




Tertiary lymphoid structures in nasopharyngeal carcinoma: A multi-institutional study

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ABSTRACT

Background: Tertiary lymphoid structures (TLSs) associate with prognosis of many malignancies. However, the clinical significance of TLSs is not well-elucidated in nasopharyngeal carcinoma (NPC) patients.

Material and methods: In this whole population-based multicenter study, a total of 115 patients treated for NPC were included. The patients were treated at the five Finnish university hospitals. TLSs were assessed in routine hematoxylin and eosin (HE)-stained sections.

Results: Presence of TLSs associates significantly with improved survival in NPC. Absence of TLSs had a significant association with a poor disease-specific survival of NPC with a hazard ratio (HR) of 1.96 (95 % CI 1.09–3.53, $P = 0.025$) in the multivariable analysis. Similarly, absence of TLSs associated with worse overall survival with a HR of 1.68 (95 % CI 1.02–2.75, $P = 0.040$).

Conclusion: TLSs seem to be associated with prognosis of NPC patients. Having TLSs in NPC tumors correlates with good survival. The assessment of TLSs could aid in understanding the clinical behavior and in planning the treatment of NPC.

1. Introduction

Nasopharyngeal carcinoma (NPC) is one of the aggressive tumors of the head and neck region and it arises from the surface epithelium of the nasopharynx. NPC is closely associated with Epstein-Barr Virus (EBV) infection, particularly in East Asian endemic regions. There were 120,416 new cases of NPC diagnosed around the world in 2022 and it caused 73,476 deaths that year according to the GLOBCAN report [1]. NPC is distinguished by a geographic heterogeneity and it is endemic in

Southern China and South-East Asia where large cohort studies have been reported. In non-endemic European and North American populations, however, the NPC studies are usually based on small cohorts [2]. Thus, large cohort studies from the latter regions are warranted.

NPC is characterized by a high rate of locoregionally advanced disease and distant metastasis due to the adjacent rich lymphatic network [3]. Risk stratification of NPC is predominantly guided by the extent of the primary tumor, the nodal status and distant metastasis, i.e. TNM staging system. Unfortunately, NPC tumors of the same TNM stage may

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present with a dramatic variation in clinical behavior indicating that further prognostic classifiers are needed to supplement the TNM staging system [4,5]. In addition, the TNM staging system does not include molecular or histopathological parameters implicated in the pathogenesis of NPC. Therefore, it is necessary to identify prognostic biomarkers to guide the selection of patients for best treatment choices. In the last two decades, the assessment of immune-related parameters/biomarkers for treatment planning and immunotherapy has received more attention [6].

Among the immune-related parameters, tertiary lymphoid structures (TLSs) have been studied recently in solid tumors showing powerful prognostic value [7]. TLSs are ectopic lymphoid aggregates which consist of rich areas of T and B lymphocytes, dendritic cells, and plasma cells [8]. Of note, they have a prominent role in the adaptive antitumor immune response [8]. It is of importance that TLSs can be assessed in routine hematoxylin and eosin (HE)-stained sections. In NPC, however, the clinical impact of TLSs in HE-stained samples has not yet been well-documented. In this multicenter study, we aimed at analyzing the prognostic impact of TLSs in a large whole population-based NPC cohort from a non-endemic country (Finland).

2. Material and methods

Our whole population-based patients material included 115 patients treated primarily for NPC at one of the five Finnish University Central Hospitals (Helsinki, Turku, Tampere, Oulu, and Kuopio). Ethical permissions of the research ethics committee of the Hospital District of Southwest Finland and the Finnish National Supervisory Authority for Welfare and Health (VALVIRA) were obtained.

The assessment of TLSs was conducted as previously described [9]. With regard to the presence of TLSs, there were tumors with no TLSs (i.e.

no lymphoid structures found); tumors with lymphoid aggregates (i.e. vague, ill-defined clusters of lymphocytes); tumors with primary follicle/s (rounded clusters of lymphocytes without formation of germinal centers); and tumors with secondary follicles (i.e. follicles with germinal center formation). Two observers (AA, IL) blinded to follow-up data assessed the TLSs in HE-stained sections (Fig. 1).

Statistical analysis: All statistical analyses were conducted using IBM SPSS Statistics (version 25.0). Univariable and multivariable analyses were conducted to assess the relationship between prognostic parameters and survival. We used also Kaplan-Meier curves for disease-specific and overall survival analysis. The log-rank test was used to assess the statistical significance between the survival curves of patients in the various groups of TLSs. We categorized the tumors into four groups (no TLSs; lymphoid aggregates; primary follicles; secondary follicles) as explained in description of the scoring method. We then categorized the tumors into two groups: those having TLSs vs. those with no TLSs as previously reported [10–12]. Disease-specific survival was defined as the period from the date of diagnosis to the date of death of NPC or to the date of last follow-up. Overall survival was defined as the period from diagnosis until death of any cause or last follow-up.

