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Original research

*L*ongitudinal assessment of plasma *E*BV-DN*A* in non-endemic EBV-related nasopharyngeal cancer (NPC): the *LEA* study

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ABSTRACT

Background: Plasma Epstein Barr Virus (EBV)-DNA is an established biomarker for endemic EBV-related nasopharyngeal carcinoma (NPC). Its relevance in non-endemic regions is less understood. This study longitudinally assessed plasma EBV-DNA (LEA study) throughout the curative management of non-endemic EBV-related NPC to evaluate its prognostic value.

Materials and methods: Between 2012 and 2023, patients with non-endemic, non-metastatic, histologically confirmed EBER+ NPC treated at a tertiary center were retrospectively analyzed. Plasma EBV-DNA levels were measured at pre-treatment (T1), early (T2a, ≤6 weeks) and late (T2b, ≤14 weeks) post-treatment, during follow-up (T3), and after induction chemotherapy (T4), when applicable. EBV-DNA was analyzed for its association with recurrence-free survival (RFS).

Results: At a median follow-up of 60 months (range: 9–134), 167 patients were included. Median age was 50 years (range: 22–75), with 72 % male; 84 % had stage III-IV disease (TNM VIII Edition). Pre-treatment EBV-DNA was detected in 96 % of patients, with no predictive cut-off (median RFS: 43 vs. 62 months for detectable vs. undetectable EBV-DNA). Post-treatment undetectable EBV-DNA correlated with better RFS, also with no cut-off. Follow-up EBV-DNA anticipated recurrence in 71 % of cases by a median of 38 days (range: 11–365). Persistently negative follow-up EBV-DNA was observed in 91 % of non-recurrent patients; isolated positive spikes lacked prognostic significance. Post-induction chemotherapy EBV-DNA demonstrated a 92 % negative predictive value for recurrence.

Conclusions: Plasma EBV-DNA is a valuable prognostic biomarker for non-endemic NPC. Pre- and post-treatment undetectable EBV-DNA holds a positive prognostic value. Post-induction EBV-DNA is the most informative timepoint. Longitudinal EBV-DNA monitoring is warranted in clinical practice.

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1. Introduction

Plasma Epstein Barr Virus (EBV)-DNA is a well-established biomarker for EBV-Encoded RNA (EBER) positive nasopharyngeal carcinoma (NPC) in endemic areas[1], employed to personalize curative treatment decisions[2–5] and to monitor disease during follow-up and recurrence. Endemic countries assess plasma EBV-DNA via multiple copy gene-based assay (*Bam*HI-W), the acknowledged gold standard, validated in the US[6] and largely employed in endemic clinical trials.

In Europe, a non-endemic area, plasma EBV-DNA is measured via European Conformity (CE)-marked, single copy gene-based tests. These assays, originally designed for post-transplantation EBV monitoring, reduce inter-laboratory variability. Currently, the prospective harmonization between *Bam*HI-W and CE-marked methods has not been demonstrated, limiting the routine use of plasma EBV-DNA in non-endemic NPC clinical management. In this regard, our institution attempted to overcome this limitation, demonstrating retrospectively the agreement among CE-marked and *Bam*HI-W assays[7]. Other barriers for broader implementation of non-endemic EBV-DNA include limited comparability with endemic data (e.g., different detection limits/cut-offs)[8], NPC's rarity, poor research funding, and lack of widespread access to EBV-DNA assays[7]. Our institution previously attempted to 'fill the gap' in non-endemic plasma EBV-DNA: *Alfieri et al* [9] retrospectively described the positive prognostic role of negative baseline plasma EBV-DNA in 130 locally advanced, EBER+ NPC patients, consistent with previous data[10]. However, plasma EBV-DNA performance throughout the full trajectory of non-endemic NPC curative management remains unexplored[11].

This study aims to provide a longitudinal assessment of plasma EBV-DNA (LEA study) in a single-institution cohort of non-endemic, EBV-related NPC patients throughout curative management.

2. Materials and methods

This is a retrospective, single-institution, observational study including EBER+ NPC patients curatively managed at Fondazione IRCCS Istituto Nazionale dei Tumori di Milano (INT) from January 2012 to January 2023. The study received INT Ethics Committee Approval (n. 150/23); written informed consent was obtained from all alive participants. Patients were treated according to main international clinical practice guidelines recommendations[12–16]: for details concerning chemotherapy and radiotherapy in TNM stage I-IV [American Joint Committee on Cancer (AJCC) VIII Edition[17]] NPC patients, see Figure 1A. Patients treated with a palliative intent or with upfront recurrent/metastatic stage were excluded. Although oligo-metastatic patients – especially in case of bone-only metastases – are amenable

