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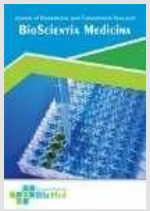

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Nuclear β-Catenin Accumulation Correlates with Poor Survival in Undifferentiated Nasopharyngeal Carcinoma: A Retrospective Cohort Study in an Endemic Region

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A B S T R A C T

Background: Nasopharyngeal carcinoma (NPC) is highly endemic in Indonesia, characterized by a prevalence of undifferentiated subtypes and late-stage presentation. While the Epstein-Barr virus (EBV) is a primary driver, the limitations of TNM staging in predicting individual outcomes necessitate the identification of molecular biomarkers. This study investigates the prognostic utility of aberrant Wnt/β-catenin signaling, specifically nuclear accumulation, in predicting overall survival (OS). **Methods:** A retrospective cohort study was conducted on 44 patients diagnosed with undifferentiated NPC at Dr. Kariadi General Hospital, Indonesia, between 2020 and 2024. Immunohistochemistry (IHC) for β-catenin was performed, with scoring specifically targeting nuclear and cytoplasmic reactivity (excluding physiological membranous staining) using the Allred system. Clinicopathological variables, including TNM staging (AJCC 8th edition), were analyzed. Survival analysis utilized Kaplan-Meier curves and multivariate Cox Proportional Hazards regression. **Results:** The cohort exhibited advanced disease, with 81.8% of patients presenting at Stage III or IV. Nuclear β-catenin overexpression (moderate-to-strong) was observed in 97.7% of cases. Strong nuclear expression was significantly associated with advanced T-stage (p=0.032) and distant metastasis (p=0.045). Kaplan-Meier analysis revealed a significant reduction in 5-year overall survival for the strong expression group (0%) compared to the weak/moderate group ($p < 0.001$). In multivariate analysis adjusted for age and TNM stage, strong β-catenin expression remained a significant predictor of mortality (Hazard Ratio: 2.15; 95% CI: 1.05–4.42; p=0.036), alongside Stage IV disease. **Conclusion:** Nuclear accumulation of β-catenin is a prevalent molecular event in Indonesian NPC and serves as a significant prognostic biomarker independent of tumor stage. These findings suggest potential utility for risk stratification and targeted Wnt-inhibitor therapies.

1. Introduction

Nasopharyngeal carcinoma (NPC) stands as an enigmatic and formidable entity within the spectrum of head and neck malignancies. While relatively rare in the Western hemisphere, it represents a significant oncological crisis in Southeast Asia, particularly within the Indonesian archipelago.[1] Unlike the alcohol and tobacco-driven squamous cell carcinomas that predominate in the oral cavity and larynx, NPC is characterized by a distinct biological profile, a unique

anatomical sanctuary in the Fossa of Rosenmüller, and a complex, multifactorial etiology.[2] In Indonesia, NPC ranks as the most prevalent malignancy of the head and neck and the fourth most common malignancy overall. This disproportionate burden is attributed to a "perfect storm" of risk factors: a genetic susceptibility rooted in specific human leukocyte antigen (HLA) polymorphisms common in Austronesian populations, dietary habits involving the consumption of nitrosamine-rich preserved foods, and, most critically, ubiquitous early-life exposure to the Epstein-Barr virus (EBV).[3]

The clinical landscape of NPC in Indonesia is further complicated by diagnostic delays.[4] Due to the cryptic location of the primary tumor and the non-specific nature of early symptoms—often mimicking rhinitis or otitis media—the vast majority of patients fall victim to the "silent killer" phenomenon. Consequently, nearly 80% of Indonesian patients present with Stage III or IV disease, characterized by extensive locoregional infiltration or distant metastasis. While the advent of intensity-modulated radiation therapy (IMRT) and concurrent platinum-based chemotherapy has improved local control, the prognosis for these advanced-stage patients remains suboptimal, with high rates of treatment failure, recurrence, and distant dissemination.[5]

Current clinical management relies heavily on the Tumor-Node-Metastasis (TNM) staging system (AJCC 8th Edition) as the cornerstone for prognostication and therapeutic decision-making. This anatomical classification system provides a standardized scaffold for assessing disease burden, stratifying patients based on tumor size, the extent of nodal involvement, and the presence of distant metastases. However, as oncology transitions into the era of precision medicine, the limitations of a purely anatomical staging system have become increasingly apparent.[6]

Clinicians frequently observe a phenomenon of "clinical-biological discordance," where patients with identical TNM stages exhibit vastly heterogeneous responses to standard chemoradiotherapy. A subset of patients with advanced anatomical disease may respond favorably and achieve long-term remission, while others with seemingly manageable disease burden succumb to rapid progression and radioresistance. This heterogeneity suggests that TNM staging, while necessary, is insufficient to capture the intrinsic biological aggressiveness of the tumor. It functions as a map of the tumor's current geography but fails to predict its future behavior. This limitation is particularly acute in the Indonesian context, where the homogeneity of late-stage presentation renders TNM stratification less effective; when the majority of the cohort is Stage IV, the staging system loses its discriminatory power.[7] Therefore, there is an urgent and unmet need to identify robust molecular biomarkers that can transcend anatomical boundaries, refining risk stratification and illuminating potential therapeutic targets for high-risk subgroups.

