Long-Term Monitoring of Dynamic Changes in Plasma EBV DNA For Improved Prognosis Prediction of Nasopharyngeal Carcinoma

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Research

Abstract

Background: This study was performed to investigate whether long-term monitoring of dynamic changes in plasma Epstein-Barr virus (EBV) DNA could improve prognosis prediction of nasopharyngeal carcinoma (NPC).

Methods: 1077 non-metastatic NPC patients were recruited to retrospectively analyze the prognostic value of plasma EBV DNA load pre-treatment and 3, 12, 24, and 36 months post-treatment. We also examined the prognostic value of dynamic changes in plasma EBV DNA at various time points.

Results: Patients with plasma EBV DNA load above optimal pre- and post-treatment cut-offs had significantly worse five-year progression-free survival, distant metastasis-free survival, locoregional relapse-free survival, and overall survival(OS) at all time points, excluding only OS at 36 months post-treatment due to limited mortalities. Patients with persistently undetectable plasma EBV DNA at the first four time points had the best prognosis, followed by those with positive detection pre-treatment and consistently negative detection post-treatment, those with negative detection pre-treatment and positive detection at one time point post-treatment, and those with positive detection pre-treatment and at one time point post-treatment, whereas patients with positive detection at ≥2 time points post-treatment had the worst prognosis. Cox proportional hazard models identified the dynamic change pattern as an independent prognostic factor, and ROC curve analysis demonstrated that the dynamic change at four time point was more valuable than any single time point for predicting disease progression, distant metastasis, locoregional relapse, and mortality.

Conclusions: Dynamic changes in plasma EBV DNA pre- and post-treatment could predict the long-term survival outcome and provide accurate risk stratification in NPC.

Background

Nasopharyngeal carcinoma (NPC) is a malignant head and neck tumor originating from the nasopharyngeal epithelia that is highly prevalent in southeast Asia, especially in southern China [1]. With the development of radiotherapy and chemotherapy regimens [2], the survival outcomes of NPC patients have greatly improved. However, about 5%-15% of NPC patients develop nasopharynx or regional lymph node recurrence after treatment, and up to 10%-30% patients develop distant metastasis[3]. Therefore, identification of biomarkers to predict locoregional relapse and distant metastasis would be of great clinical value for improved survival and better prognosis.

Endemic NPC is strongly associated with Epstein-Barr virus (EBV) infection [4]. Cell-free EBV DNA from NPC tumor cells can be detected in the plasma of NPC patients [5], and the plasma DNA load can reflect the tumor load and residual disease or subclinical metastases [6]. Therefore, EBV DNA is widely considered as a reliable biomarker for early screening, clinical staging, prognosis prediction, individualized treatment and follow-up monitoring in NPC [7-13].While multiple studies have reported that plasma EBV DNA could be used as a predictive marker for NPC prognosis [5, 14-18], however, these studies only measured plasma EBV DNA load at limited time points, such as pre-treatment and 3 months post-treatment. We suggested that a long-term monitoring of dynamic changes of plasma EBV DNA could improve its prognostic value in NPC.In a meta-analysis by Qu et al [19], plasma EBV DNA was measured primarily from day 1 to 3 months post-treatment. Therefore, the prognostic value of plasma EBV DNA at other time points, such as more than 3 months post-treatment, requires further study.

In addition, dynamic changes of plasma EBV DNA pre- and post- treatment may also be useful for prognosis prediction and treatment evaluation. Recently, several studies have reported the potential prognostic value of dynamic changes in plasma EBV DNA in NPC patients at different time points, including during treatment [20, 21], pre- and early post-treatment[22, 23], and within 3 months post-treatment [24-26]. However, long-term monitoring of dynamic changes in plasma EBV DNA after 3 months post-treatment might also provide prognostic value. Therefore, this study is to further examine the predictive value and dynamic changes of plasma EBV DNA load at multiple time points pre- and post-treatment in NPC.

Materials And Methods

Patients

From January 2005 to December 2015, 1077 non-metastatic NPC patients treated in Nanfang Hospital were enrolled in this study. All cases were confirmed by pathological examinations and staged according to the 7th edition American Joint Committee on Cancer

(AJCC) [27]. The exclusion criteria consisted of non-WHO pathological types, distant metastasis at initial diagnosis, prior malignancy, no plasma EBV DNA records. The work flow was shown in **Figure 1**.

Treatment

All patients received intensity-modulated radiotherapy (IMRT) at a total dose of 66-74 Gy for 6-8 weeks at target areas located by CT. In the patient cohort, 76 patients (7.1%) were at stage I and received IMRT treatment alone, 145 patients (13.4%) were at stage II and received concurrent chemoradiotherapy, and 856 patients (79.5%) were at stages III/IV and received concurrent chemoradiotherapy, induction chemotherapy, and/or adjuvant chemotherapy**(Additional file 1)**.

Follow-up

Patients were followed up at 3, 6 and 12 months in the first year after therapy, every 6 months during the second and third year, and yearly thereafter. Follow-up evaluation included physical examination of the head and neck, nasopharyngeal endoscopy, chest radiography, abdominal ultrasound, EBV DNA serological testing, MRI of nasopharynx and neck, and whole-body PET. Patients with locoregional relapse and distant metastasis were confirmed by fine needle aspirationand pathological examination of biopsy.The primary study endpoint was progression-free survival (PFS), and the secondary endpoints included distant metastasis-free survival (DMFS), locoregional relapse-free survival (LRFS), and overall survival (OS). **(Additional file 2).**

Quantification of Plasma EBV DNA

Measurements of plasma EBV DNA were performed in the Laboratory Medicine Center of Nanfang Hospital, Southern Medical University. Venous blood samples (5 ml/each case) were collected before treatment and 3, 12 (±1 months), 24 (±3 months), and 36(±3 months) months after treatment, placed in ethylenediaminetetraacetic acid (EDTA) tubes, and centrifuged at 1500×g for 5 min at 4°C.

The plasma total DNA was extracted and EBV DNA concentration was determined by real-time quantitative polymerase chain reaction (RT-qPCR, **Additional file 3**). In this study, the optimal cutoff levels chosen to classify the patients into low and high EBV DNA groups were 1500 copies/mL pre-treatment and 0 copies/mL at each time point post-treatment.

