American Journal of Otolaryngology and Head and Neck Surgery

Research Article
Published: 17 Jan, 2022

The Role of Topoisomerase (TOPO2A) as Prognostic Factor in Nasopharyngeal Carcinoma

Bandera A[1], Cazorla O[2], Perez-Villa L[3], García-Anaya MJ[4], Alvarez M[5], Toledo I[4], Medina JA[4] and Gómez-Millán J[4]*

[1]Department of Otorhinolaryngology, Agencia Sanitaria Costa del Sol., Spain

[2]Department of Otorhinolaryngology, Instituto de investigaciones biomédicas de Malaga (IBIMA), Hospital Universitario Virgen de la Victoria, Spain

[3]Department of Pathology, Instituto de investigaciones biomédicas de Malaga (IBIMA), Hospital Universitario Virgen de la Victoria, Spain

[4]Department of Radiation Oncology, Instituto de investigaciones Biomédicas de Malaga (IBIMA), Hospital Universitario Virgen de la Victoria, Spain

[5]Department of Pathology, Hospital Universitario Virgen de la Victoria, Instituto de Investigación Biomédica de Málaga (IBIMA) Spain

Abstract

Background: DNA topoisomerase has an essential role in cell division.

Aims: We investigated the role of Topoisomerase-2A as a prognostic biomarker in nasopharynx carcinoma.

Study Design: Retrospective cohort study.

Methods: We retrospectively reviewed 58 with nasopharynx cancer. Immunohistochemistry was performed and a validated score was calculated. The role of TOPO2A as a survival prognostic factor was studied.

Results: Topo2A expression was inversely correlated with stage, presenting early stage a 35.6% expression compared with 76.9% in advance stage (p=0.01). In the logistic regression analysis, stage (HR 7.94; 0.01 to 0.46; p=0.02) and age (HR 0.26; 0.08 to 0.92; p=0.04) were independently associated with TOPO2A expression. Patients with topoisomerase expression presented a significant decrease in OS (p=0.05) and PFS (p=0.03). In the Cox regression analysis for OS and PFS, the prognostic value of TOPO2A was confirmed.

Conclusion: Topoisomerase-2A expression may identify a subgroup of patients with worse prognosis in which new molecular therapies, or chemotherapy intensification could be tested.

Keywords: Topo2A; Nasopharynx; Radiotherapy; Chemotherapy

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Correspondence:
Martina Álvarez Pérez, Laboratorio de Biología Molecular del Cancer, Centro de Investigaciones Médico-Sanitarias (CIMES), Universidad de Málaga, Department of Pathology, Hospital Universitario Virgen de la Victoria, Instituto de Investigación Biomédica de Málaga (IBIMA). Campus Teatinos s/n, 29010 Malaga, Spain, E-mail: martina@uma.es

Received Date: *21 Dec 2021*
Accepted Date: *11 Jan 2022*
Published Date: *17 Jan 2022*

Citation:
Bandera A, Cazorla O, Perez-Villa L, García-Anaya MJ, Alvarez M, Toledo I, et al. The Role of Topoisomerase (TOPO2A) as Prognostic Factor in Nasopharyngeal Carcinoma. Am J Otolaryngol Head Neck Surg. 2022; 5(1): 1171.

Introduction

Nasopharyngeal Carcinoma (NPC) is a relative uncommon epithelial carcinoma, with a strong pathogenic association with Epstein Bar Virus (EBV). Moreover, a different histology distribution between endemic and non-endemic areas has been observed, with a predominance of undifferentiated histology subtype (WHO type III) in 95% and 63% of cases respectively [1]. During the last years, the combination of concomitant Chemotherapy and Radiotherapy (CRT) [2,3], and the incorporation of IMRT technique have significantly improved the prognostic of this disease [4-6]. However, in locally advanced cases the rate of treatment failure is still high, mainly due to metastatic recurrent disease. Moreover, although intensification with CT has shown to improve the outcome of patients in some studies [7,8], the prognosis of this disease has not significantly changed during the last the last years. Thus, investigation of new molecular biomarkers and targets is mandatory to improve the prognosis of this disease.

