bFGF in Tumor Tissue Independently Prognosticates Disease Outcome of a Natural Course of Invasive Breast Cancer

Nataša Todorović-Raković, Marko Radulovic, Tijana Vujasinović, Zaki Abu Rabi, Jelena Milovanović, Dragica Nikolić-Vukosavljević

Department of Experimental Oncology, Institute for Oncology and Radiology of Serbia, Belgrade, Serbia

Correspondence:
Marko Radulovic
Department of Experimental Oncology
Institute for Oncology and Radiology of Serbia
Pasterova 14
11000 Belgrade
Serbia
Tel: + 381 11 20 67 213
Fax: + 381 11 2685 300
E-mail: marko@radulovic.net

Abstract.

BACKGROUND: Basic fibroblast growth factor (bFGF) is a potent angiogenic and mitogenic factor that has been functionally predisposed to promote tumorigenesis, while literature data also associate bFGF with a favorable outcome of breast cancer.

OBJECTIVE: In order to help resolve such controversy, this study set out to investigate the role of bFGF in breast cancer for the first time by use of the node-negative patient group with smaller tumors and without any systemic adjuvant therapy. This has allowed an increased homogeneity of the group and a far more reliable interpretation of results.

METHODS: The study included 133 node-negative breast cancer patients with 33 distant metastasis events. bFGF levels were determined by ELISA in primary tumor tissue homogenates.

RESULTS: The study demonstrated that bFGF in primary tumor tissue associated with favorable breast cancer outcome. bFGF levels significantly and positively correlated with ER levels.

CONCLUSIONS: The obtained results are relevant for the future prognostic research aimed at surpassing the currently achievable prognostic accuracies which are by far inadequate to allow reliable therapeutic decision making in breast cancer.

Keywords: breast cancer; bFGF; estrogen receptor; prognosis; metastasis

1. Introduction

Inflammatory cytokines have been intensively examined in recent years as potential prognostic biomarkers in different types of human cancer, as it is assumed that inflammation and inflammatory cytokines may be the critical components of tumor development.

Basic fibroblast growth factor (bFGF, FGF2 or FGFβ) is a cytokine with pleiotropic effects that belongs to the family of fibroblast growth factors. The family is involved in several processes including the cell proliferation, motility, differentiation, angiogenesis, wound healing and tumorigenesis. It comprises 18 secreted FGF ligands, together with the four signaling tyrosine kinase FGF receptors expressed in specific spatial and temporal patterns [1]. Binding of bFGF to FGFR1, FGFR2 and FGFR3 results in auto-phosphorylation of intracellular tyrosine residues which are involved in initiating tumor cell proliferation and invasion [2].

Several studies have indicated a potential prognostic value of bFGF in different types of human cancers. As a potent angiogenic and mitogenic factor bFGF is often associated with a promotion of tumorigenesis and metastasis, while literature data related to its prognostic significance in early breast cancer remain contradictory [3].

The most reliable way to evaluate the prognostic significance of potential biomarkers is by following the course of a disease that had not been interrupted by any adjuvant (postoperative) therapy, the so-called “natural course of disease”. Literature investigating the

potential biomarkers for a natural course of disease is generally scarce and none exists for the bFGF.

In this study, we have explored whether bFGF levels in breast carcinoma cytosols correlated with the occurrence of distant metastases in a breast cancer patient group not treated with systemic adjuvant therapy. We report that bFGF does exert an independent prognostic value by associating with favourable disease outcome.

2. Materials and methods

2.1. Experimental subjects

For this type of retrospective study on archived histology samples, formal consent is not required. Patient data were received by the pathology unit in a de-identified form, not including direct and indirect identifiers which could enable reidentification, in adherence to the Safe-Harbor methodology of the 2012 Health Insurance Portability and Accountability Act (HIPAA). The study was approved by the Institutional Review Board. The study conforms with The Code of Ethics of the World Medical Association (Declaration of Helsinki), printed in the British Medical Journal (18 July 1964) and its 7th revision in 2013.