Statistical analysis

Survival outcomes were estimated using the Kaplan-Meier method and compared by the log-rank test. The Cox proportional hazard model was used for multivariate analysis including the following variables: sex, age (≥45 vs.<45 years), T stage (T4 vs. T1-3), N stage (N2-3 vs. N0-1), pre-treatment EBV DNA load (≥1500 vs.<1500copies/mL), and the patterns of dynamic change in plasma EBV DNA pre- and post-treatment. Receiver operating characteristic (ROC) curve analysis was performed to calculate the optimal cutoff value of plasma EBV DNA at each time point and compare the different prognostic values of dynamic change patterns and a single time point of plasma EBV DNA level. All statistical analyses were conducted using SPSS21.0 (IBM Corporation, Armonk, NY, USA).A P<0.05 (two tailed) was considered statistically significant for all tests.

Results

Patient characteristics

The characteristics of 1077 NPC patients were listed in **Table 1**. The median follow-up time was 54 months. During the long-term follow-up, 331 patients (30.7%) experienced disease progression, including 76 cases of locoregional recurrence (7.1%), 172 cases of distant metastasis (16.0%), 40 cases of both locoregional recurrence and distant metastasis (3.7%), and 147 deaths (13.6%). The 5-year PFS, DMFS, LRFS, and OS rates were 68.4%, 79.2%, 87.7% and 84.8%, respectively.

Pre- and post-treatment plasma EBV DNA levels at various time points

A total of 1077 patients received plasma EBV DNA tests at a single time point pre- and post-treatment (**Figure 1**), and 252 patients received plasma EBV DNA tests at 4 time points (pre-treatment, 3, 12, and 24 months post-treatment). The results showed that the positive rate and viral load of pre-treatment plasma EBV DNA were significantly higher than those at each time point post-treatment (**Figure 2A and 2B**). Among 252 patients receiving plasma EBV DNA tests at 4 time points pre- and post-treatment, post-treatment EBV-DNA positivity was significantly lower than pre-treatment, and post-treatment plasma EBV DNA seemed to show an increase over time

(Figure 2C). As shown in **Figure 2D**, the changes of plasma EBV DNA load pre- and post-treatment were quite different among these 252 NPC patients. Some patients maintained plasma EBV DNA baseline negativity (0 copies/ml) at all 4 time points, some patients showed plasma EBV DNA above baseline at only a single time point (pre- or post-treatment), and some patients rebounded above the baseline level after treatment (single or multiple time points), while some patients maintained plasma EBV DNA positivity at all 4 time points.

Patients with pre-treatment plasma EBV DNA load ≥1500 copies/mL had worse 5-year PFS, DMFS, LRFS, and OS than those with plasma EBV DNA load <1500 copies/mL (all P<0.001). Patients with plasma EBV DNA levels >0 copies/mL at 3 different time points post-treatment (3, 12, and 24 months) had a significantly lower 5-year PFS, DMFS, LRFS, and OS than those with negative plasma EBV DNA (all P<0.001). There were also significant differences in 5-year PFS, DMFS, and LRFS between patients with EBV DNA levels >0 copies/mL and EBV DNA levels <0 copies/mL at 36 months post-treatment (all P<0.001, **Additional file 4, Figure 3**). However, it was difficult to compare OS between these two groups at 36 months post-treatment as there was only 1 death among these patients.

Dynamic changes in plasma EBV DNA at 4 time points pre- and post-treatment

The positive rate and viral load of plasma EBV DNA fluctuated markedly at the 4 time points pre- and post-treatments (**Figure 2C and 2D**). To examine the prognostic value of dynamic changes in plasma EBV DNA, we divided the 252 patients receiving tests at all 4 time points (pre-treatment and 3, 12, and 24 months post-treatment) into 5 subgroups according to the dynamic change pattern observed in Figure 2D. Pattern 1 (Pre- & Post 0+) consisted of 82 patients (82/252, 32.5%) with persistently undetectable plasma EBV DNA at all 4 time points. Pattern 2 (Pre+ & Post 0+) included 104 patients (104/252, 41.3%) with positive detection pre-treatment but negative detection at all time points post-treatment. Pattern 3 (Pre- & Post 1+) included 18 patients (18/252, 7.2%) with negative detection pre-treatment and positive detection at one time point post-treatment. Pattern 4 (Pre+ & Post 1+) contained 17 patients (17/252, 6.7%) with positive detection pre-treatment and at one time point post-treatment. Pattern 5 (Pre-/+ & Post ≥2+) included 31 patients (31/252, 12.3%) with positive detection at ≥2 time points post-treatment and negative or positive detection pre-treatment (**Table 2**).

There were significant differences in the rates of disease progression, distant metastasis, locoregional relapse, and mortality among these 5 patterns (all P<0.001, Figure 4A-4D). Similarly, the 3-year PFS, DMFS, LRFS, and OS also differed significantly among these 5 patterns (all P<0.001, Figure 4E-4H). The patients of Pattern 1 had the best prognosis, followed by Patterns 2, 3 and 4, while the patients of Pattern 5 had the worst prognosis (**Table 2, Figure 4**).

Multivariate analysis using a Cox hazard ratio model

Multivariate analysis identified the pattern of dynamic changes in plasma EBV DNA (defined by 4 time points pre- and post-treatment) as an independent predictor of PFS, DMFS, LRFS, and OS in NPC patients (**Table 3**). Pattern 4 and Pattern 5 were independent risk factors for worse PFS, DMFS, LRFS, and OS (all P<0.05), however, Pattern 3 was an independent risk factor for poorer PFS and DMFS (all P<0.01).

ROC curve analysis

ROC analyses demonstrated that dynamic changes in plasma EBV DNA showed larger area under the curve (AUC) values than plasma EBV DNA level at any single time point for predicting NPC disease progression (AUC=0.805; 95%CI, 0.740-0.869), distant metastasis (AUC=0.804; 95%CI, 0.730-0.879), locoregional relapse (AUC=0.704; 95%CI, 0.606-0.802), and mortality (AUC=0.817; 95%CI, 0.726-0.908) (all P<0.001) (**Figure 5**).

Discussion

Multiple reports have shown that pre- and post-treatment plasma EBV DNA are prognostic factors for NPC progression and survival [5, 14-18], and these findings are also confirmed in this study. However, these previous studies mainly focused on few time points, for example, pre-treatment and 3 months post-treatment [19]. In this study, we investigated the prognostic value of plasma EBV DNA load at multiple time points during a long-time follow-up, and examined the patterns of dynamic changes in plasma EBV DNA across 4 time points (pre-treatment, 3 month, 12 month, and 24 month post-treatment). We found that Dynamic changes in plasma EBV DNA pre- and post-treatment could predict the long-term survival outcome and provide accurate risk stratification in NPC.

