Predictive Value of ERCC1 mRNA Level and Pretreatment EBV-DNA Virus Load in Stage II Nasopharyngeal Carcinoma Patients Receiving Intensity-Modulated Radiotherapy with Concurrent Cisplatin

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Abstract

Background: The protein expression of ERCC1 in DNA repair genes was related to resistance platinum and predicting treatment outcomes in various malignant carcinoma ,the level of plasma Epstein-Barr virus EBV-DNA concentrations is positively correlated with clinical stages of nasopharyngeal carcinoma(NPC), but the predictive value of ERCC1 mRNA and EBV-DNA level for stratified treatment with stage II NPC is unclear precisely. This study aimed to assess the predictive value of combined EBV-DNA and ERCC1 in stage II NPC patients treated with intensity-modulated radiotherapy (IMRT) with concurrent cisplatin and provide guidance for future stratified treatment.

Methods: A total 78 stage II NPC patients who received IMRT and concurrent cisplatin-based chemotherapy had measurements of ERCC1 mRNA and pre-treatment EBV DNA levels by real-time PCR (RT-PCR) analysis were analyzed. Associations of ERCC1 mRNA and pre-treatment EBV DNA levels with clinical characteristics and survivals were evaluated.

Results: Cut-off value of ERCC1 mRNA obtained from ROC curve was used and there were significant differences in progression-free survival (PFS) and overall survival (OS) between high expression group as compared to low expression group ($P=0.021$ and 0.030 , respectively). Patients with pretreatment EBV-DNA<2000 copies/ml had significantly better PFS ($P= 0.024$) than those with pretreatment EBV-DNA $\geq$ 2000 copies/ml, but there was no significant difference in OS ($P= 0.062$). Patients were divided into three groups by combination of ERCC1 mRNA and EBV-DNA level ERCC1 mRNA low expression/pre EBV-DNA<2000 copies/ml, ERCC1 mRNA low expression/pre EBV-DNA $\geq$ 2000 copies/ml, ERCC1 mRNA high expression/pre EBV-DNA $\geq$ 2000 copies/ml. In these groups, 1-year, 3-year, 5-year OS were 100%, 100%, 100%; 100%, 94.1%, 90.9%; 100%, 85%, 72.9%, respectively ($P=0.038$); 1-year, 3-year, 5-year PFS were 100%, 100%, 100%; 97.1%, 91.2%, 84.8%; 95%, 85%, 71.4%, respectively ($P=0.028$). Multivariate analysis showed combination of ERCC1 mRNA and EBV-DNA levels remained independent prognostic factor but not ERCC1 mRNA and EBV-DNA alone.

Conclusion: Combined ERCC1 mRNA and pre EBV-DNA is a better prognostic factor in stage II NPC patients treated with concurrent chemoradiation. Patients with ERCC1 mRNA high expression/pre EBV-DNA $\geq$ 2000 copies/ml should be treated with more aggressive regimen.

Background

Nasopharyngeal carcinoma (NPC) is one of the most common malignancies in Southern China with an incidence rate of 20–30/10000 [1, 2]. Non-keratinizing carcinoma (WHO 2005 classification), especially the undifferentiated type of Non-keratinizing carcinoma, is the major pathological type in regions with high incidence [3], which is more sensitive to chemoradiotherapy. Cisplatin is the standard concurrent chemotherapeutic agent in advanced NPC(stage III-IV)[4], due to its inhibitory effect on the tumor cells recovery from sub-lethal and potentially lethal radiation damage [5].

NCCN guidelines recommend concurrent chemoradiation for stage II NPC, however the role of chemotherapy in stage II NPC is still controversial. A phase III randomized controlled trial revealed improved 5-year overall survival (OS) rates in stage II NPC who received concurrent chemoradiotherapy compared to radiotherapy alone (94.5% vs 85.8%; $P=0.007$) [6]. In contrast, data from the Real-World Study (RWD) showed that patients with stage II NPC did not benefit much with additional chemotherapy; those treated with radiotherapy alone had excellent long-term survival [7]. It is possible selection of patients for chemoradiation is important. The clinical stage II NPC includes three subgroups: T2N0M0, T2N1M0, and T2N1M0. Long-term follow-up data showed that the survivals of these subgroups were different [8]. However, the current staging system based solely on anatomical information cannot fully reflect the risk factors stratification of stage II NPC; there is biological heterogeneity among tumor individuals. Therefore, there are some limitations in guiding stage II NPC treatment through clinical staging system.