This report was written according to REMARK recommendations for tumor marker prognostic studies [4]. Selection of invasive breast tumor histology specimens was retrospective in node-negative breast cancer patients, based on the absence of hormonal, chemotherapeutic or any other systemic treatment that could interfere with the natural course of metastasis occurrence. We assembled this very specific patient group from a period of over 20 years ago when low metastasis risk patients were not prescribed systemic therapy at our institution. This was in line with recommendations valid in the year 1993 for the lower-risk pT1/2 and N0M0 patients. The prospective power calculation rested on a pilot experiment which included 30 patients. The parameters for the sample size calculation were: target power of 0.8, the effect size by hazard ratio (HR) of 0.40, alpha 0.05, the variability of 0.58 standard deviations (SD) and the event rate of 23%. For clarification, the variability was calculated for bFGF as the distance in standard

deviations between the averages of the low- and high-risk groups stratified per actual metastasis outcome. The required numbers were 121 patients with 28 events, as calculated by the *stpower cox* function, a two-sided test (Stata/MP 13 software, StataCorp, College Station, TX). The final sample size amounted to 133 patients with 32 events. The actual average SD distance between groups with and without metastasis was 0.59, the event rate was 25% and effect size 0.35, resulting in the actual power of 0.94. The median follow-up period for patients without metastasis occurrence was 145 months, while the median time to distant metastasis occurrence from the date of primary tumor removal surgery was 61 months. The average age ± SD at diagnosis was 58 ± 10 years. Based on the 11 fmol/mg and 20 fmol/mg respective cutpoints, 71% of patients were estrogen receptor (ER) positive (median of 31 fmol/ml) and 24% were progesterone receptor positive (median of 6 fmol/ml). Estrogen and progesterone receptors were determined by a dextran-coated charcoal method as described [5]. HER2 status was available for 84 out of the 133 patients, among which 23% were found to overexpress HER2.

2.2 Preparation of tumor extracts

Cytosol tumor extracts were prepared from frozen tumors in 10 mM Tris buffer pH 7.4, containing 1.5 mM ethylenediamine tetraacetic, 10 mM monothioglycerol and 10 mM sodium molybdate. The estrogen receptors, the progesterone receptors (PR) and bFGF were measured from the same samples. Tumor extract protein concentrations were assayed using the Lowry method. Aliquots were stored at -80°C until the measurement of bFGF.

2.3 bFGF assay

The bFGF assay system was based on a solid phase ELISA employing a highly specific monoclonal antibody for FGF bound to the wells of a microtitre plate, together with a polyclonal antibody to bFGF conjugated to horseradish peroxidase (Human FGF basic Quantikine ELISA Immunoassay; R&D Systems, Minneapolis, MN). The bFGF immunoassay contains recombinant human bFGF as the standard, and it has been shown to quantitate accurately both the natural human bFGF and the recombinant human bFGF. Cytosol extracts were diluted to achieve a protein concentration of 0.25 mg/ml and the assays were performed in duplicate. The linearity of the assay ($r = 0.99$) was determined using biological samples with high concentrations of bFGF diluted with the calibrator diluent. The bFGF amounts were normalized against cytosol protein content as pg/mg of protein.

2.4 Data categorisation

Categorization of the continuous values was achieved by use of the outcome-oriented and data-oriented approaches. The log-rank test was employed for the outcome-oriented optimal cutpoint selection with the minimal *P*-value by use of the X-tile 3.6.1 software from Yale University, New Haven, CT [6]. The data-oriented categorization was based on the median cutpoint.

2.5 Prognostic performance evaluation

The proportional hazards assumption was tested for each feature by use of the Cox proportional hazards test for the time-dependent covariates (SPSS version 20, Chicago, IL). The assumption was satisfied if the interaction of the feature (F) with its product with time (F*T) revealed $p>0.05$

for F*T. Proportionality assumption was further confirmed based on the Schoenfeld residuals and graphical evaluation as the second opinion tests, *etat phtest* and *stphplot,* respectively, in Stata/MP 13.

