



HAL
open science

Predicting acute severe toxicity for head and neck squamous cell carcinomas by combining dosimetry with a radiosensitivity biomarker : a pilot study

Sophie Deneuve, Thierry Bastogne, Mirlande Duclos, Céline Mirjolet, Pascaline Bois, Patrick Bachmann, Lara Nokovitch, Pierre-Eric Roux, Didier Girodet, Marc Poupart, et al.

► To cite this version:

Sophie Deneuve, Thierry Bastogne, Mirlande Duclos, Céline Mirjolet, Pascaline Bois, et al.. Predicting acute severe toxicity for head and neck squamous cell carcinomas by combining dosimetry with a radiosensitivity biomarker : a pilot study. *Tumori*, 2022, 10.1177/03008916221078061 . hal-03718454

HAL Id: hal-03718454

<https://hal.science/hal-03718454>

Submitted on 8 Jul 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Predicting acute severe toxicity for head and neck squamous cell carcinomas by combining dosimetry with a radiosensitivity biomarker : a pilot study.

**Sophie Deneuve, MD PhD^{1,2}; Thierry Bastogne, PhD^{3,4,5}; Mirlande Duclos, BSc⁶ ; Céline Mirjolet^{7,8}
Pascaline Bois¹, dietetician ; Patrick Bachmann¹, MD ; Lara Nokovitch ¹, MD ; Pierre-Eric Roux¹, MD
; Didier Girodet¹, MD ; Marc Poupart¹, MD ; Philippe Zrounba¹, MD ; Line Claude^{1,2}, MD ; Letizia
Ferella, MD⁹ ; Nicola Alessandro Iacovelli, MD⁹ ; Nicolas Foray, PhD^{2*}, Tiziana Rancati , MS^{10*} and
Sandrine Pereira, PhD^{2,6*}**

¹ *Centre Regional de Lutte Contre le Cancer Léon-Bérard, Département de Chirurgie Oncologique, Lyon, France*

² *INSERM, U1296 Unit, Centre Léon Bérard, Lyon, France*

³ *Institut De Cancérologie de Lorraine – Alexis Vautrin, Vandoeuvre-lès-Nancy, France*

⁴ *CRAN, CNRS UMR 7039, INRIA BIGS, Vandoeuvre-lès-Nancy, France,*

⁵ *CYBERnano, 193 av. Paul Muller, Villers-lès-Nancy, France*

⁶ *Neolys Diagnostics, Lyon, France*

⁷ *Centre Georges François Leclerc, UNICANCER, Dijon*

⁸ *INSERM UMR 1231, Cadir Team, Dijon*

⁹ *Departement of Radiation Oncology 2, Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy*¹⁰*Prostate Cancer Program, Scientific Directorate Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy*

*These authors contributed equally to the work

Corresponding author address: tiziana.rancati@institutotumori.mi.it

Supplementary Figure S1 and table S1 contains online supplementary data.

Reducing acute severe toxicity for head and neck squamous cell carcinomas by combining dosimetry with a radiosensitivity biomarker

ABSTRACT

Introduction. Radiotherapy (RT) against head and neck squamous cell carcinomas (HNSCC) may lead to severe toxicity in 30-40% patients. The normal tissue complication probability (NTCP) models, based on dosimetric data refined the normal tissue dose/volume tolerance guidelines. In parallel, the radiation-induced nucleoshuttling of the ATM protein (RIANS) is a predictive approach of individual intrinsic radiosensitivity. Here, we assessed whether the combination of NTCP with RADIODETECT®, a blood assay derived from the RIANS model, can improve post-RT toxicity prediction in HNSCC patients.

Methods. A first cohort of 53 HNSCC was previously used to determine two cutoffs, i.e. 57.8 ng/mL for grade \geq 2 toxicity and 46 ng/mL for grade \geq 3 toxicity. Validation was performed on a retrospective cohort of 36 HNSCC patients treated with postoperative RT. Toxicity was graded with CTCAE scale and two criteria were considered: grade \geq 2 oral mucositis (OM2), grade \geq 3 mucositis (OM3) and grade \geq 2 dysphagia (DY2), grade \geq 3 dysphagia (DY3). pATM quantification was assessed in lymphocytes of HNSCC patients. The discrimination power of the pATM assay with the selected cutoffs with respect the four toxicity endpoints was evaluated through the area under the receiver operator characteristics curve (AUC-ROC). Two previously described NTCP models were considered, including the dose to the oral cavity and the mean dose to the parotid glands (OM3) and the dose to the oral cavity, to the larynx and the volume of pharyngeal constrictor muscles (DY3).

Results. Considering OM2, the $AUC-ROC^{NTCPmodel}=0.55$, $AUC-ROC^{RADIODETECT®}=0.75$ and $AUC-ROC^{NTCP+RADIODETECT®}=0.80$. For OM3, $AUC-ROC^{NTCPmodel}=0.61$, $AUC-ROC^{RADIODETECT®}=0.67$ and $AUC-ROC^{NTCP+RADIODETECT®}=0.78$. Considering DY2, $AUC-ROC^{NTCPmodel}=0.64$, $AUC-ROC^{RADIODETECT®}=0.57$ and $AUC-ROC^{NTCP+RADIODETECT®}=0.71$. For DY3, $AUC-ROC^{NTCP}=0.61$, $AUC-ROC^{RADIODETECT®}=0.57$ and $AUC-ROC^{NTCP+RADIODETECT®}=0.65$.

Conclusions. Combination of NTCP and RADIODETECT® models might significantly improve the prediction of toxicities for HNSCC patients.

KEY WORDS

Reducing acute severe toxicity for head and neck squamous cell carcinomas by combining dosimetry with a radiosensitivity biomarker

Head and neck squamous cell carcinomas, radiation-induced toxicity, predictive models, biological marker, pATM, normal tissue complication probability.

