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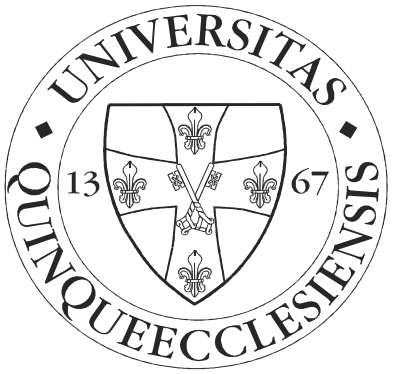

Clinical applications of combined PET/MRI in oncology, head and neck cancer.

Ph.D. thesis summary

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1. Introduction:

Head and neck cancer originates in the upper aerodigestive tract, from the level From the skull base to the thoracic outlet. Head and Neck cancers are a heterogeneous group of cancers that existed anatomically close to each other, but different in terms of etiology, histology, diagnostic, and treatment approaches. (Society for Medical Oncology 2017) About 91% of all H&N cancer are squamous cell carcinomas, 2% are sarcomas and the other 7% are adenocarcinomas, melanomas, and not well-specified tumors. (European crude and age-adjusted incidence by cancer, years of diagnosis 2000 and 2007 analysis based on 83 population-based cancer registries * 2014). Head and neck squamous cell carcinoma (HNSCC) evolves an assortment of cancers arising in the lip, oral cavity, hypopharynx, oropharynx, nasopharynx, or larynx.(Society for Medical Oncology 2017) Alcohol and tobacco are known risk factors for most head and neck cancers, and incidence rates are found to be higher in regions with high rates of alcohol and tobacco consumption. (Hashibe et al. 2011)

Smoking, alcohol consumption are the most important risk factors for developing cancer (Blot et al. 1988), human papillomavirus (HPV) infection, and Epstein-Barr virus (EBV) infection (nasopharyngeal cancers in Asia) are also associated risk factors with head and neck cancer. (Cruz et al. 1997; Sankaranarayanan et al. 1998). Herpes simplex virus (HSV) is another risk factor but is less strongly correlated with the development of oral carcinomas than EBV or HPV. (Larsson et al. 1991) Immunodeficiency is also found to be an important risk factor for developing head and neck cancer, which may increase the incidence by threefold. (Deeken et al. 2012; Grulich et al. 2007) As well as other but less relevant risk factors that may play a role in HNC development such as occupational exposure, radiation, genetic factors, betel nut chewing, poor oral hygiene and periodontal disease, which has been linked with carcinoma of the oral cavity. (Guha et al. 2014; Hashim et al. 2016; Lacko et al. 2014; Sale et al. 2004; Vaughan et al. 1997).

According to the American Joint Committee on Cancer (AJCC) (Edge and Compton 2010), head and neck cancer arises in the upper aerodigestive tract lining membranes. The TNM classifications described as; T which represents the extent of the primary tumor for each site in the neck area, size, or both, generally the T classifications are similar from different sites with minimal differences in some specific details for each localization, the major sites include (1) the oral cavity, (2) the oropharynx, (3) the hypopharynx, (4) the larynx, (5) the nasopharynx, and (6) nasal and

paranasal sinuses, N classification, which describes the lymph nodes metastatic in the adjacent lymphatic system. Regional cervical lymph node status is a very important prognostic factor in head and neck cancer and must be evaluated and assessed in each tumor. M classification describes the absence or presence of distant metastases in other organs in the body, most commonly lungs, liver, and bone. After gathering this clinical information, the specific TNM status of each patient is then tabulated to give a numerical status of Stage I, II, III, or IV.
For a better estimation and assessment of the disease extent before the treatment, imaging modalities and techniques such as computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET) alone or combined (PET/CT or PET/MRI), as well as Ultrasonography (US), may be applied and, in advanced tumor stages, have added to the accuracy of the primary tumor and lymph node staging, primary localization and regional lymph node involvement. As another useful technique for primary tumor evaluation, Endoscopy, is preferable for a more detailed assessment for T staging. Biopsy, by fine-needle aspiration (FNA) may confirm the presence of the tumor and it's helpful to understand the histopathologic nature of the disease, but, in another context, it can't alone rule out the presence of the disease.

