Plasma EBV DNA Clearance Rate as a Novel Prognostic Marker for Metastatic/Recurrent Nasopharyngeal Carcinoma

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Abstract

Purpose: To investigate the prognostic effect of the concentrations and clearance rates of plasma EBV DNA in metastatic/recurrent nasopharyngeal carcinoma (NPC).

Experimental Design: Thirty relapsed and four previously nontreated metastatic NPC patients were treated according to the consensus guidelines of the head and neck cancer team in our hospital (i.v. chemotherapy first, followed by local irradiation boost and oral maintenance chemotherapy where applicable). Multiple plasma samples were collected during the first month of chemotherapy. Circulating EBV DNA concentrations were measured by a real-time quantitative PCR. The half-life values (t1/2) of plasma EBV DNA clearance were calculated. The associations between clinical outcome and plasma EBV DNA assays were analyzed.

Results: Tumor response evaluated after 12 weeks of treatment showed 14 complete responses (41.2%), 12 partial responses (35.3%), 7 stable diseases (20.6%), and 1 progression disease (2.9%). The plasma EBV DNA concentrations have no significant effects on outcome prediction. The t1/2 of plasma EBV DNA clearance ranged from 1.85 to 28.29 days (median, 3.99). Patients with a short t1/2 of plasma EBV DNA clearance have significantly higher complete response rate and overall survival than those with long t1/2. Multivariate analysis revealed a significant effect of the t1/2 of plasma EBV DNA clearance on survival.

Conclusions: The clearance rates of plasma EBV DNA during the first month of chemotherapy can predict tumor response and patient survival. Early change of chemotherapy regimen may be considered for patients with slow plasma EBV DNA clearance rate.

Nasopharyngeal carcinoma (NPC) is distinct from other cancers of the head and neck by its epidemiology, histopathology, clinical characteristics, methods of treatment, and patterns of failure. Because of the inherent anatomic constraints and a high degree of radiosensitivity, radiotherapy has been the primary treatment for NPC patients without distant metastasis. Treatment failure in the past was due to a high rate of local recurrence and/or distant metastasis. However, advances in radiation oncology have improved the locoregional control, and treatment failure is now due mainly to distant metastasis. To date, the outcome of salvage treatment for relapse is still very poor, with the 5-year survival rates after local recurrence and distant metastasis being 9.4% to 30% and ≤5%, respectively (1–6). Because most metastatic/recurrent patients will succumb rapidly to the disease, the development of a quick and precise marker to predict treatment response is urgently needed.

NPC has been proven as an EBV-associated cancer. EBV genome is present in the cells from almost every primary and metastatic NPC, regardless of the degree of tumor differentiation or the geographic origin of the patients. Recently, the quantification of plasma EBV DNA by the real-time quantitative PCR has been shown as a useful marker in the detection, monitoring, and prognostic prediction for previously nontreated NPC (7–12). However, the predictive/prognostic value of the clearance rate of plasma EBV DNA during the chemotherapy period has never been reported. Thus, we investigated whether the clearance rate of plasma EBV DNA as well as pretreatment plasma EBV DNA levels and various other clinicopathologic factors could serve as an early predictive marker for NPC patients with metastatic/recurrent diseases.

Materials and Methods

Patients. The inclusion criteria for this prospective study were biopsy-proven NPC patients with (a) relapse after
EBV DNA Clearance in Metastatic/Recurrent NPC

Translational Relevance

Circulating EBV DNA levels have been shown to correlate with the tumor load in NPC. We hypothesize that the decline in plasma EBV DNA during chemotherapy represents the decrease of cancer cells, and therefore, the rate of decline reflects the chemosensitivity of the tumor. Our new findings indicate for the first time that the clearance rate of circulating EBV DNA is superior to the absolute concentrations of EBV DNA or other clinicopathologic factors in predicting the tumor response and patient survival for metastatic/recurrent NPC. Specifically, the results show that the EBV clearance rate within the first month of the treatment is the most important independent predictive/prognostic biomarker. Clinically, this can be translated into an earlier tumor response evaluation, thus providing oncologists a timely change of chemotherapy regimen in patients with slow EBV clearance rate.

