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KAREN PATRICIA DOMINGUEZ GALLAGHER

ANÁLISE CLINICOPATOLÓGICA, IMUNOISTOQUÍMICA E MOLECULAR DOS RABDOMIOSSARCOMAS EM REGIÃO DE CABEÇA E PESCOÇO

CLINICOPATHOLOGICAL, IMMUNOHISTOCHEMICAL AND MOLECULAR ANALYSIS OF HEAD AND NECK RHABDOMYOSARCOMAS

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Tese apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutora em Estomatopatologia, na Área de Patologia.

Thesis presented to the Piracicaba Dental School of the University of Campinas in partial fulfillment of the requirements for the degree of Doctor in Oral Medicine and Oral Pathology, in the Pathology area.

Orientador: Prof. Dr. Alan Roger dos Santos Silva.

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RESUMO

Este estudo abrangente sobre rabdomiossarcomas de cabeça e pescoço visa aprimorar a compreensão dessas neoplasias malignas de alto grau, especialmente prevalentes em crianças e adolescentes, representando aproximadamente 50% dos sarcomas de tecidos moles na população pediátrica. A complexidade clínica e morfológica desses tumores demanda abordagens multidisciplinares para diagnóstico, tratamento e prognóstico eficazes, justificando o desenvolvimento de estudos clínicos, patológicos e biológicos. O presente estudo, dividido em três capítulos, tem como objetivo analisar as características clínicopatológicas, imuno-histoquímicas e moleculares dos rabdomiossarcomas de cabeça e pescoço. No primeiro capítulo, uma revisão sistemática destaca as alterações genéticas desses tumores, enfatizando a prevalência do tumor primário nas localizações parameníngeas em pacientes pediátricos. A variante alveolar foi a mais comum e está caracterizada pela fusão *PAX-FOXO1* em aproximadamente 80% dos casos. A identificação da mutação *MYOD1* na variante de células fusiformes/esclerosantes correlacionou-se com uma taxa de mortalidade mais elevada, enquanto a sobrevida global em 5 anos foi de 61,3%. O segundo capítulo apresenta três novos casos de rabdomiossarcoma com rearranjos *TFCP2*, um subtipo raro que afeta predominantemente a região maxilofacial em adultos jovens. Esses casos revelaram uma notável agressividade do tumor, especialmente nos ossos craniofaciais. O diagnóstico foi estabelecido por meio de uma abordagem integrada envolvendo correlação clinicopatológica e análise molecular confirmada por hibridização *in situ* por fluorescência (FISH). O terceiro capítulo, um estudo colaborativo internacional retrospectivo, analisa 44 casos pediátricos de rabdomiossarcoma de cabeça e pescoço em países em desenvolvimento. Ressaltou-se a prevalência da variante embrionária e a expressão variável para desmina, miogenina e Myo-D1. Diferenças na expressão de marcadores substitutos, como AP2β e HMGA2, sugerem subgrupos de rabdomiossarcomas com fusão positiva e negativa, porém são necessários mais estudos para validá-los. Em conjunto, esses estudos destacam a complexidade desses tumores, enfatizando a necessidade de abordagens multidisciplinares e pesquisas adicionais para validar marcadores prognósticos e aprimorar a compreensão dessas neoplasias raras, fornecendo insights cruciais para estratégias terapêuticas mais eficazes.

Palavras-chave: Rabdomiossarcoma. Cabeça. Pescoço. Pediatria. Crianças. Adolescentes. Molecular.

ABSTRACT

This comprehensive study on head and neck rhabdomyosarcomas aims to enhance the understanding of these high-grade malignant neoplasms, particularly prevalent in children and adolescents, representing approximately 50% of soft tissue sarcomas in the pediatric population. The clinical and morphological complexity of these tumors necessitates multidisciplinary approaches for effective diagnosis, treatment, and prognosis, justifying the development of clinical, pathological, and biological studies. The present study, divided into three chapters, analyzes the clinicopathological, immunohistochemical, and molecular characteristics of head and neck rhabdomyosarcomas. In the first chapter, a systematic review highlights the genetic alterations of these tumors, emphasizing the primary tumor location in parameningeal tissues in pediatric patients. The most common variant is alveolar rhabdomyosarcoma, characterized by *PAX-FOXO1* fusion in approximately 80% of cases. Identifying the *MYOD1* mutation in the spindle cell/sclerosing variant correlates with a higher mortality rate, while the 5-year overall survival is 61.3%. The second chapter presents three new cases of rhabdomyosarcoma with *TFCP2* rearrangements, a rare subtype predominantly affecting the maxillofacial region in young adults. These cases reveal remarkable tumor aggressiveness, especially in the craniofacial bones. The diagnosis is established through an integrated approach involving clinicopathological correlation and molecular analysis, confirmed by fluorescence *in situ* hybridization (FISH). The third chapter, an international collaborative retrospective study, analyzes 44 pediatric head and neck rhabdomyosarcoma cases in developing countries. The prevalence of the embryonal variant and variable expression for desmin, myogenin, and Myo-D1 are noteworthy. Differences in surrogate marker expressions, such as AP2β and HMGA2, suggest subgroups of rhabdomyosarcomas with positive and negative fusion. However, more studies are needed to validate them. In summary, these studies underscore the complexity of these tumors, emphasizing the need for multidisciplinary approaches and additional research to validate prognostic markers and enhance the understanding of these rare neoplasms, providing crucial insights for more effective therapeutic strategies.

Keywords: Rhabdomyosarcoma. Head. Neck. Pediatric. Children. Adolescents. Molecular.

SUMARIO

1 INTRODUÇÃO

O rabdomiossarcoma (RMS) é uma neoplasia maligna originada de células mesenquimais primitivas com diferentes graus de diferenciação miogênica (Saab et al., 2011). Sua incidência é de 4,5 a 4,9 pacientes/milhão em indivíduos com idade <20 anos nos Estados Unidos e <15 anos na Europa, representando cerca de 7% das malignidades pediátricas e menos de 1% em adultos (Dasgupta et al., 2016; Skapek et al., 2019). Apesar da baixa incidência, o RMS é o sarcoma de tecidos moles mais comum em crianças e adolescentes, com leve predileção pelo sexo masculino. A apresentação ocorre bimodalmente, com o primeiro pico entre 2 e 6 anos e o segundo na adolescência, enquanto a faixa etária varia em adultos (Dziuba et al., 2018; Ruiz-Mesa et al., 2015; Skapek et al., 2019).

Embora a maioria dos casos de RMS se origine de forma esporádica, a etiologia deste tumor pode estar relacionada a fatores genéticos observados em síndromes como Li-Fraumeni, neurofibromatose, Beckwith-Wiedemann e Costello, associados a uma maior probabilidade de desenvolvimento de RMS. Além disso, exposições ambientais como tabagismo, idade materna avançada, exposição à radiação, uso materno de antibióticos e uso recreativo materno de drogas também foram associadas à ocorrência da doença (Martin-Giacalone et al., 2021; Radzikowska et al., 2015; Skapek et al., 2019).

O RMS pode originar-se em diversas partes do corpo, e a localização anatômica mais comum do tumor varia conforme o grupo etário. Em pacientes pediátricos, a região de cabeça e pescoço é o local mais frequentemente afetado, seguido pelo trato geniturinário e extremidades (Dasgupta et al., 2016; Saab et al., 2011). Em contraste, o RMS em adultos ocorre mais frequentemente nas extremidades, seguido pelo tronco, trato geniturinário e, por último, na região de cabeça e pescoço (Chen et al., 2017; Chen et al., 2022; Sultan et al., 2009). Com base na localização anatômica do tumor primário, os RMSs de cabeça e pescoço podem ser classificados em: a) orbital, b) parameníngeo (ouvido médio, nasofaringe, cavidades nasais, seios paranasais, fossas: infratemporal e pterigopalatina) e c) não-parameníngeo (cavidade oral, nariz, orelha, face, couro cabeludo, pescoço e glândulas salivares) (Turner e Richmon, 2011).

A última classificação da Organização Mundial da Saúde (OMS) para tumores ósseos e de tecidos moles divide o RMS em quatro variantes histopatológicas: embrionário, alveolar, de células fusiformes/esclerosantes e pleomórfico (WHO, 2020). No entanto, os avanços moleculares dos últimos anos também contribuíram para a subclassificação dos RMSs com base em alterações genéticas. Do ponto de vista molecular, a presença ou ausência da fusão dos genes

PAX3/7-FOXO1 permite subdividir os RMSs alveolares como RMS de fusão positiva ou negativa. Adicionalmente, os RMSs de células fusiformes/esclerosantes são divididos com base em suas alterações genéticas em três grupos: a) congênito ou infantil com fusões genéticas associadas aos genes *VGLL2, NCOA1/2 e SRF*, b) RMS com mutação do gene *MYOD1* e c) o RMS com rearranjo do gene *TFCP2* (Agaram et al., 2019; Agaram, 2022).

A abordagem terapêutica para o RMS é multimodal, envolvendo cirurgia, quimioterapia e radioterapia conforme os protocolos estabelecidos por ensaios clínicos desenvolvidos por grupos europeus e norte-americanos que trabalham em colaboração para estudar o RMS (International Society of Pediatric Oncology–Malignant Mesenchymal Tumor Committee (SIOPMMT), Soft Tissue Sarcoma Cooperative Group (Cooperative Weichteilsarkomen Studie–CWS), e Italian Soft Tissue Sarcoma Committee (STSC), North American Intergroup Rhabdomyosarcoma Study Group (IRSG) e Children's Oncology Group (COG)). Os pacientes recebem tratamento de acordo com o risco, determinado pela estratificação em grupos de risco, considerando as características clínicopatológicas (Arndt et al., 2018; Darwish et al., 2020; Hettmer et al., 2022).

Durante várias décadas, os estudos colaborativos internacionais, incluindo ensaios clínicos randomizados e não randomizados, bem como estudos observacionais e epidemiológicos em diversas instituições de referência em vários países, contribuíram para uma melhor compreensão do comportamento biológico do RMS. Apesar dos avanços e dos conhecimentos gerados sobre este tumor, ainda representa um desafio diagnóstico devido às variabilidades morfológicas e de apresentação clínica, que podem resultar em diagnóstico tardio, levando a tratamentos mais radicais, pior prognóstico e baixa qualidade de vida para os pacientes (Darwish et al., 2020; Ruiz-Mesa et al., 2015). Portanto, este trabalho teve como objetivo analisar as características clínicopatológicas, imuno-histoquímicas e moleculares dos RMS que afetam a região de cabeça e pescoço por meio de uma revisão sistemática, três casos clínicos e um estudo observacional retrospectivo, os quais serão apresentados como artigos científicos.

2 ARTIGOS

2.1 ARTIGO 1

Molecular profile of head and neck rhabdomyosarcomas: A systematic review and meta-analysis.

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Abstract

Objective: This systematic review aimed to identify the molecular alterations of head and neck rhabdomyosarcomas (HNRMS) and their prognostic values. **Study Design:** An electronic search was performed using PubMed, Embase, Scopus, and Web of Science with a designed search strategy. Inclusion criteria comprised cases of primary HNRMS with an established histopathological diagnosis and molecular analysis. Forty-nine studies were included and were appraised for methodological quality using the Joanna Briggs Institute Critical Appraisal tools. Five studies were selected for meta-analysis. **Results:** HNRMS predominantly affects pediatric patients (44.4%), and the parameningeal region (57.7%) is the commonest location. The alveolar variant (43.2%) predominates over the embryonal and spindle cell/sclerosing types, followed by the epithelioid and pleomorphic variants. *PAX-FOXO1* fusion was observed in 103

(79.8%) cases of alveolar RMS. *MYOD1* mutation was found in 39 cases of sclerosing/spindle cell RMS (53.4%). *FUS/ EWSR1-TFCP2* gene fusions were identified in 21 cases of RMS with epithelioid and spindle cell morphology (95.5%). The 5-year overall survival rate of patients was 61.3%, and *MYOD1* mutation correlated with significantly higher mortality. **Conclusion:** The genotypic profile of histologic variants of HNRMS is widely variable, and *MYOD1* mutation could be a potential prognostic factor, but more studies are required to establish this.

Keywords: Rhabdomyosarcoma, head and neck, molecular, systematic review.

Introduction

Rhabdomyosarcoma (RMS) is an aggressive malignant neoplasm microscopically characterized by skeletal myoblast-like cells [1,2]. It is the most common soft tissue sarcoma in children and adolescents, with 35-40% involving the head and neck region compared to adult tumors that represent less than 1% of all malignancies [3-5].

There are four histopathological subtypes of rhabdomyosarcoma, including embryonal, alveolar, pleomorphic, and spindle cell/sclerosing [6]. These tumors may be further subdivided based on their genetic alterations and expression profiles. Approximately 80% of alveolar RMSs present with chromosomal translocations that result in *PAX3/7–FOXO1* fusion genes, which is important since fusion positive neoplasms exhibit different clinical and biological behavior. Hibbitts et al. [7] found that the 5-years overall survival was 65% in *FOXO1* positive RMS in a series of patients from the Children's Oncology Group and confirmed that after metastatic status, *FOXO1* fusion is the most important prognostic factor in childhood RMS. Moreover, several genetic abnormalities in spindle cell/ sclerosing RMS described in recent years have led to a sub-classification of this tumor type into three groups: congenital spindle cell RMS with *VGLL2/NCOA2/CITED2* rearrangements, *MYOD1*-mutant spindle cell/sclerosing RMS, and intraosseous RMS with *EWSR1/FUS-TFCP2 or MEIS-NCOA2 fusions* [8,9].

Molecular profiling of tumors to identify targetable alterations has led to rapid development and evolution in precision oncology with widespread and mainstream clinical use [10]. In RMS, these molecular investigations can complement clinical and microscopic analysis to corroborate or discover molecular markers allowing risk and treatment stratification of patients [11]. This systematic review aimed to analyze the molecular features of head and neck RMS (HNRMS) variants and their correlation with prognosis.

Methods

Protocol and registration

The present systematic review followed the PRISMA guidelines [12]. A protocol on its methodology was registered on the platform of the International Prospective Register of Systematic Reviews (PROSPERO) [13] under the number CRD42020172454. The review question was, "Are there molecular prognostic factors in the different histopathological variants of rhabdomyosarcomas that arise in the head and neck region?"

Eligibility criteria

The inclusion criteria were as follows: a) articles appraising cases of primary HNRMS with a well-described histopathological and molecular diagnosis; b) randomized and non-randomized clinical trials, cohort, cross-sectional and case-control studies, case series or case reports published in the English, Portuguese, or Spanish language.

The exclusion criteria encompassed: a) reviews, congress annals, conference abstracts; b) full texts articles not available; c) studies including RMS located in different body sites, and did not individualize data for HNRMS.

Information sources and search strategy

Electronic searches without publication date restriction were undertaken on October 20, 2020. The following databases: PubMed/MEDLINE, Scopus, Embase, and Web of Science were assessed using a developed search strategy **(Supplement Table S1).** An additional hand-screen across reference lists of included studies was carried out for potentially relevant studies. Rayyan Qatar Computing Research Institute (QCRI) [14] was used to remove duplicate references. An updated search was conducted on April 6, 2021, using the same strategy as the original search; however, it was limited by the date of publication from October 2020 to April 2021.

Study selection

All articles were reviewed in two phases by two authors independently. First, the titles and abstracts were reviewed, and the studies that met the inclusion criteria were selected. Then, the full-text of the studies included in phase one was assessed, and those that fulfilled the eligibility criteria were included for the qualitative synthesis. The disagreement between the reviewers was resolved through discussion or consultation with a third author.

Data collection process

The following data were obtained for each included study: author(s), year of publication, country, sample size, type of study, sex and age of patients, site, size, histopathologic variant, immunohistochemical profile, molecular studies, genetic alterations, stage of disease, treatment, presence or absence of recurrence/metastasis, time of follow-up, and status of the patient at last follow-up.

Risk of bias between studies

Two reviewers who used the Joanna Briggs Institute (JBI) Critical Appraisal tools [15] assessed the risk of bias in the included articles and categorized it as high, moderate, or low. Disagreements between reviewers were resolved through discussion or consultation with a third author.

Quantitative synthesis

Qualitative and quantitative data were descriptively presented. All cases of HNRMS with documented patients' follow-up time and status were selected for statistical analysis. The clinicopathological and molecular features (age, sex, site, size, histopathological variant, genetic profile, stage, presence of recurrence and/or metastasis, and treatment) were correlated with the patients' status (dead or alive) using the Chi-square or Fisher's exact test. The Kaplan-Meier method was used to calculate overall survival (OS) rates, while the difference between survival curves was investigated using the univariate Log-Rank test. In addition, a univariate Cox proportional hazard regression model was employed to identify potential prognostic factors. A multivariate Cox regression model was created using all variables that reached a *p*-value < 0.10. The analyses were carried out using SPSS software version 22.0 (IBM Corporation, Armonk, NY), and a *p*-value ≤ 0.05 was considered statistically significant.

Additionally, the associations between odds of death, recurrence, and metastasis with molecular alterations of HNRMS were assessed through meta-analyses following the appropriate Cochrane Guidelines [16]. The forest plots were performed using the Review Manager® 5.4 software (RevMan 5.4, The Nordic Cochrane Centre, Copenhagen, Denmark), including the total number of patients with molecular alterations and the number of patients who succumbed to death, presented recurrence and/or metastasis. The outcomes were measured using the dichotomous analysis of odds ratio (OR), considering 95% CI. The statistical heterogeneity was determined using the inconsistency index (I^2), and a random-effect model was applied due to the heterogeneity of the studies.

Results

Study selection

In the original search, 859 records were collected from the electronic databases. After removing duplicates, 498 articles were screened by title and abstract, resulting in 101 studies that underwent full-text assessment. On search update, 27 references were identified from the databases; after duplicate removal, the title and abstract of 15 studies were screened, and full-text reading was conducted in seven studies. Seventy-one articles were excluded, and the reasons for exclusion are listed in **Supplement Table S2.** Additionally, 12 studies [17-28] were included from the manual search across reference lists of selected studies. Forty-nine articles were included in this review, and five of them were selected for meta-analysis [21, 29-32]. The flowchart summarizing this process is illustrated in **Figure 1**.

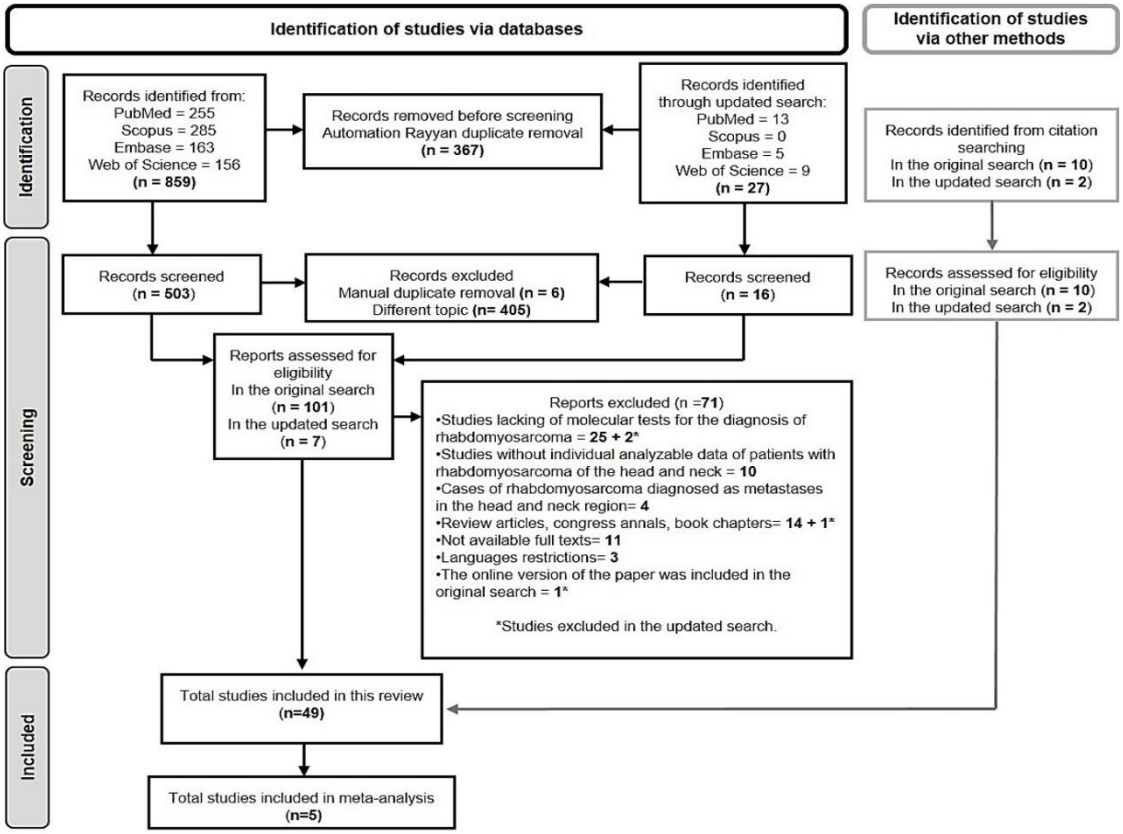

Figure 1. Flow diagram of literature search and selection criteria. *Adapted from Page MJ. et al. 2020* [12]

Studies characteristics

The 49 papers included in this systematic review were published between 1994 and 2021. Twenty-three were case reports [19, 20, 23-25, 27, 33-49] and twenty-six were case series' [17, 18, 21, 22, 26, 28-32, 50-65]. Forty-six studies were from fifteen countries: Australia [25], Belgium [20], China [26,

[30] France [21, 33, 38, 57], India [61], Israel [41], Japan [40], Malaysia [45], México [35], Spain [48], Sweden [63], Taiwan [24, 31], The Netherlands [65], UK [23, 37], USA [17-19, 22, 27-29, 32, 34, 36, 39, 42-44, 46, 47, 49-52, 55, 56, 58-60, 64]. The remaining three were international collaboration between USA and Canada [53, 54, 62].

Risk of bias within studies

Two checklists of JBI's critical appraisal tools were used: for case series and case reports. Thirty-three articles showed a low risk of bias. Moreover, eight case series were judged as moderate risk [28, 32, 51, 53, 56, 63-65] due to the unclear description of the participant's inclusion. Only one presented a high risk of bias [57] because it did not provide the inclusion criteria, diagnostic methods, and follow-up data. Among the case reports, two were classified with high risk [20, 41] and four with moderate risk [37, 42, 43, 48]. Most studies did not report chronologic clinical data, follow-up, and outcomes information. The risk of bias assessment of the 49 included articles is presented in **Supplement Table S3.**

Synthesis of results

The summary of 49 articles included in this systematic review is described in **Supplement Table S4.** The demographic and clinicopathological features of 324 HNRMS are presented in **Table 1**. The age of patients varied widely from 0.2 to 87 years old (mean age: 23.3 years), affecting predominantly pediatric patients (144 cases, 44.4%) compared to adults (104 cases, 32.1%) and showed slight male predilection. The most commonly affected site was the parameningeal region (187 cases, 57.7%), followed by non-parameningeal sites (102 cases, 31.5%). Paranasal sinuses (29/187; 15.5%); nasopharynx/parapharyngeal areas (24/187; 12.83%) and infratemporal fossa (14/128; 10.9%) were the commonest location for parameningeal RMS, while the masseter region (17/102; 16.7%) and the neck (13/102; 12.7%) were more affected by non-parameningeal tumors. Additionally, 51 (15.7%) HNRMS cases involved the oral cavity being the buccal mucosa, the most affected location.

The alveolar variant was the most frequent subtype identified in 140 (43.2%) cases Followed by the embryonal and spindle cell/sclerosing variants (23.8% each, n=77) cases, respectively. Thirty (9.3%) cases were described as unusual subtypes, including 12 cases of epithelioid RMS, 10 cases of *FUS/TFCP2* RMS, two pleomorphic RMS, 3 cases with mixed features, and another three as not otherwise specified (NOS) **(Table 1).**

Most patients (144 cases, 44.4%) presented with a high-stage disease (III-IV). Of the 114 (35.2%) cases containing tumor size information, 63 (55.3%) cases measured ≤5 cm.

Regardless of the therapeutic approach; multimodal treatment was the most prevalent option, including surgery with radiotherapy and chemotherapy. The presence of recurrence and metastasis was observed in 16.4% and 29.6% of cases, respectively. The average follow-up time was at 35.9 months (ranging from 1 to 259 months). One hundred and fifty-seven (48.5%) patients were alive at their last follow-up, and 92 (28.4%) were dead. It is important to note that not all of the information detailed above was available in all cases **(Table 1).**

Molecular studies were performed in 129 (92.1%) cases of alveolar RMS, and 103 (79.8%) patients expressed *PAX3-FKHR/FOXO1* or *PAX7-FKHR/FOXO1* gene fusions from *t(2;13) or t(1;13)*, respectively **(Supplement Table S4)**. Forty-nine cases of alveolar RMS demonstrated the *PAX-FKHR/FOXO1* gene fusion, while the *PAX3-FKHR/FOXO1* fusion was identified in 44 alveolar RMS, and only two cases showed the fusion variation *PAX7-FKHR/FOXO1*. Chromosomal translocation in the *FKHR* gene was observed in six cases.

The features of 30 alveolar RMSs were described in this study because they presented well-documented and individualized data for characterization. Twenty-eight (93.3%) of these cases presented with a *PAX3/7-FOXO1* fusion **(Table 2)**. The frequency of this fusion was similar in pediatric and adult patients, although it was absent in two RMSs in children. Sixteen cases of fusion-positive alveolar RMS affected the parameningeal region, eight cases in non-parameningeal areas, and four in the orbit. Metastasis was observed in 10/30 cases (33.3%), eight of which expressed the *PAX-FOXO1* gene fusion.

Only 24 (31.2%) cases of Embryonal RMS were investigated for molecular alterations. Variable chromosomic rearrangement was identified in 12 cases, and chromosome 11 was most predominantly affected (**Supplement Table S4**).

