Development and clinical applications of the time intensity curve shape analysis in dynamic contrast enhanced MRI: a pixel-by-pixel approach
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CHAPTER 8

TIC Shape and model based analysis of DCE-MRI data: a comparison in patients undergoing anti-angiogenic treatment for recurrent glioma.

Lavini C, Verhoeff JJC, Majoie CB, Stalpers LJ, Richel DJ, Maas M.

ABSTRACT

Purpose:
To compare Time Intensity Curve (TIC)-shape analysis of DCE-MRI data with model-based analysis and semi-quantitative analysis in patients with high-grade glioma treated with the anti-angiogenic drug bevacizumab.

Material and Methods:
Fifteen patients had a pre-treatment and at least one post-treatment DCE-MRI. We applied a pixel-by-pixel TIC shape analysis, where TICs are classified into 5 different types according to their shape, and calculated the occurrence of each TIC type in the ROI. The results were compared to the pharmacokinetic model (PKM) parameters $K_{\text{trans}}$, $K_{\text{ep}}$, $V_e$ and $V_i$, and with the semi-quantitative parameters Maximum Enhancement ($ME$) and Initial Slope of Increase ($ISI$).

Results:
The relative amount of type 2 and 4 TIC shape significantly correlated with the parameter $K_{\text{ep}}$ but not with $K_{\text{trans}}$ or $V_e$.
The PKM parameter $V_e$ and the semi-quantitative parameters $ME$ and $ISI$ showed significant changes after treatment. None of the TIC shapes individually showed significant changes.

Conclusion:
The semi-quantitative parameters $ME$ and $ISI$ are more sensitive to the effect of the bevacizumab than $K_{\text{trans}}$ and $V_e$. The pixel-by-pixel TIC shape analysis parameters are not sensitive to the effect of bevacizumab, though they can be seen as surrogate for the PKM parameter $K_{\text{ep}}$. 
INTRODUCTION

Dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) using small molecular weight, gadolinium-chelate based contrast media is widely accepted as a valuable diagnostic aid in cancer imaging, as a mirror of the effect of anti tumour drugs, and possibly also as a prognostic tool (1). Several analysis methods can be applied to DCE-MRI, varying from a simple by-eye observation of the time-dependent variation in signal intensity after contrast delivery, to more sophisticated methods that make use of theoretical pharmacokinetic (PK) models. The application of PK models to the analysis of DCE-MRI data allows the extraction of physiologically relevant quantities that reflect intrinsic properties of the tissue, such as vascular permeability, blood flow, extracellular-extravascular and vascular volume. Because the quantities measured reflect properties of the tissue and are independent of the MRI settings and parameters which are used to generate them, this analysis method is meant to be a truly quantitative method. First proposed, independently, by Tofts, Larsson and Brix in the early 1990 and later refined (2), this PK model is still widely used in cancer imaging: it generates the parameters $K^{\text{trans}}$ (the wash-in rate), which describes the forward leakage rate of the contrast medium, $K_{\text{ep}}$ (the wash-out constant unit), $V_e$ (the extracellular-extravascular space) and $V_i$ (the plasma volume). These PK parameters are useful in the differentiation between infective and neoplastic brain lesions (3) and glioma grading (4, 5). A plethora of different other models have been proposed (6, 7), but Tofts’ model remains the most used, because of its relative simplicity. Its most important parameter, $K^{\text{trans}}$ was proposed as a surrogate marker of the expression of VEGF in high grade glioma and of the metalloproteinase 9 in brain tuberculomas (8). Recently, it was shown to be a sensitive marker of response to the angiogenesis inhibitors bevacizumab in gliomas (9,10).

Despite the proven advantages, one of the greatest practical limitations of PK modelling in DCE-MRI, and the main reason for which it is not widely applied in routine clinical settings even 20 years after its introduction, is that its implementation is far from straightforward. Firstly, for a correct implementation of the model, the absolute contrast agent concentrations must be calculated from the MR Signal, this implying the non-straightforward calculation of the native $T_1$ of the tissue. Secondly, the results are dependent on the chosen model. Besides, noise and inadequate temporal resolution severely limit the robustness of the (non linear) fitting procedure. The choice of the arterial input function (AIF) and a number of
other parameters used in the model are pivotal (they largely determine the final results) but are at the same time very difficult to measure accurately. Furthermore, the pharmacokinetic model which should be applied to a certain patient or patient population can be more or less complex, depending on the pathology, the way of delivery of the contrast agent and the dosage, and there is no agreement yet on which model is the most suited in the various pathologies. Finally, the ability of Tofts’ model to correctly describe low molecular weight contrast kinetics in most tumours has been questioned (11).

