Prediction and evaluation of response to breast cancer chemotherapy by use of multifractal analysis

Jelena Vasiljevic, Jelena Pribic, Ksenija Kanjer, Wojtek Jonakowski, Jelena Sopha, Dragica Nikolic
Vukosavljevic and Marko Radulovic

Abstract—Neoadjuvant chemotherapy increases survival of patients with locally advanced breast cancer. Very accurate predictors of chemotherapy response are essential for effective chemotherapeutic management due to the pronounced individual heterogeneity in breast cancer. Predictive molecular determinants for conventional chemotherapy are only emerging and still incorporate a high degree of predictive variability. Taken together, there is a pressing need for improvements of predictive performance. We addressed this issue by exploring the value of tumour histology image analysis as a novel tool for prediction and evaluation of chemotherapy response. Fractal analysis was applied to hematoxylin/eosin stained archival diagnostic breast tumour biopsies derived from 106 patients diagnosed with invasive breast cancer and treated with anthracycline. Based on the results it is concluded that multifractal analysis of breast tumour tissue prior to chemotherapy can predict the pathological complete response, partial pathological response and progressive/stable disease with accuracies ranging from 82% - 91%. Multifractal comparison of tumour sections before and after chemotherapy, also based on 350 representative histology images for each group, has achieved a discrimination accuracy between the groups of 73%. This study indicates for the first time the potential value of multifractal analysis as a simple and cost-effective quantitative clinical tool for prediction and evaluation of chemotherapy response.

Keywords—breast cancer, chemotherapy, histology, prediction

I. INTRODUCTION

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 the aim to reduce the risk of recurrence and death through eradicating distant metastatic deposits. Patients with advanced breast cancer are additionally administered a preoperative (neoadjuvant) chemotherapy. Due to the high heterogeneity of breast cancer, the individual sensitivity to a particular chemotherapy is also highly heterogeneous. By determining the right treatment for each individual patient, predictive markers reduce relapse rates and prolong survival in invasive breast cancer.

Hormonal receptors and c-erbB2 expression present obvious markers for decisions on hormonal and erbB2-targeted therapies [1]. Unfortunately, the choice of a predictive marker is far less straightforward for conventional chemotherapy

A number of molecular and pathological predictive tools are used in both prediction and evaluation of breast cancer chemotherapy response, including the proliferation marker Ki67 [2], PET/CT imaging [3], MRI imaging [4], histopathological tumour examination, and residual tumor size [5]. Even the most recent candidate predictors of tumour chemotherapy response such as microRNAs, proliferation index, TIMP-1, Lin-28 and gene panels Oncotype DX, MammaPrint and immune-related gene signatures still exhibit predictive variability with consequent uncertain therapeutic guidance [6-8].

In parallel to these molecular predictive methods, digital pathology emerges as a tool to analyse histology images, based on the fact that morphological changes of tumour tissue reflect the sum effect of a very large number of molecular changes that may be difficult or even impossible to fully acquire and interpret by use of available molecular methods. Tumour tissue histology image analysis may thus present a convenient readout of the molecular alterations in breast cancer. Multifractal analysis is one of the digital pathology approaches, known as powerful morphometry tool for quantitative assessment of complex pathological structures [9]. However, its potential use in breast cancer therapy prediction and evaluation has not been investigated. We thus hypothesized that multifractal analysis of tumour histology may prove useful for improvement of the currently poor accuracy of chemotherapy efficacy prediction and evaluation methods. This task was approached by a neoadjuvant therapy model which has been accepted as an ideal in vivo assessment of therapy response because the tumour remains in situ throughout treatment, thus allowing the exact evaluation of the chemotherapy response [8].

II. METHODS

Criteria for the selection of patients for this retrospective study were as follows: 1) an incisional biopsy of the primary breast cancer confirming invasive carcinoma before commencing the treatment and 2) primary locally advanced breast cancer that was strictly not operable.

