

## ORIGINAL PAPER



## Comparative clinicopathological aspects of chronic tonsillitis and adenoiditis in children

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### Abstract

Chronic palatal and nasopharyngeal inflammations are common lesions in pediatric pathology, with major effects on children's development. The study included 34 cases of chronic tonsillitis and adenoiditis for which we quantified immunohistochemically and analyzed the distribution of inflammatory elements in the follicular, extrafollicular and epithelial compartments, in relation to the composite histological scores and the clinico-epidemiological profile of the lesions. The cases were more frequent under the age of 10, in female patients, coming from urban areas, with the diagnosis of tonsillitis. B-lymphocytes have been associated with follicular areas in tonsillitis and epithelial areas in adenoiditis. In all compartments, T-lymphocytes were more frequently associated with tonsillitis and plasma cells associated with adenoiditis. Macrophages and dendritic cells had a relatively uniform distribution for the three compartments in all cases. The results obtained indicate different inflammatory phenotypes for chronic tonsillitis and adenoiditis, an aspect that may be useful for stratifying patients for optimal therapy.

**Keywords:** chronic inflammation, tonsillitis, adenoiditis, lymphocytes, plasma cells, macrophages.

### Introduction

The Waldeyer's ring is a complex structure belonging to the mucosa-associated lymphoid tissue (MALT), with the role of defense and immunological regulation, located at the entrance to the respiratory and digestive tract and formed by Luschka's tonsils (adenoids), Gerlach's tonsils (tubal), palatine tonsils and lingual tonsil [1, 2].

Chronic inflammation of the Waldeyer's lymphatic ring, especially those located in the palatal and nasopharyngeal levels are common lesions in pediatric pathology, with effects on somatic and functional development of the child and influencing the quality of life [3–5]. In the case of persistent chronic inflammation with periods of exacerbation and obstructive sleep apnea syndrome and sometimes in the case of hyponasal speech, otitis media, dental malocclusion and cardiopulmonary complications, surgical excision of the affected lymphoid tissue is indicated [5, 6]. Some studies indicate a different immunological reactivity of lymphoid structures in the Waldeyer's ring, which are attributed to more frequent exposure of the nasopharyngeal area by respiration, antigenic exposure on a wider area of the palatine area, differences in the structure of the covering epithelium or certain factors, as is the age of the patients [7–11]. There are relatively few studies that have analyzed the histological and immunohistochemical (IHC) distribution

of inflammatory elements in these lymphoid structures, on the one hand due to the tendency to avoid histological evaluation in the absence of risk factors, and on the other hand the heterogeneity of histological criteria used [7, 12]. However, some authors also indicate advantages of histological examination represented both by identifying some particular aspects of the chronic inflammation (tuberculosis, Crohn's disease, storage diseases, etc.) and by the fact that these surgical specimens can be used to investigate the immune response in the sense of mechanisms involved in the initiation and persistence of chronic inflammation [7, 11]. Also, functional heterogeneity of the tonsils appears to be related to the distribution of T- and B-lymphocytes that cooperate in a complex way with macrophages and epithelial cells [13].

### Aim

In this study, we quantified and analyzed the distribution of inflammatory elements associated with chronic inflammation of the Waldeyer's lymphatic ring in children, in relation to the clinicopathological parameters of the lesions.

### Materials and Methods

The study included 34 cases of chronic inflammation

located in the Waldeyer's lymphatic ring, investigated and operated within the Ear, Nose, and Throat (ENT) Department, Emergency County Hospital of Craiova, Romania, within the period 2016–2019. All cases in this study presented the association of recurrent chronic inflammation and respiratory disorders, which were indications for surgery [4, 6]. The biological material was represented by palatine tonsillectomy or adenoidectomy specimens from patients less than 15 years old, which have been fixed in 10% neutral buffered formalin, processed by classical technique of paraffin embedding and Hematoxylin–Eosin (HE) staining. The diagnosis of the lesions was made within the Department of Pathology of the same Hospital, according to the existing data in the specialized literature [7, 11, 14].

The histopathological (HP) analysis aimed to establish a composite histological score (CHS), which took into account the following parameters and scores, adapted from the data in the literature [3, 9]: inflammation of the covering epithelium (score 1 – mild, score 2 – moderate, score 3 – intense), reticulation of the crypts (score 1 – mild, score 2 – moderate, score 3 – increased), lymphoid hyperplasia (score 1  $\leq$  3 lymphoid follicles, score 2  $>$  3 lymphoid follicles), collagen sclerosis (score 1 – focal, score 2 – diffuse), CHS with values 1–5 being considered low (LCHS), and those with values between 6–10 were considered high (HCHS). In cases with multiple surgical pieces, such as bilateral palatal tonsillectomy or the association of tonsillectomy with adenoidectomy in mixed inflammations, the most histologically affected specimen in terms of chronic inflammation was included in the study.

For the IHC analysis were used monoclonal mouse antihuman markers addressed to inflammatory elements associated with chronic processes (Table 1). After the primary processing that included dewaxing in xylene, rehydrating in alcohols, endogenous enzymatic blocking and unspecific blocking, the antigens retrieval was performed by microwaving in citrate buffer for 20 minutes or by incubation with pepsin for 10 minutes.

**Table 1 – The antibodies and immunostaining protocol**

Antibody	Clone	Dilution	Pretreatment	External positive control
CD20	L26	1:200	Microwaving in citrate buffer (HIER), pH 6	Spleen
CD45RO	UCHL1	1:100	Microwaving in citrate buffer (HIER), pH 6	Spleen
CD138	MI15	1:100	Pepsin	Nasal mucosa (rhinitis)
CD68	PG-M1	1:50	Microwaving in Tris-EDTA buffer (HIER), pH 9	Kidney

CD: Cluster of differentiation; EDTA: Ethylenediaminetetraacetic acid; HIER: Heat-induced epitope retrieval.

