Immunophenotyping of palatine tonsils in children with OSAS versus recurrent tonsillitis

Original Article

Authors

Tiago Chantre
Serviço de Otorrinolaringologia, Centro Hospitalar Universitário Lisboa Central, Portugal

Juliana Gonçalves
Human Immunobiology and Pathogenesis Laboratory, iNOVA4Health, Nova Medical School, Portugal

Sofia A. Cerqueira
Human Immunobiology and Pathogenesis Laboratory, iNOVA4Health, Nova Medical School, Portugal

João Nascimento
Human Immunobiology and Pathogenesis Laboratory, iNOVA4Health, Nova Medical School, Portugal

Helena Soares
Human Immunobiology and Pathogenesis Laboratory, iNOVA4Health, Nova Medical School, Portugal

Inês Alpoim Moreira
Serviço de Otorrinolaringologia, Centro Hospitalar Universitário Lisboa Central, Portugal

Herédio Sousa
Serviço de Otorrinolaringologia, Centro Hospitalar Universitário Lisboa Central, Portugal

Correspondence:
Tiago Chantre
tiagomendeschantre@gmail.com

Article received on July 13, 2023.
Accepted for publication on August 1, 2023.

Abstract

Introduction - Obstructive Sleep Apnea Syndrome (OSAS) and Recurrent Tonsillitis (RA) are the main indications for performing tonsillectomy in children. Despite the growing knowledge in tissue immunology of palatine tonsils, the pathophysiology that leads to the development of OSAS or RA is not fully known.
Objectives - Comparative immunophenotypic analysis of children with OSAS versus RA. The epidemiological study of the selected patients was also carried out.
Material and Methods - Analysis by flow cytometry of palatine tonsils of children with OSAS versus RA. In processing the palatine tonsils, mononuclear cells are isolated and, from these, CD4+ T cells and follicular T cells. Cell size and viability and the Inducible T-cell costimulator (ICOS), a marker for the binding of follicular T cells to B cells, during antibody production, were also evaluated. 69 patients from Hospital Dona Estefânia, aged less than 18 years, between November 2018 and November 2022 were included.
Results - Palatine tonsils were removed by extracapsular dissection of 54 children diagnosed with OSAS and 15 with RA. In patients with OSAS, the mean age was 4.7 (± 2.2) years, with 24 males and 30 females. In patients with RA, the mean age was 6.1 (± 2.5) years, with 7 males and 8 females. Children submitted to tonsillectomy for OSAS were significantly younger than those for RA ($p = 0.035$). There were no significant differences between genders in the two groups ($p = 0.881$). Significant differences were found in the grade of the palatine tonsils, according to the Brodsky Classification, between the two groups of patients ($p = 0.031$). Children with OSAS were more likely to have otitis media with effusion requiring myringotomy and placement of transtympanic ventilation tubes at the same surgical time ($p = 0.034$). There were no statistically significant differences in operative complications of tonsillectomy between the two patient groups ($p = 0.456$).
By flow cytometry analysis, the count of MNCs and CD4 T cells was not changed between the two groups. Palatine tonsils from patients with RA showed less TFH cells when compared

with tonsils from patients with OSAS, but not significantly (p = 0.07). The size of CD4 T cells did not show significant differences between groups (p=0.840), however, the viability of these cells was significantly higher in patients with RA (p=0.015). In the patients studied, there is a greater intensity in the expression of ICOS in patients with RA compared to patients with OSAS.
Conclusions - Our results point to differences in the local lymphocyte response between patients with OSAS and RA, however, additional flow cytometry studies are still needed to investigate the immunological mechanisms underlying these two pathologies.
Keywords: Obstructive sleep apnea syndrome, recurrent tonsillitis, palatine tonsils, immunophenotyping, pediatrics.

Introduction

Tonsillectomy is one of the most frequently performed surgeries in the pediatric population worldwide[1]. The most common indication for this surgery is obstructive sleep apnea syndrome (OSAS), followed by recurrent tonsillitis (RT)[1,2]. Hypertrophy of the palatine tonsils is the most common cause of childhood OSAS, affecting between 1% and 5% of all children between two and six years of age[3]. The palatine tonsils act as the first site of immune response to inhaled and ingested microorganisms, together with the lymphoid tissue of the Waldeyer's ring[4]. Luminal antigens are taken up by specialized M cells and transported to the lymphoid tissue, which is characterized by a large number of lymphoid follicles containing differentiated B cells and T follicular helper cells (TFH)[5].

