

Original Article

Correlations between apparent diffusion coefficient values and histopathology classification of breast invasive ductal carcinoma

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Abstract: Objective: The purpose of this study was to evaluate the correlation between apparent diffusion coefficient (ADC) values and histopathologic classification of breast cancer. Methods: Sixty-six cases of breast cancer confirmed by histopathology with histopathologic classification were recruited in this retrospective study. ADC values of lesions were measured; the mean (ADC_{mean}) and minimum (ADC_{min}) were extracted from regions of interest (ROIs). The cases were divided into two groups, histopathologic classification and prognostic recurrence, according to the treatment and prognosis (histopathologic classification, lymph node status). Results: According to the analysis, ADC_{mean} had no correlation with either histopathologic classification group or prognostic recurrence group. There was negative correlation between ADC_{min} and histopathologic classification group ($r=-0.615$, $P=0.000$) as well as prognostic recurrence group ($r=-0.754$, $P=0.000$). Conclusion: The ADC_{min} can be considered as an optimal DWI single parameter of medical plan before breast cancer surgery and the preliminary assessment of prognostic recurrence.

Keywords: Breast cancer, histopathologic classification, apparent diffusion coefficient

Introduction

Breast MR has been improving rapidly since it was coming into existence. As the technology that has been widely used for diagnosis of breast cancer, diffusion-weighted imaging (DWI), which has been integrated into standard breast cancer for discrimination of breast lesions, possesses high sensitivity and specificity for cancer detection. DWI is currently the only technique used for detecting Brownian motion of bulk water molecules in vivo, and it values the limitation of Brownian motion on these molecules through ADC values [1]. Breast cancer is a highly heterogeneous malignancy. The density, atypia of tumor cells and extracellular volume in different histopathologic grades will affect the Brownian motion of water molecules [2-5]. Currently, the correlation between the different histopathologic grades and ADC values has been discussed in literatures at home and abroad. Yet, since the regions of interest (ROIs) vary and method of measure-

ment differs, there are distinct differences between the conclusions. This study aimed to figure out the ADC value, which was a more reflection of histopathologic classification in breast cancer, by discussing whether different classification of breast cancer was correlated to either average or minimum ADC value.

Materials and methods

Patients

This study was approved by the Institution Review Board of Guangxi Medical University and an informed consent was obtained from each patient. A total of 66 lesions were consecutively recruited from May 2011 to May 2013 at our Hospital. All of the patients were female and underwent preoperative breast MRI with DWI (age range: 32-76 years; mean age: 51.61). All of them had met the following criteria: (a) Without any biopsy or interventional therapy or medical treatment performed on breast lesions

ADC for breast invasive ductal carcinoma

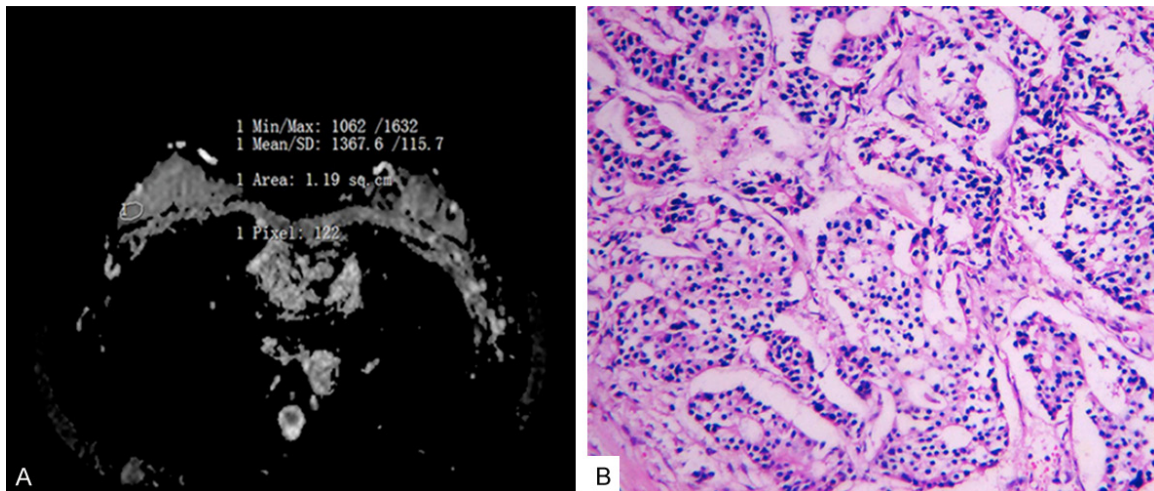


Figure 1. A: $\text{ADC}_{\text{mean}} = 1.368 \times 10^{-3} \text{ mm}^2/\text{s}$, $\text{ADC}_{\text{min}} = 1.062 \times 10^{-3} \text{ mm}^2/\text{s}$; B: Photomicrograph (hematoxylin-eosin staining, original magnification 100 \times) showing pathological of grade I invasive ductal carcinoma in the right breast.

