Treatment-related outcome of oropharyngeal cancer patients differentiated by HPV dictated risk profile: a tertiary cancer centre series analysis

P. Bossi1*, E. Orlandi2, R. Miceli3, F. Perrone4, M. Guzzo5, L. Marianii2, R. Granata1, L. Locati1, C. Fallai2, B. Cortelazzi4, S. Pliotti2, G. Scaramellini5, A. Gloghini4 & L. Licitra1

1Head and Neck Cancer Medical Oncology Unit; 2Radiotherapy Unit; 3Clinical Epidemiology and Trial Organization Unit; 4Laboratory of Experimental Molecular Pathology, Department of Pathology; 5Otorhinolaryngology Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy

Received 31 July 2013; revised 30 December 2013; accepted 31 December 2013

Background: To date, no treatment modality has been identified as more effective for oropharyngeal cancer (OPC), and no predictive factors are known to guide treatment decision for this disease. This retrospective study evaluates the differential effects of diverse treatment options for OPC according to patient risk profiles.

Patients and methods: We considered two series of locally advanced squamous cell OPC patients treated with either surgery followed by radiotherapy (surgical series) or chemoradiation (CRT) with/without induction docetaxel, cisplatin and 5-fluorouracil (TPF) chemotherapy (CRT series). Smoking habits, tumor p16 expression/human papillomavirus (HPV) status and T and N stage were analyzed to stratify the patients according to Ang’s risk profile (low, intermediate and high risk). Overall survival (OS) and disease-free survival were calculated with the Kaplan–Meier method.

Results: Globally, 171 patients were considered, 56 in surgical and 115 in CRT series. Patients were stratified in low- (20% of surgical and CRT groups), intermediate- (23% and 41%) and high-risk (57% and 39%) groups. In the surgical series, 5-year OS was 54.5%, 46.9% and 40.0% in low, intermediate and high Ang’s risk profiles, respectively, whereas in the CRT series those were 100%, 78.9% and 46.7%, respectively. In the multivariable analyses, adjusting for inhomogeneity between the treatment group, the CRT effect was significantly higher in the low- and intermediate-risk groups (P-value for the interaction treatment risk group = 0.034 in the OS analysis).

Conclusions: In this retrospective analysis, low- and intermediate-risk OPC patients had a better survival when treated with CRT compared with open surgery followed by radiation therapy. These data suggest that different treatment approaches might be essential in determining outcome results.

Key words: oropharyngeal cancer, human papilloma virus, chemoradiation, risk profile, survival

*Correspondence to: Dr Paolo Bossi, Head and Neck Cancer Medical Oncology Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Via Venezian 1, 20133 Milan, Italy.
Tel: +39-02-23902150; Fax: +39-02-23903353; E-mail: paolo.bossi@istitutotumori.mi.it

© The Author 2014. Published by Oxford University Press on behalf of the European Society for Medical Oncology. All rights reserved. For permissions, please email: journals.permissions@oup.com.
introduction

Over the last decades, the epidemiology of oropharyngeal cancer (OPC) has been characterized by an increased incidence paralleled by an increase in survival, which have been attributed to higher incidence and favorable prognostic contribution of human papillomavirus (HPV) infection [1]. Smoking has also been identified as a prognostic factor, regardless of HPV status [2].

In locally advanced disease, treatment strategies consist of either surgery followed by postoperative radiation therapy (RT) with or without adjuvant chemotherapy, or curative chemoradiotherapy (CRT) [3]. However, no treatment modality has been identified as more effective, and no predictive factors guide treatment decision. Accordingly, current guidelines suggest both treatment strategies as equally acceptable for advanced OPC therapy [4], but further evidence on this issue is required.

To evaluate the effects of different treatment options for OPC according to patient risk profiles, we retrospectively analyzed two series of OPC patients treated during the last 20 years at the National Cancer Institute of Milan (Italy) with either surgery followed by postoperative RT or curative CRT, stratified according to p16 expression/HPV status and smoking [2, 5].

patients and methods

treatment approach

Until 2000, patients with advanced OPC were treated with surgery followed by RT. Over the following years, CRT gradually became the standard treatment, associated with induction chemotherapy in patients at an increased risk of metastatization (bulky nodes or T4 disease).

