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Role of perfusion CT in the evaluation of functional primary tumor response after radiochemotherapy in head and neck cancer: preliminary findings

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Abstract:	Objectives To report initial results of a prospective study aimed to evaluate the CT perfusion parameter changes (ΔPCTp) of the primary tumor after radiochemotherapy (RCT) in head and neck cancer (HNC) and to correlate with PET/CT response. Methods Eligibility criteria included HNC (Stage III-IV) candidates to RCT. Patients underwent a PCT at baseline and at 3 weeks and 3 months after treatment. Blood volume (BV), blood flow (BF), mean transit time (MTT) and permeability surface product (PS) were computed. Moreover, a PET/CT was performed at baseline and at 3 months after treatment. The ΔPCTp was evaluated between baseline and the 3 week/3month evaluations whereas PET/CT response was based on the SUVmax change values according to EORTC criteria. Results Between July 2012 and July 2015, 25 patients were enrolled.

	A significant reduction of all PCTp was observed from the baseline to after RCT (p<0,001). Specifically, a significant reduction was shown at 3 weeks for all PCTp except MTT (from 6,18 sec to 5,14 sec; p=0,722). Differently, a significant reduction of all PCTp (p<0,001) including MTT (from 6,18 sec to 2,24 sec; p=0,001) was shown at 3 months. Moreover, the reduction of PS resulted to significantly predict the PET/CT response at 3 months (p=0,037) with a trend also at 3 weeks (p=0,099) at the multivariate analysis. Conclusions Our preliminary findings seem to show that almost all PCTp are significantly reduced after RCT whereas PS seems to come out as the strongest factor in predicting the PET/CT response. Advances in knowledge: This article provides information on the potential useful role of perfusion CT in evaluating tumor response both early and late after radiochemotherapy.

Dear reviewer,

we do apologize for our inadequate corrections following your comments and suggestions

Again, you can find the editings in italics and underlined in the text.

- The paragraph about the reliability of PET/CT within 10-12 weeks has been modified (sorry I didn't understand the meaning of your comment last time) as well as a paragraph about the justification for the use of PCT has been added in the introduction together with the corresponding literature references.

- The two late frames mentioned in the PCT protocol section are two stacks of axial images acquired on the lesion site with the same z-axis coverage and other scanning parameters as first-pass frames. Such late scans were performed with a 15-second time interval from one another during the recirculation phase of intravenously administered iodinated contrast medium (CM) so as to obtain data for calculation of the PS parameter, which reflects CM backflow from the interstitial to the intravascular space after the first pass phase due to increased leakiness of neoangiogenetic vessels compared with normal ones (ref 21). We modified the text of our revised manuscript in order to make those points as clear as possible to readers.

- Furthermore, a large comment regarding the pathophysiological background of our findings and a comparison with those reported by Pietsch et al. has been added in the discussion section.

  Finally, we clearly stated that the correlation of ΔPCTp with SUV_{max} changes was a major limitation of our study and we hope, with a longer follow up, to correlate the ΔPCTp with more important oncological outcomes such as local recurrence free survival and/or cancer specific survival.

  In this regard, the structure of the discussion section has been slightly edited in order to properly insert these comments.

We hope that our corrections satisfied your requests.

Role of perfusion CT in the evaluation of functional primary tumor response after radiochemotherapy in head and neck cancer: preliminary findings

Short Title**: Perfusion CT and radiochemotherapy in head and neck cancer**

Type of Manuscript**:** full paper

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ABSTRACT

Objectives

To report initial results of a prospective study aimed to evaluate the CT perfusion parameter changes (ΔPCTp) of the primary tumor after radiochemotherapy (RCT) in head and neck cancer (HNC) and to correlate with PET/CT response.

Methods

Eligibility criteria included HNC (Stage III-IV) candidates to RCT. Patients underwent *a* PCT at baseline and at 3 weeks and 3 months after treatment. Blood volume (BV), blood flow (BF), mean transit time (MTT) and permeability surface product (PS) were computed. Moreover, a PET/CT was performed at baseline and at 3 months after treatment. The ΔPCTp was evaluated between baseline and the 3-week/3-month evaluations whereas PET/CT response was based on the SUV_{max} change values according to EORTC criteria.

Results

Between July 2012 and July 2015, 25 patients were enrolled. A significant reduction of all PCTp was observed from the baseline to after RCT ($p<0.001$). Specifically, a significant reduction was shown at 3 weeks for all PCTp except MTT (from 6.18 sec to 5.14 sec; $p=0.722$). Differently, a significant reduction of all PCTp ($p<0,001$) including MTT (from 6.18 sec to 2.24 sec; $p=0.001$) was shown at 3 months. Moreover, the reduction of PS resulted to significantly predict the PET/CT response at 3 months ($p=0.037$) with a trend also at 3 weeks ($p=0.099$) at the multivariate analysis.

Conclusions

Our preliminary findings seem to show that almost all PCTp are significantly reduced after RCT whereas PS seems to come out as the strongest factor in predicting the PET/CT response.

Advances in knowledge: This article provides information on the potential useful role of perfusion CT in evaluating tumor response both early and late after radiochemotherapy.