Repair of DNA damages is a key process after cancer cells exposed to radiotherapy and chemotherapy [9]. Mediators that are associated with DNA damage repair process may affect the outcomes of radiotherapy and chemotherapy [9]. Excision repair cross-complementation group 1 (ERCC1) act as a rate-limiting enzyme in the nucleotide excision repair (NER) pathway [10–11]. Recently, increasing experimental and clinical evidences demonstrated that high ERCC1 expression was associated with resistance to platinum-based chemotherapy in gastric cancer, ovarian cancer, and lung cancer, and low ERCC1 expression predicted good survival of these patients [12–17]. In locoregionally advanced NPC treated with chemoradiotherapy, there were several studies used immunohistochemical techniques (IHC) to determine the expression of ERCC1 and found that high tumor ERCC1 expression predicted poor treatment response and survival [18–21]. However, the relationship between ERCC1 mRNA and treatment outcomes in NPC has not been established.

Epstein-Barr virus (EBV) DNA concentrations correlate positively with disease stage as well as exhibit prognostic importance in NPC [22]. Many published studies have shown that EBV DNA is an important biomarker for monitoring survival for all stages of disease [23–25]. In stage II NPC, our previous study found that the plasma EBV-DNA level one week after radiotherapy completion could predict 3-year and 5-year recurrence and metastasis rate [26], but the predictive value of pre-treatment plasma EBV-DNA level have not been studied.

Understanding the underlying intrinsic factors would help us to predict the prognosis and select optimal treatments. However, whether combined ERCC1 and EBV-DNA is associated with prognosis in the prediction of concurrent chemoradiotherapy (CCRT) treatment outcomes and provide clinical guidance remains unclear. The subject of this study was to retrospectively assess the prognostic value of combined ERCC1 mRNA with the level of pre-treatment the plasma EBV-DNA and survival for stage II NPC.

Methods

Human NPC tissue samples

All procedures performed in this study involving human participants were approved by Ethics Committee of the participating hospitals, and were in accordance with the 1964 Declaration of Helsinki. Informed consent was obtained from each patient. Human NPC tissues were collected from 78 patients who underwent CCRT between June 2012 and June 2015. All cases were biopsy-proven NPC and stage II disease according to the seventh edition TNM staging system. The median age was 46 years old (18–73 years old) and the gender ratio (male vs. female) was 3.1:1. The clinicopathological characteristics of the NPC patients were presented in Table 1.

Table 1
Characteristics of the patients with stage II NPC patients

Patient characteristic	Number (%)
Median follow-up (months, rang)	62 m(22 m-84 m)
Sex	
Male	59
Female	19
Age (year)	
<60	64
≥ 60	14
T stage	
T1	15
T2	63
N stage	
N0	23
N1	55
TN stage	
T2N0	23
T1N1	16
T2N1	39
Median ERCC1mRNA (Rang)	1.119 (0.456–1.411)
Median EBV-DNA load (Rang)	4089 (582 – 2.02 × 105)

Chemoradiation treatment

All patients were treated with intensity-modulated radiotherapy (IMRT) given once daily 5 times per week. The radiation dose was as follow: PGTV_{nx} 70.06 Gy/31 times, PGTV_{nd} 70.06 Gy/31 times, PTV₁ 66.03 Gy/31 times and PTV₂ 54.25 Gy/31 times. All volumes were treated simultaneously.

Cisplatin (80 mg/m²) was given via intravenous infusion every 21 day for three cycles, beginning on the first day of radiotherapy.

Response evaluation and follow-up studies

At 12 weeks after completion of CCRT, magnetic resonance imaging (MRI) of nasopharynx and neck was obtained in all patients and the tumor response was evaluated according to the Response Evaluation Criteria in Solid Tumors (Version 1.1). Based on the tumor response, patients were divided into complete response group (CR), partial response group (PR), stable disease group (SD), or progressive disease (PD) group. The response rate (RR) was calculated as number of CR plus PR cases divided by all cases. All patients were followed every 3 months in the first year and every 6 months for the following years.

Real-time PCR analysis

RNA was extracted from the cancerous tissues using TRIzol reagent (Takara, Dalian, China) according to manufacturer's instructions, and cDNA was synthesized using PrimeScript 1st strand cDNA Synthesis Kit (Takara). ERCC1 expression level was detected by using SYBR Green real-time PCR in ABI7900 system (Applied Biosystems, Foster City, USA). GAPDH was used as an internal reference for ERCC1 mRNA expression. The primers for ERCC1: F, 5'-CCGCCAGCAAGGAAGAAA-3', R, 5'-CTGCCGAGGGCTCACAAT-3'; the primers for GAPDH: F, 5'-GAGATCCCTCCAAAATCAAGT-3', R, 5'-CTTCCACGATACCAAAGTTGT-3'. The mRNA expression of ERCC1 was calculated by using compared Ct method.