In this study, we found that positive rate of plasma EBV DNA decreased significantly post-treatment, but increased again slowly over time, with a peak at 24 month post-treatment (Figure 2C). Comparisons of 5-year survival rates at different time points pre- and post-treatment showed that the differences of PFS and DMFS were bigger at 24 month post-treatment than at several other time points. This was consistent with Liu et al [28] and Wu et al [29], who found a sharp peak in the risk curve of treatment failure and NPC-related death at 2 years post-treatment. Plasma EBV DNA level at 2 years post-treatment appeared to be critical for disease surveillance of NPC patients. Furthermore, this peak in treatment failure risk coincided with the peak of disease progression observed post-treatment seen in our daily clinical practice. Therefore, monitoring and evaluation of plasma EBV DNA was particularly important at 2 years post-treatment, which could effectively predict disease progression and treatment failure.

Many studies have reported that plasma EBV DNA loads and its dynamic changes could be used as a predictive marker for NPC prognosis at limited time points [20-26], such as pre-treatment and 3 months post-treatment, however, the prognostic value and dynamic changes of plasma EBV DNA at other time points, such as more than 3 months post-treatment, are not be fully understood yet. Notably, in this study, we enrolled 252 NPC patients who received plasma EBV DNA tests at 4 time points (pre-treatment, 3, 12, and 24 months post-treatment). We showed the dynamic changes in plasma EBV DNA at 4 different time points. Based on this dynamic change, we divided 252 patients into 5 subgroups/patterns and performed survival analysis. This was different from the previous reports [20-26], which mainly focused on the following time points primarily from pre-treatment to 3 months post-treatment.

We found that there was significant difference on prognosis among 5 patterns of dynamic changes in plasma EBV DNA, which suggested that the more positive time points of plasma EBV DNA detection, the higher the risk of disease progression, distant metastasis, locoregional recurrence and death. The patients of Pattern 5 had the worst prognosis on progression, metastasis, recurrence and mortality, and the patients of Pattern 1 had the best prognosis. This conclusion was similar to other studies [30, 31]. As to pattern 2 and pattern 3, the patients of pattern 2 had a better prognosis than those of pattern 3, consistent with Qu's meta-analysis that post-treatment EBV DNA level had greater impact than pre-treatment EBV DNA on clinical outcomes of NPC [19]. Moreover, the patients of pattern 4 had a worse prognosis than those of pattern 2 and 3, the possible explanation was that these patients had a higher baseline and residual tumor load [15].

The results of multivariate analysis showed that dynamic change in plasma EBV DNA pre- and post-treatment was an independent prognostic biomarker for NPC patients (P<0.001). Compared with pattern 1, the risk stratification of disease progression, distant metastasis, locoregional relapse, and mortality were gradually increased from Pattern 2 to Pattern 5, while the hazard ratio of disease progression, distant metastasis, locoregional relapse and mortality of Pattern 5 were as high as 30.637, 31.581, 8.176 and 43.442, respectively. Therefore, pattens of dynamic changes in plasma EBV DNA pre- and post-treatment could provide more accurate and in-depth information for risk stratification of NPC patients and helps to distinguish patients with poor prognosis for further treatment.

In daily clinical practice, it is very important to early detect locoregional tumor recurrence post-treatment, because timely salvage treatment could improve survival outcome of these patients [32]. Chen's findings showed that positive cell free EBV DNA results preceded radiological and/or clinical evidence of disease recurrence by a median of 2.3 months [31]. These findings confirmed that plasma EBV DNA was a significant sensitive and non-invasive predictor of tumor recurrence. Based on our results, we suggested a long-term follow up program according to dynamic changes in plasma EBV DNA. We could examine plasma EBV DNA load and evaluate the risk of disease progression, distant metastasis, locoregional recurrence and mortality. The patients with positivity detection of plasma EBV DNA at any time point post-treatment need to be adjusted to a closer follow-up surveillance strategy, while patients with positive plasma EBV DNA at two or more points post-treatment should be give much attention, and necessary radiological and/or physical examinations should be performed to exclude locoregional recurrence or distant metastasis.

This study also has several limitations. Firstly, the retrospective design might introduce selection bias. Secondly, the sample size (252 cases) has been the largest to date for assessment of dynamic changes in plasma EBV DNA over multiple time points, however, it was still insufficient for investigating more complex change patterns. Finally, the long-term follow up program we proposed can't be further verified in this study. Therefore, a prospective, multicenter, randomized clinical trial with larger sample sizes for a long-term follow-up are needed in future.

Conclusions

Plasma EBV DNA load at multiple time points pre- and post- treatment (up to 36 months) was a significant predictor for NPC prognosis, and the dynamic change pattern in plasma EBV DNA at these time points could provide much accurate predictive value for NPC

prognosis and risk stratification. Therefore, serial plasma EBV DNA measurements extending for several years post-treatment and assessment of dynamic changes in plasma EBV DNA load should be conducted routinely in clinical practice to identify high-risk patients for further treatment.

Abbreviations

EBV: Epstein-Barr virus; NPC: nasopharyngeal carcinoma; ROC: receiver operating characteristic; MRI: magnetic resonance imaging; PET: positron emission tomography; AJCC: American Joint Committee on Cancer; IMRT: intensity-modulated radiotherapy; PFS: progression-free survival; DMFS: distant metastasis-free survival; LRFS: locoregional relapse-free survival; OS: overall survival.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Nanfang Hospital of Southern Medical University(NFEC-2017-165).

Consent for publication

Not applicable.

Availability of data and materials

All data included in this study are available upon request by contact with the corresponding author.

Competing interests

The authors declare that they have no competing interests.

Author contributions

Study concept: **Xiong Liu**. Study design: **Juan Lu** and **Feipeng Zhao**. Data acquisition: **Wanxia Li, Jing Chen,** and **Bijun Liang**. Quality control of data and algorithms: **Fangfang Zeng** and **Xinyu Peng**. Data analysis and interpretation: **Zonghua Li, Junzheng Li, Xiaofei Yuan,** and **Shuting Wu**. Statistical analysis: **Yanfei Li, Junzheng Li,** and **Xiaofei Yuan**. Article preparation: **Wanxia Li**, **Jing Chen**, and **Bijun Liang**. Article editing: **Juan Lu** and **Feipeng Zhao**. Article review: All authors.