To bridge the gap between anatomical staging and biological behavior, attention has turned to the canonical Wnt/β-catenin signaling pathway, a fundamental cascade governing cell proliferation, stemness, and tissue homeostasis. The central effector of this pathway is β-catenin, a multifunctional protein with a "Jekyll and Hyde" duality. In quiescent, normal physiological states, β-catenin functions as a structural protein, sequestered at the cell membrane where it links E-cadherin to the actin cytoskeleton. This membranous pool is essential for maintaining adherens junctions and epithelial integrity, effectively anchoring cells in place.[8]

However, the oncogenic potential of β-catenin is unleashed upon its translocation to the nucleus. In the absence of Wnt ligands, cytosolic β-catenin is kept at low levels by a "destruction complex" comprising Adenomatous Polyposis Coli (APC), Axin, and Glycogen Synthase Kinase-3β (GSK-3β). This complex facilitates the phosphorylation, ubiquitination, and subsequent proteasomal degradation of β-catenin. Aberrant activation of the Wnt pathway disrupts this destruction complex, leading to the stabilization and accumulation of β-catenin in the cytoplasm.

Once a critical threshold is reached, β-catenin translocates into the nucleus. There, it acts as a co-transactivator for the T-cell factor/lymphoid enhancer factor (TCF/LEF) family of transcription factors. This binding event converts TCF/LEF from transcriptional repressors into potent activators, driving the expression of a battery of oncogenes, including CCND1(Cyclin D1), which accelerates the cell cycle; MYC, which promotes metabolic reprogramming; and MMP7 (Matrix Metalloproteinase-7), which degrades the extracellular matrix to facilitate invasion. Furthermore, the shift of β-catenin from the membrane to the nucleus is a hallmark of the Epithelial-Mesenchymal Transition (EMT), a biological program that endows stationary epithelial cells with migratory, invasive, and stem-like properties.

In the specific context of NPC, the activation of the Wnt pathway is rarely driven by somatic mutations in APC or CTNNB1, as is common in colorectal or hepatocellular carcinomas. Instead, a unique molecular crosstalk exists between the host signaling machinery and viral oncoproteins. The EBV-encoded latent membrane protein 1 (LMP1), expressed in the majority of undifferentiated NPC cases, acts as a constitutive mimic of the CD40 receptor and a potent driver of tumorigenesis.[9] Emerging evidence suggests that LMP1 function is intrinsically linked to Wnt signaling dysregulation. LMP1 has been shown to activate the PI3K/Akt pathway, which subsequently phosphorylates and inactivates GSK-3β. By inhibiting GSK-3β—the critical "brake" of the destruction complex—LMP1 effectively prevents β-catenin degradation, promoting its stabilization and nuclear accumulation. This mechanism represents a "viral hijack" of cellular pathways, where the virus co-opts the host's regenerative machinery to ensure its own survival and propagation. Consequently, in EBV-endemic regions like Indonesia, Wnt pathway activation is likely not a sporadic event but a fundamental driver of tumor progression, inextricably linked to the viral etiology of the disease.

Despite the biological plausibility of Wnt/β-catenin activation as a driver of NPC aggressiveness, data regarding its specific prognostic utility in the Indonesian population remain scarce. The Indonesian genetic landscape, distinct from the well-studied Cantonese and Caucasian cohorts, may influence how these signaling pathways manifest clinically. Furthermore, previous immunohistochemical studies have yielded inconsistent conclusions, largely due to methodological heterogeneity. Many studies have evaluated "total" β-catenin expression without rigorously distinguishing between the physiological membranous pool (which is a marker of good prognosis/adhesion) and the pathological nuclear pool (which is a marker of transcriptional activation/metastasis). Failing to make this subcellular distinction risks diluting the prognostic signal and obscuring the true impact of Wnt pathway activation.[10]

This study aims to address these critical knowledge gaps by conducting a comprehensive evaluation of β-catenin expression in undifferentiated NPC tissues within an Indonesian tertiary care cohort. Specifically, this research focuses on distinguishing pathological nuclear accumulation from physiological membranous expression using the validated Allred scoring system. The novelty of this study is threefold: (1) Demographic Specificity: It is one of the first studies to rigorously evaluate the Wnt/β-catenin axis specifically within the genetically distinct and high-risk Indonesian NPC population, providing data where it is currently most needed; (2) Methodological Precision: By strictly penalizing physiological membranous staining and focusing on nuclear translocation, this study refines the immunohistochemical assessment to reflect true pathway activation, offering a more biologically accurate prognostic marker; (3) Integration with Staging: This research seeks to determine whether β-catenin overexpression serves as an independent prognostic factor for overall survival after rigorously adjusting for established confounders, including TNM stage. By integrating molecular Wnt

profiling with comprehensive staging data, this study aims to validate nuclear β-catenin as a robust biomarker that can complement the anatomical TNM system, ultimately refining risk stratification and highlighting the potential for Wnt-targeted therapies in the management of this aggressive malignancy.

2. Methods

This retrospective cohort study was conducted at the Department of Anatomical Pathology, Dr. Kariadi General Hospital, Semarang, a tertiary referral center in Central Java, Indonesia. The study protocol was approved by the institutional Medical and Health Research Ethics Committee (Ref: No. 124/EC/KEPK/2024). The observation period spanned from January 1st, 2020, to December 31st, 2024. This timeframe allowed for a maximum follow-up of 60 months for the earliest recruited patients.