Alternative ways are therefore still being sought. Some authors propose an improvement in the accuracy and robustness of the pharmacokinetic model (12,13,14). Others focus on simpler, model independent parameters such as the Initial Area under the Gadolinium Curve (IAUGC) (9,10,15), which has been recommended as a practical substitute for $K^{\text{trans}}$ in clinical studies (16). Recent work has investigated the value of the heterogeneity of the semi-quantitative DCE-MRI parameters (17). Other authors focus on non-quantitative, “heuristic” methods such as the analysis of the shape of the native time intensity curve (TIC) (18,19,20).

The TIC-shape analysis, in which the native MR signal intensity curve is simply determined on the basis of its shape, was probably the first ever used approach in DCE-MRI analysis (21) and has proved very useful, though not always robust and reproducible (18). The technique does not always encounter the enthusiasm of MR physicists and is sometimes referred to as “curve-ology” (22). Yet it has to be recognised that a large number of papers have been published making use of this method, which has proved particularly successful in breast imaging (21,23). Its simplicity makes it available in the daily clinical practice, where there is no time for lengthy model based analysis, and offers a quick and easily interpretable answer. Though TICs are mostly classified based on an averaged signal from a region of interest, recently different authors have proposed and implemented a pixel-by-pixel approach to the TIC classification (18,19,20), developed in order to overcome the problem of the loss of information about the heterogeneity within the ROI. This approach has the advantage of giving a birds-eye view of the DCE-MRI behaviour in the region of interest, something much valued by radiologists, who are more prone to classify lesions visually, than to use absolute measures of physiological quantities. More importantly, because tumours are physiologically heterogeneous, the TIC classification of an average region of interest (ROI) is not significant, being
unable to pick up spatial changes in the tumours and therefore to assess tumour response. The pixel-by-pixel approach was developed to overcome this problem. The other advantage of this non-quantitative pixel-by-pixel approach with respect to the model-based approach lies in the easy processing of the data, the linear fitting, and the absence of any underlying model. TIC shape analysis is therefore not dependent on assumptions, needs no conversion to absolute concentration time curves, does not require the knowledge of the native $T_1$ in the tissue, it is independent on the choice of the AIF, and is robust also in case of low signal noise ratio (SNR). It also lends itself well to the creation of parameters that arise from the heterogeneity of the pattern distribution in space, such as the percentage of a certain enhancement type. Despite the advantages, it has to be recognised that the shapes of the TIC have been defined heuristically, and it is not always clear if there is an underlying physiological difference between the observed shapes. Although the shape of the TIC is somehow linked to the values that can be obtained via PK modelling (e.g. Tofts), the relation can still be dependent on the protocol chosen.

We intended to perform a impartial comparison between the TIC shape analysis, the semi-quantitative and the model based analysis. In order to understand the value and possible limitations of the TIC shape analysis, in this work we have applied the TIC shape analysis in a study investigating the effect of the angiogenesis inhibitor bevacizumab combined with high dose temozolomide in patients with recurrent high grade glioma, and we have compared it to two common semi-quantitative parameters and with the outcome of the standard (extended Tofts’) pharmacokinetic analysis.
MATERIALS AND METHODS

Patients
Fifteen patients were enrolled in the frame of a clinical phase II study investigating the effect the angiogenesis inhibitor bevacizumab (Avastin®) on recurrent high-grade glioma. They all gave informed consent to the study, which was approved by the local medical ethical committee. The precise details of the patient population are given elsewhere (24). The original study included 23 patients, but for this study only 15 were retained. Eight patients were excluded from the present study because of the quality of the pixel-by-pixel fit of the pharmacokinetic parameters. If the PK parameters maps had more than 30% pixels where the non-linear fit to Tofts model did not converge, the scan was excluded. This was done to insure a fair comparison with the other two techniques.

Of the fifteen patients, five had grade 3 and ten had grade 4 glioma. All patients underwent successive MRI scans before and during the treatment with bevacizumab combined with high dose, metronomically scheduled temozolomide (24). The first scan was obtained three days before the administration of Avastin®, the second scan three days after the administration, and other scans were planned on the 21st and 80th day after the treatment. Not all patients underwent all the series of scans. For 2 patients 5 scans were available, for 6 patients 4 scans, for 6 patients 3 scans, and for 1 patient only 2 scans were available.