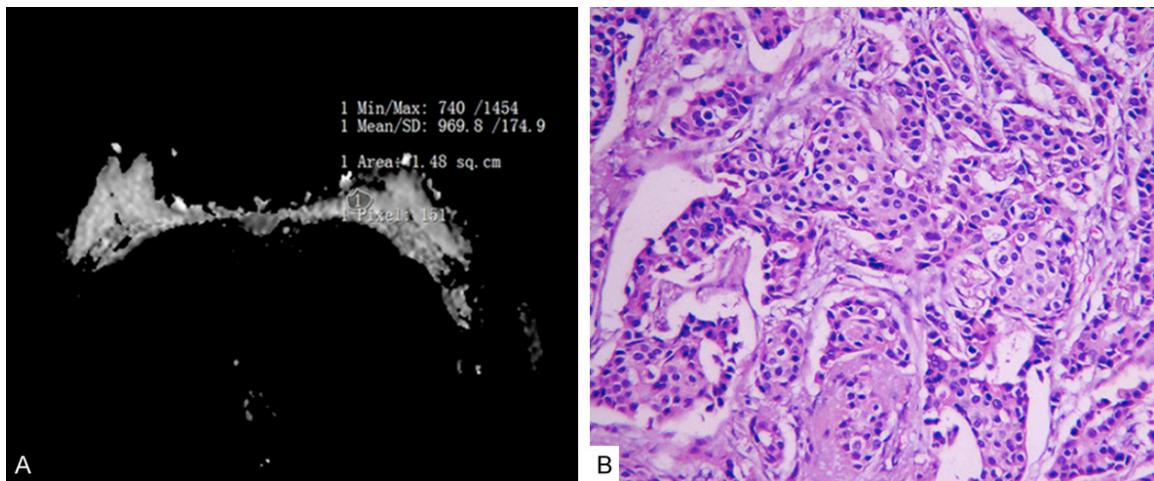


Figure 2. A: $\text{ADC}_{\text{mean}} = 0.97 \times 10^{-3} \text{ mm}^2/\text{s}$, $\text{ADC}_{\text{min}} = 0.74 \times 10^{-3} \text{ mm}^2/\text{s}$; B: Photomicrograph (hematoxylin-eosin staining, original magnification 100 \times) showing pathological of grade II invasive ductal carcinoma in the left breast.

before the MR imaging scan; (b) The breast lesions were confirmed by histopathological examination of specimens obtained by excision biopsy, core biopsy, or fine-needle aspiration.

MRI imaging protocol

MR imaging was performed with a 1.5 Tesla (T) clinical MR imaging system (MagnetomAvanto, Siemens Healthcare, Erlangen, Germany) equipped with a dedicated eight-channel phased array breast coil in the prone position. A transverse T2-weighted TIRM pulse sequence was performed with 5600/59/180 (repetition time/echo time/inversion time) ms, a 4 mm section thickness, a 0.8 mm intersection gap, a

field of view of 34 \times 34 cm, a matrix of 314 \times 320. A transverse T1-weighted FLASH pulse sequence was performed with 8.6/4.7 (repetition time/echo time [TR/TE]), a 1 mm section thickness, a 0.2 mm intersection gap, a field of view of 32 \times 32 cm, a matrix of 323 \times 448. DWI MR images were acquired in the axial planes by using an echo-planar imaging sequence, parallel imaging with sensitivity encoding (acceleration factor of two), fat suppression (in a spectral selective attenuated inversion-recovery sequence), volume shimming, b values of 0 and 800 s/mm^2 , TR/TE/TI=5800/86/180 ms, a 6 mm section thickness, a 0.2 mm intersection gap, a field of view of 32 \times 32 cm, and a matrix of 323 \times 448. The ADC maps were created auto-