Table 1. Patient characteristics and its associations with tumor response

<table>
<thead>
<tr>
<th>Parameters</th>
<th>No. (%)</th>
<th>No. of complete response (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤50 y</td>
<td>18 (52.9)</td>
<td>8 (44.4)</td>
<td>0.9509</td>
</tr>
<tr>
<td>&gt;50 y</td>
<td>16 (47.1)</td>
<td>6 (37.5)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>31 (91.2)</td>
<td>13 (41.9)</td>
<td>1.0000</td>
</tr>
<tr>
<td>Female</td>
<td>3 (8.8)</td>
<td>1 (33.3)</td>
<td></td>
</tr>
<tr>
<td>Karnofsky scale</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥80%</td>
<td>21 (61.8)</td>
<td>12 (57.1)</td>
<td>0.0408</td>
</tr>
<tr>
<td>&lt;80%</td>
<td>13 (38.2)</td>
<td>2 (15.4)</td>
<td></td>
</tr>
<tr>
<td>Pathology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonkeratinizing carcinoma</td>
<td>26 (76.5)</td>
<td>10 (38.5)</td>
<td>0.6892</td>
</tr>
<tr>
<td>Undifferentiated carcinoma</td>
<td>8 (23.5)</td>
<td>4 (50.0)</td>
<td></td>
</tr>
<tr>
<td>Site of disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Locoregional</td>
<td>7 (20.6)</td>
<td>4 (57.1)</td>
<td>0.4099</td>
</tr>
<tr>
<td>Distant ± locoregional</td>
<td>27 (79.4)</td>
<td>10 (37.0)</td>
<td></td>
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<tr>
<td>No. of lesions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solitary</td>
<td>10 (29.4)</td>
<td>4 (40.0)</td>
<td>1.0000</td>
</tr>
<tr>
<td>Multiple</td>
<td>24 (70.6)</td>
<td>10 (41.7)</td>
<td></td>
</tr>
<tr>
<td>Disease-free interval</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤6 mo</td>
<td>15 (44.1)</td>
<td>5 (33.3)</td>
<td>0.6350</td>
</tr>
<tr>
<td>&gt;6 mo</td>
<td>19 (55.9)</td>
<td>9 (47.4)</td>
<td></td>
</tr>
<tr>
<td>Serum LDH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤200 U/l</td>
<td>21 (61.8)</td>
<td>11 (52.4)</td>
<td>0.1840</td>
</tr>
<tr>
<td>&gt;200 U/l</td>
<td>13 (38.2)</td>
<td>3 (23.1)</td>
<td></td>
</tr>
<tr>
<td>Serum albumin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤4 g/dl</td>
<td>7 (20.6)</td>
<td>3 (42.9)</td>
<td>1.0000</td>
</tr>
<tr>
<td>&gt;4 g/dl</td>
<td>27 (79.4)</td>
<td>11 (40.7)</td>
<td></td>
</tr>
<tr>
<td>Chemotherapy regimen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEP</td>
<td>30 (88.2)</td>
<td>10 (33.3)</td>
<td>0.0216</td>
</tr>
<tr>
<td>PFL</td>
<td>4 (11.8)</td>
<td>4 (100.0)</td>
<td></td>
</tr>
</tbody>
</table>

curative treatment or distant metastasis in previously non-treated cases, (b) Karnofsky performance status of ≥60%, (c) WBC count of >3,000/μL and a platelet count of >100,000/μL, (d) serum creatinine level of <1.5 mg/dL, (e) normal liver function with total bilirubin of <2.5 mg/dL, (f) detectable plasma EBV DNA and measurable disease by imaging studies, and (g) a written informed consent.

Treatment. The treatment protocol was a relatively uniform and step-by-step process, according to the consensus guidelines of the head and neck cancer team in our hospital. They consisted first of i.v. chemotherapy of MEP (8 mg/m² mitomycin-C on day 1, 60 mg/m² epirubicin on day 1, and 60 mg/m² cisplatin on day 1, repeated every 3 wk for a maximum of six cycles) or PFL (60 mg/m² cisplatin on days 1 and 15, 2,500 mg/m² and 5-fluorouracil + 250 mg/m² leucovorin on day 8 and 22 repeated every 4 wk for a maximum of six cycles). Gemcitabine (1,000 mg/m²) on day 1 and 15, and 25 mg/m² vinorelbine on days 8 and 22 repeated every 4 wk were delivered either as a consolidation for responders or as a second line of treatment for nonresponders, but this option was
only undertaken by patients who could afford the expense of these two drugs that were not covered by public health insurance for head and neck cancer in our country. Local irradiation boost to the metastatic/recurrent sites was recommended if clinically applicable. Finally, less toxic maintenance oral chemotherapy (200 mg tegafur-uracil twice daily with or without 50 mg cyclophosphamide once daily) was prescribed for at least 1 y or until disease progression or patient's refusal to continue further.

