Plasma Epstein–Barr viral DNA load at midpoint of radiotherapy course predicts outcome in advanced-stage nasopharyngeal carcinoma

S. F. Leung¹*, K. C. A. Chan², B. B. Ma¹, E. P. Hui¹, F. Mo¹, K. C. K. Chow², L. Leung¹, K. W. Chu¹, B. Zee³, Y. M. D. Lo² & A. T. C. Chan¹

Departments of ¹Clinical Oncology; ²Chemical Pathology; ³School of Public Health, State Key Laboratory in Oncology in South China, Sir YK Pao Center for Cancer, The Chinese University of Hong Kong, Shatin, Hong Kong

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Background: To test the hypothesis that prognostication of treatment outcome is feasible by biomarker response at midcourse of chemoradiotherapy (CRT)/radiotherapy (RT), with respect to the plasma load of Epstein–Barr viral (EBV) DNA in nasopharyngeal carcinoma (NPC).

Patients and methods: One hundred seven patients with stage IIB–IV NPC were prospectively studied. Plasma EBV DNA load was measured by quantitative PCR before therapy (pre-DNA), at completion of 4 weeks of CRT/RT (mid-DNA), and within 3 months of completion of therapy (post-DNA). The end points are post-DNA load, a recognized surrogate of survival, and clinical outcome.

Results: Ninety-three percent of patients had detectable EBV DNA before therapy (median load = 972 copies/ml). EBV DNA became undetectable in 55 (51%) patients at the end of week 4 of therapy. Detectable mid-DNA was associated with worse clinical outcome (median follow-up time, 6.2 years), for distant failure [hazard ratio (HR) 12.02, 95% confidence interval (CI) 2.78–51.93; \( P < 0.0001 \)], progression-free survival (PFS; HR 4.05, 95% CI 1.89–8.67, \( P < 0.0001 \)), and overall survival (OS; HR 3.29, 95% CI 1.37–7.90, \( P = 0.0077 \)). Seventy-four percent of all failures were associated with detectable mid-DNA, whereas 34% of all failures were associated with detectable post-DNA. Stratification by tumor stage (IIB, III, IV) has no significant prognostic effect.

Conclusions: Unfavorable EBV DNA response at midcourse of RT/CRT is an adverse prognosticator for treatment outcome, is linked to majority of all failures, and discriminates outcome better than tumor stage. The data could provide a basis for trial design that addresses alteration of therapy intensity during the latter phase of CRT, and adjuvant therapy. Validation studies are awaited.

Key words: EBV DNA nasopharyngeal carcinoma

Introduction

Plasma Epstein–Barr viral DNA load (EBV DNA) is one of the most well-recognized biomarker for nasopharyngeal carcinoma (NPC) [1]. An expanding body of data suggests that EBV DNA load correlates with tumor load in NPC [2–6]. An important aspect of such correlation is that, for patients with detectable residual EBV DNA soon after completion of a full course of radiotherapy (RT) or chemoradiotherapy (CRT), a very high risk of tumor recurrence was encountered during the early follow-up period [4–7]. It had also been observed that, throughout a course of curative-intent RT for NPC, the EBV DNA load declined in an exponential fashion, and in some patients with early-stage disease, the decline had resulted in an undetectable level by the fourth week of a 6- to 8-week course of RT [2]. Given such observations, it is logical to hypothesize that patients who have a high EBV DNA load at midcourse of therapy are more probable to have residual EBV DNA at the completion of RT/CRT, and hence also an adverse clinical outcome. There is prospect that the powerful prognostication by post-therapy EBV DNA load could be made earlier at midcourse of therapy. With the expanding spectrum of systemic therapies [8–11], including targeted therapy agents [12, 13], being actively explored for NPC, the availability of predictive biomarkers at midcourse of RT/CRT may provide an additional dimension for risk stratification and individualized therapy.
patients and methods

study objectives and hypotheses

The primary objective is to test the hypothesis that a high EBV DNA load at midcourse of RT/CRT (mid-DNA) correlates with detectable EBV DNA after completion of RT/CRT (post-DNA). The secondary objective is to test the hypothesis that mid-DNA load is a determinant of clinical outcome.