series considered

We considered stage III–IV squamous cell OPC patients [6]. The first series consisted of patients with available pathologic specimens and complete follow-up information undergoing surgery followed by RT between 1990 and 1999. These subjects had already been included in a previous study about the HPV prognostic role [7]. The second series consisted of all patients with locally advanced OPC treated between 2004 and 2010 with CRT. We did not include patients treated between 2000 and 2003, since during that period surgical approach and CRT were both adopted.

surgery

Surgery consisted of resection of the lateral wall of the oropharynx, the close portion of the soft palate and partial glossectomy. For advanced tumors, a composite resection of soft tissue ‘en bloc’ along with mandibulectomy (‘commando’ operation) was employed. Tumors not involving the midline were treated with unilateral neck dissection. Contralateral neck dissection was planned when the primary tumor extended over the midline. Clinically involved nodes required the dissection of all the five nodal levels. In the clinically N0 neck, the V level was usually spared.

radiation therapy

Patients in the first series received postoperative conventional (two-dimensional) RT, consisting of 45–66 Gy, according to the specific risk of the irradiated region, with standard fractionation (1.8–2 Gy daily), within 6–10 weeks after surgery. Of note, in 1990s, the addition of chemotherapy to postoperative RT was not part of the standard treatment. Patients in the second series received either three-dimensional RT (3DRT) or intensity-modulated RT (IMRT). With the 3DRT approach, consisting of 3–5 treatment fields, either conventional fractionation (66–70 Gy/33–35 fractions/6.5–7 weeks) or accelerated fractionation with concomitant boost (72 Gy/40 fractions/6 weeks/twice-a-day in the last 10 days) were used. IMRT treatment consisted of sequential IMRT based on conventional (70 Gy/35 fractions) or accelerated (66 Gy/30 fractions/6 weeks or 70 Gy/33 fractions/6.5 weeks) fractionation.

chemotherapy

Cisplatin 100 mg/sm every 3 weeks was administered to patients in the second series during RT, for an intended total dose of 300 mg/sm. Induction chemotherapy, when employed, consisted of two or three cycles of TPF scheme (docetaxel 75 mg/sm, cisplatin 75 mg/sm on day 1 and 5-fluorouracil 750 mg/sm/day continuous infusion for 4 days).

risk factor stratification

Patients were stratified into three classes (low, intermediate and high risk) according to p16 expression/HPV status, smoking status and tumor stage, as already found by Ang et al. [5] and validated by our group [8], p16 expression was determined using the CIN tec p16INK4a Histology kit in all cases [9].

HPV status was also assessed by HPV DNA in situ hybridization using a Ventana Benchmark ULTRA automated immunostainer and the Inform HPV III family probe (a cocktail of probes recognizing 12 high-risk HPV genotypes) [9] and/or by E6 and E7 mRNA expression using real-time PCR [10].

For smoking status, a cutoff of 10 packs/year was employed.

statistical analysis

Comparison between the two groups was carried out using the Mann–Whitney–Wilcoxon test. Categorical variables were analyzed with the χ² test or the Fisher’s exact test with mid-p correction, as appropriate; the Cochran–Armitage trend test was used for ordered categories.

The study end points were overall survival (OS) and disease-free survival (DFS). The initial date was the date of surgery for group 1 and the beginning of chemotherapy or RT for group 2. The follow-up time of event-free patients was censored at 60 months. OS was calculated from initial date to the date of death, or as equal to censoring time for living patients. DFS time was calculated from initial date to the date of disease relapse, second primary tumor or death without evidence of disease, whichever occurred first; DFS time was equal to censoring time for disease-free patients.

OS and DFS curves were estimated by the Kaplan–Meier method and compared with the log-rank test. Multivariable analyses of OS and DFS were carried out using Cox models; the covariates were treatment (CRT and surgery alone), the Ang’s risk profile (low or intermediate and high) and their interaction. To adjust for confounding factors, we also included a ‘propensity score’ in the Cox models, which estimated the tendency to perform CRT. The propensity score was the linear predictor from a multivariate binary logistic model in which the response variable was treatment and the covariates were patient’s age, T and N stage, and Ang’s risk profile. The Cox model results are shown in terms of hazard ratios (HRs; surgery alone versus CRT), with two-sided Wald P-values; confidence intervals including one indicate a higher risk with surgery than with CRT. From the multivariate Cox models, adjusted OS and DFS were calculated by averaging the curves estimated for each treatment group and the observed combinations of the covariates, i.e. Ang’s risk score and propensity score.

