Radiotherapy and Oncology xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Radiotherapy and Oncology

journal homepage: www.thegreenjournal.com

Original article

Prognostic impact of HPV-associated p16-expression and smoking status on outcomes following radiotherapy for oropharyngeal cancer: The MARCH-HPV project

Pernille Lassen [a], Benjamin Lacas [b,c], Jean-Pierre Pignon [b,c], Andy Trotti [d], Bjorn Zackrisson [e], Qiang Zhang [f], Jens Overgaard [a], Pierre Blanchard [g,c,*], on behalf of the MARCH Collaborative Group

[a] Department of Experimental Clinical Oncology, Aarhus University Hospital, Denmark; [b] Gustave-Roussy, Paris-Saclay University, Biostatistics and Epidemiology Department; [c] INSERM U1018, CESP, Université Paris-Sud, Université Paris-Saclay, Villejuif, France; [d] Moffitt Cancer Center, Department of Radiation Oncology, Tampa, USA; [e] Department of Radiation Sciences – Oncology, Umeå University, Umeå, Sweden; [f] NRG Oncology Statistics and Data Management Center (formerly RTOG), Philadelphia, USA; and [g] Gustave-Roussy, Paris-Saclay University, Radiotherapy Department, Villejuif, France

ARTICLE INFO

Article history:
Received 28 September 2017
Received in revised form 16 October 2017
Accepted 16 October 2017
Available online xxxx

Keywords:
HPV
Smoking
Oropharynx carcinoma
Prognostic
Radiotherapy
Altered fractionation

ABSTRACT

Background and purpose: Evaluate the prognostic and predictive impact of HPV-associated p16-expression and assess the combined prognostic impact of p16 and smoking on altered fractionated radiotherapy (AFRT) for oropharyngeal cancer (OPC) within the frames of the update of the Meta-Analysis of Radiotherapy in Carcinomas of Head and neck (MARCH).

Materials and methods: Patients with OPC, known tumor p16-status and smoking history were identified from the MARCH update, resulting in a dataset of 815 patients from four randomized trials (RTOG9003, DAHANCA6&7, RTOG0129, ARTSCAN). Analysis was performed using a Cox model stratified by trial and adjusted on gender, age, T-stage, N-stage, type of radiotherapy fractionation, p16, smoking. Primary endpoint was progression-free survival (PFS).

Results: In total, 465 patients (57%) had p16-positive tumors and 350 (43%) p16-negative. Compared to p16-negative, p16-positive patients had significantly better PFS (HR = 0.42 [95% CI: 0.34–0.51], 28.9% absolute increase at 10 years) and OS (HR = 0.40 [0.32–0.49], 32.1% absolute increase at 10 years). No interaction between p16-status and fractionation schedule was detected. Smoking negatively impacted outcome; in the p16-positive subgroup, never smokers had significantly better PFS than former/current smokers (HR = 0.49 [0.33–0.75], 24.2% survival benefit at 10 years).

Conclusions: No predictive impact of p16-status on response to AFRT could be detected but the strong prognostic impact of p16-status was confirmed and especially p16-positive never smoking patients have superior outcome after RT.

Human papillomavirus (HPV) is a well-established cause of oropharyngeal cancer (OPC) [1,2] and although the proportion of OPC attributable to HPV shows geographical variation [3], the incidence of HPV-associated OPC has increased at an epidemic rate over the past 40 years in many western countries [4–7]. HPV-positive OPCs are distinct in terms of epidemiological, clinical and molecular features when compared to HPV-negative OPC, and tumor HPV-status is recognized as the strongest independent prognostic factor for radiotherapy (RT) outcome in OPC, in favor of HPV-positivity [8–12]. These observations are explained in part by a higher sensitivity of HPV-positive tumors to RT [13,14] combined with a different and more favorable risk factor profile and better general health status in the group of patients with HPV-positive disease [15].

Tobacco smoking, along with alcohol consumption, remains the main etiological factor in squamous cell carcinoma of the head and neck (HNSCC) worldwide and smoking independently affects treatment response and survival in a negative way for patients with OPC, regardless of tumor HPV-status [8,16,17]. Thus, smoking patients with HPV-positive disease have intermediate prognosis, and besides the excess co-morbidity [18] and risk of secondary cancers caused by significant lifetime exposure to smoking, the presumed dual HPV/tobacco etiology may result in different mutational profiles between HPV-positive never smokers and HPV-positive ever smokers [19]. Moreover, smoking during RT has been shown to compromise treatment outcome for patients with head

* Corresponding author at: Department of Radiation Oncology, Gustave Roussy Cancer Campus, 114 rue Edouard Vaillant, 94800 Villejuif, France.
E-mail address: pierre.blanchard@gustaveroussy.fr (P. Blanchard).

https://doi.org/10.1016/j.radonc.2017.10.018
0167-8140/

and neck cancer [20,21]. Although it has been shown repeatedly that the proportion of HPV-associated OPC is higher among never smokers than former/current smokers, still the majority of HPV-positive disease is found among patients with a history of either current or former smoking [22,23]. Thus, smoking continues to be of utmost clinical importance, modifying prognosis also for the group of patients with HPV-associated OPC, for whom clinical trials investigating de-intensified therapies are currently ongoing.