statistical considerations

The estimated sample size was that required to demonstrate the correlation between mid-DNA load and post-DNA load. We estimated that about 20% of NPC patients with stage IIB–IV disease would have detectable post-DNA at 6 weeks after RT/CRT [4]. We hypothesized that the patient population could be dichotomized at the end of the fourth week of RT/CRT into a good-risk, and a poor-risk subgroup with high mid-DNA. We also assumed that there is a 90% probability the poor-risk group would capture all patients with elevated post-DNA while the good-risk group would have no patient with elevated post-DNA; that is to say, a high mid-DNA load has a 90% prediction sensitivity for elevated post-DNA. Taking the lower bound of the confidence interval (CI) of this prediction sensitivity as 70%, in order to have 80% power using a 5% type I error test for this postulated difference, we need 95 patients for the study. An additional 20% of patients were included in the planned sample to allow for dropoff.

patients

Eligible patients have stage IIB–IVB NPC (UICC stage-classification, 1997) [14] of World Health Organisation type II or III histology. Staging workup included, in all patients, MRI of head–neck, chest radiograph, and serum alkaline phosphatase. Further systemic imaging was carried out for patients with abnormal baseline assessment as indicated. Patients with known distant metastatic disease (stage IVC) and those planned to receive neoadjuvant chemotherapy were not eligible. The study protocol was approved by the Institutional Review Board of the investigators’ affiliated institution, and written informed consent was obtained from all subjects at recruitment. Consecutive consenting patients (n = 120) were recruited between June 2004 and April 2006. Patients who missed blood sampling at any of the three study time-points (n = 12), and non-eligible histology (n = 1), were excluded from the analyses, which included 107 patients. All patients who had completed blood sampling were available for assessment to the last follow-up visit, except for three patients: one emigrated after 23 months of follow-up, another one was lost to follow-up after 37 months, and another one after 8 months of documented distant failure.

oncology treatment and clinical outcome assessment

Seventy-eight patients were treated with CRT and 29 patients with RT alone. Generally, stage IIB patients were treated by RT alone (92%), as were patients with stage III–IV disease who were considered to have medical risks for chemotherapy, or who declined chemotherapy (10% and 3% of stage III and IV patients, respectively). RT was delivered by either conventional 2D RT mode (66 Gy in 33 fractions, with nasopharyngeal brachytherapy boost for T1, T2a tumors, and ‘parapharyngeal’ boost for parapharyngeal extension of tumor), or intensity-modulated RT mode (66 Gy/33 fractions with boost of 8 Gy/4 fractions). Concurrent chemotherapy was delivered by weekly cisplatin 40 mg/m² during the course of RT. No adjuvant chemotherapy was given. Post-therapy nasopharyngoscopic assessment was carried out at 6–12 weeks after RT/CRT for all patients, accompanied by nasopharyngeal biopsy for all except five patients. Patients were followed up once every 3 months during the first 3 years of follow-up and at longer intervals thereafter.

plasma EBV DNA sampling and assay

Plasma EBV DNA load was measured by quantitative PCR before commencement of therapy (pre-DNA), at completion of 4 weeks of CRT/RT (mid-DNA), and on the day of post-therapy nasopharyngoscopy (post-DNA) which was within 3 months post-therapy. The details of the assay had been previously published [1].

study end points

The end points are post-DNA load and clinical outcome at follow-up. Clinical outcome was based on clinical follow-up data as available on 31 December 2011, which was 66 months after recruitment of the last patient in the study cohort. Patients who had positive biopsies within 8 weeks post-RT/CRT, but had negative biopsies by 12 weeks post-RT/CRT (n = 4) were not reckoned as failures.