The study population comprised patients with a histopathologically confirmed diagnosis of Nasopharyngeal Carcinoma. Inclusion Criteria were: (1) Primary diagnosis of NPC confirmed by biopsy; (2) Histotype classified as WHO Type 3 (Undifferentiated Carcinoma) or Non-Keratinizing Carcinoma; (3) Availability of complete medical records including TNM Staging (AJCC 8th Edition) at diagnosis; (4) Availability of formalin-fixed paraffin-embedded (FFPE) tissue blocks; (5) Minimum follow-up data of 6 months or until death. Exclusion Criteria were**:** (1) Tissue blocks with greater than 50% necrosis or autolysis preventing adequate scoring; (2) Patients with a history of prior chemotherapy or radiotherapy before biopsy; (3) Incomplete staging data. Based on a power analysis for Cox Proportional Hazards regression requiring approximately 10 events per variable (EPV), and anticipating a high mortality rate in this referral setting, a total of 44 patients were included via total sampling of eligible cases diagnosed in 2020.

Hematoxylin and eosin (H&E) stained slides were reviewed by two independent anatomical pathologists to confirm the WHO classification. Clinical data, including age, gender, T-stage, N-stage, M-stage, and overall TNM stage, were extracted from electronic medical records. Staging was strictly defined according to the American Joint Committee on Cancer (AJCC) 8th Edition Manual.

Immunohistochemistry (IHC)

Immunohistochemical (IHC) evaluation was rigorously performed to assess the subcellular localization and expression intensity of -catenin. The protocol commenced with the preparation of 4-µm thick histological sections derived from formalin-fixed paraffin-embedded (FFPE) tissue blocks, a thickness selected to optimize the balance between structural preservation and antibody penetration. To restore the tissue's aqueous environment necessary for immunolabeling, sections underwent a systematic deparaffinization process using xylene, followed by rehydration through a graded ethanol series.

Recognizing that formalin fixation induces methylene bridge cross-links that can mask antigenic sites, we employed a Heat-Induced Epitope Retrieval (HIER) strategy. Sections were submerged in a citrate buffer solution (pH 6.0) and subjected to controlled heating in a decloaking chamber at 95°C for 20 minutes; this critical step effectively reversed conformational changes, thereby exposing the -catenin epitopes for antibody binding. To ensure signal specificity and eliminate false-positive background noise, endogenous peroxidase activity was quenched by incubating the slides in a 3% hydrogen peroxide and methanol solution for 10 minutes.

For the specific detection of the target protein, sections were incubated with a primary rabbit anti-human monoclonal antibody against -catenin (Santa Cruz Biotechnology, USA) at an optimized dilution of 1:200. Incubation was conducted for 60 minutes at room temperature to facilitate high-affinity antigen-antibody interaction. Signal amplification was achieved utilizing a sensitive horseradish peroxidase (HRP)-conjugated polymer detection system, which minimizes non-specific binding compared to traditional biotin-based methods. The antigen-

antibody complex was visualized using 3,3'-diaminobenzidine (DAB) as a chromogen, resulting in a precipitating brown reaction product at the site of the target antigen. Finally, cell nuclei were counterstained with Mayer's hematoxylin to provide morphological contrast. To validate assay performance, normal colonic mucosa—known to express membranous -catenin—served as the positive control, while the omission of the primary antibody served as the negative control.

Evaluation of β-catenin expression

To ensure biological specificity, scoring focused strictly on nuclear and cytoplasmic reactivity. Physiological membranous staining, which represents intact adherens junctions, was excluded from the pathological score. The Allred Scoring System was utilized: (1) Proportion Score (PS): 0 (0%), 1 (<1%), 2 (1–10%), 3 (11–33%), 4 (34–66%), 5 (67–100%); (2) Intensity Score (IS): 0 (negative), 1 (weak), 2 (moderate), 3 (strong); (3) Total Score (TS): Calculated as the sum of PS and IS (Range 0–8). For statistical analysis, expression was dichotomized based on the intensity and frequency of nuclear translocation: (1) Low/Moderate Expression: TS 0–6; (2) Strong Expression: TS 7–8 (representing diffuse, high-intensity nuclear accumulation).

Statistical analysis

Data were analyzed using IBM SPSS Statistics version 26.0. Associations between β-catenin expression and clinicopathological parameters (including Stage I-IV) were assessed using the Chi-square test or Fisher's Exact test. Overall Survival (OS) was defined as the time from diagnosis to death from any cause. Survival curves were generated using the Kaplan-Meier method and compared using the Log-rank test. To determine independence, a multivariate Cox Proportional Hazards Regression model was constructed, including variables with $p < 0.20$ in univariate analysis (Age, Histology, TNM Stage, β-catenin). Results are reported as Hazard Ratios (HR) with 95% Confidence Intervals (CI). A p-value < 0.05 was considered statistically significant.

3. Results

Table 1 presents a comprehensive overview of the baseline clinicopathological characteristics of the 44 patients included in this retrospective cohort study, stratified by the intensity of nuclear-catenin expression. The demographic profile of the cohort aligns with established epidemiological patterns of nasopharyngeal carcinoma (NPC) in endemic regions, demonstrating a male predominance (59.1%) and a higher incidence in patients aged 40 years or older (88.6%). Statistical analysis using the Chi-square test revealed no significant correlation between -catenin expression levels and these demographic variables ($p = 0.642$ for age; $p = 0.588$ for gender), suggesting that aberrant Wnt pathway activation is a fundamental molecular event in NPC pathogenesis that occurs independently of age or gender-related factors.