Altered fractionated RT (AFRT) regimens for the treatment of HNSCC have been investigated in numerous clinical trials, leading to the provision of conflicting results regarding tumor control and survival, mostly due to trial heterogeneity and limited sample size. However, the Meta-Analysis of Radiotherapy in Carcinomas of Head and neck (MARCH) has demonstrated that altered fractionation RT was associated with improved overall survival and progression-free survival when compared to conventional fractionation RT [24]. Moreover, altered fractionation significantly improved locoregional tumor control, predominantly by reducing the risk of local failure whereas the benefit on nodal control was less pronounced. These findings were recently confirmed in an update of the Meta-Analysis based on more patients and longer follow up [25]. Whether tumor HPV status is associated with a differentiated response to altered fractionation RT is less well investigated. During the update of the MARCH-analysis, data on HPV-associated p16-expression and smoking status have been collected from trials where this information was available. With this study, we aimed to evaluate the prognostic and predictive impact of p16-status and to assess the combined prognostic impact of p16 and smoking in altered fractionated RT of OPC within the frames of the update of the Meta-Analysis of Radiotherapy in Carcinomas of Head and neck.

Material and methods

Patients and trials

Search strategy, selection criteria and data collection for the update of the Meta-Analysis of Radiotherapy in HNSCC (MARCH) are described elsewhere, alongside with the checking procedure of individual patient data [25]. Among the 33 trials included only five collected p16-status [26–30]. Within those trials, p16-status was available for 999 OPC. The adjustment on smoking status led to the exclusion of one more trial (65 patients) [28] and additional 119 patients from the remaining trials due to missing values. Our analysis was restricted to trials where RT was given as the primary treatment modality excluding studies with postoperative RT, and only patients with OPC, known tumor HPV-status and smoking history were included, in turn yielding a dataset consisting of 815 patients from 4 different randomized trials (RTOG9003 [26], DAHANCA6&7 [27], RTOG0129 [29], ARTSCAN [30] (Fig. 1, Supplementary Table 1). In all trials HPV-association was assessed by use of p16-expression, an established surrogate for tumor HPV in OPC, and tumors were classified as p16-positive in case of strong and diffuse nuclear and cytoplasmic staining in >70% of tumor cells, evaluated by immunohistochemistry [31]. Smoking history was not reported uniformly between trials and no consistent information on lifetime exposure and pack-years was available. Thus, smoking was reported as never, former or current apart from in the ARTSCAN trial where smoking was collected as never/former or current smoking.

Endpoints

The primary endpoint was progression-free survival (PFS), defined as time from randomization to first failure (loco-regional or distant) or death from any cause. Secondary endpoints were overall survival (OS), overall survival after first failure, loco-regional control (LRC), cancer and non-cancer mortality. OS was defined as time from randomization to death from any cause. Overall survival after first failure was defined as time from first failure to death from any cause, with the exclusion of patients without failure. Events considered for LRC were local failure, regional failure or synchronous regional and local failures. Since only the first event was collected, patients with distant failure were censored at that time. Patients alive without the events corresponding to each endpoint were censored at their date of last follow-up. Non-cancer mortality was defined as deaths without failure and resulting from known causes other than the treated head and neck cancer. Cancer mortality included deaths from any cause with previous failure and deaths from the treated head and neck cancer. Deaths without failure and from unknown cause were considered as cancer mortality if they occurred within 5 years after randomization and as non-cancer mortality [32] otherwise.

Statistical analysis

The prognostic effect of p16 was estimated on all endpoints. Hazard ratios (HR) and 95% confidence intervals (CI) were estimated with a Cox model stratified by trial and adjusted on T-stage (T1–2, T3–4), N-stage (N0, N+), gender, age (<50, [50–60[, [60–70[, ≥70), treatment arm (standard or modified fractionation) and p16-status (positive, negative). In this model smoking status was coded as never/former vs current, in order to enable inclusion of ARTSCAN in the analysis. Survival rates were estimated for the control group every 3 months with the Kaplan–Meier method and calculated for the experimental group with the HR from the Cox model, based on the formula described by Stewart and Parmar [33], for PFS, OS and overall survival after first failure. For cancer and non-cancer mortality, the Fine & Gray model was used to estimate sub-distribution HRs (sub-HRs), adjusted on the same covariates than the Cox model. Survival rates were estimated with incidence curves [34]. LRC was analyzed using both methods. As recommended, estimations of HRs with cause-specific models were also performed [35] using the adjusted Cox model previously described. Adjusted and unadjusted cumulative incidences were calculated. All survival and cumulative incidence curves were truncated at five, eight or 12 years, depending on numbers of patients left at those times.

The predictive effect of p16-status was estimated only for PFS, OS and cancer mortality, since a lack of power was expected for the other endpoints. The same models, including smoking as never/former vs current were used for the evaluation of the prognostic effect of p16-status, with the addition of an interaction between p16-status and RT fractionation.

The prognostic effect of the combination between p16-status and smoking was studied for all endpoints. For this analysis, smoking status was classified as never smokers vs. former/current smokers since the group of never smokers is presumably distinct both in terms of tumor biology and mutational profile but also with regard to patient related co-morbidity and risk profile. Consequently, this led to the exclusion of the 166 patients from the ARTSCAN trial leaving 649 patients available for analysis. Otherwise, the same models were used, with an adjustment on the combination (p16-negative and never smokers, p16-negative and former/current smokers, p16-positive and never smokers, p16-positive and former/current smokers).

Chi^2 heterogeneity tests and I^2 statistic were used to investigate heterogeneity between trials [36]. In case of significant heterogeneity ($p < 0.10$), a random-effects model was used. Median follow-up time was estimated with the reverse Kaplan–Meier method [37]. Hazard proportionality was assessed with Schoenfeld residuals [38]. With 525 events, it would be possible to detect with

Please cite this article in press as: Lassen P et al. Prognostic impact of HPV-associated p16-expression and smoking status on outcomes following radiotherapy for oropharyngeal cancer: The MARCH-HPV project. Radiother Oncol (2017), https://doi.org/10.1016/j.radonc.2017.10.018