Quantitative detection of EBV-DNA in plasma

3 ml of blood was collected each time, added into the EDTA anticoagulant tube, centrifuged at 2000 R / min for 5 min, and 200 μ l of plasma was frozen at -20 °C for standby. Extraction and amplification steps of EBV-DNA: put frozen plasma at room temperature, extract DNA in strict accordance with the instructions of EBV fluorescence quantitative polymerase chain reaction (RT-PCR) detection kit; detect gene amplification with mixed PCR reaction solution on ABI 7300 PCR instrument, PCR reaction conditions: 93 °C pre denaturation for 2 min, 93 °C 45 s, 55 °C 60 s. 10 cycles; 93 °C 30 s, 55 °C 45 s, 30 cycles. The calculation formula of plasma EBV-DNA concentration: $C = q \times (v_{dn} / V_{PC}) \times (1 / v_{ext})$.

Statistical analysis

All the data analysis was performed with SPSS software (Version 23.0, SPSS Inc., Chicago, USA). The survival curves, the receiver-operator characteristic (ROC) curve and Forest map were drawing in GraphPad Prism 8.3 (GraphPad Software Inc., San Diego, CA, USA). The overall survival was defined as the time from start of treatment to death from any cause. The progression free survival was defined as the time from start of treatment to the observation of disease progress. Association between ERCC1 mRNA expression, the level of EBV-DNA and clinicopathological characteristics were assessed by Chi-square

test. The survival difference in OS and PFS between different groups was assessed by Kaplan-Meier plots. All statistical tests were two-sided, and P values less than 0.05 were considered as statistically significant.

Results

ERCC1 mRNA expression in NPC tissues and treatment response

The mRNA expression levels of ERCC1 in NPC tissues determined by RT-PCR ranged from 0.456 to 1.412 with the median value of 1.119.

To determine the diagnostic value of the ERCC1 mRNA to response, the data from 78 cases were analyzed by ROC curve analysis. The results showed that the area under the curve (AUC) was significantly larger than the area expected by chance alone ($P < 0.0001$, 95% CI = 0.917–0.960; Fig. 1). The optimal cut-off value was 1.295; with sensitivity of 87.5% and a specificity of 81.4%.

Using this cut-off value, ERCC1 mRNA expression was found significantly associated with N stage and TN stage but not significantly associated with gender, age or T stage (Table 2). Furthermore, using this cut-off value as dichotomy, 40/41 had complete response or partial response in the low expression group (< 1.295) and 30/37 had complete response or partial response in the high expression group (≥ 1.295). Those with low expression had significant higher treatment response rate than those with high expression (97.65% vs 81.08%; $P = 0.043$).

Table 2
The relationship between ERCC1 mRNA expression and clinical characteristics in stage II NPC patients

Variables	Case	ERCC1mRNA	ERCC1mRNA	P value
	n (%)	low expression	high expression	
Sex				0.199
Male	59	46	13	
Female	19	12	7	
Age (year)				0.341
<60	64	49	15	
≥ 60	14	9	5	
T stage				0.329
T1	15	13	2	
T2	63	45	18	
N stage				0.012
N0	23	23	0	
N1	46	35	11	
TN stage				0.000
T2N0	23	23	0	
T1N1	16	14	2	
T2N1	39	21	18	

General treatment outcomes

With median follow up of 62 months (range 22–84), the 1-year, 3-year and 5-year OS rates were 98.72%, 93.59% and 91.03%, respectively, and the 1-year, 3-year and 5-year progression-free survival (PFS) rates were 97.4%, 92.3% and 86.5%, respectively.

The relationship between ERCC1 mRNA expression levels and treatment outcomes

The Kaplan-Meier survival curves for OS and PFS comparing high ERCC1 mRNA expression and low ERCC1 mRNA expression using the cut-off value were shown in Fig. 2A and 2B. There were statistically significant difference in both OS and PFS. The 1-year, 3-year and 5-year OS were 100%, 85.0%, 72.9% in the high expression group versus 100%, 96.6%, 94.7% in the low expression group ($P = 0.021$). The 1-year,

3-year and 5-year PFS rates were 95%, 85%, 71.4% in the high expression group versus 98.3%, 94.8%, 91.1% in the low expression group ($P=0.030$).