Prior to surgery, all patients were treated with standard anthracycline-based chemotherapy (5-
fluorouracil 500 mg/m², doxorubicin 50 mg/m² and cyclophosphamide 500 mg/m² intravenously). Breast tumour response was evaluated after chemotherapy completion by pathohistological examination of the resected surgical material including measurement of the residual tumor size, optical microscopy and immunohistohemical analysis according to recommendations of International Expert Panel [5], as previously described in detail [8].

Tumour tissue sections were cut at 5-μm thickness from the paraffin blocks and stained with haematoxylin/eosin stain as described [8]. Representative tissue sections were selected for each patient by a pathologist and digital microscopic images acquired at x400 magnification using Olympus BX-51 light microscope and a mounted Olympus digital camera. Multifractal analysis of binary digital medical images was performed by use of FracLac software and the obtained parameters subsequently analysed statistically for differences between the three groups of tissues by use of DTREG 10.3.0. A model of Single Tree of the classification type was performed.

Split-sample cross-validation was performed by randomly splitting the original sample into training set and validation sets in ten validation cycles by use of DTREG software.

### III. RESULTS AND DISCUSSION

The value of multifractal analysis in prediction and evaluation of the chemotherapy response was approached by a neoadjuvant chemotherapy (CT) model as presented on Fig. 1.

**Fig. 1** the conceptual scheme of patient group comparisons by use of multifractal analysis

Due to the fact that this study was retrospective, histology images of tumours before the therapy were divided in three response categories as indicated on Fig. 1 according to their actual response to chemotherapy: pathological complete response (pCR), partial pathological response (pPR) and progressive/stable disease (PD/SD). Significant discrimination between such groups by multifractal analysis indicates its potential in important clinical tasks of chemotherapy prediction and evaluation. Tumour samples of patients with pCR and PD/SD were not available as these patients did not undergo surgery. The pPR group underwent a tumour extraction surgery and was analysed both before and after chemotherapy in order to test the value multifractal analysis in evaluation of the tumour chemotherapy response (Fig. 1). Multifractal analysis was performed on binary black and white digital images, derived from original colour images. Examples of the typical analysed histology sections are shown on Fig. 2, indicating the absence of obvious visual differences between the three response groups before therapy. Even a detailed pathological microscopic analysis or molecular predictor tools are often unable to provide clues which could serve as the basis for a reliable prediction of chemotherapy response [2].

**Fig. 2** comparison of pre-therapy breast tumor histological images by multifractal analysis: a) pathological complete response, b) partial pathological response and c) progressive/stable disease groups.

Table 1 indicates that multifractal analysis is able to distinguish between chemotherapy response groups with good accuracy and thus may indeed become useful for the prediction of individual response to chemotherapy. Accuracy represents the percentage of times that the predicted and observed outcomes match [10]. Prediction accuracies of over 82% achieved for the three chemotherapy responder groups (Table 1) are comparable with those previously obtained by Ki67 as the standard
prediction marker [2] and the PET/CT system which even had an advantage of making predictions according to the actual response to the first cycle of chemotherapy [3].

Table 1: Comparison of chemotherapy responder groups by multifractal analysis

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<tr>
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<th>pCR</th>
<th>pPR</th>
<th>PD/SD</th>
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<tr>
<td>Accuracy (%)*</td>
<td>83/75</td>
<td>91/87</td>
<td>82/73</td>
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</table>

* training/validation groups

A ten-fold internal split-sample cross-validation was performed as the established internal indicator of model's prognostic overoptimism and stability.

Fig. 3: Comparison of the partial response group: a) before and b) after chemotherapy.

Examples of binarized histology images of the same breast tumour from the pPR group are shown on Fig. 3, prior and after chemotherapy (Figs. 3a and 3b, respectively).

It has been established that pathologic complete response (pCR) points to a favourable prognosis [11]. However, within the partial response group the prognosis of disease progression is variable and the extent of chemotherapy response is hard to determine reliably by visual microscopic inspection. We thus examined whether multifractal analysis has the potential to sub-stratify this largest group of chemotherapy responders. Table 2 indicates the potential for use of multifractal analysis in evaluation of chemotherapy response as it differentiates between tumour histology sections before and after therapy with the accuracy in the training group of 74%.

