Head and Neck Pathology (2019) 13:722–726
https://doi.org/10.1007/s12105-018-0960-0

CASE REPORT

Laryngeal Epstein–Barr Virus-Associated Smooth Muscle Tumor in an Undernourished Child

Ciro Dantas Soares[1] · Roman Carlos[2] · Juan Pablo Díaz Molina[2] · Thayná Melo de Lima Morais[1] · Oslei Paes de Almeida[1]

Received: 23 June 2018 / Accepted: 16 August 2018 / Published online: 17 August 2018

Abstract
Smooth muscle tumors associated with Epstein–Barr virus infections (EBV–SMT) of laryngeal origin are exceedingly rare and have been reported in few adult patients, but not in children. This reported case describes a lesion found in the larynx of an 8-year-old Guatemalan undernourished girl. Microscopically, the lesion showed a highly cellular mesenchymal spindle cell tumor, containing frequent lymphocytes. The immunohistochemical analysis revealed positivity for α-smooth muscle actin and h-caldesmon. In addition, most of the tumor cells were positive for EBV by in situ hybridization. To the best of the author's knowledge, this is the first literature-reported case of laryngeal EBV–SMT occurring in an undernourished child.

Keywords Epstein–Barr virus infections/pathology · Smooth muscle tumor/pathology · Pediatric tumors

Introduction

The Epstein–Barr virus (EBV) plays an important role in the pathogenesis of a few neoplasias, such as specific types of lymphomas and nasopharyngeal carcinomas [1–3]. EBV-associated smooth muscle tumors (EBV–SMT) occur most commonly in immunocompromised patients with AIDS, congenital or acquired immunodeficiencies and patients with previous history of organ transplantation [4, 5]. EBV–SMT is exceedingly rare in larynx, with only 4 cases reported to date in adults of no gender predilection (male:female ratio 1:1) and mean age 47.5 years, as described in Table 1 [6–8].

This study reports a case of laryngeal EBV–SMT in an undernourished 8-year-old girl, emphasizing its clinical, histological and immunohistochemical features. To the best of the authors' knowledge, this is the first reported case of laryngeal EBV–SMT in a pediatric patient.

Case Report

An 8-year-old Guatemalan girl presented with a supraglottic laryngeal nodule located at the left ventricular band. The tumor was incidentally discovered during intubation for general anesthesia to perform a laparoscopy related to a recent history of a gastric tumor resected in another institution, for which the diagnosis and histopathologic tissue blocks, or slides, were not available for review. The tumor was of nodular appearance, smooth surface, and erythematous color (Fig. 1).

Grossly, multiple soft tissue fragments with a rubbery consistency and tan color were received, measuring $2.4 \times 1.1 \times 0.5$ cm in aggregate. On histopathological analysis, the tumor revealed irregularly-oriented smooth muscle fascicles containing spindle or plump cells with hyperchromatic nuclei with an eosinophilic cytoplasm. There was no marked cellular atypia and no tumor necrosis. The mitotic index was very low. In addition, a lymphocytic infiltrate permeating the neoplastic cells was observed throughout the tumor, as well as the absence of other inflammatory cells (Fig. 2).

The antibodies used for the immunohistochemical studies are listed in Table 2. Neoplastic cells were positive for smooth muscle actin (SMA), muscle actin-specific (HHF35) and h-caldesmon (Fig. 2). T-lymphocytes were positive for CD3, most CD8+ and occasional CD4+. B-lymphocytes

✉ Ciro Dantas Soares
ciro.dss@gmail.com; c162617@g.unicamp.br

[1] Oral Pathology, Piracicaba Dental School, University of Campinas (UNICAMP), Av. Limeira 901, PO Box 52, Piracicaba, São Paulo 13414-903, Brazil

[2] Centro Clínico de Cabeza y Cuello/Hospital Herrera Llerandi, Ciudad de Guatemala, Guatemala