Although the immunological effects of tonsillectomy have been widely debated in the last few decades, two recent systematic reviews concluded that tonsillectomy does not have a negative impact on humoral and cell-mediated immunity in children[6,7]. Because OSAS and RT have different presentation and epidemiology, it has been suggested that the underlying immunological changes are also distinct[8]. Previous studies have reported that there is increased tonsillar inflammation in patients with OSAS, including increased proliferation of T cells[4], elevated levels of proinflammatory cytokines[9] and substance P[10], and positive regulation of cysteinyl-leukotriene receptors[11]. However, anti-inflammatory drugs, such as corticosteroids and leukotriene receptor antagonists, have been shown to have only mild effects in patients with OSAS[12]. These clinical observations warrant a more in-depth study of the immunopathology of palatine tonsil hyperplasia in children with OSAS and a comparison with patients with RT. Flow cytometry allows the counting of mononuclear cells (MNCs) in the palatine tonsils and evaluation of innate and acquired immunity[13], thereby facilitating the comparison of cell populations between patients with OSAS and those with RT.

The objective of this study was to describe the clinical characteristics, concomitant surgical interventions, and postoperative complications in two groups of children who underwent tonsillectomy for OSAS or RT. The palatine tonsils of the individuals in the sample were subjected to immunophenotyping by flow cytometry.

Materials and Methods

Selection of patients

Between November 2018 and November 2022, 69 patients from the otorhinolaryngology department of the Hospital Dona Estefânia (HDE) were included in the study. The inclusion criteria consisted of pediatric patients with a diagnosis of OSAS or RT whose legal guardians agreed to their participation in the different stages of the study by reading and signing the consent form. All patients were under 18 years of age and underwent tonsillectomy. The exclusion criteria were the presence of autoinflammatory diseases such as the PFAPA (Periodic Fever, Aphthous stomatitis, Pharyngitis, and Adenitis) syndrome, patients tonsillectomized due to a diagnosis of cancer or blood disorders, patients with simultaneous diagnoses of OSAS and RT, and patients lacking legal consent for data collection and/or analysis of the palatine tonsils. All patients tonsillectomized for RT met the Paradise criteria[14]. The diagnosis of OSAS was made based on the clinical history, objective

examination, and home video recordings of sleep showing snoring with apnea. Polysomnography (PSG) was not routinely performed in all patients with a diagnosis of OSAS who underwent tonsillectomy.

The following demographic data were collected: age, sex, size of palatine tonsils, delayed speech development, asthma, otitis media with effusion (OME) requiring myringotomy, placement of tympanostomy tube (TT), other comorbidities, cycles of antibiotic therapy in the year before surgery, indication for tonsillectomy, other surgeries performed at the same time, and surgical complications. The size of the palatine tonsils was defined based on the Brodsky classification[15], in which the tonsils receive a score from 1 to 4 according to the percentage of airway occupied by the tonsils (Grade 1 ≤25%; grade 2 from 26% to 50%; grade 3 from 51% to 75%, and grade 4 >75%).

Palatine tonsil preparation and flow cytometry analysis

The surgically removed tonsils were placed in RPMI medium (Gibco, 21875-034) and kept at 4°C until they were processed. They were cut into small pieces with surgical scissors and forceps. The pieces were subsequently transferred to a 70-µm membrane where they were crushed to release the MNCs. These cells were diluted in 1 phosphate buffered saline (PBS; VWR Chemicals, K813) and transferred to Biocoll solution (BioWest, L0560-500) in a ratio of 2:1. The cells were centrifuged at 1200 g for 30 minutes, without a break. Next, the layer of MNCs was transferred to an empty tube with a Pasteur pipette. These cells were washed twice with 1PBS by centrifuging at 700 g for 10 minutes. The cells were counted and resuspended in 1 Mojo buffer and incubated for 15 minutes in ice with CD4 (1:20) magnetic nanobeads to separate the CD4+ T cells by positive selection. The cells were subsequently transferred to a tube that was placed inside a magnet and incubated for 5 minutes. Then, the supernatant was discarded and the cells were washed twice with 1 Mojo buffer. The purified CD4+ T cells were resuspended in 1 Mojo buffer, counted, and centrifuged (700 g, 5 minutes). Half of the CD4+ T cells were placed in RPMI culture media supplemented with 10% fetal bovine serum (FBS; BioWest, S1780-500) and 1% antibiotic-antimycotic (Gibco, 15240096) at a concentration of 610^6/mL for 5 days. On day 5, the cells were labelled with a viability marker and the inducible T-cell co-stimulator (ICOS), which binds TFH cells to B cells during antibody production. The other half was labelled with CD4, PD-1, and CXCR-5, markers that allow the identification and sorting of TFH cells typically found in the tonsils. All data were acquired in a BD FACSCanto II flow cytometer (BD Bioscience) and analyzed using the FlowJo software, version 10.