References

1. Chen YP, Chan ATC, Le QT, Blanchard P, Sun Y, Ma J. Nasopharyngeal carcinoma. Lancet. 2019;394(10192):64-80.
2. Chen L, Zhang Y, Lai SZ, Li WF, Hu WH, Sun R, et al. 10-Year Results of Therapeutic Ratio by Intensity-Modulated Radiotherapy Versus Two-Dimensional Radiotherapy in Patients with Nasopharyngeal Carcinoma. Oncologist. 2019;24(1):e38-e45.
3. Lee AW, Ma BB, Ng WT, Chan AT. Management of Nasopharyngeal Carcinoma: Current Practice and Future Perspective. J Clin Oncol. 2015;33(29):3356-64.
4. Tsao SW, Tsang CM, Lo KW. Epstein-Barr virus infection and nasopharyngeal carcinoma. Philos Trans R Soc Lond B Biol Sci. 2017;372(1732).
5. Lin JC, Wang WY, Chen KY, Wei YH, Liang WM, Jan JS, et al. Quantification of plasma Epstein-Barr virus DNA in patients with advanced nasopharyngeal carcinoma. N Engl J Med. 2004;350(24):2461-70.

6. Peng L, Yang Y, Guo R, Mao YP, Xu C, Chen YP, et al. Relationship between pretreatment concentration of plasma Epstein-Barr virus DNA and tumor burden in nasopharyngeal carcinoma: An updated interpretation. Cancer Med. 2018;7(12):5988-98.

7. Chan KCA, Woo JKS, King A, Zee BCY, Lam WKJ, Chan SL, et al. Analysis of Plasma Epstein-Barr Virus DNA to Screen for Nasopharyngeal Cancer. N Engl J Med. 2017;377(6):513-22.

8. Guo R, Tang LL, Mao YP, Du XJ, Chen L, Zhang ZC, et al. Proposed modifications and incorporation of plasma Epstein-Barr virus DNA improve the TNM staging system for Epstein-Barr virus-related nasopharyngeal carcinoma. Cancer. 2019;125(1):79-89.

9. Wang WY, Twu CW, Chen HH, Jan JS, Jiang RS, Chao JY, et al. Plasma EBV DNA clearance rate as a novel prognostic marker for metastatic/recurrent nasopharyngeal carcinoma. Clin Cancer Res. 2010;16(3):1016-24.

10. Chan ATC, Hui EP, Ngan RKC, Tung SY, Cheng ACK, Ng WT, et al. Analysis of Plasma Epstein-Barr Virus DNA in Nasopharyngeal Cancer After Chemoradiation to Identify High-Risk Patients for Adjuvant Chemotherapy: A Randomized Controlled Trial. J Clin Oncol. 2018:JCO2018777847.

11. Liang H, Lv X, Wang L, Wu YS, Sun R, Ye YF, et al. The plasma Epstein-Barr virus DNA level guides precision treatment for nasopharyngeal carcinoma in the intensity-modulated radiotherapy era: a large population-based cohort study from an endemic area. Ther Adv Med Oncol. 2018;10:1758835918782331.

12. Hsu CL, Chan SC, Chang KP, Lin TL, Lin CY, Hsieh CH, et al. Clinical scenario of EBV DNA follow-up in patients of treated localized nasopharyngeal carcinoma. Oral Oncol. 2013;49(6):620-5.

13. Li WF, Zhang Y, Huang XB, Du XJ, Tang LL, Chen L, et al. Prognostic value of plasma Epstein-Barr virus DNA level during posttreatment follow-up in the patients with nasopharyngeal carcinoma having undergone intensity-modulated radiotherapy. Chin J Cancer. 2017;36(1):87.

14. Chan AT, Lo YM, Zee B, Chan LY, Ma BB, Leung SF, et al. Plasma Epstein-Barr virus DNA and residual disease after radiotherapy for undifferentiated nasopharyngeal carcinoma. J Natl Cancer Inst. 2002;94(21):1614-9.

15. Hou X, Zhao C, Guo Y, Han F, Lu LX, Wu SX, et al. Different clinical significance of pre- and post-treatment plasma Epstein-Barr virus DNA load in nasopharyngeal carcinoma treated with radiotherapy. Clin Oncol (R Coll Radiol). 2011;23(2):128-33.

16. Wang WY, Twu CW, Chen HH, Jiang RS, Wu CT, Liang KL, et al. Long-term survival analysis of nasopharyngeal carcinoma by plasma Epstein-Barr virus DNA levels. Cancer. 2013;119(5):963-70.

17. Zhao FP, Liu X, Chen XM, Lu J, Yu BL, Tian WD, et al. Levels of plasma Epstein-Barr virus DNA prior and subsequent to treatment predicts the prognosis of nasopharyngeal carcinoma. Oncol Lett. 2015;10(5):2888-94.

18. Huang CL, Sun ZQ, Guo R, Liu X, Mao YP, Peng H, et al. Plasma Epstein-Barr Virus DNA Load After Induction Chemotherapy Predicts Outcome in Locoregionally Advanced Nasopharyngeal Carcinoma. Int J Radiat Oncol Biol Phys. 2019;104(2):355-61.

19. Qu H, Huang Y, Zhao S, Zhou Y, Lv W. Prognostic value of Epstein-Barr virus DNA level for nasopharyngeal carcinoma: a meta-analysis of 8128 cases. Eur Arch Otorhinolaryngol. 2020;277(1):9-18.

20. Leung SF, Chan KC, Ma BB, Hui EP, Mo F, Chow KC, et al. Plasma Epstein-Barr viral DNA load at midpoint of radiotherapy course predicts outcome in advanced-stage nasopharyngeal carcinoma. Ann Oncol. 2014;25(6):1204-8.

21. Lv J, Chen Y, Zhou G, Qi Z, Tan KRL, Wang H, et al. Liquid biopsy tracking during sequential chemo-radiotherapy identifies distinct prognostic phenotypes in nasopharyngeal carcinoma. Nat Commun. 2019;10(1):3941.

22. Peng H, Guo R, Chen L, Zhang Y, Li WF, Mao YP, et al. Prognostic Impact of Plasma Epstein-Barr Virus DNA in Patients with Nasopharyngeal Carcinoma Treated using Intensity-Modulated Radiation Therapy. Sci Rep. 2016;6:22000.

23. Prayongrat A, Chakkabat C, Kannarunimit D, Hansasuta P, Lertbutsayanukul C. Prevalence and significance of plasma Epstein-Barr Virus DNA level in nasopharyngeal carcinoma. J Radiat Res. 2017;58(4):509-16.

24. Zhang Y, Li WF, Mao YP, Guo R, Tang LL, Peng H, et al. Risk stratification based on change in plasma Epstein-Barr virus DNA load after treatment in nasopharyngeal carcinoma. Oncotarget. 2016;7(8):9576-85.

25. Zhang Y, Tang LL, Li YQ, Liu X, Liu Q, Ma J. Spontaneous remission of residual post-therapy plasma Epstein-Barr virus DNA and its prognostic implication in nasopharyngeal carcinoma: A large-scale, big-data intelligence platform-based analysis. Int J Cancer. 2019;144(9):2313-9.