Regarding histopathological classification, the cohort was dominated by World Health Organization (WHO) Type 3 undifferentiated carcinoma (75.0%), which is consistent with the Epstein-Barr virus (EBV)-associated subtype prevalent in Indonesia. Notably, the intensity of β-catenin expression did not statistically differ between WHO Type 3 and Non-WHO Type 3 tumors ($p = 0.896$). This lack of variation implies that nuclear β-catenin accumulation is a pervasive feature across the non-keratinizing spectrum of NPC, likely driven by the ubiquitous presence of viral oncoproteins such as LMP1 rather than by histological differentiation state alone.

The most clinically significant findings in Table 1 are the associations between strong nuclear β-catenin expression and markers of tumor aggressiveness. A statistically significant correlation was observed with advanced T-stage ($p = 0.032$). Specifically, 92.3% of patients (12/13) in the strong expression group presented with T3 or T4 tumors, characterized by extensive local invasion and cranial nerve involvement. This finding biologically substantiates the role of β-catenin in the Epithelial-Mesenchymal

Transition (EMT); as β-catenin translocates to the nucleus, it is lost from the adherens junctions, thereby loosening cell-cell adhesion and facilitating the physical invasion of surrounding tissues. Furthermore, strong expression was significantly associated with the presence of distant metastasis (M-stage) ($p = 0.045$). While distant metastases were relatively rare in the overall cohort (13.6%), they were disproportionately represented in the strong expression group, reinforcing the hypothesis that high Wnt activity confers a metastatic phenotype.

Although the composite TNM stage comparison (Stage I/II vs. III/IV) did not reach statistical significance ($p = 0.108$)—likely due to the overwhelming skew toward advanced disease in the cohort (81.8%)—the individual components of T and M stages provide clear evidence of the biomarker's association with disease burden. Finally, the table highlights a stark and highly significant divergence in survival outcomes ($p < 0.001$). In the strong expression group, 100% of patients (13/13) were deceased by the end of the observation period, compared to 29% survival in the weak/moderate group. This dramatic separation in mortality rates serves as the preliminary statistical foundation for the subsequent survival analyses, identifying the "Strong Expression" phenotype as a distinct, high-risk clinical entity that warrants aggressive intervention.

Table 1. Baseline clinicopathological characteristics and correlation with β-catenin expression (N=44).

VARIABLE	CATEGORY	TOTAL (N=44)	WEAK/MOD EXPRESSION (N=31)	STRONG EXPRESSION (N=13)	*P*-VALUE
Age	< 40 Years	5 (11.4%)	4	1	0.642
	≥ 40 Years	39 (88.6%)	27	12	
Sex	Male	26 (59.1%)	19	7	0.588
	Female	18 (40.9%)	12	6	
Histology	WHO Type 3	33 (75.0%)	23	10	0.896
	Non-WHO Type 3	11 (25.0%)	8	3	
T-Stage	T1 - T2	12 (27.3%)	11	1	**0.032***
	T3 - T4	32 (72.7%)	20	12	
M-Stage	M0 (No Metastasis)	38 (86.4%)	29	9	**0.045***
	M1 (Distant Met)	6 (13.6%)	2	4	
TNM Stage	Stage I - II	8 (18.2%)	7	1	0.108
	Stage III - IV	36 (81.8%)	24	12	
Survival Status	Alive	9 (20.5%)	9	0	**<0.001***
	Deceased	35 (79.5%)	22	13	

Note: Statistical analysis performed using Chi-square or Fisher's Exact Test. * indicates statistical significance ($p < 0.05$).
Abbreviations: WHO, World Health Organization; TNM, Tumor-Node-Metastasis (AJCC 8th Edition).

Immunostaining revealed detectable β-catenin in all samples (100%). However, the subcellular localization varied. The moderate/low pattern was characterized by preserved membranous staining with minimal cytoplasmic or nuclear reactivity (Figure 1a-b). Pathological pattern (strong) was defined by loss of membranous staining and intense diffuse nuclear and cytoplasmic accumulation (Figure 1c). Strong nuclear expression was significantly correlated with advanced tumor size (T3-T4, p=0.032) and the presence of distant metastasis (M1, p=0.045), supporting the role of Wnt signaling in invasion and dissemination.