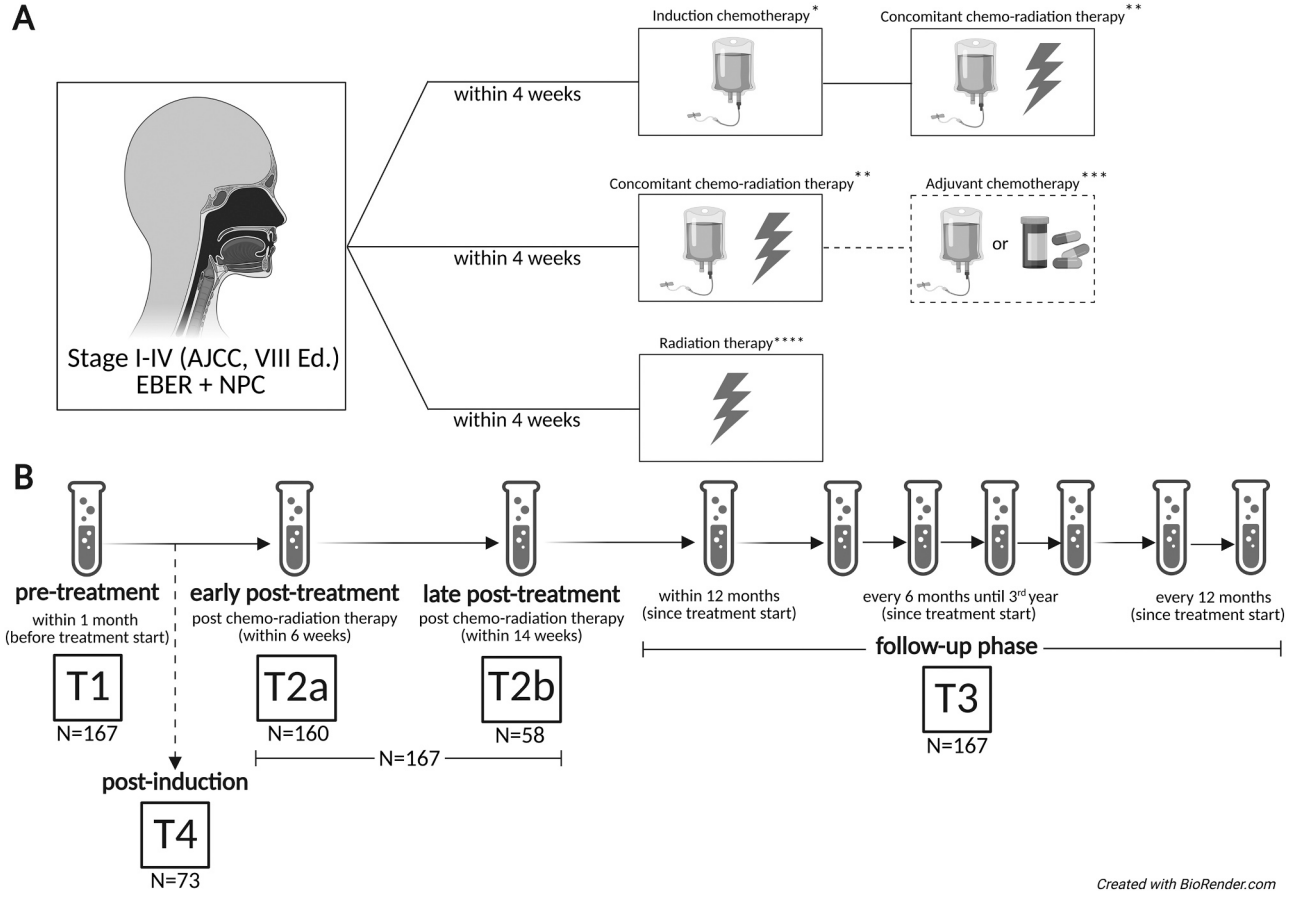

Fig. 1. Therapeutic algorithms for patients with locally-advanced EBER+ nasopharyngeal carcinoma (NPC) and timepoints of longitudinal plasma EBV-DNA sampling. A. Curative management of locally advanced EBER+ NPC patients. *3-weekly (day 1,8) platinum salts + gemcitabine or 3-weekly platinum salts + 5-fluorouracil + /- taxane (up to 3 cycles). **weekly or 3-weekly platinum salts concomitant to radiation therapy. All patients were treated with Volumetric Modulated Arc Therapy (VMAT). Total prescription dose was 70 Gy either by conventional fractionation (2 Gy per fraction, 5 fractions per week) or by moderately hypo-fractionated regimen (2.12 Gy per fraction, 5 fractions per week), using a sequential or simultaneous integrated boost approach. ***3-weekly (day 1–14) non-metronomic oral capecitabine (up to 6 months) or metronomic (daily) oral capecitabine (up to 1 year) or i.v. 3-weekly (day 1,8) gemcitabine + platinum salts (up to 6 cycles) or i.v. 4-weekly 5-fluorouracil + platinum salts (up to 3 cycles). **** All patients were treated with VMAT as described above.**B.** Longitudinal assessment of plasma EBV-DNA. Concerning T3 specifically, EBV-DNA plasma sampling was performed within 12 months since treatment start, every 6 months until the 3rd year since treatment start, and every 12 months from the 4th year onwards.

for potentially curative treatment[18,19], we excluded them from analyses, to limit potential confounding and sample heterogeneity.