Analyses were carried out using the SAS® and R software [11]. P-values of <0.05 were considered statistically significant.
results
The surgical and CRT series consisted of 56 and 115 patients, respectively.

p16 and HPV analysis
The incidence of p16 expression changed from 39% (22 of 56) in the surgical series to 59% (68 of 115) in the CRT series (Table 1). HPV status was assessed in all the patients in the surgical group, whereas biopsies were adequate for HPV testing in 93 of 115 patients in the CRT group. HPV positivity shifted from 20% (11 of 56) of the surgically treated series to 57% (53 of 93) of the more recent CRT series (Table 1). Noteworthy, among the p16-positive cases, HPV infection was detected in 84% of the cases. All HPV-positive cases showed the punctuate labeling characteristic of integrated HPV DNA, while some cases also showed diffuse signals consistent with episomal DNA.

Consistently with an epidemiological shift, high-risk profile was observed in 57% of patients in the surgical series and in 39% of the CRT group; intermediate-risk patients were 23% and 41%, respectively, and low-risk patients were 20% in both groups.

No tumor was p16 and HPV positive in surgical and CRT high-risk groups. In contrast, all tumors were p16 positive in the low-risk group with ascertained HPV positivity in 82% and 100% of surgical and CRT series, respectively.

In the intermediate-risk group, p16 positivity reached 85% in surgical and 94% in CRT series, whereas HPV positivity was ascertained in 46% of surgically treated patients and in the majority of the CRT patients (84%) (Table 1).

OS, DFS and treatment-related effect
Induction TPF chemotherapy was carried out in 40% of patients in the CRT group and was associated with T or N tumor stages. The median duration of RT treatment was 42 days in post-operative series and 48 days in CRT series.

Surgically treated patients exhibited a higher number of local and regional relapses, whereas there was no difference in distant metastatization and second primaries between the two groups (supplementary Table S1, available at Annals of Oncology online).

Five-year OS and DFS without differentiating according to risk factors are reported in supplementary Figure S1, available at.

Table 1. Main patient and disease characteristics according to the treatment group

<table>
<thead>
<tr>
<th></th>
<th>Surgery</th>
<th>CRT</th>
<th>P-value</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
<td>115</td>
<td>171</td>
<td></td>
</tr>
<tr>
<td>Patient’s age (years)</td>
<td>56</td>
<td>115</td>
<td>0.201</td>
<td>171</td>
</tr>
<tr>
<td>Median (IQ range)</td>
<td>58 (51.8–63.5)</td>
<td>60 (53.5–66.5)</td>
<td>0.940</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>10</td>
<td>17.9</td>
<td>20</td>
<td>17.4</td>
</tr>
<tr>
<td>Male</td>
<td>46</td>
<td>82.1</td>
<td>95</td>
<td>82.6</td>
</tr>
<tr>
<td>T stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>–</td>
<td>16</td>
<td>13.9</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>28.6</td>
<td>31</td>
<td>27.0</td>
</tr>
<tr>
<td>3</td>
<td>24</td>
<td>42.9</td>
<td>14</td>
<td>12.2</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>28.6</td>
<td>54</td>
<td>47.0</td>
</tr>
<tr>
<td>N stage</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>7</td>
<td>12.5</td>
<td>6</td>
<td>5.2</td>
</tr>
<tr>
<td>N1</td>
<td>6</td>
<td>10.7</td>
<td>10</td>
<td>8.7</td>
</tr>
<tr>
<td>N2a</td>
<td>6</td>
<td>10.7</td>
<td>4</td>
<td>3.5</td>
</tr>
<tr>
<td>N2b</td>
<td>32</td>
<td>57.1</td>
<td>49</td>
<td>42.6</td>
</tr>
<tr>
<td>N2c</td>
<td>3</td>
<td>5.4</td>
<td>35</td>
<td>30.4</td>
</tr>
<tr>
<td>N3</td>
<td>2</td>
<td>3.6</td>
<td>11</td>
<td>9.6</td>
</tr>
<tr>
<td>P16 status</td>
<td></td>
<td></td>
<td>0.030</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>34</td>
<td>60.7</td>
<td>48</td>
<td>41.7</td>
</tr>
<tr>
<td>Positive</td>
<td>22</td>
<td>39.3</td>
<td>67</td>
<td>58.3</td>
</tr>
<tr>
<td>HPV status (by ISH or RT-PCR)</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>45</td>
<td>80.4</td>
<td>40</td>
<td>34.8</td>
</tr>
<tr>
<td>Positive</td>
<td>11</td>
<td>19.6</td>
<td>53</td>
<td>64.2</td>
</tr>
<tr>
<td>Not available</td>
<td>0</td>
<td>–</td>
<td>22</td>
<td>19.1</td>
</tr>
<tr>
<td>Risk profile</td>
<td></td>
<td></td>
<td>0.048</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>11</td>
<td>19.6</td>
<td>23</td>
<td>20.0</td>
</tr>
<tr>
<td>Intermediate</td>
<td>13</td>
<td>23.2</td>
<td>47</td>
<td>40.9</td>
</tr>
<tr>
<td>High</td>
<td>32</td>
<td>57.1</td>
<td>45</td>
<td>39.1</td>
</tr>
</tbody>
</table>

