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Comparison of Two Mathematical Models of Cellularity Calculation

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Abstract

OBJECT: Nowadays, there is increasing evidence that functional magnetic resonance imaging (MRI) modalities, namely, diffusion-weighted imaging (DWI) and dynamic-contrast enhanced MRI (DCE MRI), can characterize tumor architecture like cellularity and vascularity. Previously, two formulas based on a logistic tumor growth model were proposed to predict tumor cellularity with DWI and DCE. The purpose of this study was to proof these formulas. *METHODS:* 16 patients with head and neck squamous cell carcinomas were included into the study. There were 2 women and 14 men with a mean age of 57.0 ± 7.5 years. In every case, tumor cellularity was calculated using the proposed formulas by Atuegwu et al. In every case, also tumor cell count was estimated on histopathological specimens as an average cell count per 2 to 5 high-power fields. *RESULTS:* There was no significant correlation between the calculated cellularity and histopathologically estimated cell count by using the formula based on apparent diffusion coefficient (ADC) values. A moderate positive correlation (r=0.515, P=.041) could be identified by using the formula including ADC and V_e values. *CONCLUSIONS:* The formula including ADC and V_e values is more sensitive to predict tumor cellularity than the formula including ADC values only.

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Introduction

Nowadays, there is a changing behavior regarding clinical oncologic imaging techniques and their possible role in daily routine. Previously, radiologic imaging like computed tomography (CT) and magnetic resonance tomography (MRI) was only used for tumor detection and tumor staging. However, emergent functional imaging modalities like diffusion-weighted imaging (DWI) and dynamic contrast enhanced MRI (DCE-MRI) can not only detect malignant lesions but also characterize tumor microstructure [1–5].

DWI measures the random water movement in tissues, the so-called Brownian motion, which can be quantified by apparent diffusion coefficient (ADC) [2]. The underlying principle is that the free movement is hindered by cells and, therefore, ADC may predict cell density [2,4,6].

Another imaging modality is DCE MRI, which can measure the perfusion in tissue using contrast media agents [8]. Several parameters can be obtained with this technique, namely, K_{trans}, K_{ep}, and V_e [8]. K_{trans} is the volume transfer constant, V_e is the extravascular extracellular volume fraction, and K_{ep} is the flux rate constant [8]. It is widely acknowledged that DCE parameters, especially K_{trans}, are associated with microvessel density in tissues, [8,9]. Interestingly, V_e as a parameter reflecting the extracellular volume fraction might also be linked to cell count [9,10]. In fact, previously, it has been shown that V_e correlated with ADC in head and neck cancer [11]. Furthermore, some studies indicated that V_e correlated with cellularity [9,10].

Prediction of tumor behavior by imaging modalities is of increasing interest. Atuegwu et al. proposed formulas by which cellularity might be calculated by using of ADC values (formula 1) and ADC and V_e values (formula 2) [12]. However, the authors only used breast cancer patients to evaluate their results [12]. Recently, the results of cellularity calculation based on ADC values (formula 1) were analyzed in different tumors [13]. It has been shown that this formula did not apply for all lesions [13].

Therefore, the aim of this study was to compare results of both formulas for cellularity calculation with the histopathologically estimated cell count.

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Material and Methods

Patients

Sixteen patients with head and neck sqamous cell carcinoma (HNSCC) were included into the study. There were 2 women and 14 men with a median age of 57 years, mean age of 57.0 ± 7.5 years, and age range 49-79 years. In 11 cases, primary HNSCC and, in 5 patients, local tumor recurrences were diagnosed by histopathology.

DWI

DWI was obtained with an axial DWI-EPI sequence (TR/TE 8620/73 milliseconds, slice thickness 4 mm, voxel size 3.2 × 2.6 × 4.0 mm, *b*-values of 0 and 800 s/mm^2). ADC maps were automatically generated by the implemented software. Regions of interest were manually drawn on the ADC maps along the contours of the tumor on each slice. In all lesions, minimal ADC values (ADC_{min}), mean ADC values (ADC_{mean}), and maximal ADC values (ADC_{max}) were estimated.