The sections were subsequently incubated with the primary antibody, at 4°C, overnight. The working system used in this study was represented by Labeled Streptavidin–Biotin 2 (LSAB2) system (Dako, Redox, Romania, code K0675), and the chromogen was 3,3'-Diaminobenzidine (DAB) tetrahydrochloride (Dako, Redox, Romania, code K3468). Validation of IHC results was achieved by using external positive controls and external negative controls by omitting the primary antibody. IHC reactions were investigated both semi-quantitatively and by descriptive

analysis of the distribution of inflammatory elements in different compartments. The quantification of the immunostainings was done by obtaining the positivity index (PI), resulting by reporting the number of labeled cells to the total number of cells counted at 40 $\times$  microscope objective. For each compartment of each case were counted 10 microscopic fields (MFs) from areas with the most immunosignals, the average values of the cases and groups being subsequently compared. The quantification of the immunostained elements was performed for each case in three compartments, respectively epithelial, extrafollicular (which included the interfollicular and subepithelial areas) and follicular (which included the germinal centers and the mantle areas).

For the statistical analysis, we used the Student's *t*-test,  $\chi^2$  (chi-squared), one-way analysis of variance (ANOVA) and Pearson's comparative tests within Statistical Package for the Social Sciences (SPSS) 10 software, the results being considered significant for values of  $p < 0.05$ . For the images acquisition was used the Nikon Eclipse E600 microscope equipped with Lucia 5 software.

The study included only nonspecific chronic inflammation, excluding patients with other associated acute or chronic inflammatory processes, as well as those who have received drug treatments, nutritional supplements in the past three months, or with an immunocompromised status. The local Ethical Committee approved the study, and informed consent was obtained for all cases.

## Results

The 34 cases included in the study belonged to patients aged between 5–15 years, with a mean diagnosis age of 9.7 $\pm$ 3.4 years and who had recurrent chronic nonspecific inflammation, associated with persistent respiratory manifestations, and located in the palatine tonsils and adenoids.

In this study predominated the patients under the age of 10 (55.9%), females (52.9%), who came from urban areas most often (70.6%) and who were diagnosed with chronic tonsillitis (47.9%) (Table 1). Tonsils (67.6%) also predominated as investigated histological specimens. CHS revealed more frequently high values in patients less than 10 years old ( $p=0.002$ ,  $\chi^2$  test), in males ( $p=0.028$ ,  $\chi^2$  test), from urban areas ( $p=0.362$ ,  $\chi^2$  test), in both tonsils and adenoids surgical specimens ( $p=0.877$ ,  $\chi^2$  test) (Table 2).

**Table 2 – Distribution of cases in relation to the analyzed clinicopathological parameters**

Parameter	No. of cases	CHS (No. of cases)	
		LCHS	HCHS
Age [years]	$\leq 10$	19	3
	$> 10$	15	5
Gender	Male	16	3
	Female	18	8
Environment	Urban	24	8
	Rural	10	5
Surgical specimen	Tonsil	23	9
	Adenoids	11	4
Clinical diagnosis	Tonsillitis	16	–
	Adenoiditis	7	–
	Mixed	11	–

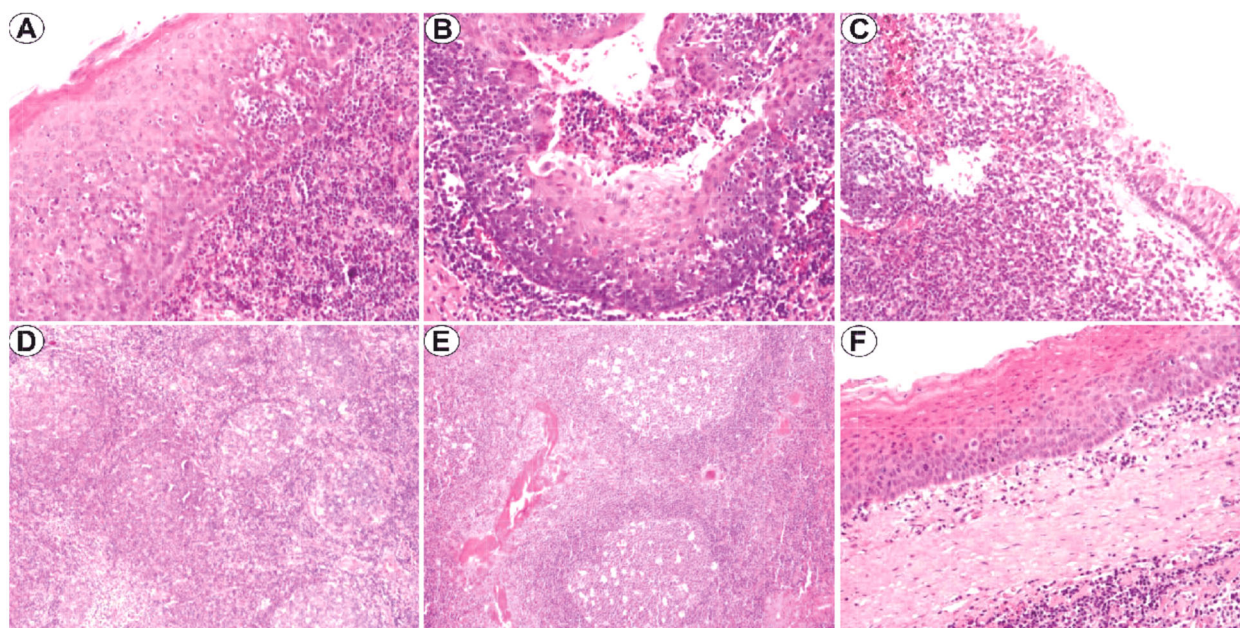
CHS: Composite histological score; HCHS: High composite histological score; LCHS: Low composite histological score.

HP analysis of the cases indicated both in the investigated tonsils and adenoids changes in the covering epithelium and in the crypts. Thus, in the case of tonsils and adenoids, the covering epithelium presented variable degrees of infiltration with inflammatory elements, represented predominantly by lymphocytes with diffuse or focal disposition (Ugras abscesses) (Figure 1A). The non-keratinized and keratinized epithelium of the tonsils presented focal acanthosis, hyperkeratosis, focal papillary projections, and basal hyperplasia, and in the crypts the inflammatory epithelial infiltration (reticulation) was variable, including with the formation of abscesses (Figure 1B). The non-keratinized and cylindrical ciliated epithelium of the adenoids showed other changes represented by basal hyperplasia, focal goblet cell hyperplasia, papillary projections. In all cases, we found the presence of an inflammatory infiltrate in the subepithelial extrafollicular compartment that infiltrated the basement

membrane and led to defects of the epithelia covering the tonsils and adenoids, sometimes to the surface, with the presence of ulcerations, especially in the case of adenoids (Figure 1C).

