The addition of pretreatment plasma Epstein-Barr virus DNA into the 8th edition of nasopharyngeal cancer TNM stage classification

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Abstract

The 8th edition of the American Joint Committee on Cancer (AJCC)/Union for International Cancer Control (UICC) stage classification (TNM) for nasopharyngeal carcinoma (NPC) was launched. It remains unknown if incorporation of non-anatomic factors into the stage classification would better predict survival. We prospectively recruited 518 patients with non-metastatic NPC treated with radical IMRT +/- chemotherapy based on the 8th edition TNM. Recursive partitioning analysis (RPA) incorporating pretreatment plasma EBV DNA derived new stage groups. Multivariable analyses to calculate adjusted hazard ratios (AHRs) derived another set of stage groups. 5-year progression-free survival (PFS), overall survival (OS) and cancer-specific survival (CSS) were: stage I (PFS 100%, OS 90%, CSS 100%), II (PFS 88%, OS 84%, CSS 95%), III (PFS 84%, OS 84%, CSS 90%) and IVA (PFS 71%, OS 75%, CSS 80%) ($p < 0.001$, $p = 0.066$, and $p = 0.002$ respectively). RPA derived 4 new stages: RPA-I (T1-T4N0-N2 & EBV DNA <500 copies/ml) (PFS 94%, OS 89%, CSS 96%), RPA-II (T1-T4N0-N2 & EBV DNA ≥500 copies/ml) (PFS 80%, OS 83%, CSS 89%), RPA-III (T1-T2N3) (PFS 64%, OS 83%, CSS 83%) and RPA-IVA (T3-T4N3) (PFS 63%, OS 60% and CSS 68%) (all with $p < 0.001$). AHR using covariate adjustment also yielded a valid classification (I: T1-T2N0-N2; II: T3-T4N0-N2 or T1-T2N3, and III: T3-T4N3) (all with $p < 0.001$). However, RPA stages better predicted survival for PFS and CSS after bootstrapping replications. Our RPA-based stage groups revealed better survival prediction compared to the 8th edition TNM and the AHR stage groups.

What's new?

Do newly proposed stage groups incorporating pretreatment plasma EBV DNA better predict survival than the 8th edition of AJCC/UICC TNM stage classification for non-metastatic NPC? We prospectively measured pretreatment plasma EBV DNA in 518 completely staged non-metastatic NPC patients who were later treated with IMRT with/without adjunct chemotherapy. We proposed new stage groups which incorporated pretreatment plasma EBV DNA into recursive partitioning analyses demonstrated significantly better survival prediction as compared to the 8th edition TNM.

Nasopharyngeal carcinoma (NPC) is endemic in southeast Asia including Hong Kong.[1] Radiation therapy with or without adjunct chemotherapy is considered standard radical treatment for non-metastatic disease.[2] Intensity-modulated radiation therapy (IMRT) is the most effective contemporary radiation technique for NPC based on level I evidence.[3-5] Indeed, the latest 8th edition of the American Joint Committee on Cancer (AJCC)/Union for International Cancer Control (UICC) stage classification (TNM) relies on the improved locoregional control by IMRT, in addition to more detailed pretreatment imaging with magnetic resonance imaging (MRI) leading to more homogeneous definitions of T2 (vs. T4) and N3 disease.[6] The AJCC and UICC have been striving to introduce non-anatomic factors to further segregate prognosis in certain disease areas while maintaining anatomic stage groups. So far only a few diseases including prostate cancer, esophageal cancer, cutaneous melanoma, breast cancer, etc have incorporated non-anatomic factors into recent modifications of their respective staging systems.[7,8]

Plasma EBV DNA has also been shown to be an accurate surrogate marker and has been adopted widely in screening, diagnosis, treatment monitoring and posttreatment surveillance from relapse for NPC for the past two decades.[9-15] There is a paucity of information on the use of circulating tumor DNA in the past editions of TNM. However, accumulating evidence suggests that plasma EBV DNA carries important prognostic value and that the current TNM staging system is not suited for outcome prediction in patients who are continuously monitored by plasma EBV DNA.[12,16] Though nomograms have been developed which give important information on survival outcomes on NPC, they are less easy to use, are designed for individual patients, and correspondingly cannot offer precise information on survival differences for treatment comparison and surveillance purposes among different jurisdictions for different stage groups.[17–19] A new pretreatment staging system comprising both anatomic and non-anatomic factors is warranted in this era of precision medicine using modern radiation techniques to improve survival prediction and better stratify high-risk patients for more intensified treatment while sparing low-risk groups from unnecessary therapy.

Materials and Methods

Study population

This prospective observational study received prior institutional review board approval and recruited previously

untreated patients with non-metastatic (M0) NPC (histologically confirmed with positivity on Epstein-Barr encoded RNA by in-situ hybridization) planned to receive IMRT with or without adjunct (concurrent +/– induction or adjuvant) chemotherapy from October 2010 to May 2016 (ClinicalTrials.gov NCT02476669). The study protocol and treatment details are described elsewhere.[20] All patients provided written informed consent before study commencement. They underwent complete pretreatment investigations including MRI and positron-emission tomography with contrast-enhanced computed tomography (PET-CT) using the same scanners, together with serum hematology, biochemistry, lactate dehydrogenase (LDH), hepatitis B serology, plasma EBV DNA, fiberoptic nasopharyngoscopy and nasopharyngeal biopsy at the same treating institution. Two independent specialist head and neck radiologists, blinded to treatment details reviewed all PET-CT and MRI images to determine the disease stage based on 7th edition TNM before treatment and then re-staged each case blindly based on 8th edition TNM. Excellent agreement (Cohen's Kappa 0.83) was observed and any discrepancies were resolved by consensus.

Determination of plasma EBV DNA titers

Plasma EBV DNA before treatment was measured in a single laboratory with the assay method described previously (Supporting Information).[20] Four milliliters (ml) of peripheral blood was drawn for DNA extraction by a QIAamp Blood Kit (Qiagen, Hilden, Germany) within 4 hours of collection. All plasma DNA samples were also subject to real-time polymerase chain reaction (PCR) analysis for the β-*globin* gene, which gave a positive signal on all tested samples. Multiple controls without templates were also included in each analysis as negative controls. All results were expressed as genome copies per ml with accuracy to the nearest 0.1 copy/ml. This study compiled with the REMARK recommendations for tumor marker prognostic studies using biological material.