Seventy-three (94.8%) spindle cell/sclerosing RMS cases were tested for molecular alterations (**Supplement Table S4**). *MYOD1* mutations were present in 26 cases (35.6%), while *MYOD1/PIK3CA* mutations were seen in 15 (20.5%) spindle cell/sclerosing RMSs. The fusion of *EWSR1* or *FUS* with the *TFCP2* gene was identified in three cases. Additionally, one case was *SRF-NCOA2* fusion-positive, and three cases presented with *PIK3CA* mutation. The remaining cases did not exhibit any molecular alterations.

Individualized data were available for 63 cases of spindle cell/sclerosing RMSs **(Table 3).** Genetic alterations were observed in 25/33 (75.8%) pediatric patients compared to 19/30 (63.3%) adults. Most cases occurred in females. The non-parameningeal region was frequently involved (32 cases, 50.8%), and 19 (30.2%) were located in parameningeal sites. *MYOD1*

mutation occurred in 38 (60.3%) spindle cell/sclerosing RMSs and 15 (39.5%) of them presented with *PIK3CA* mutation.

Less than 40 intraosseous RMS, diagnosed as epithelioid RMS or *FUS/EWSR1-TFCP2* RMS, have been reported. Twenty-three of them affected the head and neck region, and 22 were included in this systematic review. One was excluded because it did not meet the inclusion criteria. The mean age at diagnosis was 29.7 (±17.9) years with a slight male predilection. Twenty-one (90%) cases were intraosseous, with the mandible being the most affected location. Only one case involved the soft tissues, specifically the neck. Local or regional pain and cortical destruction were observed in most cases. Microscopically, epithelioid and spindle cell components were described in these tumors. The *FUS-TFCP2* positive fusion was found in 14 (63.3%) cases, and the *EWSR1-TFCP2* gene fusion was seen in seven cases (31.8%) (**Supplement Table S4**).

Additionally, three cases of RMS NOS displayed *SRF-NCAO1* or *SRF-FOXO1* fusion, and two cases identified as hybrid RMS with mixed morphology had *MYOD1* mutation (**Supplement Table S4**).

With regards to the immunohistochemical (IHC) profile, most cases were tested for Desmin, Myogenin, and Myo-D1; the results were heterogeneous but almost all cases were positive for at least one myogenic marker. The spindle cell/sclerosing RMS showed intense positivity for Myo-D1, independently of the *MYOD1* mutation presence or absence. Furthermore, those cases diagnosed as epithelioid RMS or *FUS/EWSR1-TFCP2 RMS* were diffusely positive for cytokeratins AE1/AE3, and some of them presented a positive reaction for ALK protein as well **(Supplement Table S4).**

Sixty-nine HNRMS cases included patients' follow-up and status data, although only 62 cases displaying genetic alterations were included in the statistical analysis. The analysis showed that patients with parameningeal RMS (p= 0.02), spindle cell/sclerosing type with *MYOD1* mutation (p= 0.04), presence of metastasis (p= 0.03), and those not treated with multimodal therapy (p= 0.01) displayed significantly higher mortality **(Table 4).** The 5-year overall survival (OS) rate was 61.3% **(Supplement Figure S5)**. Log-rank analysis showed significant association between parameningeal location (p= 0.01), advanced stage [III- IV] (p= < 0.01), presence of recurrence (p= 0.04) and metastasis (p= < 0.01) **(Table 5; Supplement Figure S6)** and a lower survival rate. In the univariate Cox regression analysis, the same variables, except the presence of recurrence, were significantly correlated with patient survival.

The multivariate Cox regression model showed the parameningeal location as the only independent prognostic factor for a lower survival rate **(Table 6).**

Five studies were included for the quantitative synthesis, i.e., spindle cell/ sclerosing RMS cases with or without *MYOD1* mutation and those with *FUS-TFCP2* vs. *EWSR1-TFCP2* fusion with complete information about the presence of recurrence and metastasis, and live status at last follow-up were grouped for meta-analysis. However, no significant associations between molecular alterations and odds of death, recurrence, or metastasis were observed **(Supplement Figure S7).**

Discussion

Several genetic abnormalities have been identified in different subtypes of RMS; consequently, this tumor can exhibit diverse growth patterns and cellular morphology that characterize its biological behavior [8]. This systematic review assembles the information available in the literature regarding the genetic alterations reported in HNRMSs, helps understand this neoplasm's molecular profile, and its correlation with prognosis.

The findings of the present study corroborate the well-known demographic and clinicopathological characteristics of RMSs. This rare sarcoma comprises approximately 5% of all pediatric neoplasms and 50% of all soft tissue sarcomas in children and adolescents, and represents <1% of all solid tumor malignancies in adults [3-4]. Some authors report a slight male predilection [3, 66], as we confirmed in this study. Parameningeal locations were identified most often, accounting for nearly 60% of tumors. Railley et al. [67] also reported a remarkable predilection for this location with 76.5% of frequency, in contrast to Turner and Richmon,[5] who found 49.1% of cases arising in the parameningeal region. Despite the variable frequency, the worst prognosis and highest treatment failure rate are associated with this anatomical region [51, 52]. Our study shows that parameningeal involvement was significantly associated with low survival rates (p=0.02) of HNRMS.

The most common histologic variants encountered in children and adolescents are the embryonal and alveolar subtypes, whereas pleomorphic RMS is more common in adults [4, 67]. In the present study, alveolar RMS predominated over the embryonal and spindle cell/sclerosing types. Although the spindle cell/sclerosing RMS has, a low frequency compared to alveolar and embryonal types [6, 29], efforts to study its molecular profile have gained more attention during

recent years [18-20, 50] which probably explains why the number of spindle cell/sclerosing RMS cases included in this study was the same as the number of embryonal RMS.

Regarding molecular features, alveolar RMS can undergo specific chromosomal translocations detected in 70%–80% of cases [57, 64]. The translocations *t(2;13)(q35;q14)* or *t(1;13)(p36;q14)* produce chimeric transcription factors that result in *PAX3-FKHR (PAX3-FOXO1)* or *PAX7-FKHR (PAX7-FOXO1)* gene fusion, respectively [5,68]. Some studies report that fusion status is associated with distinct clinical phenotypes and has a prognostic impact [7, 60, 66-68]. Kubo et al. [69] conducted a systematic review and meta-analysis and concluded that no difference in overall survival between patients with fusion-positive and negative alveolar RMS was observed. However, *PAX3–FOXO1* fusion appears to carry an unfavorable outcome, unlike those seen with *PAX7–FOXO1* fusion [7, 68, 69]. The present study shows that 79.8% of alveolar HNRMSs were fusion-positive however, no prognostic correlation was observed. This could be due to insufficient individualized data available for alveolar HNRMS.

With regard to genotyping, the last 2020 WHO classification subdivides the spindle cell/sclerosing RMS into three groups: congenital/infantile spindle cell RMS associated with various gene fusions involving *VGLL2*, *NCOA1/2*, and *SRF* genes, spindle cell/sclerosing RMS with *MYOD1* mutations, and the last intraosseous RMS with *EWSR1/FUS-TFCP2* or *MEIS-NCOA2* fusions [6, 32]. Agaram et al. [50] reported that spindle cell/sclerosing RMS presenting with *MYOD1* mutation is associated with poor outcomes irrespective of age and can present with concomitant *PIK3CA* mutations, especially those with sclerosing morphology. A recent study carried out by Shern et al.[70], showed that 3% of fusion-negative RMS are *MYOD-1* mutant tumors, and 88% of these were observed in either the head and neck or parameningeal region. In addition, they found that 53% of cases presented concomitant *PIK3CA* and *MYOD1* gene mutations and concluded that *MYOD-1* mutation is an indicator of poor prognosis. The present study supports these results showing that *MYOD1* mutation displayed a significant correlation with higher mortality rate (p=0.04) in HNRMS and 39% of them displayed additional *PIK3CA* mutation.

Intraosseous RMS has a remarkable predilection for craniofacial bones, especially the mandible causing pain and cortical destruction. Females are more affected in some series [21, 32, 72], but we observed a slight male predilection and more cases in adults in our study. Myogenic differentiation, cytokeratins, and ALK expressions characterize this variant [21, 32]. The *FUS/EWSR1-TFCP2* gene fusions are hallmarks of this neoplasm with a highly aggressive

clinical behavior [71, 73]. However, the meta-analysis results of the present review did not show an association between odds of death, recurrence, or metastasis when *TFCP2* has fusion with *FUS* or *EWSR*. Furthermore, we noted that some studies diagnosed this neoplasm as "epithelioid RMS" [27, 71, 72], especially before publication of the recently WHO classification which may have influenced some of the results.

Karanian et al. [38] reported three RMS NOS cases localized in the neck that exhibited *SRF-NCOA1* or *SRF-FOXO1* fusions and were included in this systematic review. The authors suggested that this novel fusion might assemble this group as a different variant within the molecular classification of RMS. However, Shern et al., 2021[70], observed some cases of RMS NOS with *MYOD-1* mutations. Although RMS NOS is not a recognized entity, the findings mentioned before, emphasizes the necessity for molecular analysis and characterization for precise and accurate classification and diagnosis.

As a consequence of advances in treatment modalities, the 5-year OS rate for RMS has improved to over 70%. Curry et al., [74] reported a 5-year OS of 73.2 % in pediatric HNRMS and Turner et al., [5] reported a 5-year OS of 62.8%. The outcome of adolescents and adult RMSs appears to be worse than in children. The 5-year OS rate is 27% in adults compared to 61% in pediatric patients [3]. The survival rate of individuals with HNRMS presenting genetic alterations were 61.3% in this series.

It is essential to highlight the limitations of our study. Several analyzed studies did not provide complete demographic information, clinical data analysis, and status at the last follow-up. These data were necessary to correlate genetic alterations with the mortality rate and clinicopathological features. Inclusion of this information in future publications would help improve understanding of the prognostic implications of molecular markers in HNRMS.

In summary, the present study does not confirm a correlation between molecular alterations and the overall prognosis of HNRMS. Nonetheless, this systematic review gathers important and relevant evidence published in the literature showing that different histopathological subtypes of HNRMS present with several molecular alterations that result in the variable morphology of this tumor. The *MYOD1* mutation, frequently observed in the spindle cell/sclerosing type, appears to be associated with higher mortality rates.

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Figures

Figure 1. Flow diagram of literature search and selection criteria. *Adapted from Page MJ. et al. 2020* [12]

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Tables

Table 1. Demographic and clinicopathological features of 324 cases of head and neck rhabdomyosarcomas.

	n= 324	**%**
Demographic variables		
Sex		
Male	161	49.7
Female	155	47.8
Not available data	8	2.5
Age (mean age 23.3 ±20.2; range 0.2 - 87 y.o)		
Pediatric (≤ 19 years old)	144	44.4
Adults (> 19 years old)	104	32.1
Not available data	76	23.5
Clinicopathological variables		
Site		
Parameningeal	187	57.7
Non -parameningeal	102	31.5
Orbit	19	5.9
Head and neck region without specification	16	4.9
Size		
≤5 cm	63	19.4
>5 cm	51	15.7
Not available data	210	64.8
Histopathological variant		
Alveolar RMS	140	43.2
Embryonal RMS	77	23.8
Spindle cell/sclerosing RMS	77	23.8
FUS/EWSRT1-TFCP2; Epithelioid RMS	22	6.8
*Others	8	2.4
Stage of disease		
I – II	79	24.4
III - IV	144	44.4
Not available data	101	31.2

*Others: RMS with mixed histopathologic features = 3; RMS NOS = 3; Pleomorphic RMS = 2

Abbreviations: NOS= no other specified; RMS= rhabdomyosarcoma.

Table 1. (Continued) Demographic and clinicopathological features of 324 cases of head and neck rhabdomyosarcomas.

	n= 324	**%**
Clinicopathological variables		
Therapeutic approach		
Surgery	39	12.0
Chemotherapy	16	4.9
Radiotherapy	4	1.2
Surgery + Chemotherapy or Radiotherapy	11	3.4
Chemotherapy + Radiotherapy	87	26.9
Surgery + Chemotherapy + Radiotherapy	122	37.7
Not available data for categorization	45	13.9
Recurrence		
Yes	53	16.4
No	165	50.9
Not available	106	32.7
Metastasis		
Yes	96	29.6
No	121	37.4
Not available data for categorization	107	33.0
Follow up (mean 35.9; range 1 – 259 mo)		
< 12 months	18	5.6
12 – 60 months	51	15.7
> 60 months	14	4.3
Not available data for categorization	241	74.4
Vital status		
Alive	157	48.5
Dead	92	28.4
Not available	75	23.1

Abbreviations: mo= months

Table 2. Demographic and clinicopathological features of 30 cases of alveolar rhabdomyosarcomas in head and neck region.

	Molecular alterations				
	PAX 3/7 - FOXO 1				
	Positive		*Negative*		**Total**
	n	*%*	n	*%*	***n = 30***
Demographic features					
Age group					
Pediatric (≤ 19 y.o.)	14	87.5	2	12.5	16
Adults (> 19 y.o.)	14	100	-		14
Sex					
Male	8	88.9	1	11.1	9
Female	15	93.8	1	6.2	16
Not available	5	100	-		5
Clinicopathological features					
Site					
Parameningeal	16	100	-		16
Non -parameningeal	8	88.9	1	11.1	9
Orbit	4	80	1	20	5
Size					
≤5 cm	5	100	-		5
>5 cm	2	100	-		2
Not available	21	91.3	2	8.7	23
Stage of disease					
I - II	3	100	-		3
III - IV	5	100	-		5
Not available	20	90.9	2	9.1	22
Treatment					
Multimodal	10	83.3	2	16.7	12
No multimodal	4	100	-		4
Not available	12	85.7	2	42.9	14
Recurrence					
Yes	1	100	-		1
No	2	100	-		2
Not available	23	85.2	4	14.8	27
Metastases					
Yes	8	80	2	20	10
No	2	100	-		2
Not available	16	88.8	2	11.	18

Table 3. Demographic and clinicopathological features of 63 cases of spindle cell/ sclerosing rhabdomyosarcomas in the head and neck region.

	Molecular alterations						
	MYOD1		***MYOD1-PIK3CA***		***Other genes***		
	Positive	Negative	Positive	Negative	*Positive	Negative	**Total**
	n= 23 %	n= 5 %	n= 15 %	n=8 %	n=4 %	n=8 %	**n = 63**
Demographic features							
Age group							
Pediatric	15 65.2	4 80.0	7 46.7	4 50.0	1 25.0	2 25.0	33
Adults	8 34.8	1 20.0	8 53.3	4 50.0	3 75.0	6 75.0	30
Sex							
Male	9 39.1	4 80.0	2 13.3	5 62.5	3 75.0	5 62.5	28
Female	14 60.9	1 20.0	13 86.7	3 37.5	1 25.0	3 37.5	35
Clinicopathological features							
Site							
Parameningeal	5 21.7	2 40.0	6 40.0	2 25.0	1 25.0	3 37.5	19
Non -parameningeal	11 47.8	3 60.0	4 26.7	6 75.0	3 75.0	5 62.5	32
Head and neck region	7 30.4	-	5 33.3				12
Histopathological variant							
Spindle cell	8 34.8	4 80.0	5 33.3	7 87.5	4 100	3 37.5	31
Sclerosing	11 47.8	1 20.0	8 53.3	1 12.5	-	5 62.5	26
Spindle cell / sclerosing	4 17.4	-	2 13.3	-	-		6
Stage of disease							
I - II	8 34.8	3 60.0	5 33.3	6 75.0	-	1 12.5	23
III - IV	2 8.7	-	4 26.7	2 25.0	-	1 12.5	9
Not available	13 54.2	2 40.0	6 40.0	-	4 100	6 75.0	31

* *EWSR1-TFCP2* positive fusion= 1 case; *FUS-TFCP2* positive fusion= 2 cases; *SRF-NCOA2* positive fusion = 1 case

Table 3. (Continued) Demographic and clinicopathological features of 63 cases of spindle cell/ sclerosing rhabdomyosarcomas in head and neck region.

	Molecular alterations						
	MYOD1		***MYOD1-PIK3CA***		****Other genes = 12***		
	Positive	Negative	Positive	Negative	*Positive	Negative	**Total**
	n= 23 %	n=5 %	n= 15 %	n= 8 %	n=4 %	n=8 %	**n = 63**
Clinicopathological features							
Treatment							
Multimodal	9 39.1	4 80.0	4 26.7	5 62.5	2 50.0	4 50.0	28
No multimodal	11 47.8	1 20.0	9 60.0	3 37.5	1 25.0	-	25
Not available	3 13.0	-	2 13.3	-	1 25.0	4 50.0	10
Recurrence							
Yes	10 43.5	-	4 26.7	-	1 25.0	-	15
No	12 52.2	4 80.0	8 53.3	6 75.0	1 25.0	2 25.0	33
Not available	1 4.3	1 20.0	3 20.0	2 25.0	2 50.0	6 75.0	15
Metastases							
Yes	2 8.7	-	5 33.3	-	1 25.0	2 25.0	10
No	14 60.9	2 40.0	4 26.7	2 25.0	1 25.0	-	23
Not available	7 30.4	3 60.0	6 40	6 75.0	2 50.0	6 75.0	30
Live status							
Alive	13 54.2	3 60.0	4 26.7	5 62.5	4 100	1 12.5	30
Dead	9 39.1	-	9 60.0	1 12.5	-	2 25.0	21
Not available	1 4.3	2 40.0	2 13.3	2 25.0	-	5 62.5	12

* *EWSR1-TFCP2* positive fusion= 1 case; *FUS-TFCP2* positive fusion= 2 cases; *NCOA2-SRF* positive fusion = 1 case

Table 4. Association analysis between the clinicopathological and molecular features of 62 cases of head and neck rhabdomyosarcomas with genetic alterations.

Clinicopathological variables	**Alive N (%)**	**Dead N (%)**	***p*-value**
Age group			0.52
Pediatrics (≤19 years old)	19 (50.0 %)	10 (41.7 %)	
Adults (>19 years old)	19 (50.0 %)	14 (58.3 %)	
Sex			0.66
Male	18 (47.4 %)	10 (41.7 %)	
Female	20 (52.6 %)	14 (58.3 %)	
Site			**< 0.01**
Parameningeal	6 (15.8 %)	12 (50.0 %)	
Non-parameningeal	32 (84.2 %)	12 (50.0 %)	
Size			0.07
≤ 5 cm	18 (69.2 %)	7 (41.2 %)	
> 5 cm	8 (30.8 %)	10 (58.8 %)	
Histopathological variant			0.28*
Alveolar RMS	5 (13.2%)	1 (4.2 %)	
Spindle cell/ sclerosing RMS	21 (55.3 %)	18 (75.0 %)	
[a] Others	12 (31.6 %)	5 (20.8 %)	
Molecular alterations			**0.04***
PAX 3/7 - FOXO1 positive fusion	5 (13.2 %)	1 (4.2 %)	
MYOD1 positive mutation	16 (42.1 %)	18 (75.0 %)	
*Others	17 (44.7 %)	5 (20.8 %)	
Stage			0.35*
Stages I - II	6 (85.7 %)	8 (61.5 %)	
Stages III - IV	1 (14.3 %)	5 (38.5 %)	
Recurrence			0.09
Yes	8 (28.6 %)	11 (52.4 %)	
No	20 (71.4 %)	10 (47.6 %)	
Metastasis			**0.03**
Yes	7 (25.9 %)	10 (58.8 %)	
No	20 (74.1 %)	7 (41.2 %)	
Treatment			**0.01**
Multimodal	26 (70.3 %)	8 (36.4 %)	
No multimodal	11 (29.7 %)	14 (63.6 %)	

*Exact Fisher´s Test

[a] Others: Epithelioid RMS and *FUS/EWSR1-TFCP2* RMS (14 cases), RMS NOS (3 cases).
[b] Others: *FUS/EWSR1-TFCP2* positive fusion: (17 cases); *SRF-NCOA1-2* positive fusion (4 cases); *PIK3CA* mutation (1 case).
Abbreviations: NOS= no other specified; RMS= rhabdomyosarcoma.

Table 5. Log-rank univariate analysis of the clinicopathological and molecular features of 62 cases of head and neck rhabdomyosarcomas with genetic alterations.

Clinicopathological variables	**Log-rank univariate analysis**			
	5-year survival (%)	**Estimative (95% CI*)**	**Chi-square**	***p*-value**
Age group				
Pediatrics (≤19 years old)	72.4 %	125.3 (68.4 – 182.2)	2.62	0.10
Adults (>19 years old)	63.6 %	50.8 (29.9 – 71.6)		
Sex				
Male	75.0 %	88.9 (48.4 – 129.4)	0.28	0.59
Female	61.8 %	101.3 (46.7 – 155.9)		
Site				
Parameningeal	38.9 %	38.3 (22.4 – 54.1)	6.64	**0.01**
Non-parameningeal	79.5 %	130.1 (77.5 – 182.6)		
Size			2.26	
≤ 5 cm	72.0 %	147.8 (80.6 – 215)		0.13
> 5 cm	61.1 %	45.6 (27.1 – 64.1)		
Histopathological variant				
Alveolar RMS	83.3 %	31 (7.4 – 54.6)	0.65	0.42
Spindle cell/ sclerosing RMS	64.1 %	99.4 (58.5 – 140.2)		
[a] Others	70.6 %	64.8 (34.7 – 94.5)		
Molecular alterations				
PAX 3/7 - FOXO1 positive fusion	83.3 %	31 (7.4 – 54.5)	0.005	0.94
	58.8 %	90.9 (50.8 – 130.9)		
MYOD1 positive mutation	77.3 %	71 (43.7 – 98.3)		
[b] Others				
Stage				
Stages I - II	64.3 %	108.9 (51.2 – 166.6)	7.16	**< 0.01**
Stages III - IV	16.7 %	25.5 (8.3 – 42.7)		
Recurrence				
Yes	52.6 %	38.9 (27.4 – 50.5)	3.93	**0.04**
No	73.3 %	127.5 (73.2 – 181.7)		
Metastasis				
Yes	41.1 %	28.1 (10.4 – 45.7)	11.48	**< 0.01**
No	81.5 %	141.8 (79.5 – 204.3)		
Treatment				
Multimodal	82.4 %	78.8 (48.9 – 108.6)	0.98	0.32
No multimodal	52.0 %	89.1 (44.3 – 133.9)		

[a] Others: Epithelioid RMS and *FUS/EWSR1-TFCP2* RMS (14 cases), RMS NOS (3 cases).
[b] Others: *FUS/EWSR1-TFCP2* positive fusion: (17 cases); *SRF-NCOA1-2* positive fusion (4 cases); *PIK3CA* mutation (1 case).
Abbreviations: NOS= no other specified; RMS= rhabdomyosarcoma.

Table 6. Association analysis between the clinicopathological and molecular features of 62 cases of head and neck rhabdomyosarcomas with genetic alterations – univariate Cox regression and multivariate Cox regression model created using all variables that achieved a *p*-value < 0.10.

Variables	**Univariate analysis**		**Multivariate analysis**	
	HR (95% CI)	***p*-value**	**HR (95% CI)**	***p*-value**
Age group				
Pediatrics (≤19 years old)	1			
Adults (>19 years old)	1.92 (0.85 – 4.35)	0.12		
Sex				
Male	1.23 (0.55 – 2.85)	0.6		
Female	1			
Site				
Parameningeal	2.77 (1.23 – 6.26)	**0.01**	9.94 (1.58 – 62.51)	**0.01**
Non-parameningeal	1		1	
Size				
≤ 5 cm	1			
>5 cm	2.06 (0.78 – 5.42)	0.14		
Histopathological variant				
Alveolar RMS	1			
Spindle cell/ sclerosing RMS	1.12 (0.77 – 2.02)	0.38		
[a] Others	1			
Molecular alterations				
PAX 3/7 - FOXO1 positive fusion	1			
MYOD1 positive mutation	1.01 (0.63 – 1.62)	0.95		
[b] Others	1			
Stage				
I – II	1		1	
III – IV	5.22 (1.36 – 20.06)	**0.02**	2.93 (0.11 – 81.71)	0.53
Recurrence				
Yes	2.40 (0.98 – 5.92)	0.06	1.97 (0.12 – 31.41)	0.63
No	1		1	
Metastasis				
Yes	4.77 (1.77 – 12.83)	**< 0.01**	9.18 (0.28 – 292.87)	0.21
No	1		1	
Treatment				
Multimodal	1			
No multimodal	1.55 (0.64 – 3.72)	0.33		

[a] Others: Epithelioid RMS and FUS/EWSR1-TFCP2 RMS (14 cases), RMS NOS (3 cases).
[b] Others: *FUS/EWSR1-TFCP2* positive fusion: (17 cases); *SRF-NCOA1-2* positive fusion (4 cases); PIK3CA mutation (1 case).
Abbreviations: NOS= no other specified; RMS= rhabdomyosarcoma.

Supplement materials

Supplement 1. Search strategies with appropriated key words and Mesh terms.