Univariate Cox proportional hazards regression test was subsequently employed for statistical comparison of the prognosticated and actual metastasis outcomes. The hazard ratio (HR) designates the effect size of the Cox proportional hazards regression, corresponding to metastasis rates in the high- and low-risk groups of patients (SPSS).

Multivariate Cox proportional hazards regression analysis was performed to test for independence of each prognostic factor. Variables categorized by outcome were added to a full model using forward selection entry criterion of $P<0.20$ in univariate analysis and removed using backwards elimination per selection stay criterion of $P<0.05$ (SPSS).

The Rate-of-Change (ROC) analysis by the area under the ROC curve (AUC) was employed as a quantitative measure of discrimination efficiency. Discrimination is the capability to stratify patients who experience the event and patients who do not experience the event. AUC ranges from 0.5 (chance accuracy) to 1.0 (perfect accuracy), with the intermediate benchmarks of 0.6 (fair), 0.7 (good), 0.8 (excellent) and 0.9 (almost perfect). Accuracy was an additional prognostic measure, representing the percentage of times that the predicted and observed outcomes match. Kaplan–Meier analysis was executed for the period from tumor extraction surgery until the occurrence of metastasis (SPSS).

2.6 Validation strategies

A bootstrap with 1000 random data resamples was performed to quantify the overoptimism [7] by correction of the *P*-values and confidence intervals (95 % CI) of hazard risks (SPSS) and AUCs (Stata/MP 13).

3. Results

Table 1 shows the prognostic evaluation of bFGF in comparison to clinicopathological parameters: age, histological grade, pathological tumor size (pT) and estrogen receptor status (ER). The evaluation was performed by the Cox proportional hazards regression, AUC and accuracy criteria. Cox regression was performed by use of continuous and categorized values while AUCs were calculated only by continuous data, both against the actual metastasis outcome, as indicated in Table 1.

pT and ER significantly associated with the metastasis outcome already by their continuous values (prior to any categorization) based on the Cox univariate regression *P*-values. On the other hand, ROC analysis of the continuous values indicated the significance of pT and bFGF (Table 1). The confidence intervals of hazard ratios (HRs) and AUCs were corrected for bias by use of the bootstrap internal validation as indicated in Tables 1 and 2.

Categorization of data was necessary for the calculation of prognostic accuracy, multiparametric Cox regression and Kaplan-Meier analyses (Tables 1 and 2, Fig. 1). pT was categorized by the clinically established criteria for this parameter (<2cm, pT = 1; 2-5 cm, pT = 2; Table 1, Fig. 1), while histological grade is intrinsically a categorical variable. Other variables were categorized by both the outcome- and data-oriented approaches. The outcome-oriented categorization of continuous values resulted in improvement of the prognostic performance for all relevant features (Table 1). In contrast, the data-oriented categorization only improved the prognostic

performance of bFGF, while it had no effect on the age variable and it worsened the performance of ER. The prognostic significance of bFGF was independent of the categorization approach (data- or outcome-oriented) which additionally supported its prognostic value (Table 1). By the criteria of prognostic accuracy, bFGF was the best performer, followed by pT, ER, age and histological grade (Table 1).

To evaluate the relative prognostic value of bFGF, it was compared to pT which was chosen based on its best prognostic performance among the clinicopathological variables. Kaplan-Meier plots were produced for this purpose by use of data categorized by outcome for both pT and bFGF (Fig. 1). The plots indicate a distinct separation between good and poor prognosis groups. The metastasis incidence was 19% and 39% in the low- and high-risk groups for bFGF, while for pT it was 15% and 37%, respectively. This figure together with the data presented in Table 1 indicates that the prognostic value of bFGF is comparable to pT as the established and the best-performing clinicopathological parameter in terms of disease outcome prognosis. The association of bFGF with the favorable disease outcome can also be illustrated by the bFGF median levels of 76 pg/mg in tumors of the high-risk group and 112 pg/mg measured in tumors of the low-risk group.