INTRODUCTION

Most head and neck cancers are squamous cell carcinomas (HNSCC) arising in the stratified epithelium of the oral cavity, the oropharynx, the pharynx, or the larynx [1]. With 55,000 new cases per year in the USA, HNSCC incidence has been estimated at about 600,000 new cases per year worldwide [2], making HNSCC one of the most frequent cancer in the world. HNSCC are usually diagnosed at locally advanced stage [3]. While early stage HNSCC are usually treated with surgery and/or radiotherapy (RT), the treatment of locally advanced HNSCC depends on primary site and stage but usually includes radiation therapy[4]. Early complications of the RT are observed from the beginning of the treatment to a few months after its end. Among them, oral mucositis and dysphagia are the most frequently observed in patients treated for HNSCC [5]. Those complications decrease patients' quality of life [5], and may interfere with or even permanently interrupt treatments. [6]. RT-induced toxicity (RTT) results from a complex interplay of patient- tumor- and treatment-related factors [7-9], as for examples concomitant chemoradiotherapy [10-12], altered fractionations [13] and dosimetric parameters [14]. The impossibility to spare the oral and oropharyngeal mucosa with doses lower than 32-39 Gy or 9.5 Gy per week [15-17] leads to severe mucositis. Similarly, the impossibility of sparing the constrictor muscles with doses lower than 51- 62 Gy leads to swallowing dysfunction, that decreases patients' quality of life [18]. Emami *et al.* were the first to propose a predictive approach to the RTT, based on the combination of 3D dosimetry and tissue tolerance data in 1991[19]. These normal tissue complication probability (NTCP) models allow to precise the normal tissue dose/volume tolerance guidelines. For HNSCC, Orlandi *et al.*, 2018; and Cavallo *et al.*, 2017 have developed specific NTCP models that predict mucositis and dysphagia [20, 21]. However, the NTCP approach used alone remains insufficient to predict RTT [22].

Beyond dosimetric factors, the risk of RTT also depends on individual radiosensitivity. Using a *DNA sequencing* approach, some trials have attempted to link genetic polymorphisms to RTT [23]. An alternative approach to DNA sequencing is to quantify a biological function such as apoptosis or DNA repair/ signaling through assays involving irradiated and non-irradiated cells (functional approach) [24-25]. DNA double-strand breaks (DSBs) are key-lesions leading to radiation-induced chromosomal aberrations and consequently to clonogenic cell death. The study of their repair and signaling have

been shown to allow the prediction of individual radiosensitivity [26]. We previously proposed a reliable predictive assay using the assessment of the radiation-induced nucleo-shuttling of the ATM protein (RIANS), a major contributor to DSB repair and signaling [24, 26-29]. A quantitative correlation was found between the maximal number of nuclear pATM foci assessed with immunofluorescence after irradiation and the severity of the RT-induced toxicities assessed with the Common Terminology Criteria for Adverse Events (CTCAE) [27, 30]. This first predictive assay based on the RIANS model requiring a time-consuming step of cellular amplification, we developed a faster assay assessing the quantity of nuclear pATM molecules with the ELISA method at 10 minutes and 1 hour after 2 Gy and compared with pATM immunofluorescence data [30]. Both immunofluorescence or ELISA RIANS assays gave the highest statistical performances to predict radiosensitivity [31]. Further, to create a simpler biological test to be proposed in clinical practice, the RADIODETECT® approach was investigated [32]. This blood assay is an ELISA assay on fibroblasts first and then proposed on lymphocytes by Pereira et al [30, 32].

The present pilot study aims to create a new assay by combining the RADIODETECT® assay with two previously developed NTCP models [20-21]. Prediction of acute RT-induced mucositis and dysphagia was tested in a cohort of HNSCC patients treated with post-operative radiotherapy with this combined approach.

MATERIALS AND METHODS

Patients' population

We used an observational cohort of 36 non-metastatic HNSCC patients treated with postoperative radiotherapy between 1st January 2017 and 1st June 2017. Radiation therapy was delivered either by Intensity Modulated Radiotherapy (IMRT), Volumetric Modulated Arc Therapy (VMAT) or helical tomotherapy, with or without concurrent chemotherapy.

Reducing acute severe toxicity for head and neck squamous cell carcinomas by combining dosimetry with a radiosensitivity biomarker

A six-month prospective observational study was initially planned, evaluating the side-effects of RT on their nutritional status. CTCAE grades concerning dysphagia, mucositis and also dosimetric data were prospectively collected for 36 patients of the cohort.

Among them, all accepted to enter the Collection number: 2017-A00086-47 and gave a blood sample, collected during a follow-up consult 3 to 6 months after the end of treatment. The study was approved by the local Ethical Committee (number: 2017-A00086-47). All patients were informed and signed consent.

The following data were collected: age, sex, primary site of HNSCC, radiotherapy details including dosimetry and technique and chemotherapy details such as type of chemotherapy and doses.

Toxicity endpoint definition

Patients were evaluated for toxicity at baseline and weekly during RT treatment according to the Common Terminology Criteria for Adverse Events (CTCAE) scale version 4.03 (CTCAE 2010).

Four different endpoints were considered: 1) grade \geq 2 early oral mucositis; 2) grade \geq 2 early dysphagia; 3) grade \geq 3 early oral mucositis; 4) grade \geq 3 early dysphagia.