Treatment

All patients received radical IMRT (70 Gy to primary tumor and positive cervical nodes and 66 Gy to the high-risk nodal areas) in 33 to 35 fractions over 6.5 to 7 weeks. The details of target volume and organ-at-risk (OARs) delineation, IMRT planning, as well as chemotherapy schedules (concurrent, adjuvant and induction) are described in the Supporting Information.[20] Treatment was based on the 7th edition TNM, representing the

operational stage classification when the patients were referred for treatment; Eastern Cooperative Oncology Group (ECOG) performance status and medical comorbidities of each patient were also used. In general, patients with stage I and II disease received IMRT alone while stage III to IVB received concurrent chemoradiation with either adjuvant or induction chemotherapy. Those with bulky cervical nodal (≥ 3cm in diameter) stage II disease received concurrent chemoradiation without adjunct chemotherapy, at the discretion of the treating oncologist.

Post-treatment follow-up

All patients had 6-site random nasopharyngeal biopsies and plasma EBV DNA titres assessed 8 weeks after IMRT, as our routine practice.[21] Local salvage/adjuvant treatment was given to patients who still had persistent primary tumor at 12 weeks after IMRT. If the plasma EBV DNA remained > 0 copy/ml at 8 weeks after IMRT, it would be repeated 4 weeks thereafter until it was undetectable or locoregional disease was proven to exist or clinically or radiologically metastasis was evident. Patients with complete locoregional remission after treatment had regular clinical and imaging follow-up. The clinical surveillance protocol was described in the Supporting Information.

Survival endpoints

We pre-specified progression-free survival (PFS), overall survival (OS) and cancer-specific survival (CSS) as the most appropriate endpoints to evaluate the performance of the current and proposed stage groups on survival prediction.[27] Local failure-free survival, regional failure-free survival and distant metastasis-free survival have been reported as 3-year endpoints in a previous publication.[20]

Statistical analysis

All pre-specified survival endpoints were calculated by Kaplan-Meier methods with log-rank tests for comparisons. While treatment was based on 7th edition TNM, all statistical analyses on survival endpoints and evaluation of performance for survival prediction among different sets of stage groups were based on 8th edition TNM. Recursive partitioning analysis (RPA) for PFS, OS and CSS was conducted with ordinal T (T1, T2, T3, T4) and N (N0, N1, N2, N3) categories based on 8th edition TNM and pretreatment plasma EBV DNA to derive

a new set of RPA stages objectively (Supporting Information). Additionally, Cox regression with multivariable analyses was performed to calculate adjusted hazard ratios (AHR) for the following variables: age, sex, ACE-27,[22] smoking status, treatment modality [concurrent chemoradiation +/− induction/adjuvant vs radiation therapy alone], pre-treatment lactate dehydrogenase (LDH), and all the above with and without pretreatment plasma EBV DNA. The regressions addressed risk of progression, cancer-related death and death from any cause with various T and N combinations considering the order of T and N categories among the stage groups. The stage groups derived using AHR were collectively termed AHR stages. Subsequently the performances of RPA stages and AHR stages were evaluated against the 8th edition TNM in predicting PFS, CSS and OS, by using the survival curve of each stage group for assessment of hazard consistency, hazard discrimination and outcome prediction (in terms of percentage of variation explained) based on the method described by Groome et al.[23] To evaluate the performance of each stage group on survival prediction, the normalized scores which compared each stage schema among all evaluating stage schemas were calculated. The normalized scores range from 0 to 1, with 0 as the best performance and 1 the worst.[23] The normalized scores from all criteria were then summed to an overall score which presented the overall performance of the measured stage schema. Lower overall score indicated better overall performance on survival prediction. Internal validation was addressed using bootstrapping evaluation methods to validate the ranking of different stage schemas. The bootstrap scores and ranks were determined for each stage schema after 1000 bootstrap replications. Database lock was performed on 30th July 2017. Statistical significance was defined as $p < 0.05$ (two-sided).

Results

Patient characteristics

A total of 551 patients were prospectively recruited and 33 patients were excluded from further assessment because of ineligibility (Fig. 1). The dispositions of the remaining 518 eligible patients are listed in Table 1. The demographics and stage distribution based on AJCC/UICC 7th edition and 8th edition TNM are also displayed (Table 1 and Supporting Information Tables S1-S6). The PFS, OS and CSS based on 7th edition and 8th edition TNM were shown as well (Supporting Information Fig. S1). Essentially the 8th edition TNM demonstrated better stage segregation compared to the 7th edition. The pretreatment plasma EBV DNA by stage is also shown (Supporting Information Fig. S2), which was undetectable in 38 patients (7.3%). Their

characteristics were shown (Supporting Information Table S7) and were also included in subsequent statistical analyses.

Performance of the current 8th edition TNM

The median follow-up duration was 5.2 years (range 1.2-6.4 years). Based on 8th edition TNM, the 5-year PFS for stage I, II, III and IVA were 100%, 88%, 84% and 71% (p < .001). The 5-year OS for stage I, II, III and IVA were 90%, 84%, 84% and 75% respectively (p = .066). The 5-year CSS for stage I, II, III and IVA were 100%, 95%, 90% and 80% respectively (p = 0.002) (Supporting Information Fig. S1).