Statistical Analysis

All data were analyzed using the SPSS software, version 25 (Statistical Package for the Social Sciences; SPSS Inc., Chicago, IL). Data were expressed as means ± standard deviation. The normality of distribution was assessed using D'Agostino–Pearson's K^2 test (n>6) or Shapiro–Wilk test (n ≤ 6). The P values were calculated using the Mann-Whitney test for non-normal data and the normal t-test for non-paired samples. The agreement between the means of the three treatment groups (tonsillectomy, adenotonsillectomy, and adenotonsillectomy and myringotomy/ placement of a TT) was assessed using the chi-square test. Two-tailed p values < 0.05 were considered significant.

Results

Epidemiological characteristics

The palatine tonsils were removed by extracapsular dissection in 54 patients with a diagnosis of OSAS and in 15 patients with RT. The study participants' demographic and clinical characteristics are summarized in Table 1. The mean age of the patients with OSAS was 4.7 (± 2.2) years; 24 patients (44.4%) were boys and 30 patients were (55.6%) girls. The mean age of the patients with RT was 6.1 (± 2.5) years; seven patients (46.7%) were boys and eight (53.3%) were girls. The children who underwent tonsillectomy for OSAS were

Table 1
Epidemiological characteristics of the patients who underwent tonsillectomy for obstructive sleep apnea syndrome and recurrent tonsillitis.

	OSAS (n = 54)	RT (n = 15)	*p* value
Age (years)	4.7 ± 2.2	6.1 ± 2.5	0.035
Sex (female)	30 (55.6%)	8 (53.3%)	0.881
Sex (male)	24 (44.4%)	7 (46.7%)	
Size of the palatine tonsils (Brodsky Classification)	3.0 ± 0.6	2.6 ± 0.6	0.031
Delayed Speech Development	5 (9.3%)	0 (0%)	0.114
Asthma	3 (5.6%)	0 (0%)	0.179
OME requiring myringotomy and placement of TT	26 (48.1%)	4 (26.7%)	0.031
Other comorbidities	5 (9.3%)	4 (26.7%)	0.828
Cycles of antibiotic therapy in the year before the surgery	4.4 ± 1.8	6.2 ± 2.3	0.559
Tonsillectomy	1 (1.9%)	2 (13.3%)	0.081
Adenotonsillectomy	27 (50%)	9 (60%)	
Adenotonsillectomy and myringotomy placement of TT	26 (48.1%)	4 (26.7%)	
Surgical complications	2 (3.7%)	0 (0%)	0.456

Abbreviations: OSAS, obstructive sleep apnea syndrome; RT, recurrent tonsillitis; TT, tympanostomy tube.

significantly younger than those operated for RT (p = 0.035). There were no significant differences between the sexes in both groups (p = 0.881). A significant difference was found (p = 0.031) in the tonsil grade, according to the Brodsky classification, between the two groups (OSAS 3.0 ± 0.6; RA 2.6 ± 0.6). In the RT group, only 46.7% of patients (n = 7) had grade 3 or 4 tonsils, whereas this percentage in the OSAS group was 85.2% (n = 46). The children with OSAS were not statistically more prone to having a history of delayed speech development compared to those with RT (OSAS 9.3% [n = 5] vs RT 0% [n = 0]; p = 0.114). However, they had a higher likelihood of having OME requiring myringotomy and placement of TT during the same surgery (OSAS 48.1% [n = 26] vs RT 26.7% [n = 5]; p = 0.031). There were no differences in the occurrence of other comorbidities between the groups (p = 0.828). The patients with OSAS received 4.4± 1.8 cycles of antibiotic therapy in the year before the surgery, while those with RT received 6.2± 2.3 cycles, and there was no significant difference between the groups (p = 0.559). Furthermore, there were no statistically significant differences in the need for other surgeries between the two groups (p = 0.081). However, 48.1% (n = 26) patients with OSAS required adenoidectomy and myringotomy with placement of TT during the same operation, versus only 26.7% (n = 4) of patients in the RT group. There were no statistically significant differences in surgical complications after tonsillectomy between the two groups (p = 0.456). Postoperative complications were only reported in the OSAS group (n = 2; 3.7%), namely a case of post-tonsillectomy hemorrhage one day after the surgery that required a hemostasis procedure in the operating room, and another case of hemorrhage on the second day after adenoidectomy that also required the hemostasis procedure.