Complete blood count, platelet count, and body weight were checked and patients examined clinically every week during the i.v. chemotherapy period. Liver and renal function tests were repeated every 3 to 4 wk.

Tumor response was first evaluated after 12 wk of chemotherapy according to the WHO criteria (13). We routinely used computerized tomography (CT) scan and/or magnetic resonance imaging to assess the treatment results. Whole body (18)F-fluorodeoxyglucose (FDG) positron emission tomography (PET) scan or PET/CT scan were also done for 30 of 34 patients before salvage chemotherapy as a reference. PET/CT scan was repeated when a response judgment was equivocal by conventional imaging studies. In these circumstances, a complete response was made only if follow-up PET/CT scan showed complete disappearance of all previously FDG-uptake lesions.

**Quantification of plasma EBV DNA.** During the first month of chemotherapy, we collected multiple blood samples for EBV DNA measurement along with other routine blood tests. Plasma DNA was extracted and subjected to a real-time quantitative PCR assay. The sequences of forward and reverse primers and the probe were 5′-CCCCACACTCCACACC-3′, 5′-TCTTAG-GAGCTGTCCGAGGG-3′, and 5′-(FAM)CACACACTACA-CACACCCACCCGTCTC(TAMRA)-3′. The principles of the real-time quantitative PCR and reaction setup were as previously described (7, 8).

**Determination of clearance rates of the plasma EBV DNA.** Assuming an exponential decay model, when the natural logarithm of the plasma EBV DNA concentration was plotted against time, a straight line with a slope of \(-k\) would be observed. The half-life value \(t_{1/2}\) of plasma EBV DNA clearance was calculated using the equation of \[t_{1/2} = 0.693/k\].

**Statistical analyses.** The relationship between tumor response and various clinical parameters, pretreatment plasma EBV DNA concentration, and the \(t_{1/2}\) of plasma EBV DNA clearance were evaluated by using the \(\chi^2\) test or the Fisher's
exact test. Overall survival was calculated from the first day of chemotherapy to the date of death or the last follow-up visit. The Kaplan-Meier product-limit method was used to estimate overall survival. Univariate comparison of survival curves was done by using the log-rank test. Multivariate Cox proportional hazards model was used to estimate the hazard ratios (HR) and 95% confidence intervals (CI). All analyses were two sided and a \( P \) value of <0.05 was considered statistically significant.

### Table 2. Comparison of absolute concentrations and clearance rates of the plasma EBV DNA in predicting tumor response

<table>
<thead>
<tr>
<th>Plasma EBV DNA</th>
<th>No. of complete response/no. total (%)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretreatment level (copies/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \leq 1,000 ) vs &gt;1,000</td>
<td>3/6 (50.0) vs 11/28 (39.3)</td>
<td>0.6722</td>
</tr>
<tr>
<td>( \leq 1,500 ) vs &gt;1,500</td>
<td>5/9 (55.6) vs 9/25 (36.0)</td>
<td>0.4351</td>
</tr>
<tr>
<td>( \leq 5,000 ) vs &gt;5,000</td>
<td>8/16 (50.0) vs 6/18 (33.3)</td>
<td>0.5244</td>
</tr>
<tr>
<td>( \leq 10,000 ) vs &gt;10,000</td>
<td>9/19 (47.4) vs 5/15 (33.3)</td>
<td>0.6350</td>
</tr>
<tr>
<td>( \leq 50,000 ) vs &gt;50,000</td>
<td>13/28 (46.4) vs 1/6 (16.7)</td>
<td>0.3636</td>
</tr>
<tr>
<td>( t_{1/2} ) of clearance rate (d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \leq 4 ) vs &gt;4</td>
<td>12/17 (70.6) vs 2/17 (11.8)</td>
<td>0.0017</td>
</tr>
<tr>
<td>( \leq 5 ) vs &gt;5</td>
<td>12/18 (66.7) vs 2/16 (12.5)</td>
<td>0.0043</td>
</tr>
<tr>
<td>( \leq 6 ) vs &gt;6</td>
<td>12/20 (60.0) vs 2/14 (14.3)</td>
<td>0.0208</td>
</tr>
<tr>
<td>( \leq 7 ) vs &gt;7</td>
<td>13/23 (56.5) vs 1/11 (9.1)</td>
<td>0.0110</td>
</tr>
<tr>
<td>( \leq 8 ) vs &gt;8</td>
<td>14/25 (56.5) vs 0/9 (0.0)</td>
<td>0.0044</td>
</tr>
</tbody>
</table>
considered statistically significant. Analyses were done by using SAS (version 8.0, SAS Institute, Inc.).