Refined TNM stage by RPA and AHR

The RPA algorithm classified NPC into the following four RPA stages, namely RPA-I (T1-T4N0-N2 & EBV DNA < 500 copies/ml) (PFS 94%, OS 89%, CSS 96%, at 5 years), RPA-II (T1-T4N0-N2 & EBV DNA ≥ 500 copies/ml) (PFS 80%, OS 83%, CSS 89%, at 5 years), RPA-III (T1-T2N3) (PFS 64%, OS 83%, CSS 83%, at 5 years) and RPA-IVA (T3-T4N3) (PFS 63%, OS 60%, CSS 68%, at 5 years) (p < 0.001 for PFS, OS and CSS) (Fig. 2). RPA-IVB would designate metastatic (M1) disease at presentation. Multivariable analyses adjusted for age, sex, smoking, ACE-27 scores, LDH and treatment modality confirmed that higher RPA stage carried an increased risk of disease progression (RPA-II vs. I: HR = 3.07; 95% CI: 1.47–6.43; p = 0.003; RPA-III vs. I: HR = 6.37; 95% CI: 2.79–14.40; p < 0.001, and RPA-IVA vs. I: HR = 6.67; 95% CI: 3.05–14.58; p < .001), death from any cause (RPA-II vs. I: HR = 1.62; 95% CI: 0.82–3.20; p = 0.16; RPA-III vs. I: HR = 2.33; 95% CI: 0.89–6.11; p = 0.09, and RPA-IVA vs. I: HR = 6.18; 95% CI: 2.95–12.97; p < 0.001), and cancer-related death (RPA-II vs. I: HR = 3.81; 95% CI: 1.27–11.48; p = 0.017; RPA-III vs. I: HR = 8.93; 95% CI: 2.44–32.71; p = 0.001, and RPA-IVA vs. I: HR = 16.86; 95% CI: 5.22–54,44; p < 0.001) (Supporting Information Table S8).

AHR modelling also produced the following three AHR stage groups: AHR-I (T1-T2N0-N2), AHR-II (T3-T4N0-N2 or T1-T2N3) and AHR-III (T3-T4N3), with corresponding 5-year PFS rates of 91%, 80% and 63%, respectively (p < 0.001), OS rates of 89%, 83% and 60% (p < 0.001), respectively and CSS rates of 96%, 89% and 68%, respectively (p < 0.001) (Fig. 3). AHR-IV would designate metastatic (M1) disease. These AHR stage groups remained the same, regardless of whether pretreatment plasma EBV DNA was incorporated into

multivariable analysis, as reflected by similar HR within each respective AHR stage (Supporting Information Table S9). Likewise, a higher AHR stage demonstrated an increased risk of disease progression, death from any cause and cancer-related death (Supporting Information Table S10).

Performance of survival prediction of RPA stage against AHR stage groups and 8th edition TNM

We compared our RPA and AHR stages against the 8th edition TNM. RPA stage group performed the best in PFS and CCS prediction, followed by AHR and 8th edition TNM stage groups (Table 2). On the other hand, OS was better predicted by AHR compared to RPA. Both RPA and AHR performed much better than the 8th edition TNM and carried > 80% chance of having higher rankings and superiority compared to the 8th edition. The bootstrap validations also yielded the same findings.

Treatment modality within each RPA stage group

We also analyzed if treatment modality (concurrent chemoradiation +/− induction/adjuvant chemotherapy vs radiation therapy alone) would affect survival difference within each RPA stage. There was no difference in PFS (p = 0.272) and CSS (p = 0.493) among patients in RPA-I who received chemoradiation +/− induction/adjuvant chemotherapy or radiation therapy alone, while the OS difference was just borderline significant (p = 0.046) (Supporting Information Fig. S3 and Table S11). No conclusion can be drawn for higher RPA stages because the majority of patients received more intensive treatment including concurrent chemoradiation for their advanced disease as classified according to the 7th edition TNM.

Discussion

The AJCC/UICC TNM staging classification is the most widely accepted lingua franca to describe tumor extent, which is essential to treatment planning, prognostication, stratification into clinical trials, treatment response evaluation, population surveillance and facilitation of international communication and global cancer control.[24] This study, to the best of our knowledge, is the largest prospective study of unselected population which has employed non-anatomic factor within the UICC framework for NPC, and in particular with emphasis on the recently developed 8th edition TNM. Our results provide robust evidence for its use with better performance to predict survival as compared to the current edition of TNM.

For the past 20 years, the DNA fragment corresponding to the BamHI-W region in the EBV genome has been extensively studied in PCR for quantification of plasma EBV DNA in NPC.[9–15] Lo et al devised one of the first methodologies of quantifying plasma EBV DNA in 1999 with continuous refinement which is also used in their recent NPC screening study and also our current study.[25] It has been the most accurate biomarker for screening, diagnosis, treatment response monitoring, surveillance for recurrence and prognostication of NPC in endemic areas.[9,11-15,25] Two recent meta-analyses, with overlapping prospective and retrospective studies included in each respective meta-analysis, demonstrated that pretreatment plasma EBV DNA titer was a significant prognostic factor.[26,27] Different pretreatment cut-off values of the titres (including 1500 copies/ml and 4000 copies/ml in different studies) have been shown important. Nevertheless, almost all these trials as well as the meta-analyses were conducted using older editions of TNM stage groups and prior to the advent of IMRT. Those trials suggesting a higher cutoff at 4000 copies/ml as prognostic factor had comparable stage distribution (stage III/IV disease 58% and 84.1% respectively) with ours (stage III/IVA 79.9%).[12,16] Such a discrepancy of the plasma EBV DNA cutoff between their studies and our cohort are most liklely attributed to the stage migration after the launch the latest edition of TNM, more dedicated and comprehensive staging workup including PET-CT and MRI and the much improved accuracy of plasma EBV DNA enumeration in our study. Indeed, the assays for plasma EBV DNA have also improved to enhance sensitivities and specificities over the past 15 years. Notwithstanding, harmonization among international institutions is extremely difficult to achieve, as plasma EBV DNA assays are laboratory-developed tests with a diverse heterogeneity of different DNA extraction, purification and stabilization methods, different instruments, different primers and probes that target different regions of the EBV genome, different quantification controls and different reporting units for the results, as commented by Kim et al.[28,29,30] Posttreatment titers are considered more prognostic of OS and recommendations for additional treatment after chemoradiation for patients with persistently elevated titers after treatment have recently been made;[26,27,29] however a significant proportion of patients are vulnerable following intensive radical chemoradiation that often renders them physically intolerant of complete additional treatment. So far there is also no concrete evidence of additional benefit following adjuvant chemotherapy after concurrent chemoradiation.[31,32]