As a new imaging modality, PET/MRI has been had emerged as an effective and accurate imaging modality in oncology. (Partovi et al. 2014) The PET/MRI is expected to be more valuable than PET or CT alone or combined because PET/MRI involves better contrast in soft tissues from the MRI and a lower radiation dose than CT. (Pace L, Nicolai E, Aiello M, Catalano OA 2013) The superior role of the PET/MRI over other imaging modalities is its ability to perform many functional imaging techniques. (Queiroz et al. 2014) These include DWI which is a widely used technology as a noninvasive diagnosis technology of tissue biology. (Yamauchi H 2014)

In the clinical field, to discriminate different types of cervical lymph nodes and monitor treatment response imaging technologies have been used, for example, to determine primary tumor and lymph nodes characteristics; such as morphology (shape and size), presences of necrosis, internal cellularity, stage and grade of the primary tumor. (De Bondt et al. 2009; King et al. 2004) These criteria have been succeeded in providing information about the cellularity and the functional aspect of the tumor with the anatomical information, not only for providing information about location and the size of the tumor but also for the tumor functional and biological aspects by

monitoring the hypermetabolic areas inside the tumor (Sauter et al. 2013; Veit-Haibach et al. 2013).

2. Aims

2.1. Diffusion-Weighted Imaging (DWI) derived from PET/MRI for lymph node assessment in patients with Head and Neck Squamous Cell Carcinoma (HNSCC). (I)

- Our aim in this study was to evaluate the efficacy of DWI-ADC to differentiate between benign and malignant lymph node in Head and Neck.
- To assess also the efficacy of DWI-ADC in differentiating sub-centimeters lymph nodes.
- We also studied the ability of DWI-ADC in differentiating different tumor grades and tumor localization.

2.2. Pre-treatment PET/MRI based FDG and DWI imaging parameters for predicting HPV status and tumor response to chemoradiotherapy in primary oropharyngeal squamous cell carcinoma (OPSCC). (II)

- Our aim was to assess the ability of DWI-ADC and 18F-FDG metabolic imaging parameters in predicting HPV status.
- We also studied the role of DWI-ADC and 18F-FDG imaging parameters in predicting treatment response to chemo/radiotherapy.

2.3. Correlation between tissue cellularity and metabolism represented by diffusion weighted imaging (DWI) and 18F-FDG PET/MRI in head and neck cancer (HNC)

- Our aim was to investigate the correlation between tissue cellularity and metabolism represented by ADC and FDG metabolic parameters.
- We also studied the ability of the imaging parameters in predicting tumor aggressiveness. (III)

3. METHDOS

3.1. DWI role in differentiating between benign and malignant lymph nodes (I).

We included 90 subjects, 65 patients were recruited with confirmed primary HNSCCs underwent 18F-FDG PET/MRI (3T) for staging and clinical assessment. All 65 patients were confirmed with metastatic lymph nodes due to HNSCC (time between imaging scan and biopsy ranged between 1-3 days), and 25 healthy subjects were randomly chosen from the radiology department available database, one node was selected from the neck region for evaluation. The inclusion criteria used for the patients in the study were: (1) Confirmed primary HNSCC malignancy by biopsy; (2) Multiparametric MR imaging (DWI); (3) PET/MRI for initial staging prior to primary treatment (Surgery or/and radio/chemotherapy); (4) Histopathological results were available for comparison; (5) No previous neck surgery, chemotherapy or (chemo)-radiotherpay. The criteria used for choosing healthy subjects were: (1) Free history of malignancy; (2) No previous neck surgery; (3) No previous treatment by chemoradiotherapy; and (4) No head and neck lesions, inflammation or abscess or any abnormalities. The criteria used for considering lymph nodes as metastatic was confirmed after (1) Biopsy (gold standard); (2) High FDG accumulation was considered as indication for malignancy in the <10mm group (biopsy/FNA were taken). Metastatic lymph nodes with short-axis diameter <10mm (n = 17) and short-axis diameter >10mm (n = 48). The criteria used for considering lymph nodes as normal were: (1) Free history of malignancy; (2) Ovoid or smooth in shape; and (3) Short-axis diameter <10mm.