analysis

The prediction sensitivity for elevated post-DNA by elevated mid-DNA was computed. The proportions of patients with elevated post-DNA were compared between the subgroups with elevated mid-DNA and undetectable mid-DNA. Kaplan–Meier plots of probability of locoregional failures, distant failures, PFS, and OS were established for different patient subsets, and the log-rank test used to assess the differences among them. Multivariate analyses using the Cox proportional hazard model was carried out including the following variables: mid-DNA, pre-DNA, tumor stage, and use of concurrent chemotherapy and gender. A separate analysis was carried out including also post-DNA.

results

The median follow-up time was 73 months (range: 49–89.6 months). Ninety-three percent of patients had detectable EBV DNA before therapy (median load = 972 copies/ml). At the end of week 4 of therapy, EBV DNA became undetectable in 55 (51%) patients. At post-therapy assessment, 17 (16%) patients had detectable residual EBV DNA.

relation between mid-DNA and post-DNA

Detectable mid-DNA has a prediction sensitivity of 94.1% for detectable post-DNA (95% CI 76.3% to 100%). The probabilities of detectable post-DNA were 16 of 52 and 1 of 55, respectively, for patients with and without detectable mid-DNA (P < 0.0001, Fisher’s exact test). Thus, all cases of detectable post-DNA were preceded by detectable mid-DNA, with the exception of one case.

relation between mid-DNA and clinical outcome

Detectable mid-DNA correlates with significantly worse clinical outcome, for distant failure [hazard ratio (HR) 12.02, 95% CI 2.78–51.93, P = 0.0009], PFS (HR 4.05, 95% CI 1.89–8.67, P = 0.0003), and OS (HR 3.29, 95% CI 1.37–7.89, P = 0.0077), but not locoregional failure (Table 1, Figure 1). When multivariate analysis was carried out incorporating the variables of pre-DNA, mid-DNA, tumor stage, use of chemotherapy and gender, mid-DNA was the only significant prognosticator (P = 0.0035 for distant failure, P = 0.0029 for PFS). Post-DNA was not incorporated into this multivariate analysis, as the prime consideration in the present study was to explore a new time-point of prognostication, at midcourse, without post-DNA data. A repeat
analysis incorporating post-DNA also showed that post-DNA was the only independent variable governing prognosis.

proportions of failures with unfavorable EBV DNA loads
There were 35 patients with failures. High pre-DNA, detectable mid-DNA, and detectable post-DNA were found in 46%, 74%, and 34% of these 35 failure cases, respectively.

comparison between mid-DNA and post-DNA
Table 1 shows that both mid-DNA and post-DNA were significant prognosticators for distant failure, PFS, and OS, and that detectable post-DNA was largely a subset of detectable mid-DNA. It is relevant to ask the question: for patients with detectable mid-DNA (an adverse factor) which disappeared after RT (a favorable factor), did they have a more favorable prognosis that is comparable with those patients who had undetectable mid-DNA? Table 2 showed that the former group had a significantly worse prognosis than the latter.

risk stratification by tumor stage
Within the sample size of the present study, there was no significant difference in outcome when stratification is based on tumor stage (IIB, III, IV) (Table 3).

discussion
There has been no precedent model of using biomarker response at midcourse of RT to predict clinical outcome. This hampers the design of trials to evaluate risk-adapted differential treatment intensities at different phases of a course of RT or CRT. In the current era of IMRT-based treatment of NPC, the optimal integration of systemic therapies to radiation is a key research question, in terms of choice of chemotherapy and targeted therapy [12, 13], the

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Table 1. Clinical outcome of patient subsets segregated by EBV DNA loads of different time-points