The EBV-DNA viral load and treatment outcomes

Using pretreatment EBV-DNA load 2000 copies/ml as a cut-off [22], EBV-DNA viral load was found to be significantly associated with T stage and TN stage but not significantly correlated with gender, age or N stage (Table 3).

Table 3
The relationship between pre EBV-DNA level and clinical characteristics in stage II NPC patients

Variables	Case	EBV-DNA	EBV-DNA	P value
	n (%)	<2000 copies/ml	≥ 2000 copies/ml	
Sex				0.395
Male	59	20	39	
Female	19	4	15	
Age (year)				1.000
<60	64	20	44	
≥ 60	14	4	10	
T stage				0.035
T1	15	8	7	
T2	63	16	47	
N stage				0.619
N0	23	8	15	
N1	55	16	39	
TN stage				0.018
T2N0	23	8	15	
T1N1	16	9	7	
T2N1	39	7	32	

The Kaplan-Meier survival curves for OS and PFS comparing high EBV-DNA load (≥ 2000 copies/ml) and low EBV-DNA load (2000 copies/ml) were shown in Fig. 3A and 3B. There was a trend although not statistically significant difference in OS. The 1-year, 3-year and 5-year OS were 100%, 90.7%, 85.7% in the high EBV-DNA load group versus 100%, 100%, and 100% in the low EBV-DNA load group ($P=0.062$). But

there was statistically significant difference in PFS. The 1-year, 3-year and 5-year PFS rates were 96.3%, 88.9%, 80.1%, in the high EBV-DNA load group versus 100%, 100% and 100% in the low EBV-DNA load group ($P = 0.024$).

Combination of ERCC1 mRNA expression and EBV DNA viral load improved prognostic stratification

Patients were stratified into three groups: low ERCC1 mRNA expression and low EBV-DNA load, low ERCC1 mRNA expression and high EBV-DNA load, high ERCC1 mRNA expression and high EBV-DNA load. There was no patient with high ERCC1 mRNA expression and low EBV-DNA. For these three groups, the 1-year, 3-year, 5-year OS rates were 100%, 100%, 100%; 100%, 94.1%, 90.9%; 100%, 85%, 72.9%, respectively ($p = 0.038$); the 1-year, 3-year, 5-year PFS rates were 100%, 100%, 100%; 97.1%, 91.2%, 84.8%; 95%, 85%, 71.4% respectively ($P = 0.028$). Figure 4A and 4B showed OS and PFS for these three groups.

Univariate and multivariate analysis

In univariate analysis, ERCC1 mRNA based on cut-off value and ERCC1 mRNA expression/pre EBV-DNA viral load were significant prognostic factors for OS ($P = 0.037$, $P = 0.023$) and PFS ($P = 0.043$, $P = 0.014$) (Fig. 5). However, after adjusting for covariates, multivariable analysis indicated only ERCC1 mRNA expression/pre EBV-DNA viral load was independent and significant negative prognostic factors for OS and PFS in patients stage II NPC. (HR: 4.290, 95%CI 1.220-15.082, $P = 0.023$; HR: 3.461, 95%CI 1.292–9.270, $P = 0.014$).

Discussion

In the present study, the ERCC1 mRNA expression level in NPC tissues determined by RT-PCR was examined rather than the ERCC1 protein level determined by the immunohistochemistry (IHC); the latter is more commonly used in most studies. Each method has its advantages and disadvantages. IHC procedure is easier to perform but with poorer reproducibility. In comparison, RT-PCR is more sensitive. In addition, ERCC1 has four protein subtypes 201, 202, 203 and 204. Only subtype 202 is related to the efficacy of platinum chemotherapy. However, the commonly used protein antibody detection method of ERCC1 cannot distinguish different subtypes [27, 28]. A meta-analysis by Song et al suggested that RT-PCR method is superior to IHC in term of detecting ERCC1 expression level to predict the outcomes of platinum-based chemotherapy in gastric carcinoma [29]. However, this is still controversial due to the differences in experimental reagents and criteria for assessment.

Our study showed that ERCC1 expression level was strongly correlated with N stage and TN stage but not significantly associated with gender, age or T stage. This is different from the results by Olausson et al that found ERCC1 expression significantly correlated with age, gender and histological type, but not associated with tumor size and TNM stage [30]. Our study also showed that ERCC1 mRNA level was significantly associated with treatment outcomes in stage II NPC. Using the cut-off value obtained from

ROC curve, ERCC1 mRNA can be divided into high expression and low expression groups. The OS and PFS of high expression group were significantly worse than that of low expression group. It is possible that the cancer cells with high ERCC1 expression have better ability to repair DNA damages, which is associated with resistance to platinum radio-chemotherapy leading to poor response to initial radiotherapy and chemotherapy.