DCE

DCE imaging was performed using T1w DCE sequences according to a protocol reported previously [9]. The following pharmacokinetic parameters were calculated:

- K_{trans}: volume transfer constant which estimates the diffusion of contrast medium from the plasma through the vessel wall into the interstitial space, representing vessel permeability;
- V_e: volume of the extravascular extracellular leakage space;
- K_{ep}: parameter for diffusion of contrast medium from the extracellular leakage space back to the plasma. It is in close relation with K_{trans} and V_e and is calculated by the formula:

$$K_{ep} = K_{trans} \times V_e^{-1}$$

Calculation of Cellularity

As previously described by Atuegwu et al. (2013) [12], the number of tumor cells can be calculated from ADC values taking into account tumor volume fractions estimated from extended Tofts model (ETM) analysis of DCE-MRI data. For the cell number calculation, the following relationship has been used:

$$N = \theta\left(\frac{ADC_w - ADC_{mean}}{ADC_w - ADC_{min}}\right) v_{TC} \quad (1)$$

Where ADC_w is the ADC of free water ($ADC_w = 3 \times 10^{-3}$ mm^2/s) and ADC_{min} is the minimum and ADC_{mean} is the mean ADC value within the region of interest, respectively. θ is the carrying capacity, i.e., maximum number of cells within a given volume [12]. To calculate θ, we converted the given volumes to a standard volume of 1 mm^{3}and used the tumor cell volume of 4189 μm^3 [12]. Tumor volume fractions v_{TC} can be calculated from the extravascular extracellular (v_e) and plasma volume (v_p) fractions using the equation:

$$v_{TC} = 1 - v_e - v_p \quad (2)$$

v_e and v_p can be estimated from ETM. In our study, we used the Tofts model (TM), which assumes negligible plasma volume ($v_p = 0$).

We then computed the number of tumor cells per cubic millimeter in two ways: 1) using ADC values only, i.e., assuming $v_{TC} = 1$, and 2) taking into account volume fractions $v_{TC} = 1 - v_e$.

Estimation of Cellularity

For this study, we reanalyzed our previous data regarding associations between ADC parameters and histopathological findings [9]. Here, KI 67 antigen stained specimens (MIB-1 monoclonal antibody, Dako Cytomation, Denmark) were used as reported previously [9]. In every case, cellularity was estimated as an average cell count per 2 to 5 high-power fields (×400; 0.16 mm^2 per field). All images were analyzed by using a research microscope, Jenalumar, with camera Diagnostic instruments 4.2 as reported previously [9].

Statistical Analysis

Because the fact that the formula calculated cells in a volume and previously reported data were based on cell count on high-power fields, a correlation analysis between the calculated and estimated cellularity was performed. Spearman's correlation coefficient was used, and *P* values <.05 were taken to indicate statistical significance in all instances.

Results

Table 1 displays the correlation coefficients between calculated and estimated cell count. There was no significant correlation between the calculated cellularity and histopathologically estimated cell count by using the formula based on ADC values (formula 1) (Figure 1*A*). A moderate positive correlation of r=0.515, P=.041 could be identified by using of the formula including both ADC and V_e values (formula 2) (Figure 1*B*).

Discussion

The present study identified a statistically significant correlation between the calculated cellularity using the formula based on ADC and V_e values and the estimated cellularity using histopathology specimens in HNSCC.

Recently, there has been increasing evidence that MRI, using functional imaging modalities, namely, DWI and DCE, can predict tumor behavior and microstructure [1–5]. Especially ADC values acquired by DWI correlate with cellularity [2,4,7]. In a recent meta-analysis, a moderate correlation coefficient of r=−0.56 between ADC values and cell count could be identified [4,7]. However, this association seems to be different in different tumor entities [4,7]. For example, in gliomas, the correlation coefficient was higher (r=−0.66), whereas in lymphomas, it was −0.25 [4]. This seems to be related to the fact that ADC values are mainly influenced by cellularity, but also, other cellular structures such as [15] extracellular matrix can also cause diffusion restriction in tissues [6,13,14].

The underlying hypothesis is that due to increasing cell density, the free diffusion of protons is hindered and therefore the ADC is lowered [2,6]. Another aspect seems to be that the intracellular protons have a slower diffusion than the extracellular protons due to higher viscous intracellular milieu [6]. As a recent example, different correlation coefficients between ADC values and various histopathology parameters in a murine prostate model could be identified [16]. The values ranged from r=−0.23 with nuclear spaces up to r=0.74 with extracellular spaces [16]. Furthermore, a strong inverse

Table 1. Correlation between the calculated cellularity and histopathologically estimated cell count.

	Correlation with Histopathologically Estimated Cell Count
ADC only (formula 1)	r=0.243, P=.365
ADC and V_e (formula 2)	r=0.515, P=.041