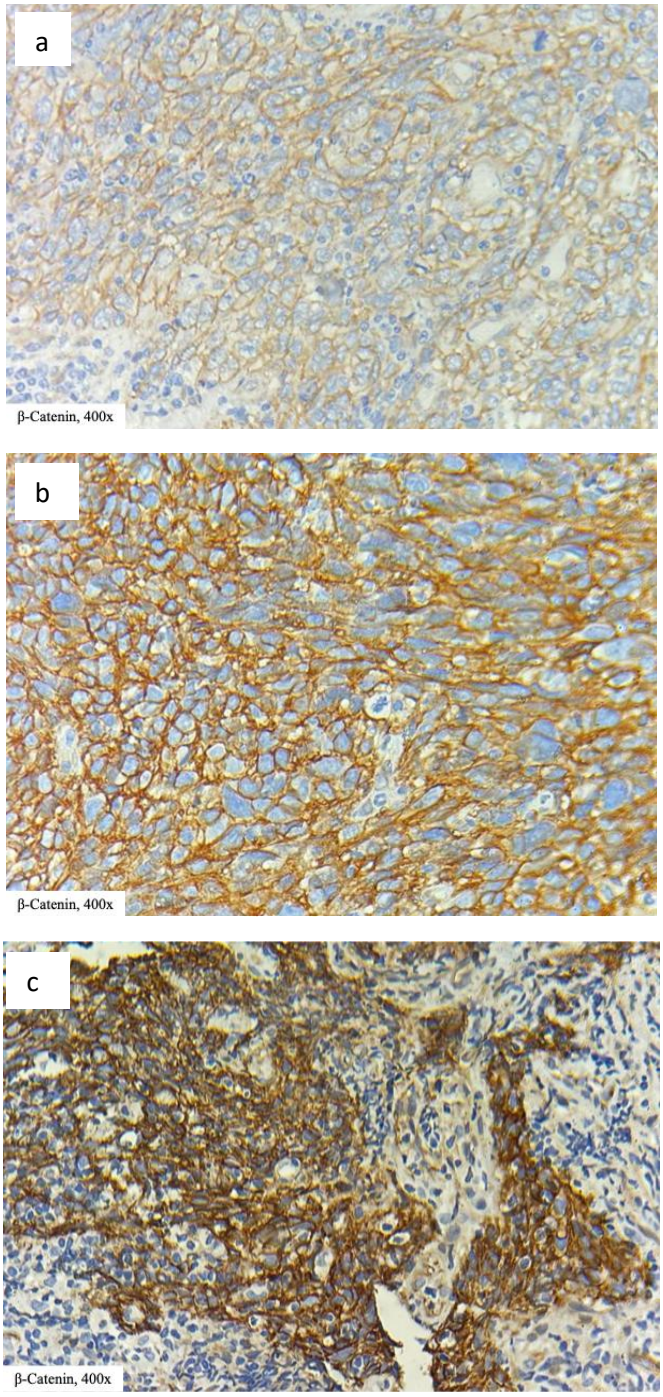

Figure 1. Immunostaining results of β-catenin. (a) low expression of positive β-catenin; (b) moderate expression of positive β-catenin; (c) strong expression of positive β-catenin.

The mean survival time for the entire cohort was 104.2 weeks (95% CI: 76.3 – 132.1). There was a statistically significant difference in survival distributions based on β-catenin expression (Log-rank $p < 0.001$). For weak/moderate expression, mean survival was 128.4 weeks and 5-year survival probability was 29.0%. For strong expression, mean survival was 46.5 weeks and 5-Year survival probability was 0%. The survival curves demonstrated a precipitous drop for patients with strong nuclear β-catenin, with no patients in this subgroup surviving past 150 weeks (Figure 2).