Grade 2 mucositis was defined as “Moderate pain, not interfering with oral intake”, while grade 3 as “Severe pain, interfering with oral intake” and grade 4 if life-threatening consequences or urgent intervention indicated. Grade 2 dysphagia was defined as “Symptomatic and altered eating/swallowing”, while grade 3 as “Severely altered eating/swallowing; tube feeding or total parental nutrition or hospitalization indicated” and grade 4 if life-threatening consequences or urgent intervention indicated. In all cases peak toxicity was considered, i.e. at least one grade \geq 2 or one grade \geq 3 event occurring at any time during treatment

RADIODTECT® approach

The RADIODTECT® assay is an ELISA-based RANS assay based on three steps, which have been published elsewhere [32]: (1) isolation of cells, (2) cells lysis and (3) ELISA assay.

(1) Isolation and Treatment of Human Lymphocytes

Lymphocytes isolated from the blood of patients were stored at room temperature. Following dilution with equal volume phosphate buffer saline (PBS), blood was poured on 3 mL Ficoll-Paque and centrifuged at 2000g for 20 min. Transferred cells were diluted and then washed twice with PBS, and approximately $1-1.5 \times 10^6$ cells were suspended in 1 mL of RPMI1640 containing 10% FBS and 1% antibiotic (penicillin/streptomycin) for use in further tests.

(2) Cells lysis

Cells were pelleted by centrifugation at 500 g for 5 min at 4°C and washed twice with ice-cold PBS1X. The dry pellet was resuspended in 250 µl of RIPA extraction buffer and then incubated on ice for 10 min. The lysed cells were then centrifugated at 15000 g for 15 min at 4°C, and the supernatant collected in prechilled tubes.

(3) ELISA assay

pATM was quantified in total cell protein fraction by applying an ELISA commercial kit and protocol (#NR-E10877-4, NOVATEIN Biosciences, Woburn, USA) with a similar phosphospecific ser1981pATM antibody which was used for immunofluorescence experiments [27]. The ELISA plates were analyzed with a spectrophotometer (TECAN, Lyon,France) at 450 nm.

NTCP approach

The NTCP models were previously described [20-21].

Definition of organs at risk

The oral cavity was contoured including the surface of inner lips, buccal mucosa, tongue, base of tongue, floor of mouth and palate [33]. Minor salivary glands were included in the contoured oral cavity volume. Parotid glands were outlined according to van de Water et al. [33] and were considered together as a single organ. Pharyngeal constrictors included superior, medial, and inferior constrictors; while the glottic larynx and supraglottic larynx were considered separately.

The contouring used for this study was performed by two radiotherapists expert in head and neck radiotherapy.

Contouring was

NTCP models

Two previously developed NTCP models were considered [20-21]: the dose-response relationship for the selected endpoints was described through a logistic sigmoid-shaped curve. Non-uniform dose distribution to organs at risk was reduced either to mean dose, or to Equivalent Uniform Dose (EUD). A dose-volume histogram (DVH) cutpoint (V_{xGy} , the fractional organ volume receiving at least X Gy) was used as dosimetric descriptor when relevant. Details are reported in the original papers.

We here report only the EUD definition. EUD has the capability of taking the whole DVH into account, while simultaneously accounting for organ architecture:

$$EUD = \left(\sum_i v_i D_i^{\frac{1}{n}} \right)^n$$

where v_i is the volume fraction that receives the bin dose D_i , $\{D_i, v_i\}$ are the points of a differential DVH and the parameter n is a volume effect factor. The best volume parameters n were determined through numerical optimization for each organs at risk and for each toxicity endpoint.

Reducing acute severe toxicity for head and neck squamous cell carcinomas by combining dosimetry with a radiosensitivity biomarker

The model for grade \geq 3 oral mucositis included the EUD to the oral cavity calculated with $n=0.05$ (Odds Ratio, OR=1.06 for 1 Gy increase) and the mean dose to the parotid glands (OR=1.02 for 1 Gy increase). The single patient probability was in this case determined by:

$$NTCP_{mucositis} = \frac{1}{1 + e^{-(-3.5+0.055 \cdot \text{Mean Dose}_{\text{Parotids Gland}} + 0.021 \cdot \text{EUD}_{\text{Oral Cavity}})}}$$

The model for grade \geq 3 dysphagia included the EUD to the oral cavity calculated with $n=0.15$ (OR=1.04 for 1 Gy increase), the EUD to the glottic larynx calculated with $n=0.35$ (OR=1.02 for 1 Gy increase) and the $V_{50\text{Gy}}$ (the fractional organ volume receiving at least 50 Gy) to the pharyngeal constrictor muscles (OR=1.02, for 1% increase). The single patient probability was in this case determined by:

$$NTCP_{dysphagia} = \frac{1}{1 + e^{-(-5.233+0.019 \cdot V_{50\text{Gy,Costrictors}} + 0.019 \cdot \text{EUD}_{\text{Glottic Larynx}} + 0.042 \cdot \text{EUD}_{\text{Oral Cavity}})}}$$

Statistical analyses

Patients were classified as radioresistant when their expected or observed toxicity was below the chosen toxicity endpoint, and were classified as radiosensitive when their observed toxicity was above the chosen toxicity endpoint.

RadioDtect© assay

Determination of threshold

The training cohort described elsewhere [32] was used to determine the threshold i.e. 57.8 ng/mL for grade \geq 2 toxicity [32]. However, this cohort with different types of cancer included only 23 patients with grade \geq 3 toxicity [32]. Because grade \geq 3 toxicity are more frequent in HNSCC patients, we used another cohort including 53 HNSCC patients to verify the threshold for grade \geq 2 toxicity obtained previously [32] and determine the threshold for grade \geq 3 toxicity in HNSCC.

After the determination of the pATM concentration in peripheral blood, the 53 HNSCC patients were classified as radiosensitive vs radioresistant using the two determined thresholds, i.e. 57.8 ng/mL for grade \geq 2 toxicity and 46 ng/mL for grade \geq 3 toxicity (Figure S1). The discrimination power of the pATM assay with the selected thresholds with respect the four toxicity endpoints was evaluated through the Area Under the receiver operator characteristics Curve (AUC-ROC).