Multivariate Cox regression analysis of the metastasis risk has highlighted bFGF as the independent prognostic factor, after the adjustment for age, histological grade, pT and ER (Table 2). This was in line with the Spearman correlation analysis which for bFGF only indicated

significant interaction with ER. The prognostic value of bFGF was also supported by its most pronounced HR in the multivariate test which narrowly surpassed the ER (Table 2).

4. Discussion

Our results for the first time establish the association of bFGF with good prognosis in the group of patients who did not receive any kind of adjuvant systemic therapy. This result is important as it effectively resolves the lasting controversy on whether bFGF associates with favorable or unfavorable breast cancer outcome.

Several previous studies equally indicate the association of bFGF with the favorable disease outcome in breast cancer. Analyses of the bFGF levels in breast tumor cytosols showed that patients with higher levels of bFGF had smaller tumors, an absence of axillary metastasis, low S-phase incidence, longer recurrence-free and overall survival [8, 9]. The immunohistochemical study of the breast tumors has come to the similar conclusion [10], while the study investigating levels of bFGF in the serum of patients with metastatic breast cancer also showed that median time to progression was worse in patients with low bFGF expression [11]. The *in vitro* study likewise demonstrated that bFGF expression can cause a less malignant phenotype in breast cancer cells, possibly as a result of decreased motility and invasion and that bFGF expression in breast cancer cells *in vivo* can reverse phenotypic features of malignancy, including migration, invasion, and tumor formation [12]. bFGF was also indicated as a positive prognostic factor for the pre-invasive ductal carcinoma in situ (DCIS) where it was expressed in 12% of subjects [13].

Such association of bFGF with a favorable outcome is somewhat surprising since it is generally considered as a factor promoting endothelial cell motility and proliferation. A number of studies are in line with this tumor-promoting role of bFGF showing that increased bFGF expression is a marker for worse prognosis in nodal-negative breast cancer patients [3] and operable lung cancer [14]. Besides, many FGF receptor inhibitors have been developed as candidates for anti-tumor therapy [15].

Loss of bFGF expression may not actively contribute to increased metastatic potential of breast cancer cells but rather only present a marker of less differentiated cancers in view of the report that breast cancer cells with lower epithelial markers exert higher invasiveness as they might have lost their dependence on bFGF for survival [16]. This is consistent with immunochemical studies showing that bFGF is produced by malignant cells in early breast cancer, while it gradually disappears at more advanced stages [17].

On the other hand, bFGF expression in a tumor might reflect its active inhibitory role in tumor progression as it was shown that bFGF inhibits proliferation in a dose-dependent manner [18] and promotes apoptosis [19] in several human breast cancer cell lines, such as MCF-7 and MB-134. Moreover, the tumor-protective role of bFGF also fits the current knowledge in view of its pleiotropic effects derived from binding to three types of FGF receptors [1]. The multimodality of bFGF functionality is also based on the fact that intermediate concentrations induce the

maximal stimulation of migration and growth while high levels of bFGF elicit weak responses [20]. bFGF within the tumor microenvironment has been accordingly reported to exert inhibitory as well as stimulatory effects, depending on the cell type evaluated, the experimental design used and the context in which it was tested [21]. The phenomenon of bFGF correlating either positively or negatively with disease outcome could be further based on the immunomodulatory effects, because the expression of endothelial cell adhesion molecules is up-regulated in the inflamed tissues by bFGF, thus potentiating leukocyte recruitment [22]. This scenario is consistent with the hypothesis that one of the major escape mechanisms of tumors is the avoidance of an effective immune infiltrate by downregulation of endothelial adhesion molecules [23]. Accordingly, the functional result of the action of bFGF in cancer could be dependent on the balance between its opposite roles in tumors including the promotion of angiogenesis and formation of an efficient leukocyte infiltrate in tumors. It was even postulated that the observed conflicting effects of a bFGF ligand might derive from a differential expression of its isoforms within breast cancer cells, resulting in either growth-promoting or growth-inhibitory outcomes, depending on the individual isoforms expressed [24].