Follicular hyperplasia was invariably present in all cases, in the case of adenoids predominating the increase in the number of follicles, and in the case of tonsils, the increase in volume with the appearance of germinal centers (Figure 1, D and E). The follicular mantle areas were rarely visible in both adenoids and tonsils. Areas of collagenous sclerosis of varying degrees have been identified in all specimens, most common subepithelial in the case of tonsils (Figure 1F).

The immunorexpression of the analyzed markers was identified in all the analyzed cases, with some differences of the PI values in relation to the location of the inflammation and the compartments of the affected structures.



**Figure 1** – (A) Chronic tonsillitis, epithelial infiltration with lymphocytes forming Ugras abscesses; (B) Chronic tonsillitis, reticulation of the cryptic epithelium and abscess formation; (C) Chronic adenoiditis, inflammatory infiltration of the epithelium and ulceration; (D) Chronic tonsillitis, follicular hyperplasia; (E) Chronic adenoiditis, follicular hyperplasia; (F) Chronic tonsillitis, subepithelial collagen sclerosis. Hematoxylin–Eosin (HE) staining: (A–C and F)  $\times 100$ ; (D and E)  $\times 40$ .

### CD20 immunorexpression

Cluster of differentiation 20 (CD20) immunoreactions were identified in the membrane and apical cytoplasmic level, with a mean PI value for the entire analyzed group of  $35.7 \pm 18.1$  in the epithelial compartment,  $46.9 \pm 14.8$  at the extrafollicular level and  $52.9 \pm 16.6$  at the follicular level.

In the case of tonsillitis, the highest PI values were identified at the follicular level, compared to the other two compartments ( $p < 0.001$ , ANOVA test) (Table 3), while in the case of adenoiditis, the PI values did not present significant differences compared to the investigated compartments.

CD20 reactions were higher at the limit of statistical significance in the epithelial compartment of the adenoids ( $p = 0.080$ , Student's *t*-test) and significantly higher ( $p = 0.005$ , Student's *t*-test) in the follicular compartment of the tonsils (Figure 2, A–D). In the case of the extra-

follicular compartment, the immunosignals predominated at the interfollicular level in the case of tonsillitis and subepithelial in the case of adenoiditis (Figure 2, E and F).

### CD45RO immunorexpression

Cluster of differentiation 45RO (CD45RO) immunorexpression was identified at the membrane and apical cytoplasmic level, the mean PI value for the whole group being  $40 \pm 20.6$  in the epithelial compartment,  $50.2 \pm 13.7$  at the extrafollicular level and  $60.5 \pm 14$  at the follicular level. In the case of both tonsillitis and adenoiditis, the values were significantly higher ( $p = 0.005$ , ANOVA test, respectively  $p = 0.002$ , ANOVA test) at the level of the follicular compartment compared to the extrafollicular and epithelial ones (Table 3; Figure 3, A and B).

Immunoreactions were significantly higher in tonsillitis compared to adenoids in the epithelial compartment

( $p=0.006$ , Student's  $t$ -test) but also in the extrafollicular ( $p=0.021$ , Student's  $t$ -test) and follicular ( $p=0.003$ , Student's  $t$ -test) compartments (Figure 4, A–F).

### CD138 immunoeexpression

Cluster of differentiation 138 (CD138) immunoeexpression was also present in the membrane and apical cytoplasm of the plasma cells, but also in epithelial cells, especially in the lower half of the cylindrical ciliated epithelium of the adenoids and the cryptic epithelium in the tonsils. The mean value of CD138 PI for the whole group was  $9.5\pm 9.5$  at the epithelial compartment,  $43.5\pm 21.8$  at the extrafollicular level and  $6.9\pm 3$  at the follicular level. Both in the case of tonsillitis ( $p<0.001$ , ANOVA test) and adenoiditis ( $p<0.001$ , ANOVA test), the PI values were significantly higher at extrafollicular level (both interfollicular, but especially subepithelial), compared to the epithelial and follicular compartments (Table 3; Figure 5, A and B).

PI values of CD138 were higher in the case of adenoiditis compared to tonsillitis in all investigated compartments, the appearance being statistically significant only in the extrafollicular compartment ( $p<0.001$ , Student's  $t$ -test). Most of the positive elements for CD138 were subepithelial located, but also at the interfollicular level, especially in the case of adenoids. For tonsillitis, the reactions were mainly associated with the cryptic epithelium. We found a relatively low number of CD138-positive elements at the epithelial and follicular level (Figure 6, A–D).

### CD68 immunoeexpression

Cluster of differentiation 68 (CD68) immunoeexpression was identified in the cytoplasm of macrophage cells, the mean PI values for the analyzed group being  $13.1\pm 4.9$  in the epithelial compartment,  $44.4\pm 8.1$  in the extrafollicular level and  $23.2\pm 8.1$  in the follicular level. CD68 PI values

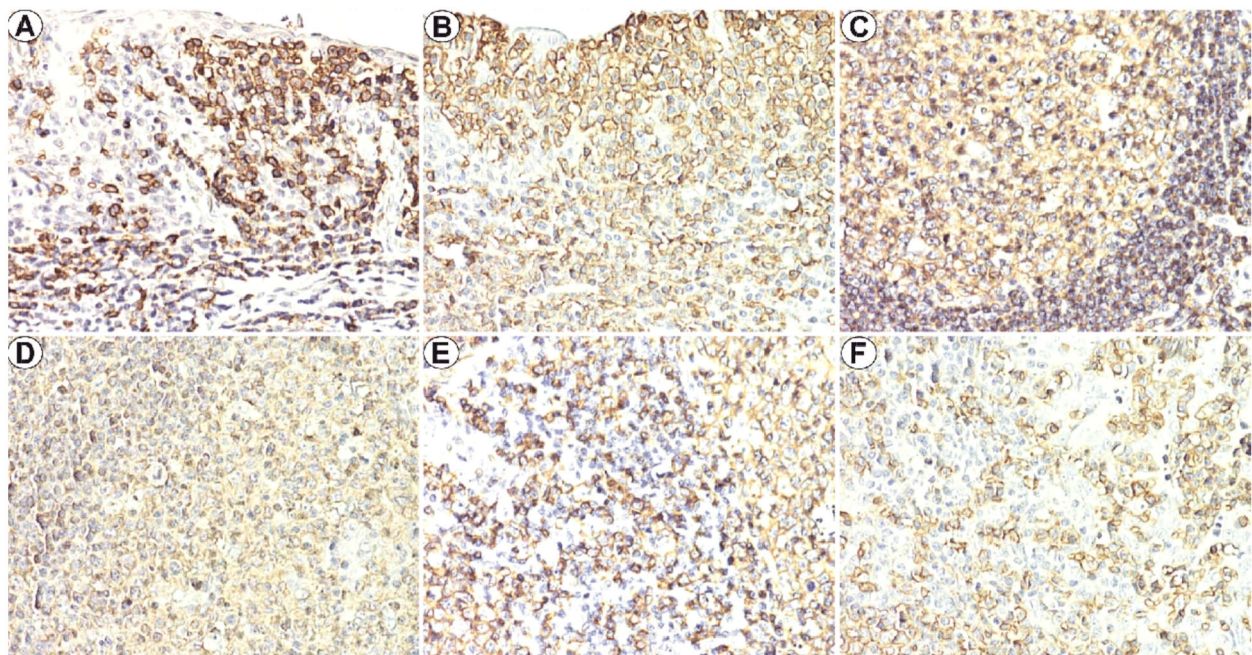
were significantly higher at the extrafollicular level, compared to the epithelial and follicular areas, both in the case of tonsillitis ( $p<0.001$ , ANOVA test) and adenoiditis ( $p<0.001$ , ANOVA test) (Table 3; Figure 7, A and B).