Figure 1B describes study design and longitudinal data collection methods of plasma EBV-DNA at the different timepoints: T1 (pre-treatment), T2 (post-treatment), T3 (follow-up). T2 was subdivided into early (T2a, ≤6 weeks) vs. late (T2b, ≤14 weeks) post-treatment according to literature[20]; the authors updated the sampling timing schedule[21] after the completion of our study procedures: when both timepoints were available, only T2b was considered. T4 quantification was performed for patients undergoing induction chemotherapy. T3 had more than one sample collected. Patients lacking one or more of the three crucial timepoints (T1, T2, T3) were excluded. For details, see Supplementary Figure 1.

2.1. Biological Sample Collection

From 2012–2023, we collected peripheral blood (PB) samples from all EBER+ NPC patients treated with curative intent at our institution (INT). PB was collected in 2x6mL K2EDTA tubes, and plasma was obtained by centrifugation at 2000xg for 10 min. Plasma samples were re-identified, aliquoted and stored at −80°C until EBV-DNA quantification.

2.2. EBV-DNA detection

EBV-DNA viral loads were measured on plasma samples by quantitative real-time polymerase chain reaction (RT-PCR). Four different methods were used from 2012 to 2023; all are CE-IVD marked and target a single-copy gene. Plasma EBV-DNA quantification assays were performed according to manufacturer's instructions. Results were calculated in International Unit per milliliter (IU/mL) or copies/mL, previously shown to be comparable[7]. For the extraction and assays' characteristics, see Supplementary Table 1.

2.3. Data analysis

Main baseline patient (sex, age), disease (TNM AJCC VIII Edition stage, locoregional and/or distant recurrence) and treatment characteristics [radiation (RT), concomitant chemo-radiation (cCRT) with/without induction chemotherapy (ICT) and/or adjuvant chemotherapy (CT), diagnostic neck surgery including either excisional lymph node biopsy or latero-cervical lymph node dissection] were profiled using descriptive statistics.

The analysis focused on EBV-DNA's longitudinal evolution in single NPC patients. EBV-DNA loads, according to our previous results[7], were classified as EBV negative (EBV-) if EBV-DNA was not detectable, and EBV positive (EBV+) if EBV-DNA was detectable and quantifiable, or detectable but unquantifiable (EBV-UQ, i.e., below the lower limit of assay detection). In our previous study, patients with EBV-UQ had a similar behavior as those with detectable and quantifiable EBV-DNA[9].

At pre-treatment (T1), we characterized the baseline EBV-DNA load and its correlation with recurrence-free survival (RFS). Non-parametric statistics were used (Kruskal-Wallis or Mann-Whitney test) with $p < 0.05$ as significance threshold. We investigated pre-treatment EBV-DNA quantification as a predictor of recurrence estimating the area under the Receiver Operating Curve (ROC).

At each timepoint (T2a and T2b, T3, T4), we assessed EBV-DNA's (EBV+, EBV-) predictive ability for recurrence. Confusion matrices were used to calculate accuracy, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). The lead time (number of days elapsing between the first positive follow-up quantification and the clinical/radiological diagnosis of disease recurrence) was estimated. EBV-DNA status (EBV+, EBV-) and EBV-DNA viral load were stratified according to disease and therapy characteristics.

At follow-up (T3), we considered EBV-DNA load at the last timepoint before last follow-up or recurrence, identifying three patterns: i) *persistently negative*, including consistent EBV- at all T3 timepoints; ii) *last positive*, including EBV+ at the last T3 timepoint(s) before recurrence or at last follow up; iii) *positive spike*, including one or more EBV+ followed by re-negativization (i.e., EBV- at the last time point before recurrence or last follow-up).

Data were reported as median (range); lead time was also reported as mean.

3. Results

3.1. Study population

As in Table 1, 167 patients were retrieved for this retrospective analysis, most being male (120/167, 72 %) with a median age at diagnosis of 50 years (range: 22–75). Most presented with stage III (48/167, 29 %) or IV (92/167, 55 %) disease. Overall, 29/167 patients (17 %) underwent diagnostic neck surgery, and 87/167 (52 %) were treated with ICT followed by cCRT.

At a median follow-up of 60 months (range: 9–134; 5 % of patients had <1 year of follow-up), 42/167 patients recurred (25 %) with a median RFS of 46 months (range: 1–130), and 139/167 (83 %) were alive at data cut-off.

Disease recurrence occurred at a median of 13 months (range: 1–116). Post-induction sampling was available for 73/167 patients, mostly after 2 (39/73, 53 %) or 3 (33/73, 46 %) ICT cycles.

3.2. Pre-treatment EBV-DNA (T1)

Median EBV-DNA load was 2.55 log IU/mL (range: 0–5.09), while being undetectable in 6/167 (4 %) patients. Of them, 2 were stage II, 1 was stage III, and 3 were stage IVA. Median plasma EBV-DNA was

Table 1
Baseline patients' characteristics.