The observed conflict in the prognostic association of tumor bFGF levels with the cancer outcome may be also caused by the heterogeneity of patient groups used and their insufficient size. Our study thus boasts improved homogeneity and size of the patient group in comparison to previous studies and accordingly provides a more dependable insight into the prognostic relevance of bFGF.

Although there is strong evidence that invasive breast cancer is promoted by angiogenesis [25], several studies question the angiogenic role of bFGF in tumors as its expression was found dissociated or even inversely associated with microvessel density (MVD) [26]. Visscher et al. proposed that biologic significance of elevated bFGF expression may be related to extracellular matrix remodeling rather than to induction of prominent neovascularization [27]. These observations are consistent with bFGF association with good rather than poor prognosis in patients with invasive breast cancer.

Except for ER, no correlation was found between the extent of bFGF tumor tissue levels and prognostic parameters. In line with this observation is the previous study indicating that bFGF levels in breast tumor tissue positively correlated with a high expression of the estrogen receptor [17]. Furthermore, the recent study demonstrated that treatment of the ER+ breast cancer cell line MCF-7 with estrogen led to an increased production of numerous FGF ligands including bFGF [28].

Improved screening and increased use of adjuvant systemic therapy are considered as the main factors responsible for the observed improvements in breast cancer overall survival over the last two decades [29]. The current clinical significance of the prognosis of disease risk derives from the fact that despite such significant benefit of the adjuvant cytotoxic therapy, almost two-thirds of the patients would have survived without it, thus avoiding unnecessary harsh toxic side effects which decrease the quality of life. Therefore, the therapeutic concept for the breast cancer has been shifting from “maximally tolerated treatment” to the “minimally necessary treatment”

considering that systemic therapy should be administered only to patients who would surely benefit from it [30, 31]. Such individual therapeutic optimization may become a reality once the elusive goal of highly accurate individual prognosis of disease outcome becomes available. Unfortunately, the current clinical pathologic and molecular prognostic approaches do not deliver sufficient accuracy to achieve this goal [32, 33]. The prognostic independence of bFGF demonstrated in this study indicates the possibility that bFGF indeed meets the requirements for the multi-marker protein prognostic score which may be the most promising strategy to sufficiently exceed the currently achievable prognostic accuracies.

In conclusion, the prognostic significance of bFGF in cancer was here investigated for the first time by use of the optimized patient group which was larger in comparison to the previous prognostic studies of bFGF, node-negative, had smaller tumors (pT1/2) and did not receive any systemic therapy. This has allowed an increased group homogeneity and a far more reliable interpretation of results. The study demonstrated that bFGF associates with favorable breast cancer outcome. This may be explained by the active role of bFGF within the tumor microenvironment, or alternatively, bFGF could be only a passive marker reflecting the changes in the malignant potential of a tumor. By use of the optimal patient group, the current report resolves the long-standing controversy on whether bFGF is a marker of high- or low- risk in the early breast cancer. The obtained results are highly relevant for the future prognostic research involving the development of composite prognostic scores which seem to be the most substantial approach to surpass the obtainable prognostic accuracies which are currently by far inadequate to allow reliable therapeutic decision making.

Acknowledgements: This study was funded by the research Grant No. 175068 from the Fund for Basic Science of the Ministry of Education and Science of the Republic of Serbia.

Conflict of interest: The authors declare that they have no conflict of interest.