Table S1. Search strategies with appropriated key words and Mesh terms	
Database	***Search strategy***
PubMed	("Rhabdomyosarcoma" OR Rhabdomyosarcomas) AND ("Head" OR "Neck" OR "Orbital" OR orbit OR "head and neck" OR "non-parameningeal" OR parameningeal OR oral OR "oral cavity" OR maxillofacial)) AND ("Mutation" OR "Gene fusion" OR "Translocation, Genetic" OR cytogenetic OR alterations OR "molecular" OR "fusion status" OR "PAX-FOXO1" OR "PAX3–FOXO1" OR "PAX7–FOXO1" OR "PAX3/7–FOXO1" OR "PAX3–FKHR" OR "PAX–FKHR" OR "MYOD1" OR "TFCP2" OR "VGLL2" OR "NCOA2" OR "DICER1")
Scopus	(TITLE-ABS-KEY ("Rhabdomyosarcoma" OR rhabdomyosarcomas) AND TITLE-ABS-KEY ("Head" OR "Neck" OR "Orbital" OR "head and neck" OR "non-parameningeal" OR "parameningeal" OR oral OR "oral cavity" OR maxillofacial) AND TITLE-ABS-KEY ("Mutation" OR "Gene Fusion" OR "Translocation,Genetic" OR "Fusion Status" OR cytogenetic OR alterations OR molecular OR "PAX-FOXO1" OR "PAX3--FOXO1" OR "PAX7--FOXO1" OR "PAX3/7-FOXO1" OR "PAX3-FKHR" OR "PAX-FKHR") OR TITLE-ABS-KEY ("MYOD1" OR "TFCP2" OR "VGLL2" OR "NCOA2" OR "DICER1"))
Web of Science	ALL FIELDS: ("Rhabdomyosarcoma" OR Rhabdomyosarcomas) AND ALL FIELDS: ("Head" OR "Neck" OR "Orbital" OR orbit OR "head and neck" OR "non-parameningeal" OR parameningeal OR oral OR "oral cavity" OR maxillofacial) AND ALL FIELDS: ("Mutation" OR "Gene Fusion" OR "Translocation, Genetic" OR cytogenetic OR alterations OR molecular OR "Fusion Status" OR "PAX-FOXO1" OR "PAX3FOXO1" OR "PAX7FOXO1" OR "PAX3/7FOXO1" OR "PAX3FKHR" OR "PAXFKHR" OR "MYOD1" OR "TFCP2" OR "VGLL2" OR "NCOA2" OR "DICER1")
Embase	('rhabdomyosarcoma':ti,ab,kw OR rhabdomyosarcomas:ti,ab,kw) AND (('head':ti,ab,kw OR 'neck':ti,ab,kw OR 'orbital':ti,ab,kw OR orbit:ti,ab,kw OR head:ti,ab,kw) AND neck:ti,ab,kw OR 'non-parameningeal':ti,ab,kw OR parameningeal:ti,ab,kw OR oral:ti,ab,kw OR 'oral cavity':ti,ab,kw OR maxillofacial:ti,ab,kw) AND ('mutation':ti,ab,kw OR 'gene fusion':ti,ab,kw OR 'translocation, genetic':ti,ab,kw OR cytogenetic OR alterations:ti,ab,kw OR molecular:ti,ab,kw OR 'fusion status':ti,ab,kw OR 'pax-foxo1':ti,ab,kw OR 'pax3–foxo1':ti,ab,kw OR 'pax7–foxo1':ti,ab,kw OR 'pax3/7–foxo1':ti,ab,kw OR 'pax3–fkhr':ti,ab,kw OR 'pax–fkhr':ti,ab,kw OR 'myod1':ti,ab,kw OR 'tfcp2':ti,ab,kw OR 'vgll2':ti,ab,kw OR 'ncoa2':ti,ab,kw OR 'dicer1':ti,ab,kw)

Updated search April 6, 2021= 27 papers from databases.

- Pubmed: 13
- Scopus: 0
- Embase: 5
- Web of science: 9

Supplement 2. Excluded articles and reasons for exclusion

Table S2. Excluded articles and reasons for exclusion

Number	*Reference*	*Reason for exclusion*
1	Agrawal, M.; Malathi, M.; Nargund, A.; Agrawal, M.; Padma, M.; Kapali, A. Diagnosis Of Solid Tumors In Infants By Fine Needle Aspiratin Cytology(Fnac).Pediatric Hematology Oncology Journal. 2018; 3: S48.	**Only abstract available**
2	Ahmad Z, Din NU, Ahmad A, Imran S, Pervez S, Ahmed R, Kayani N. Rhabdomyosarcoma--an epidemiological and histopathologic study of 277 cases from a major tertiary care center in Karachi, Pakistan. Asian Pac J Cancer Prev. 2015;16(2):757-60.	**No molecular tests**
3	Albalawi ED, Alkatan HM, Elkhamary SM, Safieh LA, Maktabi AMY. Genetic profiling of rhabdomyosarcoma with clinicopathological and radiological correlation. Can J Ophthalmol. 2019 Apr;54(2):247-257.	**Without individual analyzable data**
4	Armstrong SJ, Duncan AW, Mott MG. Rhabdomyosarcoma associated with familial adenomatous polyposis. Pediatr Radiol. 1991;21(6):445-6.	**No molecular tests**
5	Arnold MA, Anderson JR, Gastier-Foster JM, Barr FG, Skapek SX, Hawkins DS, Raney RB Jr, Parham DM, Teot LA, Rudzinski ER, Walterhouse DO. Histology, Fusion Status, and Outcome in Alveolar Rhabdomyosarcoma With Low-Risk Clinical Features: A Report From the Children's Oncology Group. Pediatr Blood Cancer. 2016 Apr;63(4):634-9.	**Without individual analyzable data**
6	Aye JM, Chi YY, Tian J, Rudzinski ER, Binitie OT, Dasgupta R, Wolden SL, Hawkins DS, Gupta AA. Do children and adolescents with completely resected alveolar rhabdomyosarcoma require adjuvant radiation? A report from the Children's Oncology Group. Pediatr Blood Cancer. 2020 May;67(5):e28243.	**Without individual analyzable data**
7	Bagdonaite L, Jeeva I, Chang BY, Kalantzis G, El-Hindy N. Multidisciplinary management of adult orbital rhabdomyosarcoma*. Orbit. 2013 Jun;32(3):208-10.	**No molecular tests**
8	Bahrami A, Gown AM, Baird GS, Hicks MJ, Folpe AL. Aberrant expression of epithelial and neuroendocrine markers in alveolar rhabdomyosarcoma: a potentially serious diagnostic pitfall. Mod Pathol. 2008 Jul;21(7):795-806.	**Without individual analyzable data**
9	Barr FG. Gene fusions involving PAX and FOX family members in alveolar rhabdomyosarcoma. Oncogene. 2001 Sep 10;20(40):5736-46.	**Review article**
10	Barr FG. Fusions involving paired box and fork head family transcription factors in the pediatric cancer alveolar rhabdomyosarcoma. Curr Top Microbiol Immunol. 1997;220:113-29.	**Book chapter**

11	Barr FG. The role of chimeric paired box transcription factors in the pathogenesis of pediatric rhabdomysarcoma. Cancer Res. 1999 Apr 1;59(7 Suppl):1711s-1715s.	**Review article**
12	Bhurgri Y, Bhurgri A, Puri R, Ashraf S, Qidwai A, Ashraf K, Ahmed N, Mazhar A, Bhurgri H, Usman A, Faridi N, Malik J, Ahmed R, Muzaffar S, Kayani N, Pervez S, Hasan SH. Rhabdomyosarcoma in Karachi 1998-2002. Asian Pac J Cancer Prev. 2004 Jul-Sep;5(3):284-90.	**No molecular tests**
13	Bhurgri Y, Mazhar A, Bhurgri H, Usman A, Malik J, Bhurgri A, Ahmed R, Muzaffar S, Kayani N, Pervez S, Hasan SH. Orbital embryonal rhabdomyosarcoma in Karachi (1998-2002). J Pak Med Assoc. 2004 Nov;54(11):561-5.	**No molecular tests**
14	Bhutoria S, Oneil C. Embryonal rhabdomyosarcoma of the adult soft palate. Indian J Pathol Microbiol. 2011 Jan-Mar;54(1):136-7.	**Not available article**
15	Bradley PJ. Head and neck oncology. Curr Opin Otolaryngol Head Neck Surg 2002;10(2):67-68.	**Not available article**
16	Brigger MT, Cunningham MJ. Malignant cervical masses in children. Otolaryngol Clin North Am. 2015 Feb;48(1):59-77.	**Review article**
17	Casiraghi O, Lefèvre M. Tumeurs malignes indifférenciées à cellules rondes des cavités naso-sinusiennes et du nasopharynx [Undifferentiated malignant round cell tumors of the sinonasal tract and nasopharynx]. Ann Pathol. 2009 Sep;29(4):296-312. French.	**Language restrictions**
18	Carroll SJ, Nodit L. Spindle cell rhabdomyosarcoma: a brief diagnostic review and differential diagnosis. Arch Pathol Lab Med. 2013 Aug;137(8):1155-8.	**Review article**
19	Cescon M, Grazi GL, Assietti R, Scanni A, Frigerio F, Sparacio F, Ercolani G, Cavallari A. Embryonal rhabdomyosarcoma of the orbit in a liver transplant recipient. Transpl Int. 2003 Jun;16(6):437-40.	**No molecular tests**
20	Chi AC, Barnes JD, Budnick S, Agresta SV, Neville B. Rhabdomyosarcoma of the maxillary gingiva. J Periodontol. 2007 Sep;78(9):1839-45.	**No molecular tests**
21	Chong DY, Demirci H, Ronan SM, Flint A, Elner VM. Orbital rhabdomyosarcoma in Li-Fraumeni syndrome. Arch Ophthalmol. 2007 Apr;125(4):566-9.	**No molecular tests**
22	Chowdhury T, Barnacle A, Haque S, Sebire N, Gibson S, Anderson J, Roebuck D. Ultrasound-guided core needle biopsy for the diagnosis of rhabdomyosarcoma in childhood. Pediatr Blood Cancer. 2009 Sep;53(3):356-60.	**Without individual analyzable data**
23	Cordes B, Williams MD, Tirado Y, Bell D, Rosenthal DI, Al-Dhahri SF, Hanna EY, El-Naggar AK. Molecular and phenotypic analysis of poorly differentiated sinonasal neoplasms: an integrated approach for early diagnosis and classification. Hum Pathol. 2009 Mar;40(3):283-92.	**Without individual analyzable data**
24	Cortes Barrantes P, Jakobiec FA, Dryja TP. A Review of the Role of Cytogenetics in the Diagnosis of Orbital Rhabdomyosarcoma. Semin Ophthalmol. 2019;34(4):243-251.	**Review article**

25	Darouichi M. Embryonic rhabdomyosarcoma of the oral floor. Feuill Radiol 2015;55(1):37-43.	**Language restrictions**
26	Das K, Mirani N, Hameed M, Pliner L, Aisner SC. Fine-needle aspiration cytology of alveolar rhabdomyosarcoma utilizing ThinPrep liquid-based sample and cytospin preparations: a case confirmed by FKHR break apart rearrangement by FISH probe. Diagn Cytopathol. 2006 Oct;34(10):704-6.	**Metastatic RMS**
27	Deyrup A.T., Thway K., Fisher C., Wang W.-L., Lazar A.J., Jones R.L., Tighiouart M., Weiss S.W. 2 Clinicopathologic analysis of adult alveolar and embryonal rhabdomyosarcoma: A study of 62 cases. Lab Invest. 2009; 89 Suppl. 1 (13A-).	**Only abstract available**
28	Dodd LG, Hertel J. Needle biopsy of mesenchymal lesions of the head and neck: Evolving concepts and new strategies for diagnosis. Semin Diagn Pathol. 2015 Jul;32(4):275-83.	**Review article**
29	Downing JR, Khandekar A, Shurtleff SA, Head DR, Parham DM, Webber BL, Pappo AS, Hulshof MG, Conn WP, Shapiro DN. Multiplex RT-PCR assay for the differential diagnosis of alveolar rhabdomyosarcoma and Ewing's sarcoma. Am J Pathol. 1995 Mar;146(3):626-34.	**Not available article**
30	Dugo R., Gutierrez M.F., Cores M., Nana M., Urbieta M., De Matteo E., Colli S., Garcia Lombardi M. Rhabdomyosarcomas in infants younger than 1 year of age. A 30 years experience in a single pediatric institution. Pediatr Blood Cancer. 2018;65:e27455.	**Congress annals**
31	Ehlers JP, Penne RB, Eagle RC Jr, Carrasco JR. Alveolar rhabdomyosarcoma presenting as an acute orbital mass in the medial rectus muscle. Ophthalmic Plast Reconstr Surg. 2007 Mar-Apr;23(2):149-51.	**No molecular tests**
32	Folpe AL, McKenney JK, Bridge JA, Weiss SW. Sclerosing rhabdomyosarcoma in adults: report of four cases of a hyalinizing, matrix-rich variant of rhabdomyosarcoma that may be confused with osteosarcoma, chondrosarcoma, or angiosarcoma. Am J Surg Pathol. 2002 Sep;26(9):1175-83.	**No molecular tests**
33	Foong S., Eykman E., Arbuckle S. Spindle cell rhabdomyosarcoma in a young adult: Classification and immunohistochemistry. Pathology. 2019; 51 Supplement 1 (S147-S148).	**Only abstract available**
34	Fu L, Jin Y, Jia C, Zhang J, Tai J, Li H, Chen F, Shi J, Guo Y, Ni X, He L. Detection of FOXO1 break-apart status by fluorescence in situ hybridization in atypical alveolar rhabdomyosarcoma. Sci China Life Sci. 2017 Jul;60(7):721-728.	**Without individual analyzable data**
35	Galili N, Davis RJ, Fredericks WJ, Mukhopadhyay S, Rauscher FJ 3rd, Emanuel BS, Rovera G, Barr FG. Fusion of a fork head domain gene to PAX3 in the solid tumour alveolar rhabdomyosarcoma. Nat Genet. 1993 Nov;5(3):230-5.	**Review article**
36	Gallego Melcón S, Sánchez de Toledo Codina J. Molecular biology of rhabdomyosarcoma. Clin Transl Oncol. 2007 Jul;9(7):415-9.	**Review article**
37	Gordón-Núñez MA, Piva MR, Dos Anjos ED, Freitas RA. Orofacial rhabdomyosarcoma: report of a case and review of the literature. Med Oral Patol Oral Cir Bucal. 2008 Dec 1;13(12):E765-9.	**No molecular tests**

38	Guram S., Dirks J., Barot S., Griffin A., Weinreb I., Demicco E., Shultz D.B., Razak A.R.A., Gladdy R.A., Gupta A.A. Is PAX3-FOXO1 associated with worse outcome in adults with rhabdomyosarcoma (RMS)?. Journal of Clinical Oncology. 2019; 37 Supplement 15.	**Not available article**
39	Hassold N, Warmuth-Metz M, Winkler B, Kreissl MC, Ernestus K, Beer M, Neubauer H. Hit the mark with diffusion-weighted imaging: metastases of rhabdomyosarcoma to the extraocular eye muscles. BMC Pediatr. 2014 Feb 27;14:57.	**No molecular tests**
40	Heathcote JG. Changing patterns in orbital pathology. Saudi J Ophthalmol. 2018 Jan-Mar;32(1):1-2.	**Review article**
41	Jadali, F; Pour, KG; Aghakhani, R; Khoddami, M; Ahmadi, MA; Behnam, B. The frequency of PAX3 and PAX7 Mutations in Children with Rhabdomyosarcoma. J Ped Hematol Oncol. 2016; 6: 100-105	**Not available article**
42	Jivraj I, Somers GR, Belliveau MJ, Malkin D, DeAngelis DD. Management of orbital rhabdomyosarcoma in a child with Li-Fraumeni syndrome. J AAPOS. 2019 Jun;23(3):182-185.	**No molecular tests**
43	Kazanowska B, Reich A, Stegmaier S, Békássy AN, Leuschner I, Chybicka A, Koscielniak E. Pax3-fkhr and pax7-fkhr fusion genes impact outcome of alveolar rhabdomyosarcoma in children. Fetal Pediatr Pathol. 2007 Jan-Feb;26(1):17-31.	**Without individual analyzable data**
44	Kerbrat A, Beaufrere A, Neiva-Vaz C, Galmiche L, Belhous K, Orbach D, Gauthier-Villars M, Picard A, Kadlub N. Rhabdomyosarcoma and rhabdomyoma associated with nevoid basal cell carcinoma syndrome: Local treatment strategy. Pediatr Dermatol. 2018 Jul;35(4):e245-e247.	**No molecular tests**
45	Kohashi K, Kinoshita I, Oda Y. Soft Tissue Special Issue: Skeletal Muscle Tumors: A Clinicopathological Review. Head Neck Pathol. 2020 Mar;14(1):12-20.	**Review article**
46	Kumar A, Singh M, Sharma MC, Bakshi S, Sharma BS. Pediatric sclerosing rhabdomyosarcomas: a review. ISRN Oncol. 2014 Mar 5;2014:640195.	**No molecular tests**
47	Kumar P, Surya V, Urs AB, Augustine J, Mohanty S, Gupta S. Sarcomas of the Oral and Maxillofacial Region: Analysis of 26 Cases with Emphasis on Diagnostic Challenges. Pathol Oncol Res. 2019 Apr;25(2):593-601.	**No molecular tests**
48	Lamovec J, Volavsek M. Sclerosing rhabdomyosarcoma of the parotid gland in an adult. Ann Diagn Pathol. 2009 Oct;13(5):334-8.	**No molecular tests**
49	Li A, Blandford A, Chundury RV, Traboulsi EI, Anderson P, Murphy E, Parikh S, Perry J. Orbital rhabdomyosarcoma in a child with Leigh syndrome. J AAPOS. 2018 Apr;22(2):150-152.e1.	**No molecular tests**
50	Lin XY, Wang Y, Yu JH, Liu Y, Wang L, Li QC, Wang EH. Sclerosing rhabdomyosarcoma presenting in the masseter muscle: a case report. Diagn Pathol. 2013 Feb 4;8:18.	**No molecular tests**

51	Liu J, Guzman MA, Pezanowski D, Patel D, Hauptman J, Keisling M, Hou SJ, Papenhausen PR, Pascasio JM, Punnett HH, Halligan GE, de Chadarévian JP. FOXO1-FGFR1 fusion and amplification in a solid variant of alveolar rhabdomyosarcoma. Mod Pathol. 2011 Oct;24(10):1327-35.	**Metastatic RMS**
52	Mastrangelo, D; Hadjistilianou, T; Sappia, F. Embryonal rhabdomyosarcoma of the orbit: A molecular study of chromosome 11.Vision Research. 1996;36: 4145	**Not available article**
53	Martinez AP, Fritchie KJ, Weiss SW, Agaimy A, Haller F, Huang HY, Lee S, Bahrami A, Folpe AL. Histiocyte-rich rhabdomyoblastic tumor: rhabdomyosarcoma, rhabdomyoma, or rhabdomyoblastic tumor of uncertain malignant potential? A histologically distinctive rhabdomyoblastic tumor in search of a place in the classification of skeletal muscle neoplasms. Mod Pathol. 2019 Mar;32(3):446-457.	**No molecular tests**
54	Nangalia R, Shah N, Sheikh MA, Pal M. Rhabdomyosarcoma involving maxilla mimicking gingival enlargement: A diagnostic challenge. BMJ Case Rep. 2019 Nov 26;12(11):e230692.	**No molecular tests**
55	Pondrom M, Bougeard G, Karanian M, Bonneau-Lagacherie J, Boulanger C, Boutroux H, Briandet C, Chevreau C, Corradini N, Coze C, Defachelles AS, Galmiche-Roland L, Orbach D, Piguet C, Scoazec JY, Vérité C, Willems M, Frebourg T, Minard V, Brugières L. Rhabdomyosarcoma associated with germline TP53 alteration in children and adolescents: The French experience. Pediatr Blood Cancer. 2020 Sep;67(9):e28486.	**No molecular tests**
56	Radzikowska J, Kukwa W, Kukwa A, Czarnecka AM, Kawecki M, Lian F, Szczylik C, Krzeski A. Management of pediatric head and neck rhabdomyosarcoma: A case-series of 36 patients. Oncol Lett. 2016 Nov;12(5):3555-3562.	**No molecular tests**
57	Rekhi B., Singhvi T. Clinicopathological spectrum, including molecular cytogenetic analysis of a series of spindle cell/sclerosing rhabdomyosarcomas. Virchows Arch. 2014; 465 (Suppl 1):S1–S379	**Only abstract available**
58	Rekhi B, Upadhyay P, Ramteke MP, Dutt A. MYOD1 (L122R) mutations are associated with spindle cell and sclerosing rhabdomyosarcomas with aggressive clinical outcomes. Mod Pathol. 2016 Dec;29(12):1532-1540.	**Without individual analyzable data**
59	Rudzinski E., Anderson J., Moore J., Skapek S., Hawkins D., Parham D. A re-review of alveolar rhabdomyosarcoma: Looking back at COG study D9803. Pediatric and Developmental Pathology. 2011;14(6):502-516.	**Congress annals**
60	Styczewska M, Krawczyk MA, Bień E. Misdiagnosis of a chin abscess in a teenager with rhabdomyosarcoma – Consequences for the patient's health and quality of life. Pediatr Pol 2020;95(2):132-136.	**No molecular tests**
61	Sugimoto T, Hosoi H, Matsumura T, Shirai C, Mine H, Sawada T, et al. Cellular and molecular biological diagnosis of rhabdomyosarcoma originating in the head and neck. Pract Otol 1996;89(3):365-370.	**Language restrictions**
62	Thompson JC, Woods GM, Arnold MA, et al. Pediatric Oral/Maxillofacial Soft Tissue Sarcomas: A Clinicopathologic Report of Four Cases. Case Rep Oncol. 2016;9(2):447-453.	**Metastatic RMS**

63	Tsai J.-W., Liang C.-W., Lee J.-C., Li W.-S., Huang H.-Y. Histological diversity of spindle cell/sclerosing rhabdomyosarcomas: Clinicopathological, immunohistochemical, and molecular analysis of 7 cases. Lab Invest. 2018; 98, 18–41.	**Congress annals**
64	Teot LA, Schneider M, Thorner AR, Tian J, Chi YY, Ducar M, Lin L, Wlodarski M, Grier HE, Fletcher CDM, van Hummelen P, Skapek SX, Hawkins DS, Wagers AJ, Rodriguez-Galindo C, Hettmer S. Clinical and mutational spectrum of highly differentiated, paired box 3:forkhead box protein o1 fusion-negative rhabdomyosarcoma: A report from the Children's Oncology Group. Cancer. 2018 May 1;124(9):1973-1981.	**Without individual analyzable data**
65	Wang J, Tu X, Sheng W. Sclerosing rhabdomyosarcoma: a clinicopathologic and immunohistochemical study of five cases. Am J Clin Pathol. 2008 Mar;129(3):410-5.	**No molecular tests**
66	Waldman LE, Williamson AK, Amodio JB, Collins L. Congenital Rhabdomyosarcoma Presenting as a Neck Mass at Birth. Case Rep Pediatr. 2018 Jul 30;2018:1243436.	**Metastatic RMS**
67	Zurac, S.; Zanfir, D.; Iorgulescu, A.; Toader, M.; Gramada, E.; Socoliuc, C.; Popp, C.; Nichita, L.; Cioplea, M.; Stanga, P.; Cioroianu, A.; Suiaga, D.; Marinescu, I.; Dumitru, C. "outburst" of embryonal rhabdomyosarcoma-series of 5 cases. Virchows Arch. 2018; 473: 310.	**Not available article**
68*	Iqbal HA, Anjum R, Naseem N. Rare Variant of Adult Rhabdomyosarcoma Presenting as a Palatal Swelling. Pak J Med Sci. 2021;37(3):922-925.	**No molecular tests**
69*	de Aguiar MCF, de Noronha MS, Silveira RL, Araújo JAD, Werkema FS, Bell D, Caldeira PC. Epithelioid rhabdomyosarcoma: Report of the first case in the jaw. Oral Surg Oral Med Oral Pathol Oral Radiol. 2020;130(5):e308-e315.	**No molecular tests**
70*	Milman T, Ida CM, Zhang PJL, Eagle RC Jr. Gene Fusions in Ocular Adnexal Tumors. Am J Ophthalmol. 2021; 221:211-225.	**Review article**
71*	Koutlas IG, Olson DR, Rawwas J. FET(EWSR1)-TFCP2 Rhabdomyosarcoma: An Additional Example of this Aggressive Variant with Predilection for the Gnathic Bones. Head Neck Pathol. 2021; 15(1):374-380.	**The online version of the paper was included in the original search**

*Artcicles excluded in the updated search

Supplement 3. Risk of bias assessed by The Joanna Briggs Institute Critical Appraisal tools.

Table S3. Summary of the Risk of Bias assessment*

Case series	Risk of Bias	**Case reports**	Risk of Bias
Agaram, et al., 2019 [17]	🟢	Dashti, et al., 2018 [19]	🟢
Alaggio, et al., 2016 [18]	🟢	Debiec-Rychter, et al., 2003 [20]	🔴
Le Loarer, et al., 2020 [21]	🟢	Neffendorf , et al., 2014 [23]	🟢
Marburger et al., 2012 [22]	🟢	Win et al., 2013 [24]	🟢
Liu et al., 2014 [26]	🟢	Wong, et al., 2019 [25]	🟢
Zhu et al., 2019 [28]	🟡	Bowe et al., 2019 [27]	🟢
Owosho et al., 2016 [29]	🟢	Brunac, et al., 2020 [33]	🟢
Wang et al., 2018 [30]	🟢	Eftekhari, et al., 2015 [34]	🟢
Tsai et al., 2019 [31]	🟢	Eguía-Aguilar, et al., 2016 [35]	🟢
Xu, et al., 2021 [32]	🟡	Gui, et al., 2019 [36]	🟢
Agaram, et al., 2019 [50]	🟢	Houreih, et al., 2009 [37]	🟡
Ahmed & Tsokos, 2007 [51]	🟡	Karanian, et al., 2020 [38]	🟢
Bradley JA, et al. 2020 [52]	🟢	Koutlas, et al 2020 [39]	🟢
Bridge et al., 2000 [53]	🟡	Kusafuka, 2018 [40]	🟢
Bridge, et al., 2002 [54]	🟢	Manor, et al. 2012 [41]	🔴
Chiles et al., 2004 43 [55]	🟢	Manucha, et al., 2006 [42]	🟡
Gollin & Janecka, 1994 [56]	🟡	McInturff, et al., 2017 [43]	🟡
Hostein, et al., 2004 [57]	🔴	Mentrikoski, et al., 2013 [43]	🟢
Montone, et al., 2009 [58]	🟢	Nordashima, et al., 2019 [45]	🟢
Mosquera, et al., 2013 [59]	🟢	Pennington JD, et al. 2018 [46]	🟢
Owosho et al., 2016 [60]	🟢	Robinson, et al., 2013 [47]	🟢
Rekhi et al., 2014 [61]	🟢	Sabater-Marco, et al., 2014 [48]	🟡
Thompson, et al., 2018 [62]	🟢	Wessinger, et al., 2021 [49]	🟡
Tostar , et al., 2006 [63]	🟡		
Yasuda, et al., 2009 [64]	🟡		
Tomassen, et al., 2021 [65]	🟡		

*** Risk of bias assessed by The Joanna Briggs Institute Critical Appraisal Checklist for Case Series and Quasi-Experimental Studies [non-randomized experimental studies].**

🔴 High = up to 49% score "yes"; 🟡 Moderate = 50% to 69% score "yes"; 🟢 Low = more than 70% score "yes".