26. He SS, Wang Y, Bao Y, Cai XY, Yang XL, Chen DM, et al. Dynamic changes in plasma Epstein-Barr virus DNA load during treatment have prognostic value in nasopharyngeal carcinoma: a retrospective study. Cancer Med. 2018;7(4):1110-7.

27. Edge SB, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol. 2010;17(6):1471-4.

28. Liu X, Tang LL, Du XJ, Li WF, Chen L, Zhou GQ, et al. Changes in Disease Failure Risk of Nasopharyngeal Carcinoma over Time: Analysis of 749 Patients with Long-Term Follow-Up. J Cancer. 2017;8(3):455-9.

29. Wu SG, Lian CL, Wang J, Zhang WW, Sun JY, Lin Q, et al. The effect of histological subtypes on survival outcome in nasopharyngeal carcinoma after extensive follow up. Ann Transl Med. 2019;7(23):768.

30. Lo YM, Chan LY, Chan AT, Leung SF, Lo KW, Zhang J, et al. Quantitative and temporal correlation between circulating cell-free Epstein-Barr virus DNA and tumor recurrence in nasopharyngeal carcinoma. Cancer Res. 1999;59(21):5452-5.

31. Chen FP, Huang XD, Lv JW, Wen DW, Zhou GQ, Lin L, et al. Prognostic potential of liquid biopsy tracking in the posttreatment surveillance of patients with nonmetastatic nasopharyngeal carcinoma. Cancer. 2020.

32. Hu J, Bao C, Gao J, Guan X, Hu W, Yang J, et al. Salvage treatment using carbon ion radiation in patients with locoregionally recurrent nasopharyngeal carcinoma: Initial results. Cancer. 2018;124(11):2427-37.

Tables

Table 1 Clinical characteristics of NPC patients (n=1077)

Characteristic	N (%)
Sex	
Female	284 (26.4%)
Male	793 (73.6%)
Age (years)	
<45	469 (43.5%)
≥45	608 (56.5%)
Smoking	
Yes	657 (61.0%)
No	420 (39.0%)
WHO pathologic type[1]	
Keratinizing carcinoma	6 (0.6%)
Differentiated non-keratinizing carcinoma	79 (7.3%)
Undifferentiated non-keratinizing carcinoma	992 (92.1%)
Overall stage[2]	
I	76 (7.1%)
II	145 (13.5%)
III	344 (31.9%)
IV	512 (47.5%)
Tumor stage[2]	
T1	222 (20.6%)
T2	213 (19.8%)
T3	205 (19.0%)
T4	437 (40.6%)
Node stage[2]	
N0	162 (15.1%)
N1	311 (28.9%)
N2	512 (47.5%)
N3	92 (8.5%)

[1] Pathologic type according to the 2005 World Health Organization (WHO) classification of tumors.

[2] According to the 7th edition of the UICC/AJCC staging system.

Table 2 Comparisons of 3-year survival rates among groups defined by plasma EBV DNA dynamic change patterns across 4 time points pre- and post-treatment

Subject	N (%)	PFS	DMFS	LRFS	OS
EBV-DNA Dynamic change pattern	252	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$
Pattern 1 (Pre- & Post 0+)	82 (32.5%)	91.4%	97.5%	95.0%	96.2%
Pattern 2 (Pre+ & Post 0+)	104 (41.3%)	86.2%	92.0%	92.1%	99.0%
Pattern 3 (Pre- & Post 1+)	18 (7.2%)	66.7%	77.8%	88.2%	94.4%
Pattern 4 (Pre+ & Post 1+)	17 (6.7%)	39.7%	70.6%	69.5%	87.4%
Pattern 5 (Pre-/+ & Post≥2+)	31 (12.3%)	6.5%	17.9%	49.1%	55.3%
Abbreviations: PFS=progression-free survival; DMFS=distant metastasis-free survival; LRFS=locoregional relapse-free survival; OS=Overall survival.					

Table 3 Multivariate analysis of prognostic risk factors in NPC patients receiving 4 plasma EBV DNA tests (n=252)

| Variable	PFS		DMFS		LRFS		OS	
	HR(95%CI)	*P* value	HR(95%CI)	*P* value	HR(95%CI)	*P* value	HR(95%CI)	*P* value
Sex (male vs. female)	1.828 (1.001-3.337)	0.050	2.079 (0.977-4.425)	0.057	0.578 (0.241-1.385)	0.219	2.213 (0.689-7.112)	0.182
age (≥45 vs. <45)	0.988 (0.635-1.536)	0.957	0.783 (0.448-1.367)	0.389	0.929 (0.477-1.808)	0.828	1.220 (0.588-2.530)	0.594
Smoking (Yes vs. No)	0.764 (0.458-1.274)	0.302	0.588 (0.310-1.116)	0.104	1.592 (0.714-3.550)	0.256	0.977 (0.431-2.217)	0.956
T stage (T4 vs. T1-3)	2.071 (1.308-3.277)	0.002	2.268 (1.261-4.077)	0.006	1.301 (0.665-2.542)	0.442	1.788 (0.837-3.822)	0.134
N stage (N2-3 vs. N0-1)	1.192 (0.706-2.012)	0.511	1.767 (0.845-3.698)	0.131	0.872 (0.412-1.844)	0.719	1.269 (0.485-3.321)	0.628
Pre-EBV (≥1500 vs. <1500)	0.737 (0.401-1.354)	0.325	0.667 (0.315-1.411)	0.290	1.624 (0.607-4.343)	0.334	0.636 (0.256-1.583)	0.331
EBV DNA Dynamic change pattern		<0.001		<0.001		<0.001		<0.001
Pattern 1 (Pre- & Post 0+)	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Pattern 2 (Pre+ & Post 0+)	1.436 (0.636-3.244)	0.384	1.847 (0.625-5.454)	0.267	0.919 (0.256-3.297)	0.897	1.617 (0.345-7.588)	0.542
Pattern 3 (Pre- & Post 1+)	4.296 (1.699-10.863)	0.002	6.591 (1.910-22.744)	0.003	2.176 (0.534-8.878)	0.278	3.403 (0.546-21.225)	0.190
Pattern 4 (Pre+ & Post 1+)	10.409 (4.043-26.794)	<0.001	7.865 (2.242-27.593)	0.001	5.131 (1.213-21.708)	0.026	11.076 (2.238-54.823)	0.003
Pattern 5 (Pre-/+ & Post≥ 2+)	30.637 (12.456-75.355)	<0.001	31.581 (10.154-98.220)	<0.001	8.176 (2.193-30.485)	0.002	43.442 (9.850-191.603)	<0.001