### Results

**Patients, treatment, and outcome.** From March 2005 to May 2008, 34 eligible patients (30 relapsed and 4 newly diagnosed cases with distant metastasis) were obtained. Table 1 lists the patient characteristics. All relapsed patients had received cisplatin-based chemoradiotherapy as their initial treatment. The disease-free interval, calculated from the last day of the initial treatment to the date of documented failures, for the 30 relapsed patients ranged from 1 to 85 months (median, 10 months; mean, 19 months). The disease-free interval of the four previously nontreated patients was counted as 0 month.

The site of disease recurrences of the 30 relapsed patients before salvage chemotherapy consisted of 7 local and/or regional disease, 21 distant metastases, and 2 distant plus neck failures. The clinical stages of the four newly diagnosed NPC were T3N3bM1, T4N3bM1, T3N3bM1, and T2bN3bM1, respectively. Of the 34 patients, solitary disease site was noted for 10 patients (29.4%), whereas the remaining 24 patients (70.6%) had multiple active lesions. These 34 metastatic/recurrent patients were first treated by MEP (30 cases) and PFL (4 cases) as per the primary treating physicians. Eleven patients also received chemotherapy with gemcitabine + vinorelbine after MEP or PFL. Local irradiation boosts to the metastatic/recurrent sites were given to 17 patients and maintenance oral chemotherapy was prescribed for 24 patients.

Tumor responses after 12 weeks of chemotherapy were 14 complete responses (41.2%), 12 partial responses (35.3%), 7 stable diseases (20.6%), and 1 progression disease (2.9%). As of the cutoff date of May 2009, for collection of survival data, there were 17 deaths, 4 live with disease, and 13 live with no evidence of disease, with a median follow-up of 30 months (range, 18-50 months). The 1-, 2-, and 3-year overall survival rates were 73.5%,

### Table 3. Univariate analysis of clinical factors for overall survival

<table>
<thead>
<tr>
<th>Parameters</th>
<th>n</th>
<th>Overall survival</th>
<th>HR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td></td>
<td></td>
<td>2-y rate (%)</td>
<td>Median (mo)</td>
</tr>
<tr>
<td>≤50</td>
<td>18</td>
<td>58.3</td>
<td>36.0</td>
<td>0.67 (0.25-1.75)</td>
</tr>
<tr>
<td>&gt;50</td>
<td>16</td>
<td>51.6</td>
<td>25.0</td>
<td>0.89 (0.19-4.21)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>31</td>
<td>53.4</td>
<td>28.0</td>
<td>0.89 (0.19-4.21)</td>
</tr>
<tr>
<td>Female</td>
<td>3</td>
<td>66.7</td>
<td>25.0</td>
<td>0.89 (0.19-4.21)</td>
</tr>
<tr>
<td>Karnofsky scale</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>≥80%</td>
<td>21</td>
<td>71.1</td>
<td>Not reached</td>
<td>0.37 (0.10-0.89)</td>
</tr>
<tr>
<td>&lt;80%</td>
<td>13</td>
<td>17.6</td>
<td>22.0</td>
<td></td>
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<tr>
<td>Pathology</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonkeratinizing carcinoma</td>
<td>26</td>
<td>47.5</td>
<td>23.0</td>
<td>2.95 (0.79-6.80)</td>
</tr>
<tr>
<td>Undifferentiated carcinoma</td>
<td>8</td>
<td>75.0</td>
<td>Not reached</td>
<td></td>
</tr>
<tr>
<td>Site of disease involvement</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Locoregional alone</td>
<td>7</td>
<td>75.0</td>
<td>43.0</td>
<td>0.35 (0.15-1.31)</td>
</tr>
<tr>
<td>Distant ± locoregional</td>
<td>27</td>
<td>48.9</td>
<td>22.0</td>
<td></td>
</tr>
<tr>
<td>No. of lesions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solitary</td>
<td>10</td>
<td>85.7</td>
<td>Not reached</td>
<td>0.20 (0.11-0.79)</td>
</tr>
<tr>
<td>Multiple</td>
<td>24</td>
<td>42.3</td>
<td>18.0</td>
<td></td>
</tr>
<tr>
<td>Disease-free interval</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤6 mo</td>
<td>15</td>
<td>37.3</td>
<td>16.0</td>
<td>2.45 (0.97-7.07)</td>
</tr>
<tr>
<td>&gt;6 mo</td>
<td>19</td>
<td>69.2</td>
<td>36.0</td>
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<tr>
<td>Serum LDH</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>≤200 U/l</td>
<td>21</td>
<td>66.7</td>
<td>32.0</td>
<td>0.58 (0.20-1.52)</td>
</tr>
<tr>
<td>&gt;200 U/l</td>
<td>13</td>
<td>36.9</td>
<td>14.0</td>
<td></td>
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<tr>
<td>Serum albumin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤4 g/dl</td>
<td>7</td>
<td>28.6</td>
<td>22.0</td>
<td>2.65 (0.98-16.4)</td>
</tr>
<tr>
<td>&gt;4 g/dl</td>
<td>27</td>
<td>60.2</td>
<td>36.0</td>
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<tr>
<td>Chemotherapy regimen</td>
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</tr>
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<td>MEP</td>
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<td>48.4</td>
<td>23.0</td>
<td>Undefined</td>
</tr>
<tr>
<td>PFL</td>
<td>4</td>
<td>100.0</td>
<td>Not reached</td>
<td></td>
</tr>
</tbody>
</table>
54.9%, and 38.1%, respectively. The median survival was 28 months for the whole series and 22 months for 27 patients with distant metastasis.