MRI Protocol
All MRI scans were performed on a 1.5 T MRI scanner (Siemens, Erlangen, Germany). A series of 2D T₁-weighted IR scans (TI= 50, 300, 600, 1500, 3000 ms, 20 slices, 230 mm, TR/TE=6000/11 ms) was performed to calculate T₁ maps. We chose this protocol over the more common multiple flip angle approach because of its higher accuracy. A 2D T₁-weighted GRE Dynamic Contrast Enhanced scan was performed during delivery of 0.1 mM/Kg contrast medium (Gadovist®) followed by a chase of 12 ml saline, delivered intravenously through an injection pump with a 20 Gauge needle at a speed of 5 ml/sec. The dynamic scan parameters (from two slightly different protocols) were as follows: 20 slices (the same as the IR scans) in the axial plane, FOV 230 mm, phase FOV 87.5%, thickness 3.0 (protocol 1) or 4.0 mm (protocol 2), 28 dynamic acquisitions, each lasting 15.5 sec seconds (protocol 1) or 13.7 (protocol 2), TR/TE= 140/3.31, flip angle 70 degrees, BW 260Hz/pix.
CHAPTER 8  TIC Shape analysis and PK modelling in glioma

Selection of the ROIs

All the ROIs were drawn around the tumour on the maximum enhancement images by the same radiotherapist. For each measurement the ROIs were drawn again, so that, as a consequence of the therapy, the extent of the lesion changed on the ME images, resulting in different ROIs size during the course of the treatment.

Dynamic Contrast Enhanced MRI Analysis

All the DCE-MRI images were analysed using in-house written software (25) developed on Matlab®. They were analysed in a quantitative (model based) and semi-quantitative fashion and using a pixel by pixel TIC shape analysis.

Semi-quantitative Analysis

For the semi-quantitative analysis we calculated the Net Enhancing Volume, $ME$ and $ISI$, as described in (18). Briefly, the maximum enhancement ($ME$) is defined as the ratio between the maximum signal difference ($MSD$) (which is the difference between the signal intensity at its maximum $S(max)$ and the Signal Baseline ($SB$)), and the Signal Baseline: $ME = MSD/SB$.

Quantitative Analysis

For the quantitative analysis, we calculated the parameters $K^{trans}$, $K ep$ $V_e$ and $V_i$ by solving the standard extended Tofts’ model (2) in an analytical fashion using an Arterial Input Function (AIF) measured in the superior sagittal sinus. In order to obtain the absolute concentrations of Gd needed for the pharmacokinetic modelling we calculated pixel-by-pixel maps of absolute $T_1$ values using signal intensities from a set of IR T1-w scans, and fitting the intensities to the Bloch equation

$$M(TI) = M0 \left(1 - 2e^{-\frac{TI}{T_1}}\right)$$

in a pixel-by-pixel fashion.

The $T_1$ maps were used to transform the DCE-MRI signal intensities into concentration-time curves using

$$TR \cdot \Re \cdot [Gd] = -\ln\left\{\frac{S10 - S10 \cdot E - 1 + a \cdot E}{E \cdot (S10 \cdot a \cdot E - S10 \cdot a + 1 - a \cdot E)}\right\}$$

(26) where $E = \exp(-TR/T_{10})$ and $a = \cos(\alpha)$, and $T_{10}$ is the tissue $T_1$ value before contrast delivery. We used the assumption $\frac{1}{T_1} = \frac{1}{T_{10}} + \Re[Gd]$ with $\Re$ set at 4.5 l/(s*mMol), where [Gd] is the Gadolinium concentration in mMol/liter.
The AIF was measured for each individual in the sagittal sinus and averaged over different slices (in the middle of the scan section to avoid inflow effects). A method was applied to correct for the low temporal resolution of the scan (=13.7 or 15.5 seconds) as described previously (26). We used Equation 2 to convert the blood signal to Gd concentration, and a fixed value of blood $T_1=1700$ ms was used, as the $T_1$ measured with the IR method is not reliable in flowing spins. The resulting concentration was multiplied by an arbitrary scaling factor ($sf=4.0$) to compensate for different haematocrit in vein and capillaries and other possible sources of uncertainties (such as unknown blood $\Re$), and then fitted to equation 3 (27):

$$C_p(t) = C_b(t) + C_b(t) \otimes B(t)$$ \hspace{1cm} [3]

$$C_b(t) = b \cdot mb^2 \cdot t \cdot e^{-mb \cdot t}$$

$$B(t) = a \cdot e^{-ma \cdot t}$$

where $t$ is time, and $a$, $ma$, $b$ and $mb$ are the fitting parameters. Once the concentration-time curve (CTC) in every pixel $C_i(t)$ was calculated, the pharmacokinetic parameters maps of $K_{trans}$, $K_{ep}$, $v_e$ and $v_i$ were obtained by solving (pixel-by-pixel)

$$C_i(t) = v_i C_p(t) + \int_0^t C_p(u) \cdot e^{-K_{ep} \cdot (t-u)} \, du$$ \hspace{1cm} [4]

with $K_{trans} = K_{ep} v_e$ being the volume transfer constant, $C_i(t)$ the Concentration in the tissue and $C_p(t)$ being the AIF (2).