The distribution of CD68-positive elements did not present significant differences in the tonsil compartments compared to those of the adenoids (Figure 8, A–D).

**Table 3 – Distribution of the PI values for the analyzed markers in relation with the inflammation type and epithelial, extrafollicular and follicular compartment and their statistical significance**

PI values / Compartments / Lesions		Chronic tonsillitis	Chronic adenoiditis	p-value (Student's t-test)
CD20 PI	Epithelial	$31.7\pm 16.7$	$44\pm 18.8$	0.080
	Extrafollicular	$47.6\pm 14.7$	$45.4\pm 15.5$	0.705
	Follicular	$58.9\pm 13$	$40.4\pm 16.9$	0.005
	p-value (ANOVA test)	<0.001	0.781	–
CD45RO PI	Epithelial	$48.6\pm 18.5$	$28.1\pm 18.4$	0.006
	Extrafollicular	$55\pm 8.6$	$40.4\pm 17.3$	0.021
	Follicular	$63.9\pm 13.3$	$53.4\pm 13.2$	0.003
	p-value (ANOVA test)	0.005	0.002	–
CD138 PI	Epithelial	$7.1\pm 6.3$	$14.5\pm 13.1$	0.102
	Extrafollicular	$34.1\pm 16.9$	$63.1\pm 17.7$	<0.001
	Follicular	$6.4\pm 2.8$	$7.9\pm 3.4$	0.251
	p-value (ANOVA test)	<0.001	<0.001	–
CD68 PI	Epithelial	$13.6\pm 5.3$	$12.2\pm 4$	0.430
	Extrafollicular	$42.6\pm 7.6$	$48.1\pm 8.1$	0.072
	Follicular	$23.2\pm 7.7$	$23.1\pm 9.2$	0.980
	p-value (ANOVA test)	<0.001	<0.001	–

ANOVA: Analysis of variance; CD: Cluster of differentiation; PI: Positivity index.



**Figure 2 – (A) Chronic tonsillitis, epithelial compartment; (B) Chronic adenoiditis, epithelial compartment; (C) Chronic tonsillitis, follicular compartment; (D) Chronic adenoiditis, follicular compartment; (E) Chronic tonsillitis, extrafollicular compartment; (F) Chronic adenoiditis, extrafollicular compartment. Anti-CD20 antibody immunostaining: (A–F)  $\times 200$ . CD20: Cluster of differentiation 20.**

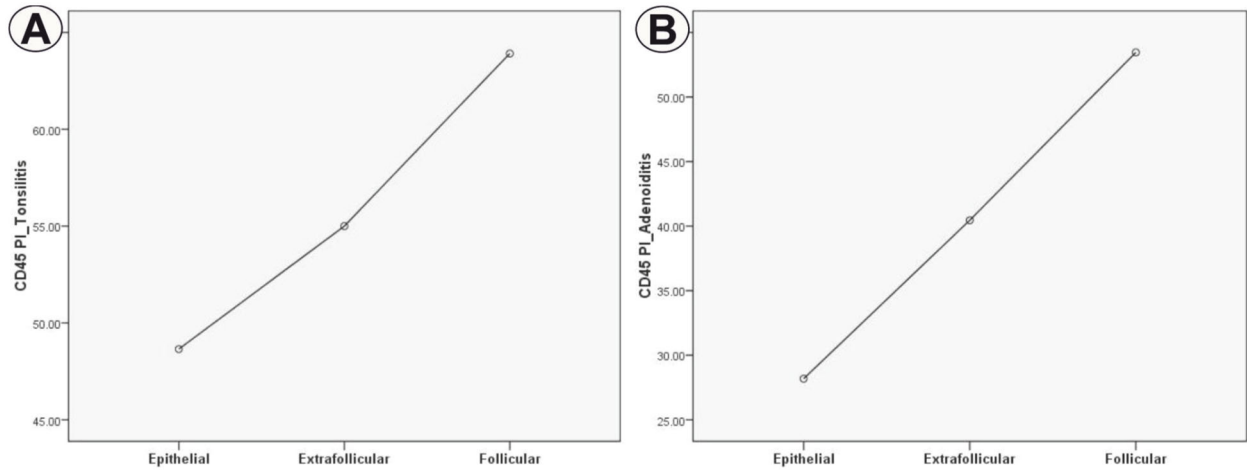


Figure 3 – Distribution of CD45RO PI values in tonsillitis (A) and adenoiditis (B). CD45RO: Cluster of differentiation 45RO; PI: Positivity index.