DNA topoisomerases are enzymes with an essential role in cell division, sorting out problems during DNA replication and transcription, in which DNA is not free rotated around its axis, inducing transient DNA breaks using a transesterification mechanism, minimizing the risk of genetic instability [9,10]. The prognostic value of Topoisomerase-2A (TOPO2A) expression has been

investigated in several retrospective studies, showing that expression is correlated with a worse prognosis [4,11,12]. Moreover, TOPO2A has been identified as a molecular target, showing different studies with breast cancer a higher response to anthracyclines in patients with amplification-driven HER overexpression, probably explained by the proximity of both genes on chromosome 17q [13]. NPC is considered as highly proliferative tumors, with a high capacity of locoregional and distant growth in which DNA topoisomerase might play an important role. However, only one study has investigated the prognostic value of TOPO2A in NPC [14]. In this investigation a retrospective analysis of 124 NPC of an endemic area, showed that patients with TOPO2A overexpression presented lower disease specific survival and distant metastasis-free survival compared with patients that showed an absence in the expression of TOPO2A. To the best of our knowledge there are not investigations that had studied the role of TOPO2A in non-endemic areas.

The aim of this retrospective study is to analyze the prognostic value of TOPO2A expression in NPC in one Centre in the south of Spain.

Material and Methods

Patient data and treatment characteristics

Between January 1995 and December 2011, 60 patients with newly diagnosed NPC were treated RT or CRT. Of the 60 patients, 58 were fully assessable related to the availability of the pathological specimen. Pretreatment evaluation consisted in physical examination, a complete laryngopharynx endoscopy, chest X-ray, blood test, Epstein Barr virus serology and computed tomography of the neck. Computed tomography of the chest was performed when more advances cases, such as T4 or N3, were detected. Eight weeks after treatment, response assessment was performed under RECIST criteria. After treatment, patients underwent regular clinical and radiological examination to check for the occurrence of relapse or death.

Different chemotherapy regiments were utilized. In patients treated with concomitant chemo-radiotherapy, chemotherapy was administered with Cisplatin, to a dose of 100 mg/m^2 dose every 3 weeks. In patients treated with induction chemotherapy (patients treated from 1995 to 2000), 3 cycles of CDDP (100 mg/m^2 iv day 1) and iv infusion of 5-Fluouracil (1000 mg/m^2/days 1 to 5). Radiotherapy was planned with three-dimensional conformal treatment (3D) in patients treated before 2011 or Intensity Modulated Radiation Therapy (IMRT) in the rest of patients. In the 3D group, patients were treated with conventional fractionation of 2 Gy per fraction, five days a week, to 50 Gy in areas of subclinical disease (nasopharynx and potential local dissemination sites, cervical levels II to V bilateral and retropharyngeal nodes) and 70 Gy over the macroscopic disease. In patients treated with IMRT, a dose of 65.10 Gy with 2.17 fractions were delivered over macroscopic disease and 54 Gy/1.8 Gy over subclinical disease.

Immunohistochemistry

For the immunohistochemical study, consecutive sections of paraffin of 4 μm to 5 μm in thickness were mounted on coated slides (DAKO ChemMate, Copenhagen, Denmark). Prior to immunostaining, the tissue sections were subjected to antigen retrieval (by means of heat in PT Link at 95°C for 20 min in a Tris/EDTA buffer, PH9). Slides were incubated with rabbit monoclonal Anti-Topoisomerase II alpha antibody (clone EP1102Y, Epitomics) dilution 1:200 during 30 min at room temperature. The immunohistochemical technique was carried out with an automated immunostainer (DAKO TechMate Horizon) using the EnVision system (DAKO) for visualization and slides were counterstained with hematoxylin. As a positive control, a human breast tissue sample with known reactivity was used. As a negative control, the same sample in which the primary antibody was substituted for non-immune serum from the same species was used. For the immunohistochemical evaluation, without prior knowledge of the clinical information, the immunostaining were visualized by two pathologists.