As Eq 3 can be rewritten as

$$C_p(t) = \left(\alpha \cdot t \cdot e^{-mb \cdot t} - \beta \cdot e^{-mb \cdot t} + \beta \cdot e^{-ma \cdot t}\right)$$

with $\alpha = \left[ b \cdot mb^2 + \frac{a \cdot b \cdot mb^2}{(ma - mb)} \right]$ and $\beta = \frac{a \cdot b \cdot mb^2}{(ma - mb)^2}$ (26),

Eq 4 can be solved in closed form to obtain Eq 5

$$C_i(t) = v_i \left\{ a_1 \cdot e^{-mbt} \cdot t + a_2 \left( e^{-mbt} + e^{-K_{ep} t} \right) + a_3 \left( e^{-mbt} + e^{-K_{ep} t} \right) + a_4 \left( e^{-mat} - e^{-K_{ep} t} \right) \right\}$$ \hspace{1cm} [5]
\[ a_1 = \frac{\alpha}{(k_{ep} - mb)} \quad a_3 = \frac{\beta}{(k_{ep} - mb)} \]

\[ a_2 = \frac{\alpha}{(k_{ep} - mb)^2} \quad a_4 = \frac{\beta}{(k_{ep} - ma)} \]

In order to avoid fitting of noisy pixels, and therefore unstable parameter fitting, we excluded from the PK analysis all pixels with a low relative enhancement. The enhancement threshold was set to 30% enhancement based on the observation in (18).

**TIC Shape Analysis**

The shape of the TIC was classified in a pixel-by-pixel fashion according to the algorithm described in (18). Briefly, TICs were classified as belonging to any shape from 1 to 7 as described in figure 1, where 1 represents non-significant enhancement (where the no-enhancement threshold was set to 30% enhancement), 2 represents slow enhancement, 3 a quick enhancement followed by a plateau, 4 quick enhancement followed by washout, 5 is a quick enhancement followed by slow enhancement, 6 represents arterial enhancement and 7 is all remaining pixels that cannot be classified as any of the previously described shapes (Figure 1a).

In order to create a quantitative measure of the TIC classification that could be tested against the results of the PK modelling, we calculated images statistics by counting the relative occurrence (expressed as a fraction of 1) of each shape within the ROI.

**Median and Average Values**

The PK and semi-quantitative parameters were non-normally distributed across the ROI. From all the scans we calculated median values of the PK model parameters \( v_e, K_{ep}, K^{trans} \) and \( v_i \), and of the semiquantitative parameters ME and ISI. Of the same parameters we also calculated the mean, standard deviation, the skew and the kurtosis of the parameters distribution across the ROI.

We also calculated median values of the PK modelling parameters across pixels placed in the same category by the TIC shape classification algorithm (Figure 2).

We also calculated average values of all the median parameters across the whole study, pooling all patient scans together, separating pre-treatment and the post-treatment values (Table 2).

**Statistical Analysis**
To evaluate the statistical significance of changes in the measured parameters as an effect of the treatment we used the Wilcoxon signed-rank test (SPSS Chicago). To this end, we compared the median value across the ROI of the parameters as evaluated 3 days after and 3 days before treatment (Table 1). Correlation between the median PK modelling parameters and the shape analysis parameters across the study (Figure 4), and between the semiquantitative and PK modelling analysis parameters within the same ROI (figure 5) was measured using the Spearman correlation test.

**Figure 2** (left) Median values of $K_{ep}$, $K^{\text{trans}}$, $v_e$ and $v_i$ in the different shape types described in figure 1a-b, averaged over the whole study population. The arterial type (type 6) is not represented here, as the number of pixels classified as “arterial” in the tumours is negligible. Type 1, or “no enhancement” was excluded from this analysis. **Figure 3.** (right) Scatter plot of the $K_{ep}$ and $v_e$ values in each shape type in one patient (logarithmic scale on the X axis).