Our study measured plasma EBV DNA consistently by using the same assay in the same institution for all patients. Le et al previously showed that different PCR assays using primer/probe sets for polymerase-1, latent membrane protein-2 (LMP-2) and BamHI-W may yield slightly different plasma EBV DNA concentrations, even when the samples came from endemic regions.[28] Calibration with World Health Organization (WHO) International Standard is not practicable, since it was mainly developed based on the specimens from post-transplant lymphoproliferative disorders and infectious mononucleosis which consisted of both whole-virus and naked EBV DNA, in contrast to NPC in which EBV DNA is usually naked and fragmented.[33,34] Fryer et al also commented that the majority of the current nucleic acid amplification techniques do not distinguish between EBV subtypes.[33] In addition, the recent NPC screening program in Hong Kong revealed that NPC patients have longer EBV DNA fragment lengths compared to non-NPC individuals.[35] The same NPC screening program also noted that the presence of detectable plasma EBV DNA was significantly associated with increasing age, current smoking status and the ambient temperature when plasma sampling was performed ($p < 0.001$ for all factors).[36] This is one of the limitations in our study. Hopefully future concerted efforts can be made to derive a standard quantification assay applicable to NPC, with determination of the fragment lengths as well. Another limitation of our study is the absence of external validation using data from other institutions. We acknowledge this although inherent discrepancy can still exist even if the same assay is used but performed at different time intervals after sample collection and at different institutions based on the reasons mentioned above. Our assay has been the most commonly used method for EBV DNA quantification in endemic areas and internal validation by 1000 bootstrapping replications has confirmed that EBV DNA at 500 copies/ml is a robust cut-off value for prognostication and survival prediction in our RPA model.

We have demonstrated that the stage segregation by the 8th edition of TNM was modestly better than the 7th edition. However, the 8th edition TNM was inferior when compared to our RPA or AHR stage groups in predicting PFS, OS and CSS. While AHR stage groups performed better than RPA in OS prediction, both had at least 80% of chance of superiority to the 8th edition TNM stage groups. When comparing the performance of each staging schema, we considered hazard consistency, hazard discrimination, and outcome prediction. Unlike the approach used by Groome et al,[23] we did not include "balance" as a criterion of performance. The "balance" criterion, which compares the observed and expected proportions of the stage groups, is formulated to help

improve the statistical power but posing no direct clinical relevance. Therefore, we focused on the three mentioned criteria to assess and compare the performance of each staging schema which demonstrated more clinical impact.

Our RPA stage groups provide insights on future treatment strategies for patients in the relatively low-risk RPA-I stage group. No difference in PFS and CSS was found in our patients in RPA-I who received chemoradiation +/− induction/adjuvant chemotherapy or radiation therapy alone for their NPC while there was just only a borderline OS advantage in patients treated with chemoradiation. Though the result may be confounded by the imbalance of number of patients in each treatment modality, it may be inferred here that these RPA-I patients may be managed without chemoradiation. Future prospective clinical trials are warranted to confirm the validity of such treatment strategy when patients are staged based on our proposal.

In conclusion, our proposed RPA staging system was found superior to the 8th edition of AJCC/UICC staging system. Future treatment strategies based on our proposed system can be considered so that a more precise and individualized treatment can be better tailor-made. External validation of our proposed staging system with other institutions is awaited.

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Figure legends

Figure 1. Enrollment of the participants. A total of 518 patients were recruited into the study. They had all complete pretreatment investigations including magnetic resonance imaging and positron-emission tomography with contrast-enhanced computed tomography by the same scanners, together with serum hematology, biochemistry, lactate dehydrogenase (LDH), hepatitis B serology, plasma EBV DNA, fiberoptic nasopharyngoscopy and nasopharyngeal biopsy before receiving intensity-modulated radiation therapy with or without concurrent chemoradiation and/or adjunct chemotherapy.

Figure 2. The proposed stage grouping derived by recursive partitioning analysis (RPA) (*a*). Progression-free survival (*b*), overall survival (*c*) and cancer-specific survival (*d*) for proposed stage groups derived by RPA. Grid for our proposed stage grouping based on RPA (*e*).

Figure 3. The proposed stage grouping derived by adjusted hazard ratios (AHR) (*a*). Progression-free survival (*b*), overall survival (*c*) and cancer-specific survival (*d*) for proposed stage groups derived by AHR. Grid for our proposed stage grouping based on AHR (*e*).

Table 1. Patient characteristics at baseline based on 8th edition of AJCC/UICC staging classification

Characteristic	*n* = 518 (%)
Mean age in years	53
Male/female	385 (74.3) / 133 (25.7)
ECOG performance status	
0	80 (15.4)
1	438 (84.6)
T-classification	
T1	147 (28.4)
T2	72 (13.9)
T3	234 (45.2)
T4	65 (12.5)
N-classification	
N0	60 (11.6)
N1	127 (24.5)
N2	201 (38.8)
N3	130 (25.1)

Overall stage	
I	30 (5.8)
II	74 (14.3)
III	234 (45.2)
IVA	180 (34.7)
Laterality of primary tumor	
Midline	231 (44.6)
Left	160 (30.9)
Right	127 (24.5)
Involvement of retropharyngeal node	388 (74.9)
Median pretreatment plasma EBV DNA in copies/milliliter (range)	588.5 (0–1143750)
Stage I (n = 30)	12 (0–315)
Stage II (n = 74)	321 (0–8850)
Stage III (n = 234)	494 (0–175000)
Stage IVA (n = 180)	2012.5 (0–1143750)
Median pretreatment serum lactate dehydrogenase in international units/liter (range)	
Stage I (n = 30)	179.5 (121–310)
Stage II (n = 74)	185.5 (140–275)
Stage III (n = 234)	197.5 (109–521)
Stage IVA (n = 180)	200 (125–688)
Radical IMRT only	71 (13.7)
Concurrent chemoradiation	91 (17.6)
Induction chemotherapy then concurrent chemoradiation	165 (31.9)
Concurrent chemoradiation then adjuvant chemotherapy	191 (36.9)

AJCC: American Joint Committee on Cancer; EBV DNA: Epstein-Barr virus deoxyribonucleic acid; ECOG: Eastern Cooperative Oncology Group; IMRT: intensity-modulated radiation therapy; UICC: Union for International Cancer Control.