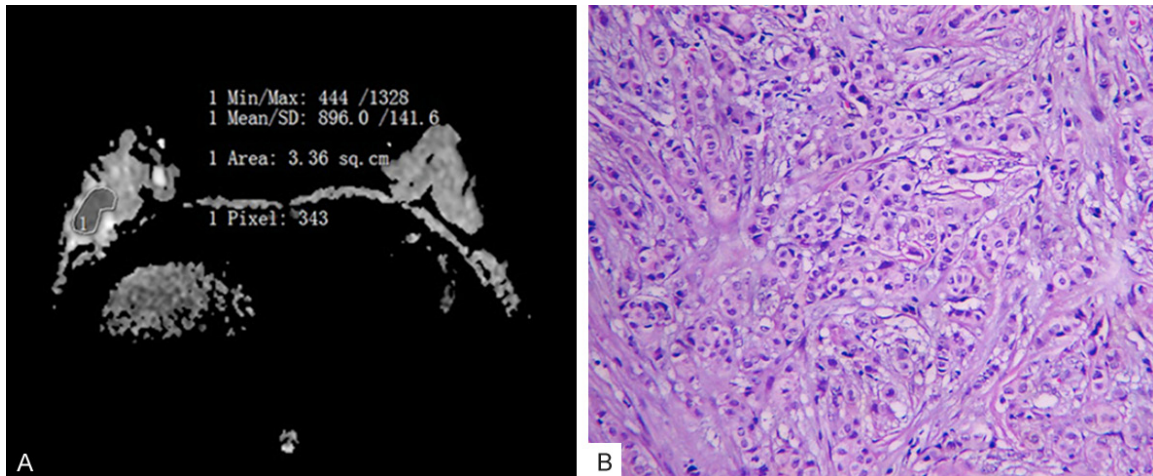


Figure 3. A: $ADC_{mean} = 0.896 \times 10^{-3} \text{ mm}^2/\text{s}$, $ADC_{min} = 0.444 \times 10^{-3} \text{ mm}^2/\text{s}$; B: Photomicrograph (hematoxylin-eosin staining, original magnification 100 \times) showing pathological of grade III invasive ductal carcinoma in the right breast.

matically by the system from the trace-weighted images with b values of 0 and 800. ADC values were calculated according to the following formula: $ADC = -(1/b) \ln (S2/S1)$, where the S2 and S1 are the signal intensities at b value of 800 and 0, respectively.

MR imaging analysis

ROIs were freehanded along the border of tumor on ADC figures in order to cover the entire lesion areas, while the obviously necrotic, liquescent, hemorrhagic, cystic, or calcified areas were excluded (based on T1WI, T2WI, and dynamic contrast-enhanced MRI figures). Mean ADC (ADC_{mean}) and minimum ADC (ADC_{min}) values of ROIs were figured out (Figures 1-3).

Histopathological assessment and analysis of MR imaging

The Bloom-Richardson semiquantitative classification method improved by Elston and Ellis was used for the assessment of histological classification using a numerical scoring system according to tubule formation, nuclear pleomorphism and mitotic count: (1) Tubule formation: Scored 1 point when it was of >75%, scored 2 when its range is 10%~75% and scored 3 when its range below 10%. (2) Nuclear pleomorphism: Scored 1 point when its shape was of normal pancreatic ductal epithelial, regular and fairly uniform; scored 3 when it was obviously pleomorphic and 2.5 times of the normal ductal epithelial; scored 2 if the size and

pleomorphism was moderate. (3) Mitotic count: In the area where cells grew briskly (diameter 0.44 mm, area 0.152 mm²), mitotic count (/10 HPF) ranged between 0 and 5 scored 1 point, 6 to 10 scored 2 and scored 3 when it exceeded 11. All the scores were added up and the total score could range from 3 to 9, with a total score of 3~5 representative of grade I, a total score of 6-7 representative of grade II and a total score of 7-9 representative of grade III, which indicated well-differentiated, moderately differentiated and poorly differentiated respectively. Firstly, the ADC values corresponded to grade I, II, and III in the classification above were divided into group I, II and III. Secondly, according to the assessment of prognosis and recurrence, the grade I patients without lymph node metastasis into were assigned into group A and those grade II patients without lymph node metastasis in were assigned into group B. Patients with lymph node metastasis in any histopathologic grade were assigned into group C [5-8].

Statistical analysis

Results were expressed as mean \pm standard deviation ($\bar{x} \pm SD$) and A p-value of less than 0.05 was judged as statistically significant. All the data were analyzed by One-way analysis of variance (One-way ANOVA), LSD test and Spearman' rank correlation coefficients were used to evaluate the correlation between the ADC values and histopathologic classification and prognostic recurrence with SPSS 19.0 software.

