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# Proving of a Mathematical Model of Cell Calculation Based on Apparent Diffusion Coefficient

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## Abstract

*OBJECTIVES:* Recently, Atuegwu et al. proposed a mathematical model based on $ADC_{mean}$ and $ADC_{min}$ to calculation of cellularity. Our purpose was to compare the calculated cellularity according to the formula with the estimated cell count by histopathology in different tumors. *METHODS:* For this study, we re-analyzed our previous data regarding associations between ADC parameters and histopathological findings. Overall, 134 patients with different tumors were acquired for the analysis. For all tumors, the number of tumor cells was calculated according to Atuegwu et al. 2013. We performed a correlation analysis between the calculated and estimated cellularity. Thereby, Pearson's correlation coefficient was used and $P < .05$ was taken to indicate statistical significance in all instances. *RESULTS:* The estimated and calculated cellularity correlated well together in HNSCC ($r = 0.701$, $P = .016$) and lymphomas ($r = 0.661$, $P = .001$), and moderately in rectal cancer ($r = 0.510$, $P = .036$). There were no statistically significant correlations between the estimated and calculated cellularity in uterine cervical cancer, meningiomas, and in thyroid cancer. *CONCLUSION:* The proposed formula for cellularity calculation does not apply for all tumors. It may be used for HNSCC, cerebral lymphomas and rectal cancer, but not for uterine cervical cancer, meningioma, and thyroid cancer. Furthermore, its usefulness should be proved for other tumors.

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## Introduction

Magnetic resonance imaging (MRI) is used as a staging investigation in numerous malignant diseases. Some MRI techniques, for instance, diffusion weighted imaging (DWI), are influenced by histological composition of investigated tissue, and, therefore, can be used as marker of tissue architecture [1–4]. DWI measures the random motion of water molecules in tissues [1,2]. The water diffusion can be quantified by a parameter, defined as the apparent diffusion coefficient (ADC) [1–4]. ADC reflects the mobility of water within tissues and documents quantitatively restriction of water diffusion by several barriers, such as cell membranes [1–4]. Therefore, ADC can indirectly provide information about cell density [1].

Previously, numerous experimental and clinical studies reported data regarding associations between DWI and histopathological features in different tumors and tumor like lesions [3–7]. In most publications, different ADC fractions, especially minimum ADC ($ADC_{min}$) and mean ADC ($ADC_{mean}$) showed statistically significant inverse correlations with cell count in several tumors [5–7].

Recently, Atuegwu et al. proposed a mathematical model based on $ADC_{mean}$ and $ADC_{min}$ to calculation of cellularity [8]. The authors observed a strong and significant Pearson correlation and a strong concordance correlation between the estimated and the simulated number of tumor cells [8]. However, the proposed formula was not proven by histopathological examination, and, therefore, it is unclear, if the mathematical model provides real cell count or not.

Therefore, our purpose was to compare the calculated cellularity according to the formula with the estimated cell count by histopathology in different tumors.

## Materials and Methods

### *Estimated Cellularity*

For this study, we re-analyzed our previous data regarding associations between ADC parameters and histopathological findings

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**Table 1.** Primary Tumors, Number of Patients, and MRI Technique

| Entity | n | Magnetic Field | b values |
|---|---|---|---|
| Uterine cervical cancer | 21 | 3 T | b0 and b1000 |
| HNSCC | | 3 T | b0 and b800 |
| Cerebral lymphoma | 21 | 1.5 T | b0 and b1000 |
| Meningioma | 49 | 1.5 T | b0 and b1000 |
| Rectal cancer | 17 | 3 T | b0 and b1000 |
| Thyroid cancer | 15 | 3 T | b0 and b800 |

HNSCC, head neck squamous cell carcinoma.

**Table 2.** Correlations Between the Estimated and Calculated Cellularity

| Entity | n | Correlation Coefficients |
|---|---|---|
| Uterine cervical cancer | 21 | r = −0.245, $P$ = .285 |
| HNSCC | 11 | **r = 0.701** ***P* = .016** |
| Cerebral lymphoma | 21 | **r = 0.661** ***P* = .001** |
| Meningioma | 49 | r = −0.110 $P$ = .450 |
| Rectal cancer | 17 | r **= 0.510** ***P* = .036** |
| Thyroid cancer | 15 | r = 0.350 $P$ = .202 |
| Total | 134 | r = 0.119 $P$ = .190 |

HNSCC, head neck squamous cell carcinoma.
Significant correlations are highlighted in bold.

[9–14]. Overall, 134 patients with different tumors were acquired for the analysis (Table 1). In all cases, the diagnosis was confirmed by histopathological examination. For every tumor entity, cellularity was estimated as an average cell count per 2–5 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 [6,9].

