Fractal Analysis of Breast Tumour Microscopic Images in Prognosis of Distant Metastasis Risk

JELENA PRIBIC[1], JELENA VASILJEVIC[*, 2], KSENIJA KANJER[1], NEBOJSA T. MILOSEVIC[3], DRAGICA NIKOLIC VUKOSAVLJEVIC[1] and MARKO RADULOVIC[1]

[1] Department of Experimental Oncology
Institute of Oncology and Radiology of Serbia
Pasterova 14, Belgrade
SERBIA

[2] Institute "Mihajlo Pupin"
Volgina 15, Belgrade
SERBIA

[3] Department of Biophysics
School of Medicine, University of Belgrade
Visegradska 26/2**,** Belgrade,
SERBIA

*jvasiljevic74@gmail.com

Abstract: Invasive breast cancer exerts an exceptional outcome heterogeneity. For this reason, highly accurate tools for prognosis of distant metastasis are necessary for effective therapeutic management. However, current markers still exert insufficient accuracy thus prompting the need for novel prognostic strategies. Based on this pressing need and the fact that primary tumour of patients with early breast cancer is the main source of information for the individual characterization of this disease, we aimed to explore the prognostic value of tumour histology image analysis. This retrospective study was done on a group of 92 patients, selected on the basis of invasive breast cancer diagnosis without systemic treatment. Median follow-up was 147 months. The standard box-counting fractal analysis was performed on digital images of primary tumour tissue sections stained with haematoxylin/eosin. Patients with low fractal dimension had significantly higher risk of distant metastasis (57%) than those with high fractal dimension (24%), $p < 0.001$. Log rank (Mantel Cox) regression analysis and Kaplan Meier plots revealed that the fractal dimension equalled or exceeded the prognostic performance of the most significant clinicopathological prognostic parameters such as pathological tumour size (P=0.004) and even the clinicopathological Adjuvant! composite score (P=0.004). This is the first report of association between fractal dimension of the whole H&E tumour tissue microscopic histology and disease outcome. Fractal analysis of the primary breast tumour histology may thus provide a simple, cost-effective and highly prognostically significant indicator of distant metastasis risk. High clinical relevance of the obtained results is based on the role of prognosis in early breast cancer treatment decisions and the resulting impact on quality of life and survival.

Key-Words: breast cancer; prognosis; histology; chemotherapy; fractal dimension; multifractal

1 Introduction

Fractal geometry was formulated by mathematician Benoît Mandelbrot as he extended the existing concepts of fractional dimensions for geometric patterns in nature whose complex geometry cannot be characterized by traditional Euclidean geometry. Its capacity to describe the irregular shapes renders it particularly useful in medicine for the quantitative characterization of complex normal and pathological microscopic tissue structures [1-3]. In the field of breast cancer research, direct association was demonstrated between fractal dimension and breast tumour grade, suggesting that

fractal geometry may be a good surrogate measure of tumour differentiation [4].

The primary tumour of patients with early breast cancer is the main source of information for critical clinical decisions such as the assessment of the risk of distant metastasis and the choice of systemic treatment. Application of fractal geometry to analysis of primary tumours is thus a major opportunity in cancer research. Fractals cannot be measured in traditional ways because they are not limited to geometric patterns. The main tool of fractal geometry is fractal dimension, describing the morphologic complexity of the images. As an orientation, a fractal dimension of the straight line has a value of FD=1, while the more complicated structures with rough edges have values of FD>1 [5]. It is estimated by three classes of methods: box-counting, fractional Brownian motion and area measurement.

2 Problem Formulation

Death by breast cancer is mainly caused by a metastatic relapse at distant sites. For that reason, besides local surgery and radiotherapy, patients are also administered postoperative systemic therapy with an aim to reduce the risk of recurrence and death through eradicating distant metastatic deposits. The downside of this strategy is that patients which do not develop distant metastases needlessly suffer from systemic toxic side effects of chemotherapy [6]. However, such therapeutic bias towards over-treatment is still necessary due to a high prognostic variability of the current risk prognosis based on standard clinicopathological features such as tumour size, nodal status, metastasis (TNM stage), histological grade, steroid receptor status, age and menopausal status [7; 8]. Risk prognosis has been a field of intensive research in the past decade with a focus on molecular prognosticators including transcriptional profiling [9], microRNA analysis [10], detection of circulating tumour cells in blood [11], proliferation [12] and stem cell markers [13]. These novel prognostic tools often outperform the established clinicopathological prognosticators, but regrettably still exhibit high prognostic variability with consequent uncertain therapeutic guidance. Taken together, there is an urgent need for the improvement of risk assessment in breast cancer.