3. Results

This is a multi-institutional study including a homogenous Finnish non-endemic cohort with only two patients from Southeast Asia (one from Hong Kong and the other from Vietnam). The patients were diagnosed in the period between 1990 and 2009. There were 80 (69.6 %) male and 35 (30.4 %) female patients with histologically confirmed diagnosis of NPC. The median age of patients at the time of diagnosis was 58 years (range 12–85). Their median follow-up time was 60 months. According to histologic typing, there were 87 (75.7 %) non-

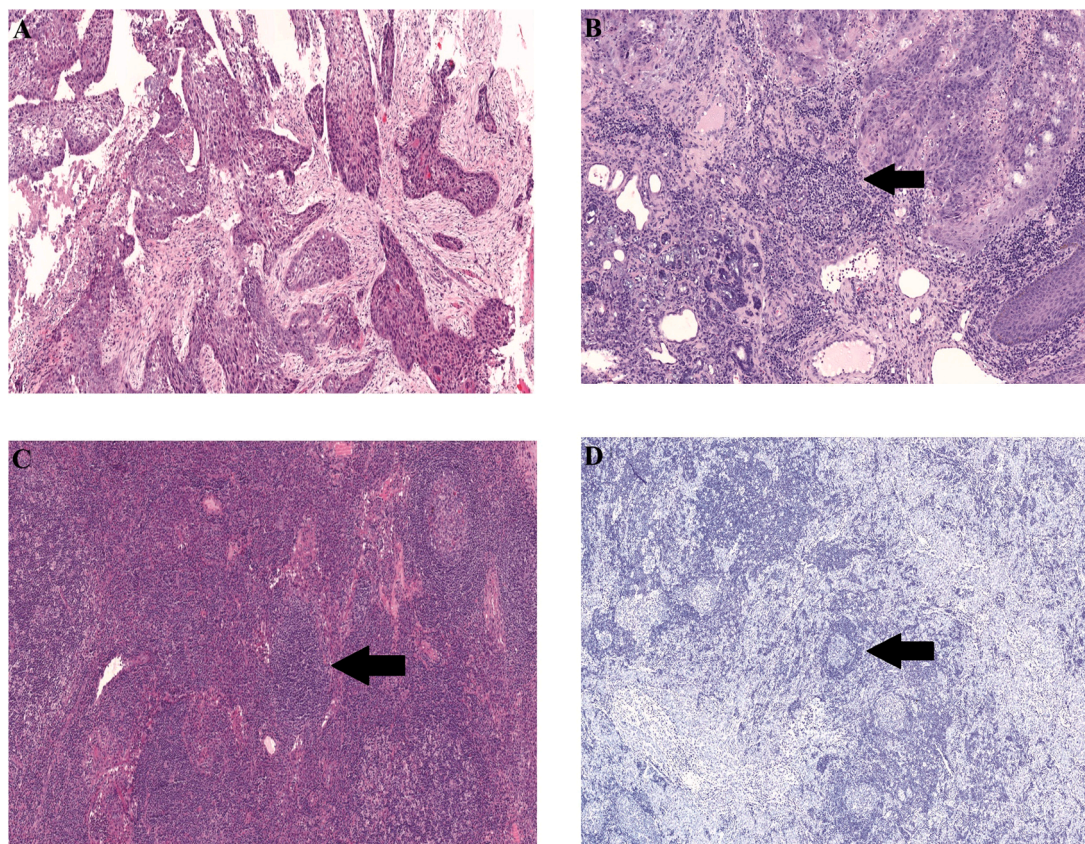


Fig. 1. Sections of nasopharyngeal carcinoma stained with hematoxylin and eosin. A: Tumor with no tertiary lymphoid structures. B: Tumor with tertiary lymphoid structure present as lymphoid aggregate (arrow). C: Tumor with primary follicle (arrow). D: Tumor with secondary follicle (arrow).

keratinizing tumors and 28 (24.3 %) keratinizing tumors. Fifteen (13 %) cases were of stage I, 29 (25.2 %) of stage II, 40 (34.8 %) of stage III, and 31 (27 %) of stage IV. A total of 50 (43.5 %) patients were treated with radiotherapy and 60 (52.2 %) patients received chemoradiotherapy, while 5 (4.3 %) patients received palliative treatment.