Furthermore, in all cases the tumors were investigated by DWI. Thereby, different equipment and b values were used (Table 1).

### *Cell Number Calculation*

For all tumors, the number of tumor cells was calculated according to Atuegwu et al. 2013 [8]. In this study, ADC values were converted to tumor cell number N using following equation:

$$N = \theta\left(\frac{ADC_w - ADC_{mean}}{ADC_w - ADC_{min}}\right) \quad (8)$$

Where $ADC_w$ is the ADC of free water ($ADC_w = 3 \times 10^{-3}$ $mm^2/s$); $ADC_{min}$ is the minimum and $ADC_{mean}$ the mean ADC value within the ROI, respectively. θ is the carrying capacity which can be interpreted as the maximum number of cells that can be contained within a given volume [15]. Due to varied imaging voxel sizes for different entities, we converted the given volumes to a standard volume of 1 $mm^3$. To calculate θ, we used the tumor cell volume of 4189 $\mu m^3$ [8].

### *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, we performed a correlation analysis between the calculated and estimated cellularity. Thereby Pearson's correlation coefficient was used and $P < .05$ was taken to indicate statistical significance in all instances.

## Results

Table 1 shows the results of the performed correlation analysis between the calculated and estimated cellularity. In the total sample, the calculated cellularity did not correlated with the estimated cell count. The subgroup analysis showed the following. Both parameters correlated well in HNSCC and lymphomas, and moderately in rectal cancer (Table 2). There were no statistically significant correlations between the estimated and calculated cellularity in uterine cervical cancer, meningiomas, and in thyroid cancer.

## Discussion

Our study provides data about calculated and estimated cellularity in different tumors.

As seen, the estimated and calculated cellularity correlated statistically significant in HNSCC, lymphoma and rectal cancer. Therefore, the proposed formula for cellularity calculation [8] can be used in clinical practice for these entities. However, there were no significant correlations between the estimated cell count and calculated cellularity in uterine cervical cancer, meningioma, and thyroid cancer.

It is unclear, why the formula reflects the real cell count in some tumors, whereas in other does not. Several causes of this phenomenon are possible. The formula is based on ADC values, namely $ADC_{mean}$ and $ADC_{min}$ and assumes that the ADC fractions correlate with cell count [8]. Recently, two meta-analyses regarding associations between $ADC_{mean}$ and $ADC_{min}$ and cellularity were published [16,17]. These articles identified the following: firstly, $ADC_{min}$ did not better correlate with cellularity in comparison to $ADC_{mean}$ [17]. Secondly, different tumors showed also different associations between ADC and cell count [16]. In detail, it has been shown that correlation between ADC and cellularity ranged in different tumors [16]. Overall, the identified correlation coefficients for the analyzed tumors were as follows: glioma (ρ = −0.66), ovarian cancer (ρ = −0.64), lung cancer, (ρ = −0.63), uterine cervical cancer (ρ = −0.57), prostatic cancer (ρ = −0.56), renal cell carcinoma (ρ = −0.53), squamous cell carcinoma of head and neck (ρ = −0.53), breast cancer (ρ = −0.48), meningioma (ρ = −0.45), and lymphoma (ρ = −0.25) [16].

Another cause of our controversial results is the fact that not only cell count can influence water diffusion and ADC. It is well known that other histological factors, such as cell size and nucleic-cytoplasma ratio play a role in restriction of water diffusion [1–3]. According Matsumoto et al., increase of cell size decreased water diffusion in vitro [18]. Furthermore, it has been shown that nucleic size also affected water diffusion in cell culture [19].

The proposed formula does not consider the fact that several tumors and tumor-like lesions have different cell and nucleic sizes. Therefore, it cannot be used for all tumors. However, our study showed that it provides results, which are concordant with the estimated cell count for HNSCC, cerebral lymphoma and rectal cancer. Clearly, further investigations with different tumors are needed to proof the usefulness of the formula in other malignancies. We hypothesize that in future, more sensitive ADC-based mathematical models adjusted for every tumor entity may better reflect cellularity than a general formula. Furthermore, these models may include other ADC parameters than $ADC_{mean}$ and/or $ADC_{min}$. Recently, some reports showed that histogram analysis of ADC maps provided other parameters, which better correlated with tumor cell count [19,20].

The present study has several limitations. Firstly, it is retrospective. Secondly, the analyzed tumor groups had small number of patients.

In conclusion, our results suggested that the proposed formula for cellularity calculation does not apply for all tumors. It may be used for HNSCC, cerebral lymphomas and rectal cancer, but not for uterine cervical cancer, meningioma, and thyroid cancer. Furthermore, its usefulness should be checked for other tumors.

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