Figure 1

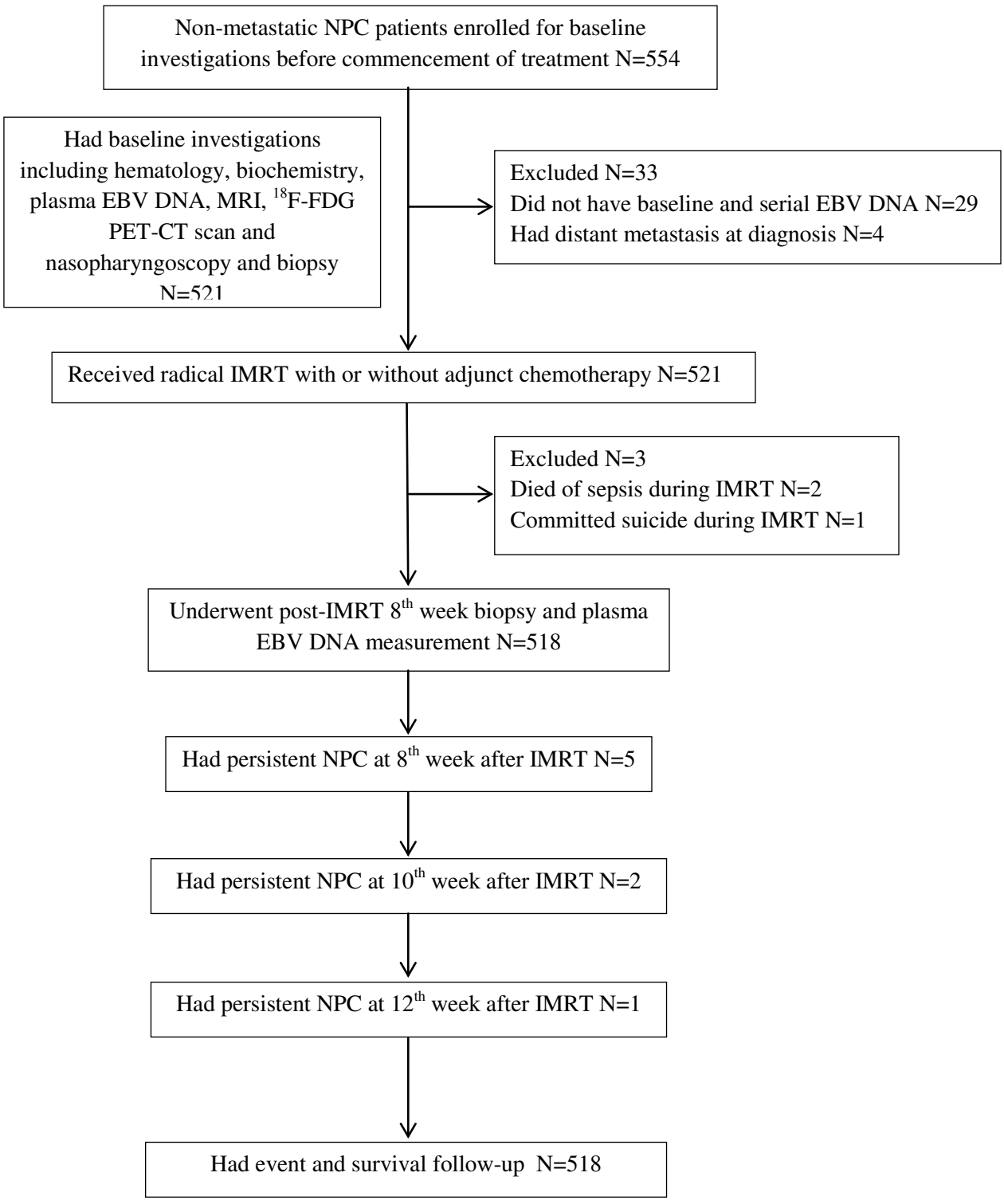
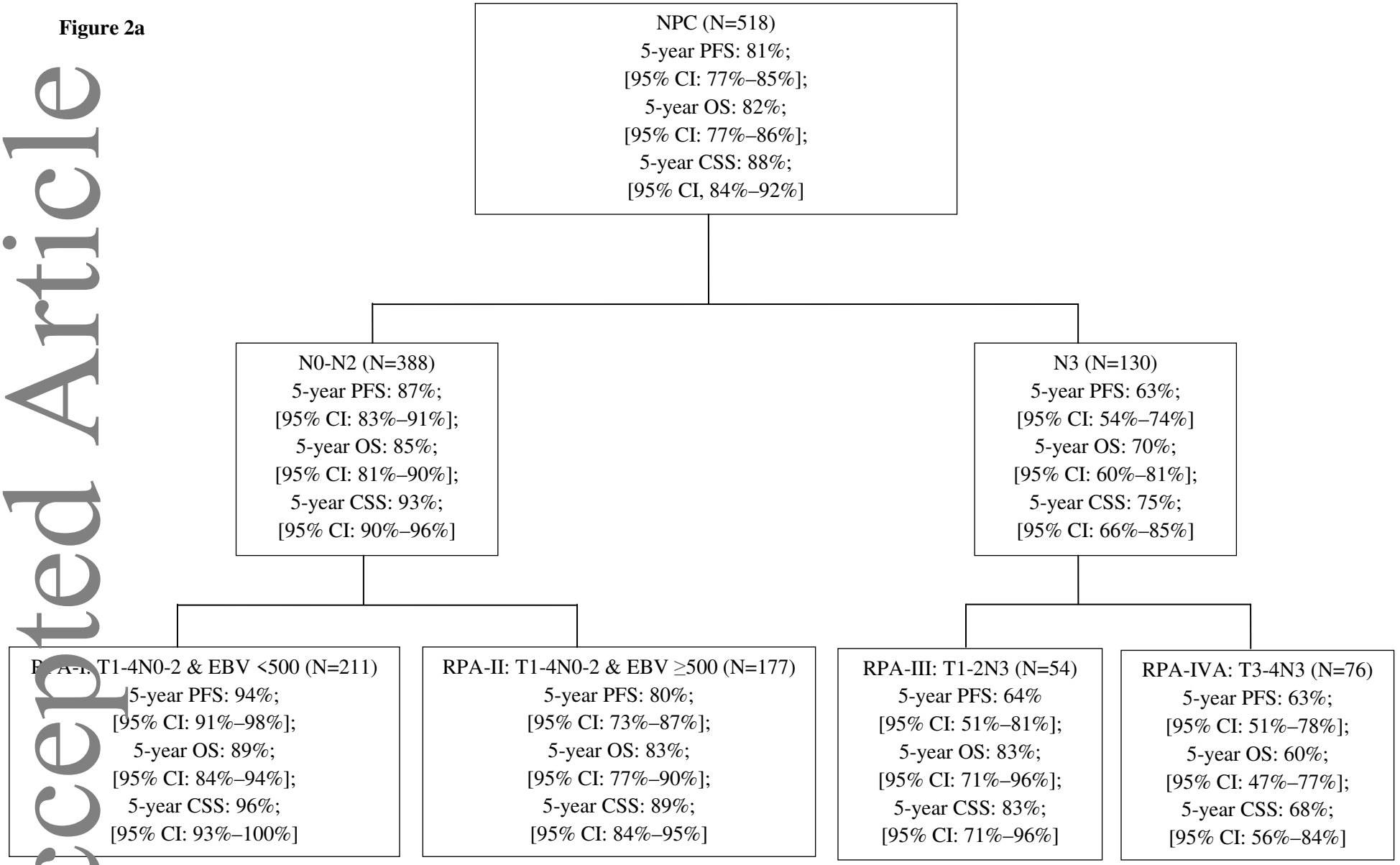
