Evaluation of Anti-vascular Therapy with Texture Analysis

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Abstract. Background: Texture analysis can be used to identify and discriminate between objects in an image and has already been used for the characterization of diseases. The present study evaluated the potential of texture analysis for monitoring tumor cell viability during treatment with an anti-vascular drug. Materials and Methods: Texture analysis of T2-weighted magnetic resonance images of C3H mammary carcinomas in mice was used to study tumor cell viability during treatment with the anti-vascular drug combretastatin A-4 disodium phosphate (CA4DP). Texture analysis was done by MaZda®, and boot strap statistics were used to analyze the results. Results: The results revealed significant differences in the textures of T2-weighted images when non-tumor tissue and tumor tissue were compared. Furthermore, selected texture parameters were significantly different when image data from before and after CA4DP administration were analyzed. Conclusion: Texture analysis is helpful in the diagnosis of different types of tissues, especially when these tissues can not be recognized by human observers. Some functional changes, such as perfusion changes in the tumor, can also be detected by texture analysis.

Magnetic resonance imaging (MRI) is one of the most important ways to evaluate the viability of tumor cells following anti-vascular drug therapy, and allows for the monitoring of diseased tissue in a non-invasive and non-destructive fashion, especially following administration of a contrast agent. However, human observers can only recognize a limited part of the textural information in an image and, subsequently, clinical diagnosis may become inadequate. Digital texture analysis, based on advanced mathematical and statistical methods (3, 4), could supplement the work of radiologists.

Texture analysis can be used to identify and discriminate between objects in an image (4). Different methods for the quantification of textures related to spatial variations in an image are available and can be divided into statistical or structural approaches. The first method, the one employed here, includes co-occurrence matrices for the spatial distribution of gray tones, mean and variance values of the pixel intensity distribution, gradient parameters, run-length matrix parameters, etc. (5). Because histological alterations may result in texture changes in the MR image, texture analysis has already been successfully used for the characterization of diseased skeletal muscle (6), multiple sclerosis (7), macroscopic lesions and microscopic abnormalities in the hippocampus (8), for the monitoring of cell therapy in vivo (9), assessment of regional demyelination and remyelination in mouse brain (10) and for automatic segmentation in the cerebellum (11), tibia, femur and knees (12). Also, it has been applied to the classification of pathological tissues from liver, thyroid, breasts, kidneys, prostate, heart, brain and lungs (13-21).

The present study evaluated the potential of texture analysis for monitoring tumor cell viability during treatment with an anti-vascular drug.

Materials and Methods

Animal model. C3H mouse mammary carcinomas were implanted in the right rear foot of 10 to 14-week-old-female CDF1 mice. The maintenance of this tumor type has been described in detail.
elsewhere (22). The experiments were started approximately 3 weeks after implantation, when the tumor volume had reached 200±50 mm³. The tumor volume was determined according to the formula: D1×D2×D3×n/6, where D1, D2 and D3 represent three orthogonal diameters. The animals were awake during the complete procedure, but restrained in a plastic jig. The right foot was exposed and secured with tape, and the tail was put through a hole in the rear of the jig, helping to secure the mouse in a fixed position. The tumor was positioned inside a 9-mm-diameter radiofrequency surface coil. A 25-G butterfly needle connected with a polythene tube (Ø: 0.38 mm) was inserted in the tail vein.

Furthermore, an 18-G venflon connected with a polythene tube (Ø: 0.38 mm) was inserted i.p. Body temperature was maintained at 37±0.5°C throughout the experiment (rectal temperature was monitored) using a plastic tube with circulating warm water that was wrapped around the body. MRI was performed 30 min after positioning the mouse in the magnet.

Thirteen mice were divided into two groups: i) eight mice received 250 mg/kg combretastatin A-4 disodium phosphate (CA4DP); ii) five mice served as a control group and received saline only. Both the solutions were administered at a dosage of 0.02 ml/kg. CA4DP was kindly provided by the manufacturer (Oxigene Europe AB, Lund, Sweden). The drug was dissolved in 0.9% NaCl and stored in the dark at +4°C and used within a week.