Keywords: Head and neck cancer; Perfusion Computed Tomography; Positron Emission Tomography; Radiochemotherapy;

List of abbreviations: HNC: head and neck cancer; HPV: human papilloma virus; RCT: radiochemotherapy; IMRT: intensity and modulated radiotherapy; IGRT: image guided radiotherapy; PCT: Perfusion Computed Tomography; PET: Positron Emission Tomography; CTV: Clinical target volume; PTV: planning target volume; CBCT: cone beam CT; BF: blood flow; BV: blood volume; MTT: mean transit time; PS: permeability surface; ROI: region of interest; TOF: time of fly; CR: complete response; PR: partial response; SD: stable disease; PD: progression disease; GLM: general linear model

INTRODUCTION

In Western countries head and neck cancer (HNC) account for about 5% of all tumors.[1,2] Squamous cell carcinoma is the most common histotype (about 90%), and generally arises from the mucosa of the upper aerodigestive tract.

The etiology of these tumors has traditionally been related to tobacco and alcohol consumption, whereas in the last decade infection from the human papilloma virus (HPV) has been identified as an emerging cause; most of the HPV-related HNC arise in the oropharynx subsite, and they generally carry a better prognosis than the non-HPV-related cancers.[3]

HNC constitute a heterogeneous clinical setting including five main different subtypes according to the site of the primary tumor: nasopharynx, oropharynx, oral cavity, hypopharynx, and larynx. Nevertheless, they are all characterized by a predominant route of loco-regional metastasis through the lymphatic route and by a relatively low proportion of distant hematogenous metastasis.

Therefore, prognosis and therapeutic options are strongly related both to the subsite of origin and to the clinical stage at diagnosis; these features heavily affect the probability of control both for the primary tumor and for lymph node disease.

In the last few decades, due to technological improvements in treatment delivery and set-up control systems such as intensity and modulated radiotherapy (IMRT) and image-guided radiotherapy (IGRT), RCT has emerged as a valid alternative to radical surgery, resulting in similar rates of local control.[4] As a consequence, interest is rising on new imaging technologies with the potential of properly evaluating tumor response after treatment in order to select, as soon as possible during treatment, "non-responders" patients who could benefit from salvage surgery.[5]

In the current clinical practice, evaluation of tumor response is based on fiberoptic endoscopy of the HN region, combined with "morphological" CT/MR based-imaging. However, CT and MR imaging are often inadequate to discriminate between post-actinic tissue changes and persistence of tumor (i.e., in presence of post-RT scarring areas) and often require confirmation by biopsy.[6]

By adding functional metabolic information to standard radiological imaging, [^{18}F]FDG-PET/CT has emerged in the last few years as an important advancement for assessing tumor response to treatment, based on its excellent negative predictive value (95.1%).

The main drawback of this imaging approach is the non-negligible rate of *early* false positive results (resulting in a 58.6% positive predictive value), due to local enhanced glucose consumption induced by post-actinic inflammation *tough an high reliability of [^{18}F]FDG-PET/CT has been reported within 10-12 weeks after completing RT*.[7]

Nevertheless, efforts are continuing to exploit the full potential of molecular imaging both to

improve the prediction of tumor response and to shorten timing of the imaging procedure for therapy response evaluation relative to treatment.[8]

More recently, perfusion computed tomography (PCT) obtaining quantitative information on the status of tumor microcirculation that might reflect early treatment response or predict outcome, has been proposed as a potential additional tool for noninvasive functional assessment of treatment response.[9,10,11,12,13,14] *Though no consensus has been reached so far about the optimal timing of PCT imaging for the assessment of early treatment response, several experimental studies on both humans and animal models have shown the ability of PCT to detect changes of PCT parameters less than one month after or even within days of the beginning of therapy (i.e. well before a reduction in tumour size can be demonstrated on conventional morphological CT imaging*) [15,16,17]

In this study we prospectively evaluated the changes in perfusion parameters (ΔPCTp) of the primary tumor, measured both at 3 weeks and at 3 months after RCT, compared to the pre-treatment values. The ΔPCTp values so obtained were correlated to the changes in [^{18}F]FDG uptake in the primary tumor measured three months after RCT versus the pre-treatment values.

MATERIALS AND METHODS

This study was carried out as a collaboration involving the Divisions of Radiation Oncology, Diagnostic Radiology and Nuclear Medicine and was conducted according to the Declaration of Helsinki. All patients gave their written consent to all diagnostic and therapeutic procedures.

Study Design

We prospectively enrolled patients affected by intermediate to advanced HNC (Stage III-IV), candidate to RCT with curative intent. The eligibility criteria were:

- histologically proven undifferentiated nasopharyngeal-type carcinoma or squamous cell carcinoma.
- tumor arising from one of the following subsites: nasopharynx, oropharynx, oral cavity, larynx and hypopharynx.
- no contraindication to concurrent chemotherapy.
- absent distant metastases.
- no prior induction chemotherapy or HN oncologic treatment (surgery and/or RT).
- no contraindication to iodinated medium contrast administration.
- absence of metal dental implants possibly affecting quality of the CT image.

All patients underwent a complete pretreatment diagnostic and staging workup including panendoscopy of the HN region with biopsy of the primary tumor, contrast-enhanced CT (including a perfusion study of the primary tumor as described in more detail in the dedicated subsection), and [^{18}F]FDG-PET/CT of the total body.