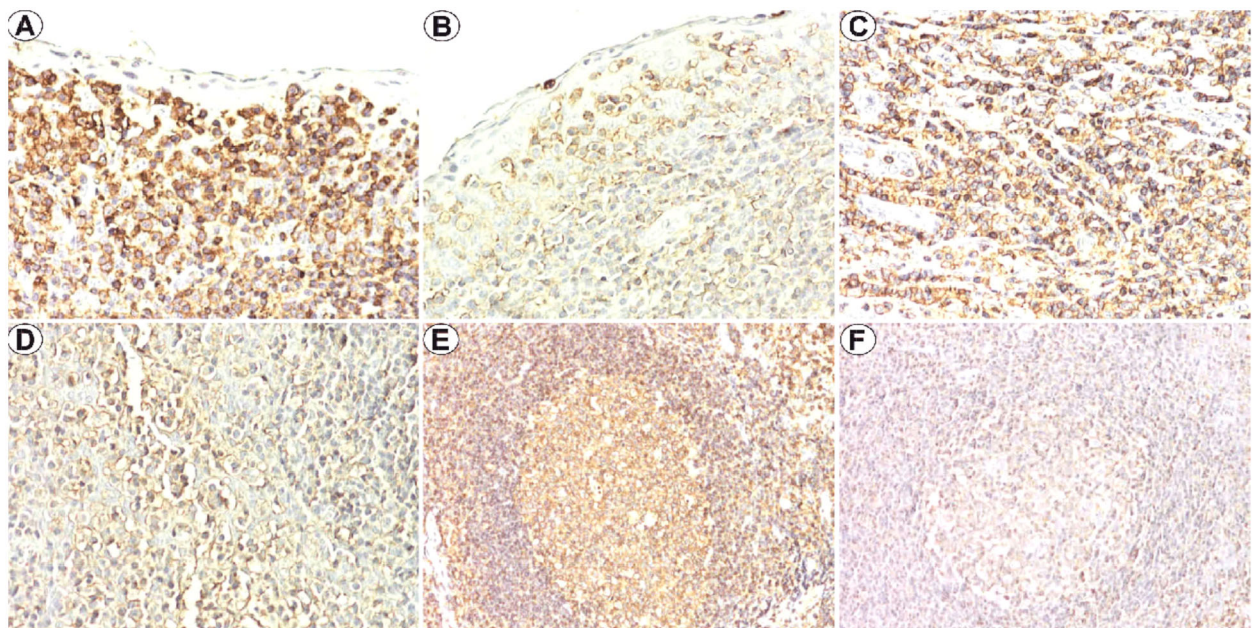


Figure 4 – (A) Chronic tonsillitis, epithelial compartment; (B) Chronic adenoiditis, epithelial compartment; (C) Chronic tonsillitis, extrafollicular compartment; (D) Chronic adenoiditis, extrafollicular compartment; (E) Chronic tonsillitis, follicular compartment; (F) Chronic adenoiditis, follicular compartment. Anti-CD45RO antibody immunostaining: (A–D and F) ×200; (E) ×100. CD45RO: Cluster of differentiation 45RO.