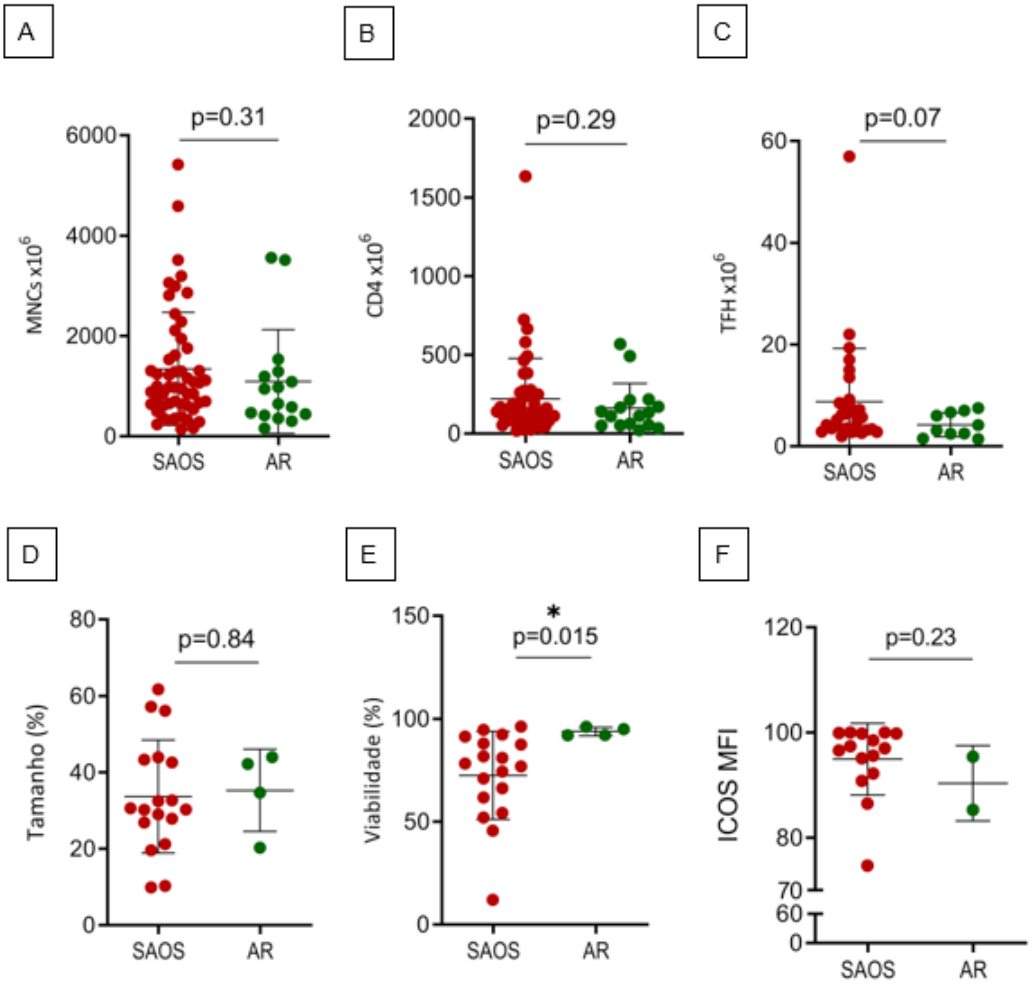
Immunophenotypic analysis

Figure 1A shows a wide variation in the number of MNCs (in millions) present in each tonsil; there were no significant differences in the number of cells between patients with OSAS (n = 54) and those with RT (n = 15) (p = 0.31). Figure 1B shows a comparison of the total number of purified CD4+ T cells obtained from the palatine tonsils of all patients (OSAS

n = 54, RT n = 15), and no statistically significant differences (p = 0.29) were observed. Figure 1C demonstrates a comparison of the total number of TFH cells obtained from the tonsils of patients with OSAS (n=30) and RA (n=10). Although the difference was not significant, the palatine tonsils of patients with RA tended to have less TFH cells than the tonsils of patients with OSAS (p = 0.07). Figure 1D shows a comparison of the size of the CD4+ T cells obtained through Forward Scatter-A (FSC-A) and Side Scatter (SSC-A) in flow cytometry; the cells were extracted from the tonsils of patients with OSAS (n=18) and RT (n=4). There were no significant differences in the CD4+ T cell size between the two groups (p = 0.84).