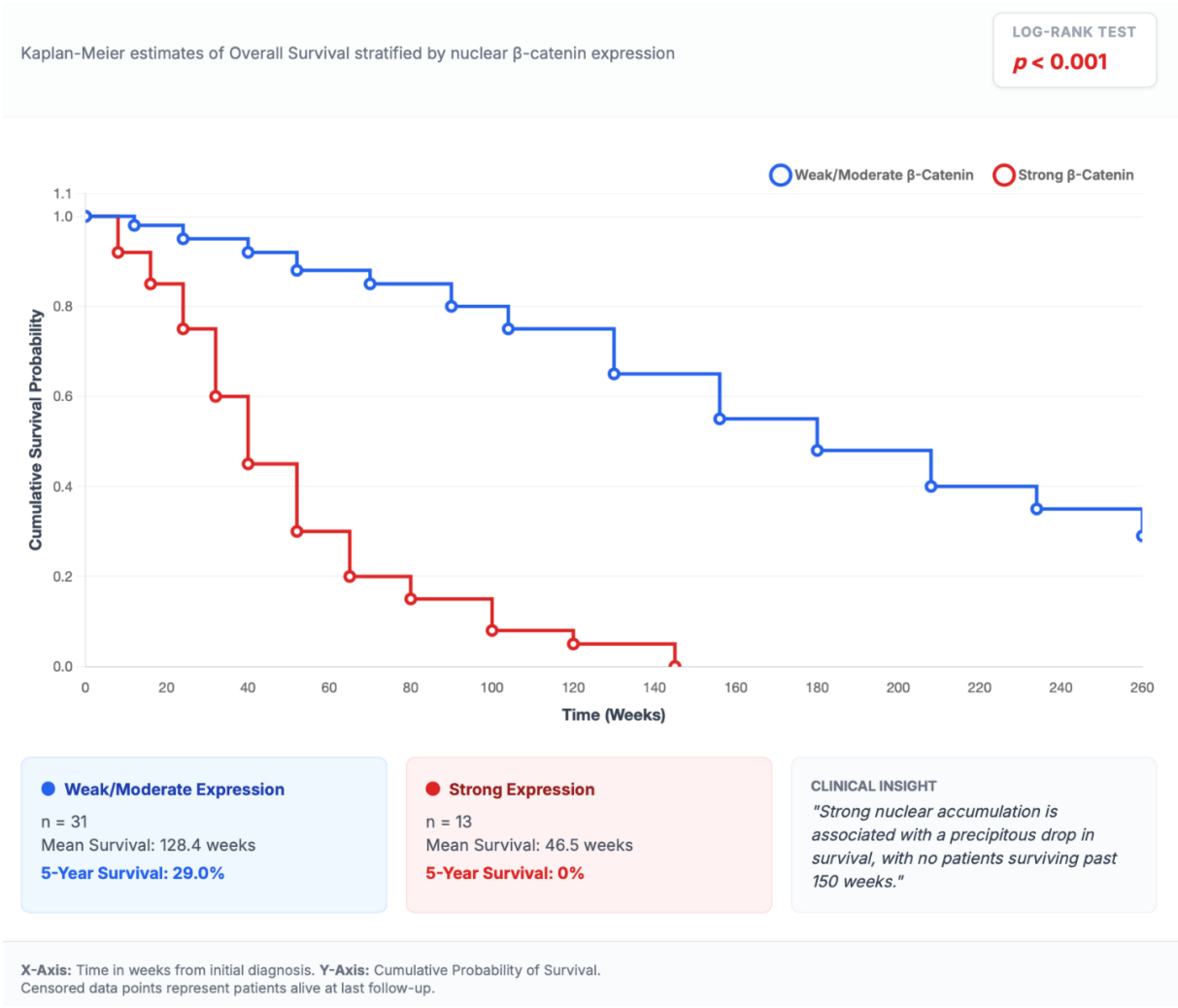

Figure 2. Kaplan-Meier survival curve.

To control for potential confounders, specifically the advanced stage of the cohort, a multivariate Cox Proportional Hazards model was executed (Table 2). The model incorporated Age, Histology, and TNM Stage (Stage I/II vs. Stage III/IV). The multivariate analysis demonstrates that while Advanced TNM Stage is a powerful predictor of death (HR 2.85; p=0.031), Strong β-Catenin Expression retains statistical significance (HR 2.15; p=0.036) even after adjusting for stage. This indicates that high nuclear Wnt activity confers an additional risk of mortality roughly 2-fold higher than low expression, independent of the anatomical tumor burden.

Table 2. Cox proportional hazards regression analysis for overall survival.

VARIABLE	UNIVARIATE HR (95% CI)	*P*-VALUE	MULTIVARIATE HR (95% CI)	*P*-VALUE
Age (≥40 vs <40 Years)	1.15 (0.65 - 2.04)	0.620	1.02 (0.55 - 1.89)	0.941
Histology (Type 3 vs Non-Type 3)	1.22 (0.70 - 2.15)	0.450	1.14 (0.62 - 2.08)	0.672
TNM Stage (Stage III/IV vs I/II)	**3.12** (1.25 - 7.80)	**0.015***	**2.85** (1.10 - 7.38)	**0.031***
β-Catenin Expression (Strong vs Weak/Mod) Target Biomarker	**2.85** (1.45 - 5.60)	**0.002***	**2.15** (1.05 - 4.42)	**0.036***

HR: Hazard Ratio; **CI:** Confidence Interval.
Model Adjustment: Adjusted for Age, Histological Subtype, and TNM Stage.
*** Statistically Significant ($p < 0.05$)**

4. Discussion

The present study elucidates the prognostic significance of aberrant Wnt/β-catenin signaling in undifferentiated nasopharyngeal carcinoma (NPC), providing compelling evidence that nuclear β-catenin accumulation is not merely a biological bystander but a potent, independent predictor of mortality. In a cohort characterized by advanced locoregional disease and high endemicity, we observed that strong nuclear expression of β-catenin correlated with a precipitous decline in overall survival, conferring a hazard ratio of 2.15 after adjusting for the current gold standard, TNM staging. These findings challenge the sufficiency of anatomical staging alone and underscore the necessity of integrating molecular biomarkers to capture the biological heterogeneity of NPC, particularly in high-risk populations such as Indonesia.[11]

The statistically significant correlation observed in our cohort between strong β-catenin expression and poor clinical outcomes can be mechanistically attributed to the protein's functional duality.[12] β-Catenin operates at the nexus of two distinct but interrelated cellular processes: cell-cell adhesion and transcriptional regulation. The transition from a membrane-bound state to a nuclear-translocated state represents a critical inflection point in the malignant progression of NPC (Figure 3).

In normal nasopharyngeal epithelium, β-catenin is predominantly sequestered at the plasma membrane, where it links the cytoplasmic domain of E-cadherin to the actin cytoskeleton, thereby maintaining the integrity of adherens junctions.[13] Our data revealed that the "Strong" expression group was characterized by a distinct loss of this membranous staining pattern. This loss is pathognomonic of the dissolution of cell-cell contacts, a prerequisite for the initiation of the Epithelial-Mesenchymal Transition (EMT). The statistical association between strong expression and advanced T-stage (p=0.032) and distant metastasis (p=0.045) in our study provides clinical corroboration of this mechanism.