Baseline patients' characteristics (N = 167)		N (%)
Sex	Male	120 (72 %)
	Female	47 (28 %)
Age	50 yo (median)	IQR: 22–75 yo
Stage (AJCC VIII Edition)	I	2 (1 %)
	II	25 (15 %)
	III	48 (29 %)
	IVA	87 (52 %)
	IVB	5 (3 %)
Diagnostic neck surgery	No	138 (83 %)
	Yes	29 (17 %)
Disease recurrence	No	125 (75 %)
	Yes	42 (25 %)
Status	Alive	139 (83 %)
	Dead	17 (10 %)
	Lost to FUP	11 (7 %)
Curative treatment	ICT + cCRT	87 (52 %)
	cCRT	71 (43 %)
	RT	2 (1 %)
	cCRT + adj CT	7 (4 %)
Type of recurrence	Local	13 (31 %)
	Regional	4 (10 %)
	Locoregional	3 (7 %)
	Distant	18 (42 %)
	Distant + locoregional	4 (10 %)
RFS (since curative treatment end)	46 mo (median)	IQR: 1–130 mo
ICT	Yes	73 (44 %)
	No	94 (56 %)
N of ICT cycles at post-induction sampling	1	1 (1 %)
	2	39 (53 %)
	3	33 (46 %)

AJCC, American Joint Committee on Cancer; IQR, interquartile range; FUP, follow-up; ICT, induction chemotherapy; cCRT, concomitant chemo-radiation; RT, radiotherapy; adj CT, adjuvant chemotherapy; mo, months; yo, years old; RFS, recurrence-free survival; N, number.

2.69 log IU/mL (range: 1.59–5.09) in recurrent vs. 2.45 log IU/mL (range: 0–4.94) in non-recurrent patients, with no significant difference considering all quantification assays (p = 0.23, Figure 2A), and analyzing them separately (Supplementary Figure 2). However, none of the six patients with EBV- at T1 recurred, and they always showed EBV- values throughout follow-up; these patients had a median RFS of 62 months (range: 51–65), higher than the overall study population (median RFS: 43 months, range 1–131). RFS did not correlate with pre-treatment EBV-DNA load (Figure 2B).

The ROC curve was not significant either considering all patients (AUC = 0.570), or considering only stage III-IV (AJCC VIII Edition [17]) patients (AUC = 0.573), in lack of a definite predictive cut-off value of baseline EBV-DNA (Supplementary Figure 3). Moreover, significance was not reached (all patients AUC = 0.547; stage III-IV AUC = 0.550) with plasma EBV-DNA levels expressed either in IU/mL (Abbott and Elitech), or in copies/mL (other methods).

3.3. Post-treatment EBV-DNA (T2)

We collected samples from 160 patients at T2a and 58 at T2b: 48 patients had samples collected at both T2a and T2b.

Considering T2a and T2b separately, NPV and PPV were equivalent (81 % and 19 %, respectively). Figure 3A shows the EBV-DNA dynamic load between T2a and T2b in 48 patients with both samples. Of these, excluding 27 patients who were EBV- at T2a, remained EBV- at T2b and did not recur, and one who was EBV+ both at T2a and T2b and recurred, the other 20 showed mixed patterns, with an equal recurrence distribution between EBV+ and EBV- at T2a or T2b.

Considering the T2 timepoint overall (T2a + T2b), 148/167 patients had EBV- and 19/167 had EBV+ . Of these 19 patients, 11 recurred. Out of all 42 recurrent cases, the remaining 31 patients were EBV-. Prediction accuracy was 77 %, with a sensitivity of 26 %, and a specificity of 94 %. PPV and NPV were 58 % and 79 %, respectively (Figure 3B). NPV increased when considering shorter recurrence time windows (6 months: 98 %; 36 months 82 %, Table 2). In recurrent patients with EBV+ at T2, the median time to recurrence was 5 months (range 0–21).

Most of 19 patients with EBV+ at T2 (N = 16/19, 84 %) had an EBV-UQ load, without any difference in median pre-treatment EBV-DNA viral load between non-recurrent vs. recurrent patients (median log IU/mL EBV-DNA= 3.1 vs. 3.2).

Of the 16 patients with EBV-UQ at T2, 8 patients recurred while 8 did not; all patients with a quantifiable EBV-DNA load (N = 3) recurred. Concerning RFS, the 3 patients with EBV+ quantifiable viral load recurred at a mean of 10.3 months, with an inferior RFS compared to patients with EBV- (mean 26.8 months, p = 0.07); patients with EBV-UQ had an intermediate RFS (15.9 months, p = 0.07).

EBV-DNA at T1 was higher in patients with EBV+ at T2 (median 3.10 log IU/mL) compared to patients with EBV- at T2 (median: 2.53 log IU/mL, p = 0.022). In recurrent patients, RFS was higher in patients with EBV- at T2 (median: 20 months) compared to patients with EBV+ at T2 (median: 12 months, p = 0.01).