Table 1 Cases reported of EBV–SMT in larynx

Author and year	Age and sex	Cause of immunodeficiency	Site in the larynx	Multifocal disease
Deyrup 2006	50 yof	Post-transplant	Vocal cord	Yes
	31 yom	Post-transplant	Subglottis	Yes
Gan 2008	36 yof	Post-transplant (renal)	Vocal cord	Yes
Huang 2010	54 yom	Post-transplant (renal)	Vocal cord	Yes
Present case	8 yof	Unknown, undernourished	Supraglottis	Yes

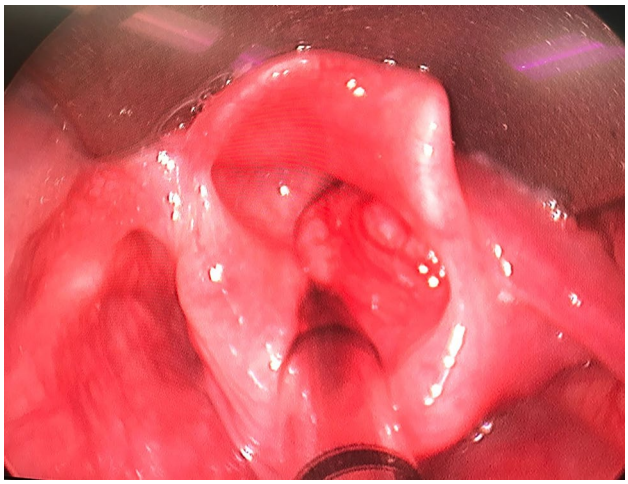

Fig. 1 Initial presentation of the laryngeal tumor diagnosed as EBV-smooth muscle tumor in an 8-year-old girl discovered occasionally during videolaryngoscopy for general anesthesia

(CD20+) were rare. The proliferative index assessed by Ki67-positive nuclei was 24%. Desmin, pan-cytokeratin, S-100 protein and CD34 were negative. In situ hybridization for EBV-encoded RNA (EBER) revealed positivity in about 80% of neoplastic cells (Fig. 3).

Based on clinical, histopathological and immunohistochemical findings, a diagnosis of EBV–SMT was established. No additional GI lesions were identified, but close follow-up is maintained. The patient was treated with a conservative, complete endoscopic resection. No evidence of recurrence arose during 1-year follow-up. The patient's nutritional condition was greatly improved, and instructions were provided to the family, to maintain proper nutrition, yet compatible with the family's low socio-economic conditions.

Discussion

EBV plays a key role in the pathogenesis of certain human conditions, including lymphoproliferative disorders, Hodgkin and non-Hodgkin lymphomas, oral hairy leukoplakia, nasopharyngeal carcinomas, and a specific variant of smooth muscle tumors. A known reservoir for EBV is the lymphoid tissue of the oropharynx [1–3].

EBV-associated smooth muscle tumors (EBV–SMT) are rare, affecting mainly immunocompromised patients. The mechanisms eliciting smooth muscle cells to proliferate under EBV stimuli are not well understood. It was suggested that an abnormal proliferation of myogenous vascular wall cells may play a role in the initiation of EBV–SMT development [6]. Additionally, it is well-accepted that in some EBV-driven lesions, epithelial cells and lymphocytes (B and T cells) are EBV-target cells, acting as initiators or playing a central role in the pathogenesis of such lesions [9, 10].

EBV–SMT tumor cells are permeated by a variable number of lymphocytes and few studies had determined the phenotype of these reactive lymphocytes. In this case, it was demonstrated by IHC that the substantial majority of lymphocytes present were CD3+ and CD8+, with less frequent CD20+ and CD4+ lymphocytes. Considering that none of these lymphocytes revealed positivity for EBV, they are most likely reactive, and predominantly composed of cytotoxic T cells (CD8+). These results suggest that B or T cells do not play a vital role in the development of these lesions, given that the EBV+ cells were the smooth muscle cells themselves, corroborating the findings of Deyrup et al. [6].