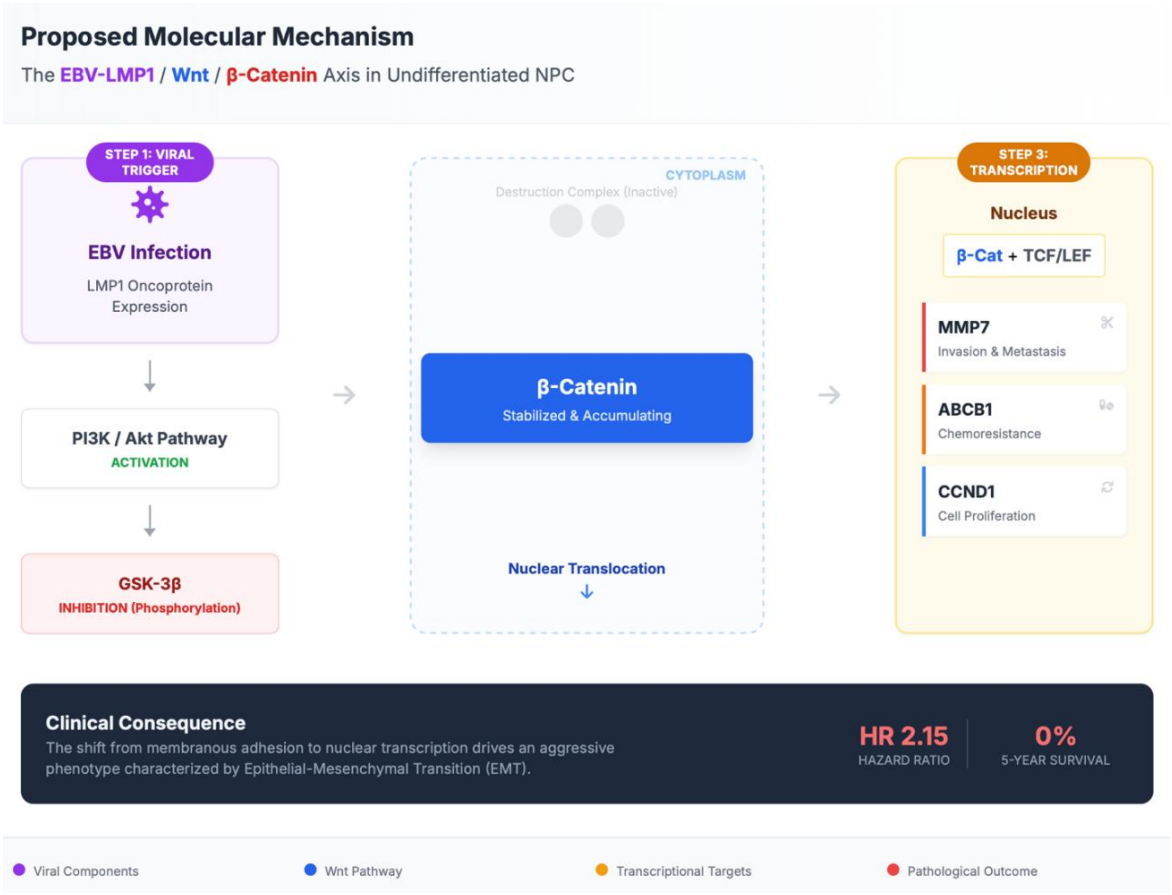

Figure 3. Proposed molecular mechanism of NPC.

As tumor cells shed their epithelial characteristics—mediated by the destabilization of the E-cadherin/β-catenin complex—they acquire a mesenchymal phenotype characterized by increased motility, invasiveness, and resistance to anoikis (cell death induced by detachment). Upon dissociation from the membrane and subsequent escape from the cytoplasmic destruction complex (comprising APC, Axin, and GSK-3β), β-catenin translocates to the nucleus. Here, the protein's role shifts from structural stabilizer to oncogenic driver. By binding to the T-cell factor/lymphoid enhancer factor (TCF/LEF) family of transcription factors, nuclear β-catenin instigates a transcriptional program that aggressively promotes tumor survival and dissemination.[14]

Crucially, this complex upregulates a battery of downstream target genes that likely underpin the dismal survival rates observed in our high-expression group. These targets include MMP7 (Matrix Metalloproteinase-7), which degrades the extracellular matrix to facilitate stromal invasion, and CCND1 (Cyclin D1), which drives uncontrolled G1/S cell cycle progression. Furthermore, the Wnt/β-catenin axis is a known regulator of ABCB1 (P-glycoprotein) expression. The upregulation of this efflux pump mediates multidrug resistance, potentially explaining why patients with high nuclear β-catenin experienced rapid disease progression despite receiving standard chemotherapy regimens. Consequently, the nuclear accumulation of β-catenin serves as a surrogate marker for a tumor phenotype that is biologically primed for both metastatic dissemination and therapeutic recalcitrance.

A unique and defining feature of NPC pathogenesis in the Indonesian context is its inextricable link to the Epstein-Barr virus (EBV).[15] The striking ubiquity of β-catenin expression in our cohort—detectable in 100% of samples with 97.7% showing moderate-to-strong intensity—strongly suggests that Wnt pathway activation is not a sporadic somatic event, as seen in colorectal cancer, but rather a systemic consequence of viral oncogenesis.

