

# Correlation of a priori DCE-MRI data with Ki-67 and HIF-1 $\alpha$ expression levels in neck nodal metastases: Initial analysis

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## Introduction

No molecular markers or genes have been proven to contribute substantially to the clinical decision-making process in head and neck (HN) cancers. Preliminary evidence supports the potential role of such markers but patient and treatment heterogeneity detracts from the ability to draw definitive conclusions [1]. The precise role of the clinical application of molecular prognostic markers in HN cancers remains elusive. We investigated whether the expression levels of selected molecular markers for proliferation (Ki-67) and hypoxia (HIF-1 $\alpha$ ) correlate with non invasive, in vivo dynamic contrast enhanced (DCE) MRI data in neck nodal metastases.

## Material and Methods

**Patients** 12 newly diagnosed head and neck squamous cell carcinoma (HNSCC) patients (M/F: 8/4, age: 57 $\pm$ 12y) with metastatic nodes were included (Table 1). After a minimum of 12 months post treatment, an overall survival assessment was performed. **MRI** MRI was performed on a 1.5 Tesla GE Excite scanner using a 4 or 8-channel neurovascular phased-array coil. The protocol consisted of standard clinical MR imaging covering the entire neck using T2-weighted and T1-weighted images. DCE- MRI studies were acquired on the neck nodes using a fast multiphase spoiled gradient echo sequence. Antecubital vein catheters delivered a bolus of 0.1mmol/kg Gd-DTPA (Magnevist) at 2 cc/s, followed by saline flush. The entire node was covered contiguously with 5-7 mm thick slices, zero gap, yielding 3-6 slices with 3.75-7.5 sec temporal resolution. Acquisition parameters included TR 9 ms, TE 2 ms, flip angle 30 $^\circ$ , bandwidth 15.63 kHz, FOV 18-20 cm, time course data points 40-80, and matrix 256x128. **Surgery** All patients underwent a standardized modified radical neck dissection. At the time of excision the surgeon marked those nodes studied with DCE-MRI with a unique identifier. This was possible as the surgeon was provided before the surgery with high-resolution anatomical MRI images in three planes with exact location provided by an experienced neuroradiologist of the neck nodes studied by DCE-MRI. After excision, the surgical specimens were immediately sent to the pathologist for appropriate processing, maintaining specimen orientation and lymph node designation. Immunohistochemistry (IHC) assays were done using standard monoclonal antibodies available by the institutional IHC core facility.

**Analysis** MRI data was analyzed with IDL 5.4. ROIs were manually drawn by the experienced neuroradiologist. The total number of pixels within the entire ROI was converted into the tumor volume (mm<sup>3</sup>). Quantitative DCE-MRI analyses of the tumor tissue time course data was done using the two-compartment Tofts model in all ROIs [2], as well as each pixel within the ROI using histogram analysis. A population based arterial input function was used [3]. The latter analyses calculated the pixel  $K^{trans}$  (volume transfer constant),  $v_e$  (extravascular-extra-cellular volume fraction), and  $k_{ep}$  (redistribution rate constant). A histogram analysis was performed on all pixels within the ROI, which yielded median and standard deviation (std) of the distribution of all pixels. The std is indicative of the tumor heterogeneity [4]. **IHC** Pathological data was reviewed by a single pathologist (> 10 yrs experience). All lymph nodes demonstrated squamous cell carcinoma. Analysis for molecular markers was performed with standard IHC techniques for Ki-67 (cellular proliferation) and HIF-1 $\alpha$  (hypoxia inducible transcription factor). IHC data were classified on an ordinal scale as follows: -, no immuno staining; +, immuno staining is less than 1% of cells; ++, immuno staining in 1-10% of cells; +++, immuno staining in 10-50% of cells; +++++, immuno staining in more than 50% of cells, according to literature [5]. Correlations between nominal DCE-MRI parameters and ordinal molecular marker expression levels were assessed using non-parametric Spearman's correlation tests. Patients were divided into groups based on overall survival (i.e. dead or alive), which was statistically tested using a 2-sided Student's t-test.