3.4. Follow-up (T3)

In recurrent patients, median EBV-DNA load at T3 was 2.3 log IU/mL (range: 0–5.9), with no difference between locoregional vs. distant recurrence (p = 0.188).

To study EBV-DNA evolution with recurrence, we considered the last follow-up samples collected either before recurrence, or before the last follow-up visit (if non-recurrent). We defined three EBV-DNA patterns: i) *persistently negative*; ii) *last positive*; iii) *positive spike* (**Data analysis**).

As shown in Figure 4A, most non-recurrent patients (N = 114/125, 91 %) showed a *persistently negative* EBV-DNA profile, with only 1 % (N = 1/125) *last positive* EBV-DNA. Conversely, 71 % (N = 30/42) of recurrent patients showed *last positive* EBV-DNA, and 17 % (N = 7/42) were *persistently negative* (Figure 4B). In recurrent patients with *last positive* EBV-DNA (excluding 9 patients with last follow-up in the range of ±1 week from recurrence), the biomarker anticipated recurrence with a mean time of 72 days (median: 38 days; range: 11–365).

3.5. Post-induction EBV-DNA (T4)

Considering post-induction EBV-DNA samples (N = 73/167), 51 % (N = 37/73) were EBV+ and 49 % (N = 36/73) EBV-, with most samples collected after 2 or 3 ICT cycles (Table 1). The overall accuracy of EBV+ as predictor of recurrence at T4 was 70 % (Figure 5A), with a sensitivity of 86 % and a specificity of 63 %. The NPV was 92 %, and the PPV was 49 %. Considering patients with EBV+ at T4 (N = 37), the evolution of EBV-DNA viral load from T4 to T2 (Figure 5B) did not add prognostic information; however, with a more longitudinal evaluation from T4 to T2 to T3, EBV+ and EBV- samples seemed to qualitatively better segregate patients into recurrent vs. non-recurrent, respectively (Figure 5C). ICT cycles number did not significantly impact post-induction EBV-DNA predictive value: at 2 ICT cycles, NPV was 94 % and PPV 52 %, while at 3 ICT cycles NPV was 90 % and PPV 38 %.

To further examine our results, we ran a multivariate logistic regression analysis including EBV-DNA load at T1, T2, and T4 as covariates to predict recurrence, considering patients who had T4 EBV-DNA sampling (Supplementary Figure 4). We found that plasma EBV-DNA load at T4 was significantly associated with recurrence (p = 0.004), whereas the value at T1 and T2 were not, which was expected since almost all patients at T1 (161/167) were EBV+ , being EBV- at T2 (148/167).

Supplementary Figure 5 depicts the overall dynamics of EBV-DNA

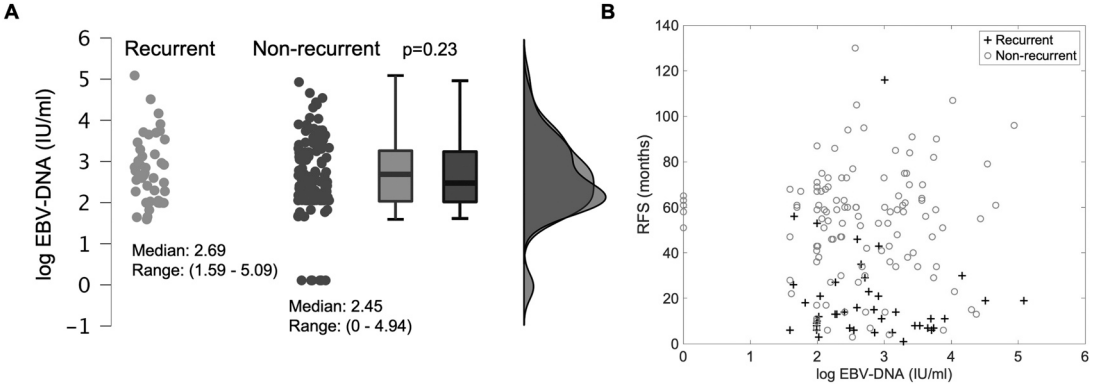

Fig. 2. Distribution and predictive value of pre-treatment (T1) plasma EBV-DNA. A. Distribution of EBV-DNA load (log IU/mL) at pre-treatment in recurrent (light grey) and non-recurrent (dark grey) patients. The plot presents individual values, the aggregated box-plot, and the superimposed probability density estimates. **B.** Scatter plot of the correlation between pre-treatment EBV-DNA load (log IU/mL), x-axis, and recurrence-free survival (RFS, months), y-axis.