**RESULTS**

**Values of PK Parameters in Each of the Heuristically Defined TIC Shape Type**

The analysis of the PK parameters medians in each TIC shape type reveals that, among the different tissue TIC types (2 to 5 as in Figure 1), type 2 curves are mostly associated with high $v_e$ and low $K_{ep}$. Conversely, type 4 curves are associated with high $K_{ep}$ and with low $v_e$. Type 3 TICs have, in general, high values of $v_e$ and intermediate $K_{ep}$ and the highest $v_i$ (probably a result of the large initial slope), whereas type 5 have low values of $v_e$ and $K_{ep}$ (Figure 2). The values of $K^{\text{trans}}$ remain the same across all the shape types. “Type 6” (arterial) and “Type 7” (undefined) TICs do not appear in this graph as we did not observe any pixel of this type within the tumours. To better picture the relation between the PK parameters
and the shape classification, in figure 3 a distribution is shown of the $K_{ep}$ and $v_e$ for the four different types of TIC shapes in one patient.

<table>
<thead>
<tr>
<th>DCE-MRI parameter</th>
<th>+3/-3 day ratio</th>
<th>Wilcoxon P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median $K_{ep}$</td>
<td>1.04 (0.966, 1.079)</td>
<td>0.59</td>
</tr>
<tr>
<td>Median $K_{trans}$</td>
<td>0.907 (0.91, 0.89)</td>
<td>0.23</td>
</tr>
<tr>
<td>Median $v_e$</td>
<td>0.84 (0.88, 0.82)</td>
<td><strong>0.027</strong></td>
</tr>
<tr>
<td>Median $v_i$</td>
<td>0.74 (0.21, 1.04)</td>
<td>0.125</td>
</tr>
<tr>
<td>Median $ME$</td>
<td>0.72 (0.69, 0.74)</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>Median $ISI$</td>
<td>0.79 (0.73, 0.82)</td>
<td><strong>0.003</strong></td>
</tr>
<tr>
<td>% Type 2</td>
<td>1.07 (1.25, 0.98)</td>
<td>0.594</td>
</tr>
<tr>
<td>% Type 4</td>
<td>1.75 (1.55, 1.84)</td>
<td>0.82</td>
</tr>
<tr>
<td>STD $ME$</td>
<td>0.62 (0.59, 0.63)</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>Skew $ME$</td>
<td>2.04 (1.7, 2.73)</td>
<td><strong>0.008</strong></td>
</tr>
<tr>
<td>STD $K_{trans}$</td>
<td>0.85 (0.71, 0.92)</td>
<td>0.140</td>
</tr>
<tr>
<td>STD $v_e$</td>
<td>0.90 (1.18, 0.76)</td>
<td>0.156</td>
</tr>
<tr>
<td>STD $K_{ep}$</td>
<td>1.07 (0.94, 1.13)</td>
<td>0.609</td>
</tr>
<tr>
<td>Net enhancing Volume</td>
<td>0.59 (0.64, 0.52)</td>
<td><strong>0.005</strong></td>
</tr>
</tbody>
</table>

Table 1: Measure of treatment response of several quantitative and semi-quantitative DCE-MRI parameters in patients with recurrent malignant glioma. The +/-3 day ratio is the ratio between the median value of the parameter (across the ROI) of each patient 3 days after and 3 days before, averaged across all the patients. Significances are marked in bold.

It can be seen that two clusters, corresponding roughly to the type 2 and type 4 TICs, can roughly be separated by using a threshold in $K_{ep}$, but share a similar distribution of $v_e$. The type 3 and type 5 cluster are more difficult to characterise in terms of $K_{ep}$ and $v_e$.

**Relation Between Shape–derived Parameters and Model-derived Parameters**

The general relationship between $K_{ep}$ and the relative amount of type 2 and 4 TIC shapes is described in figure 4 where the results of all the scans of this study are pooled together. We observed a significant positive correlation between type 4 TICs and the $K_{ep}$ and a negative correlation between type 2 TICs and $K_{ep}$.
(Spearman correlation coefficient 0.507 and -0.477 respectively, p<0.001) (Figure 4).

<table>
<thead>
<tr>
<th>DCE-MRI parameter</th>
<th>Average pre-treatment All (grade 3, grade 4)</th>
<th>Average post-treatment All (grade 3, grade 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median $K_{\text{trans}}$</td>
<td>0.152 (0.125, 0.166)</td>
<td>0.109 (0.076, 0.14)</td>
</tr>
<tr>
<td>Median $v_e$</td>
<td>0.625 (0.501, 0.680)</td>
<td>0.451 (0.345, 0.55)</td>
</tr>
<tr>
<td>Median $K_{\text{ep}}$</td>
<td>0.271 (0.268, 0.280)</td>
<td>0.265 (0.234, 0.299)</td>
</tr>
<tr>
<td>Median $v_i$</td>
<td>0.066 (0.046, 0.076)</td>
<td>0.049 (0.025, 0.064)</td>
</tr>
<tr>
<td>Median $ME$</td>
<td>0.702 (0.7288, 0.688)</td>
<td>0.482 (0.48, 0.54)</td>
</tr>
<tr>
<td>Median $ISI$</td>
<td>80.2 (81.1, 76.6)</td>
<td>60.13 (57.2, 69.3)</td>
</tr>
<tr>
<td>rel amount Type 4</td>
<td>0.044 (0.036, 0.048)</td>
<td>0.060 (0.043, 0.078)</td>
</tr>
<tr>
<td>rel amount Type 2</td>
<td>0.681 (0.663, 0.713)</td>
<td>0.689 (0.718, 0.652)</td>
</tr>
</tbody>
</table>

