Correlation of stromal area and nuclear-to-cytoplasm ratio with apparent diffusion coefficient in laryngeal and hypopharyngeal squamous cell carcinoma

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Purpose: Diffusion weighted MR Imaging (DWI) is increasingly being used in head and neck cancer, where response prediction might become an important application for treatment personalization. For head and neck squamous cell carcinoma (HNSCC), a correlation between local failure after (chemo)radiotherapy and pre-treatment diffusivity has been found [1]. DWI and the derived apparent diffusion coefficient (ADC) reflect microstructural features of tissues, as water diffusion can be restricted by cell membranes and tortuosity in the extracellular matrix. However, the histopathological basis of the association between higher ADC and local failure is not clear. Therefore, DWI was validated with automatically determined histologic features in scanned whole-mount slides. The aim of this study was to investigate the relationship between histological characteristics of HNSCC and ADC values.

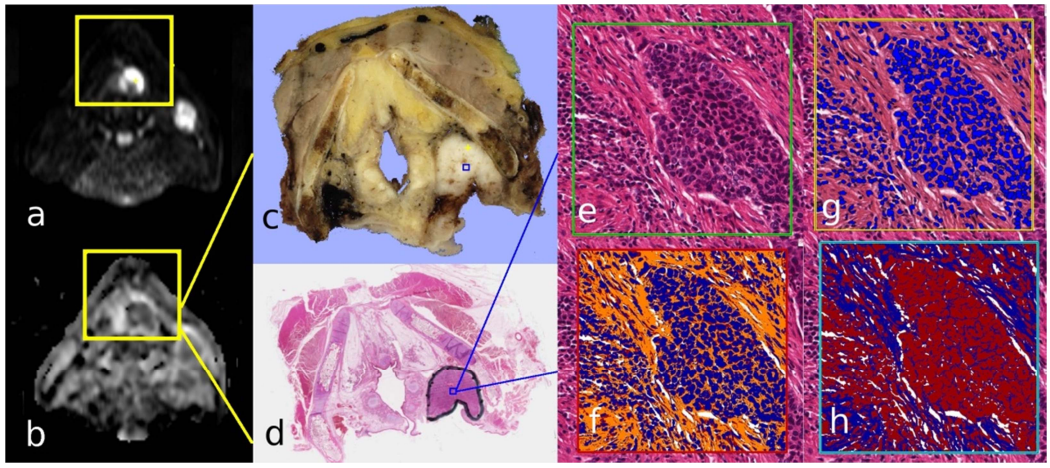

Figure 1. Registered diffusion weighted imaging with pathology and histology of a patient with a hypopharyngeal tumor. The ADC map (b) with b800 DWI (a) and the corresponding pathological slice (c) and whole mount digitized HE section (d) all show the tumor. The HE section was digitized at histological resolution, enabling color based segmentation. Nuclear density (g), regarded as cell density, percentage areas of nuclei (blue, f), cytoplasm ((red, h)-(blue, f)) and stroma (blue, h), and nuclear-to-cytoplasmic ratio (N/C) within the tumor were determined using color based segmentation on four consecutive slices.

Materials and methods: Sixteen patients with laryngeal or hypopharyngeal squamous cell carcinomas gave informed consent for this research (median age 60 years, range 49-78 years). Before having a total laryngectomy (TLE), patients underwent 1.5 Tesla MRI exam including diffusion weighted single shot spin echo echo planar imaging (DWI) with STIR fat suppression, TR/TE 5872/70ms, TI 180 ms, nsa 4, FOV 25x20cm^2, slice thickness 4mm and matrix 121x101mm^2. ADC maps were created with a linear fit of the signal intensity of the DWI with b 150 and 800 s/m^2. After resection, whole-mount heamatoxylin-eosin–stained sections were generated from the fixated specimen and digitized. The H&E sections were registered to the MRI with a registration-error less than 3 mm. Microanatomical variables were automatically determined (Aperio Technologies) (Figure 1). Spearman's correlation between tumor ADC and microanatomical variables were calculated.

TABLE 1. Microanatomical parameters and correlation with ADC

	CD (n=16)	% nuclei (n=12)	% cytoplasm (n=12)	% stroma (n=12)	N/C ratio (n=12)
Mean (range)	6406 (4806-8050)	43.1 (24.1-70.2)	19.6 (11.2-27.1)	39.6 (14.0-75.8)	2.4 (1.1-4.7)
Correlation ADC r (P value)	- 0.57 (0.02)	- 0.66 (0.02)	0.45 (0.15)	0.68 (0.02)	- 0.78 (<0.01)
Correlation % nuclei r (P value)		1.000	-0.14 (0.99)	-.965 (<0.01)	0.656 (0.26)

Results: ADC was significantly and inversely correlated to cell density, percentage area of nuclei and N/C ratio and positively to stromal area. Additionally, the very strong correlation between cell density and stromal area shows their interdependence although they result from different counting algorithms (Table 1).

Discussion/Conclusion: We found a strong relationship between ADC and both cellularity and nuclear-to-cytoplasm ratio, which has been attributed to the extracellular matrix, rather than to cellular differences [2]. Stromal ratio was indistinguishable from cellularity due to strong interdependence. Besides the association with higher ADC, poor prognosis has also been related to higher stromal fractions in breast, rectum and esophagus [3-5]. This suggests that the relationship between local failure and higher ADC might be partly attributed to the amount of tumor-stroma.

[1] Hakatenaka et al, Int J Radiat Oncol Biol Phys. 2011; [2] Zamecnik Acta Neuropathol. 2005; [3] de Kruijf et al, Breast Cancer Res Treat. 2011;[4] Mesker et al, Cell Oncol. 2009; [5] Wang et al, J Thorac Oncol. 2012.