**Plasma EBV DNA assay.** The pretreatment plasma EBV DNA levels ranged from 38 to 8,437,690 copies/mL (median, 5,758; mean, 274,344). Each patient had three to five measurements of plasma EBV DNA during the first month of chemotherapy. The raw data of serial changes of plasma EBV DNA level, slopes of the regression line, and the calculated t1/2 of plasma EBV DNA clearance rates, along with the sites of active disease and tumor response for all 34 patients are listed in the Supplementary Table. Figure 1 illustrates two examples by plotting the natural logarithm of plasma EBV DNA concentration against time and the determination the t1/2 of plasma EBV DNA clearance. The t1/2 of plasma EBV DNA clearance ranged from 1.85 to 28.29 with a median of 3.99 days (interquantile range, 2.86-8.69).

**Predictive markers analysis for tumor response.** Table 1 also summarizes the associations between complete response rates and various clinical factors. Performance status (P = 0.0408) and chemotherapy regimen (P = 0.0216) could predict tumor response significantly. The remaining clinical factors, including age, gender, histologic type, site of disease recurrence, number of lesions, disease-free interval, serum lactate dehydrogenase (LDH) concentration, and serum albumin level, have no predictive value.

Table 2 analyzes the associations between tumor response and plasma EBV DNA assays. The pretreatment plasma EBV DNA concentrations had no significant effect on tumor response using different cutoff values of 1,000, 1,500, 5,000, 10,000, and 50,000 copies/mL. However, the t1/2 of plasma EBV DNA clearance is a highly significant predictive marker for tumor response. Twelve of 17 patients (70.6%) with a t1/2 of ≤4 days reached a complete response, whereas, 2 of the 17 patients (11.8%) with a t1/2 of >4 days obtained complete response (P = 0.0017). When cutoffs of the t1/2 of plasma EBV DNA clearance are set at 5, 6, 7, and 8 days, all statistics show that patients with short t1/2 of plasma EBV DNA clearance have significantly higher complete response rate than those with long t1/2 of plasma EBV DNA clearance. There is no complete response when the t1/2 of plasma EBV DNA clearance is >8 days. Figure 1 shows two representative cases. One is a complete responder with a t1/2 of 3.66 days and the other a stable disease with a t1/2 of 8.73 days.