<table>
<thead>
<tr>
<th>Variables</th>
<th>LR failure</th>
<th>Distant failure</th>
<th>PFS</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$P$ value</td>
<td>$P$ value</td>
<td>$P$ value</td>
<td>$P$ value</td>
</tr>
<tr>
<td>Mid-DNA, &gt;0 versus 0 copy/ml</td>
<td>$P = 0.1378$</td>
<td>$P = 0.0009$</td>
<td>$P = 0.0003$</td>
<td>$P = 0.0077$</td>
</tr>
<tr>
<td>Pre-DNA, &gt;4000 versus ≤4000 copies/ml</td>
<td>$P = 0.6596$</td>
<td>$P = 0.0108$</td>
<td>$P = 0.0153$</td>
<td>$P = 0.2135$</td>
</tr>
<tr>
<td>Post-DNA, &gt;0 versus 0 copy/ml</td>
<td>$P = 0.0252$</td>
<td>$P = 0.0001$</td>
<td>$P &lt; 0.0001$</td>
<td>$P &lt; 0.0001$</td>
</tr>
</tbody>
</table>

Clinical outcome was described by Kaplan–Meier plots of locoregional failure (LR failure), distant failure, progression-free survival (PFS), and overall survival (OS).

$P$ values of <0.01 highlighted in bold type.

Pre-DNA, EBV DNA load before commencement of radiotherapy; mid-DNA, EBV DNA load at the end of the fourth week of radiotherapy/chemoradiotherapy; post-DNA, EBV DNA load within 3 months after completion of radiotherapy/chemoradiotherapy; HR, hazard ratio; CI, confidence interval.

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Table 2. Comparing clinical outcome of patient subsets with 'both mid-DNA and post-DNA undetectable' and with 'mid-DNA detectable but post-DNA undetectable'

<table>
<thead>
<tr>
<th>Variables</th>
<th>LR failure</th>
<th>Distant failure</th>
<th>PFS</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both mid-DNA and post-DNA undetectable ($n = 54$) (%)</td>
<td>11</td>
<td>4</td>
<td>85</td>
<td>91</td>
</tr>
<tr>
<td>Mid-DNA detectable but post-DNA undetectable ($n = 36$) (%)</td>
<td>18</td>
<td>25</td>
<td>64</td>
<td>83</td>
</tr>
<tr>
<td>$P$ value</td>
<td>0.4475</td>
<td>0.0025</td>
<td>0.0193</td>
<td>0.2932</td>
</tr>
</tbody>
</table>

Clinical outcome is described by 5-year actuarial rates of locoregional failure (LR failure), distant failure, progression-free survival (PFS), and overall survival (OS).
temporal relationship to radiation, and patient subsets [10–13]. This highlights the need for more biomarker integration in trial design, to allow risk stratification for individualized therapy. Avoidance of noncontributive therapy is a concern, as chemotheraphy and targeted therapy agents are not without toxicities [10, 12, 13], and cost is an especially relevant consideration for the latter. While several studies had consistently demonstrated the value of post-therapy EBV DNA load as a powerful prognosticator of clinical outcome [4–7], the present study shows that, for the first time, such prognostication could be shifted from the post-therapy time-point to midcourse of therapy. In fact, essentially all cases of residual post-DNA were predicted by residual mid-DNA, detectable mid-DNA encompassed about three-quarters of all failures, while detectable post-DNA encompassed only one-third of all failures. Furthermore, patients with detectable mid-DNA that disappeared at the end of RT still sustained a worse prognosis than those with undetectable mid-DNA. That is to say, unfavorable EBV DNA response at midcourse of therapy identifies an at-risk group (constituting about half of all patients) which encompassed the majority of failures. Those who went on to sustain detectable EBV DNA at the completion of therapy had an even higher risk of failure, though this poor-risk group accounts for only one-third of all failures. Such observations have implications for selecting patients for therapy intensification. It is tempting to postulate that the therapeutie ratio could be increased by lowering intensity of therapy in the good-risk group during the later phase of RT/CRT, while intensified therapy should be maintained, or further augmented, into the adjuvant phase in the poor-risk group. The improved risk stratification would also allow more cost-effective design of trials, so that therapy intensification trials can target at truly high-risk subjects, while truly good-risk subjects could be recruited for trials of reducing therapy intensification. The EBV DNA load at midcourse of RT is a more relevant than tumor stage for prognostication.