3 Problem Solution

In parallel to the mentioned molecular approaches, digital pathology emerges as a structure analysis tool to aid in detection, diagnosis and prognosis of cancer [12]. It is based on computational analysis of medical images by use of fractal or texture algorithms, with proven diagnostic and prognostic value in mammography [14], breast ultrasound [15], and breast magnetic resonance imaging [16].

Surprisingly, image analysis has rarely been implemented for characterization of tumours at the microscopic histology level [2]. Only two studies exploited fractal image analysis on breast tumour microarray cores for prognostic purposes [4; 5]. This study therefore approached the problem of prognosis improvement in breast cancer by exploring the prognostic value of the tumour histology fractal dimension. In order to avoid interference of chemotherapy with metastasis occurrence, the study was done on a group of low-risk patients that did not receive any systemic treatment.

3.1 Methods

This report was written according to REMARK recommendations for tumour marker prognostic studies [17]. Selection of invasive breast tumour histology samples was retrospective, based on the absence of hormonal, chemotherapeutic or any other systemic treatment that could interfere with the natural course of metastasis occurrence. The prospective power calculation was based on a pilot experiment which included 35 patients. For a target power of 0.9, standard deviation of 0.75, minimal effect size of 0.4>HR>2.5, alpha 0.05 and an event rate of 30%, the required numbers were 44 patients and 19 events (powerSurvEpi package R software). In spite of the rarity of systemically untreated patients and avoidance of missing data the final sample size was 92, twice the minimal number that was provided by the power calculation. Median follow up time by reverse Kaplan-Meier method was 147 months, ranging between 77 - 165 months, with the follow-up ending in February 2007.

Samples were obtained from surgically removed invasive primary breast tumours. Tissue was formalin-fixed, paraffin-embedded and cut to produce 4 μm whole

sections which were mounted on slides, deparaffinised and rehydrated. Storage time of archival embedded samples was up to 1 month. Slides were stained with haematoxylin-eosin (H&E). Representative histological sections for each tumour were chosen by a pathologist. Digital microscopic images were acquired at x400 magnification using Olympus BX-51 light microscope and a mounted Olympus digital camera. At least five representative non-overlapping colour images were captured from each section in 3638 × 2736 pixel resolution and saved as TIFF.

Fractal dimension (FD) is the main fractal parameter used in image analysis. Standard non-overlapping box counting was employed by use of the freely available ImageJ software (http://imagej.nih.gov/ij/) and its FracLac plugin. The the average FD from multiple box counting scans, each delivering its own FD, was used for calculations.

Continuous variables were categorized by use of "optimal" cutpoints which define the two groups with minimal *P*-value, calculated by X-tile 3.6.1 software. Survival curves for disease free interval were plotted according to the method of Kaplan and Meier. Associations between available categorized variables and the occurrence of distant metastases were assessed for statistical significance using a log -rank test. Spearman's rank correlation test was employed to calculate the correlation between variables. Analyses were performed by SPSS version 20 (Chicago, IL). Adjuvant! Online score for Breast Cancer (Version 8.0) was calculated at https://www.adjuvantonline.com/breastnew.jsp as a 10 year risk of relapse with no additional therapy based on age, tumour grade, ER status and tumour size.

3.2 Results and Discussion

This study was performed on patients with a natural course of disease due to interference of systemic therapy with metastasis occurrence. Such patients are increasingly difficult to find due to a trend of widening systemic therapy use [18]. The patient group was assembled from a period of over 20 years ago when low metastasis risk patients were not prescribed systemic therapy at our institution. Lower risk classification was based on tumour grade < 3, pT < 3 and absence of lymph node metastases.

Based on the established ability of fractal geometry to quantify irregular structures of tumour microscopic histology, we hypothesized that this methodological approach could provide a valuable addition to existing clinicopathological and molecular prognosticators in breast cancer.

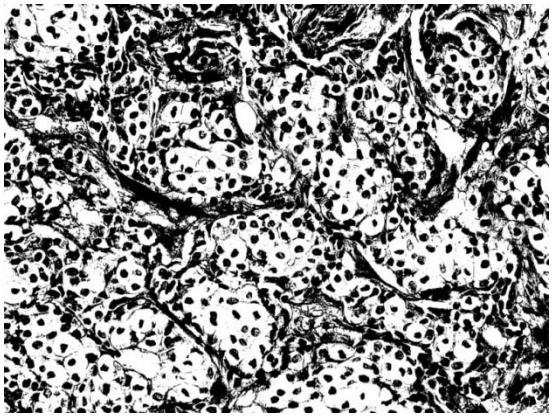

Fig.1 shows an example of a binarized tumour H&E histology image under x400 magnification as analysed by box counting in order to calculate the fractal dimension. All analysed images represented areas of tumour tissue that are predominantly populated by malignant cells as shown on Fig.1 where foci of malignant cells spread throughout the image are clearly visible.