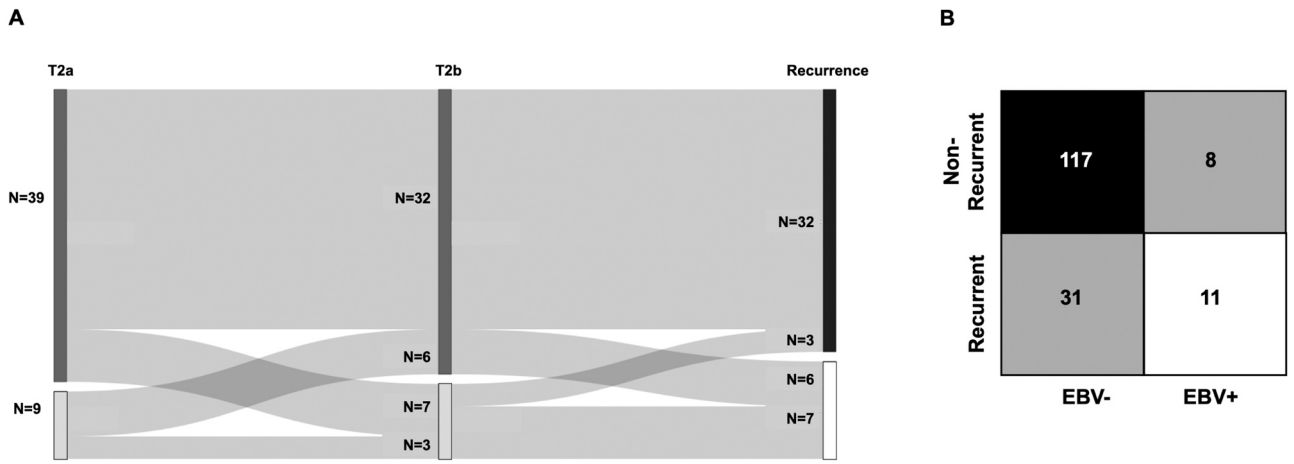

Fig. 3. Dynamics and predictive value of post-treatment (T2) plasma EBV-DNA. A. Sankey diagram (N = 48 patients with both T2a and T2b samples) representing the evolution of EBV-DNA load (EBV- in dark grey; EBV+ in light grey) during post-treatment phase, from early (T2a) to late (T2b) post-treatment assessment, in relation to disease recurrence (non-recurrent in black; recurrent in white). Apart from 27 patients who were EBV- at T2a, remained EBV- at T2b and did not recur, and one patient who was EBV+ both at T2a and T2b and recurred, all the other patients showed mixed patterns, with an equal distribution between EBV+ and EBV- at T2a and/or T2b among recurrent vs. non-recurrent. **B.** Confusion matrix (N = 167) of the predictive capability of post-treatment (T2) positive/negative EBV-DNA load for recurrence.

Table 2
Performance of post-treatment (T2) plasma EBV-DNA in predicting disease recurrence.

	6 mo	12 mo	24 mo	36 mo	overall
Accuracy	90 %	86 %	83 %	80 %	77 %
Sensitivity	63 %	40 %	34 %	30 %	26 %
Specificity	91 %	93 %	94 %	94 %	94 %
PPV	26 %	42 %	58 %	58 %	58 %
NPV	98 %	92 %	86 %	82 %	79 %

PPV, positive predictive value; NPV, negative predictive value; mo, months (after curative treatment completion).

throughout curative management in our NPC cohort.

4. Discussion

In our single-institution, non-endemic EBER+ NPC sample, we first performed a longitudinal assessment of plasma EBV-DNA (LEA study) throughout NPC curative management. To our knowledge, although single-center and retrospective, this is the largest, non-endemic, EBV-related NPC cohort in literature, with a prolonged follow-up (median: 60 months)[10]. In our analyses, undetectable pre- and post-treatment plasma EBV-DNA correlated with a favorable prognosis. Post-induction timepoint was the most informative one (NPV 92 %). During follow-up, systematic EBV-DNA monitoring provided a clinically relevant added value.

Pre-treatment EBV-DNA revealed no prognostic cut-off, regardless of the detection methods and disease stage. This may result from concurrent factors, including a lower baseline viral load[22–24]. Unlike the endemic 'biomarker-integrated' approach, non-endemic EBER+ NPC treatment algorithm remains entirely 'stage-driven', preventing biomarker-driven decisions (i.e., treatment intensification or de-escalation). In this regard, a limit of our work is the TNM staging defined as per AJCC VIII Edition, as IX Edition was only recently updated [25]. In absence of a prognostic cut-off value, only undetectable pre-treatment plasma EBV-DNA (rate: 4 %) holds a positive prognostic significance, while detectable UQ vs. quantifiable baseline values yield similar outcomes, consistent with previous findings[9].

Post-treatment evaluations demonstrated high NPV and specificity for recurrence prediction, maintained over time. Patients with EBV-UQ showed mixed outcomes (8 out of 16 patients recurred), while those with quantifiable EBV-DNA were likely to recur (3 out of 3 patients), in absence of a defined cut-off. Early (T2a) and late (T2b) post-treatment EBV-DNA yielded comparable results, diverging from the dynamic predictive behavior described in endemic data [20,21]. This discrepancy may reflect the late implementation of serial T2a-T2b testing in our cohort, due to recent endemic data on repeated late post-treatment evaluations[21]. In other words, we believe that the lack of a