Table 1. Results of ADC values in different histopathologic grades of breast cancer and the results of pairwise comparisons within groups examined by LSD test

Histological grade	Cases (n)	ADC _{mean} ($\bar{x} \pm SD$, $\times 10^{-3} \text{ mm}^2/\text{s}$)	ADC _{min} ($\bar{x} \pm SD$, $\times 10^{-3} \text{ mm}^2/\text{s}$)	p value	
				ADC _{mean}	ADC _{min}
I	10	0.993±0.108	0.849±0.165	I:II	0.678 0.000
II	22	0.969±0.184	0.660±0.146	II:III	0.316 0.001
III	34	0.926±0.143	0.535±0.106	I:III	0.231 0.000

Table 2. Results of ADC values in different prognostic groups of breast cancer and the results of pairwise comparisons within groups examined by LSD test

Grade	Cases (n)	ADC _{mean} ($\bar{x} \pm SD$, $\times 10^{-3} \text{ mm}^2/\text{s}$)	ADC _{min} ($\bar{x} \pm SD$, $\times 10^{-3} \text{ mm}^2/\text{s}$)	p value	
				ADC _{mean}	ADC _{min}
A	8	1.014±0.111	0.918±0.085	A:B	0.819 0.001
B	12	1.029±0.171	0.750±0.093	B:C	0.025 0.000
C	46	0.919±0.147	0.540±0.108	A:C	0.100 0.000

Note: A is histological grade I without lymph node metastasis into, B is histological grade II without lymph node metastasis in, C is any histopathologic grade with lymph node metastasis.

Results

All the cases underwent HE stain and histopathologic classification by pathologists who had been working in hospital for ten to fifteen years. Meanwhile, all the ADC values were measured by radiologists with working experience of ten to fifteen years. One-way ANOVA was used in group I, group II and group III. There was no statistical difference within the groups of ADC_{mean} values (F=0.963, P=0.387). On the contrary, the differences between the ADC_{min} values (F=23.793, P=0.000) were noticeable. The ADC_{min} values were compared using LSD test (Detail in **Table 1**). In the groups of prognostic recurrence (group A, B, C), One-way ANOVA was used again. The differences of ADC_{mean} values (F=3.465, P=0.037) and ADC_{min} values (F=56.726, P=0.000) within the groups were statistically significant. LSD test again used to make a series of pair wise comparisons between ADCs. While only group B and group C had distinct difference in ADC_{mean} value, all the groups differed from each other in minimum ADCs (P<0.05) (Detail in **Table 2**). The ADC_{min} values were evaluated using Spearman' rank correlation coefficient method. The correlation coefficient r of ADC_{min} values in group I to III and group A~C were -0.615 (P=0.000<0.01) and -0.754 (P=0.000<0.01) respectively.

Discussion

DWI is one of the MR functional imaging techniques. It is currently the only non-invasive technology that can detect water molecules in vivo (Brownian motion) and qualify it via ADC values. Now it has been proved that ADCs has relatively high sensitivity and specificity towards the differentiation of benign and malignant breast lesions. ADCs are affected by multiple factors, such as density, arrangement of tumor cells and extracellular volume, nucleus cytoplasm ratio, membrane structure and absorption of macromolecules [9, 10]. There is an increase in cell density

when degree of differentiation becomes lower. Accordingly, the arrangement becomes closer and its volume decreases. Nucleus of tumor cells becomes bigger, meanwhile, the amount of organelle increases. This will lead to a loss of cytoplasm and slow-down movement of water molecules. As a result, ADC values become lower.

This study showed that the differences of ADC_{mean} neither had statistical significance in histopathologic grades, nor in the prognostic recurrence groups. Above all, ADCs had no correlation with traditional prognostic factors such as tumor size, lymph node metastasis status and histopathologic classification, which was consistent with the conclusion of literatures [5, 6, 11]. Yet, the literatures had pointed out that the higher histopathologic grade is the more glandular tubes will be within per unit of area. In addition, the density of tumor cells increases and its atypia becomes more obvious. The higher grade also results in a higher nucleus cytoplasm ratio and smaller extracellular volume, and controlled diffusion of water molecules becomes more significant. The ADCs decreases accordingly. Therefore, there exists correlation between ADCs and histopathologic classification, and ADCs of breast cancer can help to

forecast the degree of differentiation. This is certainly consistent with the conclusion of this study that ADC_{min} values had correlation in both histopathologic grades and prognostic recurrence groups. The reports Diffusion-weighted imaging in breast cancer: relationship between apparent diffusion coefficient and tumour aggressiveness Costantini et al. [4] and Invasive ductal carcinoma: correlation of apparent diffusion coefficient value with pathological prognostic factors Razek, et al. [12] took ADC_{mean} as the reference value. However, in this study, the value was ADC_{min} . In terms of reference value, the two methods lead to inconsistent results. In addition, the grade I (1.25×10^{-3} mm²/s), grade II (1.02×10^{-3} mm²/s) and grade III (0.92×10^{-3} mm²/s) in the report of Costantini et al. [4] also have differences with that of our study. The reasons can be summarize into following aspects.