Performances of the assay

The discrimination power of the pATM assay with the selected cutoffs with respect the four toxicity endpoints was evaluated through the Area Under the Receiver Operator Characteristic Curve (AUC-ROC). Other performance measures were derived from the confusion matrix. Particularly, (1) Accuracy = (TP+TN)/total (where TP=True Positives and TN=True Negatives), (2) Misclassification Rate (or Error Rate) = (FP+FN)/total (where FP=False Positives and FN=False Negatives), (3) True Positive Rate (or Sensitivity) = TP/actual positives, (4) False Positive Rate = FP/actual negatives, (5) True Negative Rate (or Specificity) = TN/actual negatives, (6) Precision = TP/predicted positives and (7) Null Error Rate = actual positives/total (i.e. how often one may be wrong if one always predicts the majority class; here, these corresponds to patients showing no toxicity).

Dosimetric NTCP approach

The probability of experiencing grade \geq 3 oral mucositis or grade \geq 3 dysphagia was calculated using the previously developed NTCP models [20-21], no fitting was done in this case. The logistic regression coefficients determined by previous modelling and presented here above were directly used to compute complication probabilities for patients in the study population. The model discrimination was assessed through the AUC-ROC.

Of note prediction from models for grade \geq 3 toxicity was used to derive the “dosimetric risk” of grade grade \geq 2 side-effects, using z-standardization of predicted grade \geq 3 toxicity probabilities. In this frame the dosimetric risk for each patient was calculated using the following equation:

$$dosimetric\ risk_{mucositis/dysphagia} = \frac{NTCP_{mucositis/dysphagia} - \overline{NTCP_{mucositis/dysphagia}}}{\sigma_{NTCP}}$$

Where $NTCP_{mucositis/dysphagia}$ is the prediction for the single patients from grade \geq 3 models, $\overline{NTCP_{mucositis/dysphagia}}$ is the mean value of NTCPs in the population and σ_{NTCP} is the standard deviation of NTCPs in the population. Patients with a dosimetric risk <0 have a lower toxicity probability with respect to the “mean patient” in the population, while patients with a dosimetric risk >0 have a higher toxicity probability with respect to the “mean patient” in the population.

Combined biological-dosimetric NTCP approaches

Combined biological-dosimetric NTCP models were developed by fitting two-variable logistic regression model, including: (a) the result of the pATM assay on blood lymphocytes (using the previously determined binary classification of patients as radio-sensitive vs. radio-resistant, with a binary variable, YES=radio-sensitive, NO=radioresistant) and (b) either the prediction from the dosimetric models for grade \geq 3 toxicity (continuous variable that in principle can range from 0 to 100) or the z-standardized prediction for grade \geq 2 (continuous variable that in principle can range from -infinite to +infinite). The discrimination of the Combined biological-dosimetric NTCP models was evaluated through the AUC-ROC.

RESULTS

Population

Patients' characteristics and treatments cohort of 36 patients are detailed in Tables 1 and 2. Median RT prescription dose was 60 Gy (range 50-70 Gy) and conventional fractionation of 2 Gy/fraction was used.

Grade \geq 2 mucositis was experienced by 18/36 (50%) patients. Among them, 11 had received concurrent chemotherapy. Grade \geq 3 mucositis was experienced by 8/36 (22%) patients. Among them, 5 had received concurrent chemotherapy. Grade \geq 2 dysphagia was experienced by 20/36 (55.5%) patients. Among them, 13 had received concurrent chemotherapy. Grade \geq 3 dysphagia was experienced by 12/36 (33.3%) patients. Among them, 8 had received concurrent chemotherapy. Use of concurrent chemotherapy was not significantly associated neither with grade \geq 2 or grade \geq 3 mucositis (Fisher's exact test $p=0.52$ and 0.7) nor to grade \geq 2 or grade \geq 3 dysphagia ($p= 0.75$ and 0.15 , respectively).

RADIODETECT© data

For each considered endpoint results on the performance of the pATM assay in the classification of patients with/without toxicity are reported in Table 3, this includes AUC-ROCs, p-values for the Chi-squared test and the results for the metrics calculated from the confusion matrix. ROC curves are shown in figures 1a (grade \geq 2 oral mucositis), 2a (grade \geq 3 oral mucositis), 3a (grade \geq 2 dysphagia) and 4a (grade \geq 3 dysphagia).

Association between chemotherapy and pATM assay result

The concentration of pATM molecules was not significantly associated with use of concurrent chemotherapy, for both the two selected threshold (46 ng/mL and 57.8 ng/mL, p-values for the Chi-squared test 0.50 and 0.89, respectively).

Dosimetric NTCP models and RADIODETECT© + NTCP combined approach

For each patient predicted probabilities of grade \geq 3 oral mucositis and of grade \geq 3 dysphagia were calculated using NTCP models. From these calculated NTCPs the dosimetric risks were then computed as described in the method section and used for the dosimetric models for grade \geq 2 oral mucositis and of grade \geq 2 dysphagia.

Details on predicted probabilities from the NTCP models are reported in tables 4 and 5 together with some performance measures (AUC-ROC, Mann-Whitney for patients with and without toxicity). ROC curves are shown in figures 1a (grade \geq 2 oral mucositis), 2a (grade \geq 3 oral mucositis), 3a (grade \geq 2 dysphagia) and 4a (grade \geq 3 dysphagia). Figures from 1b to 4b reports the box-and-whisker plots for NTCP predictions of toxicity for the different endpoints.

Models resulting from the combined RADIODETECT© + NTCP approach are described in table 6, while tables 4 and 5 report their performance measures.

DISCUSSION

The aim of this pilot study was to evaluate the possible added value of a radiosensitivity biomarker (RADIODETECT©) when explicitly included in dosimetric NTCP models.