Table 2: Average values of the quantitative, semiquantitative, and heuristic parameters before and after treatment. For the parameters the average value is given (across all the patients) of the median value calculated over each ROI.

We found no significant correlation between the relative amounts of each shape type and $v_e$ or $K_{\text{trans}}$. We also investigated the correlation between model based parameters and the semi-quantitative analysis, and found that in the individual patients $v_e$ correlated with $ME$ (Figure 5). No correlation was found between the initial tumour volume, and any of the other parameters.

Figure 4. Scatter plot of $K_{\text{ep}}$ versus the relative amount of type 2 and 4 TIC shape in the tumour, and trend lines. Spearman Correlation coefficient: -0.477 and 0.507 respectively, P<0.001. Each dot represents one scan.
In the plots in Figure 6 it can be observed that, among the model-derived parameters, $V_e$ decreases after treatment in all but two cases, and present smaller changes in both direction during the treatment, whereas among the non-quantitative parameters $ME$, its STD, the $ISI$ and the Net Enhancing Volume showed a marked decrease in all the patients. In general, for the parameters that showed a marked decrease after the start of the treatment, it can be seen that there is a tendency to resume the initial (pre treatment) values. We tested the effect of the drug using the Wilcoxon signed rank test on the pre-treatment scan and the first post treatment scan on the whole patient cohort. As shown in Table 1, $v_e$, $ME$, $ISI$ and net volume of enhancing pixels are the

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**Changes of the Model-derived and TIC Shape-derived Parameters During Treatment**

In Table 1 and Figure 6 we show the changes of the model-derived parameters ($K^{\text{trans}}$, $v_e$ and $K_{ep}$), the semi-quantitative and heuristic parameters ($ME$, $ISI$ and relative amount of TIC shapes), as well as the distribution properties (STD, skew) as a result of the therapy. In the plots the values are presented during the whole course of the treatment, though for the statistical significance in Table 1 we only used the first and second scan. In Table 1, average values of the $+3/-3$ parameter ratio (ratio between the median parameter across the ROI after and before the treatment) are given for the whole group and separately for grade 3 and grade 4 patients.
parameters that are the most sensitive to the effect of bevacizumab. $K_{\text{trans}}$ also showed a trend, but this did not reach statistical significance.

Figure 6 Changes in $K_{\text{trans}}$, $V_e$, $K_{sp}$, $ME$ and relative amount of type 2 and 4 TIC shapes in 15 patients treated with bevacizumab. Each symbol represents one individual patient. Black symbols with continuous line refer to patient with grade 4 glioma, grey symbols with striped lines refer to patients with grade 3 glioma. On the x axis: visit: 1 = 3 days pre treatment, 2 = 3 days after, 3 = 30 days, 4 = 80 days, 5 = 1 year after treatment. $V_e$ larger than 1 are probably due to the underestimation of the concentration of the AIF.

The Volume of enhancing pixels in the ROI also significantly decreased after treatment, hereby confirming that this parameter is important in determining the effect of antiangiogenic treatment (28). Because of the small size of the grade 3 and grade 4 cohorts, the parametric Wilcoxon test was performed only on the whole group (glioma grade 3 and 4 together). From this analysis it appears that
neither the model derived parameter $K_{ep}$ nor the relative amount of pixels classified as type 2 or 4 were sensitive to the effect of the anti-angiogenic drug.

![Image](image_url)

**Figure 7:** Changes in $K_{trans}$ maps (above) and in the TIC shape maps (below) of a patient undergoing four successive scans: a. 3 days before treatment, b 3 days after, c 24 days after, d 80 days after the start of the treatment. Whereas $K_{trans}$ significantly changes after the start of the treatment, in this patient the TIC shape maps remained substantially the same.

The median values before and after the treatment (averaged over all the post treatment scans), and the relative variation and its standard deviation are given in Table 2. An example of the change of the Shape maps and $K_{trans}$ during treatment in one patient is given in Figure 7.