The viability of CD4+ T cells extracted from the palatine tonsils of patients with RT was higher than that of CD4+ T cells from OSAS patients (p = 0.015). This comparison (Figure 1E) was made using the viability marker in flow cytometry between cells extracted from the tonsils of patients with OSAS (n=18) and RT (n=4). Figure 1F shows the mean fluorescence intensity (MFI) of the ICOS marker in the CD4+ T cells obtained from the tonsils of patients with OSAS (n=15) and RA (n=2); a difference was noted between the RA and OSAS groups, although it was not statistically significant (p=0.23). The tonsils of RA patients exhibited a lower intensity of ICOS than those of OSAS patients.

Figure 1
Comparison of the results of flow cytometry analysis of the palatine tonsils from children with Obstructive Sleep Apnea Syndrome (OSAS) and Recurrent Tonsillitis (RT). A – Mononuclear cells (MNCs); B - CD4+ T cells; C – follicular helper T (TFH) cells; D – Size of the CD4+ T cells; E – Viability of the CD4+ T cells; F - Median fluorescence intensity of the inducible T cell co-stimulator (ICOS MFI).



Discussion

The results of the present study reflect the changes in the indications for tonsillectomy that have occurred globally in the last few decades[16,17]. More children are undergoing tonsillectomy for OSAS than for RT. Among patients with OSAS, there was a peak in procedures among preschoolers, which is the age group with the highest prevalenc of OSAS[18]. In the present study, the children in the RT group were older than those in the OSAS group, which is consistent with the findings of other studies[16,17].

In the present study, OME requiring myringotomy and placement of TT was the only comorbidity that was significantly more prevalent in one of the groups, namely the OSAS group. Obstruction of the auditory tube due to adenoid hypertrophy leads to negative pressure in the middle ear and chronic OME[19]. Johnston et al. reported that children with OSAS are more prone to having a speech development delay and this was associated with a high prevalence of OME[8]. In the present study, although all patients with delayed speech development were in the OSAS group and had OME as the comorbidity, there were no statically significant differences between the groups.

The number of hospital readmissions as a result of post-tonsillectomy hemorrhage increased five times from 1987 to 2013[20]. Some authors have suggested that the rate of post-tonsillectomy hemorrhage has increased with the increasing number of tonsillectomies for OSAS[8]. In the present study, the only two cases of post-surgery hemorrhage occurred in the OSAS group, but there was no statistically significant difference in the rate of post-surgery hemorrhage between the two groups.

Determining the total number of cells in the palatine tonsils (MNCs) was considered particularly important in the initial phase of the laboratory study because it allowed determination of the viability of the sample. The fact that the number of MNCs did not differ significantly between patients with OSAS and RT indicates that the number of cells does not vary with the surgery indication. In addition, the more inflammatory profile of patients with RT compared to those with OSAS was not associated with a change in the total number of cells in the tonsils. The analysis of the population of CD4+ T cells showed that similar to MNCs, the number of CD4+ cells was not influenced by the cause of tonsil removal. Interestingly, although the number of TFH cells was not statistically significant, the palatine tonsils of patients with RT had less TFH cells than those of patients with OSAS. These findings are in line with those in the literature: a study conducted by Crotty et al.[21] showed that the tonsils of patients with RT had a lower percentage of TFH cells in the germinal centers than those of patients without RT. These results raise the hypothesis that the tonsils of patients with RT are not ideal for the study of specific reactions in the germinal centers and in particular, the physiology of TFH cells.

The flow cytometry study of the CD4+ T cells revealed that their viability in the tonsils of the RT group was close to 100% after five days in culture. The viability of CD4+ T cells in the tonsils of patients with OSAS was more varied, between 20% and 98%, and the difference between the groups was statistically significant. These data may be extremely important for immunophenotyping studies that use long-life cell cultures. Thus, for such studies, it may be advisable to use tonsils from patients with RT than those from patients with OSAS.