Contrast-enhanced MR was performed in all patients with nasopharynx cancer (to evaluate a possible invasion of the skull base) as well as in the patients with tumors located at other sites, when deemed necessary.

PCT was repeated both three weeks and three months after completion of RCT, in order to evaluate changes in the ΔPCTp values with respect to the pre-treatment values.

[^{18}F]FDG-PET/CT was also repeated three months after completion of RCT to evaluate the metabolic response of the tumor to treatment.[18,19]

Thereafter, routine follow-up was performed according to our internal protocol based on the current international guidelines.[20]

Treatment

All radiotherapy treatments were performed using an intensity modulated radiotherapy technique with a simultaneous integrated boost (IMRT-SIB).

The clinical target volumes (CTVs) were directly delineated by the radiation oncologist according to guidelines of the Italian Association of Radiation Oncology-Head and Neck Working Group, and the corresponding planning target volumes (PTVs) were automatically created by uniform expansions of 0.3 cm.[21]

The prescribed doses were 66Gy at 2.2Gy per fraction to the high-risk gross volume PTV and 60-54Gy at 2.0-1.8Gy per fraction to the intermediate (optional) and low-risk subclinical PTVs, respectively, delivered concomitantly in 30 daily fractions (five days a week).

According to our internal image-guided radiotherapy (IGRT) protocol, the patients underwent a weekly cone beam CT (CBCT) set-up control and online correction to reduce the systematic set up errors.

Chemotherapy was given weekly using Cisplatin 40 mg/m^2 i.v. over 1 h during the 6-week RT course for a maximum of 6 cycles, or Cisplatin 100 mg/m^2 i.v. once every three weeks for a maximum of 3 cycles.

PCT protocol

All PCT studies were carried out using a high-definition 64-row CT scanner (Discovery CT750 HD®, General Electric, Milwaukee, WI) operating in axial mode with a toggling table technique, resulting in a z-axis coverage of 8 cm (i.e. 2×64 detector rows×0.625 mm detector collimation) centered on the lesion site. This latter was defined upon revision of previously acquired morphological CT or MR images, as identified on a preliminary low-dose contrast-unenhanced CT scan (axial shuttle mode, detector configuration 64×0.625 mm, 100 kV, 20 mA, tube rotation time 0.7 s, 2.5 mm slice thickness, Standard convolution kernel, and 60% iterative reconstruction strength (ASIR®, Adaptive Statistical Iterative Reconstruction).

A continuous series of 17 PCT frames was acquired starting 8 seconds after the beginning of contrast medium injection for a total acquisition time for 46.6 seconds, so as to cover the entire duration of the first pass of contrast medium through the lesion under investigation. Subsequently, *two additional late frames were acquired on the same site with a delay of 15 seconds from the end of the first pass scan and from one another, respectively to obtain data over the recirculation phase of contrast medium* for computation of the permeability-surface product (PS) parameter. Scanning parameters *for all aforementioned PCT scans* were the following: tube voltage 80 kV, tube current 300 mA, tube rotation time 0.4 s, slice thickness 2.5 mm, Soft convolution kernel, and 50% ASIR.

Temporal resolution for the axial shuttle PCT protocol was 2.8 seconds .[22]

For all PCT examinations, 50 mL of iodixanol 320 mgI/mL (Visipaque 320®, GE Healhcare, Oslo, Norway) was injected intravenously at a flow rate of 4 mL/s using a power injector (Medrad Stellant®, Bayer Healthcare, Leverkusen, Germany) via a 20G needle positioned in the antecubital vein, followed by 50 mL of saline flush administered at the same flow rate.

The mean dose-length product for the whole CT examination was 445 ± 13 mGy·cm. corresponding to an effective dose of 1.38 ± 0.04 mSv ($k=0.0031$ mSv·mGy^{-1}·cm^{-1})[23]

Post-processing of CT perfusion data

All PCT datasets were transferred via local area network to a workstation (Advantage Windows v. 4.5, General Electric) equipped with a dedicated plugin (CT Perfusion 3) for computation of the following CT perfusion parameters:

- Blood Flow (BF), i.e., the blood flow transiting from the arterial input through the tissue intravascular space, expressed as mL/min per 100 g of tissue. BF provides information on blood flow from large vessels, arterioles, capillaries, and venules as well as arteriovenous

shunts, which are typical of tumor neoangiogenesis (leading to an increase of tumor BF compared to normal tissue)

- Blood Volume (BV), i.e., the blood volume that passes through the intravascular space, expressed as mL/100 g of tissue. Along with BF, it is typically increased in tumor neoangiogenesis as a result of an enlarged neovascular bed.
- Mean Transit Time (MTT), i.e., the mean time required for the blood to travel from the arterial input to the venous end, expressed in seconds. It can be expressed as the ratio between BV and BF (central volume theorem) and tends to be reduced in tumors with neoangiogenesis due to increased perfusion pressure and presence of arteriovenous shunts.
- Permeability-surface product (PS), i.e., the product between the permeability and the total surface area of the capillary endothelium in a unit mass of tissue, expressed as mL/min per 100 g of tissue. This parameter is considered as a surrogate marker of neoplastic neoangiogenesis and represents the rate of efflux of contrast medium from the intravascular to the interstitial space during its intravascular distribution phase, which is directly related to vascular permeability.[24]

Circular regions of interest (ROI) were positioned inside the lesion, as well as on the ipsilateral common or external carotid artery for sampling of the arterial input function needed to compute the CT perfusion parameters. As tumor tissue was visible on multiple slices in all cases, ROIs were placed on the slice containing the largest tumor section, in order to maximize the amount of sampled data per patient. ROI contours were kept at a distance of at least 1 mm from the tumor border to avoid partial volume effect, and care was taken to avoid macroscopic areas of tumor necrosis or calcification.[25]

All ROIs were identified with a blinded system by two radiologists with 10 and 3 years experience in HN imaging and by a radiation therapist with 9 years experience. Each PCT parameter considered for this analysis is the average of the three measurements by the three readers.