3.2. Role of PET/MRI in OPSCC (II).

46 patients with proven OPSCC underwent 18F-FDG PET/MRI for staging and restaging, assessment of the disease, and post-therapy follow-up (5.6 months on average). The inclusion criteria were (1) proved non-treated primary OPSCC, (2) patients underwent PET/MRI including DWI sequence (3) HPV test was performed. Exclusion criteria were: (1) patients who had non-measurable ADC (2) patients with motion artifact or suboptimal image quality including motion artifacts, and patients who did not underwent posttherapy follow up. Finally, a total of 33 patients were included in our study. Final confirmation of malignancy was done after biopsy of the primary tumor and metastatic lymph nodes. PET SUVmax, TLG, MTV

parameters were measured in each patient using Siemens Syngo Via (20VB) application, which provided an automated delineated volumetric analysis based on the SUV. Using the VOI Sphere tool, the metabolic volumetric contours were segmented. VOIs have been assessed blindly to the histopathological characteristics. SUVmax represented the single voxel activity concentration of a specific tumor with the highest SUV.

To evaluate the treatment results of the primary tumor based on pre and post-therapy PET/CT and PET/MRI data, we used the European Organization for Research and Treatment of Cancer (EORTC) system. To evaluate the predictive value of the pre-treatment imaging parameters we created two patient's groups based on the PET/MRI therapeutic response and clinical follow-up. The two groups were representing complete remission (CR) which includes only patients with complete remission and non-complete remission (NCR) which includes patients with partial response, stable disease, and progressive disease patients.

Immunohistochemistry of p16 protein overexpression from tumor blocks (Ventana Medical System - p16 protocol, Roche p16 cintec histology assay antibody 1: 5 dilution) was performed by the pathology department of Csolnoky Ferenc hospital from the primary tumor to detect the presence of high-risk HPV infection. We identified cases as positive, in which we observed socalled "block positivity", meani g both the nucleus and the plasma show strong staining in the tumor cells. Additional staining patterns (e.g., cytoplasmic only) were assessed as negative.

3.3. Correlation between tissue cellularity and metabolism (III).

109 patients with proven HNC underwent 18F-FDG PET/MRI for staging and restaging, assessment of the disease. The inclusion criteria were (1) proved non-treated primary HNC, (2) patients underwent PET/CT and PET/MRI including DWI sequence (3) single tracer injection session. Exclusion criteria (1) patients who had non-measurable ADC, or FDG parameters (2) patients with motion artifact or bad image quality. Finally, a total of 71 patients were included in our study. Final confirmation of malignancy was done after PET/MRI examination of the primary tumor and metastatic lymph nodes combined with biopsy.

In each patient, the SUVmax, TLG, MTV were measured from the PET imaging; Siemens (Syngo Via 10VB) was used, which provided an automatized delineated SUV-based volumetric analysis. The metabolic volumetric contours were segmented by using the Syngo Via (VOI) Sphere tool. The single voxel activity concentration of a particular tumor with the

highest SUV was represented by SUVmax. A fixed 2.5 threshold of SUV was used for tumor SUVmax for both MTV and TLG. The volume above the given VOI was represented the MTV while the TLG represented the VOI of the average SUVmean or SULmean multiplied by the MTV. The ADC map was automatically generated and analyzed on the implemented eRAD software. DWI images were analyzed by drawing round or oval region of interest (ROI) manually on the ADC map covering the largest tumor diameter, on a single DWI slice within the center of the lesion in the most homogenous part which aswas the lowest ADC or the highest SUV reported after excluding or/and avoiding the necrotic and cystic areas.

4. Statistical analysis:

4.1. DWI role in differentiating between benign and malignant lymph nodes (I).

SPSS version 25.0 (IBM Corporation, USA) was used for the statistical analysis. Continuous variables were compared using the Student's t-test or analysis of variance and were expressed as the mean ± standard deviation for the variables with a normal distribution. The Independent sample t-test was used to compare the ADC mean values of the metastatic and the normal lymph nodes as well as between sub-groups analysis. ROC curve was applied to determine the sensitivity and specificity, AUC, and the optimal threshold to differentiate between normal and malignant nodes. The selection of the optimal threshold was chosen at the point with the highest Youden index (maximizing both sensitivity and specificity). The one-way analysis of variance (ANOVA) was applied to evaluate the coincidence between primary cancer's grade with the metastatic lymph nodes' ADC values and with the primary tumor localization. Post Hoc analysis (Scheffe) was used to compare the parameters in terms of histopathology in case of significant results.