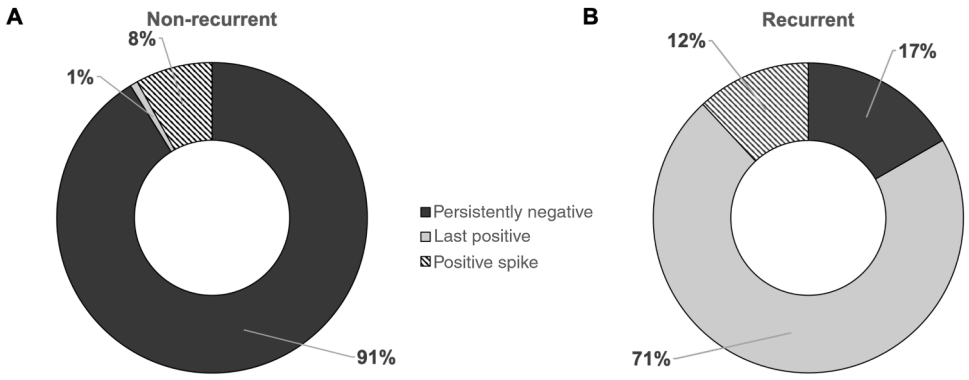

Fig. 4. Plasma EBV-DNA load predictive value during follow-up (T3). Distribution of EBV-DNA profile during follow-up (T3), in **A.** Non-recurrent and **B.** Recurrent patients.