We previously demonstrated the ability of a pATM immunofluorescence assay evaluated on skin fibroblasts to predict adverse effects after RT [25]. This assay gave accurate results, but presented with some issues which could limit its application in the clinical practice, i.e., the need of a skin biopsy, of cell amplification step and of irradiation. To cope with these issues, we first developed an ELISA assay to evaluate pATM in fibroblasts after irradiation and then adapted this assay to the quantification of pATM in blood lymphocytes without irradiation, thus coming to the RADIODETECT© [32]. This pilot study also acts as a feasibility study on use of RADIODETECT© in a clinical setting.

The RADIODETECT© assay was considered in combination with two different cutoff values which were meant to identify both patients with toxicity (grade \geq 2 side-effects, cutoff = 57.8 ng/ml, previously

determined in a larger cohort [32]) and the severity of reactions (grade \geq 3 side-effects, cutoff = 46 ng/ml). Of note both thresholds values were established on a different population (Figure S1, Table S1 and [32]) and were used in the present analysis in a validation setting.

To validate NTCP models, we used previously derived models and coefficients [35] and applied them without any change. As no models were available for grade \geq 2 toxicity, we considered the prediction of grade \geq 3 toxicity (together with z-standardization) as a surrogate of the prediction of grade \geq 2 side-effects.

The RADIODETECT $\text{\textcircled{C}}$ was better discriminating patients with toxicity for 3 out four endpoints, with only the grade \geq 2 dysphagia risk being better predicted by the dosimetric model.

Interestingly, the discriminative power was improved when using the approach combining RADIODETECT $\text{\textcircled{C}}$ and dosimetric NTCP models. The gain in AUC-ROC for the combined models ranged from 5% to 15% (when calculated with respect to the best performing single method, i.e. either RADIODETECT $\text{\textcircled{C}}$ or NTCP). This suggests both the amount of dose to specific organs at risk and the biological characterization at the individual level are responsible to the final response to radiotherapy. The main benefit was for the prediction of severe (grade \geq 3) mucositis, with an increase in the AUC-ROC of 15% with respect to the RADIODETECT $\text{\textcircled{C}}$ and of 17% with respect to the dosimetric NTCP (see figure 3 panels a and b). This result suggests a synergistic effect of biology and dose.

The main limitation of this study is the small size of the population: the results of this pilot study should be considered as a proof of concept and must be consolidated with larger cohorts.

Another possible limitation is the heterogeneity of the population, notably to the various adjuvant radiotherapy strategies. This information could not be included as covariate in models, due the small size of the population which limits the number of features that can be considered in a multivariate fashion. Despite this heterogeneity, a statistically significant association was found between toxicity and both NTCP and RADIODETECT $\text{\textcircled{C}}$ approaches for 3 out 4 endpoints.

Addressing oral mucositis and dysphagia as separated endpoints may be a further limitation, as oral mucositis often causes dysphagia. Nevertheless, dysphagia and mucositis were not completely overlapping in our population. Furthermore, due to the small population size, we did not want to implement new NTCP models, and the available models were fitted for separated endpoints.

External independent validation and assessment of clinical usefulness are needed before implementing these models in the clinical practice. Clinical usefulness can be quantified by decision-analytic methods, such as net benefit and decision curve analysis [36-37]. This process should take into account the harms and benefits of the alternative treatment and/or the possible supportive measures.

Next step will be to take into account the effect of radiosensitizers such as chemotherapy or risk factors like tobacco or comorbidities to improve prediction.

CONCLUSIONS

In this pilot study the combination of the ELISA-based RADIODETECT© assay applied to blood sampling combined with NTCP approach improved the discrimination power of both approach. The results from this pilot validation study constitute the basis of a realistic assay to be potentially proposed to every patient managed with radiotherapy. The assay could work in a synergistic way with dosimetric evaluation. Larger size cohorts, validation and assessment of the clinical usefulness are ongoing before implementing this approach in clinical practice.

Reducing acute severe toxicity for head and neck squamous cell carcinomas by combining dosimetry with a radiosensitivity biomarker

Data Availability Statement

The datasets generated for this study are available on request to the corresponding author.

Ethics Statement

The study involves human participants and it was reviewed and approved by Ethics Committees of all recruiting centers. The patients provided their written informed consent to participate in this study.

Funding: None

Conflicts of Interest: T.B. is co-founder and scientific expert in biostatistics and nano-informatics in CYBERNANO Company (Biosignal Processing & Biostatistics). T.B was employed by the company Cybernano. N.F. and S.P. reports the following patents: FR3040178A1, FR3040179A1, WO2017098190A1, EP3685163A1. S.P and M.D. are employed by the company Neolys diagnostics.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

REFERENCES

1. Leemans, CR, Braakhuis BJ and Brakenhoff RH. The molecular biology of head and neck cancer. *Nat Rev Cancer* 2011; 11, 9-22.
2. Siegel RL, Miller KD and Jemal A. Cancer statistics. *CA Cancer J Clin* 2019; 69, 7-34.
3. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A. Global cancer statistics: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*, 2018; 68, 394-424.
4. Machiels JP, René Leemans C, Golusinski W, Grau C, Licitra L, Gregoire V; EHNS Executive Board. Squamous cell carcinoma of the oral cavity, larynx, oropharynx and hypopharynx: EHNS-ESMO-ESTRO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2020; 31(11):1462-1475.
5. Bhide SA and Nutting CM. Advances in radiotherapy for head and neck cancer. *Oral Oncol* 2010; 46,439-441.
6. Sonis ST. The pathobiology of mucositis. *Nat Rev Cancer* 2004; 4, 277-284.
7. Maria OM, Eliopoulos N and Muanza T. Radiation-Induced Oral Mucositis. *Front Oncol*, 2017; 7:89.
8. Eisbruch A, Kim HM, Feng FY, Lyden TH, Haxer MJ, Feng M et al. Chemo-IMRT of oropharyngeal cancer aiming to reduce dysphagia: swallowing organs late complication probabilities and dosimetric correlates. *Crit Rev Oncol Hematol* 2015, 96(2), 372-384.
9. Orlandi E, Miceli R, Infante G, Mirabile A, Alterio D, Cossu Rocca M et al. Predictors of Patient-Reported Dysphagia Following IMRT Plus Chemotherapy in Oropharyngeal Cancer. *Dysphagia* 2019 ; 34(1), 52-62