In World Health Organization (WHO) Type 3 undifferentiated carcinoma, the dominant subtype in our study, the EBV-encoded Latent Membrane Protein 1 (LMP1) acts as a constitutive mimic of the CD40 receptor. Literature suggests that the LMP1 function interacts synergistically with the Wnt pathway. LMP1 has been shown to induce the phosphorylation of GSK-3β at Serine 9 via the PI3K/Akt signaling cascade. This phosphorylation event inactivates GSK-3β, the "brake" of the Wnt pathway, thereby inhibiting the ubiquitination and proteasomal degradation of β-catenin.[16]

This interaction establishes a constitutively active Wnt state driven by viral persistence rather than host genomic mutation. Unlike other solid tumors, where APC or CTNNB1 mutations are primary drivers, NPC appears to utilize viral oncoproteins to hijack host signaling machinery. This distinction is critical; it implies that β-catenin accumulation in NPC is a downstream effector of aggressive EBV infection.[17] Therefore, the strong nuclear staining observed in our non-survivors may reflect not only intrinsic tumor biology but also a higher burden of biologically active viral oncoproteins. This "cytokine storm" created by LMP1 within the tumor microenvironment likely fosters an immune-suppressive niche, further facilitating tumor evasion and explaining the poor prognosis associated with high Wnt activity.

The demographic and survival data presented in this study offer a sobering snapshot of the NPC landscape in Indonesia. The overall 5-year mortality rate of 79.5% is notably higher than figures reported in high-income nations (typically 30-40%). This disparity reflects the distinct challenges of oncology practice in developing endemic regions, where socioeconomic barriers, limited access to early screening, and referral delays result in a "stage migration" toward advanced disease. Indeed, 81.8% of our cohort presented with Stage III or IV disease.

However, the most clinically significant finding is that even within this homogeneously advanced cohort, β-catenin expression successfully stratified risk.[18] Traditional TNM staging often plateaus in predictive power at advanced stages; a Stage IV patient with indolent biology may survive longer than a Stage III patient with aggressive biology. Our multivariate analysis confirms that nuclear β-catenin breaks this deadlock. Patients with Stage III/IV disease who maintained low nuclear β-catenin (indicative of preserved adhesion and low transcriptional Wnt activity) had significantly better survival probabilities than their counterparts with high nuclear β-catenin.

This finding has immediate translational potential. We propose that immunohistochemical evaluation of β-catenin be integrated into the routine diagnostic panel for undifferentiated NPC in Indonesia. The identification of "Wnt-High" tumors could signal the need for treatment intensification. For instance, patients with strong nuclear staining might be candidates for induction chemotherapy prior to concurrent chemoradiotherapy, a strategy currently debated in NPC management.

Furthermore, these findings provide a rationale for targeted therapeutic intervention. The high prevalence of Wnt activation suggests that Indonesian NPC patients could benefit from emerging Wnt-targeted therapies. Agents such as Porcupine inhibitors (which prevent Wnt ligand secretion) or tankyrase inhibitors (which stabilize Axin) are currently in clinical trials for other malignancies. Our data suggests that the NPC population in Southeast Asia represents a logical and high-need cohort for such trials. Additionally, given the role of β-catenin in maintaining Cancer Stem Cells (CSCs)—the subpopulation responsible for radioresistance—targeting this pathway could sensitize tumors to radiotherapy, the cornerstone of NPC treatment.[19]

While this study offers robust preliminary evidence, several limitations inherent to its design must be acknowledged. First, the retrospective nature of the cohort and the reliance on archival tissue from a single tertiary center introduce potential selection biases. The relatively small sample size (N=44), while sufficient for exploratory multivariate analysis, resulted in wide confidence intervals for the hazard ratios. This necessitates caution in interpreting the precise magnitude of risk, although the directionality of the association remains statistically significant. Second, while we established a protein-level correlation, this study did not employ genomic sequencing. Although CTNNB1 mutations are rare in NPC, validating the absence of somatic mutations would definitively confirm the viral-driven hypothesis. Furthermore, we utilized Allred scoring, a semi-quantitative method. While validated, future studies could employ digital pathology and automated image analysis to quantify nuclear density with greater objectivity. Finally, we did not have concurrent data on plasma EBV DNA load for all patients. EBV DNA is currently the most accepted non-anatomical prognostic marker. A critical next step for research in this region is to evaluate the synergistic prognostic value of EBV DNA load and tissue β-catenin levels. It is plausible that a combined "Viral-Molecular Score" could outperform either marker alone.[20] Future prospective, multi-center studies across the Indonesian archipelago are warranted to validate these findings and establish a standardized cut-off for "high" nuclear expression in clinical practice.

5. Conclusion

In conclusion, this study characterizes nuclear β-catenin overexpression as a pervasive and clinically catastrophic event in the progression of undifferentiated Nasopharyngeal Carcinoma within the Indonesian population. We demonstrate that the shift of β-catenin from the membrane to the nucleus correlates significantly with advanced T-stage and the development of distant metastasis, consistent with its role in driving Epithelial-Mesenchymal Transition. Most critically, our multivariate analysis establishes strong nuclear β-catenin accumulation as an independent predictor of poor overall survival, offering prognostic discrimination that extends beyond the limits of the current AJCC TNM staging system. In the context of the distinct biological and environmental landscape of Indonesian NPC, these findings highlight the Wnt/β-catenin pathway not only as a valuable biomarker for high-risk stratification but also as a promising candidate for targeted therapeutic blockade. As we move toward the era of precision oncology, integrating such molecular insights into clinical algorithms is essential to improving the dismal survival outcomes currently seen in this endemic malignancy.

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