4.2. Role of PET/MRI in OPSCC (II).

Statistical analysis was performed by using SPSS 25 (IBM SPSS Statistics, Armonk, New York, USA). The data collected were evaluated using descriptive statistics (mean ± standard deviation), for variables with normal distribution, median, and interquartile range for variables

with nonnormal distribution. The normality of the measured FDG and DWI was assessed by Shapiro-Wilks test. We used the Spearman correlation coefficient to assess the correlation between FDG and DWI parameters with T stages, N stages, and graders. Mann Whitney test, Wilcoxon's rank-sum test for group comparison with variables not normally distributed; TLG, and MTV ($P<0.001$). ADCmean and SUVmax due to normally distributed were analyzed with independent sample t test for group comparison (HPV status and post-therapy results) and ANOVA test between ADC values and grades of the primary tumor. A Chi-square test was used to assess the association between categorical variables, (HPV, T stages, N stages, grades and post-therapy results). Variables for which $P<0.1$ in univariate analysis were subjected to multiple linear regression analysis to determine those that were independently associated with the imaging parameters by integrating statistical differences in the univariate analysis into the multivariate linear regression model. ROC curve was used to determine the best cut off value to differentiate between HPV+ and HPV-. A pvalue <0.05 was indicated as a statistically significant result.

4.3. Correlation between tissue cellularity and metabolism (III).

Statistical analysis was performed by using SPSS 25 (IBM SPSS Statistics, Armonk, New York, USA). The data collected were evaluated using descriptive statistics (mean ± standard deviation), for variables with normal distribution and median and interquartile range for variables with nonnormal distribution. The Spearman rank correlation (r) was used to estimate the association between 18F-FDG parameters and ADC values and tumor size (continues variable). ANOVA or Kruskal–Wallis test were performed on the clinicopathological features that may affect the 18FFDG and ADC of the tumor. Variables for which $P<0.1$ in univariate analysis were subjected to multiple linear regression analysis to determine those that were independently associated with the imaging parameters by integrating statistically significant differences in the univariate analysis into the multivariate linear regression model, we used transforming function to convert variables with non-normal distribution into a normal distribution, then the factors were added one by one (Stepwise). Mann-Whitney test and independent-sample T-test were applied to the imaging parameters after the patients were grouped based on lymph nodes involvement into positive (N+) and negative lymph nodes (N). A p-value <0.05 was indicated as a statistically significant result.

5. Results

5.1. DWI role in differentiating between benign and malignant lymph nodes (I).

5.1.1. The ADC values of normal and metastatic lymph nodes: According to the statistical analysis after comparing ADC values of the metastatic lymph nodes with ADC values of the normal lymph nodes, the ADCmean value of metastatic lymph nodes was significantly lower than the ADCmean value of normal lymph nodes. To obtain the optimal ADC value to differentiate between metastatic and normal nodes, we applied the ROC curve. The result shows that when using (1.138±0.75) as an optimal threshold value to differentiate between metastatic and normal nodes, the AUC was 98.3%, sensitivity and specificity were 92.3% and 98.6%, respectively. Furthermore, to assess the ability of DWI to discriminate small metastatic lymph. The results show that ADCmean values of the metastatic lymph nodes short-axis diameter was significantly lower than ADCmean values of the normal lymph nodes. No significant difference found between ADCmean of the metastatic lymph nodes with size <10mm and the metastatic lymph nodes with size >10mm.

5.1.2. Correlation between the primary HNSCC grade and metastatic lymph nodes' ADC values: Our results show that no significant differences between the metastatic lymph nodes' ADCmean values from poorly differentiated primary HNSCC was and the ADCmean values for the metastatic lymph nodes from well and moderately differentiated, respectively.