PET/CT protocol

PET/CT scans were acquired using a Discovery 710 PET/CT scanner (GE Healthcare, Milwaukee, WI, USA. The scanner employs 13,824 LYSO crystals in 24 detector rings to provide 47 slices per bed position. The spatial resolution is ~5mm FWHM. The system is able to record "time of fly" (TOF) information between two events in coincidence. Each patient underwent PET/CT acquisition at least 60 minutes after [^{18}F]FDG injection, following good hydration. All the patients had fasted for at least 6 hours and, at the time of [^{18}F]FDG administration, blood glucose levels were below

150 mg/dL. Injected activity was 3.7 MBq/kg. Emission imaging was performed in three-dimensional mode (3D) with an acquisition time of 2-3 min per bed position (neck-pelvis) with 5–6 bed positions per patient.

The CT acquisition protocol included a low-dose CT for anatomic localization (64 slice, 80 mAs, 120 kV, 0.5 s per rotation, 3.75 mm slice thickness) from the skull to mid-thigh, with the arms down.

Image Reconstruction

The emission data were corrected for random events, dead time, scatter, and attenuation. A 3-dimensional ordered-subsets expectation maximization iterative reconstruction algorithm (OSEM 3D) was applied with 3 iterations and 21 subsets, gaussian smoothing of 4 mm in full width at half maximum, in a 516×516 matrix and a 60 cm FOV (field of view). Attenuation maps were obtained from the *low-dose CT* data by bilinear transformation, as implemented in the post-processing software of the PET/CT scanner, and were used for attenuation correction of the PET data.

All images were uploaded onto a dedicated workstation (Advantage Workstation, 4.4; GE Healthcare, Milwaukee, WI, USA). A nuclear medicine physician analyzed the images visually and semi-quantitatively using the standardized uptake value of [^{18}F]FDG (SUV_{max} corrected for body weight); to calculate SUVs of the suspected tumor lesions, the axial slice with the maximum SUV of the lesion was first identified.

According to the EORTC criteria,[26] evaluation of metabolic response to treatment was based on the changes in SUV_{max} values as follows:

- Complete Response (CR): no uptake in the tumor, or [^{18}F]FDG uptake similar to that in the mediastinum.
- Partial Response (PR): >25% decrease in SUV_{max} of the tumor as defined on the pre-treatment scan, with no disease progression at other sites.
- Stable disease (SD): <25% increase or <15% decrease in SUV_{max} of the tumor as defined on the pre-treatment scan, and no new sites of disease.
- Progression of disease (PD): >25% increase in SUV_{max} of the tumor as defined on the pre-treatment scan, or appearance of new [^{18}F]FDG uptake in metastatic lesions.

Based on the above criteria, patients were divided into complete responders (CR), partial responders (PR) and non-responders (SD or PD).

Statistical Analysis

Before applying the inferential tests, a descriptive analysis of the quantitative variables (BF, BV, MTT, PS) was performed and the PCTp values were tested with the Kolmogorov-Smirnov normality test.

Assessment of PCTp

To assess the treatment-related differences of the PCTp values, a repeated measures general linear model (GLM) was adopted, followed by multiple comparisons with Bonferroni's method. Eight new variables, two for each PCTp, were calculated as the difference between the baseline and, respectively, the 3-week and the 3-month values.

Evaluation of predictive factors of PET/CT response

The variable "PET/CT response" (expression of the metabolic response to treatment) was included in the statistical analysis and considered as 0 for CR, 1 for PR and 2 for SD+PD.

The evaluation of PET/CT score prognostic factors (differences of the PCTp at three weeks and at three months after treatment) was performed by a "scale response" univariate GLM. Subsequently, all the significant variables were included in a "scale response" multivariate GLM to determine the contribution of each prognostic factor to the PET/CT score.

The results of the multivariate predictive model were expressed by the p-values and regression coefficients. Finally, to ensure correctness of the prediction model, histogram-based residuals analysis was performed. The significance was set at 0.05.

All statistical analyses were carried out using SPSS technology 22.

RESULTS

Patient and Tumor Characteristics

Between July 2012 and July 2015, 27 patients with intermediate and locally advanced HNC candidate to RCT were screened for possible enrollment in the study. Two of these patients were excluded because the pre-treatment [^{18}F]FDG-PET/CT disclosed the presence of hepatic metastases in one case and of a synchronous primary colon tumor in the other case.

Overall, 25 patients were definitively enrolled in the study;19 patients were affected by intermediate or locally advanced HNC and 6 by nasopharyngeal cancer (4 WHO III and 2 WHO II).