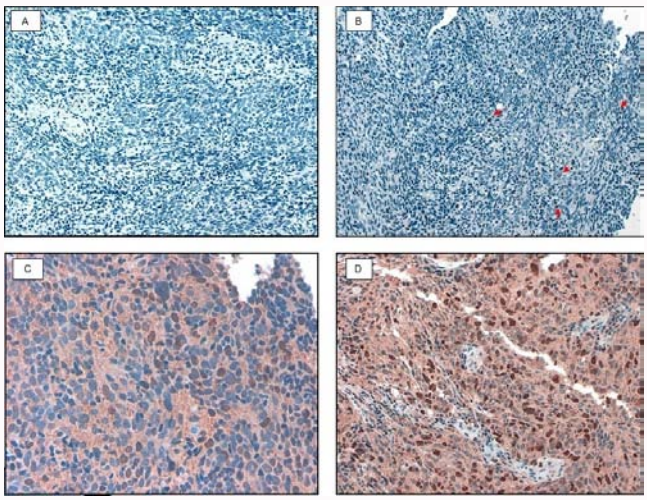

Figure 1: (A) Immunohistochemistry rated as negative, with 0% of cells stained. (B) Immunohistochemistry rated as negative, with 10% of cells stained with low intensity. Arrow indicates nucleus stained. (C) Immunohistochemistry rated as positive, with 25% of cells stained with moderate intensity. (D) Immunohistochemistry rated as positive with more than 50% of cells stained with high intensity.

Topoisomerase II alpha expression was evaluated semi-quantitatively based on two parameters: The percentage of cells with nuclear expression and the intensity of the immunostaining [14]. A score of 0 to 4 was recorded, according to the percentage of the positive neoplastic cells in each field (0<1%; 1=1% to 10%; 2=11% to 25%; 3=26% to 50%; and 4>50%). The intensity was classified from 0 to 3 (0=negative; 1= weak staining; 2= moderate staining; 3= strong staining). The upper range value of the percentage of immunostained cells was taken and multiplied by the intensity level. Thus, an interval was obtained where the values ranged from 0 to 300. We consider the interval 0 to 50 to be negative, and expression of values between 51 and 300 as positive value (Figure 1).

Statistical analysis

In this retrospective study, the association of clinical and prognostic factors with molecular characteristics was studied with chi-square test and Fisher's exact test when appropriate. The main end points of interest were Overall Survival (OS) and Progression-Free Survival (PFS). OS was measured considering the time that elapsed from the first day of treatment to the event of death due to any cause. DFS was measured from the day of the beginning of treatment to the date of relapse or death for any cause. Recurrent disease was taken into account as disease recurrence any time after treatment. The pattern of survival was estimated by Kaplan-Meier survival curves, with a threshold for significance of $p \geq 0.05$. Multivariate OS analysis was per- formed using a multivariate Cox regression model. The covariates that showed a trend to significance

(p<0.1), significance (p<0.05), or were clinically relevant, were put into a back-step multivariate Cox regression analysis. Multivariate regression logistic analysis was used to study variables associated to topoisomerase expression. All the statistic analysis was performed using SPSS version 17.0 software.

Ethics statement

This study was carried out in compliance with the Declaration of Helsinki. All subjects provided written informed consent for inclusion in the study, which was approved by the CCEIBA (Comité Coordinador de Ética de la Investigación Biomédica de Andalucía) and local CEIs (Comités de Etica de Investigación).

Results

Patient characteristics

Of the 58 patients included in the analysis, most of them were males of Caucasian race, with a diagnosis of non-keratinizing undifferentiated tumors, ECOG 0-1 Performance Status (PS) and stage III-IV at the time of diagnosis. Main characteristics of the patients included are described in Table 1.