Figures
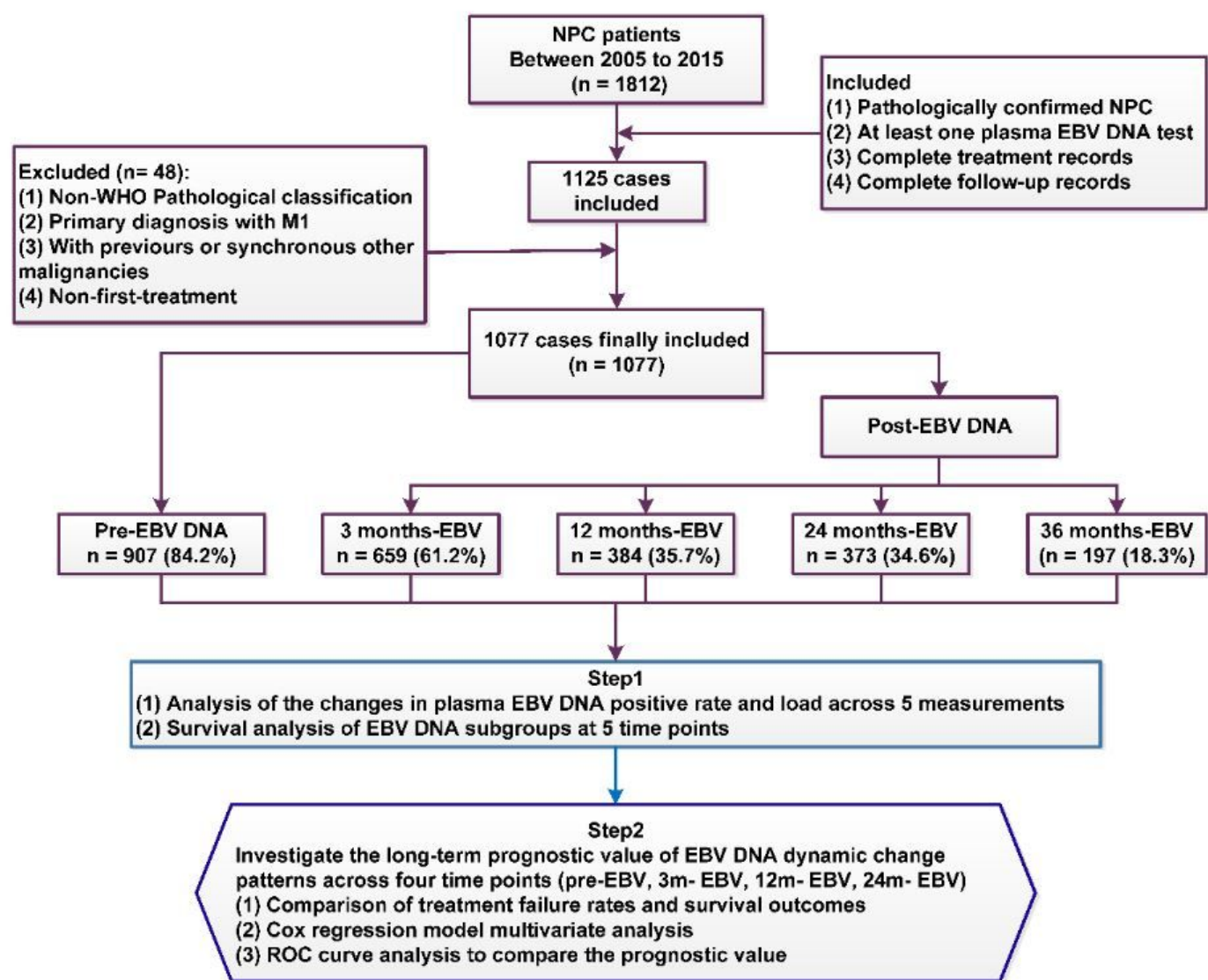
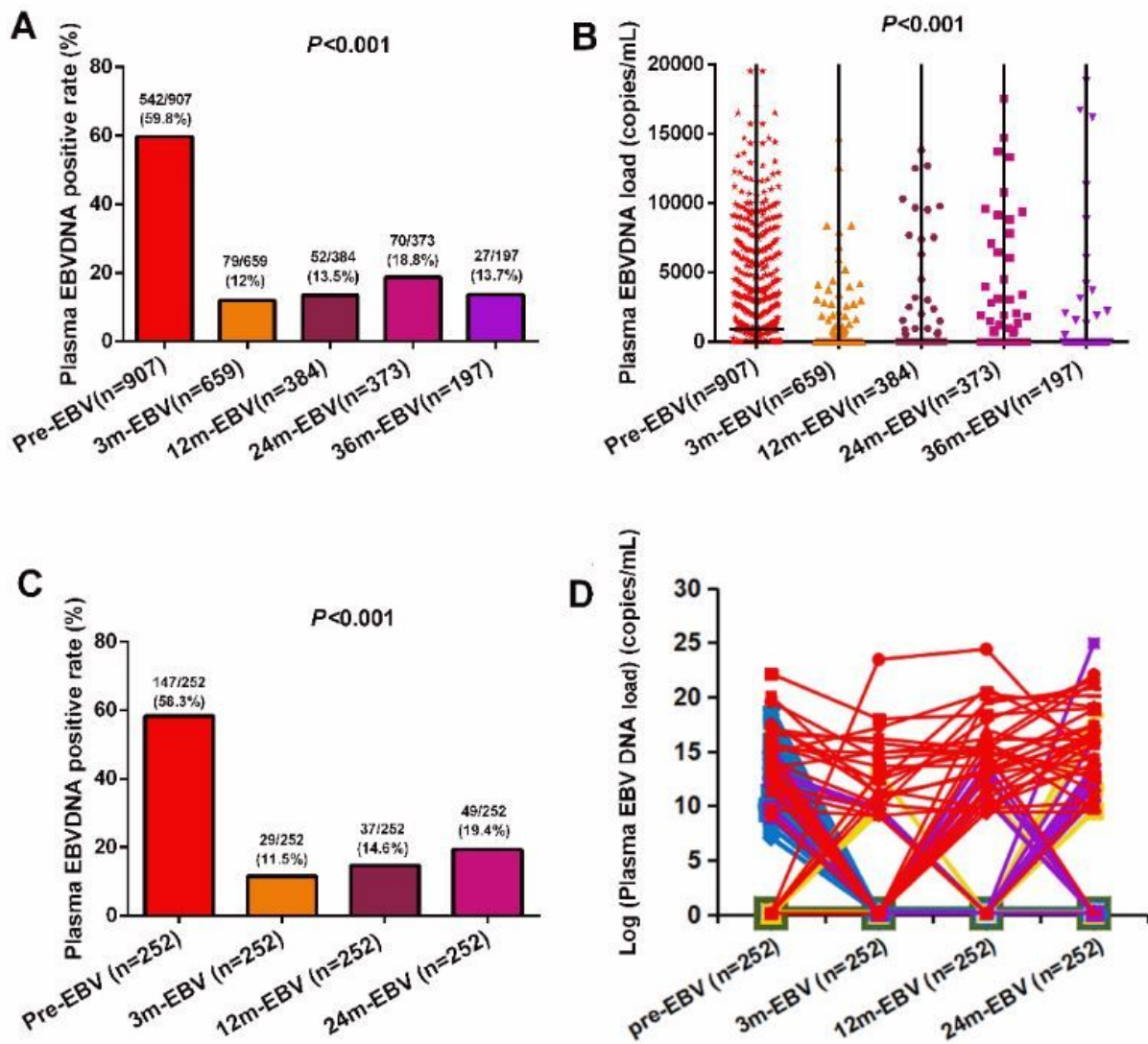