All enrolled patients completed the planned treatment and imaging procedures protocol and were therefore evaluable for the final analysis; all datasets were of sufficient quality with no or negligible artifacts due to patient motion.

Baseline patient and tumor characteristics are summarized in Table 1.

Overall, according to [^{18}F]FDG-PET/CT parameters of tumor response, CR was found in 16/25 patients (64%), PR in 7/25 patients (28%), SD in 1/25 patients (4%), and PD in 1/25 patients (4%). Moreover, according to standard radiological RECIST criteria, 17/25 patients (68%) exhibited CR while the remaining 8 patients (32%) exhibited partial response.

Variations of tumor perfusion parameters (ΔPCTp)

The primary analysis was aimed at assessing the variations of each tumor perfusion parameter in the post-treatment PCT (both at 3 weeks and at 3 months) with respect to the pretreatment values.

The mean and range values of each tumor perfusion parameter (BV, BF, MTT and PS) at different times together with the significant p-values corresponding to the variations observed at each time-point (baseline, then 3 weeks and 3 months after treatment) are reported in Tables 2a and 2b.

Overall, a significant reduction of all PCTp parameters in the post-RCT scan was observed with respect to the baseline PCT. In particular, a significant reduction of all the PCTp values except MTT was observed in the 3-week post-RCT PCT with respect to baseline. Instead, all PCTp values, including MTT, were significantly lower than the baseline values in the 3-month PCT.

As to the differences in ΔPCTp values between the 3-week and the 3-month PCT post-RCT, MTT was the only parameter with a significant reduction ($p=0.036$), while the differences were at borderline of statistical significance for BV ($p=0.066$) and for BF ($p=0.061$).

Relationship between PET/CT response and ΔPCTp

A secondary analysis was aimed at assessing the relationship between the PET/CT response and ΔPCTp both at 3 weeks and at 3 months post-treatment. The results of univariate and multivariate analysis are reported in Table 3.

The PET/CT response resulted to be significantly correlated to a reduction of all PCTp values both at 3 weeks and at 3 months, except for MTT. Multivariate analysis, which took into account only the variables found to be significant by univariate analysis, showed that PS was the only parameter to maintain a statistical significance at 3 months ($p=0.037$), with a trend towards statistical significance also at 3 weeks ($p=0.099$).

DISCUSSION

The primary aim of this study was to investigate changes in ΔPCTp of the primary tumor induced by RCT in patients affected by intermediate and locally advanced HNC. The rationale was based on the objective evaluation of tumor vascularity changes as a surrogate for the RCT effect.

For this purpose, we focused on the ΔPCTp both at 3 weeks and at 3 months, whereas the post-RCT PET/CT was performed 3 months after treatment, as the standard procedure for assessing metabolic response of the tumor after RCT.[14]

The results obtained showed a significant reduction of all PCTp values, except MTT, already as early as 3 weeks after treatment, thus demonstrating a strong effect of RCT on such parameters. Therefore, MTT was the least sensitive parameter to demonstrate the effect of RCT, as it took longer (3 months) for its reduction with respect to baseline to reach statistical significance. On the contrary, BV, BF and PS resulted highly sensitive parameters, as they exhibited a significant reduction already at 3 weeks post-RCT, with a trend towards further reduction in the 3-month PCT scan *versus* the 3-week scan.

In this regard, the radiation- and chemotherapy-induced damage on the intratumor microvasculature and low-resistance flow of neoplastic vessels explain the reduction induced by RCT in the BV and BF values, whereas the observed decline in the PS values might be explained by reduced neoangiogenesis.

Despite the relatively small sample size (25 patients), these results are promising as to the potential role of PCT to predict tumor response, consistent with the few prior reports published to date. In particular, Gandhi et al.[27] studied 9 patients, and found a linear relationship between high baseline BV values and tumor response after neoadjuvant chemotherapy. In a study including 25 patients, Petralia et al.[28] reported similar results, showing a significant reduction of both BV and BF after 3 cycles of induction chemotherapy. Similarly, Truong et al.[29] observed a positive correlation between high baseline BF values and longer locoregional control, consistent with the findings reported by Hermans et al.[30]. Finally, Surlan-Popovic et al.[31] investigated the changes of tumor perfusion parameters during the course of RT, and observed a significant reduction of BV and BF, whereas MTT and PS did not change significantly.

Although the few reports published so far on this subject include relatively small groups of patients, the overall findings consistently suggest a predictive role of high pretreatment BV and BF values as well as the reduction in the perfusion parameters induced by radiation and/or chemotherapy. Differently, most earlier studies report contradictory findings regarding the changes in PS and MTT values induced by treatment, with no significant variations in the post-treatment *versus* the

pretreatment values found in most reports.[32] It should also be noted that MTT seems to have a pivotal role in the interpretation of tumor response to treatment. In particular, Petralia et al.[25] reported a significant increase in the MTT values induced by treatment, a finding that the authors interpret as due to the reduction of the amount of arterovenous neoplastic shunts, combined with reduction of the BV and BF values.