The three most common clinical conditions associated with EBV–SMT in the pediatric population are: (a) children with congenital acquired immunodeficiency syndrome (AIDS); (b) patients on immunosuppressive treatment for organ transplantation; and (c) less commonly, patients with other congenital immunodeficiency syndromes [11–13]. Although EBV–SMT seems to occur exclusively in immunocompromised patients, the present case occurred in an undernourished 8-year-old girl without any other systemic condition related to immunosuppression. This clinical setting is unique, given that most of the pediatric reported cases refer the occurrence of EBV–SMT within the context of the three aforementioned groups [14, 15]. Although anemia is not directly related to immunodeficiency, it may cause a secondary decrease of hematopoietic stem cells and reduced production of serum proteins, which may be associated with immunosuppression

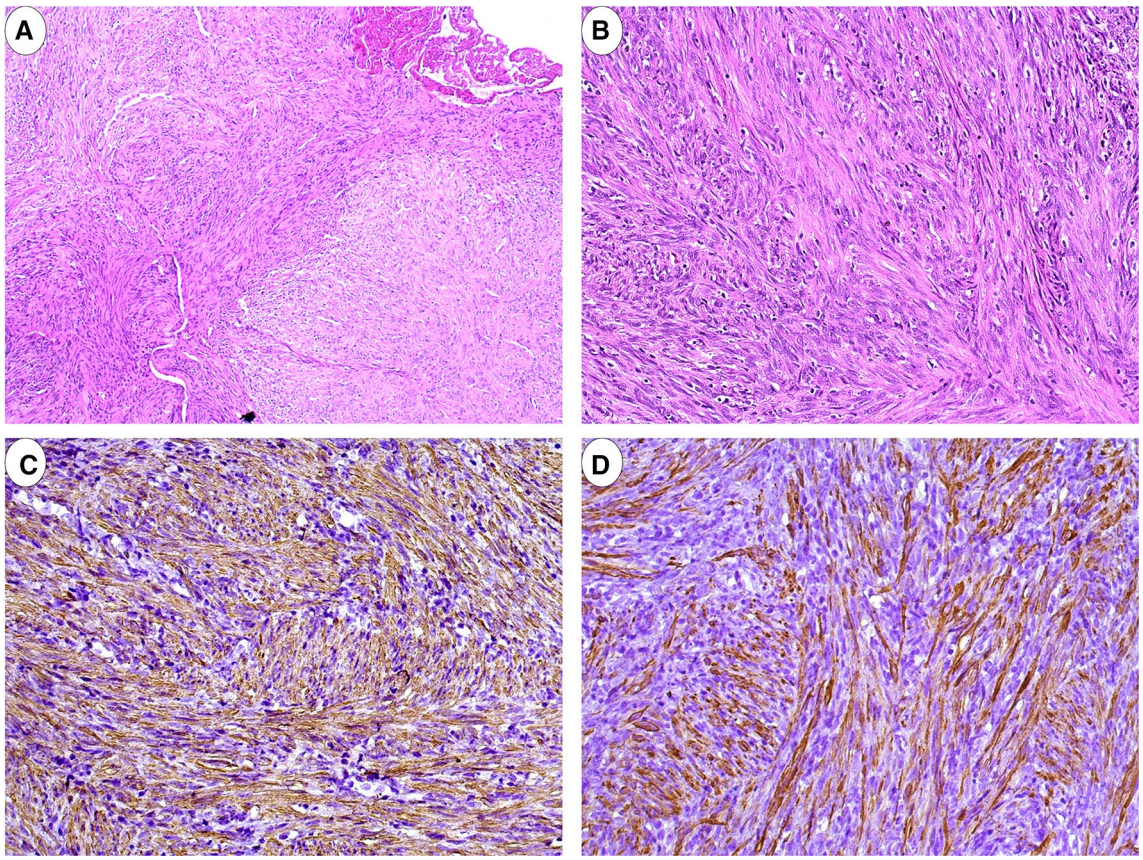

Fig. 2 Microscopic aspect of laryngeal EBV–SMT. **a** H&E stained section revealed alternating bundles and fascicles of densely packed spindle to round-shaped cells (H&E, ×100). **b** Spindle cells demonstrated abundant fibrillar eosinophilic cytoplasm and indistinct cytoplasmic borders with a moderate number of infiltrating lymphocytes (DAB, ×200). **c** Neoplastic cells were diffusely positive for α-smooth muscle actin (DAB, ×200). **d** Intense and diffuse immunopositivity for h-caldesmon (DAB, ×200)