Due to the multiple factors such as microvascular vessel density (MVD) and vascular endothelial growth factor (VEGF), the tumor cells of breast grow at different speed. Furthermore, differences are also generated in the amount of glandular ducts, nuclear pleomorphism and mitotic count, and the controlled diffusion of water molecules inside tumor shows in different degree. Hirano [13] had pointed out the maximum ADC value inside lesion reflects the highest cellular zone, and the minimum ADC value reflects the lowest cellular zone. Hence, the ADCs partially measured and ADC_{mean} of the whole lesion in some degree deviated from the extent of expression of cell density and extracellular volume in different zones. Besides, factors such as tumorous fibrosis inside lesion and tiny necrosis also affect ADCs, especially ADC_{max} , which results in the difference of pathological features reflected by ADC_{mean} . ADC_{min} , as shown in the study, has correlation with histopathologica classification, and it can accurately reflect the degree of internal pathological grading and the difference of tumor differentiation in different grades.

Both vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) increase when axillary lymph node metastasis takes place [14, 15]. Masses of new capillaries show up in tumor and internal MVD increases. This is consistent with the conclusion suggested by literatures that MVD has positive correlation with axillary lymph node metastasis [16,

17]. The MVD increases and tumor cells grow at a higher speed, accordingly, extracellular volume becomes smaller and cell density increases [11, 18, 19]. The diffusion of water molecules is limited in this case and the ADCs decline. Literatures [20-22] figured out that axillary lymph node metastasis usually take place with an increase in S-phase fraction (SPF). Phase S is the period where DNA is synthesized and duplicated. SPF is a good indicator for tumor proliferation reflection. Tumor cells actively proliferated when SPF increases. Tumor cell doubling time is short; both cell density and nucleus cytoplasm ratio increase, which result in more limitation on diffusion of water molecules and thus lower the ADC values. Generally, the breast tumor that grows too fast and larger than 5 cm in size is accompanied by axillary lymph node metastasis [11, 12, 23]. It can be seen that breast tumors with axillary lymph node metastasis, most of which are accompanied with VEGF and MVD. Their SPF values are relatively high, and tumor cells grow faster than average. Consequently, the ADCs are relatively low. Thus, the breast cancer with axillary lymph node metastasis generally has lower ADCs. This is probably the main reason why ADC_{min} had closer correlation with group A, B, C rather than histopathologic grades.

Therefore, we found that when it came to the correlation with tumor histopathologic classification and traditional prognosis factor, the choice between ADC_{mean} and ADC_{min} made a difference on the assessment. ADC_{min} could better reflect the pathological features of tumor. Moreover, many studies have attempted to predict prognosis in patients with breast cancer. It has been revealed that there are traditional prognostic factors such as tumor grade and lymph node status [5, 11-13, 17, 19]. It has been indicated that prognostic index (PI) as follows: $PI = \text{tumor size} \times 0.2 + \text{lymph node stage (1-3)} + \text{histopathological grade (1-3)}$ [7]. Of these prognostic factors, the pathological grading of the breast cancer, the SPF, VEGF and MVD reflect the cellularity [11, 14, 15, 18-23]. And our results have showed that ADC values were relative good correlation with different pathological grading and lymph node status, so ADCs may be used as surrogated marker for prognosis of breast cancer.

However, the study still had its limitations. First of all, the cases of ductal carcinoma in situ

(DCIS) and grade I was not enough. The further study shall be done when we have more cases. In addition, the patients of breast cancer with axillary lymph node metastasis who had not been immunohistochemically detected with VEGF, MVD or SPF shall be detected by immunohistochemical marking. Moreover, our study had not discussed whether different b values or equipments provided by different manufacturers had any influence on the study result [24, 25]. At last, the resolution of ADC figures was relatively low and yet there existed volume effect. Only obvious liquescent, hemorrhagic, and calcified area could be avoided. The influence made on average and minimum ADC values by tiny liquefaction, hemorrhage and calcification in the lesion was still hard to distinguish. It should be analyzed comprehensively with morphology.

Conclusions

In conclusion, ADC_{min} can be considered as an optimal DWI single parameter of medical plan before breast cancer surgery and initial assessment of prognostic recurrence. There existed correlation between ADC_{min} and both histopathologic grades and prognostic recurrence groups. Above all, ADC_{min} had strong negative correlation with group A, B and C.

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Disclosure of conflict of interest

None.

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