Adjuvant chemotherapy was not employed in the current study cohort, as per our institution’s therapy policy, which takes into account the unsettled controversy regarding the additional benefit of adjuvant therapy after CRT [8, 9, 11]. In fact, the issue of adjuvant therapy is being addressed in ongoing trials which targets at patients with residual post-therapy EBV DNA.

The alteration of therapy, according to biomarker response, during the latter phase of a course of CRT can be seen as a form of adaptive therapy—adapting to response of a biomarker with time, akin to the concept of adaptive radiation therapy which adapts to the response of physical dimension of tumor with time [16]. This model is novel and distinct from other models of biomarker kinetics as prognosticators, which either address the response to chemotherapy, as in germ-cell tumor [17] and metastatic NPC [18], or the post-RT follow-up period, as in prostate cancer [19].

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**disclosure**

YMDL is a consultant to and holds equities in Sequenom. The other authors have declared no conflicts of interest.

**references**

Noncancer health events as a leading cause of competing mortality in advanced head and neck cancer

M. Kwon¹, J.-L. Roh¹*, J. Song², S-W. Lee³, S.-B. Kim⁴, S.-H. Choi¹, S. Y. Nam¹ & S. Y. Kim¹,⁵

Departments of ¹Otolaryngology; ²Clinical Epidemiology and Biostatistics; ³Radiation Oncology; ⁴Internal Medicine (Oncology), Asan Medical Centre, University of Ulsan College of Medicine, Seoul; ⁵Biomedical Research Institute, Korea Institute of Science and Technology, Seoul, Republic of Korea

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Background: The survival of patients with head and neck squamous cell carcinoma (HNSCC) can be affected by noncancer health events (NCHE) as well as by index cancer progression and second primary cancer (SPC). This study aimed to investigate the risk factors for NCM and noncancer mortality (NCM) in patients with advanced-stage HNSCC.

Patients and methods: This cohort study involved 600 consecutive patients with overall stage III to IV HNSCC who were treated between 2001 and 2010 at our tertiary referral hospital. NCHE was defined as re-admission (i.e. after the primary treatments for the index tumors) due to noncancer-related causes. The incidences of NCHE and NCM and their risk factors were analyzed by using cumulative incidence and cause-specific hazard functions.

Results: During a median follow-up period of 54 months, 224 (37.3%) and 55 (9.2%) of the 600 patients had NCHE and NCM, respectively. The 5-year index cancer mortality, SPC mortality, and NCM rates were 23.8%, 4.2%, and 8.9%, respectively. Multivariate analyses revealed that body mass index <20 kg/m² (P = 0.018), Charlson comorbidity index (CCI) ≥1 (P < 0.001), tumor recurrence (P < 0.001), SPC occurrence (P < 0.001), and initial chemotherapy (P = 0.049) were independent NCHE predictors. Older age (P < 0.001), CCI ≥1 (P = 0.008), tumor recurrence (P < 0.001), and SPC occurrence (P = 0.047) were independent NCM predictors. Patients with respiratory NCHE were at a higher risk of NCM than patients with other NCHE types (P < 0.001).

Conclusions: One or more comorbidities, tumor recurrence, and SPC occurrence were independent predictors of both NCHE and NCM. Patients with respiratory NCHE had a particularly high risk of NCM.

Key words: head and neck squamous cell carcinoma, noncancer health event, comorbidities, competing mortalities, risk factors

Introduction

The survival of patients with head and neck squamous cell carcinoma (HNSCC) is largely dictated by the progression or recurrence of the index cancer [1, 2]. However, in recent decades, competing mortality has been recognized to also significantly affect the overall survival of patients with HNSCC [1–4]. Although recent advances in the diagnosis and treatment of HNSCC have slightly improved survival [5, 6], the overall survival of patients with HNSCC could be improved further by a better understanding of the risk factors for competing mortality, as this knowledge could improve the