T₂ measurements were performed at 20, 80, 140, 200, 260, 320, 380 min following administration of CA4DP or saline, and perfusion weighted images were acquired at 10, 90, 150, 210, 270, 330, 390 min following administration of CA4DP. The perfusion measurements were conducted by administering 0.2 mmol/kg body weight Gadolinium-DTPA (Omniscan; Nycomed, Copenhagen, Denmark) rapidly into the tail vein. The repeated perfusion measurements were performed with the assumption that no Gd-DTPA persisted in the blood stream from the prior measurement 60 min earlier.

The experimental protocol has been approved by the local committee for animal welfare and experiments conformed to Danish and European Union approved guidelines for animal welfare.

Magnetic resonance imaging. A 7 Tesla horizontal bore magnet (Oxford Instruments, Oxford, UK) interfaced to a Unity Inova console (Varian Inc, Palo Alto, CA, USA) and equipped with a 12.5 Gauss/cm gradient system (Tesla Engineering Limited, West Sussex, UK) were used for MRI. A home-made 9-mm-diameter double-turn radiofrequency surface coil served both as transmitter and receiver.

T₂-weighted images were acquired with a spin-echo sequence: TR=2.3 s, TE=0.05 s, slice thickness= 2 mm, 128x128 data matrix, 1 slice, coronal orientation and field-of-view 3×3 cm². Two images were averaged.

Perfusion weighted imaging was performed with a T₁-weighted gradient echo sequence using the following parameters: TR= 0.03 s, TE=0.008 s, flip angle=40°, 64x64 data matrix, 1 slice, coronal orientation and field-of-view=3×3 cm². Quantitative perfusion values were obtained using Peters' method (23) given as relative blood flow (rBF) with units of ml/s/cm³.

Texture analysis. MaZda® (Michal Strzelecki and Piotr Szczypinski, Institute of Electronics, Technical University of Lodz, Poland) was used for texture analysis, facilitating the calculation of more than 300 texture parameters for each digital image. MaZda allows for the definition of different regions of interest (ROI) corresponding, for example, to different tissue types (as classified by the user). Texture parameters describe a variety of features such as histogram of pixel intensity distributions, absolute gradient, run-length matrix and wavelet analysis (5). With the large number of texture parameters provided by MaZda, it is, in practice, necessary to select a subset of these. Therefore, a parametric vector, comprising only the features that discriminate most effectively between the textures, is automatically produced. One method of such feature reduction is based on maximization of the Fisher coefficient, which is defined as the ratio of between-class variance to within-class variance and another one is based on low classification error probability and average correlation coefficients between the chosen features (5). In this study, the two texture parameters providing the best classification of the data are presented.

In all animals, ROIs representing tumor tissue and non-tumor tissue were selected separately (Figure 1). The non-tumor tissue represented primarily muscle tissue on the calf of the mouse as well as bone of tibia or fibula. The tumor tissue was analyzed by dividing the tissue into: i) whole tumor ROI; ii) a region near the edge of the tumor with high intensity, defined as the tumor periphery; and finally iii) the tumor center ROI characterized by low signal intensity (Figure 1). Care was taken to select ROIs of the non-tumor tissue, tumor periphery and the central part of the tumor of approximately equal size.

The texture parameter values calculated for each time-point through more than 6-h examination were normalized by adjusting the texture parameters in the image recorded before the injection of saline or CA4DP to zero. The intensities in the following post-injection images were adjusted according to this.

Statistics. To reduce the risk of coincidental significance errors (type 1) given the large number of texture parameters, the number of texture samples were artificially increased using the bootstrap procedure (24, 25). The principle of bootstrapping is to generate a

![Figure 1. Selection of regions of interest (ROI). The non-tumor tissue includes muscle, bone, tibia etc. The edges of the tumor with high intensity were selected as the peripheral part, while the low intensity in the center area was defined as the central part.](image-url)
large amount of samples by re-sampling the original data with replacement. From the new data set, it is therefore possible to calculate confidence intervals for various estimators, without analytical knowledge of their distribution, using histogram-based methods. In our case, we performed the t-test statistics for comparison of two populations to obtain a confidence interval of their mean difference using a 95% confidence interval. The procedure was as follows: For each bootstrap sample, a test statistic $t^*$ was calculated:

$$t^* = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{s_1^2/n_1 + s_2^2/n_2}}$$

where $\bar{x}_1$ and $\bar{x}_2$ are the bootstrap empirical means, $\bar{\mu}_1$ and $\bar{\mu}_2$ are the empirical means of the original populations 1 and 2, $s_1$ and $s_2$ and denote empirical estimates of the standard deviations of the two populations. From the distribution of the bootstrap samples of $t^*$, the 2.5% and 97.5% quantiles were found by counting, and the 95% confidence interval was found in a standard way by using these quantiles in the place of the quantiles of the $t$-distribution.