5.1.3. The ADC values of lymph nodes and the correlation with the primary HNSCC tumor localization: ADCmean values of the metastatic lymph nodes from OPSCC, HPSCC, LSCC, Sinus carcinoma and Oral cavity carcinoma were calculated and assessed, No significant difference was found.

5.2. Role of PET/MRI in OPSCC (II).

5.2.1. Correlation between HPV with FDG and DWI parameters:

The results show that the ADCmean values of HPV+ were significantly lower than HPV-. Additionally, a significant inverse correlation between ADC and primary tumor grades (well, moderately and poorly differentiated tumors) was found. On the other

hand, no significant differences were found between SUVmax, TLG, and MTV with HPV status. Moreover, SUVmax was significantly higher in patients with higher N stage. Higher TLG and MTV were observed with a higher T stage.

5.2.2. Correlation between HPV and histopathological characteristics:

No significant correlations were found between HPV groups and T stages; HPV+, no significant association between HPV status and N stages, HPV+, or with primary tumor degree of differentiation.

5.2.3. Predicting treatment response:

A statistically significant difference was found between CR vs NCR pre-treatment ADC values. A significant difference was found between CR and NCR with pre-treatment TLG and MTV. No statistically significant difference was found between the two groups and SUVmax, (P=0.664). (Figure 3). Moreover, HPV+ and HPV- were significantly associated between CR and NCR patients.

5.3. **Correlation between tissue cellularity and metabolism (III).**

The results show that 18F-FDG parameters (SUVmax, TLG and, MTV) were not correlated with ADC values. 18F-FDG parameters (SUVmax, TLG and MTV) were significantly and positively correlated with tumor size. N stages were correlated with higher SUVmax. T stages and N stages were correlated with higher TLG values. T stages were correlated with higher MTV. Lower ADC values was found to be correlated with the degree of differentiation.

6. Discussion:

6.1. DWI role in differentiating between benign and malignant lymph nodes (I).

In our study, we found a statistically significant difference between metastatic and normal lymph nodes' ADC values (P=0.001); the result was in agreement with previous authors. (Goldsmid & Willis, 2016; Kwee et al., 2011; Perrone et al., 2011; Srinivasan et al., 2012; Taha Ali, 2012) Moreover, our study proposes that the best threshold to differentiate between normal and malignant nodes was (1.138+0.75*10-3mm2/sec), with (92.3%) sensitivity and

(98.6%) specificity. Our study shows that DWI might be helpful to discriminate sub-centimeters metastatic lymph nodes.

In our study we found that DWI might be useful for discriminating small lymph. According to Barchetti et al. DWI was efficient to differentiate small metastatic lymph nodes in HNSCC, (94.6%) of the measured lymph nodes were <10mm in size, a threshold of 0.96510−3mm2/sec was used as best cut-off value.(Barchetti et al., 2014) De Bondt et al. found a similar result, DWI was able to differentiate metastatic from benign lesion in HNSCC (95.4% of the measured lymph nodes were <10mm) using an optimal ADC threshold of 1.0×10−3 mm2/s. (De Bondt et al., 2009) Although, other authors found that DWI does not allow to differentiate small metastatic lymph nodes in HNSCC. (H. K. Lim et al., 2014)

6.2. Role of PET/MRI in OPSCC (II).

We analyzed the efficacy of combined PET/MRI imaging parameters to predict HPV status and local response of OPSCC treated by CRT with curative intent. We found that HPV+ lesions are associated with lower ADC values than HPV- lesions, which might be useful as a non-invasive technique to evaluate HPV status. With a value of (809±0.37*10-3mm2/s), the area under the curve (AUC) was 80.0% with 73.7% sensitivity, and 73.3% specificity was able to differentiate between HPV groups. In contrast, FDG parameters did not show any statistical significance between HPV groups, which means that FDG might be not useful for predicting HPV status. Our study has also shown that DWI, FDG volumetric metabolism parameters (TLG and MTV) are useful predictor biomarkers to assess the response before treatment in OPSCC, while SUVmax may not. Besides, the HPV+ patient's group have shown better response to therapy than HPV- patients.