10. Xu C, Zhang LH, Chen YP, Liu X, Zhou GQ, Lin AH et al. Chemoradiotherapy Versus Radiotherapy Alone in Stage II Nasopharyngeal Carcinoma: A Systemic Review and Meta-analysis of 2138 Patients. *J Cancer* 2017; 8, 287–297.
11. Tobias JS, Monson K, Gupta N, MacDougall H, Glaholm, J, Hutchison I et al. Chemoradiotherapy for locally advanced head and neck cancer: 10-year follow-up of the UK Head and Neck (UKHAN1) trial. *Lancet Oncol* 2010 ; 11, 66–74.
12. Sanguineti G, Sormani MP, Marur S, Gunn GB, Rao N, Cianchetti M et al. Effect of radiotherapy and chemotherapy on the risk of mucositis during intensity-modulated radiation therapy for oropharyngeal cancer. *Int J Radiat Oncol Biol Phys* 2012; 83, 235–242.
13. Blanchard P, Hill C, Guihenneuc-Jouyaux C, Baey C, Bourhis J and Pignon JP. Mixed treatment comparison meta-analysis of altered fractionated radiotherapy and chemotherapy in head and neck cancer. *J Clin Epidemiol* 2011; 64, 985–992.
14. Geets X, Tomsej M, Lee JA, Duprez T, Coche E, Cosnard G, Lonneux M, Grégoire V. Adaptive biological image-guided IMRT with anatomic and functional imaging in pharyngo-laryngeal tumors: impact on target volume delineation and dose distribution using helical tomotherapy. *Radiother Oncol*. 2007; 85(1):105-15.
15. Marucci L, Farneti A, Di Ridolfi P, Pinnaro P, Pellini R, Giannarelli, D, et al. Double-blind randomized phase III study comparing a mixture of natural agents versus placebo in the prevention of acute mucositis during chemoradiotherapy for head and neck cancer. *Head Neck* 2017; 39, 1761–1769.
16. Narayan S, Lehmann J, Coleman MA, Vaughan A, Yang CC, Enepekides D et al. Prospective Evaluation to Establish a Dose Response for Clinical Oral Mucositis in Patients Undergoing Head-and-Neck Conformal Radiotherapy. *Int J Radiat Oncol Biol Phys* 2008 ; 72, 756–762.
17. Wang ZH, Zhang SZ, Zhang ZY, Zhang CP, Hu HS, Tu WY, et al. Protecting the oral mucosa in patients with oral tongue squamous cell carcinoma treated postoperatively with intensity-modulated radiotherapy: A randomized study. *Laryngoscope* 2012 ; 122, 291–298.

18. Schindler A, Denaro N, Russi EG, Pizzorni N, Bossi P, Merlotti A et al. Dysphagia in head and neck cancer patients treated with radiotherapy and systemic therapies: Literature review and consensus. *Crit Rev Oncol Hematol* 2015; 96(2), 372-384.
19. Emami B, Graham MV, Purdy JA Three-dimensional conformal radiotherapy in bronchogenic carcinoma: considerations for implementation. *Int J Radiat Oncol Biol Phys* 1991; 20:1183-1190
20. Orlandi E, Iacovelli NA, Rancati T, Cicchetti A, Bossi P, Pignoli E, et al. Multivariable model for predicting acute oral mucositis during combined IMRT and chemotherapy for locally advanced nasopharyngeal cancer patients. *Oral Oncol* 2018 ; 86, 266-272.
21. Cavallo A, Rancati T, Cicchetti A, Iacovelli NA, Palorini F, Fallai C et al. Development of multivariable models for acute toxicities in nasopharyngeal cancer radiotherapy. *Radiother Oncol* 2017; 123, S858–S859.
22. Marks LB, Yorke ED, Jackson A, Ten Haken RK, Constone LS, Eisbruch A, Bentzen SM, Nam J, Deasy JO. Use of normal tissue complication probability models in the clinic. *Int J Radiat Oncol Biol Phys*. 2010; 76(3 Suppl):S10-9.
23. Rattay T and Talbot CJ. Finding the genetic determinants of adverse reactions to radiotherapy. *Clin Oncol (R Coll Radiol)* 2014; 26(5), 301-308.
24. Averbeck D, Candéias S, Chandna S, Foray N, Friedl AA, Haghdoost S, Jeggo PA, Lumniczky K, Paris F, Quintens R, Sabatier Establishing mechanisms affecting the individual response to ionizing radiation. *Int J Radiat Biol*. 2020; 96(3):297-323
25. Foray N, Bourguignon M, Hamada N. Individual response to ionizing radiation. *Mutat Res*. 2016; 770(Pt B):369-386.
26. Joubert A and Foray N. Intrinsic radiosensitivity and DNA double-strand breaks in human cells. *Cancer Radiother* 2007; 11(3), 129-142.