## Results and Discussion

A total of 14 lymph nodes had both DCE-MRI and IHC data available for analysis from 12 HNSCC patients (two patients had bilateral nodes). The pretreatment DCE-MRI parameters were correlated with Ki-67 (proliferation) and HIF-1 $\alpha$  (hypoxia) expression levels measured in surgical specimens. DCE-MRI parameters  $std(K^{trans})$  ( $\rho=-0.71$ ) and  $std(v_e)$  ( $\rho=-0.73$ ) showed strong negative correlation with proliferation measured by expression level of Ki-67 indicating that higher proliferation rate was seen in homogenous nodes. Hypoxia as measured by HIF-1 $\alpha$  exhibited strong positive correlation with median( $v_e$ ) ( $\rho=0.54$ ) showing that hypoxic cells have a higher leakage space ( $v_e$ ). MRI tumor volume correlated positively with median( $K^{trans}$ ) ( $\rho=0.54$ ) and median( $v_e$ ) ( $\rho=0.67$ ). DCE parameter  $k_{ep}$  did not show any significant correlations with the expression levels of the molecular markers. In addition, clinical follow up data was available for these 12 patients and the results showed that patients that are alive had significantly lower  $std(v_e)$  values than did deceased patients ( $p = 0.038$ ) suggesting that pretreatment tumor heterogeneity plays an important role in patient overall survival. No molecular marker data were significantly different when stratified for overall survival. Figure 1 displays MRI and IHC results for patient 7 (deceased) and patient 11 (alive). It can be appreciated that patient 7 has lower Ki-67 and higher HIF-1 $\alpha$  expression levels (Figure 1E, G), whereas patient 11 has higher Ki-67 and lower HIF-1 $\alpha$  expression levels (Figure 1F, H). The corresponding DCE-MRI parameters [ $median(K^{trans})$ ,  $std(K^{trans})$ ,  $median(v_e)$ ,  $std(v_e)$ ] are [0.35, 0.39, 0.89, 0.29], and [0.22, 0.09, 0.41, 0.20] for patient 7 and 11, respectively. The DCE-MRI and molecular marker data from these 2 patients follow the observed trends in the whole population.

## Conclusion

Correlation results between pretreatment DCE-MRI data and tumor hypoxia, proliferation status as measured by Ki-67 and HIF-1 $\alpha$  gives a better insight into tumor biology. Future studies with larger patient populations need to be carried out to confirm pretreatment DCE-MRI findings and molecular marker results in biopsy samples for better patient management.

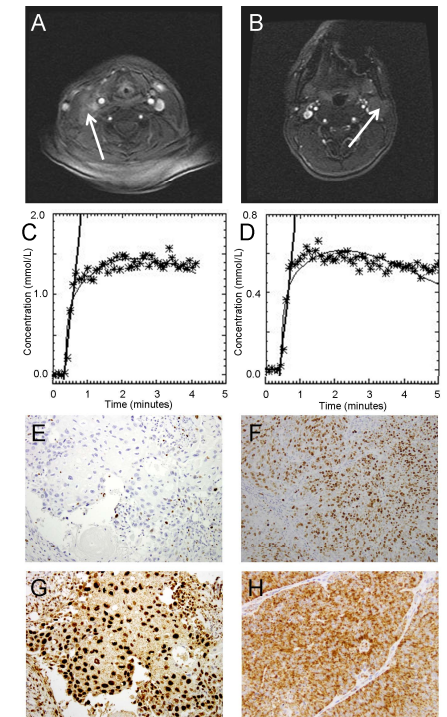
## References

[1] Silva, Oral Oncol 2004 40:688 [2] Tofts, JMRI 1999 10:223; [3] Parker, MRM 2006 56:993, [4] Lee, Ultrasound Med Biol. 2006 32:1817, [5] Zhong, Cancer Res 1999 59:5830.

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Subject	Age / sex	Primary Cancer	# Nodes	Stage	Alive	FU (m)
1	68 / f	Buccal Mucosa	2	IVa	n	15
2	47 / m	Oral Cavity	2	IVa	n	30
3	40 / f	BOT	1	IVa	y	25
4	53 / m	Oral Cavity	1	IVa	y	27
5	49 / m	Tonsil	1	IVa	y	22
6	60 / m	Unknown	1	IVa	y	27
7	79 / m	Unknown	1	IVa	n	6
8	77 / f	Unknown	1	IVa	n	17
9	56 / m	BOT	1	IVa	n	5
10	48 / m	Unknown	1	IVa	y	17
11	44 / m	Tonsil	1	III	y	14
12	65 / f	Buccal Mucosa	1	IVa	y	18

**Table 1:** Patient characteristics and long term survival. # Nodes = number of studied nodes, FU = follow up (months), BOT = Base of Tongue.



**Figure 1:** MRI and IHC data of patient 7 (left) and patient 11 (right). AB) Post contrast T1w-images, with node indicated by white arrow. CD) DCE-MRI signals, converted into Gd-DTPA concentrations, as function of acquisition time. EF) Ki-67 immuno-staining. GH) HIF-1 $\alpha$  immunostaining. Magnification is 100x.