Table 2 Antibodies utilized in the immunohistochemical study of EBV–smooth muscle tumor of the larynx

Antibody	Clone and source	Concentration	Result
Smooth muscle actin	1A4, Dako	1:400	Positive
Specific muscle actin	HHF35, Dako	1:800	Positive
h-Caldesmon	h-CD, Dako	1:400	Positive
Desmin	D33, Dako	1:800	Negative
Pan-cytokeratin	AE1AE3, Dako	1:200	Negative
S-100	Polyclonal, Dako	1:10,000	Negative
CD34	QBEnd-10, Dako	1:50	Negative
CD3	Polyclonal, Dako	1:500	Positive[a]
CD20	L 26(1,2), Dako	1:1000	Positive[a]
CD4	4B12, Dako	1:100	Positive[a]
CD8	C8/144B, Dako	1:100	Positive[a]
Ki67	MIB1, Dako	1:50	Positive[b]

[a]Positive in lymphocytes permeating the tumor cells

[b]Ki67 proliferative index was 24%

in pediatric patients. It is known that innate and adaptive immunity are impaired in undernourished children and that it can eventually cause death by infection [16–18]. Nevertheless, the involved mechanisms are not well established. In the present case, the patient's height and weight were below reference values for her age, and these were the only altered systemic conditions found. Therefore, the authors hypothesized that undernutrition could be associated with the EBV–SMT.

Pediatric EBV–SMT occurs most commonly intracranially or intraspinally in patients with immunodeficiencies, while post-transplant patients usually present liver and/or gastrointestinal tumors. Literature reviews identified four cases affecting the larynx, with a mean age of 47.5 years (ranging from 31 to 54 years of age), two cases in men, and two in women [6–8]. All cases were post-transplant related, and none occurred in pediatric patients. There are about 75 cases of EBV–SMT published in English literature, 13 involving the Head and Neck (H&N) region of adult patients and 62 cases in other bodily areas, mainly affecting liver, lungs, spleen, and kidneys. Figure 4 shows a pie chart of all