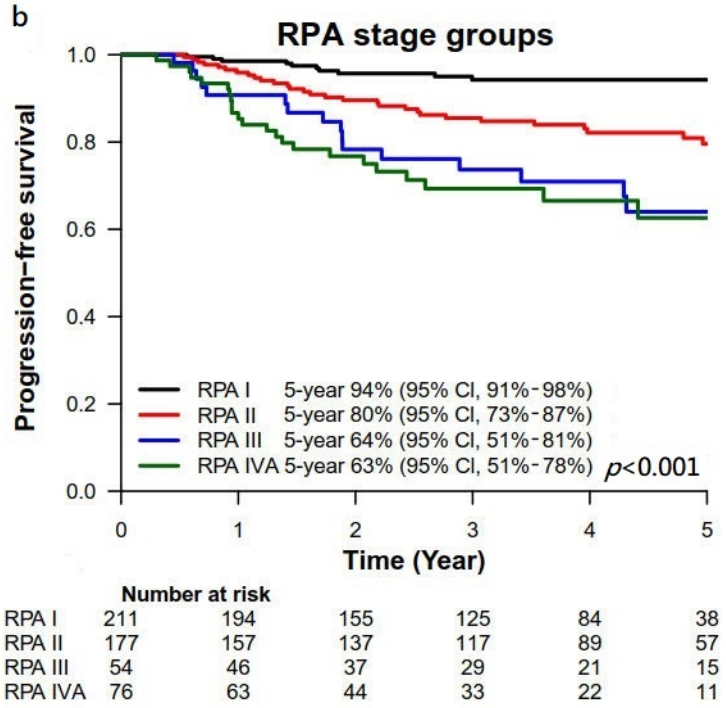
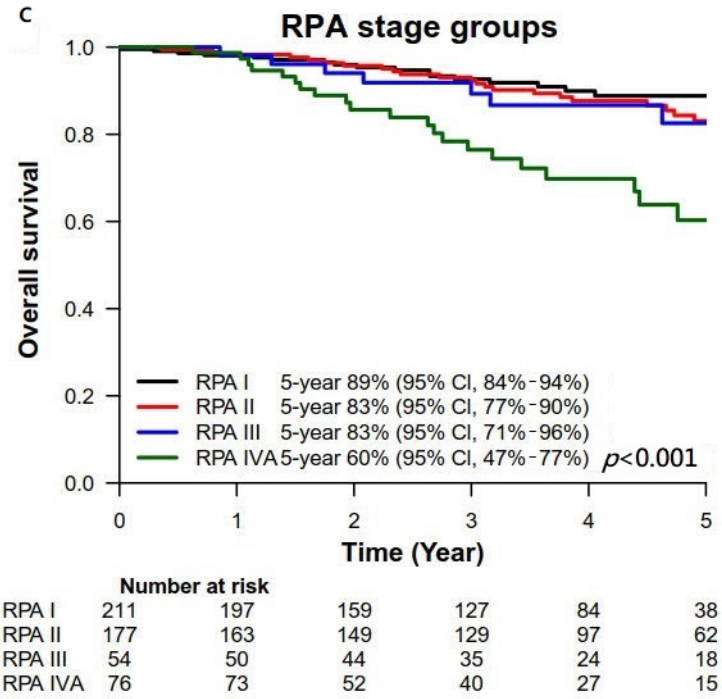
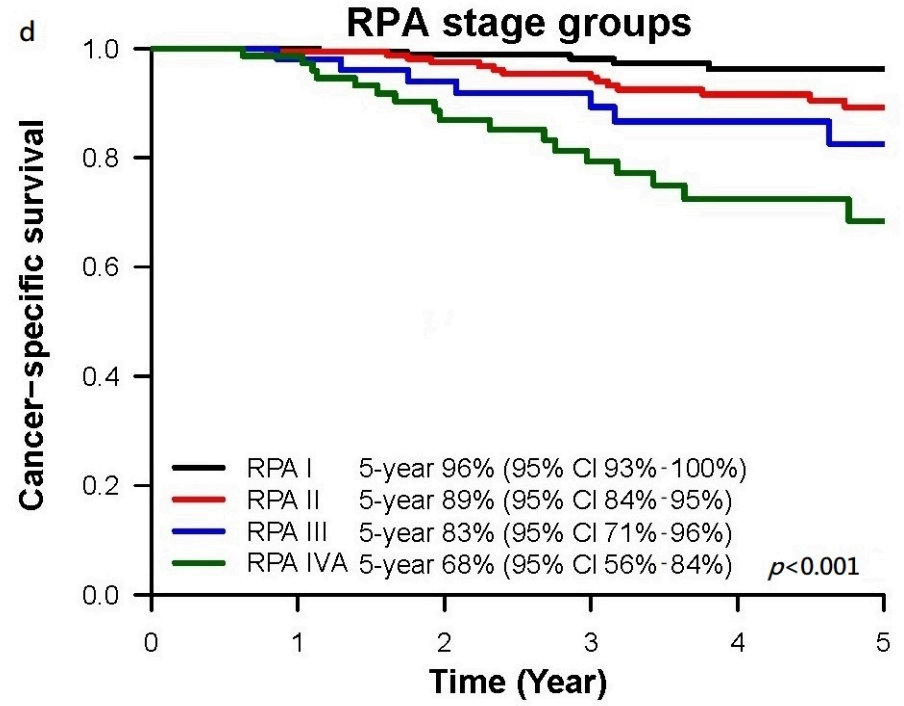