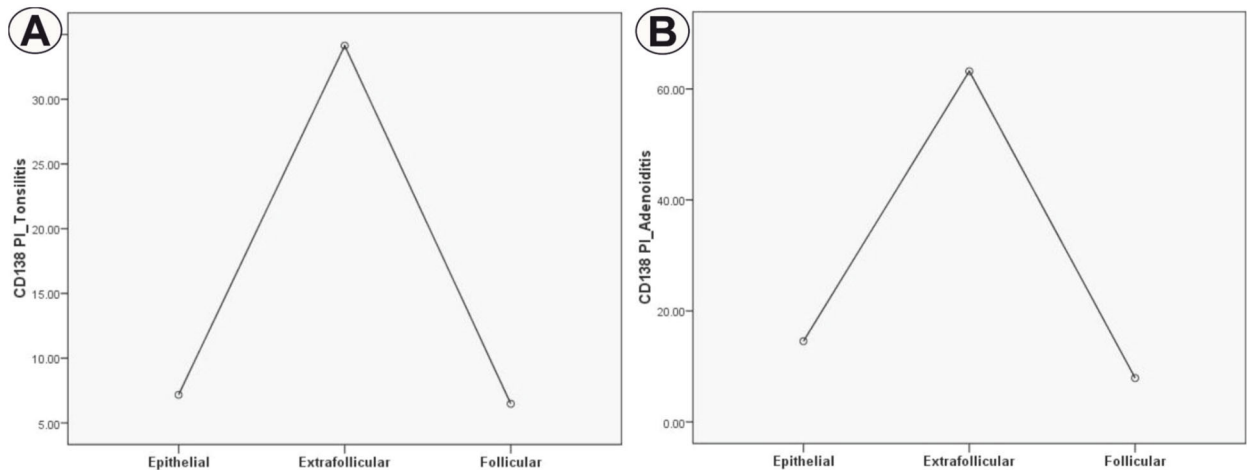
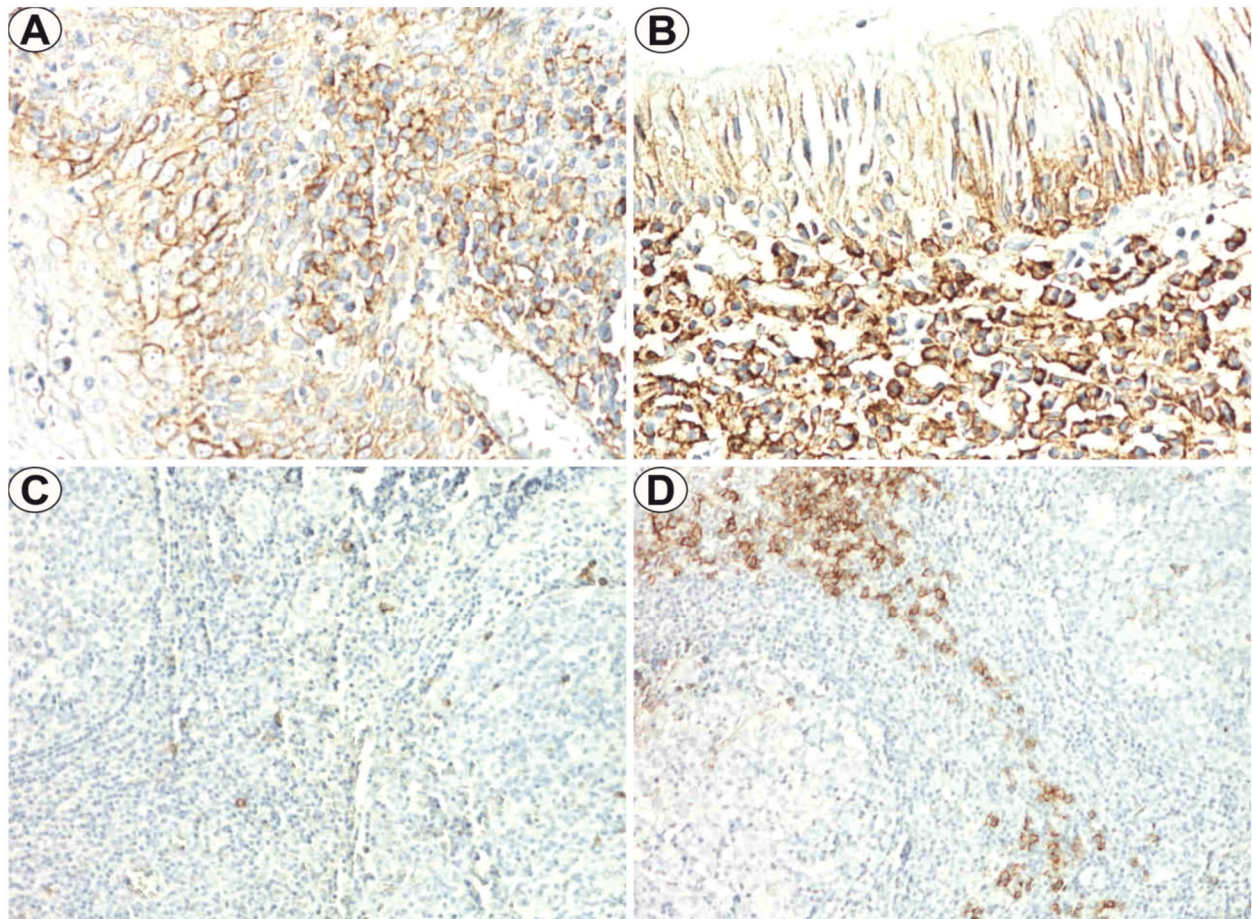
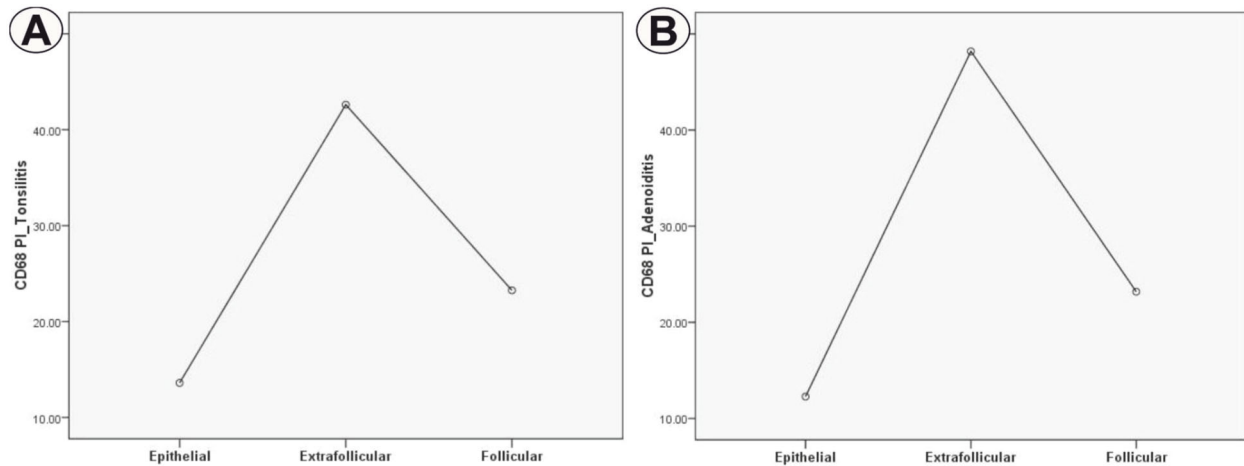


Figure 5 – Distribution of CD138 PI values in tonsillitis (A) and adenoiditis (B). CD138: Cluster of differentiation 138; PI: Positivity index.



**Figure 6** – (A) Chronic tonsillitis, cryptic epithelium and subepithelial area; (B) Chronic adenoiditis, cylindrical epithelium and subepithelial area; (C) Chronic tonsillitis, follicular and interfollicular areas; (D) Chronic adenoiditis, follicular and interfollicular areas. Anti-CD138 antibody immunostaining: (A and B)  $\times 200$ ; (C and D)  $\times 100$ . CD138: Cluster of differentiation 138.