Supplement 4. Summary of included studies

Table S4. Summary of descriptive characteristics of included studies (n = 49)

Author, year - Country	Sample size / Included	Age y.o Range (Mean)	Sex	Site	Histopathologic variant	IHC profile	Molecular studies	Genetic alterations	Stage	Treatment	Recurrence	Metastasis	Follow-up in months (Mean)	Status
Agaram et al., 2019 [17] USA	7/1	33	F	PM	SpcRMS	Desmin+; myogenin (f+); AE1/AE3+; ALK+	FISH	EWSR1-TFCP2 fusion positive	NA	Surgery	NA	Yes	108	Alive
Alaggio et al., 2016 [18] USA	26/3	9 – 11 (9.6)	F= 2 M = 1	Head and neck = 3	SpcRMS= 1 SRMS = 2	Desmin +, myogenin+, MyoD1+	PCR	MYOD1/ PIK3CA positive mutation = 1 MYOD1 positive mutation= 2	I= 1 III = 2	CT= 3	Yes= 1 No = 2	No = 3	NA= 1 36 12	Alive = 2 Dead = 1
Dashti, et al., 2018 [19] USA	*	72	M	NPM	SpcRMS	Desmin+; myogenin+; MyoD1+; ALK+; AE1/AE3+	RT–PCR and Sanger sequencing	FUS-TFCP2 fusion positive	NA	S+ CT+ RT	No	No	2	Alive
Debiec-Rychter, et al., 2003 [20] Belgium	*	18	F	NPM	SpcRMS	Desmin+; myogenin +	FISH	PAX3-FKHR; PAX7-FKHR fusion negative/ gain of chromosome 7 and loss of chromosome 14	NA	NA	NA	NA	NA	NA
Le Loarer, et al., 2020 [21] France	14/9	11 – 58 (26.3)	F= 4 M= 5	PM = NPM=	SpcRMS = 1 EpRMS= 2 EpRMS+ SpcRMS= 6	ALK + = 8 ALK - = 1	FISH/PCR	EWSR1-TFCP2 fusion positive = 3 FUS/TFCP2 fusion positive = 6	NA	CT= 1 CT+RT =3 S+ CT =3 S+ CT+ RT= 2	Yes= 3 No= 3 NA = 3	NA	6 – 21 (13.8) NA= 1	Alive= 5 Dead= 4
Marburger, et al., 2012 [22] USA	11/1	87	M	NPM	PRMS	Desmin +; Myogenin - ; MyoD1+	FISH	FOXO1A (FKHR) no rearrangements	NA	Surgery	None	NA	12	Alive
Neffendorf, et al., 2014 [23] UK	*	31	M	Orbit	ARMS	Desmin+; MyoD1+	FISH	FOXO1 Positive rearengment	NA	S+ CT+ RT	No	No	11	Alive

Win et al., 2013 [24] Taiwan	*	53	M	PM	ARMS	CD56+, desmin+, myogenin+	FISH	FKHR gene rearrangement	NA	CT+RT	NA	Yes	NA	NA
Wong et al., 2019 [25] Australia	*	23	M	PM	EpRMS	Desmin+, Myogenin+, MyoD1+; ALK+; CD99+	FISH	FUS (16p11) rearrangement	NA	CT + RT + ALK inhibitor	No	No	2	Alive
Liu et al., 2014 [26] China	39/5	3 – 56 (20.8)	F= 4 M= 1	NPM = 4 Orbit = 1	ARMS = 5	NA	PCR	PAX3-FKHR fusion positive = 4 PAX7-FKHR fusion positive = 1	NA	NA	NA	NA	NA	Dead = 4 NA = 1
Brunac, et al., 2020 [33] France	*	16	F	NPM	FUS/TFCP2 RMS	CD99+; ALK+; Desmin, myogenin, MYOD1, epithelial membrane antigen, CK7, and CD30 were heterogeneous; AE1/AE3-, S100-, chromogranin-, CD20-, CD3-, SALL4-, CD79a-, BRAF-, NTRK-.	FISH	FUS/TFCP2 fusion positive	NA	CT + RT+ ALK targeted therapies	NA	NA	19	Alive
Eftekhari, et al., 2015 [34] USA	*	3	F	Orbit	ARMS	NA	PCR	PAX-FKHR fusion positive	NA	CT+RT	No	No	48	Alive
Eguía-Aguilar, et al., 2016 [35] Mexico	*	2	M	PM	ARMS	Desmin+; myoglobin +	RT-PCR	PAX3-FKHR fusion positive	4	CT	NA	NA	NA	Dead
Gui, et al., 2019 [36] USA	*	49	M	PM	SRMS	Desmin+; myogenin +; MyoD1+; Ki67=20%	FISH	FOXO 1/ MDM2 Negative fusion	NA	Surgery + CT	NA	Yes	NA	NA

Houreih, et al., 2009 [37] UK	*	20	M	Orbit	ARMS	Desmin+; myogenin+; HHF35+; Synaptophysin +f; Chromogranin A +f	FISH/RT-PCR	PAX 3 translocation/ rearrangement of the PAX3 gene region at 2q35 without a rearrangement of FKHR at 13q14	NA	NA	Local and distance (brain, orbit, sinuses, and cheek)	NA	NA	NA
Karanian, et al., 2020 [38] France		1	M	NPM	RMS NOS	Desmin+; myogenin+; MyoD1+	RT-PCR	SRF-NCOA1 fusion positive	NA	S + CT	No	NA	108	Alive
	*	10	F	NPM	RMS NOS	Desmin+; myogenin+; MyoD1+	RT-PCR	SRF-FOXO1 fusion positive	NA	S + CT	No	NA	12	Alive
		3	M	NPM	RMS NOS	Desmin+; myogenin+; MyoD1+	RT-PCR	SRF-NCOA1 fusion positive	NA	S+ CT +RT	Yes	NA	18	Alive
Koutlas, et al 2020 [39] USA	*	15	M	NPM	EpRMS + SpcRMS	Desmin+; myogenin+; MyoD1+; AE1-AE3 +	PCR	EWSR1-TFCP2 fusion positive	NA	S+RT +CT	No	NA	7	Alive
Kusafuka et al., 2018 [40] Japan	*	19	F	NPM	SRMS	Desmin +; MyoD1++; Myogenin-; Myoglobin +; αSMA+; MSA -	FISH/PCR	FOXO1; NCOA2 fusion negative/ MYOD1 positive mutation	NA	Surgery	Local	NA	18	Alive
Manor, et al. 2012 [41] Israel	*	9	M	NPM	ERMS	NA	Cytogenetic analysis	Abnormal clone with 81–92 chromosomes, with a gain of 1–2 of each chromosome, and with 3 markers in each abnormal cell.	NA	NA	NA	NA	NA	NA
Manucha, et al., 2006 [42] USA	*	57	F	PM	ARMS	Desmin+, Myogenin+++	Cytogenetic analysis/ FISH	Karyotype of 45, XX, -5, -13, der(16)t(1;1)(q21;q13)/ complex translocation with break apart of the FKHR region	NA	NA	NA	NA	NA	NA

McInturff, et al., 2017 [43] USA	*	19	F	NPM	ERMS	Desmin+, myogenin+, MyoD1+	FISH	No rearrangement of FKHR.	NA	Surgery (Exc)	NA	NA	NA	NA
Nordashima, et al., 2019 [45] Malaysia	*	0.2	F	NPM	ARMS	Desmin+; MyoD1+	FISH	presence of translocation t(2;13) (q35;q14)	NA	CT	NA	Yes	NA	Dead
Pennington, et al. 2018 [46] USA	*	5	M	Orbit	ERMS, botryoid	Desmin+; Myogenin+	NA	PAX3/FOXO1 negative fus	1	S+ CT+RT	NA	No	NA	NA
Robinson, et al., 2013 [47] USA	*	40	M	PM	SRMS	Desmin+, Myogenin+, WT1+, CD99+, Ki67 60%	FISH	FOXO1, EWSR1 and SS18 no alterations	NA	Surgery + CT	NA	Yes	19	Dead
Sabater-Marco, et al., 2014 [48] Spain	*	65	M	NPM	PRMS	myoglobin +, MyoD1+ myogenin+, vimentin+, desmin+, SMA+, MAS+, CD10+.	FISH	FOXO1 Fusion negative	1	Surgery	Yes, local	NA	18	NA
Agaram et al., 2019 [50]	30/11	9 – 34 (18.4)	F= 9 M= 2	Head and neck = 11	SpcRMS= 2 SRMS = 5 Spc/SRMS = 4	Desmin +, myogenin+, MyoD1+	PCR	MYOD1 positive mutation/ PIK3CA negative mutation = 5 MYOD1/ PIK3CA positive mutation = 6	NA	CT = 2 CT+RT = 4 NA= 5	Yes= 7 NA= 4	NA	12 – 65 (34.6) NA = 3	Alive = 3 Dead= 5 NA = 3
Ahmed and Tsokos, 2007 [51] USA	14/4	11 – 16 (14.5)	F= 3 M= 1	PM= 4	ARMS = 4	Myogenin+ ; Desmin + =2 NA = 2	PCR	PAX3/FKHR fusion positive	NA	NA	NA	NA	NA	NA
Bradley, et al. 2020 [52] USA	24/24	3.5 (1-20)	F = 12 M = 12	PM = 24	ARMS = 24	NA	PCR	FOXO1 positive fusion = 8 FOXO1 negative fusion = 16	II = 4 III= 20	CT+RT = 24	NA	NA	0.3 - 5.6 (2.4)	NA

Bridge, et al., 2002 [53] USA & Canada	33/4	2 – 8 (5)	F= 2 M= 2	PM = 1 NPM = 2 Orbit = 1	ARMS = 2 ERMS = 2	NA	PCR	PAX3/FKHR fusion positive = 1 PAX7/FKHR fusion positive = 1 Negative fusion in both ERMS	I= 2 II = 1 IV = 1	Surgery = 4	NA	Yes = 1 NA = 3	NA	Alive = 4
Bridge, et al., 2000 [54] USA & Canada	12/2	5	M	H&N	ERMS	NA	RT-PCR/ CGH/ FISH	PAX3/7-FKHR fusion negative/ chromosomes 11;12 alterations	1	S +CT	NA	NA	NA	Alive
		4	F	H&N	ERMS	NA	RT-PCR/ CGH/ FISH	PAX3/7-FKHR fusion negative/ chromosomes alterations	1	S+CT	NA	NA	NA	Alive
Chiles, et al., 2004 [55] USA	13/2	12	M	NPM	ARMS	MyoD1 4+; Myogenin 2+; Desmin 4+	RT-PCR	PAX3-FKHR fusion negative	NA	NA	NA	NA	NA	Alive
		10	F	Orbit	ARMS	MyoD1 4+; Myogenin 2+; Desmin 4+	RT-PCR	PAX3-FKHR fusion negative	NA	NA	NA	NA	NA	Alive
Gollin and Janecka, 1994 [56] USA	18/2	3	M	PM	ERMS	Desmin+, myoglobin +	Karyotypic analysis	Structural (Chromosomes 1, 3 ,4, 5, 7, 11 14, 19), and numerical abnormalities (Chromosomes 2, 13, 15, 22).	NA	NA	NA	NA	NA	NA
		3	F	PM	ERMS	NA	Karyotypic analysis	Normal	NA	NA	NA	NA	NA	NA
Hostein, et al., 2004 [57] France	109/ 2	7	M	PM	ERMS	Myogenin+	RT-PCR	PAX3-FKHR fusion negative	NA	NA	NA	NA	NA	NA
		2	F	NPM	ARMS	Myogenin+	RT-PCR	PAX3-FKHR fusion positive	NA	NA	NA	NA	NA	NA
Montone, et al., 2009 [58] USA	13/8	18 – 53 (31.3)	NA	PM = 8	ARMS = 5 ERMS= 3	Desmin+; Myogenin+	FISH = 2 RT – PCR = 6	Positive transcription of the FKHR breakpoint = 2 Negative transcription of	NA	CT = 1 CT+RT =7	Yes= 2 NA = 6	Yes= 6 NA = 2	7 – 121 (49.8) NA = 3	Alive = 4 Dead= 2 NA = 2

								the FKHR breakpoint = 2 PAX3/7-FKHR positive fusion = 3 PAX3/7-FKHR negative fusion = 1						
Owosho, et al., 2016 [29] USA	13/10	1.75 – 72 (32.7)	F= 3 M= 7	PM = 2 NPM = 8	SpcRMS = 7 SRMS = 3	Desmin +; myogenin+ = 4 Desmin +; myogenin+; MyoD1+ = 6	RT-PCR	MYOD1; PIK3CA; SRF–NCOA2; PAX3/7-FOXO1 all negative NA= 1	I= 7 IV= 3	CT+RT = 1 Surgery = 1 S+CT= 2 S+RT= 1 S+CT+RT = 5	Yes = 1 No= 7 NA= 2	NA	4 – 94 (32) NA= 2	Alive= 5 Dead= 3 NA= 2
Owosho, et al., 2016 [60] USA	99/99	0.08 – 72 (16)	F= 47 M= 52	PM= 64 NPM=25 Orbit= 10	ERMS= 53; ARMS= 33; SpcRMS/SRMS= 13	Desmin +; myogenin +; MyoD1+	RT-PCR	ARMS FOXO1 positive fusion= 33 SpcRMS/SRMS MYOD1 positive mutation= 3; MYOD1 negative muationt = 6; SRF-NCOA2 positive fusion= 1	I=29 II=10 III=41 IV=19	S + CT+RT= 99	Yes= 22 No=75 NA= 2	Yes= 19 No=80	4-232 (83,6)	Alive= 69 Dead= 28 NA= 2
Rekhi and Singhvi, 2014 [61] India	21/1	2	M	NPM	SpcRMS + PRMS	Desmin+, Myogenin+	FISH	PAX3-FOXO1 negative fusion	NA	Surgery	NA	NA	NA	NA
Thompson, et al., 2018 [62] International colaboration	52/52	18–72 (43.2)	F=26 M=26	PM = 52	ARMS	Desmin+, myogenin+, MyoD1+	FISH PCR	(FOXO1) 17/21 (81%) (PAX3/FOXO1 rearrangement) 16/22 (73%). No PAX7/FOXO1 rearrangements were identified in this series.	II=2 III= 26 IV= 24	CT+RT = 38 CT= 7 RT= 4 NA=3	Yes= 8 No= 44	Yes= 46 No= 6	2.4–286	Alive = 25 Dead = 27
Tostar, et al., 2006 [63] Sweden	47/11	1 – 32 (10.7)	F= 4 M= 7	PM = 4 NPM = 5 Orbit = 2	ARMS = 1 ERMS = 10	NA	ISH	PTCH and GLI1 positive mutations in all cases	NA	NA	NA	NA	NA	NA

Tsai, et al., 2019 [31] Taiwan	17/9	2 – 42 (18)	F= 6 M =3	PM = 4 NPM = 5	Hybrid = 2 SpcRMS = 3 SRMS = 4	Desmin+, myogenin+, MyoD1+	PCR	MYOD 1 mutation = 7 No mutation in MYOD1 = 2	NA	CT+RT = 2 Biopsy = 1 Surgery = 4 S+CT+ RT = 2	Yes = 2 No = 6 NA= 1	Yes = 2 No = 6 NA= 1	6 – 134 (35.4) NA = 1	Alive = 8 Dead = 1
Yasuda et al., 2009 [64] USA	4/4	61 – 76 (65.5)	F= 3 M =1	PM = 3 NPM = 1	ARMS = 4	vimentin+; desmin+; myogenin+	PCR	PAX-FOXO1 positive fusion	NA	CT+RT = 4	NA	Yes = 2 NA= 2	10 – 14 (12.3)	Alive = 3 Dead = 1
Wang et al., 2018 [30] China	20/20	0.2 – 57 (20.3)	F= 10 M =10	PM = 7 NPM = 13	SpcRMS =13 SRMS = 7	Desmin+, myogenin+, MyoD1+	PCR	PIK3CA/ MYOD1 No mutations = 2 MYOD1 mutated = 7 PIK3CA mutated = 3 PIK3CA/ MYOD1 positive mutations = 8	I= 16 IV= 4	Surgery = 15 S+CT+ RT = 5	Yes = 2 No = 18	Yes = 5 No = 13 NA= 2	4 – 259 (67.7)	Alive = 8 Dead = 10 NA = 2
Mentrikoski, et al., 2013 [44] USA	*	0.6	M	NPM	SpcRMS	Desmin +; Myogenin-; smooth muscle actin+	Cytogenetic analysis	Karyotype evaluation revealed a t(6;8) (p12;q11.2) chromosomal translocation	NA	NA	NA	NA	NA	NA
Mosquera, et al., 2013 [59] USA	21/4	0.6 – 71 (30.2)	F= 2 M = 2	PM = 1 NPM = 3	SpcRMS =2 SRMS = 2	Desmin+, myogenin+	FISH/ RT-PCR	NCOA2 positive and SRF positive = 1 NCOA2 negative = 3	NA	NA	NA	NA	6 NA = 3	Alive NA= 3
**Bowe et al., 2019 [27] USA	*	72	M	NPM	EpRMS	Desmin +, myogenin+, HHF-35+, CD10+, vimentin+	FISH	PAX/FOXO1 negative	NA	Surgery + CT	No	No	12	Alive
**Tomassen, et al., 2021 [65] the Netherlands	25/1	1	M	NPM	ERMS	Desmin +, myogenin +, MyoD1+	WTS	H3K27; PRC2 No fusion	NA	Surgery + CT	No	No	20	Alive
**Wessinger, et al., 2021 [49] USA	*	73	M	PM	SpcRMS	Desmin+; MyoD1+; myogenin-	WTS	No fusion	2	Surgery + RT	NA	No	NA	NA
**Xu, et al., 2021 [32]	11/9	16 – 43 (27.7)	F= 4 M = 5	NPM = 9	FUS/EWSR1 - TFCP2 RMS	Desmin+; MyoD1+; myogenin+;	FISH; RNA/DNA sequencing	FUS- TFCP2 positive fusion = 6	NA	Surgery = 9	Yes = 1 NA = 8	Yes = 5 NA = 4	1 - 20 ()8.25)	Alive = 3 Dead = 1 NA = 5

USA						AE1/AE3+; ALK+								
**Zhu et al., 2019 [28] USA	6/1	74	F	NPM	EpRMS	Caldesmon +, SMA+, desmin+, factor XIIIa+, ALK+; MYOD1+	FISH	FUS-TFCP2 fusion positive; ALK wild type	NA	NA	NA	Yes	21	Dead

*Case Reports

**Studies included in the updated search

Abbreviations: ARMS= alveolar rhabdomyosarcoma; CT= chemotherapy; ERMS= embryonal rhabdomyosarcoma; EpRMS: epithelioid rhabdomyosarcoma; FISH: Fluorescence *in situ* hybridization; H&N: head and neck; IHQ: immunohistochemical; NPM: non parameningeal; PM: parameningeal; RT= radiotherapy; RT-PCR: Reverse transcription polymerase chain reaction; RMS NOS: not otherwise specified; SMA: smooth muscle actin; SpcRMS / SRMS: spindle cell/sclerosing rhabdomyosarcoma; WTS: whole transcriptome sequencing

Supplement 5. Survival curve of HNRMS with molecular abnormalities.

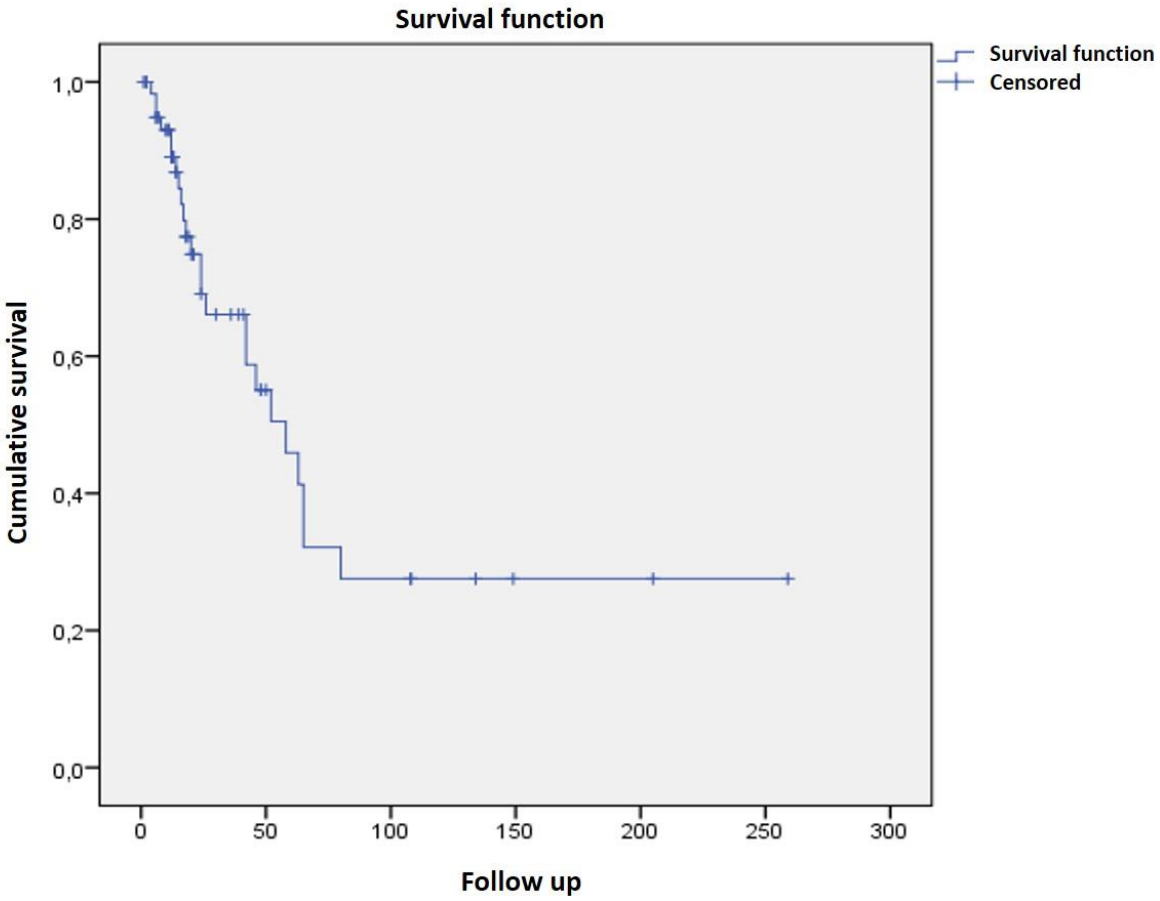

Figure S5. Survival curve: Kaplan-Meyer curve demonstrating the overall survival rate of patients affected by head and neck rhabdomyosarcoma with molecular alterations.

Supplement 6. Survival curves and prognostic variables.

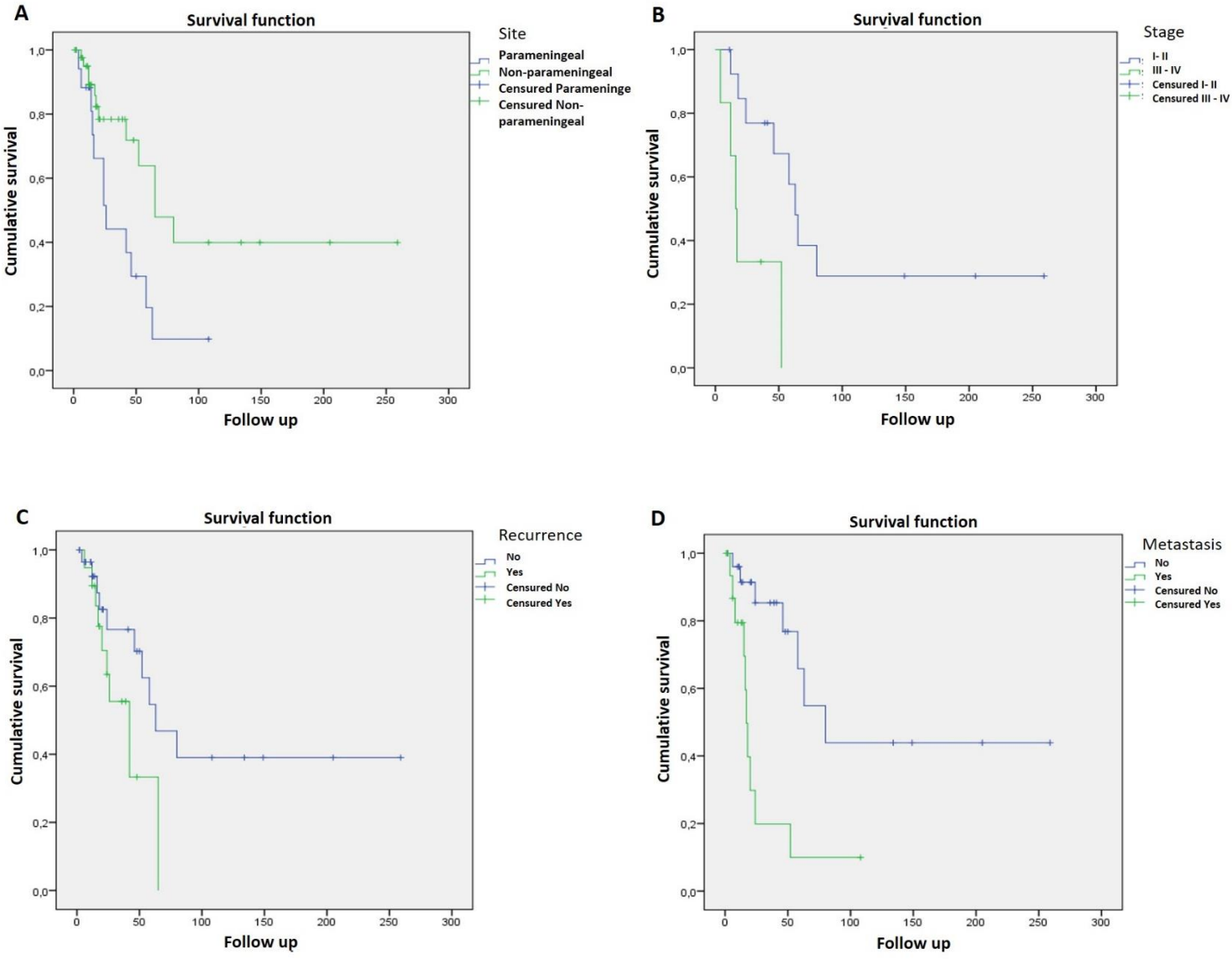

Figure S6. Survival curves and prognostic variables: The Log-Rank univariate analysis showed that PM location **(A)**, stage III-IV **(B)**, presence of recurrence **(C)** and metastasis **(D)** were factors that influenced the patient's survival. *PM= parameningeal, NPM non-parameningeal.*

2.2 ARTIGO 2

Rhabdomyosarcoma with *TFCP2* Rearrangement or Typical Co-expression of AE1/AE3 and ALK: Report of Three New Cases in the Head and Neck Region and Literature Review.