Figure 2a









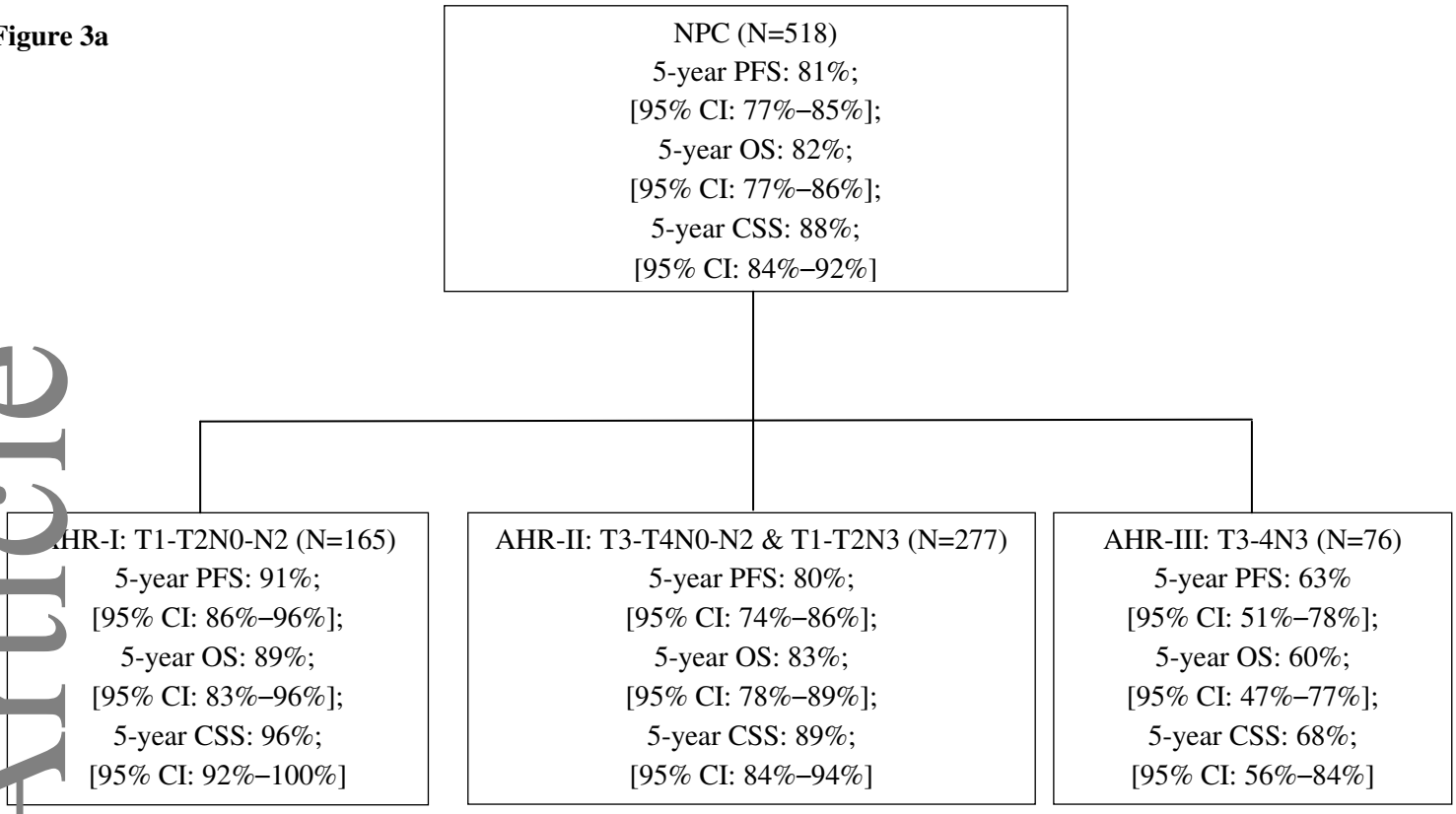
Number at risk

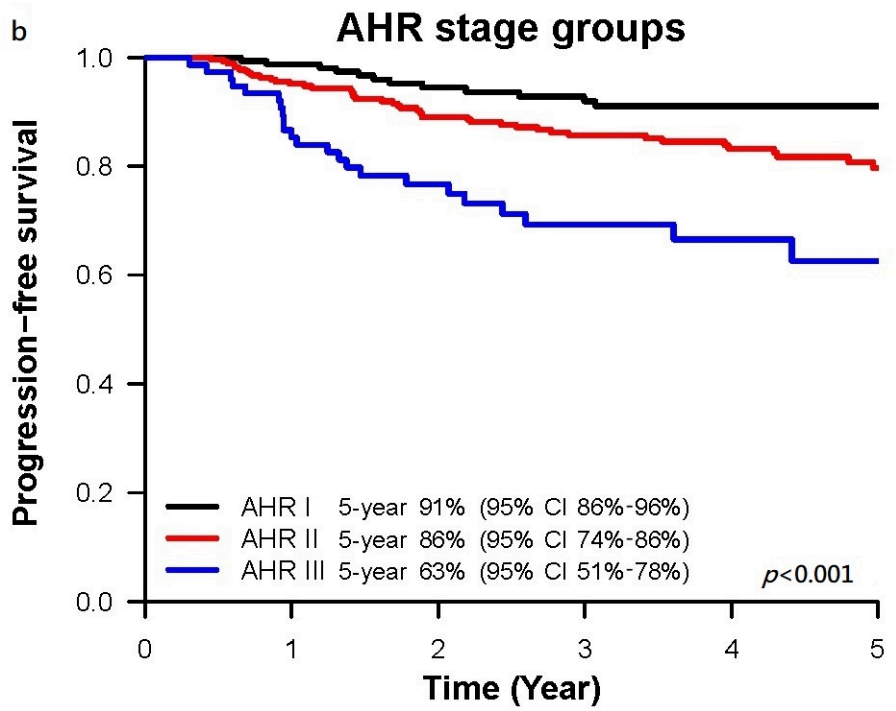
RPA I	211	197	159	127	84	38
RPA II	177	163	149	129	97	62
RPA III	54	50	44	35	24	18
RPA IVA	76	73	52	40	27	15