Several studies have shown that EBV-DNA levels in advanced stage NPC are significantly higher than those in stage I and II NPC [31–33]. Du et al observed that the level of EBV-DNA was correlated with age, T stage, N stage and total stage in stage II-IV NPC [34]. However, TWU et al reported that EBV-DNA was not correlated with clinical and pathological factors in stage III NPC [35]. In the present study with stage II diseases, we found that EBV-DNA was significantly associated with T stage and TN stage but not significantly correlated with gender, age or N stage.

Furthermore, in advanced stage NPC, patients with a high level of EBV-DNA have worse survival outcomes, and several prognostic subgroups can be classified based on their plasma EBV-DNA levels. However, in stage I and II disease, there was no study on the prognostic value of pre-therapy plasma EBV-DNA due to infrequent treatment failures. The optimal cut-off value of EBV-DNA has not been determined due to lack of harmonization of qPCR assay and different values were reported in different studies. Chai et al used 8000 copies/ml of pre-treatment plasma EBV DNA as a potential biomarker associated with OS for all stage NPC [36]. Lin et al, however reported that OS and relapse-free survival were significantly lower in patients with pretreatment plasma EBV DNA concentration ≥ 1500 copies/ml than those with < 1500 copies/ml [37]. Zhang et al in a meta-analysis of 14 prospective and retrospective control studies evaluated the relationship between EBV-DNA and half-life of EBV-DNA and survival and proposed a 4-level risk stratification model. Patients with stage II disease and pre-treatment plasma EBV-DNA < 4000 copies/ml could be down-staged to stage I and treated with radiation alone. However, patients with ≥ 4000 copies/ml pre-treatment plasma EBV-DNA should be re-classified into stage III and treated more aggressively [24]. Guo and colleague analyzed 979 newly diagnosed nonmetastatic NPC patients with RPA model and proposed a new staging model based on pre-treatment EBV DNA level using 2000 copies/ml as cut off value and anatomical information [22]. Based on Guo's study, we also used 2000 copies/ml EBV-DNA as a cut-off value in our study and found that stage II NPC also has high EBV-DNA concentrations with 70% of the patients having more than 2000 copies / ml. Using this cut-off value, we show here that higher level of pre-treatment EBV DNA was associated with an increased risk of disease recurrence. There was a trend that higher level of pre-treatment EBV-DNA was associated with poor OS but did not reach statistical significance. Leung et al [38] also did not find a significant association between pre-treatment EBV DNA levels and the probability of distant failure ($P = 0.0967$) and the probability locoregional failure ($P = 0.719$) in stage I-II NPC. The failure to detect significant association of pre-treatment EBV-DNA and OS in our study is possible due to small sample size and short follow up.

Pretreatment EBV-DNA has been shown to be an independent prognostic factor for NPC in many studies. But ERCC1 mRNA level was not an independent prognostic factor in lung cancer, gastric cancer and pancreatic cancer [39]. In our study with stage II NPC, neither pretreatment EBV-DNA nor ERCC1mRNA

was independent prognostic factors. However, combination of pretreatment EBV-DNA and ERCC1 mRNA was shown to be a significant independent prognostic factor for OS and PFS. By combining ERCC1 mRNA and pretreatment plasma EBV-DNA, stage II NPC can be divided into three subgroups: ERCC1 mRNA low expression/pre EBV-DNA < 2000copies/ml, ERCC1 mRNA low expression/pre EBV-DNA $\geq$ 2000copies/ml, ERCC1 mRNA high expression/pre EBV-DNA $\geq$ 2000copies/ml, and there were significant differences in OS and PFS between the three groups. Patients with ERCC1 mRNA high expression/pre EBV-DNA $\geq$ 2000 copies/ml did significantly worse and possibly need more aggressive treatment and closer follow-up after treatment. On the other hand, patients with ERCC1 mRNA low expression/pre EBV-DNA < 2000copies/ml did much better and their treatment could possibly be de-escalated and radiation alone without chemotherapy may be sufficient. The combination of ERCC1 mRNA and pre-treatment EBV-DNA can improve prognostic value compared to using pre-treatment EBV-DNA alone in stage II NPC patients who have relatively better survival outcomes.