The ICOS marker is one of the main markers of TFH cells, the main subset of T cells in the palatine tonsils[22]. This marker plays a key role in the production of antibodies because for this to occur the ICOS expressed on the membrane of T cells needs to bind to its ligand (ICOS-L), which is expressed on the surface of B cells[23]. Although it was only possible to assess the intensity of the ICOS marker in the samples of two patients with RT and 15 patients with OSAS, the result indicates a difference between the two study groups. In future studies, we will increase the number of

samples from patients with RT because tonsils are one of the main organs used for the in vivo study of antibody production in humans. In addition, it will be interesting to explore the variations in other markers that are also required for the production of antibodies, such as CD40L and cytokines IL-10 and IL-21[24]. This study has some limitations, namely the small size of the sample (69 patients in total and only 15 with RT). Moreover, the study population presented with heterogeneous symptoms and no subgroup analysis was conducted according to the severity of OSAS or the number of RT episodes per year. PSG was not routinely performed in these patients because it was not easily available. The strength of the study is that the flow cytometry studies were performed by a single investigator, which allowed standardization of the protocol and minimized laboratory errors.

Conclusion

This study is one of the first to use immunophenotypic analysis to compare children with OSAS to those with RT. The different proliferative rates and analysis of cell marker production in palatine tonsil cultures indicate that the hypertrophy of the lymphoid tissue in these two diseases is regulated by different mechanisms. Our results raise new hypotheses to be explored in future studies on the immunological mechanisms that underlie the differences observed between patients with OSAS and RT.

Conflict of interest

The authors declare no conflict of interest regarding this article.

Data confidentiality

The authors declare that they followed the protocols in use at their working center regarding the publication of patients' data.

Funding

Thus study did not receive any contribution, funding or grant.

Availability of scientific data

There are no publicly available datasets related to this study.

Bibliographic references

1.Johnston J, Kinzett-Carran G, Mahadevan M, Douglas RG. Our experience of the necessity of adenoidectomy or adenotonsillectomy at the time of ventilation tube insertion in 11 941 children with middle ear effusion. Clin Otolaryngol. 2018 Aug;43(4):1167-1171. doi: 10.1111/coa.13110.

2.Ramos SD, Mukerji S, Pine HS. Tonsillectomy and adenoidectomy. Pediatr Clin North Am. 2013 Aug;60(4):793-807. doi: 10.1016/j.pcl.2013.04.015.

3.Lumeng JC, Chervin RD. Epidemiology of pediatric obstructive sleep apnea. Proc Am Thorac Soc. 2008 Feb 15;5(2):242-52. doi: 10.1513/pats.200708-135MG.

4.Carrasco A, Sjölander I, Van Acker A, Dernstedt A, Fehrm J, Forsell M. et al. The tonsil lymphocyte landscape in pediatric tonsil hyperplasia and obstructive sleep apnea. Front Immunol. 2021 Oct 22;12:674080. doi: 10.3389/fimmu.2021.674080.

5.Nave H, Gebert A, Pabst R. Morphology and immunology of the human palatine tonsil. Anat Embryol (Berl). 2001 Nov;204(5):367-73. doi: 10.1007/s004290100210.

6.Bitar MA, Dowli A, Mourad M. The effect of tonsillectomy on the immune system: a systematic review and meta-analysis. Int J Pediatr Otorhinolaryngol. 2015 Aug;79(8):1184-91. doi: 10.1016/j.ijporl.2015.05.016.

7.Altwairqi RG, Aljuaid SM, Alqahtani AS. Effect of tonsillectomy on humeral and cellular immunity: a systematic review of published studies from 2009 to 2019. Eur Arch Otorhinolaryngol. 2020 Jan;277(1):1-7. doi: 10.1007/s00405-019-05672-6.

8.Johnston J, McLaren H, Mahadevan M, Douglas RG. Clinical characteristics of obstructive sleep apnea versus infectious adenotonsillar hyperplasia in children. Int J Pediatr Otorhinolaryngol. 2019 Jan;116:177-180. doi: 10.1016/j.ijporl.2018.11.004.

9.Kim J, Bhattacharjee R, Dayyat E, Snow AB, Kheirandish-Gozal L, Goldman JL. et al. Increased cellular proliferation and inflammatory cytokines in tonsils derived from children with obstructive sleep apnea. Pediatr Res. 2009 Oct;66(4):423-8. doi: 10.1203/PDR.0b013e3181b453e3.