**Survival analysis.** Table 3 lists the univariate analysis of overall survival by various clinical parameters. Performance status (P = 0.0291) and the number of lesions (P = 0.0156) were two significant prognostic factors for overall survival. The disease-free interval (P = 0.0579),

| Table 4. Univariate analysis of plasma EBV DNA assays for overall survival |
|---|---|---|---|
| Plasma EBV DNA | n | Overall survival | HR (95% CI) | P |
| Pretreatment level (copies/mL) | | 2-y rate (%) | Median (mo) | |
| ≤1,000 | 6 | 41.7 | 23.0 | 0.64 (0.19-2.41) | 0.5384 |
| >1,000 | 28 | 54.7 | 25.0 | |
| ≤1,500 | 9 | 51.9 | Not reached | 0.63 (0.22-2.01) | 0.4621 |
| >1,500 | 25 | 53.4 | 25.0 | |
| ≤5,000 | 16 | 69.6 | Not reached | 0.41 (0.16-1.10) | 0.0783 |
| >5,000 | 18 | 41.9 | 18.0 | |
| ≤10,000 | 19 | 69.1 | 36.0 | 2.08 (0.81-5.68) | 0.1253 |
| >10,000 | 15 | 38.9 | 18.0 | |
| ≤50,000 | 28 | 64.3 | 36.0 | 0.26 (0.02-0.51) | 0.0055 |
| >50,000 | 6 | 16.7* | 15.0 | |
| t1/2 of clearance rate (d) | | | |
| ≤4 | 17 | 79.4 | Not reached | 0.26 (0.09-0.66) | 0.0055 |
| >4 | 17 | 29.4 | 16.0 | |
| ≤5 | 18 | 75.0 | 43.0 | 0.31 (0.10-0.77) | 0.0139 |
| >5 | 16 | 31.6 | 16.0 | |
| ≤6 | 20 | 66.7 | Not reached | 0.35 (0.11-0.87) | 0.0257 |
| >6 | 14 | 30.6 | 16.0 | |
| ≤7 | 23 | 61.4 | Not reached | 0.38 (0.10-0.93) | 0.0371 |
| >7 | 11 | 34.1 | 11.0 | |
| ≤8 | 25 | 69.3 | Not reached | 0.20 (0.02-0.26) | 0.0001 |
| >8 | 9 | 16.7 | 10.0 | |

*Indicates an 18-mo survival rate.
serum albumin level \((P = 0.0535)\), and chemotherapy regimen \((P = 0.0600)\) had borderline significance. Other factors, including age, gender, histologic type, site of disease recurrences, and serum LDH concentration, could not predict survival.

Table 4 summarizes the univariate analysis of overall survival according to different cutoff values of the concentrations and clearance rates of plasma EBV DNA, and shows that clearance rates but not absolute concentrations are significant prognostic factors. The 2-year overall survival rates for patients with short and long \(t_{1/2}\) of plasma EBV DNA \((\leq 4 \, \text{d} \, \text{versus} \, >4 \, \text{d})\) were 79.4% and 29.4%, respectively \((P = 0.0055; \text{Fig. } 2\text{A})\). The 2-year overall survival rates for patients with a \(t_{1/2}\) of \(\leq 8 \, \text{d} \, \text{versus} \, >8 \, \text{d}\) were 69.3% and 16.7%, respectively \((P = 0.0001; \text{Fig. } 2\text{B})\). The overall survival rate at 2 years was 69.3% in patients with a pretreatment plasma EBV DNA of \(\leq 5000 \, \text{copies/mL}\) and 41.9% in those with a pretreatment plasma EBV DNA of \(>5,000 \, \text{copies/mL}\) \((P = 0.0055; \text{Fig. } 2\text{A})\). The 2-year overall survival rates of patients with a pretreatment plasma EBV DNA of \(\leq 10,000 \, \text{copies/mL}\) and \(>10,000 \, \text{copies/mL}\) were 69.1% and 38.9%, respectively \((P = 0.1253; \text{Fig. } 2\text{D})\).

Cox multivariate analysis. Variables with \(P\) values of \(<0.10\) in the univariate analysis were incorporated in the multivariate Cox proportional hazards model. After controlling for confounding factors, the effects of the \(t_{1/2}\) of plasma EBV DNA clearance \((\text{HR, 12.30; 95\% CI, 2.50-60.52; } P = 0.002)\), serum albumin level \((\text{HR, 9.11; 95\% CI, 1.73-47.98; } P = 0.009)\), performance status \((\text{HR, 4.51; 95\% CI, 1.40-14.58; } P = 0.012)\), and the number of lesions \((\text{HR, 7.80; 95\% CI, 1.12-54.24; } P = 0.038)\) on overall survival remained statistically significant.