We investigated the behaviour of the histogram of the parameters values, and observed that the histogram of $ME$ significantly narrowed after treatment, leading to a smaller standard deviation, and therefore possibly a less heterogeneous ROI. Also the STDs of $ve$, $K_{trans}$ were reduced, but changes were not significant. The STD of $K_{ep}$ did not change (Table 1).

**Differences Between Glioma Grades**

In general median values of $K_{trans}$, $ve$, $v$, and $ME$ were higher in Grade 4 than in grade 3 glioma patients. Also type 2 and type 4 TIC curves pre- and post-treatment ratios differ between patients with grade 3 and grade 4 glioma (Table 1). However, because of the small size of the grade 3 and grade 4 cohorts, it is difficult to assess the significance of this finding.
DISCUSSION

While the exact measurement of model derived parameters such as $K_{\text{trans}}$ has gained wide recognition for its ability to measure tumour grade and the effect of drugs, there is still much work going on in the search for alternative analysis methods that are less prone to errors, more robust, and easier to apply. The search is still on, and has lead to some promising alternatives, such as the use of the parameters extracted from a numerically calculated Impulse Response Function (9,10). Ferl et al. (10) investigated this non parametric analysis in high grade glioma, showing its sensitivity to the effect of bevacizumab and showing that this parameter is a viable alternative to the extended pharmacokinetic analysis. The result though is not surprising as the parameters calculated were proved to have a clear and direct relationship with the model derived parameters.

These non parametric methods are not meant to describe all the kinetic information of the tumours, but might serve as a standard reproducible measure of the effect of certain drugs (9). Among the possible non compartmental approaches, we have chosen a method which is still very commonly accepted among radiologist and widely used in clinical practice, i.e. the approach of “visually” analysing the TIC pattern after drawing ROIs: the measured TIC is judged only on the basis of its shape (29). This kind of approach, because of its simplicity and model independence has triggered researchers to work on the improvement of this eye-led analysis to try and make it user independent and more robust. Recently various versions have appeared of a computed supported classification of these curves (on a pixel-by-pixel basis), rendering the shape in a colour-coded map (18,19,20,30). Despite its popularity among radiologists, to date there is no clear evidence that this alternative technique can effectively substitute for the proper model based quantification. In an earlier study, it was shown that the shape analysis alone is a reliable marker of the disease activity in RA (31).

In this paper we have investigated TIC -shape based classification using patient data from a study using an anti-angiogenic drug (bevacizumab) and compared it to the results obtained applying Tofts’ quantitative PK model. A similar question was addressed by Hayes et. al. (32) who compared these two approaches in respondent and non-respondent breast cancer patients subjected to treatment. However in this study the TIC classification was performed on an averaged TIC from the whole ROI, therefore missing the spatial information about the tumour heterogeneity.
In a later paper from the same group, no relation was found between the quantitative model and another semi-quantitative parameter, the IAUC, in simulated data (15).

In the present study we have shown that there is a relationship between the number of pixels classified as “type 4” (positive correlation) and “type 2” (negative correlation) in the tumour and the average $K_{ep}$ value. Yet, we were not able to find a relation with the parameters $K^{\text{trans}}$ and $\nu_e$. Since the two latter parameters are the most important output of the PK model, the fact that the TIC shapes only correlate to $K_{ep}$ is somehow disappointing.

If there were an underlying relationship missed in this study, two main causes may explain this negative finding. One important factor is that $K^{\text{trans}}$ (and consequently $\nu_e$) is dependent on any error arising from the calculation of the absolute Gd concentration both of the CTC and of the AIF (as $K^{\text{trans}}$ appears as a scaling factor of the integral in equation 4). $K_{ep}$, which only appears in the fit in the exponential decay, is a parameter which is somewhat less dependent on possible inaccuracies in the concentration and wrong AIF scaling factor. For this reason the standard deviation of $\nu_e$ and $K^{\text{trans}}$ over the whole study is larger than those of $K_{ep}$, and the large STD of the data across the study might have compromised any possible underlying relationship with the outcome of the shape analysis.

The other factor is more crucial. The TIC shape is in principle only classified according to changes in the curvature of the TIC, regardless of its amplitude, which is, conversely, dependent of the contrast agent concentration. On the other hand the $K^{\text{trans}}$ is strongly modulated by the amplitude of the TIC (see Equation 4). This makes the shape analysis intrinsically less sensitive to $K^{\text{trans}}$.

This insensitivity of the shape classification to $\nu_e$ (and consequently to $K^{\text{trans}}$) can be also be seen in the scatter plot in Figure 3, where the clusters corresponding to type 2, type 3 and type 4 TICs, are divided by a threshold in $K_{ep}$ (with type 3 and type 4 being both higher then the threshold), but share all the same range of $\nu_e$.