In contrast with the above findings, our results showed a significant reduction of all perfusion parameters, including the MTT value at 3 months after treatment. A possible explanation of our findings might be the high rate (68%) of complete response based on the standard RECIST criteria that precluded a real computation of MTT value, therefore assigning a zero-value to this variable for statistical analysis. Indeed, the patients with PR to treatment exhibited an overall increase (although not statistically significant) of the MTT values (from a baseline mean value of 6.1 sec. to a mean value of 7.0 sec 3 months after RCT), thus confirming the findings reported by Petralia et al.

Therefore, an increasing interest is coming out to investigate the potential role of morphologic-functional integrated imaging technique in the interpretation of tumor response after RCT, mostly focusing on the early evaluation.

In fact, even if a real benefit of an early versus delayed salvage surgery after RCT has not been proved yet, it is likely to be.

As a consequence, a precocious identification of partial or non-responder patients might be really useful in clinical practice (probably on primary tumor more than on adenopathy) to drive them to a salvage surgery as soon as possible in order to improve oncologic outcomes.

As a secondary goal of the study, the findings regarding changes in the PCTp values induced by RCT and correlated with the PET/CT tumor response at 3 months were considered by most authors to constitute the standard of care for metabolic assessment in clinical practice.[33] Based on the EORTC criteria, the metabolic response was classified into CR, PR, SD and PD; for statistical analysis, the PET/CT response was considered as a dichotomous variable (CR *versus* PR, SD, PD).

Interestingly, the PS values at 3 months post-RCT were the strongest predictors of subsequent PET/CT response. As a matter of fact, the PS reduction at 3 months maintained statistical significance at multivariate analysis (p=0.037), with a trend toward statistical significance (p=0.099) also for the reduction at 3 weeks. Therefore, among all PCT parameters, PS emerges as the strongest predictor of metabolic tumor response to treatment.

Based on the few previous reported experiences, a possible pathophysiological explanation of our findings might be an uppermost effect of RCT on the tumor neovascularization with a consequent

early reduction of endothelium vascular permeability together with BV and BF associated with a possible unchanged or slightly increased values of mean blood transit time.

Subsequently, a delayed reduction of MTT consequent to the tumor neovasculature radiation-induced disruption together with a further significant decrease of all the others perfusion parameters could occur later.

Figures 1 and 2 report the imaging findings in a case that briefly symbolizes our results. This patient was affected by a locally advanced oral cavity tumor (Stage cT4aN1), and exhibited early radiologic PR (>50% reduction based on RECIST criteria) combined with a significant early reduction of all PCTp but MTT at 3 weeks (Fig. 1) and with a subsequent complete metabolic response 6 months after RCT (Fig .2).

These results, obtained in a relatively small sample size and with a short follow-up, require further independent confirmation in other series.

Anyway, a similar experience was recently published by Pietsch et al.[34] on 13 HNC patients treated with RCT and undergone to PCT and PET/CT both at baseline and after treatment.

The authors found out a significant correlation between high baseline values of BV, BF and tumor lesion glycolysis (TLG) with tumor recurrence whereas no statistically significant correlation between ΔPCTp and ΔPET/CTp was observed.

Interestingly, due to the short study protocol (as stated by the authors themselves),the permeability surface product was the only parameter not calculated; so, our preliminary findings might suggest a possible not previously reported prognostic role of PS.

Anyway, the correlation of ΔPCTp with PET/CT response based on EORTC criteria was a mayor limitation of our experience as the SUV_{max} changes is not really an appropriate oncological endpoint and its clinical value is limited.

Thus, longer follow-up and greater sample size *is warranted* to correlate the tumor perfusion parameter changes to the more important clinical outcomes, such as local recurrence-free survival *and/or cancer specific survival in order to properly evaluate the prognostic role of PCT in tumor response evaluation.*

Our findings are still preliminary in nature and future larger prospective studies, with a longer follow-up, are needed to confirm their validity.

In the meantime, we suggest always taking into account the functional and morphological data of PCT imaging, mostly in the presence of positive cases, in order to reduce the rate of false positivity as much as possible.

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Figure legends

Figure 1. Early radiologic response assessment in a 75-year-old patient with squamous cell carcinoma of the oral tongue treated with radiochemotherapy. (a) Pre-treatment morphologic CT image obtained from first-pass PCT dataset. (b) BV, (c) BF, (d) MTT, and (e) PS color maps show altered lesion perfusion compared to the surrounding tongue tissues. (f) Post-treatment morphologic CT image obtained from first-pass PCT dataset. Quantitative measurement of pre-treatment PCT parameters revealed BV=3.40 mL/min, BF=84 mL/min per 100 g, MTT=2.74 s, and PS=12.3 mL/min per 100 g, respectively. (g) BV, (h) BF, (i) MTT, and (j) PS color maps show normalization of lesion perfusion 3 weeks after RCT treatment. Quantitative measurement of post-treatment PCT parameters revealed BV=2.03 mL/min, BF=34 mL/min per 100 g, MTT=6.74 s, and PS=9.25 mL/min per 100 g, respectively

Figure 2: (a) metabolic [^{18}F]FDG-PET response assessment in the same patient 6 months after radiochemotherapy.. (b) Pre-treatment axial FDG PET and (c) PET/CT fused images show focal abnormal tracer uptake in the tongue. (e) FDG PET and (f) PET/CT performed 6 months after radiochemotherapy show absence of focal uptake in the tongue after complete metabolic response to treatment.