Publicado no periódico: **Head and neck pathology.** 2023 Jun;17(2):546-561. doi: 10.1007/s12105-022-01507-9. **(ANEXO 4)**

Running title: Head and neck rhabdomyosarcomas with TFCP2 rearrangement

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Abstract

BACKGROUND: Rhabdomyosarcoma (RMS) harboring *EWSR1/FUS-TFCP2* fusions has been recently described as a distinct form of RMS with an aggressive course and predilection for the craniofacial bones, especially the jaws.

METHODS: We report three new cases of this rare entity, two from Brazil and one from Guatemala, with detailed clinicopathologic, immunohistochemical, and molecular descriptions. Additionally, we explored the English-language literature searching RMS with *TFCP2* rearrangement or typical immunophenotype with co-expression of AE1/AE3 and ALK in the head and neck region.

RESULTS: Case 1 is a 58-year-old male with a 3-month history of painful swelling in the anterior maxilla. Case 2 is a 22-year-old male presenting with right facial swelling and proptosis. Case 3 is a 43-year-old female with a rapidly growing tumor located in the zygomatic region. Imaging examinations revealed highly destructive intraosseous masses in the first two cases, and a soft tissue tumor with bone invasion in case 3. Microscopically, all cases showed a hybrid spindle and epithelioid phenotype of tumor cells which expressed desmin, myogenin and/or Myo-D1, AE1/AE3, and ALK. FISH confirmed molecular alterations related to *TFCP2* rearrangement in Cases 1-2. In case 3, there was no available material for molecular analysis. The patients were subsequently referred to oncologic treatment. Additionally, we summarized the clinicopathologic, immunohistochemical, and molecular features of 27 cases of this rare RMS variant in the head and neck region reported in the English-language literature.

CONCLUSION: RMS with *TFCP2* rearrangement is a rare and aggressive tumor with a particular predilection for craniofacial bones, especially the jaws. Knowing its clinicopathologic and immunohistochemical profile can avoid misdiagnosis.

Keywords: Rhabdomyosarcoma, head and neck, *TFCP2*, jaws, AE1/AE3, ALK.

Introduction

Rhabdomyosarcoma (RMS) is a high-grade malignant neoplasm characterized by tumor cells with myogenic differentiation showing different growth patterns and morphologic features [1]. This tumor may arise in any body part; however, it mainly occurs in the trunk, genitourinary tract, extremities, and the head and neck region [2]. RMS represents the most common soft tissue sarcoma in pediatric patients, and the head and neck region is affected in approximately 40% of cases. In contrast, RMS is uncommon in adults, and only 1% occur in the head and neck region [3].

In the most recent WHO Classification of Bone and Soft Tissue Tumors (2020), RMS is divided into alveolar, embryonal, pleomorphic, and spindle cell/sclerosing types [4]. Based on molecular features the spindle cell/sclerosing RMS is subdivided into: (a) congenital/infantile spindle cell RMS harboring gene fusions of *VGLL2, NCOA1/2*, and *SRF*, (b) spindle cell/sclerosing RMS with *MYOD1* mutations; and (c) intraosseous RMS with *EWSR1/FUS-TFCP2* fusions (collectively referred to as *FET-TFCP2* fusion RMS) or *MEIS–NCOA2* fusions [4-5]. This latter variant was introduced in the current WHO Classification of Head and Neck Tumors (2022) as an independent entity in malignant maxillofacial bone tumors [6,7].

RMS with *TFCP2* rearrangement has a predilection for the craniofacial bones, most commonly the mandible, and can affect patients of all age groups with an aggressive clinical course [5,8]. Microscopically, the tumor shows a mixture of spindle and epithelioid cells with positivity for myogenic markers (desmin, myogenin, and Myo-D1), epithelial markers (pan-cytokeratins and EMA), and ALK overexpression [4, 8, 9].

Less than 30 cases of this rare variant have been reported in the head and neck region. We report three additional cases of head and neck rhabdomyosarcomas (HNRMS) with *TFCP2* rearrangement or typical immunophenotype with co-expression of AE1/AE3 and ALK. In addition, we review the literature regarding the clinicopathologic, immunohistochemical and molecular features of this rare entity.

Case Reports

Case 1

In February 2021, a 58-year-old Brazilian man presented with a 3-month history of a painful swelling in the anterior maxillary alveolar ridge. The patient's medical history was

unremarkable; however, he mentioned previous endodontic treatment of the upper right canine and extraction of the two upper central incisors. Despite treatment, the swelling and pain persisted. Intraoral examination revealed a swelling in the anterior portion of the maxilla, presenting a reddish irregular surface and ill-defined borders, extending into the palatal region (**Fig 1A**). Computed tomography (CT) demonstrated a destructive lesion in the anterior maxilla with cortical bone destruction and ill-defined borders, measuring 5.0 × 4.5 × 4.0 cm (**Fig 1B**).

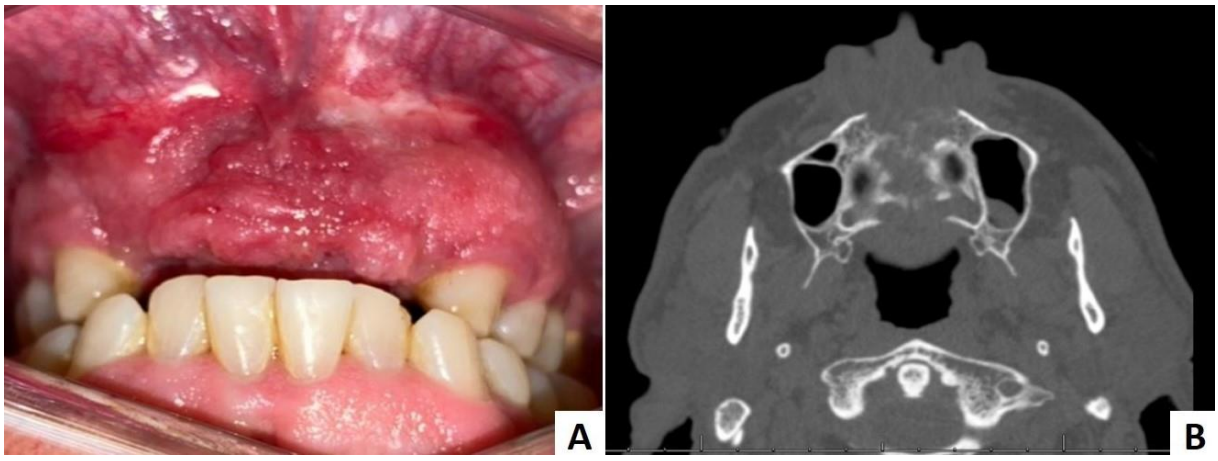
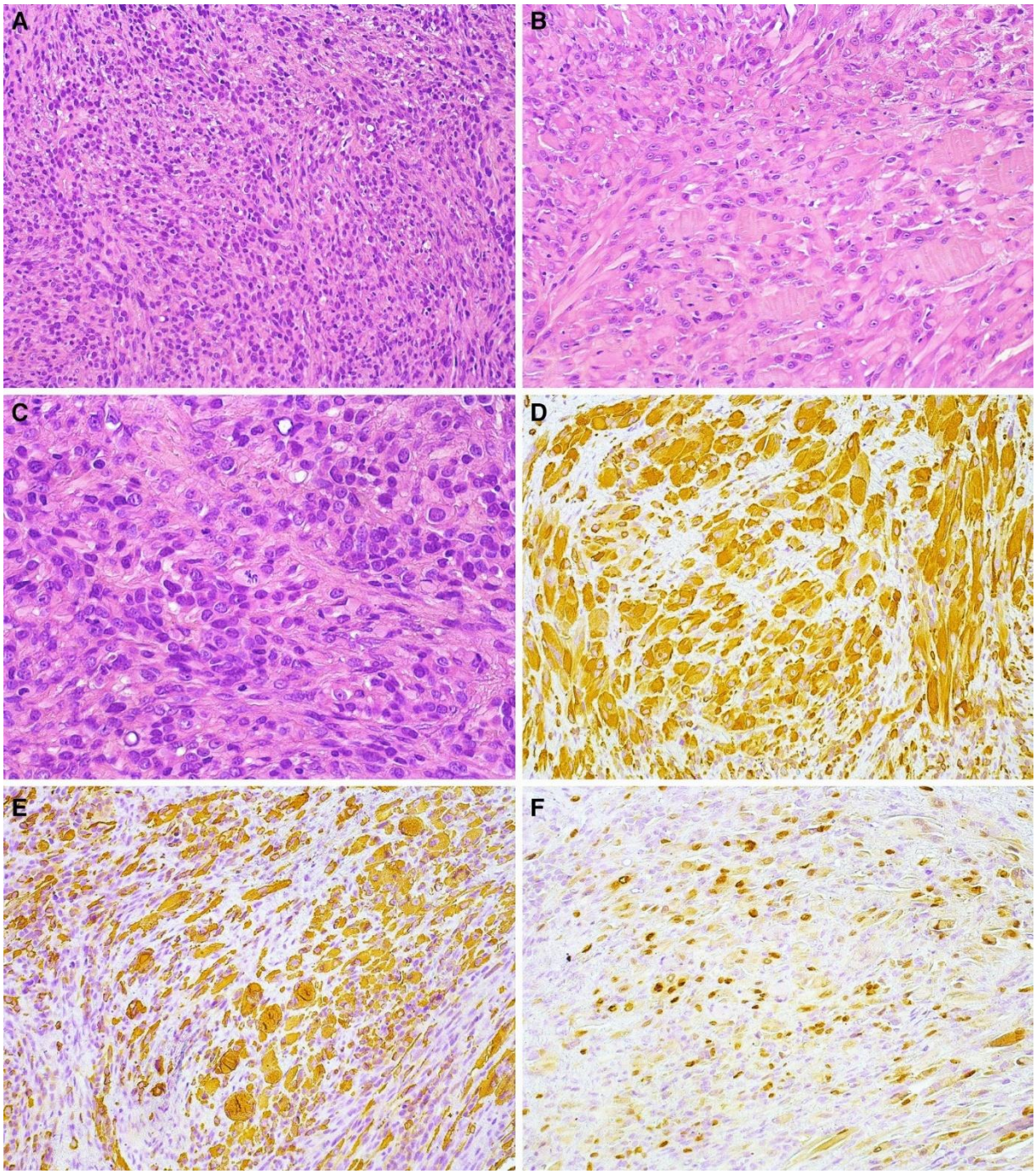


Figure 1. Case 1 – Clinical and radiographical features: a. Granulomatous swelling with irregular borders in the anterior portion of maxillary alveolar ridge. **b**. CT showing lytic lesion causing cortical destruction in the anterior maxilla.

An incisional biopsy was performed and sent for histopathologic evaluation. Gross examination showed a yellowish soft-tissue fragment measuring 0.9 × 0.6 × 0.5 cm. Microscopically, the tumor revealed a solid proliferation of neoplastic cells arranged in fascicular and storiform patterns. Tumor cells were surrounded by scarce fibrous stroma and presented spindle to epithelioid morphology with abundant eosinophilic cytoplasm and variable sized nuclei with irregular contours and prominent nucleoli. Aggregates of small, round, blue cells were observed in focal areas. Mitotic figures and apoptotic cells were also identified within the tumor (**Fig 2A-C**). Immunohistochemical evaluation showed diffuse positivity for desmin, myogenin was focally positive, and approximately 60% of cells stained for Myo-D1 (**Fig 2 E-G**). Strong and diffuse expression of AE1/AE3 (**Fig 2D**). was observed in the spindle and epithelioid areas. Cytoplasmic expression of ALK (**Fig 2H**). was focally positive in the neoplastic cells. The tumor showed a high proliferative cell index determined by the expression of Ki-67 in 90% of cells. Fluorescence in situ hybridization (FISH), using a dual color break-

apart probe, showed the translocation of *TFCP2* (**Fig 2I**). The patient was treated with three cycles of neoadjuvant chemotherapy with partial response, but unfortunately, died 3 months after the diagnosis.).



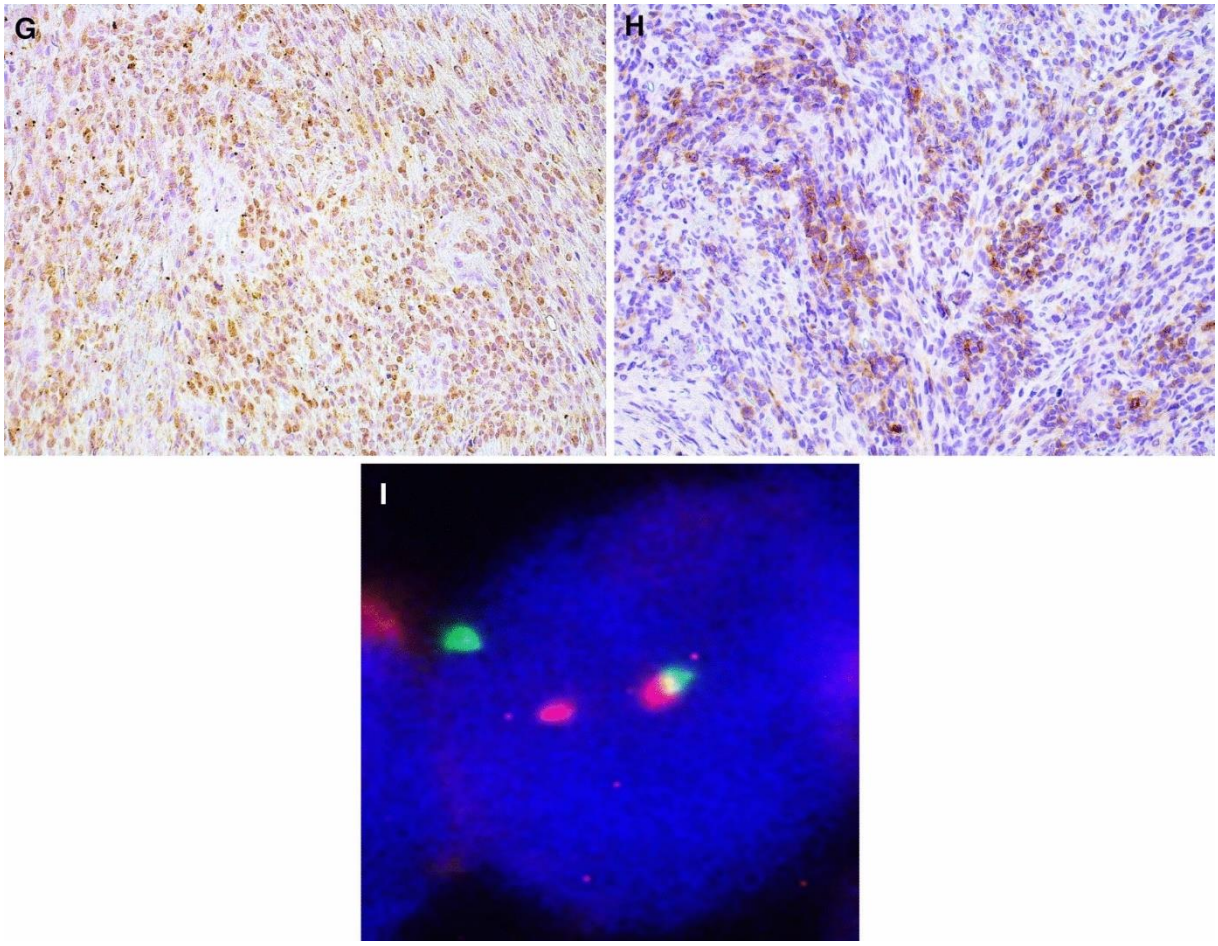


Figure 2. Case 1 – Histopathological, immunohistochemical and molecular features: a. Solid neoplasm predominantly composed of epithelioid and spindle cells (H&E, 200 ×). **b** Epithelioid cells showing abundant eosinophilic cytoplasm (H&E, 200 ×). **c** Pleomorphic and hyperchromatic nuclei of spindle-to-epithelioid cells and some atypical mitotic figures (H&E, 400 ×). Diffuse cytoplasmic positivity for AE1/AE3 (**d**; 200 ×) and Desmin (**e;** 200 ×). Strong nuclear positivity for MyoD1 (**f**; 200 ×) and myogenin (**g**; 200 ×). **h** Cytoplasmic expression of ALK (200 ×). **i** A split signal is seen with the FISH assay using a break-apart probe for *TFCP2*.

Case 2

In November 2021, a 22-year-old Brazilian male was referred for evaluation due to an expansile lesion in the right maxilla. His past medical history was unremarkable. Extraoral examination showed a diffuse swelling causing facial asymmetry on the right side and exophthalmos. Intraoral examination showed extensive ulceration with a crater-like center and irregular borders located in the posterior portion of the right buccal mucosa. CT imaging revealed an infiltrative and destructive tumor located in the right posterior maxilla with extension into the maxillary sinus, nasal cavity, infratemporal fossa, and floor of the orbit (Fig **3A, B**).

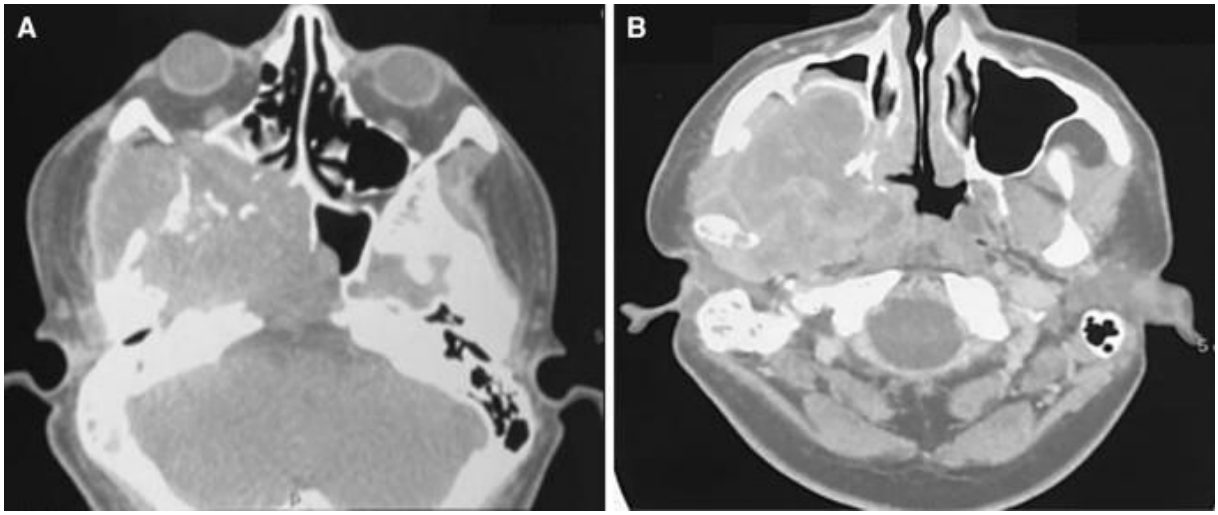


Figure 3. Case 2 – Radiographic features: a, b CT showing extensive mass in right maxilla affecting maxillary sinus and orbit, causing bone destruction and invading the surrounding soft tissue.

The patient underwent an incisional biopsy and gross examination revealed two irregular brownish soft tissues with homogenous white cut surfaces (**Fig 4A**). Microscopically, a solid neoplasm was observed within the medullary bone tissue. The tumor presented a biphasic appearance with alternating hypocellular areas of spindle cells in a myxo-collagenized stroma and hypercellular areas composed of spindled-to-epithelioid cells with abundant eosinophilic cytoplasm. Nuclei were variable in size with evident pleomorphism and prominent nucleoli. Atypical mitotic figures, foci of necrosis, and residual bone fragments were noted (**Fig 4B-D**). Immunohistochemical evaluation disclosed diffuse positivity for desmin, Myo-D1, and AE1/AE3, and focal cytoplasmic expression of ALK (**Fig 4E-H**). The cell proliferation index measured by Ki-67 was 90%. TFCP2 translocation was confirmed by fluorescence in situ hybridization (FISH) using a dual color break-apart probe (**Fig 4I**). The patient was subsequently referred to oncologic treatment, but was lost to follow-up.

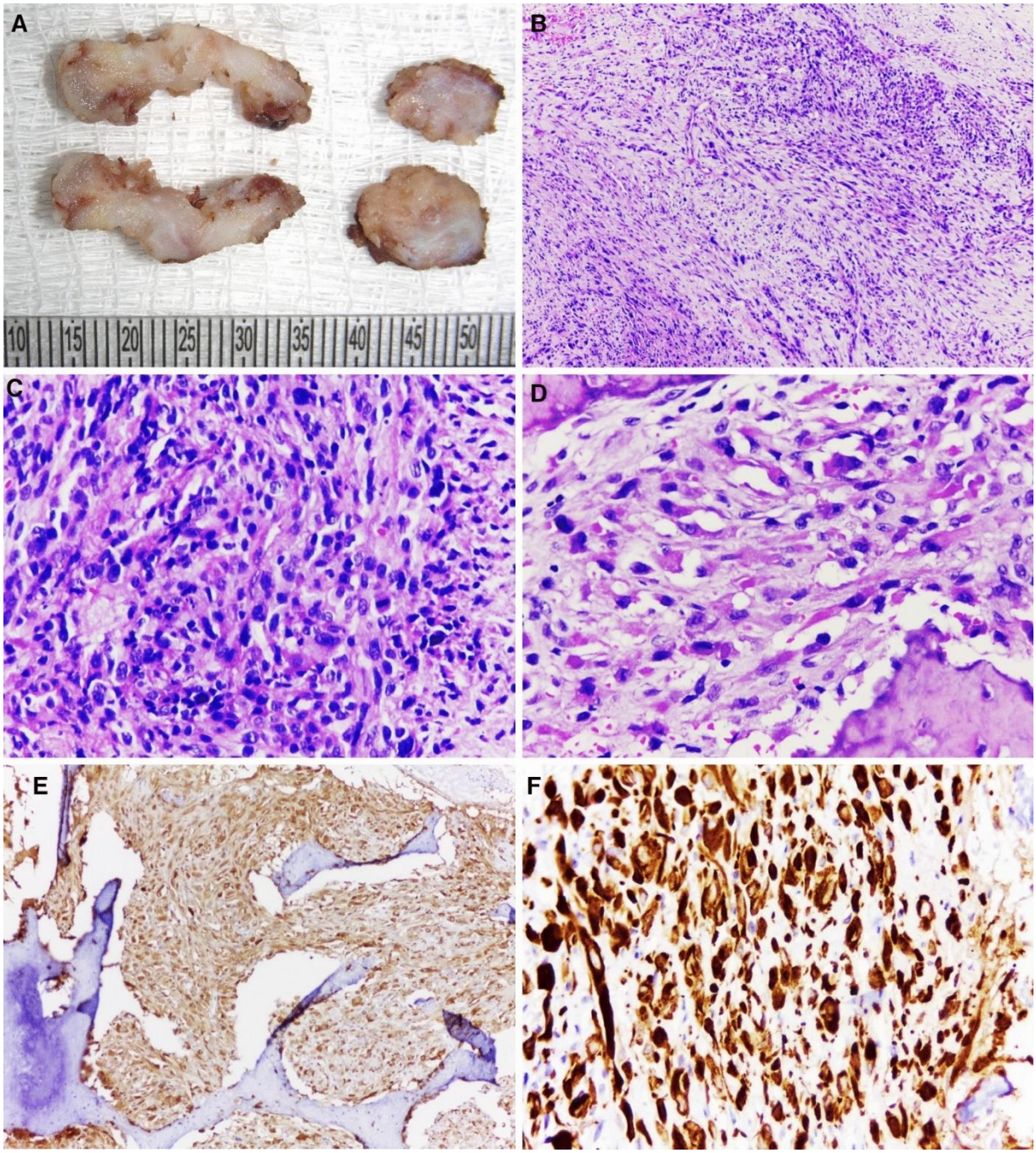


Figure 4. Case 2 – Macroscopic, microscopic and molecular features: a. Macroscopic appearance of surgical specimens. **b** Tumor composed of hypocellular and hypercellular areas (H&E, 100 ×). **c** Spindle cells with pleomorphic and hyperchromatic nuclei (H&E, 200 ×). **d** Spindled-to-epithelioid cells with abundant eosinophilic cytoplasm (H&E, 200 ×). **e** Diffuse cytoplasmic positivity for AE1/AE3 (100 ×) and desmin (**f,** 200 ×).