Correlation between perfusion and Per10-% texture parameters was done using Spearman rank correlation (non-normal distribution) and correlation between perfusion and MinNorm was made using the Pearson correlation (normal distribution). The lines were drawn using a robust fitting routine. An ANOVA was used to compare the pre- and post- injection perfusion values and $p<0.05$ was considered significant. All statistical analyses were done using Matlab (The MathWorks Inc., Natick, MA, USA).

### Results

The effect of CA4DP on blood perfusion is demonstrated in the ROI covering the whole tumor in Figure 2. For mice serving as controls, there was no change after injection of saline. On the contrary, in the eight animals which received CA4DP, blood perfusion decreased significantly ($p=0.026$) at 30 min after injection. The following measurements indicated a similar low level of blood perfusion.

ROIs representing non-tumor and tumor tissue of the control group are compared in Table I. The ROIs, which a priori had been divided into tumor tissue and non-tumor tissue, were examined in order to find the two textural parameters that most effectively discriminated the two types of tissues. These parameters were 1) WaveEnLL_s-2 originating from wavelet analysis and 2) S(0.5)SumEntrp based on the sum entropy of the co-occurrence matrix. Both parameters were significantly different ($p<0.05$) when comparing the two types of tissues.

Texture parameters for the whole tumor ROI of the control group are presented in Figure 3. The examined texture parameters demonstrated no significant difference between the pre-injection values and any of the following measurements.

ROIs representing non-tumor and tumor tissue in the CA4DP group are presented in Table II and the two most discriminating texture parameters both showed a significant difference between the tumor and non-tumor tissue ($p<0.05$).

Textural parameters from the whole tumor ROIs, before and after administration of CA4DP, are plotted in Figure 4. The two textural parameters providing the largest statistical differences between the pre- and post- CA4DP injection pixel data were a) MinNorm, which is mean pixel intensity minus 3 times the standard deviation and b) Perc.10%, or the 10-5 percentile of the pixel intensity distribution. All post-injection data showed a significant change in texture parameters when compared to the pre-injection measurement.

The same texture parameters for the whole tumor ROI as presented in Figure 4 (MinNorm and Perc.10%) were correlated to the blood perfusion in the tumor (Figure 5). The MinNorm parameter plotted against perfusion showed that the changes in MinNorm were perfusion changes

<table>
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<tr>
<th>WaveEnLL_s-2</th>
<th>S(0.5)SumEntrp</th>
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<tbody>
<tr>
<td>Tumor</td>
<td>17083.73 (14240.43-19927.04) 1.07 (0.87-1.27)</td>
</tr>
<tr>
<td>Non-tumor</td>
<td>10541.4 (9164.1-11918.71)* 0.46 (0.11-0.8)*</td>
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Figure 2. tBF estimations representing the whole tumor ROI. Results are given for the two groups of mice: those receiving saline only and those receiving CA4DP. Blood perfusion decreased significantly in the latter group after CA4DP administration.

Table I. Control group. The two most discriminating texture parameters found when comparing non-tumor and tumor tissues were: 1) WaveEnLL_s-2 (a wavelet analysis parameter) and 2) S(0.5)SumEntrp (referring to the sum entropy of co-occurrence matrix). Values are presented as mean with 95% confidence interval in parentheses. *denotes significance at $p <0.05$. 

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The correlation coefficient between the Perc.10% parameters and perfusion changes was $r=-0.4513$, $p<0.05$ (Figure 5).