27. Granzotto A, Benadjaoud MA, Vogin G, Devic C, Ferlazzo ML, Bodgi L et al. Influence of Nucleoshuttling of the ATM Protein in the Healthy Tissues Response to Radiation Therapy: Toward a Molecular Classification of Human Radiosensitivity. *Int J Radiat Oncol Biol Phys* 2016; 94, 450-460.
28. Berthel E, et al. The Nucleoshuttling of the ATM Protein: A Unified Model to Describe the Individual Response to High- and Low-Dose of Radiation? *Cancers* (Basel). 2019.
29. Belkacemi Y, Colson-Durand L, Granzotto A, Husheng S, To NH, Majdoul S, Guet S, Hervé ML, Fonteneau G, Diana C, Le Bret C, Dominique C, Fayolle M, Foray N. The Henri Mondor Procedure of Morbidity and Mortality Review Meetings: Prospective Registration of Clinical, Dosimetric, and Individual Radiosensitivity Data of Patients With Severe Radiation Toxicity. *Int J Radiat Oncol Biol Phys*. 2016; 96(3):629-36.
30. Pereira S, Bodgi L, Duclos M, Canet A, Ferlazzo ML, Devic C et al. Fast and Binary Assay for Predicting Radiosensitivity Based on the Theory of ATM Nucleo-Shuttling: Development, Validation, and Performance. *Int J Radiat Oncol Biol Phys* 2018; 100, 353-360.
31. Vogin G, Bastogne T, Bodgi L, Gillet-Daubin J, Canet A, Pereira S, Foray N. The Phosphorylated ATM Immunofluorescence Assay: A High-performance Radiosensitivity Assay to Predict Postradiation Therapy Overreactions. *Int J Radiat Oncol Biol Phys*. 2018;101(3):690-693
32. Deneuve S, Mirjolet C, Bastogne T, Duclos M, Retif P, Zrounba P, Roux PE, Poupart M, Vogin, G, Foray N and Pereira S. Proof of Concept of a Binary Blood Assay for Predicting Radiosensitivity. *Cancers* 2021; 13, 2477.
33. Eisbruch A, Kim HM, Terrell JE, Marsh LH, Dawson LA and Ship JA. Xerostomia and its predictors following parotid-sparing irradiation of head-and-neck cancer. *Int J Radiat Oncol Biol Phys* 2001; 50, 695–704.

34. DeLong ER, DeLong DM and Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics* 1988; 44, 837-845.
35. Moons KG, Kengne AP, Grobbee DE, Royston P, Vergouwe Y, Altman DG, Woodward M. Risk prediction models: II. External validation, model updating, and impact assessment. *Heart*. 2012; 98(9):691-698.
36. Steyerberg EW, Vickers AJ, Cook NR, Gerds T, Gonen M, Obuchowski N et al. Assessing the performance of prediction models: a framework for traditional and novel measures. *Epidemiology* 2010; 21, 128-138.
37. Vickers AJ and Elkin EB. Decision curve analysis: a novel method for evaluating prediction models. *Med Decis Making* 2006; 26: 565-574.

Table 1 : Patients characteristics

Patients characteristics		N=36
Gender	Female	3 (8.5%)
	Male	32 (91.5%)
Age (years)	Median	57 years old
	Range	32-85 years old
Primary HNSCC Site	Oral cavity	18 (51.5%)
	Oropharynx	5 (14%)
	Larynx	4 (11.5%)
	Hypopharynx	4 (11.5%)
	Maxillary Sinus	5 (11.5%)
Smoking history	No	5 (14%)
	Former or current	31 (86%)
Alcohol consumption	None	9 (25%)
	Occasional	17 (47%)
	Abuse*	10 (28%)
Tumor status (AJCC TNM 7 th edition,2009)	T1	2 (5.5%)
	T2	15 (41.5%)
	T3	8 (22%)
	T4	11 (30.5%)
Nodal status (AJCC TNM 7 th edition,2009)	N0	7 (20%)
	N1	5 (11%)
	N2a	7 (20%)
	N2b	8 (22%)
	N2c	5 (14%)
	N3	4 (11%)

**≥3 units per day (women); ≥5 units per day (men)*

Table 2: Oncological treatment characteristics. When considering grade \geq 3 oral mucositis, dosimetric variables were available for 35 patients. The EUD to the oral cavity (n=0.05) was ranging from 18-71 Gy, median 54 Gy; the mean dose to the parotid glands was ranging from 3.5-41.7 Gy, the median value was 22.5 Gy. When considering grade \geq 3 dysphagia, dosimetric variables were available for 33 patients. The EUD to the oral cavity (n=0.15) was ranging from 18-66 Gy, median 47 Gy; the EUD to the glottic larynx (n=0.35) was ranging from 0.5-62.3 Gy, with a median of 38.1 Gy; the V_{50Gy} to the pharyngeal constrictors was ranging from 0.5-100%, with a median of 55.4%.

Radiotherapy technique	VMAT	28 (77%)
	IMRT	7 (20%)
	Tomotherapy	1 (3%)
Prescription dose to primary tumor target	Median (range; interquartile)	60 Gy (50-70 Gy)
Homolateral nodal dose	Median (range ; interquartile)	54 Gy (0-70 Gy)
Nodal dose	Median (range; interquartile)	50 Gy (0-70 Gy)
Fractionation	2 Gy/fraction	36 (100%)
Chemotherapy	<u>Neoadjuvant</u>	
	TPF (Docetaxel, cisplatin and fluorouracil)	2 (6%)
	5FU (<i>Fluorouracil</i>) +Cisplatin	1 (3%)
	<u>Concurrent</u>	
	Cisplatin 100 mg/kg	7 (19%)
	Cisplatin 40 mg/kg	11 (31%)
	Cetuximab	1 (3%)
Radiotherapy doses to normal tissues		
EUD Oral Cavity (n=0.05)	Median (range)	54 Gy (18-71 Gy)
EUD Oral Cavity (n=0.15)	Median (range)	47 Gy (18-66 Gy)
EUD Glottic Larynx (n=0.35)	Median (range)	48 Gy (0.5-62 Gy)
Mean dose to combined parotid glands	Median (range)	22.5 Gy (3.5-42 Gy)
V50Gy to pharyngeal constrictor muscles	Median (range)	55% (0.5-100%)