Figure 2e

RPA stage	N0		N1		N2		N3
T1	Plasma EBV DNA <500 copies	Plasma EBV DNA ≥500 copies	Plasma EBV DNA <500 copies	Plasma EBV DNA ≥500 copies	Plasma EBV DNA <500 copies	Plasma EBV DNA ≥500 copies	III
	I	II	I	II	I	II	
T2	Plasma EBV DNA <500 copies	Plasma EBV DNA ≥500 copies	Plasma EBV DNA <500 copies	Plasma EBV DNA ≥500 copies	Plasma EBV DNA <500 copies	Plasma EBV DNA ≥500 copies	III
	I	II	I	II	I	II	
T3	Plasma EBV DNA <500 copies	Plasma EBV DNA ≥500 copies	Plasma EBV DNA <500 copies	Plasma EBV DNA ≥500 copies	Plasma EBV DNA <500 copies	Plasma EBV DNA ≥500 copies	IVA
	I	II	I	II	I	II	
T4	Plasma EBV DNA <500 copies	Plasma EBV DNA ≥500 copies	Plasma EBV DNA <500 copies	Plasma EBV DNA ≥500 copies	Plasma EBV DNA <500 copies	Plasma EBV DNA ≥500 copies	IVA
	I	II	I	II	I	II	

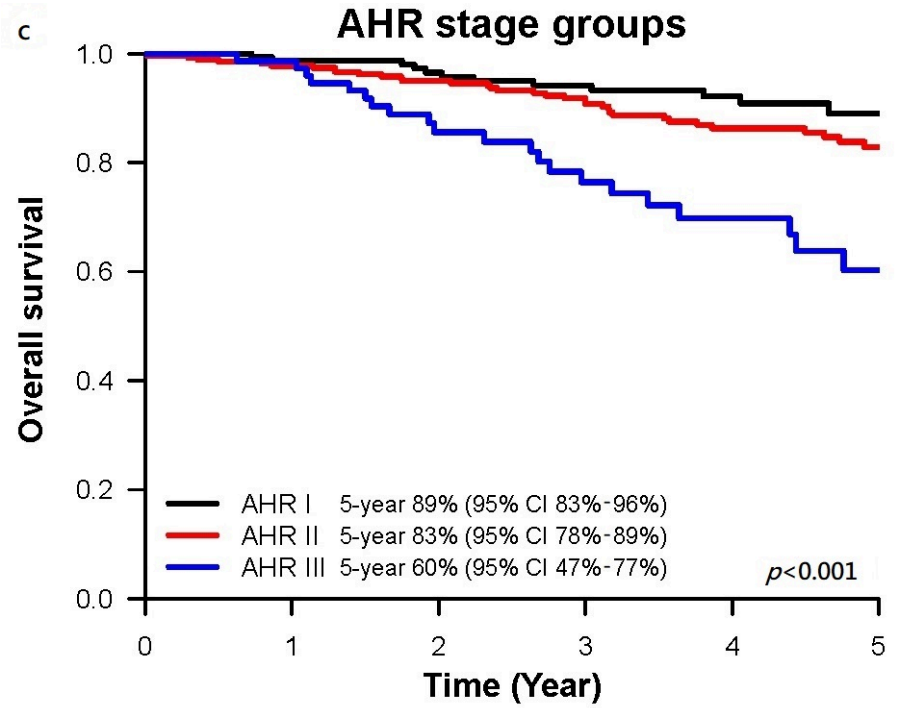
Figure 3a





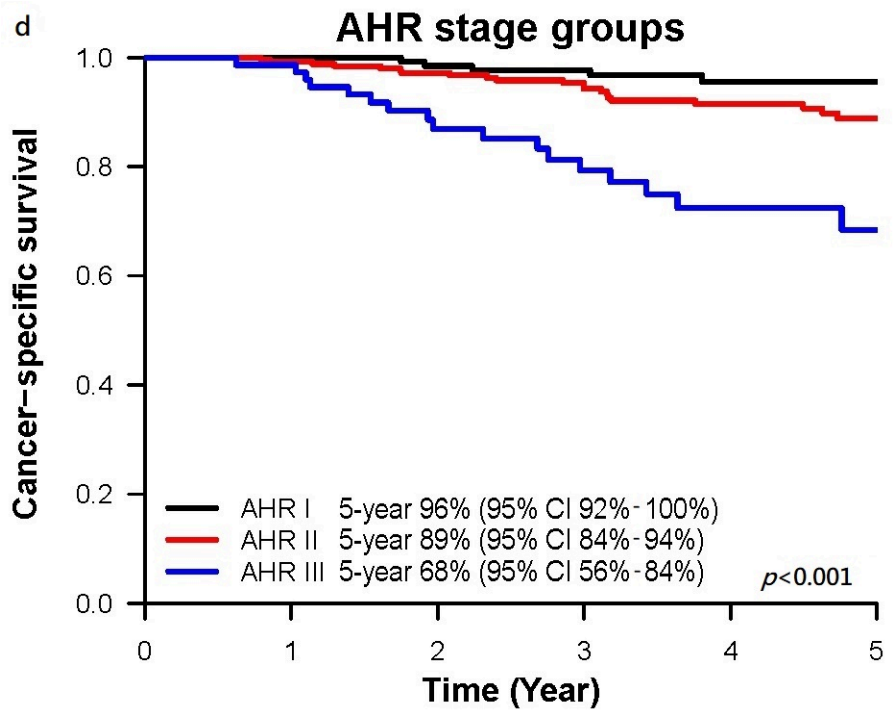
Number at risk

AHR I	165	151	122	104	71	35
AHR II	277	246	207	167	123	75
AHR III	76	63	44	33	22	11



Number at risk

AHR I	165	153	128	109	74	35
AHR II	277	257	224	182	131	83
AHR III	76	73	52	40	27	15



Number at risk

AHR I	165	153	128	109	74	35
AHR II	277	257	224	182	131	83
AHR III	76	73	52	40	27	15

Figure 3e

AHR stage	N0	N1	N2	N3
T1	I	I	I	II
T2	I	I	I	II
T3	II	II	II	III
T4	II	II	II	III

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