References

[1] D.M. Ornitz and N. Itoh, The Fibroblast Growth Factor signaling pathway, *Wiley Interdiscip Rev Dev Biol* **4** (2015), 215-66.
[2] M. Okada-Ban, J.P. Thiery and J. Jouanneau, Fibroblast growth factor-2, *International Journal of Biochemistry and Cell Biology* **32** (2000), 263-7.
[3] A. Faridi, C. Rudlowski, S. Biesterfeld, S. Schuh, W. Rath and W. Schroder, Long-term follow-up and prognostic significance of angiogenic basic fibroblast growth factor (bFGF) expression in patients with breast cancer, *Pathology, Research and Practice* **198** (2002), 1-5.
[4] D.G. Altman, L.M. McShane, W. Sauerbrei and S.E. Taube, Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK): explanation and elaboration, *PLoS Med* **9** (2012), e1001216.
[5] M.V. Brankovic-Magic, D.B. Nikolic-Vukosavljevic, Z.B. Neskovic-Konstantinovic, K.S. Kanjer and I.V. Spuzic, Variations in the content of steroid receptors in breast cancer. Comparison between primary tumors and metastatic lesions, *Acta Oncologica* **31** (1992), 629-33.
[6] R.L. Camp, M. Dolled-Filhart and D.L. Rimm, X-tile: a new bio-informatics tool for biomarker assessment and outcome-based cut-point optimization, *Clin Cancer Res* **10** (2004), 7252-9.
[7] B. Efron, Bootstrap Methods: Another Look at the Jackknife, *The Annals of Statistics* **7** (1979), 1-26.
[8] R. Colomer, J. Aparicio, S. Montero, C. Guzman, L. Larrodera and H. Cortes-Funes, Low levels of basic fibroblast growth factor (bFGF) are associated with a poor prognosis in human breast carcinoma, *British Journal of Cancer* **76** (1997), 1215-20.
[9] B.K. Linderholm, B. Lindh, L. Beckman, M. Erlanson, K. Edin, B. Travelin, J. Bergh, K. Grankvist and R. Henriksson, Prognostic correlation of basic fibroblast growth factor and vascular endothelial growth factor in 1307 primary breast cancers, *Clin Breast Cancer* **4** (2003), 340-7.
[10] C. Yiangou, J.J. Gomm, R.C. Coope, M. Law, Y.A. Luqmani, S. Shousha, R.C. Coombes and C.L. Johnston, Fibroblast growth factor 2 in breast cancer: occurrence and prognostic significance, *British Journal of Cancer* **75** (1997), 28-33.
[11] E. Alba, A. Llombart, N. Ribelles, M. Ramos, R. Fernandez, J.I. Mayordomo, I. Tusquets, M. Gil, A. Barnadas, F. Carabante, M. Ruiz, R. Vera, I. Palomero, V. Soriano, J. Gonzalez and R. Colomer, Serum endostatin and bFGF as predictive factors in advanced breast cancer patients treated with letrozole, *Clin Transl Oncol* **8** (2006), 193-9.
[12] R.M. Korah, V. Sysounthone, Y. Golowa and R. Wieder, Basic fibroblast growth factor confers a less malignant phenotype in MDA-MB-231 human breast cancer cells, *Cancer Research* **60** (2000), 733-40.