Table 2: Comparison of groups before and after chemotherapy by multifractal analysis

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<tr>
<th></th>
<th>Training</th>
<th>Validation</th>
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<td>Accuracy (%)</td>
<td>74</td>
<td>69</td>
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Multifractal analysis delivers a number of parameters, including:

$D_{\text{max}}$ - maximum of generalised fractal dimension

$f(\alpha)_{\text{max}}$ - maximum of $f(\alpha)$ multifractal spectrum

$\alpha_{\text{f max}}$ - $\alpha$ which corresponds to $f(\alpha)_{\text{max}}$

$Q_{D_{\text{max}}}$ - $Q$ which corresponds to $D_{\text{max}}$

$f(\alpha)_{\text{min}}$ - minimum of $f(\alpha)$

$\alpha_{\text{f min}}$ - $\alpha$ which corresponds to $f(\alpha)_{\text{min}}$

With many available fractal parameters, we set out to identify the one which is most based on the ability to correctly discriminate between histology images from different patient groups as on Fig. 1. This was achieved by use of variable importance classification by DTREG software. Remarkably, the most important parameters for these two tasks are different, with $f(\alpha)_{\text{max}}$ determined as crucial for the prediction and $f(\alpha)_{\text{min}}$ for the evaluation of chemotherapy response (Table 3).

Table 3: The most important multifractal parameters for the tasks of chemotherapy prediction and evaluation

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<th></th>
<th>Prediction</th>
<th>Evaluation</th>
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<tbody>
<tr>
<td>Parameter</td>
<td>$f(\alpha)_{\text{max}}$</td>
<td>$f(\alpha)_{\text{min}}$</td>
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Interpretation of this result is based on the fact that multifractal analysis describes structure features from both local and global points of view. The local regularity is described by the Hölder exponent ($\alpha$) while global regularity is reflected in the multifractal spectrum $f(\alpha)$ which describes the distribution of $\alpha$ [12]. High values of Hölder exponent ($\alpha$) reflect high local changes of the observed structure around a given point [13]. $F(\alpha)_{\text{max}}$ and $f(\alpha)_{\text{min}}$ are the parameters of the multifractal spectrum, with $f(\alpha)_{\text{max}}$ denoting the fractal dimensions with the maximum probability, while $f(\alpha)_{\text{min}}$ denotes the rarest value of $\alpha$. In view of these facts and the variable importance classification result it can be concluded that prediction and evaluation of chemotherapy response by multifractal analysis are based on distinct global properties of the tumour histology image.

The importance of quantitative imaging for the prediction of chemotherapy sensitivity is based on the fact that improvements in predicting chemotherapy complete response versus non-complete response allow more optimal chemotherapy decisions, thus affecting the quality of life and survival. On the other hand, improvements of the evaluation of chemotherapy effects facilitate clinical testing of new chemotherapeutics and enable more accurate prognosis of survival.
We assume that prognostic value of multifractal analysis derives from its capacity to extract yet unidentified microscopic qualities of a tumour. It may be speculated that among such known clues are the signs of apoptosis, occurrence and distribution of mitotic cells, vascularization, cellularity, tissue growth patterns and probably other unknown qualities [14-16]. A similar set of histological clues may also be responsible for a difference in multifractal scoring of pre- and post-chemotherapy histological images.

IV. CONCLUSION

Improvements of prediction and evaluation of chemotherapy efficacy are of high clinical relevance due to the major impact of chemotherapy on quality of life and survival. We hypothesized that multifractal analysis could provide a valuable addition to existing clinicopathological and molecular prognosticators, based on its powerful discriminating morphometric quantitative description of the irregular structures typical of tumour growth. It is here shown for the first time that multifractal analysis of breast tumour tissue has a potential of aiding both in the prediction and evaluation of response to chemotherapy. The two effects were based on the recognition of distinct global fractal properties of the tumour histological image. Cost-effectiveness of this method derives from rapid analysis of standard clinical material and presents a clear advantage over the methods which are currently used for these clinical tasks.

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