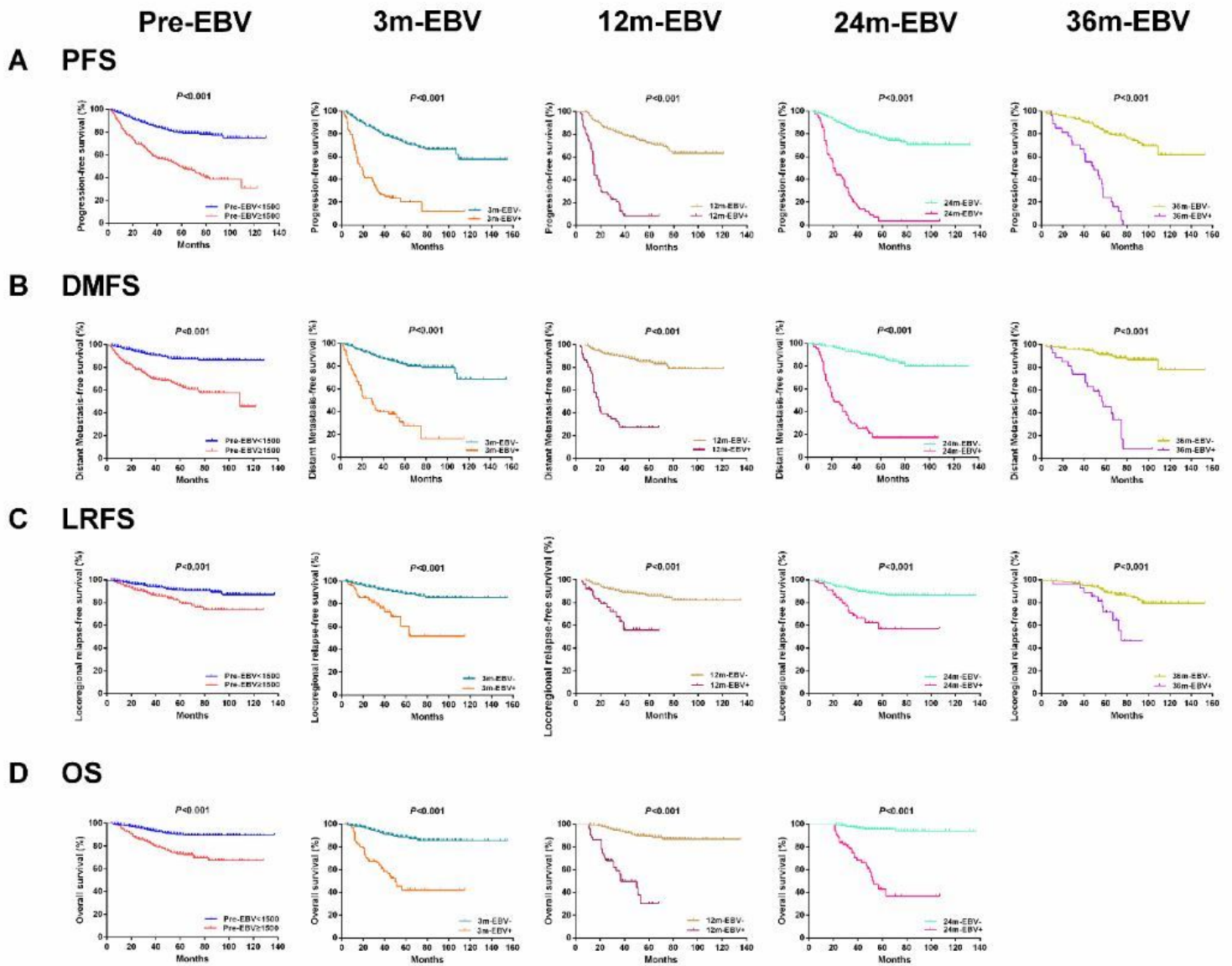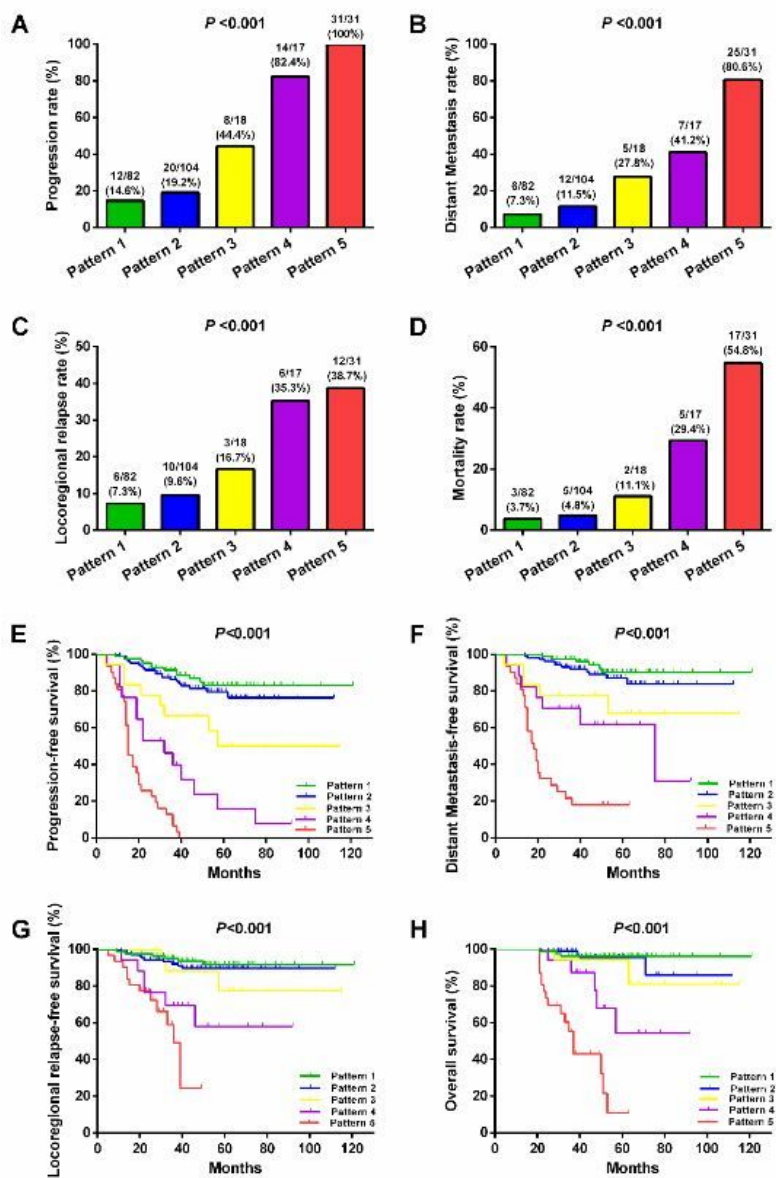**Figure 1**