EUD=Equivalent Uniform Dose; V50Gy=percent organ volume receiving > 50 Gy

Reducing acute severe toxicity for head and neck squamous cell carcinomas by combining dosimetry with a radiosensitivity biomarker

Table 3: Performance results of RadioDtect® assay

	Grade≥2 <i>oral mucositis</i>	Grade≥3 <i>oral mucositis</i>	Grade≥2 <i>dysphagia</i>	Grade≥3 <i>dysphagia</i>
Accuracy	0.75	0.58	0.58	0.58
Misclassification Rate (or Error Rate)	0.25	0.41	0.41	0.41
True Positive Rate (or Sensitivity)	0.56	0.75	0.75	0.75
True Negative Rate (or Specificity) = 0.96	0.96	0.53	0.37	0.55
Precision	0.68	0.31	0.60	0.42
Null Error Rate	0.3	0.22	0.44	0.33
AUC-ROC	0.75 95%CI 0.58 to 0.88	0.67 95%CI 0.49 to 0.81	0.57 95%CI 0.40 to 0.74	0.60 95%CI 0.51 to 0.83

Reducing acute severe toxicity for head and neck squamous cell carcinomas by combining dosimetry with a radiosensitivity biomarker

Table 4: Result on performance of NTCP models and combined RADIODETECT® + NTCP approach for grade≥3 oral mucositis and grade≥3 dysphagia

	Grade≥3 oral mucositis Dosimetric NTCP	Grade≥3 dysphagia Dosimetric NTCP	Grade≥3 oral mucositis Combined NTCP	Grade≥3 dysphagia Combined NTCP
Toxicity probability range	5-47%	5-53%	1-67%	14-65%
Toxicity probability median value (interquartile)	24% (19-34)	24% (14-37)	18% (8-31)	34% (28-46)
Toxicity probability mean value (standard deviation)	26% (10)	26% (13)	23% (18)	36% (13)
AUC-ROC	0.61 95%CI 0.43 to 0.77	0.56 95%CI 0.38 to 0.73	0.782 95%CI 0.61 to 0.90	0.65 95%CI 0.46 to 0.81
Mean predicted probability for patients with/without toxicity	29% vs 22%	27% vs 22%	19% vs 35%	34% vs 41%
Mann-Whitney test	0.36	0.47	0.02	0.15

Reducing acute severe toxicity for head and neck squamous cell carcinomas by combining dosimetry with a radiosensitivity biomarker

Table 5: Result on performance of NTCP models and combined RADIODETECT® + NTCP approach for grade≥2 oral mucositis and grade≥2 dysphagia

	Grade≥2 oral mucositis Dosimetric NTCP	Grade≥2 dysphagia Dosimetric NTCP	Grade≥2 oral mucositis Combined NTCP	Grade≥2 dysphagia Combined NTCP
Toxicity probability range			5-90%	17-88%
Toxicity probability median value (interquartile)			60% (17-76%)	51% (38-67)
Toxicity probability mean value (standard deviation)			55% (29)	52% (20)
AUC-ROC	0.55 95%CI 0.37 to 0.72	0.64 95%CI 0.45 to 0.80	0.80 95%CI 0.64 to 0.92	0.71 95%CI 0.53 to 0.86
Mean predicted probability for patients with/without toxicity	51% vs 52%	49% vs 54%	35% vs 67%	44% vs 59%
Mann-Whitney test	0.62	0.17	0.002	0.04

reducing acute severe toxicity for head and neck squamous cell carcinomas by combining dosimetry with a radiosensitivity biomarker

Table 6: Details of models from the combined RADIODETECT© + NTCP approach for all considered endpoints (grade≥2 oral mucositis, grade≥2 dysphagia, grade≥3 oral mucositis, grade≥3 dysphagia)

	Odds Ratio	p-value
Grade≥2 Oral mucositis		
<i>z-standardized prediction from NTCP model</i>	2.0	0.12
RADIODETECT©	33	0.006
Likelihood ratio test		0.001
Grade≥3 Oral mucositis		
<i>prediction from NTCP model</i>	1.14	0.06
RADIODETECT©	10.8	0.06
Likelihood ratio test		0.05
Grade≥2 Dysphagia		
<i>z-standardized prediction from NTCP model</i>	2.7	0.05
RADIODETECT©	6.8	0.07
Likelihood ratio test		0.07
Grade≥3 Dysphagia		
<i>prediction from NTCP model</i>	1.13	0.30
RADIODETECT©	3.5	0.17
Likelihood ratio test		0.32

Legends

Supplementary Figure S1: Threshold determination.

Estimated classification rule after bootstrapping resulted in a median cut-off point for pATM concentration which differs between the two cases:

- A. equal to 46 ng/ml for a classification up to CTCAE grade 3. The concentration of pATM molecules in lymphocytes predicted occurrence of grade \geq 3 with an AUC-ROC=0.74, 95% Confidence Interval 0,59 to 0,88 (significant p-value= 0,0024). Nine out 53 patients (17%) were classified as “radiosensitive” using this cut-off point.
- B. equal to 57,8 ng/ml for a classification up to CTCAE grade 2. The concentration of pATM molecules in lymphocytes predicted occurrence of grade \geq 2 with an AUC=0.74, 95% Confidence Interval 0,598 to 0,848 (significant p-value= 0,028). twenty-eight out 53 patients (52%) were classified as “radiosensitive” using this cut-off point.

Supplementary Table S1: patient’s characteristics and treatment