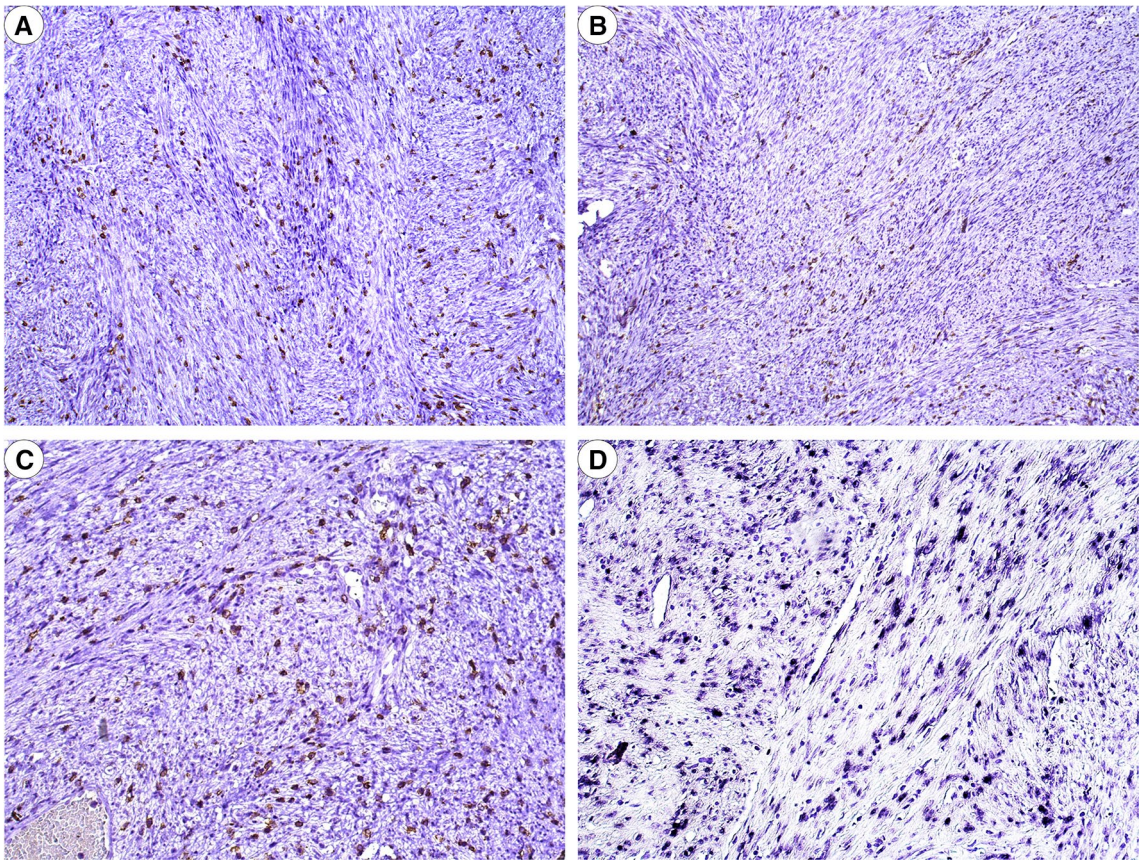

Fig. 3 Lymphocytic infiltrate permeating the tumor and EBER in situ hybridization in the laryngeal EBV–SMT. **a** T cells were evidenced by CD3 immunoexpression (DAB, ×100). **b** Few B cells immunopositive for CD20 (DAB, ×100). **c** Cytotoxic T cells within the tumor were positive for CD8 (DAB, ×200). **d** EBER in situ hybridization confirmed the EBV-positivity in the nuclei of the spindle-shaped tumor cells (EBER, in situ hybridization for EBV × 200)

H&N cases, but then again, this is the first reported case of a pediatric patient.

With regard to the differential diagnosis of EVB-SMT in the larynx, leiomyoma, low-grade leiomyosarcoma, and inflammatory myofibroblastic tumor should be considered [19]. Detection of EBV in tumor cells is fundamental for the correct diagnosis, since only EBV–SMT shows positivity for EBV in tumor cells within this context. Although some cases of EBV–SMT have been reported to mimic leiomyosarcomas or other malignant mesenchymal neoplasms, the pathobiology of these lesions is poorly understood. Currently, EBV–SMT is considered a benign tumor, with an indolent clinical course and it commonly has a multifocal presentation with no apparent metastatic potential.

The positivity for Desmin has been reported in some EBV–SMT cases. However, a small percentage of the cases were negative for this marker, and the same occurred with the present case. Indeed, negativity for Desmin is poorly discussed in the context of EBV–SMT. The authors considered that, similarly to stromal endometrial tumors with smooth muscle differentiation [20], EBV–SMT may not represent a true leiomyoma, as with variable expression of Desmin in stromal cells of GI and respiratory tract. The molecular mechanisms underlying the pathogenesis of these tumors need to be better clarified.

At present, the treatment of EBV–SMT remains controversial [8, 21]. Most patients have been treated with complete surgical tumor resection [22]. Some patients received chemotherapy or antiretroviral therapy, and in a few cases, radiotherapy [6]. In addition, improvement of the immune status seems to be very important for tumor recurrence prevention. In the present case, the patient was exclusively treated with conservative surgical excision and nutritional condition improvement, remaining disease-free after one year of follow-up.