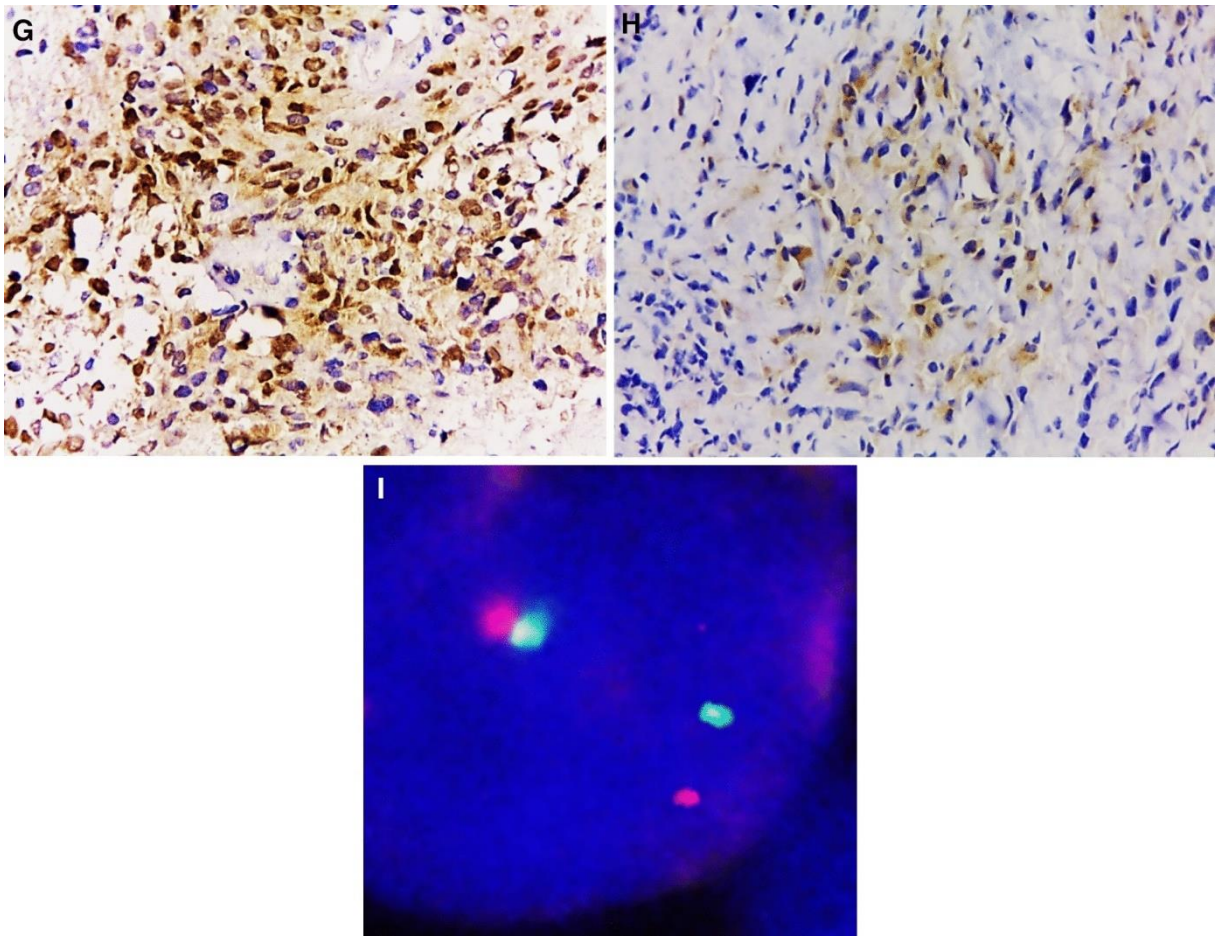


Figure 4. Case 2 – Macroscopic, microscopic and molecular features (*continue*): Strong nuclear positivity for MyoD1 (**g,** 200 ×) and focal expression of ALK (**h;** 200 ×). **i** FISH assay for *TFCP2* dual color break-apart probe showing split signals**.**

Case 3

In May 2021, a 43-year-old Guatemalan female patient with an unremarkable medical history presented with a rapidly growing tumor affecting the zygomatic region. Extraoral examination revealed an exophytic sessile mass in the right zygomatic region, causing diffuse swelling and facial asymmetry. Magnetic resonance imaging (MRI) revealed a hypo-and isointense soft tissue mass with regular borders (**Fig 5A, B**). CT evidenced an extensive ill-defined lesion causing bone destruction of the zygomatic process. An incisional biopsy was performed, and the specimen was sent for histopathologic analysis. Microscopic examination showed a malignant neoplasm characterized by a diffuse proliferation of spindle cells arranged in a fascicular pattern and intermixed with aggregates and strands of epithelioid cells showing eosinophilic cytoplasm with hyperchromatic nuclei and surrounded by a fibromyxoid stroma (**Fig 6A, B**). Multinucleated tumor cells and mitotic figures were also noted within the tumor. Immunohistochemical results revealed positivity for vimentin, AE1/AE3, desmin and Myo-D1 (Fig 6C-E), and was negative for myogenin. In addition, focal positivity for ALK was observed (Fig 6F). Considering the clinicopathologic and immunohistochemical features (AE1/AE3, ALK, desmin and Myo-D1) this tumor was diagnosed as epithelioid RMS ALK positive highly suspected of RMS with TFCP2 translocation, however it was not molecularly confirmed because of unavailability of the paraffin block sample. The patient was referred for oncologic treatment, but the follow-up information is unknown.

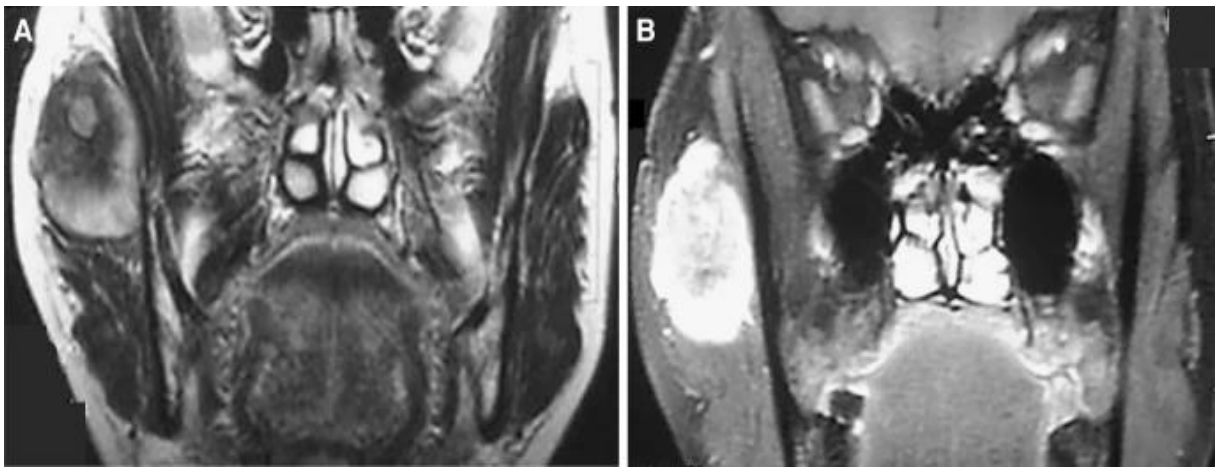


Figure 5. Case 3 – Clinical and imaginological features: **a.** MRI in T1-weighted image showed a hypo- and isointense MR signal in relation to soft tissues, and in T2-weighted image **(b)** evidenced a solid tumor component with hyperintense signal with apparently regular borders.

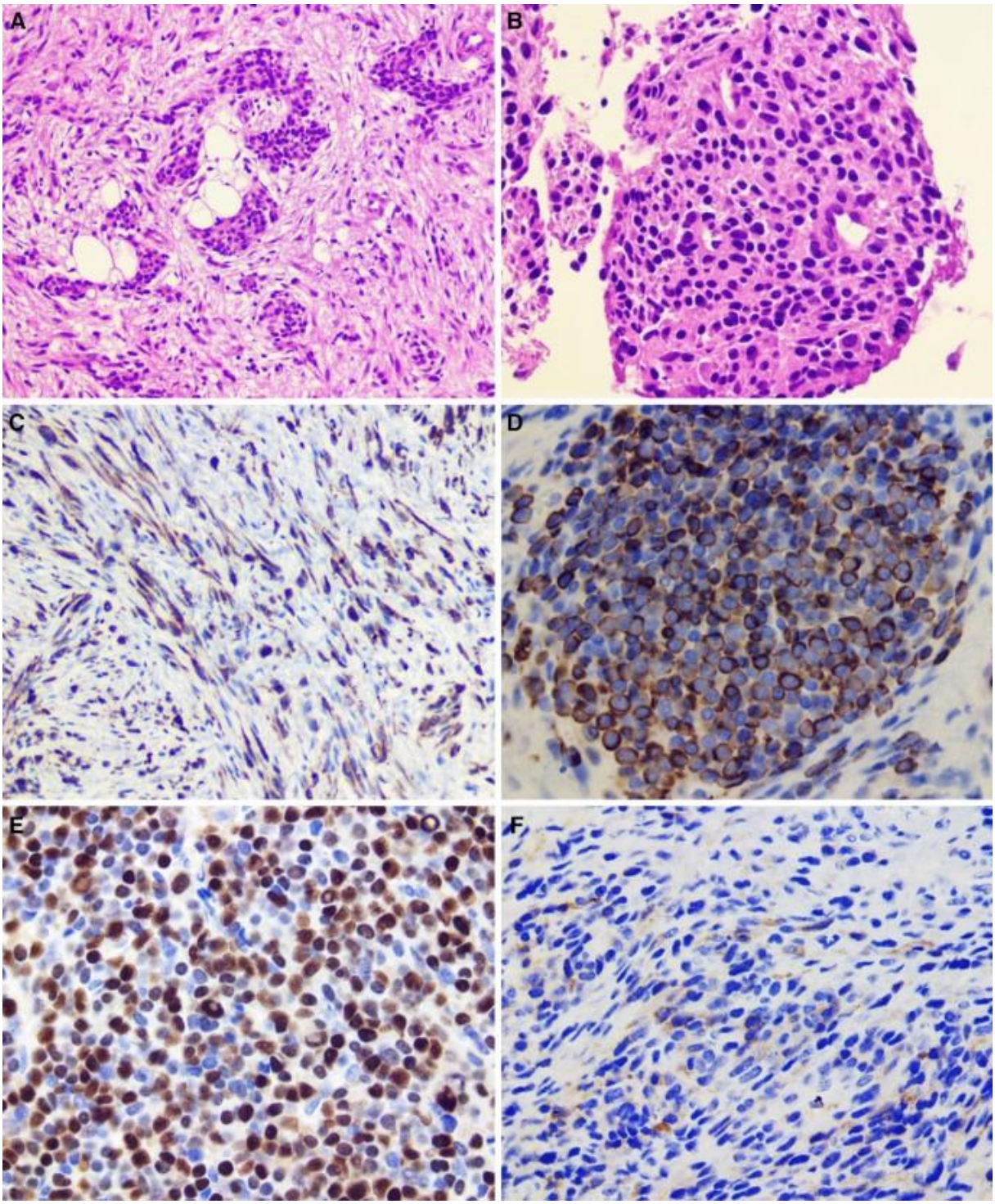


Figure 6. Case 3 – Histopathological and immunohistochemical features: **a** Tumor composed by short fascicles of spindle cells and aggregates of epithelioid cells (H&E, 200 ×). **b** Epithelioid cells with monotonous hyperchromatic nuclei and eosinophilic cytoplasm (H&E, 400 ×). Diffuse cytoplasmic positivity for AE1/AE3 (**c**; 200 ×) and desmin (**d**; 400 ×). **e** Strong nuclear positivity for MyoD1 (400 ×). **f** Focal expression of ALK (400 ×).

Table. Clinicopathological features and outcome of head and neck rhabdomyosarcomas exhibiting *TFCP2* rearrangement

Author, year	Age/ Gender	Location	Clinical Symptoms	Imaging features	Cells morphology	IHC	Molecular alteration	Metastasis	Treatment	Survival/ Follow-up (months)
Dashti et al., 2018 [12]	72/M	Mandible	Gum swelling, tingling, numbness and loosening of the teeth in anterior left mandible	Destructive lytic lesion in left parasymphyseal mandible and cortex perforation	Spindle and epithelioid cells	Desmin (+; diffuse) MyoD1 (+; diffuse) Myogenin (+; focal) AE1/AE3 (+; diffuse) ALK (+; strong)	*FUS–TFCP2* fusion	No	Mandibulectomy	ANED (2 mo.)
Wong et al., 2019 [13] & Lewin et al., 2019 [14]	23/M	Nasal cavity	Nasal congestion related	Left nasal cavity tumor of 8 cm	Spindle, epithelioid and rhabdoid cells	Desmin (+; patchy) MyoD1 (+; diffuse) Myogenin (+; rare cells) AE1/AE3 (-) ALK (+; strong)	*FUS* rearrangement *ALK* gene deletion	No	RT + CT and ALK inhibitor	AWD (2 mo.)
Agaram et al., 2019 [15]	33/F	Maxilla	NA	Large mass involving the maxilla and masticator space with extension into the maxillary and sphenoidal sinuses, orbit, and clivus	Spindle and epithelioid cells	Desmin (+; focal) MyoD1 (+; diffuse) Myogenin (+; focal) AE1/AE3 (+) ALK (+)	*EWSR1–TFCP2* fusion	Yes (femur)	Surgical resection	ANED (108 mo.)
Le Loarer et al., 2019 [17] & Watson et al., 2018 [8]	16/F	Sphenoid bone	Headache and left exophthalmos	NA	Spindle and epithelioid cells	Desmin (+; diffuse) MyoD1 (+; 75%) Myogenin (+; 10%) AE1/AE3 (+) ALK (+; 50%)	*FUS–TFCP2* fusion	Yes (right femoral bone)	CT, surgical resection and cerebral RT	DOD (15 mo.)
Le Loarer et al., 2019 [17]	32/M	Hard palate and upper lip	Nodule of gingiva and hard palate of 3 cm	NA	Spindle and epithelioid cells	Desmin (+; diffuse) MyoD1 (+; 75%) Myogenin (-) AE1/AE3 (+) ALK (-)	*EWSR–TFCP2* fusion	Yes (vertebra, ribs, pelvis)	CT	DOD (8 mo.)
Le Loarer et al., 2019 [17]	20/M	Orbito-temporo-sphenoid	Soft-tissue mass and left exophthalmos	NA	Spindle and epithelioid cells	Desmin (+; diffuse) MyoD1 (+; 65%) Myogenin (+; 15%) AE1/AE3 (+)	*FUS–TFCP2* fusion	No	CT + RT	DOD (6 mo.)

						ALK (+; <5%)				
Le Loarer et al., 2019 [17] & Brunac et al., 2019 [20]	17/F	Cervico-occipital junction	Insomnia and headaches related to neck pain for 3 months not relieved by standard painkillers. Loss- weight 3 kg.	NA	Round cells	Desmin (+; 20%) MyoD1 (+; 75%) Myogenin (-) EMA (+; focal) ALK (+; 100%)	*FUS–TFCP2* fusion	No	CT and adjuvant RT. Anti-ALK therapy	AWD (15 mo.)
Le Loarer et al., 2019 [17]	31/M	Left occipital bone	Headache	NA	Spindle, epithelioid cells	Desmin (+; diffuse) MyoD1 (+; diffuse) Myogenin (+; 15%) AE1/AE3 (+; 25%) ALK (+; 100%)	*FUS–TFCP2* fusion	Yes (lung, mediastinum)	Fragmented resection and adjuvant CT	DOD (6 mo.)
Le Loarer et al., 2019 [17]	32/M	Mandible	Toothache	NA	Spindle cells	Desmin (+; 20%) MyoD1 (+; diffuse) Myogenin (+; 15%) AE1/AE3 (+; 20%) ALK (+; 65%)	*FUS–TFCP2* fusion	Yes (lung)	Partial mandibulectomy and adjuvant CT	AWD (14 mo.)
Le Loarer et al., 2019 [17]	58/F	Mandible	NA	NA	Spindle and epithelioid cells	Desmin (+; diffuse) MyoD1 (+; 70%) Myogenin (+; 15%) AE1/AE3 (+) ALK (+; 70%)	*FUS–TFCP2* fusion	No	Surgery	ANED (21 mo.)
Le Loarer et al., 2019 [17]	12/F	Mandible	Local painful swelling for 4 months	Osteolysis of the body, angle and ramus of the mandible and extension into surrounding soft-tissue.	Spindle and epithelioid cells	Desmin (+; diffuse) MyoD1 (+; 40%) Myogenin (+; 50%) AE1/AE3 (+) ALK (+; 50%)	*FUS–TFCP2* fusion	No	Neoadjuvant CT	ANED (21 mo.)
Le Loarer et al., 2019 [17]	11/F	Maxilla	NA	NA	Epithelioid cells	Desmin (+; diffuse) MyoD1 (+; 80%) Myogenin (+; 30%) AE1/AE3 (+; weak) ALK (+; <5%)	*EWSR–TFCP2* fusion	No	CT	DOD (Unknown)

Le Loarer et al., 2019 [17]	25/M	Mandible	Local painful swelling for 1 month	NA	Epithelioid cells	Desmin (-) MyoD1 (+; 80%) Myogenin (-) AE1/AE3 (+) ALK (+; 80%)	*EWSR–TFCP2* fusion	No	Surgery	ANED (20 mo.)
Chrisinger et al., 2020 [10]	Mid-20s–30s / F	Frontal bone	Rapidly growing right scalp swelling associated with headache for 6 weeks	Destructive lesion arising from the right frontal bone with cortical breach, which measured 5 × 4.6 × 4 cm.	Spindle and epithelioid cells	Desmin (+; focal) MyoD1 (NA) Myogenin (-) AE1/AE3 (+; diffuse) ALK (+)	*EWSR–TFCP2* fusion	No	CT, RT and surgical resection	DOD (17 mo.)
Flaitz et al., 2020 [18]	15/F	Mandible	Left mandibular enlargement	NA	Spindle and epithelioid cells	Desmin (+; focal) MyoD1 (+; diffuse) Myogenin (+; focal) AE1/AE3 (NA) ALK (+; focal)	*FUS–TFCP2* fusion	NA	Surgical resection, CT and RT	Unknown
Koutlas et al., 2021 [19]	15/M	Mandible	Pain and swelling in the left posterior mandible	Destructive lesion exhibiting moth eaten-like irregular and ill-defined borders and loss of both buccal and lingual plates.	Spindle, epithelioid and round cells	Desmin (+; patchy) MyoD1 (+; diffuse) Myogenin (+; focal) AE1/AE3 (+; diffuse) ALK (-) β-catenin (+)	*EWSR–TFCP2* fusion	Yes (ipsilateral cervical lymph nodes)	Surgical resection, homolateral lymph node dissection, CT and proton beam therapy	AWD (7 mo.)
Xu et al., 2021 [9]	22/M	Mandible	NA	NA	Spindle and epithelioid cells	Desmin (+; focal) MyoD1 (+) Myogenin (+; focal) AE1/AE3 (+) ALK (+)	*FUS–TFCP2* fusion *ALK* wild type	Yes (lymph node)	NA	NA
Xu et al., 2021 [9]	34/M	Mandible	NA	NA	Spindle, epithelioid and rhabdoid cells	Desmin (+) MyoD1 (+; patchy) Myogenin (NA) AE1/AE3 (-) ALK (-)	*FUS–TFCP2* fusion *ALK* deletion	No	NA	AWD (10 mo.)
Xu et al., 2021 [9]	16/M	Mandible	NA	NA	Spindle, epithelioid	Desmin (+; focal) MyoD1 (+; focal)	*FUS–TFCP2* fusion	Yes (bone, lung,	NA	DOD (20 mo.)

					and rhabdoid cells	Myogenin (+; focal) AE1/AE3 (+) ALK (+)	*ALK* deletion	lymph node)		
Xu et al., 2021 [9]	43/F	Mandible	NA	NA	Spindle and epithelioid cells	Desmin (+) MyoD1 (+) Myogenin (+; rare cells) AE1/AE3 (+) ALK (+)	*FUS–TFCP2* fusion *ALK* not performed	NA	NA	NA
Xu et al., 2021 [9]	20/F	Maxilla	NA	NA	Spindle and epithelioid cells	Desmin (+; focal) MyoD1 (NA) Myogenin (+) AE1/AE3 (+) ALK (+)	*EWSR1–TFCP2* fusion *ALK* not performed	Yes (bone)	NA	NA
Xu et al., 2021 [9] & Zhu et al., 2019 [16]	74/F	Maxilla/ gingiva	Growing lesion on right maxillary gingiva	Expansile lytic lesion within the right maxillary alveolar ridge extending beyond midline, involving the hard palate	Spindle and epithelioid cells	Desmin (+; diffuse) MyoD1 (+; patchy) Myogenin (+; focal) AE1/AE3 (-) ALK (+)	*FUS–TFCP2* fusion *ALK* wild type	Yes (lymph node)	NA	DOD (21mo.)
Xu et al., 2021 [9] & Agaram et al., 2019 [15]	27/F	Skull	NA	NA	Spindle and epithelioid cells	Desmin (+; focal) MyoD1 (+; diffuse) Myogenin (+; focal) AE1/AE3 (+) ALK (+)	*EWSR1–TFCP2* fusion *ALK* not performed	Yes (bone)	NA	AWD (1 mo.)
Xu et al., 2021 [9]	18/M	Skull	NA	NA	Spindle and epithelioid cells	Desmin (-) MyoD1 (NA) Myogenin (-) AE1/AE3 (+) ALK (+)	*FUS–TFCP2* fusion *ALK* wild type	NA	NA	NA
Xu et al., 2021 [9]	29/M	Skull (base)	NA	NA	Spindle and epithelioid cells	Desmin (+) MyoD1 (+) Myogenin (+) AE1/AE3 (+) ALK (NA)	*EWSR1–TFCP2* fusion *ALK* wild type	Yes (Lung)	NA	AWD (2 mo.)

Xu et al., 2021 [9]	40/F	Neck superficial soft tissue	NA	NA	Spindle, epithelioid and round cells	Desmin (+) MyoD1 (NA) Myogenin (+; rare cells) AE1/AE3 (+) ALK (+)	*FUS–TFCP2* fusion *ALK* deletion	NA	NA	NA
Ochsner and Foss, 2022 [11]	48/M	Maxillary gingiva	Rapidly growing exophytic lesion with rolled borders, erythematous surface and central ulceration and necrosis located on the anterior maxillary gingiva with extension into the labial vestibule	Periapical radiograph revealed no evidence of a lytic lesion or intra-osseous involvement	Spindle, epithelioid and round cells	Desmin (+; focal) MyoD1 (+; strong and diffuse) Myogenin (-) AE1/AE3 (+) ALK (+)	*FUS* rearrangement	NA	NA	NA

Discussion

In 2017, Watson et al. described for the first time a "new epithelioid RMS" characterized by *TFCP2* rearrangement [8]. Since then, other cases of this rare entity have been reported in the literature using different terminology such as intraosseous RMS, epithelioid and spindle cell RMS with *FUS/EWSR1-TFCP2* fusion, *FET-TFCP2* RMS, RMS with *FUS* or *TFCP2* rearrangements and RMS with *TFCP2* fusions [9-11, 14, 17, 19, 20]. Despite the heterogeneous terminology, all authors agree that this is an aggressive tumor characterized by epithelioid and spindle cell phenotype with a striking predilection for the craniofacial skeleton [7-19]. However, extraosseous tumors have also been described [9-12].

So far, 27 cases of HNRMS with *TFCP2* rearrangement have been reported in the English-language literature (**TABLE**) [8-20]. Most patients were young adults in the third and fourth decades of life. Nevertheless, 29.6% of cases (8/27) occurred in pediatric patients (19 years of age or younger). The median age at diagnosis was 26 years (range, 11–74 years) with a slight male predilection. The majority of the cases were intraosseous (92.5%; 25/27), and the mandible was the most common site affected (40.7% of cases; 11/27), followed by the maxilla (14.8%; 4/27), skull, and occipital bone (11.1%; 3/27 cases each). Although, two cases affected the soft tissues of the neck [9] and oral cavity [11] without evidence of bone involvement. Similarly, our cases were in adults, two males with tumors located in the maxilla and one female presenting a zygomatic tumor. Cases 1 and 2 seem to be intraosseous lesions, but Case 3 arose in soft tissue causing bone destruction.

Clinical manifestations were described in 55.5% of cases (15/27). Most patients referred painful swelling with rapid progression (10%; 10/15). Other signs and symptoms such as headache, nasal congestion, exophthalmos, and toothache were also reported. Imaging characterization of HNRMS with *TFCP2* rearrangement was identified in 29.6% (8/27) of the cases, and most tumors were described as large osteolytic masses causing bone destruction and invasion of adjacent tissues, as also observed in our three reported cases.

Microscopically, 85.2% of tumors (23/27), showed a mixed spindle and epithelioid phenotype. Some cases also contained areas of round or rhabdoid cells (3/23; 13% for each) admixed with spindle and epithelioid cells. However, four HNRMS with *TFCP2* rearrangement (14.8%) exhibited monotonous cell morphology; two with epithelioid cells, one with spindle cells, and another with round cells. All present cases exhibited a mixture of spindle and epithelioid cytomorphology. Case 1 also showed focal areas with small round cells.

The immunohistochemical profile of this rare entity was characterized by myogenic differentiation, cytokeratins, and ALK expression. Positivity for desmin, myogenin, and MyoD1 were 92.6% (25/27), 76.9% (20/26), and 100% (23/23), respectively. Desmin and MyoD1 were more sensitive and diffusely positive in most cases when compared with myogenin, as already described by *Xu et al.* [9] and *Le Loarer et al.* [17]. In addition, to the hybrid cell morphology and positivity for myogenic markers, diffuse and strong expression of AE1/AE3 is considered a hallmark of RMS with *TFCP2* rearrangement. Of the reported cases, 84.6% (22/26) expressed diffuse positivity for AE1/AE3. Positive immunostaining for ALK was observed in 88% of cases (22/25). Similar findings of myogenic immunophenotype, pan-cytokeratin and ALK expression were observed in our cases.