[13] P. Wulfing, C. Kersting, H. Buerger, B. Mattsson, R. Mesters, C. Gustmann, B. Hinrichs, J. Tio, W. Bocker and L. Kiesel, Expression patterns of angiogenic and lymphangiogenic factors in ductal breast carcinoma in situ, *British Journal of Cancer* **92** (2005), 1720-8.
[14] M. Hu, Y. Hu, J. He and B. Li, Prognostic Value of Basic Fibroblast Growth Factor (bFGF) in Lung Cancer: A Systematic Review with Meta-Analysis, *PLoS One* **11** (2016), e0147374.
[15] M. Katoh, FGFR inhibitors: Effects on cancer cells, tumor microenvironment and whole-body homeostasis (Review), *International Journal of Molecular Medicine* **38** (2016), 3-15.
[16] E.W. Thompson, S. Paik, N. Brunner, C.L. Sommers, G. Zugmaier, R. Clarke, T.B. Shima, J. Torri, S. Donahue, M.E. Lippman and et al., Association of increased basement membrane invasiveness with absence of estrogen receptor and expression of vimentin in human breast cancer cell lines, *Journal of Cellular Physiology* **150** (1992), 534-44.
[17] K. Smith, S.B. Fox, R. Whitehouse, M. Taylor, M. Greenall, J. Clarke and A.L. Harris, Upregulation of basic fibroblast growth factor in breast carcinoma and its relationship to vascular density, oestrogen receptor, epidermal growth factor receptor and survival, *Annals of Oncology* **10** (1999), 707-13.
[18] S.W. McLeskey, I.Y. Ding, M.E. Lippman and F.G. Kern, MDA-MB-134 breast carcinoma cells overexpress fibroblast growth factor (FGF) receptors and are growth-inhibited by FGF ligands, *Cancer Research* **54** (1994), 523-30.
[19] P. Maloof, Q. Wang, H. Wang, D. Stein, T.N. Denny, J. Yahalom, E. Fenig and R. Wieder, Overexpression of basic fibroblast growth factor (FGF-2) downregulates Bcl-2 and promotes apoptosis in MCF-7 human breast cancer cells, *Breast Cancer Research and Treatment* **56** (1999), 153-67.
[20] J. Kanodia, D. Chai, J. Vollmer, J. Kim, A. Raue, G. Finn and B. Schoeberl, Deciphering the mechanism behind Fibroblast Growth Factor (FGF) induced biphasic signal-response profiles, *Cell Commun Signal* **12** (2014), 34.
[21] M. Klagsbrun, The fibroblast growth factor family: structural and biological properties, *Progress in Growth Factor Research* **1** (1989), 207-35.
[22] S.I. Zittermann and A.C. Issekutz, Basic fibroblast growth factor (bFGF, FGF-2) potentiates leukocyte recruitment to inflammation by enhancing endothelial adhesion molecule expression, *American Journal of Pathology* **168** (2006), 835-46.
[23] A.W. Griffioen, C.A. Damen, S. Martinotti, G.H. Blijham and G. Groenewegen, Endothelial intercellular adhesion molecule-1 expression is suppressed in human malignancies: the role of angiogenic factors, *Cancer Research* **56** (1996), 1111-17.
[24] M. Korc and R.E. Friesel, The role of fibroblast growth factors in tumor growth, *Curr Cancer Drug Targets* **9** (2009), 639-51.
[25] B. Uzzan, P. Nicolas, M. Cucherat and G.Y. Perret, Microvessel density as a prognostic factor in women with breast cancer: a systematic review of the literature and meta-analysis, *Cancer Research* **64** (2004), 2941-55.
[26] J. Rykala, K. Przybylowska, I. Majsterek, G. Pasz-Walczak, A. Sygut, A. Dziki and J. Kruk-Jeromin, Angiogenesis markers quantification in breast cancer and their correlation with clinicopathological prognostic variables, *Pathology Oncology Research* **17** (2011), 809-17.
[27] D.W. Visscher, F. DeMattia, S. Ottosen, F.H. Sarkar and J.D. Crissman, Biologic and clinical significance of basic fibroblast growth factor immunostaining in breast carcinoma, *Modern Pathology* **8** (1995), 665-70.
[28] N. Brady, P. Chuntova, L.K. Bade and K.L. Schwertfeger, The FGF/FGFR axis as a therapeutic target in breast cancer, *Expert Rev Endocrinol Metab* **8** (2013), 391-402.

[29] D.A. Berry, K.A. Cronin, S.K. Plevritis, D.G. Fryback, L. Clarke, M. Zelen, J.S. Mandelblatt, A.Y. Yakovlev, J.D. Habbema and E.J. Feuer, Effect of screening and adjuvant therapy on mortality from breast cancer, *New England Journal of Medicine* **353** (2005), 1784-92.
[30] C. Jackisch, N. Harbeck, J. Huober, G. von Minckwitz, B. Gerber, H.H. Kreipe, C. Liedtke, N. Marschner, V. Mobus, H. Scheithauer, A. Schneeweiss, C. Thomssen, S. Loibl, M.W. Beckmann, J.U. Blohmer, S.D. Costa, T. Decker, I. Diel, P.A. Fasching, T. Fehm, W. Janni, H.J. Luck, N. Maass, A. Scharl and M. Untch, 14th St. Gallen International Breast Cancer Conference 2015: Evidence, Controversies, Consensus - Primary Therapy of Early Breast Cancer: Opinions Expressed by German Experts, *Breast Care (Basel)* **10** (2015), 211-9.
[31] J. Diessner, M. Wischnewsky, M. Blettner, S. Hausler, W. Janni, R. Kreienberg, R. Stein, T. Stuber, L. Schwentner, C. Bartmann and A. Wockel, Do Patients with Luminal A Breast Cancer Profit from Adjuvant Systemic Therapy? A Retrospective Multicenter Study, *PLoS One* **11** (2016), e0168730.
[32] C.M. Perou, T. Sorlie, M.B. Eisen, M. van de Rijn, S.S. Jeffrey, C.A. Rees, J.R. Pollack, D.T. Ross, H. Johnsen, L.A. Akslen, O. Fluge, A. Pergamenschikov, C. Williams, S.X. Zhu, P.E. Lonning, A.L. Borresen-Dale, P.O. Brown and D. Botstein, Molecular portraits of human breast tumours, *Nature* **406** (2000), 747-52.
[33] A. Prat and C.M. Perou, Deconstructing the molecular portraits of breast cancer, *Mol Oncol* **5** (2011), 5-23.