Conclusion

In the present study, the authors reported a case of laryngeal EBV–SMT in an 8-year-old female patient. In contrast to other cases of EBV–SMT reported in literature, the only

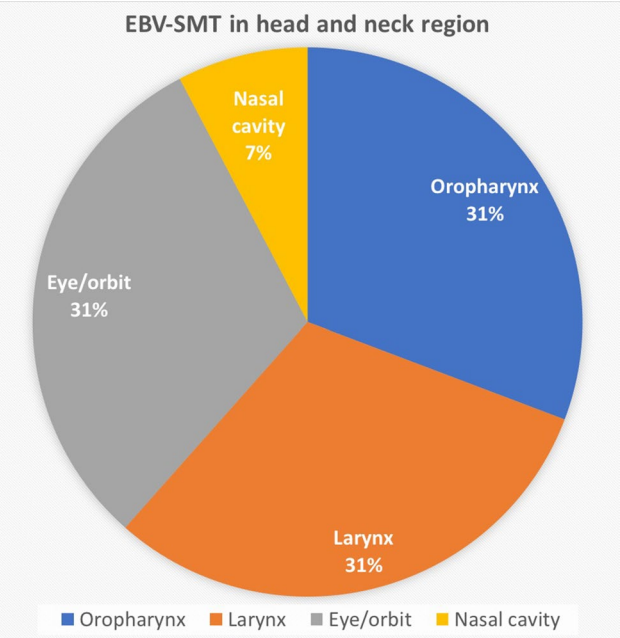

Fig. 4 Pie chart showing the distribution of head and neck EBV–SMT cases reported in literature. All cases with adults, only the present case is the report regarding a pediatric patient

systemic factor that seems to be related to the development of the present tumor is the patient's undernourishment.

Funding This work was supported by São Paulo Research Foundation (FAPESP), Grant Number #2015/25905-1.

Compliance with Ethical Standards

Conflict of interest The authors declare no potential conflicts of interest related to this case report, to this study's authorship, and/or to its publication.