The flowchart of study design.

Figure 2

Comparisons of the positive rate and viral loads of plasma EBV DNA. (A) the positive rates of plasma EBV DNA at 5 time points among 1077 NPC patients. (B) plasma EBV DNA loads at 5 time points among 1077 NPC patients. (C) the positive rates of plasma EBV DNA at 4 time points among 252 NPC patients. (D) plasma EBV DNA loads at 4 time points among 252 NPC patients.

Figure 3

Kaplan-Meier plots of survival outcomes for subgroups at different time points. (A) Progression-free survival. (B) Distant metastasis-free survival. (C) Locoregional relapse-free survival. (D) Overall survival.

Figure 4

Comparisons of treatment failure rates and survive outcomes among dynamic changes patterns in EBV DNA. (A) Progression rate. (B) Distant metastasis rate. (C) Locoregional relapse rate. (D) Mortality rate. (E) Progression-free survival. (F) Distant metastasis-free survival. (G) Locoregional relapse-free survival. (H) Overall survival.
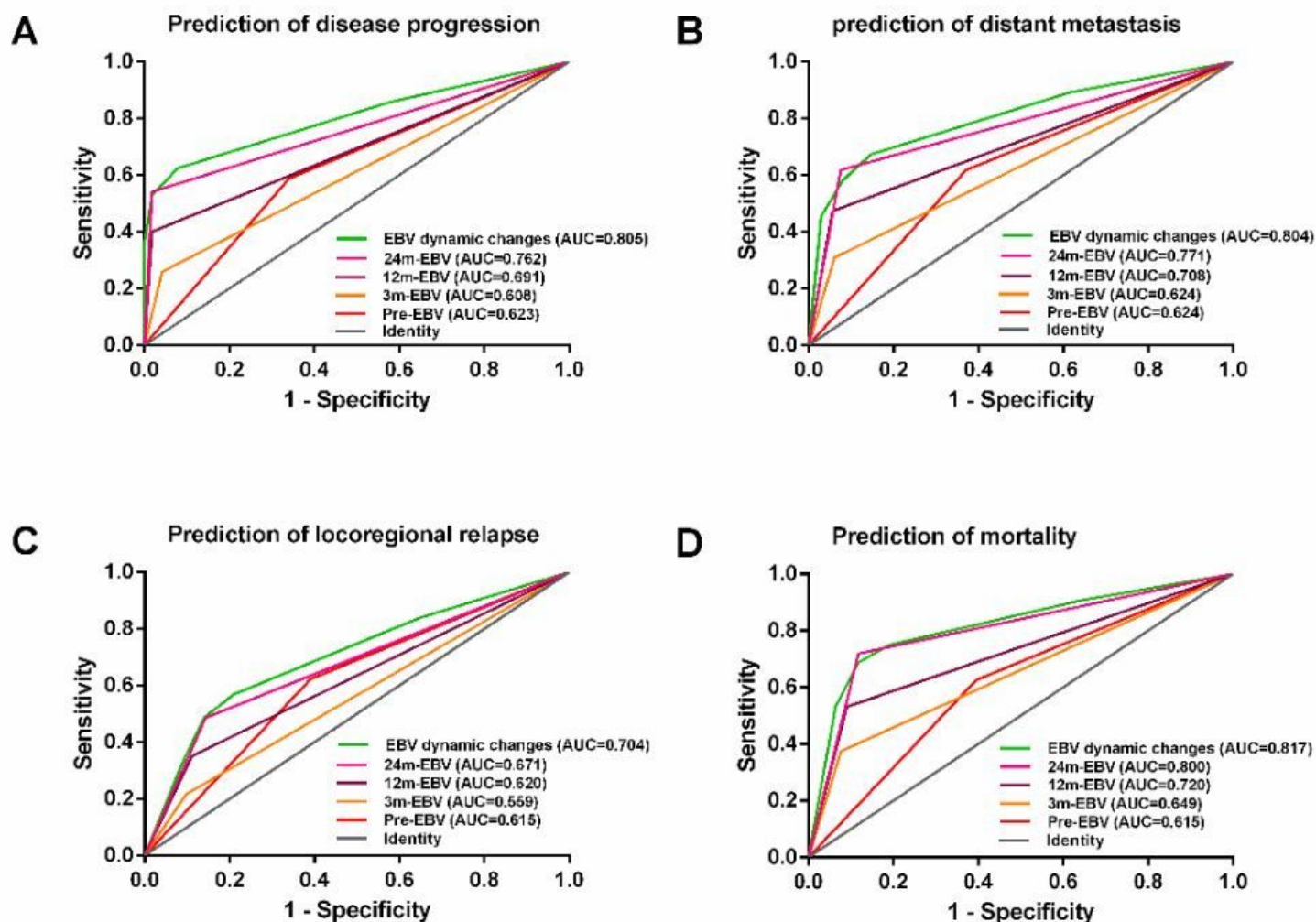
Figure 5

ROC curve analysis in comparing the different prognostic value. (A)Prediction of disease progression. (B) Prediction of distant metastasis. (C) Prediction of locoregional relapse. (D) Prediction of mortality.

Supplementary Files

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