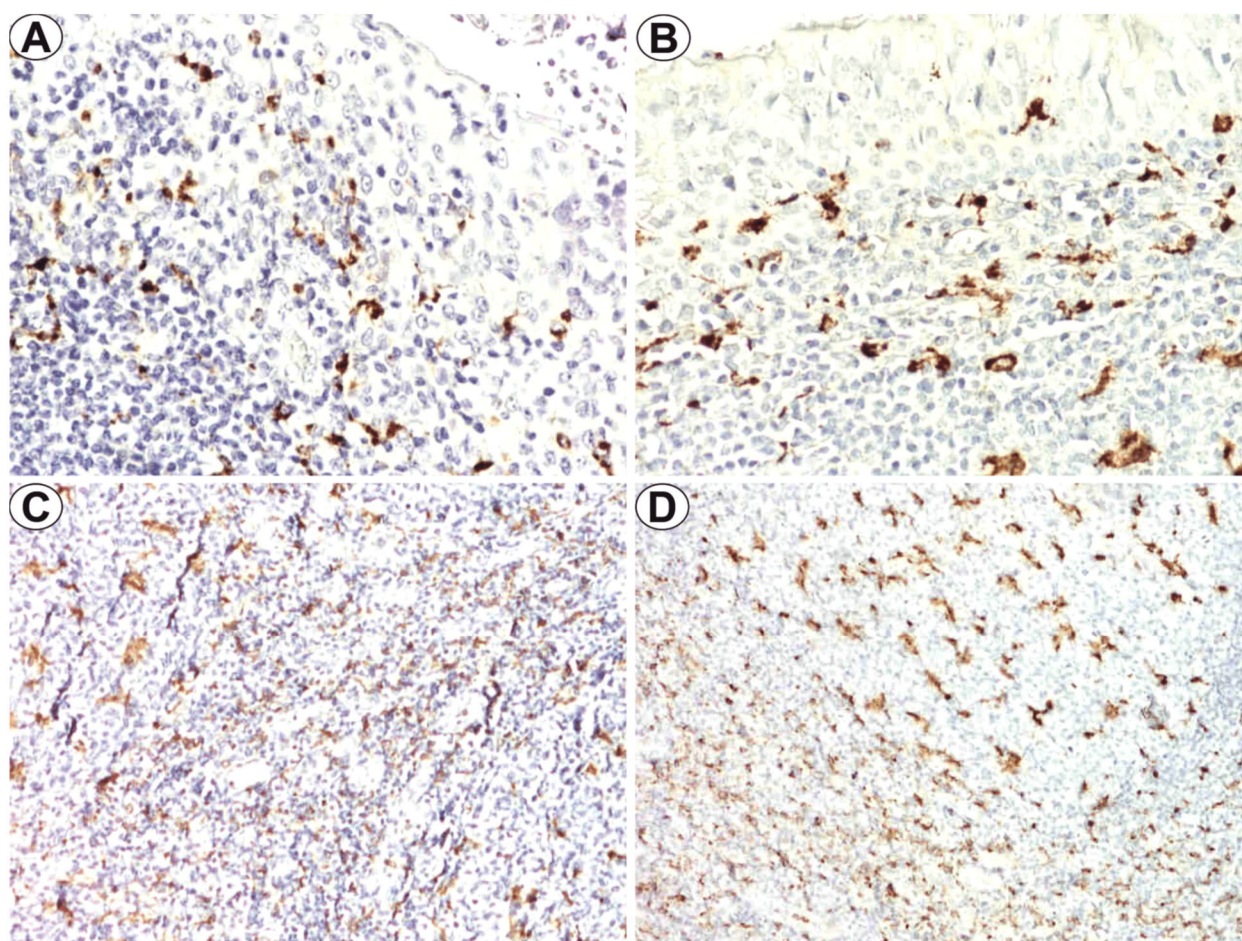
**Figure 7** – Distribution of CD68 PI values in tonsillitis (A) and adenoiditis (B). CD68: Cluster of differentiation 68; PI: Positivity index.

The analysis of PI distribution indicated a negative linear relation of CD45RO and CD138 values in all analyzed compartments, which was statistically significant in the extrafollicular one ( $p=0.020$ , Pearson's test). In the context of the obtained results, chronic tonsillitis revealed massive follicular infiltration of CD20-positive lymphocytes and in all compartments of CD45RO-positive lymphocytes, while adenoids associated the epithelial infiltration of CD20-positive lymphocytes and in all compartments of the plasmacytic elements.

For the whole investigated group, the mean PI values for the investigated markers in all tonsils and adenoids were higher in HCHS compared to LCHS, the appearance being statistically significant only in the epithelial compartment for CD45 ( $p=0.003$ , Student's  $t$ -test), in the case of the extrafollicular compartment for CD138 ( $p=0.002$ , Student's  $t$ -test) and at the limit of the statistical significance for the epithelial one in the case of CD138 ( $p=0.080$ , Student's  $t$ -test). Also, although PI values were higher in patients less than 10 years old in most compartments for the

investigated markers the aspects were only at the limit of statistical significance for follicular CD20 ( $p=0.072$ , Student's  $t$ -test), epithelial CD45 ( $p=0.083$ , Student's

$t$ -test) and extrafollicular CD138 ( $p=0.098$ , Student's  $t$ -test). We did not find other statistical relations with the investigated clinicopathological parameters.



**Figure 8** – (A) Chronic tonsillitis, cryptic epithelium and subepithelial area; (B) Chronic adenoiditis, cylindrical epithelium and subepithelial area; (C) Chronic tonsillitis, follicular and interfollicular areas; (D) Chronic adenoiditis, follicular and interfollicular areas. Anti-CD68 antibody immunostaining: (A and B)  $\times 200$ ; (C and D)  $\times 100$ . CD68: Cluster of differentiation 68.

## Discussions

Although there is ample evidence to indicate immunoregulatory activity in specific defense, the role of lymphoid structures from the Waldeyer's ring in the local and systemic defense is not fully understood [9]. The location of the Waldeyer's ring leads to a heterogeneity of clinical manifestations and complications in pediatrics, initiated and maintained by persistent antigenic stimulation, inflammation at this level being more common less than the age of 10 years old [15]. Chronic inflammations are more common in the palatine tonsils and adenoids, the clinical manifestations being variable, from persistent sore throat, to cough, snoring, pain or difficulty swallowing, fever, rhinorrhea, halitosis, oral respiration, which subsequently complicates with difficulty breathing, obstructive sleep apnea syndrome, local complications (otitis, rhinitis, sinusitis, eye infections) or at distance (glomerulonephritis, endocarditis, enteritis, etc.) [16, 17]. The aspects affect the quality of life of the child and even if the radical surgery can improve the symptoms, sometimes it has been found its reappearance having as substrate the negative psychological effects or the restoration of lymphoid structures in relation to an

allergic status [18]. On the other hand, although there is a physiological hypertrophy of the structures of the Waldeyer's ring in children, they return to normal size spontaneously until the age of 12 years [19].

In this context, it is important that therapeutic management to respond to the adapted needs of the affected child. Thus, the knowledge of the histological aspects of tonsils and adenoids and the distribution of immune elements involved in chronic inflammation may lead to the identification of pathogenic pathways of initiation, persistence or remission of lesions. Microscopic evaluation can also improve imaging techniques and treatment criteria, as well as the associated morbidity rate [20], especially in the conditions in which the impact of the deprivation of the child's organism of numerous immunocompetent cells by radical surgical technique is not yet known [5].

In our study, we included chronic inflammations of the palatine and nasopharyngeal tonsils, which were more common in children less than 10 years old, female, in urban areas. The results are relatively consistent with the literature indicating an increased reactivity of lymphoid tissue in the period 3–10 years [17] and highlight the superior urban exposure to triggers of chronic inflammation

at this level, given that it was found that in many cases, the lesions occur in a healthy infant population with a functional immune status [11].