References

1. Grywalska E, Rolinski J. Epstein–Barr virus-associated lymphomas. Semin Oncol. 2015;42(2):291–303. https://doi.org/10.1053/j.seminoncol.2014.12.030.
2. Jha HC, Pei Y, Robertson ES. Epstein–Barr virus: diseases linked to infection and transformation. Front Microbiol. 2016;7(OCT):1602. https://doi.org/10.3389/fmicb.2016.01602.
3. Rumayor Piña A, dos Santos HT, Carlos R, Altemani A, de Almeida OP. Epstein–Barr virus in nasopharyngeal carcinoma of Guatemalan and Brazilian patients. Int J Surg Pathol. 2017;25(4):304–9. https://doi.org/10.1177/1066896916679518.
4. Kazmi SAJ, Aizenberg MR, Harper JL, McComb RD. Multifocal histologically malignant Epstein–Barr virus-associated smooth muscle tumor in a pediatric transplant patient with an indolent course. Int J Surg Pathol. 2014;22(2):186–9. https://doi.org/10.1177/1066896913494793.
5. Jossen J, Chu J, Hotchkiss H, et al. Epstein–Barr virus-associated smooth muscle tumors in children following solid organ transplantation: a review. Pediatr Transpl. 2015;19(2):235–43. https://doi.org/10.1111/petr.12426.
6. Deyrup AT, Lee VK, Hill CE, et al. Epstein–Barr virus-associated smooth muscle tumors are distinctive mesenchymal tumors reflecting multiple infection events: a clinicopathologic and molecular analysis of 29 tumors from 19 patients. Am J Surg Pathol. 2006;30(1):75–82. https://doi.org/10.1097/01.pas.0000178088.69394.7b.
7. Gan EC, Lau DPC, Chuah KL. Epstein–Barr virus-associated smooth muscle tumour mimicking bilateral vocal process granuloma. J Laryngol Otol. 2008;122(1):100–4. https://doi.org/10.1017/S0022215107007682.
8. Huang J, Loh KS, Petersson F. Epstein–Barr virus-associated smooth muscle tumor of the larynx: report of a rare case mimicking leiomyosarcoma. Head Neck Pathol. 2010;4(4):300–4. https://doi.org/10.1007/s12105-010-0201-7.
9. Hatton OL, Harris-Arnold A, Schaffert S, Krams SM, Martinez OM. The interplay between Epstein–Barr virus and B lymphocytes: implications for infection, immunity, and disease. Immunol Res. 2014;58(0):268–76. https://doi.org/10.1007/s12026-014-8496-1.
10. Hutt-Fletcher LM. Epstein–Barr virus replicating in epithelial cells. Proc Natl Acad Sci USA. 2014;111(46):16242–3. https://doi.org/10.1073/pnas.1418974111.
11. Ha C, Haller JO, Rollins NK. Smooth muscle tumors in immunocompromised (HIV negative) children. Pediatr Radiol. 1993;23(5):413–4. https://doi.org/10.1007/BF02011979.
12. Hussein K, Rath B, Ludewig B, Kreipe H, Jonigk D. Clinico-pathological characteristics of different types of immunodeficiency-associated smooth muscle tumours. Eur J Cancer. 2014;50(14):2417–24. https://doi.org/10.1016/j.ejca.2014.06.006.
13. Dekate J, Chetty R. Epstein–Barr virus-associated smooth muscle tumor. Arch Pathol Lab Med. 2016;140(7):718–22. https://doi.org/10.5858/arpa.2015-0120-RS.
14. Arva NC, Schafernak KT. Rare presentations of Epstein–Barr Virus-associated smooth muscle tumor in children. Pediatr Dev Pathol. 2016;19(2):132–8. https://doi.org/10.2350/15-05-1627-CR.1.
15. Garg I, Baladron Zanetti MJ, Yasir S, Goenka AH, Kendi AT. PET/CT in an 8-year-old girl with Epstein–Barr virus-associated smooth muscle tumor. Clin Nucl Med. 2017;42(10):770–2. https://doi.org/10.1097/RLU.0000000000001794.
16. Bryce J, Boschi-Pinto C, Shibuya K, Black RE. WHO estimates of the causes of death in children. Lancet. 2005;365(9465):1147–52. https://doi.org/10.1016/S0140-6736(05)71877-8.
17. Rytter MJH, Kolte L, Briend A, Friis H, Christensen VB. The immune system in children with malnutrition—a systematic review. PLoS ONE. 2014;9(8):e105017. https://doi.org/10.1371/journal.pone.0105017.
18. Bourke CD, Berkley JA, Prendergast AJ. Immune dysfunction as a cause and consequence of malnutrition. Trends Immunol. 2016;37(6):386–98. https://doi.org/10.1016/j.it.2016.04.003.
19. Völker H-U, Scheich M, Zettl A, Hagen R, Müller-Hermelink HK, Gattenlöhner S. Laryngeal inflammatory myofibroblastic tumors: different clinical appearance and histomorphologic presentation of one entity. Head Neck. 2010;32(11):1573–1578. https://doi.org/10.1002/hed.21232.
20. Oliva E, Young RH, Amin MB, Clement PB. An immunohistochemical analysis of endometrial stromal and smooth muscle tumors of the uterus: a study of 54 cases emphasizing the importance of using a panel because of overlap in immunoreactivity for individual antibodies. Am J Surg Pathol. 2002;26(4):403–412.
21. Rogatsch H, Bonatti H, Menet A, Larcher C, Feichtinger H, Dirnhofer S. Epstein–Barr virus-associated multicentric leiomyosarcoma in an adult patient after heart transplantation: case report and review of the literature. Am J Surg Pathol. 2000;24(4):614–621.
22. Suwansirikul S, Sukpan K, Sittitrai P, Suwiwat S, Khunamornpong S. Epstein–Barr virus-associated smooth muscle tumor of the tonsil. Auris Nasus Larynx. 2012;39(3):329–32. https://doi.org/10.1016/j.anl.2011.07.013.