With respect to the treatment, 17 patients (29.3%) were treated with induction chemotherapy followed by radiotherapy alone (9 patients) or combined with chemotherapy (8 patients). Moreover, a total number of 13 patients (22.4%) were treated with RT alone or CRT in 28 patients (48.3%). Regarding the clinical and histological variables studied, invasion of retropharyngeal space, base of skull involvement and invasion of cranial nerves were detected in 27.6%, 8.6% and 25.9% respectively of the total of cases. Finally, TOPO2A expression was identified in 26 patients (44.8%).

Table 1: Patient characteristics.

Patient Characteristics		n (%)
Age	Median, range	53.5 years (17 to 82 years)
Gender	Male	47 (81)
	Female	11 (19)
Ethnic	Caucasian	46 (79.3)
	Arabic	11 (19)
	Asian	1 (1.7)
Smoking Habit	Smoker	29 (50)
	Non-smoker	29 (50)
Stage	I	9 (15.5)
	II	4 (6.9)
	III	20 (34.5)
	IV	25 (43.1)
T	T1	14 (24.1)
	T2	14 (24.1)
	T3	9 (15.5)
	T4	21 (36.2)
N	N0	16 (27.6)
	N1	6 (10.3)
	N2	21 (36.2)
	N3	15 (25.9)
Histology	Keratinized SC	12 (20.7)
	Non-keratinized SC	11 (19)
	Undifferentiated carcinoma	35 (60.3)
Topoisomerase	Low expression	32 (55,2)
	High expression	26 (44.8)

Table 2: Univariate analysis for TOPO2A overexpression.

	TOPO2A ≤ 50 N (%)	TOPO2A >51 N (%)	p
Age			
≤ 60	26 (65)	14 (35)	0.02
> 60	6 (33.3)	12 (66.7)	
Gender			
Male	26 (55.3)	21 (44.7)	0.96
Female	6 (54.5)	5 (45.5)	
WHO			
0	12 (60)	8 (40)	0.59
1-2	20 (52.6)	18 (47.4)	
Stage			
I to II	3 (23.1)	10 (76.9)	0.01
III to IV	29 (64.4)	16 (35.6)	
T			
T1 to T2	15 (53.6)	13 (46.4)	0.81
T3 to T4	17 (56.7)	13 (43.3)	
N			
N0-1	8 (36.4)	14 (63.6)	0.03
N2-3	24 (66.6)	12 (33.3)	
Histology			
Keratinized	5 (41.7)	7 (58.3)	0.29
Non-keratinized	27 (58.7)	19 (41.3)	
Ethnic			
Caucasian	25 (54.3)	21 (45.7)	0.8
Non-Caucasian	7 (58.3)	5 (41.7)	
Skull base involvement			
Yes	5 (33.3)	10 (66.7)	0.05
No	27 (62.8)	16 (37.2)	
Cranial nerve involvement			
Yes	3 (60)	2 (40)	0.8
No	29 (54.7)	24 (45.3)	
Retropharyngeal invasion			
Yes	8 (50)	8 (50)	0.63
No	24 (57.1)	18 (42.9)	

We analyzed the potential association between different clinical-pathological factors and TOPO2A overexpression (Table 2). A significant association was observed between TOPO2A expression and N stage (p=0.01), with higher expression in less advanced N stage (27.6% of N0-1 tumors with TOPO2A expression compared with 17.2%; p=0.01). Moreover, a significant association was noted between stage and TOPO2A expression, with 76.9% of the stage I-II tumors presenting expression compared with 35.6% in the more advanced tumors (p=0.01). Furthermore, tumors with skull base involvement were significantly associated with topoisomerase expression (p=0.05) (Table 2). Finally, in the logistic regression analysis, stage (HR 7.94; 0.01-0.46; p=0.02) and age (HR 0.26; 0.08-0.92; p=0.04) of the patients

Table 3: Univariate analysis of survival.

