Lane HA, Dixon JM, Jonat W and Rugo HS: Phase II randomized study of neoadjuvant everolismus plus letrozole compared with placebo plus letrozole in patients with estrogen receptor-positive breast cancer. J Clin Oncol *27*: 2630-2637, 2009.

37 Prat A and Perou CM: Deconstructing the molecular portraits of breast cancer. Mol Oncol *5*: 5-23, 2011.

38 Voduc KD, Cheang MCU, Tyldesley S, Gelmon K, Nielsen TO and Kennecke H: Breast cancer subtypes and the risk of local and regional relapse. J Clin Oncol *28*: 1684-1691, 2010.

39 Berry DA, Cirrincione C, Henderson IC, Citron ML, Budman DR, Goldstein LJ, Martino S, Perez EA, Muss HB, Norton L, Hudis C and Winer EP: Estrogen-receptor status and outcomes of modern chemotherapy for patients with node-positive breast cancer. JAMA *295(14)*: 1658-1667, 2006.

40 Cayre A, Cachin F, Maublant J, Mestas D, Feillel V, Ferriere JP, Kwiaktowski F, Chevillard S, Finat-Duclos F, Verrelle P and Penault-Llorca F: Single static view $^{99m}$Tc-sestamibi scinti-mammography predicts response to neoadjuvant chemotherapy and is related to MDR expression. Int J Oncol *20*: 1049-1055, 2002