It is important to highlight the common positivity of cytokeratins in about 50% of alveolar RMS [21], although only focally, contrasting with the diffuse expression displayed by most RMS with *TFCP2* rearrangement [17]. Moreover, epithelial membrane antigen (EMA) and other keratins, including CK7, CAM5.2, and CK5/6, can also be positive in *TFCP2* translocated RMS [6]. Therefore, it could be a potential diagnostic pitfall considering that keratin positivity is traditionally used to distinguish epithelial neoplasms from mesenchymal tumors [8, 17, 21].

The clinicopathological and immunohistochemical features of RMS with *TFCP2* rearrangements could make diagnosing this neoplasm challenging for pathologists. Therefore, the differential diagnosis, in intraosseous tumors, includes metastatic sarcomatoid carcinoma, mesenchymal chondrosarcoma, hemangioendothelioma, osteosarcoma, dedifferentiated chondrosarcoma and leiomyosarcoma [10, 11]. For soft tissue tumors, malignant peripheral nerve sheath tumors, inflammatory myofibroblastic tumors, spindle cell and round cell sarcomas with *EWSR1-PATZ1* fusion must be considered in the differential diagnosis [11].

Molecular alterations of this RMS variant are characterized by *FET-TFCP2* and *MEIS1-NCOA2* fusions [15]. HNRMS with *TFCP2* translocations displayed genetic fusions with *FUS* and *EWSR1* in 59.2% (16/27) and 3.7% (7/27) of cases, respectively. These genes are a member of the *FET (FUS, EWS, TAF15)* RNA binding protein family involved in deleterious genomic rearrangements with other transcription factor genes in some carcinomas, sarcomas, and acute leukemia [19, 22]. The gene *TFCP2* regulates the expression of epidermal growth factor receptor (EGFR) and accelerates tumor cell motility, invasion, and metastasis in breast cancer. So, Koutlas et al. postulated that mutated stem cells with *FET-TFCP2* fusion develop a myogenic phenotype through *EWSR1* or *FUS* while the *TFCP2* translocation induces invasion

and metastasis of the tumor cells, which could explain the aggressive clinical behavior of this rare variant of RMS [19]. Hence, we confirmed the *TFCP2* rearrangement in two of our cases. Unfortunately, we could not perform molecular analysis in Case 3. However, we favor clinical, microscopic, and immunohistochemical features as enough evidence to diagnose this tumor. Furthermore, the scenario of developing countries must be considered in this case due to the high cost and difficult access to molecular testing.

On the other hand, only two cases with *MEIS1-NCOA2* fusion have been reported. Both are located in the iliac bones, and are characterized by pure spindle cells without cytokeratin and ALK expression [15]. HNRMS with *MEIS1-NCOA2* fusion has not been described in the literature.

Moreover, 4/8 cases (50%) showed ALK deletion. Interestingly, ALK inhibitors have been used as potential target therapies in two patients affected by RMSs with *TFCP2* rearrangements, despite inconclusive outcome reported [9, 14, 20]. ALK expression by immunohistochemical assay does not correlate with ALK rearrangement and it seems that not all patients may benefit with use of ALK inhibitors [9, 14]. We observed focal immunopositivity for ALK in our cases.

RMS with *TFCP2* rearrangement has the potential to spread to regional lymph nodes and distant sites [9]. Among the 27 patients with *TFCP2*-translocated HNRMS, 54.5 % (12/22) developed regional and/or distant metastasis. Despite the fact that no treatment protocol has been established for this aggressive neoplasm, most patients have been treated by surgical resection which may be with chemotherapy and/or radiotherapy. Two patients were treated with ALK inhibitors, one of them treated with combined chemotherapy had a good response [20]. The median follow-up time was 20 months, ranging between 1 to 108 months, but follow-up data was not provided in all cases. The vital status of 20/27 patients (74.1%) were available, and 25% were alive without evidence of disease, 35% were alive with the tumor, and 40% were dead. We reported only one case with follow-up information of three months who died of disease.

Finally, as previously discussed by *Le Loarer et al.* [17], the so-called “epithelioid rhabdomyosarcomas” present a purely epithelioid pattern, typically affecting deep soft tissues, and scarcely express epithelial markers. This morphologic subset contrasts with features of RMS with *TFCP2* rearrangements; and only three cases have been reported presenting pure epithelioid morphology [17].

In summary, HNRMS with *TFCP2* rearrangement was recently categorized as an independent entity due to its unique predilection for craniofacial bones, immunohistochemical profile, and genetic alteration. We added 2 cases with molecular confirmation of *TFCP2* translocation affecting the maxilla and one additional suspected case in soft tissue. Microscopic evaluation of a high-grade malignant neoplasm with spindle and epithelioid cells and co-expression of myogenic markers, pan-cytokeratin, and ALK are essential diagnostic criteria. Molecular testing for *TFCP2* translocation is desirable to confirm the diagnosis [6]. Furthermore, the limited follow-up information indicates the aggressive behavior and poor prognosis for this rare variant of RMS.

Declarations/Compliance with Ethical Standards

All authors contributed substantially to the conception, draft, and design of these cases reported, as well as participation in the acquisition, analysis, and interpretation of data. The final version of this work was approved for publication by all involved participants. If there is a need, all author agrees to be accountable for any aspects of the work. We ensure that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The authors also state that the material is original and has not been published elsewhere.

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Conflicts of interest

The authors declare that they have no conflict of interest.

Availability of data and material (data transparency)

The authors declare that all data supporting the findings of this study are available in the article.

Code availability

Not applicable

Authors' contributions

All authors have contributed equally.

Ethics approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institution and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this type of study formal consent is not required**.** The tumors tissues included in the manuscript were obtained as part of the standard of care for the patient and were retrospectively collected for publication.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Consent for publication

Consent for publication was obtained for every individual person's data included in the study.

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2.3 ARTIGO 3.

Head and neck rhabdomyosarcoma in pediatric patients: an international collaborative study

Submetido no periódico: Journal of Oral Pathology and Medicine **(ANEXO 5)**

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Ethics approval statement:

All procedures performed in this retrospective observational study were in accordance with the ethical standards of the institution and/or national research committee and with the 1964 Helsinki declaration and its later amendments, ensuring patient privacy and data confidentiality. Ethical approval was obtained from the Research Ethics Committees at Piracicaba Dental School, University of Pretoria, and the University of Liverpool. Material Transfer Agreements were established between the participating institutions, formalizing the collaborative framework for this study.

Patient consent statement:

The biological specimens utilized were sourced as routine components of patient care. These tissue samples were retrospectively gathered and pose no additional burden to the individuals involved, aligning with established ethical guidelines and standards.

Statement of the authors:

The work described has not been published previously, it is not under consideration for publication elsewhere and will not be published elsewhere in the same form, or in any other language, including electronically without the written consent of the copyright holder. The final version of this work was approved for publication by all parts included.

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Abstract

Background: Rhabdomyosarcoma is a rare malignant tumor but commonly affects pediatric patients, and 35-40% of cases occur in the head and neck. This study aimed to analyze the clinicopathologic profile of pediatric head and neck rhabdomyosarcomas from Brazil, Guatemala, Mexico, and South Africa. **Methods**: 44 cases were included from ten Oral and Maxillofacial Pathology services. Clinicopathological data were reviewed, and

immunohistochemical analysis of Desmin, Myogenin, Myo-D1, and Ki67 was performed. Their expressions were quantified using the QuPath software. Cases with ≥50% of myogenin expression were tested for fusion status prediction based on AP2β, NOS-1, and HMGA2 expressions. **Results**: Most cases were from Brazil (40.9%), followed by South Africa (27.3%), Guatemala (22.7%), and Mexico (9.1%). About two-thirds of patients were diagnosed in their first decade without gender predilection. Non-para meningeal sites (45.5%) were more affected than parameningeal (40.9%) and orbit. Microscopically, embryonal rhabdomyosarcoma (77.3%) predominated over alveolar (18.2%) and spindle cell (2.3%) tumors. Immunohistochemically, Desmin was positive in 86% of cases, while myogenin and MyoD1 were expressed in 84% and 82% of tumors, respectively. The mean proliferation index measured by Ki67 decreased among alveolar > embryonal > spindle cell variants. Two alveolar rhabdomyosarcomas showed higher Ap2β/NOS-1 expression than HMGA2, indicative of fusion-positive status. Conversely, one alveolar and four embryonal cases showed opposite results, suggesting they were fusion-negative rhabdomyosarcomas. **Conclusion**: While slight clinical-demographic differences were noted among head and neck rhabdomyosarcomas in Brazil, Guatemala, Mexico, and South Africa, the fusion status identification through immunohistochemistry is still a diagnosis gap**.**

Keywords: Rhabdomyosarcoma; head and neck; pediatric; molecular; oral cavity

Introduction

Rhabdomyosarcoma (RMS) is a high-grade, malignant neoplasm originating from primitive mesenchymal cells with myogenic differentiation [1]. RMS is a rare disease, affecting ~4.5 patients per million individuals aged <20 years; however, it represents the most common soft tissue sarcoma (STS) in children, accounting for 5% of all pediatric malignancies [2,3].

About 35-40% of RMS arise in the head and neck region (HNR), where they can be subclassified into orbital, parameningeal (PM), and non-parameningeal (NPM) [4]. Based on its histopathological features, the latest WHO classification grouped RMS into four types: embryonal, alveolar, spindle cell/sclerosing, and pleomorphic [5]. Additionally, genetic alterations lead to RMS subclassifications into *PAX3/7–FOXO1* fusion-positive or fusion-negative RMS and spindle cell/sclerosing RMS with either *MYOD1*-mutations or rearrangements involving *VGLL2/NCOA2* or *TFCP2/NCOA2* genes [3,5,6].

Decades of basic research and clinical studies developed by European and North American international collaborative groups have contributed to a better understanding of RMS

pathophysiology and helped optimize clinical care [2,7]. However, some challenges remain despite improvements in RMS treatment and prognosis [8]. A recent study in Central America concluded that therapeutic standards achieved in high-income countries seem not to be reproducible in low-middle-income countries (LMIC), leading to lower survival rates related to the advanced stage of disease at diagnosis and a high rate of treatment-related mortality. They also suggested that other studies analyzing the RMS patients' profile in LMIC are necessary [9]. Therefore, this study aimed to describe the clinicopathological and immunohistochemical features of pediatric head and neck rhabdomyosarcomas (HNRMS) from LMIC, including Brazil, Guatemala, Mexico, and South Africa.

Materials and methods

This retrospective observational study was conducted in compliance with the 1964 Helsinki Declaration and its subsequent amendments, ensuring patient privacy and data confidentiality. Ethical approval was obtained from the Research Ethics Committees at Piracicaba Dental School (Ref No. 12469119.8.0000.5418), University of Pretoria (Ref No. 483/2020), and the University of Liverpool (Ref No. 12077). Material Transfer Agreements were established between the participating institutions, formalizing the collaborative framework for this study.

Pediatric patients up to 19 years old with a confirmed histopathological diagnosis of HNRMS were retrospectively retrieved from the archives of Oral and Maxillofacial Pathology (OMFP) services in ten institutions. Among these institutions, seven were located in Brazil, and three were situated in Guatemala, Mexico, and South Africa, respectively. Cases of HNRMS without available material for analysis, tumors affecting the central nervous system (CNS), or rhabdomyosarcomas diagnosed as metastatic deposits in the head and neck region (HNR) were excluded.

Demographic and clinical data were retrospectively collected from histopathological requests or patients' medical charts by a designated researcher at each participating institution. The histological type of RMS was classified by reviewing hematoxylin- and eosin-stained (HE) slides and following the WHO 2020 classification criteria [5]. Additionally, slides containing immunohistochemical markers such as Desmin, Myogenin, MyoD1, and Ki-67 were reassessed in all HNRMS. In cases with an incomplete immunohistochemical panel, with available 3µm tissue sections, additional immunohistochemical reactions were carried out, following the protocol outlined in **Supplement 1.**

Based on the cells' immunostaining pattern, positive expression for each marker was quantified using a 0 to 4+ scale as follows: (0) absent expression, (1+) <10% expression, (2+) 10-49% expression, (3+) 50-90% expression; and (4+) >90% expression [10]. The proliferation index measured by Ki67 was rated as low <10%, moderate 10–29%, or high ≥ 30% [11].

HNRMS with high myogenin expression (3+; 4+) were subjected to a gene fusion status prediction using an algorithm developed by Rudzinski et al. 10, which relies on immunohistochemical expressions of NOS-1, AP2β, and HMGA2. Additionally, some embryonal RMS cases with available tissue sections were evaluated for p53. Therefore, additional immunohistochemical using p53 (ready to use, Agilent Technologies), AP2β (1:50, Sigma-Aldrich) NOS-1 (1:100, Sigma-Aldrich), and HMGA2 (1:50, Sigma-Aldrich). were performed on 3μm tissue sections utilizing the BOND RX automated Stainer (Leica Biosystems).

For fusion status prediction, the following results were considered: stronger (3 to 4+) NOS-1 and/or AP2β with weaker HMGA2 (0 to 2+) expression supported 'fusion-positive' RMS (FPRMS). However, weaker NOS-1 and/or AP2β than HMGA2 favor 'fusion-negative' RMS (FNRMS). When NOS-1 and AP2β were discrepant, the higher score was used [10].

For immunohistochemical quantification, slides were scanned at 20× magnification using the Aperio Scan Scope CS Slide Scanner (Aperio Technologies Inc., Vista, CA), generating high-resolution whole slide images (WSI). The QuPath Bioimage analysis v0.2.0-m8 (University of Edinburgh, Scotland, UK) 12 was then applied, following a protocol proposed by Pai et al. 13, which was adapted for this study (**Supplement 2**) and is illustrated in **Figure 1**. Subsequent to automated cell counting, all relevant data were extracted from the annotation measurement tables produced by QuPath. The mean positive expression for each marker was calculated using Microsoft Excel.

Results

Forty-nine pediatric HNRMS were initially retrieved from ten OMFP services between 1998 and 2023. However, two metastatic RMS in the HNR, two affecting the CNS, and one without available tissue for further analysis were excluded. Thus, 44 HNRMS were included in this series.

A summary of the demographic and clinicopathological features of HNRMS patients is presented in **Table 1**. Most HNRMS were from Brazil (40.9%), followed by South Africa (27.3%), Guatemala (22.7%), and Mexico (9.1%). About two-thirds of patients were <10 years

old at diagnosis, with a median age of 8.1 years (range: 1-19 years) without gender predilection. The NPM sites were the most common anatomical location (45.5%), followed by PM (40.9%) sites, and the orbit was the least affected. Among NPM RMS cases, the masseter, parotid region, and oral cavity were predominantly affected. Oral RMS represented 15.9% (7 cases) of all HNRMS, with the buccal mucosa (3/7) and tongue (2/7) being the most commonly involved sites. For PM RMS, the paranasal sinuses and nasal cavity were the most frequently affected locations.

Clinical manifestations in 38.6% (17/44) of HNRMS showed that 70.5% (12/17) of patients presented with facial asymmetry (**Figure 2A-C**) caused by a painful (5/17; 29.4%) or asymptomatic (2/17; 11.8%) swelling. Other symptoms included snoring, nasal obstruction, and bleeding. In two oral RMSs, ulceration, necrosis, and tooth mobility were described. Tumor size, available for 16 (36.4%) HNRMS, ranged from 1.8 to 14 cm in the greatest dimension (mean: 6 cm), with 56.3% (9/16) of cases being ≥5 cm. The symptom duration reported in 11 (25%) HNRMS varied from 2 weeks to 8 months (mean: 3.1 months). Imaging studies were provided in three cases (**Figure 2D-F**). Still, computed tomography (CT) or magnetic resonance image (MRI) reports, available in some cases, described large lesions causing invasion, erosion, and destruction of adjacent bony structures. Previous radiotherapy in the HNR was referred in one patient's history due to a neuroblastoma ten years before the PM RMS diagnosis. Through microscopic examination, HNRMS were classified as embryonal > alveolar > spindle cell, in that order of frequency. Additionally, one mixed RMS presented alveolar and embryonal morphologies. Immunohistochemical examination indicated that all HNRMS were positive for at least two myogenic markers (Desmin, myogenin, and MyoD1).

Therapeutic approaches and outcomes were obtained in 12 (27.9%) HNRMS from two hospitals in Brazil Among them, 66.7% (8/12) of patients received combined chemotherapy and radiotherapy, while 25% (3/12) underwent chemotherapy and/or radiotherapy before surgery, and one patient was treated with chemotherapy alone. Metastases were reported in four patients, and one case experienced local recurrence two years after treatment. At the last follow-up, which ranged from 8 to 204 months (mean: 41.2 months), 66.7% (8/12) of patients were alive, and 33.3% (4/12) had passed away.

Embryonal and alveolar RMS – Clinicopathologic features

A clinicopathologic analysis showed that 77.3% (34/44) of HNRMS were embryonal, while 18% (8/44) were alveolar, with the patient's median age at diagnosis of 7 and 8 years old,

respectively. Nonetheless, PM RMS in males was remarkable among alveolar RMS, while females with NPM tumors were more frequent in the embryonal RMS group (**Table 2**).

Microscopically, within the embryonal RMS subgroup, 55.8% (19/34) exhibited hypercellular and hypocellular areas in a loose or fibro-myxoid stroma composed of primitive stellate cells or with variable degrees of muscle differentiation mixed with some undifferentiated small round cells areas (**Figure 3A**). Additionally, 20.6% (7/34) of cases displayed a dense pattern of primitive undifferentiated round-to-oval cells arranged in compact sheets (**Figure 3B**). The remaining embryonal cases exhibited anaplastic features (5/34; 14.7%) and botryoid morphology (3/34; 8.8%) (**Figure 3C-E**). Rhabdomyoblastic differentiation was noted in 61.8% (21/34) embryonal RMS (**Figure 3F**). Necrotic areas were observed in 55.8% (19/34) of cases.

In contrast, among alveolar RMS, 75% (6/8) exhibited the classic alveolar pattern characterized by small round cells adhering to fibrovascular septa, forming spaces containing discohesive cells (**Figure 3G**). The solid variant was observed in two cases (**Figure 3H**). Tumor-giant cells (**Figure 3I**) and plump-shaped rhabdomyoblasts with dark hyperchromatic nuclei and scant eosinophilic cytoplasm (**Figure 3J**) were variable within the tumors. All cases exhibited varying degrees of necrosis.

Embryonal and alveolar RMS - Immunohistochemical features

The immunophenotype HNRMS is detailed in **Table 2** and illustrated in **Figure 4**. Desmin exhibited strong and diffuse positivity in almost all cases. Myogenin expression was heterogeneous in embryonal tumors, generally quantified as moderate. In contrast, alveolar RMS demonstrated consistently high myogenin expression with intense and diffuse positivity in all cases. MyoD1 displayed variable low, moderate, or high expression in both variants. The mean proliferation index measured by Ki67 was higher (62.7%) in alveolar compared to embryonal RMS (47%).

Immunohistochemical expression of p53 was evaluated in 18/34 embryonal RMS. Tumors with dense patterns composed predominantly of small, round cells presented a mean p53 expression of 47.1%. However, for those containing more rhabdomyoblastic and differentiated cells, the mean p53 expression was 29.5%. Notably, opposite p53 results were observed in a botryoid (mean 15.1%) and anaplastic (mean 85.8%) RMSs. Finally, four cases were negative for p53, including one case each of botryoid and anaplastic subtypes.

Prediction analysis of gene fusion status

Ten HNRMS (five alveolar, four embryonal, and one mixed RMS) with high myogenin expression underwent testing for Ap2β, NOS-1, and HMGA2 expressions to predict gene fusion status. According to the proposed algorithm, 2/5 alveolar RMS might be classified as FPRMS due to higher Ap2β/NOS-1 expression than HMGA2. Conversely, all embryonal RMS and one alveolar case showed the opposite immunohistochemical results compatible with FNRMS. The fusion status in the mixed RMS was unpredictable due to unexpected expressions of Ap2β (2+), NOS-1 (3+), and HMGA2 (3+). Additionally, 2/5 alveolar RMS were negative for the mentioned markers, which led to inconclusive results.

Spindle cell RMS

The only spindle cell RMS in this series was diagnosed in a 1.7-year-old Brazilian girl with an asymptomatic tongue swelling of 6-month duration. Microscopically, the tumor was partially encapsulated and composed of fascicles of elongated spindle cells surrounded by a fibrous stroma (**Figure 2K**). Scattered rhabdomyoblasts, some pleomorphic cells (**Figure 2L**), and focal necrosis were also identified within the tumor. Immunohistochemical studies revealed positivity for Desmin, SMA, vimentin, HHF-35, myogenin and Myo-D1 (**Figure 3I-K**). However, S100, AE1-AE3, ALK, and H-Caldesmon were all negative. The proliferation index by Ki67 was 3% (**Figure 3L**). After diagnosis confirmation, the patient was referred to oncologic treatment.

Discussion

Approximately 50% of pediatric patients with STS are diagnosed with RMS, with the HNR affected in ~40% of cases 2,3. Due to RMS's relative rarity, single institutional experience is usually limited [7,14] and thus, multicenter collaborative research is highly recommended to understand this neoplasm [7,9]. The current study, involving 44 HNRMS from Brazil, Guatemala, Mexico, and South Africa, revealed a clinicopathological and immunohistopathological profile that predominantly aligns with previously published literature. Nonetheless, minor variations were identified and are further discussed below.

Our findings indicate that 68.2% of HNRMS were diagnosed in children <10 years old without a gender predilection, with most tumors affecting NPM sites over PM and the orbit. Several studies have described that RMS mainly affects children in their first decade of life, with a male-to-female ratio of 1.3-1.8:1, with PM being the most common site in the HNR [3,15-18]. Upon data comparison across countries, males were more affected in South Africa (2:1),

whereas in Brazil and Guatemala, a female predominance was observed. Notably, PM tumors were more frequent than NPM only in Guatemalan patients.

In our series, the oral cavity was affected in 35% of NPM RMS in this series, representing 15.9% of all HNRMS included. These tumors were predominantly located in the buccal mucosa and tongue. Previous studies have reported that approximately 10%–12% of HNRMS arise in the oral cavity, with varying predilection sites such as the palate, buccal mucosa, and tongue [16, 19, 20].

Most HNRMS in this study presented as painful swellings measuring ≥5 cm and causing facial asymmetry with a mean of 3 months of symptom duration. These findings vary within studies [3,16-18,21], likely influenced by sample size and data availability. However, the predominance of tumor size ≥5 cm might suggest an advanced stage of disease at diagnosis. It is essential to emphasize that some early-stage RMS signs, such as facial pain, sinonasal congestion, and ear pain, even asymptomatic swellings, can mimic more benign conditions. This may lead to misdiagnosis and inappropriate therapeutic approaches, contributing to delayed diagnosis [1-3,17]. While benign, inflammatory, and infectious diseases are more common in the pediatric population, healthcare professionals should consider RMS as a diagnostic possibility in routine practice when evaluating children, especially in cases presenting with the mentioned signs and symptoms [18].

Embryonal and alveolar RMS are the most common variants in pediatrics, with some data indicating a bimodal peak of incidence in early childhood and adolescence for embryonal RMS. Conversely, alveolar cases predominantly affect adolescents [2-6]. Our findings revealed that >70% of HNRMS were embryonal, with 41.2% of cases diagnosed in 1–5-year-old children and 32.4% in adolescents. However, the adolescent predominance among alveolar RMS was not observed in the current study, probably due to the small sample of alveolar tumors. Microscopically, they all presented classic morphological features for both variants as described by the latest WHO classification [5].

Immunophenotypic analysis of HNRMS showed, as reported previously [3,22,23], heterogeneous staining of embryonal RMS cells for Desmin, Myogenin, and MyoD1. In contrast, the stronger and more diffuse Myogenin expression was highlighted in all alveolar HNRMS compared to Desmin and MyoD1. Given that 70-80% of alveolar RMS may harbor *PAX3/7–FOXO1* fusion genes, distinguishing between FPRMS and FNRMS is essential, as the first represents a risk factor due to its more aggressive behavior [2,6,10,22]. While RT-PCR and

FISH are the gold standard for identifying gene fusions, limitations such as insufficient tissue quality/quantity for analysis can be encountered with both techniques [10]. Additionally, the high cost of molecular acts as a significant barrier, preventing the routine implementation of these ancillary tests in most OMFP laboratories in LMIC. Hence, performing immunohistochemical assays using surrogate markers for FPRMS and FNRMS identification represents a proper, cost-effective alternative for RMS subclassification [10,24].

Previous studies have indicated that AP2β and HMGA2 are considered more reliable markers for identifying FPRMS and FNRMS, respectively [10,24,25]. Moreover, when performed in combination, these markers offer >90% specificity and >60% sensitivity for fusion status subclassification [10,23]. Based on the algorithm proposed by Rudzinski et al. [10], only two alveolar cases in our series might be FPRMS, but heterogenous AP2β and NOS-1 expression were observed in one of them. In contrast, all embryonal RMS and one alveolar tumor showed higher HMGA2 expression compatible with FNRMS. It has been described that most embryonal tumors are FNRMS [2,3,6]; therefore, as Ouchi et al. [25] proposed, our results suggest that HMGA2 could be a strong candidate for identifying FNRMS due to its higher expression in RMS with embryonal morphology. Additionally, HMGA2 has also been suggested as a therapeutic target owing to its oncogenic role [25]. Nevertheless, non-molecular tests were performed to confirm the previous results due to insufficient tissue, constituting a limitation for the current study. Furthermore, considering the heterogeneous results observed across studies [10,24,25], including this series, more research is necessary to validate these markers before incorporating them as surrogate markers for fusion status prediction.

Besides embryonal and alveolar RMS, the spindle cell/sclerosing variant also exhibits a strong predilection for children, particularly those ≤1 year old [3,26,27], accounting for only 5-10% of cases, and it shows a predilection for the HNR [6,27,28]. This series included one spindle cell/sclerosing RMS, representing 2% of all HNRMS analyzed. Given its resemblance to other spindle cell neoplasms (leiomyosarcoma, synovial sarcoma, malignant peripheral nerve sheath tumor, fibrosarcoma, sarcomatoid carcinoma, and spindle cell melanoma), the diagnosis can be challenging [26,27]. Therefore, immunohistochemistry is essential. Previous studies have indicated that spindle cell/sclerosing RMS may present with *MYOD1* mutation, which is associated with poor outcomes. Consequently, identifying the *MYOD1* mutation can be used for risk stratification within the spindle/sclerosing RMS variant [28,29].