In this study, the chronic inflammation in palatine tonsils predominated both as a clinical diagnosis (47%) and as an investigated surgical specimen (67.6%). Histological evaluation was performed by CHS that considered the presence of epithelial inflammation, crypt reticulation, lymphoid hyperplasia and collagen sclerosis, high histological scores being more common (61.7%) and associated with males less than 10 years old. Data from the literature indicate a number of histological parameters considered for the evaluation of tonsils and adenoids, which in addition to those evaluated in this study include necrosis, atrophy, number and size of germ centers, subepithelial inflammatory infiltration, being recommended the use of at least two histological evaluation criteria [3, 7, 11, 14]. The invariable presence of lymphoid hyperplasia, the presence of Ugras abscesses, and sclerosis due to recurrent episodes are indicated by different authors and were observed in this study as well [3, 11, 21].

The architecture of the palatine tonsils and adenoids is similar to that of the lymph nodes, without afferent vessels, which predisposes to a direct interaction with antigens [22]. There are relatively few studies that have analyzed the distribution of immune elements in tonsils and adenoids, some of which indicate the relatively similar distribution of B- and T-lymphocytes in these structures [22].

In this study, the distribution of lymphocytes, plasma cells and macrophages indicated some differences in distribution in tonsillitis and adenoiditis. Thus, the number of CD20-positive lymphocytes predominated in all cases in the follicular compartment (in the case of tonsillitis) or extrafollicular (in the case of adenoiditis), compared to epithelial compartment, the elements being statistically more frequently associated with the epithelial level in adenoiditis and follicular level in tonsillitis. CD20 is a membrane antigen expressed in the B-lymphocyte line, starting with the late pro-B phase, and increasing in concentration with the maturation of the elements, being also involved in the process of B-lymphocytes differentiation into plasma cells and precursors, antibody secretion, presentation of antigens and activation of T-lymphocytes, as well as their subsets differentiation [23, 24]. Also, at the level of tonsils is indicated the presence of a population of memory B-cells, capable of producing specific antibodies on demand, and of establishing an activation lineage of functional T-cell lines [8]. Most studies indicate the predominance of B-lymphocytes in the follicular areas, especially in the germinal centers and less in the mantle areas or in the reticulated cryptic epithelium [11, 17, 21, 22, 25]. Other studies indicate the predominance of B-lymphocytes at the intraepithelial and subepithelial level, up to a percentage of 65% [22]. B-lymphocytes in the germinal area appear to be immunoglobulin G (IgG) positive, unlike those in the cryptic epithelium which are immunoglobulin A (IgA) positive [11].

Some studies indicate the migration of B-cells from other areas to the tonsils and adenoids to contribute to lymphoid hyperplasia, with an increase in the number of

follicles, which seems to be dependent on antigenic stimulation, more intense in adenoids, which have a less organized epithelium and in the case of crypts where the reticulation phenomenon allows an intimate contact of the antigenic, lymphoid, and epithelial elements [10, 26].

The functional association of mutual stimulation of B- and T-lymphocytes is also underlined at the level of Waldeyer's ring structures [22, 24]. CD45RO is a transmembrane enzyme protein associated with activated memory T-lymphocytes [27]. In this study, CD45RO-positive lymphocytes were statistically associated with follicular compartments for both tonsillitis and adenoiditis. There are studies that indicate a similar distribution of T-lymphocytes in the tonsils and adenoids, but most indicate that the number of T-lymphocytes appears to increase considerably following antigenic stimulation [22, 26].

Collaboration of B- and T-lymphocyte sets results in differentiation into plasma cells and antibody secretion. Studies on the migration of lymphocytes in the tonsils and adenoids have indicated a double intake of T-lymphocytes and an efferent transport four times higher in favor of T-lymphocytes, which may indicate that many of the B-lymphocytes remain confined to these structures and differentiate into plasma cells [23]. In this study, plasma cells were distributed in tonsils and adenoids, especially at the extrafollicular level (subepithelial and interfollicular), the presence of CD138-positive elements being superior and associated with adenoids. CD138 reactions were also present at the epithelial level. CD138 (syndecan-1) is a type I transmembrane protein that is found on the surface of differentiated plasma cells, not being expressed at the level of mature B-cell line, having also other roles including the organization of the epithelial cytoskeleton [28]. The data are consistent with the literature on the compartments of lymphoid structures which indicate an increase in the number of subepithelial and interfollicular plasma cells in chronic inflammation at this level [3]. Also, some authors indicate the distribution of capillary perivascular plasma cells with the release of antibodies with local and systemic effects [8].

CD68 is an intensely glycosylated transmembrane protein expressed in the macrophage monocyte system, which includes monocytes, macrophages, dendritic cells, tissue-specific macrophage cells. In our study, the number of CD68-positive elements predominated significantly in the extrafollicular compartment with relatively close distribution in tonsillitis and adenoiditis. Some studies indicate a 5% proportion of macrophages in the tonsils and adenoids [22]. The reactions at the epithelial level are largely due to the dendritic cells, which migrate into the intercellular spaces and play the role of antigen recognition and presentation to T- and B-lymphocytes [8]. In this context, some studies indicate the existence of microfold (M) cells that perform endocytosis and vesicular transcytosis of the antigen, and its presentation to dendritic cells and lymphocytes, while other studies do not support the hypothesis of their existence [8, 11]. CD68-positive macrophages also appear to be distributed in the germinal centers due to the process of lymphoblastic proliferation in which lymphocytic morphofunctional defects appear followed



by phagocytosis, but also in the epithelial crypts and subepithelial [17].

The results of this study indicate a heterogeneity of the distribution of chronic inflammatory elements involved in tonsillitis and adenoiditis depending on the compartments investigated, and the statistical associations obtained indicate different profiles of associations between lymphocytes, plasma cells and macrophages/dendritic cells for the two locations.

## ☒ Conclusions

In this study, we observed differences in the phenotype of chronic inflammation depending on the location and concordance with the obtained histological scores, B-lymphocytes being associated with follicular areas in the case of tonsillitis and epithelial areas in the case of adenoids. At the same time, in all the compartments investigated, activated T-lymphocytes were more frequently associated with tonsillitis, and plasmocytes with adenoiditis, compartments in which the two types of elements presented a negative linear relation of distribution. The results obtained may suggest different or sequential pathogenic pathways of initiation and persistence of chronic inflammation with localization in the Waldeyer's lymphatic ring, an aspect that may be useful for stratifying patients for optimal local therapy.

## Conflict of interests

The authors declare that they have no conflict of interests.

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