There were a total of 64 (55.7 %) tumors with TLSs. In these, 36 (31.3 %) cases of TLSs presented as aggregates, 21 (18.3 %) as primary follicles, and 7 (6.1 %) presented as secondary follicles. The remaining 51 (44.3 %) tumors presented no TLSs. In the assessment of the TLSs, a good level of agreement was confirmed between the observers (Kappa value = 0.76 and $P < 0.001$).

There was a significant predictive association between TLSs and the other tumor stroma-related biomarkers that we have reported in our previous studies [13,14]. Namely, with regard to tumor-stroma ratio, the presence of TLSs was associated significantly with stroma-low tumors ($P < 0.001$). In addition, presence of TLSs was significantly associated with high infiltration of stromal tumor-infiltrating lymphocytes ($P < 0.001$). No significant association was found between TLSs and other clinicopathologic factors such as age, gender, stage or histology (Table 1).

In the prognostic analyses (Tables 2 and 3), absence of TLSs

Table 1
Relationship between tertiary lymphoid structures and clinicopathologic characteristics of nasopharyngeal carcinoma.

Variable	Total N = 115	Tertiary lymphoid structures		P value (Pearson Chi-Square)
		Absent Number (%)	Present Number (%)	
		51 (44.3 %)	64 (55.7 %)	
Age				0.235
≤ 58 years	59	23 (39.0 %)	36 (61.0 %)	
> 58 years	56	28 (50.0 %)	28 (50.0 %)	
Gender				0.065
Male	80	40 (50.0 %)	40 (50.0 %)	
Female	35	11 (31.4 %)	24 (68.6 %)	
EBV status				0.078
Positive	69	26 (37.7 %)	43 (62.3 %)	
Negative	44	24 (54.5 %)	20 (45.5 %)	
Stage				0.522
I	15	4 (26.7 %)	11 (73.3 %)	
II	29	14 (48.3 %)	15 (51.7 %)	
III	40	19 (47.5 %)	21 (52.5 %)	
IV	31	14 (45.2 %)	17 (54.8 %)	
Histology				0.117
Non-keratinizing	87	35 (40.2 %)	52 (59.8 %)	
Keratinizing	28	16 (57.1 %)	12 (42.9 %)	
Tumor-stroma ratio				< 0.001
Stroma-low	67	19 (28.4 %)	48 (71.6)	
Stroma-high	48	32 (66.7 %)	16 (33.3 %)	
Stromal infiltrating lymphocytes				< 0.001
Low	31	26 (83.9 %)	5 (16.1 %)	
High	84	25 (29.8 %)	59 (70.2 %)	

Table 2
Disease-specific survival analysis of the prognostic significance of tertiary lymphoid structures and clinicopathologic characteristics in nasopharyngeal carcinoma.

Parameter	Univariable Analysis	Multivariable Analysis
	HR (95 %CI), P value	HR (95 %CI), P value
Age		
≤ 58 years	Reference	Reference
> 58 years	1.43 (0.83–2.47), P = 0.20	1.53 (0.85–2.73), P = 0.15
Gender		
Male	Reference	Reference
Female	0.99 (0.49–1.65), P = 0.73	1.07 (0.55–2.08), P = 0.85
EBV status		
Positive	Reference	Reference
Negative	2.40 (1.37–4.22), P = 0.002	1.41 (0.67–2.96), P = 0.37
Stage		
I	Reference	Reference
II	2.59 (0.55–12.24), P = 0.23	2.18 (0.44–10.64), P = 0.34
III	5.38 (1.26–23.03), P = 0.02	5.17 (1.16–22.99), P = 0.03
IV	8.88 (2.07–38.13), P = 0.003	6.94 (1.56–30.99), P = 0.01
Histology		
Non-keratinizing	Reference	Reference
Keratinizing	2.26 (1.26–4.08), P = 0.007	1.61 (0.72–3.62), P = 0.25
Tertiary lymphoid structures		
Present	Reference	Reference
Absent	2.08 (1.20–3.61), P = 0.009	1.96 (1.09–3.53), P = 0.025

Table 3
Overall survival analysis of the prognostic significance of tertiary lymphoid structures and clinicopathologic characteristics in nasopharyngeal carcinoma.