Conclusions

In summary, our study demonstrated for the first time that integrated ERCC1 mRNA and pre EBV-DNA levels may serve as a better predictor for the efficacy of CCRT treatment and long-term survival in stage II NPC patients. High ERCC1 mRNA and high pre EBV-DNA combined result in significant decrease in PFS and OS. These patients may be treated with more aggressive regimen. However, due to different methods in detecting ERCC1 expression level and various cut-off values of ERCC1 level and pretreatment EBV-DNA reported, more studies will be required to validate our results. In addition, the limitation of this study is the relatively small sample size. Harmonization of quantitative assays and large sample size will need international efforts and ultimately to achieve the goal of individualized treatment for stage II NPC patients.

Abbreviations

NPC, Nasopharyngeal carcinoma

ERCC1, Excision repair cross-complementation group 1

EBV-DNA, Epstein-Barr virus DNA

CCRT, concurrent chemoradiotherapy

OS, overall survival

PFS, progression-free survival

CR, complete response

PR, partial response

SD, stable disease

PD, progressive disease

RR, The response rate

Declarations

Acknowledgements

Not applicable.

Authors' contributions

L.H., S.C.and M.W contributed to the study design, literature research, interpretation of findings and writing of the manuscript. Y.S., J.L., Z.L.and Y.M contributed to the follow-up, data collection and analyses. P.Y. provided suggestion of statistics. H.H., X.T,and M.Y contributed to critical review of data analyses and critical edit of the manuscript. All authors reviewed and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was approval by the Ethics Committee of Guangxi Medical University and the Forth Affiliated Hospital of Guangxi Medical University, written informed consent was obtained from each patient.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

ROC curve for the cut-off points for ERCC1 mRNA that could be obtained by testing ERCC1 mRNA level to predict therapeutic response of stage II NPC patients.

Number of risk

ERCC1mRNA Low expression	58	58	56	41	3
ERCC1mRNA High expression	20	19	16	6	0

Figure 2

Kaplan-Meier analysis of ERCC1 mRNA expression based on the cut-off value for OS (A) and PFS(B) in patients with stage II NPC patients.

Number of risk

ERCC1mRNA Low expression	58	56	55	39	3
ERCC1mRNA High expression	20	17	16	6	0

Figure 3

Kaplan-Meier analysis of ERCC1 mRNA expression based on the cut-off value for OS (B) in patients with stage II NPC patients.

Number of risk

ERCC1mRNA Low expression	24	24	24	20	3
ERCC1mRNA High expression	54	54	48	27	0

Figure 4

Kaplan-Meier curves of OS (A) and PFS (B) according to pretreatment plasma EBV-DNA levels (≤ 2000 copies/ ml vs ≥ 2000 copies/ ml) for stage II NPC patients.

Number of risk

ERCC1mRNA Low expression	24	24	24	20	3
ERCC1mRNA High expression	54	49	47	25	0

Figure 5

Kaplan-Meier curves of OS (A) and PFS (B) according to pretreatment plasma EBV-DNA levels (<2000 copies/ ml vs ≥ 2000 copies/ ml) for stage II NPC patients.

Number of risk

ERCC1mRNA Low expression/
pre EBV-DNA <2000 copies/ml

24 24 24 20 3

ERCC1mRNA Low expression/
pre EBV-DNA $\geq$ 2000 copies/ml

34 34 32 21 0

ERCC1mRNA High expression/
pre EBV-DNA $\geq$ 2000 copies/ml

20 20 16 6 0

Figure 6

Kaplan-Meier survival curves of stage II NPC patients displaying different combinations of ERCC1 expressions based on the cut-off and pre EBV-DNA level. OS (A), PFS (B).

Number of risk					
ERCC1mRNA Low expression/ pre EBV-DNA <2000 copies/ml	24	24	24	20	3
ERCC1mRNA Low expression/ pre EBV-DNA $\geq$ 2000 copies/ml	34	32	31	19	0
ERCC1mRNA High expression/ pre EBV-DNA $\geq$ 2000 copies/ml	20	17	16	6	0

Figure 7

Kaplan-Meier survival curves of stage II NPC patients displaying different combinations of ERCC1 expressions based on the cut-off and pre EBV-DNA level. OS (A), PFS (B).

Figure 8

Forest plot of the association between clinicopathological variables and OS (A) and PFS (B) by subgroup for stage II NPC patients.

Figure 9

Forest plot of the association between clinicopathological variables and OS (A) and PFS (B) by subgroup for stage II NPC patients.