Table 1 Tumor and patients characteristics

Characteristics	N°	%
Risk Factors		
Smoking Status	25	
Smokers	16	64
<10 cigarettes/die	4	16
>10 cigarettes/die	12	48
Non smokers	9	36
Alcohol	25	
Potus	7	28
Non potus	18	72
Site		
Nasopharynx	6	24
Oropharynx	7	28
Oral Cavity	5	20
Hypopharynx	2	8
Larynx	5	20
Histology		
Squamous cell carcinoma	21	84
Undifferentiated carcinoma	4	16
T		
2	13	52
3	4	16
4	8	32
N		
0	7	28
1	4	16
2	12	48
3	2	8
Stage		
III	9	36
IV	16	
IVA	11	44
IVB	5	20
Concurrent chemotherapy		
Cisplatin 40 mg/mq weekly	24	96
Cisplatin 100 mg/mq every 21 days	1	4

	BV(ml/100g/min)	BF(ml/100g)	MTT(s)	PS(ml/100g/min)
Baseline				
Mean	32.53	396.76	6.18	47.63
Range	5.48-84.66	76.67-880.62	2.16-11.21	4.41-98.80
3 weeks				
Mean	13.42	192.12	5.14	24.07
Range	0-52.61	0-679.61	0-11.96	0-74.75
3 months				
Mean	5.67	74.26	2.24	11.58
Range	0-48.97	0-536.77	0-18.94	0-92.81

Table 2a. Tumor perfusion parameters variations scores from baseline to post treatment

Abbreviations: BV: Blood Volume; BF: Blood Flow; MTT: Mean Transit Time; PS: Permeability Surface

Table 2b. Statistical significance of tumor perfusion parameters variations

		p-value			
		BV	**BF**	**MTT**	**PS**
Repeated measures		<0.0001	<0.0001	0.001	<0.0001
Multiple comparison	Baseline vs 3 weeks	0.001	0.002	0.722	0.004
	Baseline vs 3 months	<0.0001	<0.0001	0.001	<0.0001
	3 weeks vs 3 months	0.066	0.061	0.036	0.089

Abbreviations: BV: Blood Volume; BF: Blood Flow; MTT: Mean Transit Time; PS: Permeability Surface

Table 3. Uni- and multivariate analysis of PET/CT response and tumor perfusion parameters variations

	Univariate Analysis	Multivariate Analysis	
	p-value	**RC**	**p-value**
PCTp baseline-3 weeks			
BV	0.030	-0.001	0.923
BF	0.042	-0.0003	0.800
MTT	0.998	---	---
PS	0.014	-0.010	0.099
PCTp baseline-3 months			
BV	0.038	0.009	0.446
BF	0.032	-0.001	0.472
MTT	0.692	---	---
PS	0.005	-0.013	0.037

Abbreviations: RC= Regression coefficient; PCTp= computed tomography tumor perfusion parameters; BV: Blood Volume; BF: Blood Flow; MTT: Mean Transit Time; PS: Permeability Surface