Parameter	Univariable Analysis	Multivariable Analysis
	HR (95 %CI), P value	HR (95 %CI), P value
Age		
≤ 58 years	Reference	Reference
> 58 years	1.88 (1.19–2.97), P = 0.007	1.93 (1.16–3.19), P = 0.011
Gender		
Male	Reference	Reference
Female	0.89 (0.54–1.46), P = 0.64	0.86 (0.49–1.52), P = 0.61
EBV status		
Positive	Reference	Reference
Negative	3.47 (2.15–5.59), P < 0.001	2.32 (1.26–4.29), P = 0.007
Stage		
I	Reference	Reference
II	2.08 (0.82–5.30), P = 0.12	1.87 (0.69–5.02), P = 0.21
III	2.71 (1.11–6.61), P = 0.03	2.84 (1.12–7.25), P = 0.02
IV	4.29 (1.74–10.53), P = 0.002	3.39 (1.32–8.73), P = 0.01
Histology		
Non-keratinizing	Reference	Reference
Keratinizing	2.34 (1.42–3.86), P < 0.001	1.37 (0.70–2.65), P = 0.36
Tertiary lymphoid structures		
Present	Reference	Reference
Absent	2.002 (1.27–3.16), P = 0.003	1.68 (1.02–2.75), P = 0.040

associated with worse disease-specific survival of NPC patients with a hazard ratio of 2.08 (95 % CI 1.20–3.61, $P = 0.009$) in the univariable analysis and a HR of 1.96 (95 % CI 1.09–3.53, $P = 0.025$) in the multivariable analysis. Similarly for overall survival, absence of TLSs associated with worse overall survival with a HR of 2.002 (95 % CI 1.27–3.16, $P = 0.003$) in the univariable analysis and with a HR of 1.68 (95 % CI 1.02–2.75, $P = 0.040$) in the multivariable analysis. In the two-group categorization Kaplan Meier survival curves confirmed the significance of TLSs indicating that cases with TLSs had a significantly better disease-specific survival ($P = 0.007$, Fig. 2A) and overall survival ($P = 0.002$, Fig. 2B) compared with cases with no TLSs. A similar two-group categorization has been used in previous studies [10–12]. On the other hand, the number of cases (and more specifically the number of events, i.e. mortality) in the alternative four-group categorization did not allow for a statistically significant correlation between the four groups of TLSs and survival ($P > 0.05$).

4. Discussion

Management of NPC remains a challenge even at experienced head and neck oncology centers. In daily practice, treatment planning of NPC is based on the TNM staging system which does not adapt well for some patients [5]. In addition, NPC cases have shown broad differences in radiosensitivity. Thus, the identification of accurate predictive and prognostic biomarkers is necessary to guide clinical trial design, to optimize risk stratification, and to personalize clinical decision making. The immune tumor microenvironment has a prominent role in head and neck cancer progression [15]. Of note, immune response is a potential source of recognizing prognostic biomarkers. While the immune cells of the tumor microenvironment have been extensively studied in head and neck cancers, less research has been focused on the clinical significance of tertiary lymphoid structures (TLSs) in NPC. Our results in this multicenter study indicate that occurrence of TLSs in these tumors is associated with a favorable clinical behavior of NPC.

TLSs have characteristics similar to secondary lymphoid organs [16]. The presence of TLSs elevates the capacity of mature dendritic cells to enhance the activation of B and T cells, and thus facilitates the onset and propagation of adaptive antitumor immune response [8,17]. Interestingly, induction of TLSs formation has been reported to enhance response to immune checkpoint blockade therapy in a mouse model of head and neck cancer [16]. Of note, TLSs in melanoma were shown to have an important role in the immune microenvironment [17]. Furthermore, TLSs had a significant role in spontaneous regression of Merkel cell carcinoma [18]. Of note, a recent study by Li and colleagues [19] has underlined the significance of TLSs in NPC. In addition, they

found that TLSs of NPC tumors resembled secondary lymphoid organs. Moreover, they found a high number of cells expressing CXCL13, IFN- γ and IL-21 around the follicular TLSs while in normal tissue they described incomplete structures with only minor expression of PD-1, CXCL13, IFN- γ and IL-21 [19].

The prognostic impact of TLSs has been reported in many cancers including digestive system carcinomas [20], pancreatic ductal adenocarcinoma [10], endometrial carcinoma [21], and lung cancer [22,23]. In head and neck cancers, TLSs have been reported as a promising prognostic factor in oral tongue cancer [9,12] and laryngeal cancer [24]. In a recent Chinese study on nasopharyngeal cancer, Chen et al. [25] reported that the signature of TLSs was associated with improved survival of NPC patients. According to the same lines, our current study found a better outcome in NPC cases with TLSs. Of note, this is the first multicenter study from a non-endemic region to analyze TLS in NPC.