	5-year OS	p	5-year PFS	p
Age				
≤ 60	72.20%	0.02	64.70%	0.06
>60	38.90%		38.90%	
Gender				
Male	54.80%	0.01	47.30%	0.01
Female	90.90%		90.90%	
Stage				
I-II	69.20%	0.4	69.20%	0.29
III-IV	59.60%		51.90%	
T				
T1-T2	67.20%	0.26	67.30%	0.3
T3-T4	56.70%		46.70%	
N				
N0-N1	68.20%	0.26	68.20%	0.18
N2-N3	57.80%		49.40%	
PS				
0	70%	0.26	68.40%	0.14
1-2	57.40%		49.30%	
Ethnic				
Caucasian	60.90%	0.7	56.50%	0.55
Non-Caucasian	64.80%		56.20%	
Topoisomerase				
≤ 50	75%	0.05	68.80%	0.03
>50	45.10%		41.20%	
Skull base involvement				
Yes	33.30%	0.01	33.30%	0.01
No	61.60%		64.60%	
Cranial nerve involvement				
Yes	20%	0.01	0%	0.01
No	65.70%		61.90%	
Retropharyngeal invasion				
Yes	43.80%	0.01	37.50%	0.09
No	68.70%		64%	

PS: Performance Status

were independently associated with TOPO2A expression.

Outcomes

After treatment, a total of 45 patients (77.6%) achieved a complete response, 4 patients (6.9%) a partial response, and 7 patients (12%) progressed. It should be noted that in 100% of patients in which a stable or progressive disease after treatment was reported, a TOPO2A overexpression was noted (p=0.02).

During the follow up 12 patients (20.7%) presented a recurrence of the disease, located locally in 1 patient, regionally in 2 patients, loco-regional in 3 patients, and distant in 6 patients. At the end of the study, 32 patients (55.1%) were still alive and 26 patients (44.9%) were death. Of these patients, 23 died as a consequence of the tumour (39.7%) and 3 secondary to other causes.

With a median follow up in alive patients of 74 months, median

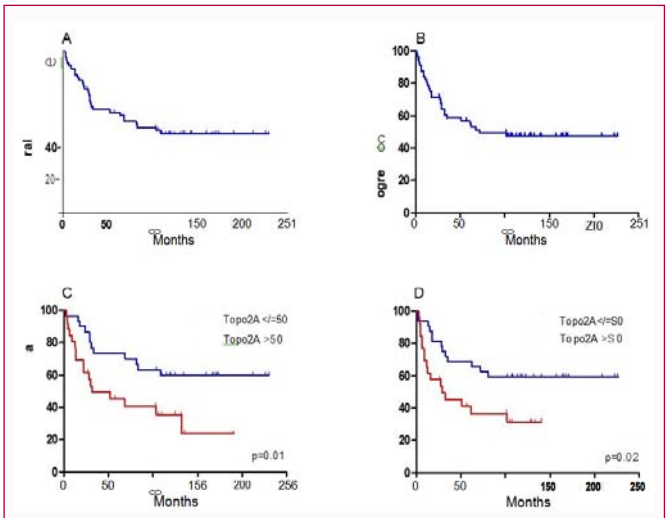

Figure 2: (A) Overall survival. (B) Progression Free Survival. (C) Overall Survival and TOPO2A expression. (D) Progression Free Survival and TOPO2A expression.

Table 4: Cox regression analysis for survival.

	OS		PFS	
	HR (95% CI)	**p**	**HR (95% CI)**	**p**
Age				
≤ 60 >60	2.63 (1.2 to 5.77)	0.02	1.93 (0.89 to 4.19)	0.1
Gender				
Female Male	7.98 (1.78 to 35.8)	0.01	6.13 (1.43 to 26.3)	0
Topoisomerase				
<50 >50	4.05 (1.72 to 9.53)	0.01	3.27 (1.43 to 7.49)	0
Stage				
Early Advanced	3.88 (1.35 to 11.16)	0.05	3.02 (1.07 to 8.5)	0