Figure 1

Click here to download Figure Fig.1.tif

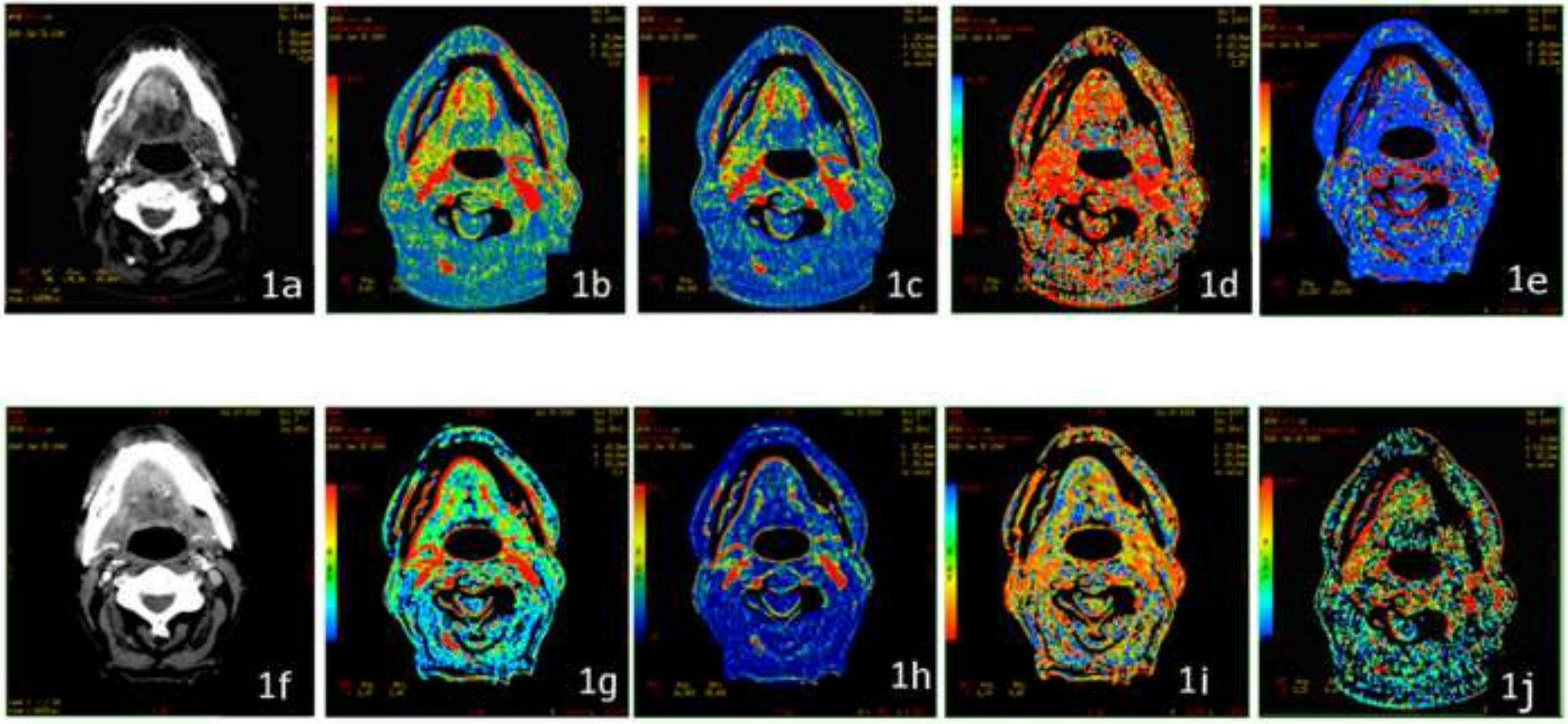

Figure 2

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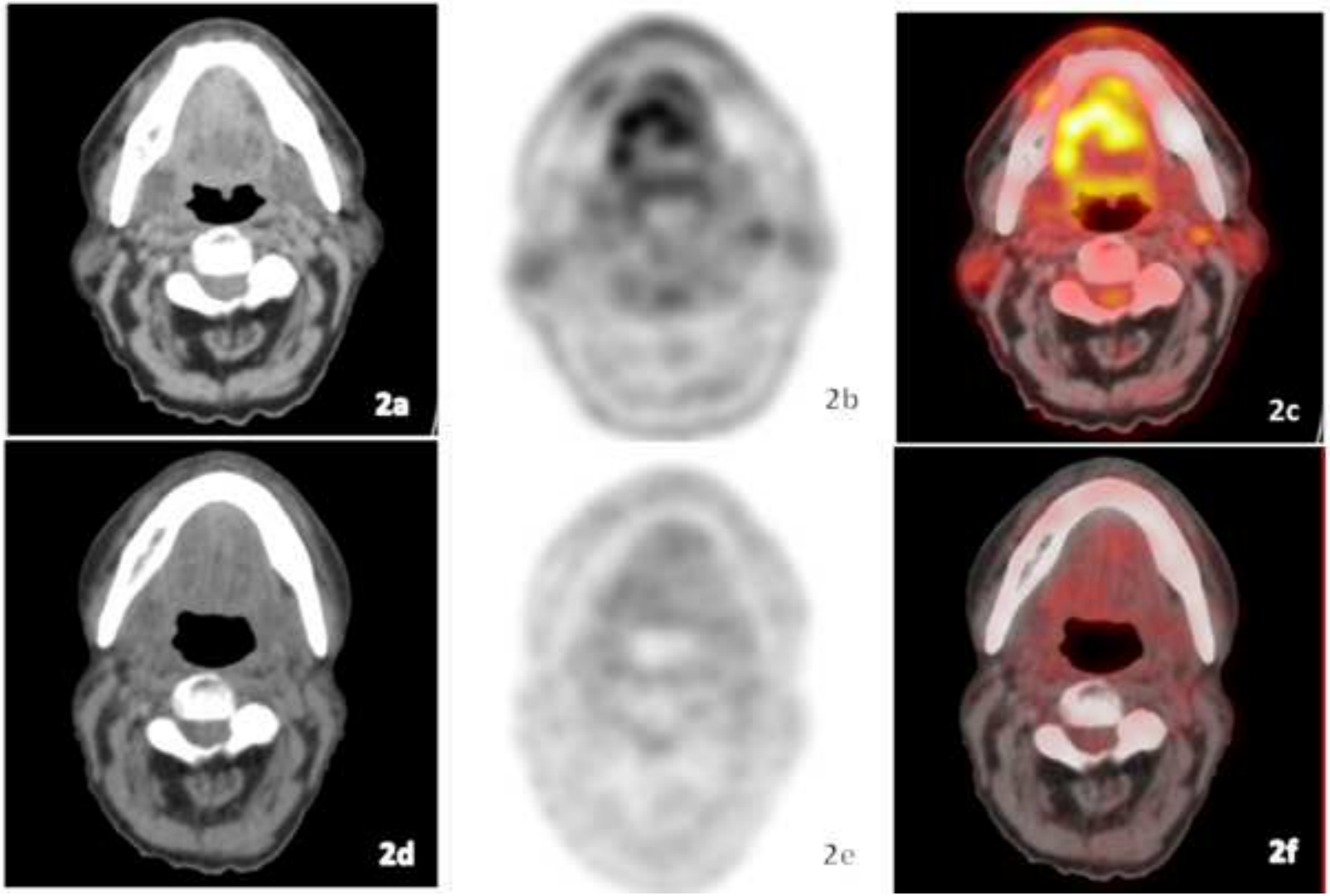

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