Of the clinicopathological parameters, a significantly association with metastasis risk was calculated for pathological tumour size (pT), age (55 years cutpoint), ER status (51 fmol/mg cutpoint) and Adjuvant! Online score (Table 1, Fig.2).

Log rank test also indicated a significant negative association of fractal dimension with the risk of distant metastasis (Table 1 and Fig.2). Fractal dimension of pan-cytokeratin stained tumour tissue sections was previously demonstrated to associate with disease specific survival in breast cancer patients [4]. Pan-cytokeratin antibody stains epithelial cells and thus focuses the fractal analysis primarily on the shapes of malignant tissue. Our study confirms the prognostic value of fractal dimension of this single previous investigation [4] in a different experimental setting. The discrepancy with the previous report is that our results indicate a negative association of the fractal dimension with outcome [4]. This divergence may be explained by a different staining of tissue

sections, as our analysis was performed on unspecifically stained H&E tissue sections, while the preceding investigation used specific staining with pan-cytokeratin. Another difference between the studies is that we only included patients without systemic treatments. Our current results are the first to indicate the association between the fractal dimension of whole H&E tumour tissue microscopic histology and disease outcome. The particular practical advantage of H&E tissue section analysis lies in cost efficiency, as such histology samples are routinely produced following a biopsy or tumour extraction surgery for standard pathological diagnostic and prognostic assessments, without any need for imunohistochemical staining and associated expenditure for chemicals.

Table 1 Clinicopathological and fractal prognostic variables

Parameter	*n*	Meta (%)	***P** value**	correlation coefficient
pT				
≤ 2 cm	55	18	**0.004**	**-0.27**
2-5 cm	37	46		
Menostatus				
pre	20	30	0.98	0.14
peri and post	72	29		
Age				
<55	37	22	**0.16**	0.02
>55	55	35		
Grade				
1	7	29	0.97	0.12
2	85	33		
ER				
<51 fmol/mg	61	20	**0.003**	0.15
≥51 fmol/mg	31	48		
ADJUVANT!				
0	66	21	**0.004**	0.14
1	26	50		
Fractal Dimension				
0	14	57	**0.001**	1.0
1	78	24		

* log-rank (Mantel Cox)
** Spearman rank order correlation

This is a tremendous cost-advantage over the widely used and expensive gene expression profiling prognostic tools such as Oncotype DX and MammaPrint [19]. Remarkably, even these expensive tests actually result in cost-effectiveness through improved low-risk stratification and the consequent reduction in chemotherapy utilization [9].

Fractal dimension correlates significantly with the pathological tumour size, but with a low coefficient of -0.27 while it does not significantly correlate with other available clinicopathological parameters (Table 1). Correlation results suggest a possible prognostic independence of the fractal dimension which is beneficial for its prognostic value.

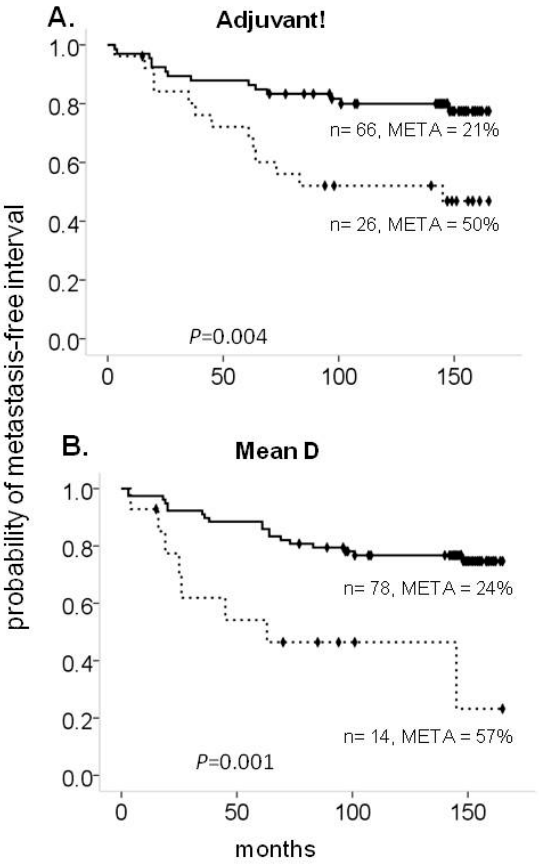

Fig.2 Probability of disease-free interval was stratified according to negative or positive fractal dimension and Adjuvant! score status for comparison of their prognostic performance. Patients were divided into poor and good prognostic subgroups according to a cutpoint with minimal *P*-value. The outcome of interest was the time interval to an event, from primary breast tumour surgery until the first distant metastasis occurrence, noted in months. The solid line designates positive and the dashed line negative fractal dimension status.