In this study, we found that DWI and metabolic imaging parameters TLG and MTV can provide more accurate information for treatment prediction since we found that higher TLG and MTV before therapy lead to a higher probability of recurrence and lower rates of response, (Paidpally et al., 2014; K Pak et al., 2014) these findings do not eliminate the role of the basic FDG parameter (SUVmax) for prediction, although, in our study, we found no significant correlation between SUVmax and response. Overall, in OPSCC, it has been reported that TLG and MTV have better results in the prediction of overall survival and disease-free survival. (Moan et al., 2019) In regard to ADC, our results revealed that ADC might be useful for

predicting response after therapy, although we have lost data for 5 patients in post-treatment examinations.

6.3. Correlation between tissue cellularity and metabolism (III).

Our results showed that FDG uptake parameters (SUVmax, TLG, and MTV) were not significantly correlated with the ADCmean value. Similar results were observed; Min et al., in their study of HNSCC, reported that there was no significant correlation between ADCmean with SUVmax and SUVmean, also no significant correlation was found between ADCmean and both MTV and TLG.

Our explanation for the lack of correlation is the fact that both imaging parameters explain different tissue microstructures characteristics, DWI assess the water molecule motion in the tissue and affected by the cellularity, proliferation rate and cell counts which in clinical use affected by ROI size placement and interobserver variability. (Lambregts et al., 2011).

Our results reveal that FDG metabolic parameters have reported different correlations; it has shown that primary tumor SUVmax and TLG were significantly correlated with the N stages; the higher N stage resulted in a higher value of SUVmax and TLG, while MTV did not show correlation with N stages. Furthermore, in our study, no significant correlation was observed between MTV and the lymph node status.

ADC, on the other hand, shows a significant correlation with tumor grades, which reflect the degree of water motion within the tumor cells, this is from the fact that higher-grade tumors (G3) show more restriction to water molecules motion (lower cellularity) which as result affect ADC. On the other side, ADC did not show significant correlations with T stages, N stages, or Tumor size although there was a slightly inverse correlation with N stages.

7. Conclusions:

Our work confirms the feasibility of DWI in differentiating between normal and metastatic lymph nodes. It's also might be useful to differentiate sub-centimeters lymph node. Additionally, we found that pre-treatment ADC was a predictor of HPV status and posttherapy results. On the other side, FDG parameters were able to predict tumor response to therapy, but they don't show a feasible role in predicting HPV status. Based on the reported results, both DWI and FDG parameters are important to assess patients with OPSCC and their role might

be complementary to each other. Finally, no linear correlation between the FDG PET and ADC MR parameters. The FDG PET-based glucose metabolic and DWI MR derived cellularity data may represent different biological aspects of HNC tumors and simultaneous PET/MR imaging could provide complementary diagnostic information. SUVmax, have shown higher accuracy in predicting tumor aggressiveness than DWI.

Limitations:

Heterogeneity of the sample, which means multiple primary tumor localization. Second, the result may not be valid for all health centers due to the variation of MRI technologies and b-value strengths (the accuracy and quality of the magnetic field depend on the vendor). Third, retrospective design of the study. single institute approach and using the conventional FDG and DWI parameters which do not include texture analysis should be noted. Although moderate sample size, we only included OPSCC patients from the HNC group to ensure a homogeneous population that may increase the reliability of our results. Focusing on the search of a correlation between 18F-FDG, ADC, and histopathological features only in HNC. Lastly, associations with other functional tumor parameters, such as apoptosis factors and were not analyzed. Fourth, the design of the study was retrospective.

Summary of novel findings:

1. DWI is an efficient non-invasive technique to differentiate between normal and malignant lymph nodes in head and neck cancer.
2. DWI is and efficient non-invasive technique to differentiate sub-centimeters <10mm short axial diameter normal and malignant lymph nodes.
3. No correlation between DWI and FDG PET imaging parameters which reflect the tumor cellularity and metabolism in head and neck cancer.
4. DWI is and efficient non-invasive technique to predict HPV status in patients with oropharyngeal squamous cell carcinoma.
5. DWI is might be helpful to predict tumor response to therapy in oropharyngeal squamous cell carcinoma.
6. FDG-PET imaging parameters did not show significant role in predicting HPV status.
7. FDG-PET imaging parameters might be helpful to predict response to therapy in oropharyngeal squamous cell carcinoma.