HPV: human papillomavirus; CRT: chemoradiation; IQ: interquartile; ISH: in situ hybridization.
In the second series, OS and DFS did not differ according to previous induction chemotherapy (data not shown).

Treatment type-related effect was more evident in the low- and intermediate-risk groups. At multivariable analysis of OS, the HR of surgery versus CRT was 4.46 [95% confidence interval (95% CI) 1.68–11.86] in the low-/intermediate-risk group and 1.34 (95% CI 0.67–2.68) in the high-risk group (P-value for the interaction treatment risk group = 0.034). At multivariable analysis of DFS, HR was 3.60 (95% CI 1.51–8.61) in the low-/intermediate-risk group and 0.85 (95% CI 0.46–1.57) in the high-risk group (P-value for interaction treatment risk group = 0.005). Figure 1 shows the adjusted OS and DFS curves according to the risk and treatment groups, respectively.

We also carried out multivariable Cox analyses including in the models p16 status as an alternative to the risk group, obtaining results coherent with those reported above. In both OS and DFS, the effect of treatment was more evident in the p16-positive subgroup [HR for OS: 5.66 (95% CI 1.94–16.48) in the p16-positive group and 1.29 (95% CI 0.66–2.53) in the p16-negative group, P-value for interaction treatment-p16 = 0.015; HR for DFS: 3.77 (95% CI 1.46–9.69) in the p16-positive group and 0.87 (95% CI 0.48–1.58) in the p16-negative group, P = 0.006]. Fifty-four percent of patients in surgical series presented pathological risk factors (microscopically involved margins in 5%, extracapsular extension in 40% and both in 9% of the cases) that according to modern standards would have required postoperative concurrent CRT. Such risk factors were present in 64%, 69% and 44% of the low-, intermediate- and high-risk groups, respectively (P = 0.233).

In a previous comprehensive analysis by tumor site [12], an absolute increase of 8.1% in the 5-year OS was observed with...
the adjunct of concomitant chemotherapy in the oropharyngeal subsite group. Thus, we carried out an OS multivariable Cox sensitivity analysis in which, for the surgical patients with adverse prognostic markers, we simulated a survival time to obtain a 60-month 8.1% OS advantage over patients with favorable prognostic markers. A significant interaction between treatment and risk group was still observed ($P = 0.044$); the HR for surgery versus CRT was 3.57 (95% CI 1.32–9.64) in the low-/intermediate-risk group and 1.13 (95% CI 0.56–2.28) in the high-risk group.

Overall, treatment type-related effect, in terms of both OS and DFS, was more evident and statistically significant in the good prognosis subgroups, i.e. intermediate- and low-risk profiles or p16-/HPV-positive status.

discussion
To our knowledge, no randomized trial has ever compared different therapeutic options such as CRT versus surgical intervention followed by postoperative (chemo)radiation for OPC treatment, and previous retrospective studies have not systematically investigated survival according to recognized risk profiles. Furthermore, comparison of survival rates among different reports is difficult because different patient populations and treatment strategies were considered.

Recently, Hong et al. [13] reported similarly improved survival rates in HPV-positive patients treated with either surgery or chemoradiation. However, in this study, the physicians’ choice of treatment modality could have biased the treatment-related results. Moreover, patients receiving curative chemoradiation were more likely to have tumors at the base of tongue, which are associated with worse outcomes [13].