Importantly, it has been proposed that TLSs have a prominent role in governing invasion and metastasis [8,26], well-recognized hallmarks of cancer. In addition, it has been speculated to administer lymphoid chemokines to induce neogenesis of TLSs which could be a strategy for the treatment of cancer [20]. Furthermore, TLSs have been reported as a reliable predictor for the efficacy of immunotherapy in head and neck cancer [27] and other cancers [17,28]. In addition, assessment of TLSs has a good interobserver agreement (i.e. reproducibility) between observers in our current study and in previous reports [29,30]. In addition, the evaluation of TLSs was simply performed using HE-stained sections. Thus, this assessment can be used for the prognostication of NPC with resources available in the conventional pathology laboratory without any additional expensive techniques.

In conclusion, the evaluation of TLSs is a valuable prognostic tool in nasopharyngeal cancer and the absence of TLSs is associated with worse prognosis. It is necessary to mention that our cohort is relatively small, and we were not able to conduct a similar analysis dividing the tumors into four groups (i.e. no TLSs, aggregate, primary TLSs, secondary TLSs) because of too small numbers of events (mortalities) in each group. To the best of our knowledge, the current study is the first multicenter study to report this finding in a non-endemic population. Indeed, validation studies are needed, and studies to further understand the beneficial role of TLSs in survival of NPC patients. In addition, further studies should consider automated assessment of TLSs which has promising results recently in gastrointestinal cancer [31] and lung cancer [32,33].

Author contributions

Conceptualization and study design: AA, AM, JH, VMK, IL.

Data production, analysis and interpretation: MR, AA, AM, IL

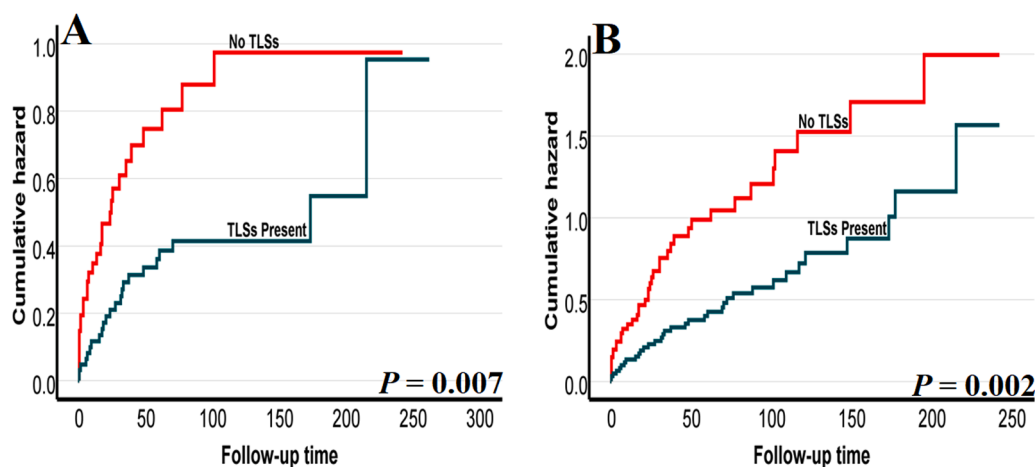


Fig. 2. Kaplan Meier survival curves for tertiary lymphoid structures in nasopharyngeal carcinoma. A: Disease-specific survival ($P = 0.007$). B: Overall survival ($P = 0.002$).

Manuscript writing: AA, MR, AM, IL

Reviewing and editing of the final manuscript: AM, VMK, JH, IL.

All authors have reviewed the manuscript and approved the final manuscript.

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Author statement

All persons listed as authors meet the criteria laid down in your guidelines and have approved the manuscript as well as its submission to *Pathology - Research and Practice* journal. We have no conflicts of interest to disclose.

CRedit authorship contribution statement

Ilmo Leivo: Writing – review & editing, Writing – original draft, Supervision, Formal analysis, Data curation, Conceptualization, Funding acquisition. **Antti Mäkitie:** Writing – review & editing, Supervision, Funding acquisition, Formal analysis, Conceptualization. **Veli-Matti Kosma:** Writing – review & editing, Data curation, Conceptualization. **Jaana Hagström:** Writing – review & editing, Data curation, Conceptualization. **Miia Ruuskanen:** Writing – review & editing, Formal analysis, Data curation. **Alhadi Almagush:** Writing – original draft, Methodology, Data curation, Conceptualization.

Declaration of Competing Interest

None declared.

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