In order to understand the sensitivity to treatment response of both approaches in the clinical practice, we have looked at whether the TIC-derived parameters as well as the PK parameters were sensitive to changes in microvascular structure as a consequence of the delivery of the anti-angiogenic agent.

In this study, the $K^{\text{trans}}$ and $\nu_e$ were reduced at the second scan (3 days after delivery of the anti-angiogenic drug), whereas there was no change in the average $K_{ep}$, confirming previous studies (10). Among the semi-quantitative parameters,
both the \textit{ME} and the \textit{ISI} were shown to be the most sensitive to the effect of the therapy. The TIC shapes-derived parameters, just as their model based counterpart $K_{ep}$, were not sensitive to the effect of bevacizumab+temozolomide. One possible hypothesis is that the TIC-shape derived parameters are the mirror of some other effects, but not the antiangiogenic effect which can be seen by the other parameters. This hypothesis needs further investigation. The lack of change in the TIC Shape parameters after treatment with bevacizumab seems rather to reflect the lack of beneficial effect of the anti-angionenic drug on the patient (24).

Our finding that the histogram of $K^{\text{trans}}$ “shrinks” after treatment reflects a similar observation made in breast patients undergoing treatment with chemotherapy (32), although in our study this effect was much stronger when looking at the histogram of \textit{ME}. This seems to confirm that heterogeneity plays an important role in the measurement of the effect of the antiangiogenic agents, as it pointed out by Alic and co-workers in (17). More research should be done to further confirm this hypothesis.

Our results that the PK parameters were sensitive to the antiangiogenic effect, but less than the semi quantitative parameters \textit{ME} and \textit{ISI}, seem to confirm earlier findings, suggesting that non-quantitative, descriptive parameters, such as that of the early signal enhancement, reach higher sensitivity and specificity than PK parameters (33). This suggestion of a possible superiority of semi- quantitative versus model-based parameter is probably a result of the still unsolved issue of the inaccuracy of the PK parameters measurement.

Some of the problems that could have affected our results are described below. In this study, we individually drew ROIs in each patient and at each visit, and calculated the parameters in these ROIs only across enhancing pixels (i.e. over the “Volume of enhancing pixels”). The ROIs were drawn based on the \textit{ME} images, and their volume could (and did) therefore vary during the course of the treatment. The fact that the ROIs where re-drawn at each visit might have influenced the results, as in the average values the information is lost about the pixels which were included in the analysis at one visit, and were not in another visit. On the other hand, we chose to draw the ROIs on the \textit{ME} images in order to avoid inclusions of pixels where no enhancement had taken place, and were therefore not suitable for the analysis (either the TIC shape analysis, or the PK analysis).

Despite the care we took to map $T_1$ accurately (by means of an IR protocol) and individually select the AIF, we were not able to completely avoid problems in the
calculation of the PKM parameters.

In this study the AIF was measured individually in the superior sagittal sinus. Despite the fact that earlier results showed the signal in the sinus to be well reproducible within patients (26), and therefore possibly also a valid source of an AIF, the absolute value of the AIF had to be multiplied by an arbitrary scaling factor to correct for an underestimation of the AIF. The correct scaling of the AIF remains a problem, both because it is severely affected by the actual (unknown) hematocrit (34) (hematocrit is known to change significantly between male (37-47%) and female (42-52%), and between different vessels, with a consequent change in the native T\_1 value) (10) and because the calculation of the concentration in the AIF is affected by flow problems, that can result in an inaccurate estimate of the Gadolinium concentration. The addition of a scaling factor does not affect the results presented here, as this scaling factor only resulted in a general rescaling of $K^{\text{trans}}$ (and consequently $v_e$), as the factor was the same across the whole study.

**In conclusion**, we found a significant correlation between the parameters derived from the TIC shape analysis and the parameter $K_{ep}$. The amount of type 2 and 4 TIC shapes can therefore be considered a reliable surrogate for the model-derived parameter $K_{ep}$. We did not find any significant correlation between these parameters and $K^{\text{trans}}$ or $v_e$. The quantitative parameter $v_e$ and the semi-quantitative parameter ME and ISI are sensitive to the effect of the anti-angiogenic agent, while $K^{\text{trans}}$ only showed a trend. The parameter $K_{ep}$, and the shape-derived parameters did not clearly show any significant change during the treatment.

These results do not support the evidence that the TIC shape alone can represent a mirror of the activity of anti-angiogenic treatment. To improve its sensitivity, further studies should focus on the combination of the TIC shape analysis with the semi-quantitative analysis and with an appropriate analysis of the parameters heterogeneity.

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