10. Gozal D, Kim J, Bhattacharjee R, Goldman JL, Kheirandish-Gozal L. Substance P and neurokinin 1 receptors as potential therapeutic targets in children with OSA. Chest. 2014 May;145(5):1039-1045. doi: 10.1378/chest.13-2026.

11. Kheirandish-Gozal L, Kim J, Goldbart AD, Gozal D. Novel pharmacological approaches for treatment of obstructive sleep apnea in children. Expert Opin Investig Drugs. 2013 Jan;22(1):71-85. doi: 10.1517/13543784.2013.735230.

12. Kuhle S, Hoffmann DU, Mitra S, Urschitz MS. Anti-inflammatory medications for obstructive sleep apnoea in children. Cochrane Database Syst Rev. 2020 Jan 17;1(1):CD007074. doi: 10.1002/14651858.CD007074.pub3.

13. Assadian F, Sandström K, Laurell G, Svensson C, Akusjärvi G, Punga T. Efficient isolation protocol for B and T lymphocytes from human palatine tonsils. J Vis Exp. 2015 Nov 16;(105):53374. doi: 10.3791/53374.

14. Paradise JL, Bluestone CD, Colborn DK, Bernard

BS, Rockette HE, Kurs-Lasky M. Tonsillectomy and adenotonsillectomy for recurrent throat infection in moderately affected children. Pediatrics. 2002 Jul;110(1 Pt 1):7-15. doi: 10.1542/peds.110.1.7.
15. Brodsky L. Modern assessment of tonsils and adenoids. Pediatr Clin North Am. 1989 Dec;36(6):1551-69. doi: 10.1016/s0031-3955(16)36806-7.
16. Borgström A, Nerfeldt P, Friberg D, Sunnergren O, Stalfors J. Trends and changes in paediatric tonsil surgery in Sweden 1987-2013: a population-based cohort study. BMJ Open. 2017 Jan 13;7(1):e013346. doi: 10.1136/bmjopen-2016-013346.
17. Hallenstål N, Sunnergren O, Ericsson E, Hemlin C, Hessén Söderman AC, Nerfeldt P. et al. Tonsil surgery in Sweden 2013-2015. Indications, surgical methods and patient-reported outcomes from the National Tonsil Surgery Register. Acta Otolaryngol. 2017 Oct;137(10):1096-1103. doi: 10.1080/00016489.2017.1327122.
18. Ward SL, Marcus CL. Obstructive sleep apnea in infants and young children. J Clin Neurophysiol. 1996 May;13(3):198-207. doi: 10.1097/00004691-199605000-00003.
19. Bluestone CD. Pathogenesis of otitis media: role of eustachian tube. Pediatr Infect Dis J. 1996 Apr;15(4):281-91. doi: 10.1097/00006454-199604000-00002.
20. Østvoll E, Sunnergren O, Stalfors J. Increasing readmission rates for hemorrhage after tonsil surgery: a longitudinal (26 Years) national study. Otolaryngol Head Neck Surg. 2018 Jan;158(1):167-176. doi: 10.1177/0194599817725680.
21. Dan JM, Havenar-Daughton C, Kendric K, Al-Kolla R, Kaushik K, Rosales SL. et al. Recurrent group A Streptococcus tonsillitis is an immunosusceptibility disease involving antibody deficiency and aberrant TFH cells. Sci Transl Med. 2019 Feb 6;11(478):eaau3776. doi: 10.1126/scitranslmed.aau3776.
22. Brenna E, Davydov AN, Ladell K, McLaren JE, Bonaiuti P, Metsger M. et al. CD4+ T follicular helper cells in human tonsils and blood are clonally convergent but divergent from non-Tfh CD4+ Cells. Cell Rep. 2020 Jan 7;30(1):137-152.e5. doi: 10.1016/j.celrep.2019.12.016.
23. De Silva NS, Klein U. Dynamics of B cells in germinal centres. Nat Rev Immunol. 2015 Mar;15(3):137-48. doi: 10.1038/nri3804.
24. van Kempen MJ, Rijkers GT, Van Cauwenberge PB. The immune response in adenoids and tonsils. Int Arch Allergy Immunol. 2000 May;122(1):8-19. doi: 10.1159/000024354