Parameter	*n*[c]	Meta (%)	*Cox P-value*	Hazard Ratio[e]	95% CI[a]	AUC / *P-value*	95% CI[a]	*Accuracy* (%)
Age								
Under CP	19	5	0.16 [b] 0.06 [c]	- 5.3	- 1.5 - 28.8	0.57 / 0.22 [b]	0.47 - 0.68	- [b] 37 [c]
Above CP	114	28	0.16 [d]	1.6	0.80 - 3.7			56 [d]

Tables

Histologic grade								
1	58	14						
2	39	32	0.11 [c]	1.4	0.92 - 2.0	0.59 / 0.15 [c]	0.48 - 0.70	54[c]
3	36	26						
pT								
1	75	15	0.002 [b]	-	-			- [b]
			0.001 [c]	3.9	1.9 - 7.8	0.65 / 0.003 [b]	0.56 - 0.76	71 [c]
2	58	37	0.005 [d]	2.7	1.4 – 5.9			62 [d]
ER								
Under CP	102	29	0.02 [b]	-	-			- [b]
				2.7	1.2 - 6.1	0.58 / 0.20 [b]	0.45 - 0.70	69 [c]
Above CP				1.5	0.72 - 3.5			54 [d]
bFGF								
Under CP	41	39	0.15 [b]	-	-			- [b]
			0.003 [c]	0.35	0.18 - 0.74	0.64 / 0.02 [b]	0.52 - 0.76	72 [c]
Above CP	92	19	0.03 [d]	0.46	0.19 - 0.96			57 [d]

	***P*-value[a]**	HR	95% CI[a]
ER	0.001	3.3	1.7 – 7.4
bFGF	0.001	0.32	0.14 –0.63

Table 1 The prognostic performance of clinicopathological, ER and bFGF features

[a] bootstrap corrected
[b, c, d] type of data: [b]*continuous,* [c]*categorized by outcome,* [d]categorized by data
Abbreviations: n = number of patients; CP = cutpoint; pT = pathological tumour size; meta = with an actual metastasis occurrence; CI = confidence interval.

Table 2. Multivariable Cox proportional hazards regression analysis

[a] bootstrap corrected
Abbreviations: HR = hazard ratio; CI = confidence interval; ER estrogen receptor.

Figure caption

Fig. 1. Kaplan-Meier analysis of the prognostic performance of pT and bFGF. (A) pT categorized by the clinical criteria of pT = 1 for tumor size <2cm and pT = 2 for tumor sizes 2-5

cm. This categorization almost ideally overlapped with the outcome-based cutpoint of 19 mm. (B) bFGF values categorized by the outcome-oriented approach. Cases classified as the low-risk are plotted on the black solid line and the high-risk on the dotted line. The numbers of patients and incidence of metastasis occurrence in the high- and low-risk groups are listed beneath the curves. The time in months refers to the interval from primary breast tumor surgery until the occurrence of the first distant metastasis; *P*-values were calculated by the log-rank test (Mantel–Cox).