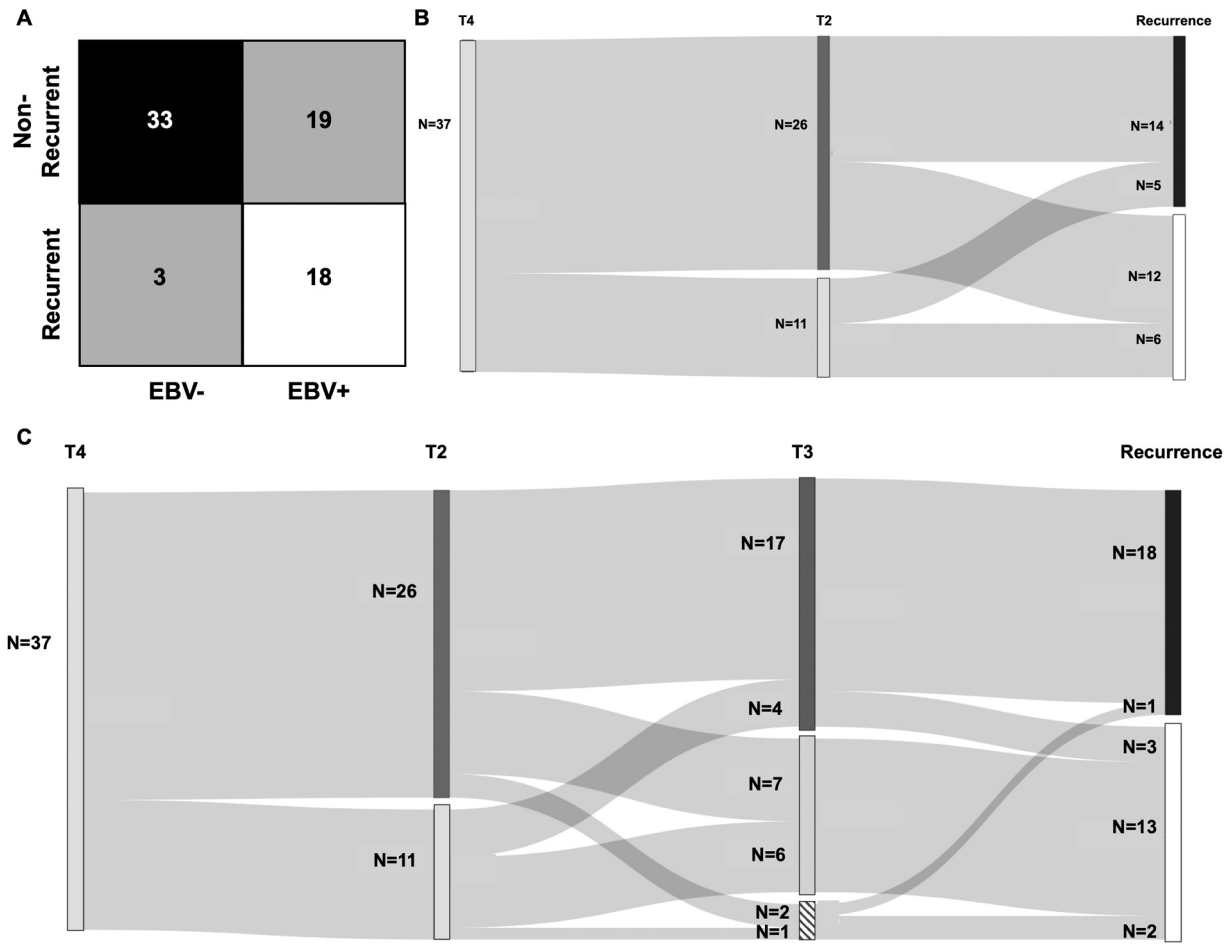

Fig. 5. Predictive value and dynamics of post-induction (T4) plasma EBV-DNA. A. Confusion matrix of the predictive capability of post-induction positive/negative EBV-DNA load for recurrence **B.** Sankey diagram representing the evolution of EBV-DNA load (EBV- in dark grey, EBV+ in light grey) from post-ICT (T4) to post-treatment (T2), in relation to recurrence (non-recurrent in black, recurrent in white). The diagram focuses on subjects with EBV+ at post-ICT (T4; N = 37). **C.** Sankey diagram representing the evolution of EBV-DNA load (EBV- in dark grey, EBV+ in light grey) from post-ICT (T4) to post-treatment (T2), to follow-up (T3: *persistently negative* in dark grey, *last positive* in light grey, and *positive spike* in pattern filling) in relation to recurrence (non-recurrent in black, recurrent in white). The diagram focuses on subjects with EBV+ at post-ICT (T4; N = 37).

significant difference in predictive accuracy between T2a and T2b may reflect the limited size of our sample with both early and late post-treatment assessments. Therefore, given the strong rationale supporting the value of longitudinal EBV-DNA monitoring also during follow-up[26], we continue to recommend serial assessments in post-treatment as well, whenever feasible.

During follow-up, *last positive* plasma EBV-DNA anticipated clinical and/or radiological recurrence in 71 % (N = 30/42) of cases, with a median and mean lead time of 38 and 72 days, respectively, similar to previous studies[27]. This predictive capability could enable earlier detection of metastatic disease, eventually improving treatment outcomes[28], though potential lead time bias should be considered. *Persistently negative* plasma EBV-DNA predicted a 91 % (N = 114/125) probability of non-relapse, potentially influencing follow-up strategies (e.g., less intensive surveillance). Isolated *positive spikes* lacked predictive significance. These findings support plasma EBV-DNA serial monitoring throughout EBER+ NPC follow-up[26].

Post-induction plasma EBV-DNA showed high NPV (92 %), with a lower PPV (49 %). The skewed timing of our sample collections (i.e., most samples obtained after 2–3 ICT cycles) prevented patients' stratification into early/intermediate/late responders, as seen in endemic areas [29]. Nonetheless, the concordance of NPV after 2 (94 %) or 3 (90 %) ICT cycles highlights the robustness of our results. In this respect, all 3 patients who recurred despite post-induction EBV- had a very high-risk disease (i.e., cT4N+ as per TNM AJCC VIII Edition). Accordingly, one European (Interlayer trial) and at least four Asian clinical trials (NCT05517135, NCT04448522, NCT04072107, NCT05304468) involving treatment escalation/de-escalation strategies based on post-induction plasma EBV-DNA were designed.

We described a different behavior of plasma EBV-DNA in non-endemic vs. endemic EBER+ NPC (e.g., lower viral load, lack of baseline/post-treatment predictive cut-off values; lack of early vs. late post-treatment dynamics). Discrepancies, as previously shown[7], are unlikely entirely due to assay differences, potentially reflecting distinct virus-host interactions, environmental factors, and NPC biology[30,31].

In conclusion, dedicated research on non-endemic EBV-related NPC is required, as patients experience poorer outcomes than their endemic counterparts[30]. Our study clarifies plasma EBV-DNA's utility in this setting, underscoring the need for biomarkers to improve prognostication and personalize treatment. With emerging therapies in advanced and curative settings (e.g., immunotherapy[32–37], anti-angiogenics [38–40]), further reproducible biomarkers could refine risk stratification, optimize therapy/follow-up adjustments, and address late toxicities, ultimately enhancing patient outcomes[30].

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CRediT authorship contribution statement

Lisa Licitra: Writing – review & editing. **Stefano Cavalieri:** Data curation. **Paolo Bossi:** Writing – review & editing. **Imperia Nuzzolese:** Data curation. **Elena Colombo:** Data curation. **Francesca Taverna:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Methodology, Investigation, Data curation, Conceptualization. **Cristiana Bergamini:** Data curation. **Laura Deborah Locati:** Writing – review & editing. **Ester Orlandi:** Writing – review & editing. **Monica Zucchini:** Writing – original draft, Visualization, Resources, Data curation. **Marco Guzzo:** Writing – review & editing. **Walter Ferrari Bravo:** Data curation. **Maria Luigia Piscitelli:** Writing – original draft, Resources, Data curation. **Loris De Cecco:** Writing – review & editing. **Rebecca Romanò:** Writing – review & editing, Writing – original draft, Visualization, Supervision. **Arianna Ottini:** Data curation. **Salvatore Alfieri:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Nicola Alessandro Iacovelli:** Writing – review & editing. **Carolina Sciortino:** Data curation. **Deborah Lenoci:** Writing – review & editing. **Sara Marceglia:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Prof. Laura Deborah Locati received conference honoraria/advisory board fees from: Lilly, MSD, EISAI, Roche, Bayer, Merck Serono, Istituto Gentili Srl, New Bridge.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejca.2025.115625.

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