High fractal dimension and structural complexity of H&E stained tumour sections thus indicates low distant metastasis risk, in disagreement with the fact that high tumour grade also reflects high histomorphological disorder and complexity but associates with high risk [5]. Fractal dimension and tumour grade thus both indicate increased disorder whilst providing opposite prognosis. A possible explanation for this discrepancy is

ISBN: 978-1-61804-266-8

that these two parameters depend on different types of risk clues. We assume that prognostic value of fractal analysis derives from its capacity to extract yet unidentified microscopic qualities of a tumour, such as tissue growth patterns, signs of apoptosis, occurrence and distribution of mitotic cells, vascularization and cellularity, in a different way when compared to the visual grading by a pathologist [20-22]. Although the exact fractal risk clues remain unknown, in addition to risk prognostication, this type of analysis may become a useful tool for identification of novel visually discernible structural signs of cancer risk.

As judged by *P*-values of a Log-rank test and Kaplan Meier survival curves (Table 1 and Fig.2), the prognostic performance of the fractal dimension (P=0.001) equalled or exceeded the most significant clinicopathological prognostic parameters, such as pathological tumour size (P=0.004) and even the clinicopathological Adjuvant! composite score (P=0.004).

4. Conclusion

Improvement of breast cancer risk prognosis is relevant for individualized chemotherapy decisions. High clinical relevance of prognostic indicators stems from a major impact of chemotherapy on quality of life and survival. This study indicates for the first time the prognostic value of the fractal dimension measured from standard whole H&E breast tumour histology sections. The patient group employed in this study is particularly well suited for assessment of prognostic markers as no systemic treatment which could interfere with the occurrence of distant metastases was applied. The potential clinical use of the described fractal dimension for prognostic purposes is based on its association with distant metastasis occurrence and its cost-effectiveness which is derived from rapid analysis of standard clinical material.

References

[1] Pantic I, Basta-Jovanovic G, Starcevic V, Paunovic J, Suzic S, Kojic Z, Pantic S, Complexity reduction of chromatin architecture in macula densa cells during mouse postnatal development. *Nephrology (Carlton)* 18**,** 2013, 117-124.

[2] Pantic I, Nesic D, Stevanovic D, Starcevic V, Pantic S, Trajkovic V, Effects of ghrelin on the structural complexity of exocrine pancreas tissue architecture. *Microsc Microanal* 19**,** 2013, 553-558.

[3] Braverman B, Tambasco M, Scale-specific multifractal medical image analysis. *Comput Math Methods Med* 2013**,** 2013, 262931.

[4] Tambasco M, Eliasziw M, Magliocco AM, Morphologic complexity of epithelial architecture for predicting invasive breast cancer survival. *J Transl Med* 8**,** 2010, 140.

[5] Tambasco M, Magliocco AM, Relationship between tumor grade and computed architectural complexity in breast cancer specimens. *Human Pathology* 39**,** 2008, 740-746.

[6] Lonning PE, Knappskog S, Staalesen V, Chrisanthar R, Lillehaug JR, Breast cancer prognostication and prediction in the postgenomic era. *Annals of Oncology* 18**,** 2007, 1293-1306.

[7] Edge SB, American Joint Committee on Cancer., American Cancer Society. (2010) *AJCC cancer staging handbook : from the AJCC cancer staging manual*, 7th edn. New York: Springer

[8] Guiu S, Michiels S, Andre F, Cortes J, Denkert C, Di Leo A, Hennessy BT, Sorlie T, Sotiriou C, Turner N, Van de Vijver M, Viale G, Loi S, Reis-Filho JS, Molecular subclasses of breast cancer: how do we define them? The IMPAKT 2012 Working Group Statement. *Annals of Oncology* 23**,** 2012, 2997-3006.