OS of all patients was 109 months with and actuarial 3-year and 5-year OS of 60% and 63% respectively. Median DFP was 73 months, with a 3-year and 5-year of 70.2% and 55.8 % respectively (Figure 2). Regarding investigation of prognostic factors associated with survival (Table 3), a significant decrease in OS was observed in patients with more than 60 years old (p=0.02), males (p=0.01), and patients with invasion of skull base, retropharyngeal space, or cranial nerves (p=0.01). Regarding PFS, a significant decrease in PFS was observed in male patients (p=0.01), and cranial nerve or skull base involvement (p=0.01). Moreover, a trend to significant decrease in PFS was observed in patients with more than 60 years (p=0.06), and retropharyngeal invasion (p=0.09). Interestingly, patients with topoisomerase expression presented a significant decrease in OS (p=0.05) and PFS (p=0.03) compared with those that presented absence or low expression. Finally, in the Cox regression analysis for OS, age of more than 60 Gy (p=0.02), male gender (p=0.01), topoisomerase expression (p=0.01) and advanced stage (p=0.05) were independently associated with a decrease in survival (Table 4).

Discussion

NPC is a highly proliferative tumour, with a high sensivity to chemotherapy and radiotherapy. However, a suboptimal rate of cure is obtained in advanced tumors. In this study we investigated the prognostic role of a potential biomarker (TOPO2A), an enzyme that play a role in chromosome replication and is usually elevated in

active proliferating cells [15]. In this study patients with TOPO2A overexpression presented a significant lower survival than with those that did not express TOPO2A, with mean OS and PFS of 64 months compared with 133 months (p=0.04), and 51 months compared with 91 months respectively (p=0.09). Increased TOPO2A has been associated with a lower survival in patients with other tumors such as small cell lung cancer [16], hepatocellular carcinoma [4], and colorectal cancer [12]. In the only published study regarding the prognostic role of topoisomerase II alpha in NPC, Lan et al. [12] identified TOPO2A as a differentially upregulated gene in NPC cancer, showing for the first time in an endemic area that TOPO2A overexpression independently predicts DSS and DMFS.

Although the cause of the negative prognostic impact of TOPO2A overexpression is not known, preclinical studies has with small cell lung cancer cell lines have shown an increase in the resistance to radiation and cisplatin associated with an increase in topoisomerase II alpha expression [17,18]. As a consequence, it has been hypothesized that increased topoisomerase II alpha may promote DNA repair, inducing drug and radiation resistance [17].

Our results have shown a higher TOPO2A expression in early stage tumors. Interestingly, Lan et al. have shown opposite findings, with TOPO2A overexpression was significantly higher in advanced compared with early stages. Although we cannot explain the discrepancy in these results, our findings might be explained by the potential abrogation during tumor progression of different molecular processes that prevent genomic instability, such as TOPO2A [19,20]. In this manner, a lack of topoisomerase activity has been shown to induce genetic instability, resulting in failure to complete replication, excess supercoiling, persistent catenation and even knots in DNA, inducing genetic instability [21]. Moreover, recent evidence supports an association between genetic instability and disease relapse (Chan N, Koritzinsky M. Chronic hypoxia decreases synthesis of homologous recombination proteins to offset chemoresistance and radio resistance. Cancer res 2008;68:605-614). This finding might identify “early” stage tumors that are going to be controlled suboptimally with RT, refining the prognosis especially in stage II.

This investigation has several limitations. Most important limitation derives from the retrospective nature of the study, and the scarce number of patients. Moreover, there are no standardized methods to measure TOPO2A immunohistochemistry.

Although these results must be prospectively confirmed, TOPO2A expression is a potential biomarker for NPC that may identify a subgroup of patients with worse prognosis in which new molecular therapies, or chemotherapy intensification, should be investigated. Moreover, it might help to homogeneously stratify NPC tumors in future clinical trials.

In conclusion, TOPO2A overexpression is associated with a decrease of OS and DFS in NPC, conferring tumor aggressiveness. Moreover, TOPO2A expression correlates with less advanced stages and may identify early tumors with different prognosis. This results may justify the prospective investigation of TOPO2A as an NPsC biomarker.

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