Tumor regional differences in texture parameters in the CA4DP group were also examined. The two most discriminatory texture parameters found in the ROI, representing tissue from the peripheral part of the tumor, are shown in Figure 6. Each of the calculated texture parameter values from the periods following CA4DP administration were significantly different from those of the pre-injection period. The two most discriminatory texture parameters from the ROIs located in the central part of the tumors are presented in Figure 7. The texture parameter in a) was significantly different the first five hours after the injection, whereas the parameter plotted in b) was significantly different only up to the first four hours after injection (only marginally different at 1 h).

Table II. The two most discriminating texture parameters found when comparing non-tumor and tumor tissue of the CA4DP group: Variance is the variance of pixel intensity distributions. *denotes significance at $p<0.05$, and $S(4,0)$Entropy refers to entropy parameters from co-occurrence matrix.

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<tr>
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<th>Variance</th>
<th>$S(4,0)$Entropy</th>
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<tr>
<td>Tumor</td>
<td>1316.68 (667.46-1965.9)</td>
<td>1.77 (1.57-1.97)</td>
</tr>
<tr>
<td>Non-tumor</td>
<td>286.34 (14.29-558.4)*</td>
<td>1.26 (1.01-1.51)*</td>
</tr>
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($r=-0.5719$ and $p<0.05$). The Per10-% parameter plotted against perfusion revealed that the changes in Perc.10% were caused by perfusion changes too. The correlation coefficient between the Perc.10% parameters and perfusion changes was $r=-0.4513$, $p<0.05$ (Figure 5).
Discussion

CA4DP is a frequently used potent anti-cancer compound that specifically targets tumor vasculature, in contrast to the more traditional therapeutic approach, where the rapidly dividing tumor cells are the target. CA4DP is a tubulin-binding agent that has been shown to depolymerize the tubulin cytoskeleton and induce apoptosis in proliferating endothelial cells (26). The loss in integrity of the endothelial cell layer is thought to result in increased permeability of the blood vessels and, thus, increasing the high tumor interstitial fluid pressure. These two factors acting together may lead to widespread vascular collapse within the tumor (27). The effect of CA4DP on tumor blood flow is thought to result from the drug specifically targeting the more penetrable endothelium and basal layer in the proliferating tumor blood vessel undergoing angiogenesis. Our perfusion measurements seemed to confirm a significant effect on the tumor blood supply by CA4DP, and this effect appeared to persist for at least six hours.

A number of earlier studies have proven texture analysis to be a useful tool in discriminating between various tissue types (6-21). This is in accordance with the present study, where non-tumor tissue could be distinguished from tumor tissue using this method. However, it should be emphasized that analysis of the image data was initiated with a supervised segmentation of the various ROI's. The next step was to find those textural parameters which were most different in one ROI as compared to another ROI. Nonetheless, the results indicate the potential of texture analysis in significantly aiding semi-automatic classification or diagnostics.

It should be noted that the texture found in MR images are the sum of complex visual patterns composed of entities, or sub-patterns, that have characteristic brightness, color, slope, size, etc. (5). The textures of MRI data reflect
complex structural and biochemical tissue properties and are also influenced by relatively fast changing physiological factors, such as blood flow and pH. Furthermore, it was assumed that, in the present study, tissue necrosis may be an important factor for the texture calculation.

In the early stage of vascular targeting therapy in tumors, a decrease in blood perfusion is the major factor causing necrosis in the tumor. The effect is probably due to increased hemodynamic pressure inside the encapsulated tumor. This phenomenon appears when the permeability of the tumor capillaries is increased even further by administration of a drug such as CA4DP, which tends to destroy the supporting matrix of the endothelium cells. Factors other than changes in blood flow (e.g. the relative distribution of intracellular and extracellular water) are probably important in determining the MR signals. However, this might be an indirect effect of blood flow decrease after administration of CA4DP. In the later phase, necrosis might take over as a more important determinant for texture parameter values. This condition, with a variety of interdependent factors, is complicated further by tumor regional differences in both flow and the extent of necrosis. The present texture parameter data of central and peripheral tumor areas indicate such regional differences.

In conclusion, texture analysis is helpful in the diagnosis of different types of tissues, especially when these tissues can not be recognized by human observers. Some functional changes such as perfusion changes in the tumor can also be detected by texture analysis.

Acknowledgements

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References