**Discussion**

To date, several retrospective analyses have identified some unfavorable prognostic factors, including older age \((4)\), anemia \((5, 6)\), poor performance status \((5, 6, 14)\), short disease-free interval \((4, 5)\), prior chemotherapy \((14)\), presence of liver metastasis \((4-6, 14, 15)\), and multiple lesions \((4, 6, 14)\). However, the application of these
pretreatment markers into clinical practice with consequent adjustment of the treatment regimen has never been shown to be feasible. Therefore, the search for new markers that could predict tumor response early will have a great effect in patient management.

The pretreatment plasma EBV DNA concentrations have been shown to correlate with the clinical stages of NPC (9, 10, 16). Ma et al. (17) further analyzed the relationship between the plasma EBV DNA level with the magnetic resonance imaging–delineated tumor volume and with tumor metabolic activity by PET/CT scan, and showed that circulating EBV DNA level reflects overall tumor load. However, the predictive/prognostic value of the clearance rate of plasma EBV DNA in the management of metastatic/recurrent patients has never been investigated. Thus far, only two articles reported the elimination kinetics of plasma EBV DNA in NPC (18, 19). Investigators from the Chinese University of Hong Kong measured serial plasma EBV DNA levels during radiotherapy for previously non-treated NPC (18) or after surgical resection for recurrent NPC (19), and observed an initial increase followed by a rapid decline in plasma EBV DNA concentration. The calculated median t1/2 of plasma EBV DNA decay was 3.8 days for 14 irradiated patients in the period between the third and seventh week of radiotherapy (18) and 139 minutes for 9 recurrent patients after surgical resection (19). However, they did not analyze the predictive/prognostic effect of the t1/2 of plasma EBV DNA decay on the treatment outcome, and no investigation on the detailed elimination kinetics of plasma EBV DNA in patients treated with chemotherapy was reported in the literature. Ngan et al. (20) evaluated the role of serum EBV DNA concentrations before, during, and after salvage chemotherapy on 19 patients with metastatic/recurrent NPC and found that 4 patients exhibiting clinical complete remission were in the group with serum EBV DNA that dropped to background level. However, they had no data regarding the correlation between EBV DNA elimination rates and clinical outcome.

In the current study, we postulate that the decline in plasma EBV DNA during chemotherapy represents the decrease of cancer cells, and therefore, the rate of decline reflects the chemosensitivity of the tumor. We collected three to five blood samples within the first month of chemotherapy and measured the EBV clearance rate. We observed that the t1/2 of plasma EBV DNA clearance is the most significant factor to predict tumor response. Furthermore, the determination of the clearance rate of plasma EBV DNA within 1 month after chemotherapy could provide oncologists the data for considerations of changing the chemotherapy regimen in patients with slow clearance rate of plasma EBV DNA. One of seven nonresponders to MEP with slow EBV clearance rate (t1/2 = 28.3 days) who could afford the expense shifted to receive gemcitabine + vinorelbine got symptoms improvement. Although follow-up imaging studies showed <50% decrease in tumor size, the plasma EBV DNA dropped from several hundred copies per milliliter to <100 copies/mL and was kept in this low viral load. The consequent effect by this early predictive marker needs to be studied further.

In survival analyses, our results confirmed some favorable prognostic factors reported previously, such as good performance status and a solitary lesion. In addition, the clearance rate of plasma EBV DNA clearance is better than the concentration of plasma EBV DNA in predicting outcome by both univariate and multivariate analyses. Of course, we could not exclude the possibility that the level of plasma EBV DNA is a significant prognostic factor if the sample size increases. Note that one limitation of our study is that only patients with elevated plasma EBV DNA levels before salvage treatment could be applied.

In a previous study, we shown that a consistent genotyping of EBV DNA between paired samples of plasma and primary tumor suggested that circulating cell–free EBV DNA may originate from the primary tumor, and plasma EBV DNA levels before or 1 week after treatment highly correlated with subsequent tumor relapse and survivals in 99 previously nontreated and stage III/IV (M0) NPC patients (7). Furthermore, results of the current study show that the EBV DNA clearance rate during the first month of chemotherapy is a novel and clinically useful biomarker that could predict tumor response and patient survival in 34 metastatic/recurrent patients. We will initiate a multicenter trial to validate the predictive/prognostic effect of the EBV clearance rate and its consequent effect on early-changing regimen with a larger sample size in the near future.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

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