In our analysis of a monoinstitutional series, we examined two different periods during which a single therapeutic strategy was employed. Overall, high-risk patients who underwent surgery or CRT treatment had similar survival, whereas CRT was associated with a better prognosis in patients with intermediate- or low-risk profiles. A similar trend was observed when stratifying patients according only to p16: surgery and CRT treatment had a similar prognosis in p16-negative patients, whereas CRT was associated with a better prognosis in patients with p16-positive profile.

To assess the transferability of our CRT results, we compared them with the subgroup of OPC patients treated with induction chemotherapy and CRT within the TAX 324 trial [14]. Five-year OS was 87% versus 82% in HPV-positive series and 35% versus 44% in HPV-negative cases in our analysis and in the TAX 324 trial, respectively.

The survival benefit observed in intermediate- and low-risk patients treated with a non-surgical approach is likely related to HPV positivity despite the low rate of HPV-associated OPC in the 90s series (20%). This underlines the importance of biological and immunologic factors dictating tumor responsiveness.

HPV-positive tumors have less genotypic alterations than negative ones [15], which may increase their sensitivity to DNA-damaging agents [16]. Conversely, HPV-negative cancers carry TP53 mutations or loss of checkpoint integrity that confer chemoradioresistance [10]. In our study, the non-surgical group received full dosage RT and concurrent cisplatin, whereas the surgical group received a postoperative RT dosage and no chemotherapy. This difference could partly explain the improved survival observed in p16- and HPV-positive patients treated with CRT, thus challenging the current concept of treatment de-escalation in patients with HPV-positive tumors. However, postoperative chemoradiation may not provide an advantage over radiotherapy alone in p16-positive OPC with extracapsular spread [17].

Furthermore, viral antigens, particularly E6 and E7, could elicit an immune response against HPV-induced cancer cells. Animal studies indicate that immune competence is required for tumor clearance, and that combined cisplatin and RT may increase the immune response toward HPV-positive cancer [18]. Accordingly, chemoradiation may induce tumor antigen-specific T-cell responses in esophageal squamous cell cancer patients [19]. Thus, we speculate that CRT could increase the synergy between treatment and immune response more potently than surgical removal and postoperative RT. In addition, surgery itself can reduce immunocompetence, possibly decreasing the effect of the immune system on residual disease.

Surgery can also trigger some processes that stimulate malignant cell growth, in particular for tumors overexpressing epidermal growth factor receptors [20–22]. In our analysis, surgically treated patients were submitted to open approaches, i.e. mandibulectomy and pharyngotomy. New transoral, minimally invasive surgeries (TransOral Robotic Surgery, TORS and Transoral Laser Microsurgery, TLM) could yield more favorable outcomes, in particular in HPV-positive tumors [23]. Moreover, they could minimize toxicity in low-risk patients through a more appropriate use of postoperative adjuvant therapies based on pathologic risk factors, and increase efficacy in high-risk subjects by intensifying local therapy within a multimodal approach. A phase II randomized trial comparing RT and TORS for OPC treatment is currently ongoing [24]. According to our data, the favorable outcome observed in low-risk patients with less invasive surgery could have been obtained by a limited surgical-induced immunosuppression.

Our study has some limitations, including the absence of chemotherapy in patients presenting high-risk features in the surgical group. However, it should be recognized that the newest strategies for treating HPV-positive tumors move toward a de-intensification of the radiation dose to be delivered (ECOG 1308 trial, NCT01084083) or the removal of concomitant systemic treatment (NCT01687413).

Taken these limitations into account, our data should be considered as hypothesis-generating, as we suggest that risk profiling may help guide treatment decisions, particularly in low- and intermediate-risk patients, and we believe that this issue should be included into future research. Indeed, tumor biology itself may be sufficient to explain the reasons of such intriguing results.

acknowledgements
Editorial assistance was provided by Ambra Corti and Luca Giacomelli, PhD; this assistance was supported by internal funds. We thank Dr Chiara Costanza Volpi for her helpful support.
This work was supported in part by an Institutional grant from the Fondazione IRCCS Istituto Nazionale dei Tumori Milano (‘Validation of a new algorithm for HPV status assessment in head and neck carcinoma’, to AG).

LL served as a consultant for BMS, GSK, Eli-Lily, Merck Serono, Amgen, Boheringer Ingelheim, Debiopharm and VentiRX. She also received research funds from Eisai, Eli-Lily, Merck Serono, Amgen, Boheringer Ingelheim and Pfizer. The other authors have declared no conflicts of interest.