[9] Rouzier R, Pronzato P, Chereau E, Carlson J, Hunt B, Valentine WJ, Multigene assays and molecular markers in breast cancer: systematic review of health economic analyses. *Breast Cancer Research and Treatment* 139**,** 2013, 621-637.

[10] Perez-Rivas LG, Jerez JM, Carmona R, de Luque V, Vicioso L, Claros MG, Viguera E, Pajares B, Sanchez A, Ribelles N, Alba E, Lozano J, A microRNA Signature Associated with Early Recurrence in Breast Cancer. *PLoS One* 9**,** 2014, e91884.

[11] Neumeister V, Agarwal S, Bordeaux J, Camp RL, Rimm DL, In situ identification of putative cancer stem cells by multiplexing ALDH1, CD44, and cytokeratin identifies breast cancer patients with poor prognosis. *American Journal of Pathology* 176**,** 2010, 2131-2138.

[12] Laurinavicius A, Plancoulaine B, Laurinaviciene A, Herlin P, Meskauskas

R, Baltrusaityte I, Besusparis J, Dasevi Ius D, Elie N, Iqbal Y, Bor C, Ellis IO, A methodology to ensure and improve accuracy of Ki67 labelling index estimation by automated digital image analysis in breast cancer tissue. *Breast Cancer Res* 16**,** 2014, R35.

[13] Giordano A, Gao H, Anfossi S, Cohen E, Mego M, Lee BN, Tin S, De Laurentiis M, Parker CA, Alvarez RH, Valero V, Ueno NT, De Placido S, Mani SA, Esteva FJ, Cristofanilli M, Reuben JM, Epithelial-mesenchymal transition and stem cell markers in patients with HER2-positive metastatic breast cancer. *Mol Cancer Ther* 11**,** 2012, 2526-2534.

[14] Mohd Khuzi A, Besar R, Wan Zaki W, Ahmad N, Identification of masses in digital mammogram using gray level co-occurrence matrices. *Biomed Imaging Interv J* 5**,** 2009, e17.

[15] Gomez W, Pereira WC, Infantosi AF, Analysis of co-occurrence texture statistics as a function of gray-level quantization for classifying breast ultrasound. *IEEE Transactions on Medical Imaging* 31**,** 2012, 1889-1899.

[16] Holli K, Laaperi AL, Harrison L, Luukkaala T, Toivonen T, Ryymin P, Dastidar P, Soimakallio S, Eskola H, Characterization of breast cancer types by texture analysis of magnetic resonance images. *Academic Radiology* 17**,** 2010, 135-141.

[17] Altman DG, McShane LM, Sauerbrei W, Taube SE, Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK): explanation and elaboration. *PLoS Med* 9**,** 2012, e1001216.

[18] Mirza AN, Mirza NQ, Vlastos G, Singletary SE, Prognostic factors in node-negative breast cancer: a review of studies with sample size more than 200 and follow-up more than 5 years. *Annals of Surgery* 235**,** 2002, 10-26.

[19] Stec J, Wang J, Coombes K, Ayers M, Hoersch S, Gold DL, Ross JS, Hess KR, Tirrell S, Linette G, Hortobagyi GN, Fraser Symmans W, Pusztai L, Comparison of the predictive accuracy of DNA array-based multigene classifiers across cDNA arrays and Affymetrix GeneChips. *J Mol Diagn* 7**,** 2005, 357-367.

[20] Pantic I, Pantic S, Basta-Jovanovic G, Gray level co-occurrence matrix texture analysis of germinal center light zone lymphocyte nuclei: physiology viewpoint with focus on apoptosis. *Microsc Microanal* 18**,** 2012, 470-475.

[21] Losa GA, Castelli C, Nuclear patterns of human breast cancer cells during apoptosis: characterisation by fractal dimension and co-occurrence matrix statistics. *Cell and Tissue Research* 322**,** 2005, 257-267.

[22] Huang W, Li X, Chen Y, Chang MC, Oborski MJ, Malyarenko DI, Muzi M, Jajamovich GH, Fedorov A, Tudorica A, Gupta SN, Laymon CM, Marro KI, Dyvorne HA, Miller JV, Barbodiak DP, Chenevert TL, Yankeelov TE, Mountz JM, Kinahan PE, Kikinis R, Taouli B, Fennessy F, Kalpathy-Cramer J, Variations of dynamic contrast-enhanced magnetic resonance imaging in evaluation of breast cancer therapy response: a multicenter data analysis challenge. *Transl Oncol* 7**,** 2014, 153-166.

Acknowledgement This work was supported by grants 175068, III 45005 and TR 32037 from the Ministry of Education and Science of the Republic of Serbia.