Regarding RMS treatment and prognosis, a combination of chemotherapy and radiotherapy was the prevalent treatment modality for HNRMS in the current series, with the

majority of patients being alive at the last follow-up. It has been described that anatomical limitations in HNRMS could prevent complete surgical resection, especially in advanced-stage tumors [18,30], potentially explaining the treatment strategy adopted for these patients. However, due to limitations in sample size and the absence of comprehensive follow-up data, statistical analysis was not feasible in the current study to identify potential associations, prognostic factors, and survival rates.

In summary, HNRMS from Brazil, Guatemala, Mexico, and South Africa generally exhibit similar features, with only slight clinical-demographic differences compared to previous publications. The potential addition of immunohistochemistry for fusion status identification could be a valuable tool in the diagnostic armamentarium. Currently, AP2β and HMGA2 appear to play predictive roles in recognizing RMS fusion status, but further studies are required to validate them as surrogate markers.

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Figure legends

Figure 1. QuPath procedures and settings: a. At 10× viewing magnification, using the polygon annotation tool, the region of interest (ROI) was outlined in selected tumor areas; **b, c.** RGB pixel depth stain vectors recalibration setting the "Estimate Stain Vectors function" with the default "auto" detection; **d.** "Positive Cell Detection" function used for automated cell counting; **e, f.** after computation, the tumor cells within the fixed-shaped annotations (ROIs) were automatically counted and visualized as red (positive) or blue (negative).

Figure 2. **Clinical and imaging features of HNRMS: a, b.** two male patients presenting facial asymmetry due to marked and discrete swellings, respectively; **c**. extensive mass presenting an ulcer-necrotic surface; **d-f.** imaging findings analysis of computerized tomography in each case, showing an expansive destructive mass invading adjacent tissue.

Figure 3. **Microscopic features of HNRMS:** *Embryonal RMS* **a.** hypercellular and hypocellular areas of primitive stellate and small round cells arranged within a fibro-myxoid stroma (HE 5X); **b.** dense pattern of primitive undifferentiated round-to-oval cells (HE 10X); **c**. tumor cells showing anaplastic features (HE 10X); **d.** botryoid morphology in a low-power view (HE); **e.** "*cambium layer"* – a hypercellular zone immediately beneath the epithelial surface (HE 10X); **f.** rhabdomyoblasts with variable grades of differentiation exhibiting eosinophilic cytoplasm (HE 20X). *Alveolar RMS* **g.** classic (HE 5X) and solid (**h;** HE 10X) patterns, **i**. Multinucleated tumor cells signalized with head arrows (HE 20X); **j.** rhabdomyoblasts with dark hyperchromatic nuclei and scant eosinophilic cytoplasm (HE 20X). *Spindle cell RMS* **k.** spindle cells arranging in fascicles (HE 10X); **l.** some tumor cells with rhabdomyoblastic differentiation exhibiting pleomorphic hyperchromatic nuclei (HE 20X).

Figure 4. **Immunohistochemical features of HNRMS:** ***Embryonal RMS*** **a.** diffuse cytoplasmatic stain for desmin (100X magnification); **b.** positivity for myogenin (100X magnification) and Myo-D1 (**c;** 100X magnification) with nuclear staining patterns; **d.** HMGA2 showing nuclear expression. ***Alveolar RMS*** **e.** tumor cells showing cytoplasmatic positivity for desmin (100X magnification); **f.** strong and diffuse nuclear myogenin expression (100X magnification); **g.** FPRMS immunophenotype determined by Ap2β (100X magnification) and

NOS-1 (**h;** 100X magnification) expressions. *Spindle cell RMS* showing strong and diffuse cytoplasmatic expression for desmin (**i;** 100X magnification); **j.** nuclear expression of MyoD1 and myogenin (**k;** 100X magnification); **l.** low Ki67 expression (100X magnification**)**.

Tables

Table 1. Clinicopathologic features of 44 head and neck rhabdomyosarcomas in paediatric patients.

	Brazil	**Guatemala**	**Mexico**	**South Africa**	**Total**
	n (%)	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Study period	2006-2021	1998-2016	2012-2019	2002-2021	1998-2021
Sample size	18 (40.9)	10 (22.7)	4 (9.1)	12 (27.3)	44 (100)
Demographic variables					
Median age (range)	8.8 (1-19)	6.5 (3-13)	2.5 (1-12)	7.5 (1-15)	8.1 (1-19)
Age groups					
1-9 years old	11 (61.1)	6 (60)	3 (75)	10 (83.3)	30 (68.2)
10-19 years old	7 (38.9)	4 (40)	1 (25)	2 (16.7)	14 (31.8)
Gender					
Male	8 (44.4)	4 (40)	2 (50)	8 (66.7)	22 (50)
Female	10 (55.6)	6 (60)	2 (50)	4 (33.3)	22 (50)
Clinicopathologic variables					
Tumor location					
Non - parameningeal	8 (44.4)	4 (40)	2 (50)	6 (50)	20 (45.5)
Parameningeal	8 (44.4)	6 (60)	1 (25)	3 (25)	18 (40.9)
Orbit	2 (11.1)	-	1 (25)	2 (16.7)	5 (11.4)
H&N without specification	-	-	-	1 (8.3)	1 (2.3)
Histopathological variant					
Embryonal RMS	15 (83.3)	5 (50)	3 (75)	11 (91.7)	34 (77.3)
Alveolar RMS	2 (11.1)	4 (40)	1 (25)	1 (8.3)	8 (18.2)
Spindle cell RMS	1 (5.5)	-	-	-	1 (2.3)
*Mixed RMS	-	1 (10)	-	-	1 (2.3)

Notes: *RMS showing mixed alveolar and embryonal features
Abbreviations: RMS= rhabdomyosarcoma

Table 2. Clinicopathologic and immunohistochemical features of 44 HNRMS in pediatric patients.

	Histopathological variant			
	Embryonal RMS n= 34 (%)	**Alveolar RMS n= 8 (%)**	**Spindle cell RMS n= 1 (%)**	**Mixed RMS* n= 1 (%)**
Demographic variables				
Median age (range)	7 (1 – 19)	8 (1 – 13)	-	-
Age groups				
1-9 years old	23 (67.6)	5 (62.5)	1 (100)	-
10-19 years old	11 (32.4)	3 (37.5)	-	1 (100)
Sex				
Male	16 (47.1)	5 (62.5)	-	1 (100)
Female	18 (52.9)	3 (37.5)	1 (100)	-
Clinicopathologic variables				
Tumor location				
Non - parameningeal	16 (45.5)	3 (37.5)	1 (100)	-
Parameningeal	12 (36.4)	5 (62.5)	-	1 (100)
Orbit	5 (15.1)	-	-	-
H&N without specification	1 (3)	-	-	-
Immunohistochemical features				
Desmin				
Negative	3 (8.8)	1 (11.1)	-	-
Low (1+)	1 (2.9)	-	-	1 (100)
Moderate (2+)	13 (38.2)	1 (11.1)	-	-
High (3+); (4+)	17 (50)	6 (75)	1 (100)	-
Myogenin	**n= 33 (%)**			
Negative	4 (12.1)	-	-	-
Low (1+)	2 (6.1)	-	-	-
Moderate (2+)	17 (51.5)	-	1 (100)	1 (100)
High (3+); (4+)	10 (30.3)	8 (100)	-	-
MyoD1	**n= 33 (%)**	**n= 7 (%)**		
Negative	3 (9.1)	1 (12.5)	-	-
Low (1+)	3 (9.1)	2 (25)	-	-
Moderate (2+)	13 (39.3)	3 (37.5)	-	1 (100)
High (3+); (4+)	14 (42.4)	1 (25)	1 (100)	-
Ki67	**n= 34 (%)**	**n= 7 (%)**		
Mean % of expression	47	62.7	3.2	21.6
Negative	1 (2.9)	-	-	-
Low <10%	3 (8.8)	-	1 (100)	-
Moderate 10 – 29%	9 (26.5)	-	-	1 (100)
High ≥ 30%	21 (61.7)	7 (100)	-	-

Notes: *RMS showing mixed alveolar and embryonal features
Abbreviations: NP= not performed; RMS= rhabdomyosarcoma

Figures

Figure 1

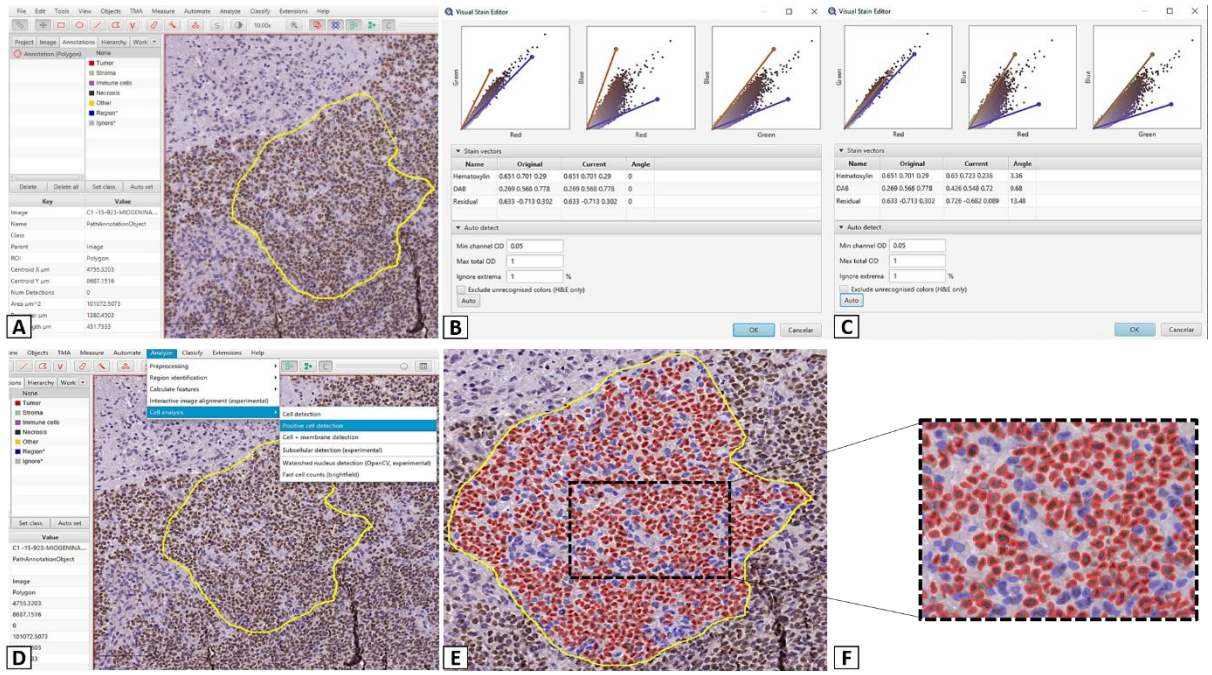


Figure 2

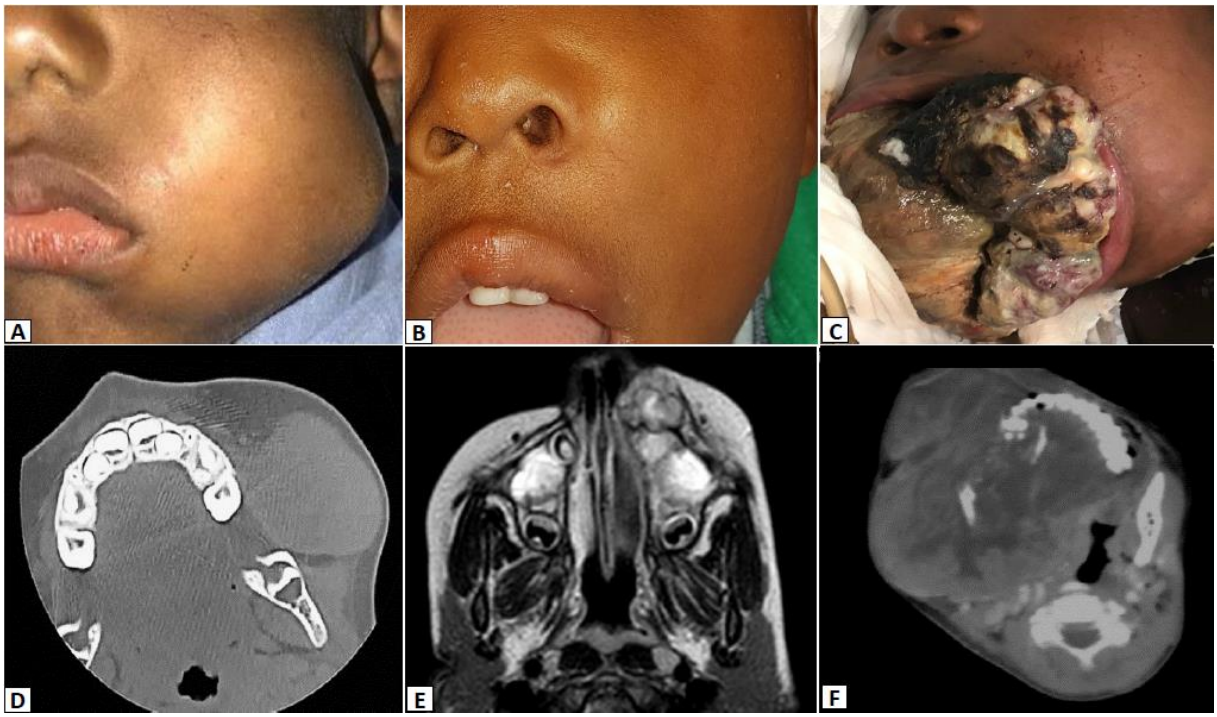


Figure 3

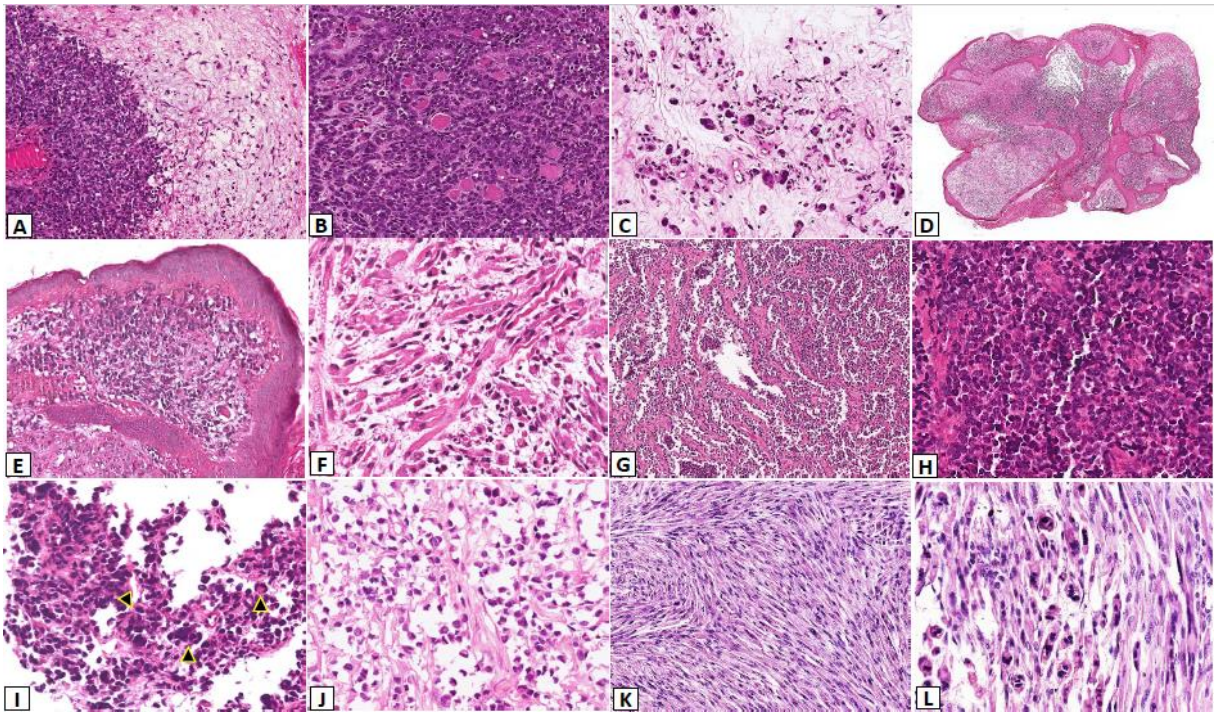
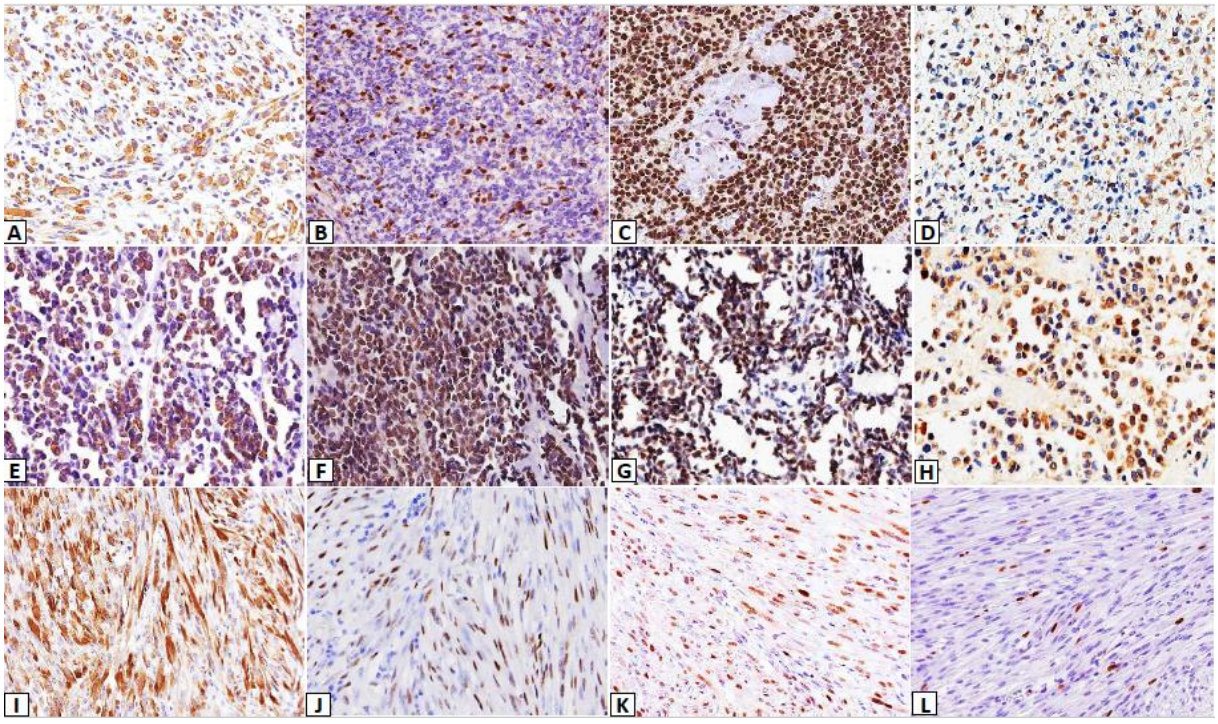


Figure 4



Supplement materials

Supplement 1. Immunohistochemistry Protocol

1. Sections were deparaffinized with xylene and hydrated in descending ethanol's baths.
2. The antigen retrieval was performed for 15 min in an electric pressure cooker.
3. Endogenous peroxidase activity was blocked by immersion in 6% H2O2 for 15 min
4. Sections were incubated with the primary antibody: Desmin (1:300, DAKO), Myogenin (1:100, DAKO), MyoD1 (1:100, DAKO), Ki67 (1:100, DAKO) for two hours at room temperature.
5. IHC staining was performed according to the manufacturer's protocol (DAKO, Carpinteria, CA, USA), followed by exposure to diaminobenzidine tetrahydrochloride (Sigma-Aldrich, St Louis, USA) for 5 min.
6. Slides were counterstained with Carazzi's hematoxylin for 5 min followed by dehydration in ascending ethanol's baths and diaphanization in xylene.
7. Finally, the slides were cover slipped using Entellan™ (Sigma Chemical CO., St Louis,MO/USA).
8. Appropriate control tissue was used for each antibody.

Supplement 2. QuPath procedures and settings

For immunohistochemical quantification, using QuPath Bioimage analysis v0.2.0-m8 (University of Edinburgh, Scotland, UK) the following protocol was used:

1. A new "Project" was created within QuPath for each immunohistochemical marker analyzed.
2. Then, WSIs in ScanScope Virtual Slide (.svs) format were imported into each project file setting as "Heme/DAB brightfield" images for further analysis.
3. For the automated cell counting, the steps detailed in **Figure 1** were sequentially followed.
4. The procedures and settings in QuPath software using the "Positive Cell Detection" function for the digital counting method is detailed below:
   - **Image file type**: ScanScope Virtual Slide (.svs)
   - **Image set (upon import to QuPath)**: Heme/DAB brightfield
   - **Representative tumor areas (n=5):** Selection at 10x magnification
   - **Pixel depth separation vectors:** "Estimate Stain Vectors" function with "Auto" calibration.
   - **Heme threshold for counterstain (default: 0.1):** 0.20 in areas with low cellularity and increased stroma/ 0.10 or 0.01 in areas with high cellularity and scarce stroma
   - **"Threshold Compartment" (depends on the antibody staining patterns):** Nucleus: DAB OD mean or Cytoplasm: DAB OD mean
   - **"Threshold Positive 1":** 0.2 (default)

3 DISCUSSÃO

Os resultados deste estudo proporcionaram uma descrição e análise abrangentes das principais características clínicopatológicas, imuno-histoquímicas e moleculares dos RMS que afetam a região de cabeça e pescoço, contribuindo significativamente para a compreensão desta doença complexa. Muitas das características clínicopatológicas identificadas, como tumores parameníngeos, tamanho > 5cm, variante alveolar, estágio avançado, margens cirúrgicas comprometidas, recorrência e metástases, estão alinhadas com critérios considerados na estratificação de risco, indicando uma maior probabilidade de uma doença mais agressiva e prognóstico desfavorável para os pacientes (Darwish et al., 2020; Radzikowska et al., 2015; Rudzinski et al., 2021; Skapek et al., 2019; Sultan et al., 2009).

Nas últimas décadas, os avanços na caracterização molecular dos RMS possibilitaram integrar as alterações genéticas às características clínicopatológicas para estratificar o risco individual dos pacientes (Turner e Richmon, 2011; Hettmer et al., 2022; Shern et al., 2022). A fusão dos genes *PAX3/7-FOXO1*, associada à variante alveolar, tem sido relacionada a uma maior agressividade e pior prognóstico (Heske et al., 2021; Hettmer et al., 2022; Hibbitts et al., 2019). Recentemente, um consenso europeu recomendou incluir a mutação de *MYOD1* como fator de risco em futuros ensaios clínicos devido à robustez das evidências associando-a a um mau prognóstico (Hettmer et al., 2022). Em nossa revisão sistemática, não observamos correlação prognóstica para a fusão de *PAX3/7-FOXO1* em RMSs alveolares de cabeça e pescoço; no entanto, a mutação de *MYOD1* em RMSs de células fusiformes/esclerosantes associou-se a uma maior taxa de mortalidade (Gallagher et al., 2022). Cerca de 90% dos RMSs com mutação em *MYOD1* afetam a região de cabeça e pescoço, e atualmente se descreve que além dos RMSs de células fusiformes/esclerosantes, os tumores com morfologia típica de RMS embrionário também podem apresentar essa mutação (Alaggio et al., 2016; Agaram et al., 2019; Hettmer et al., 2022; Shern et al., 2021).

Dentro de nossos resultados, a identificação do RMS de células fusiformes/esclerosantes com fusão dos genes *EWSR1/FUS-TFCP2*, frequentemente afetando os ossos gnatios, foi um achado significativo que contribuiu para o relato de três casos latino-americanos desse subtipo de RMS (Gallagher et al., 2022; Gallagher et al., 2023). O RMS com rearranjo do gene *TFCP2* é notável por sua extrema raridade, agressividade e predileção por adultos jovens, afetando especialmente ossos craniofaciais, predominantemente a maxila e mandíbula (Gallagher et al., 2023; WHO, 2020; WHO, 2022). Até hoje, 44 casos foram reportados na região de cabeça e pescoço, incluindo 15 na mandíbula e 14 na maxila (Bradová et al., 2023; Dehner et al., 2023).

Apesar da preferência por tecido ósseo, evidências recentes indicam que também pode ocorrer em tecidos moles tais como língua, orofaringe e pescoço (Bradová et al., 2023). Microscopicamente, a morfologia fusocelular/epitelioide e a positividade difusa para citoqueratinas tornam o diagnóstico desafiante, especialmente quando afeta a região de cabeça e pescoço, onde neoplasias epiteliais são mais frequentes que as mesenquimais (Dehner et al., 2023; Gallagher et al., 2023; WHO, 2022). A doença caracteriza-se por um curso clínico rápido e prognóstico desfavorável com taxa de sobrevida global em 3 anos de 28%. Portanto, o conhecimento de suas características clinicopatológicas distintivas, associadas à expressão de marcadores rabdomioblásticos e positividade frequente para citoqueratinas e ALK, é fundamental para um diagnóstico correto e de ser possível, a realização de testes moleculares, é desejável para confirma-lo (Bradová et al., 2023; WHO 2022).

Em linhas gerais, os resultados deste estudo ressaltaram a relevância da histopatologia e da expressão imuno-histoquímica dos marcadores miogênicos - Desmina, Miogenina e MyoD-1 - no diagnóstico do RMS e suas variantes. Embora a abordagem diagnóstica baseada em alterações genéticas prometa melhorar a acurácia, o acesso limitado a testes moleculares em alguns países indica a necessidade de buscar alternativas diagnósticas (Rudzinski et al., 2014).

A imuno-histoquímica, reconhecida como uma ferramenta auxiliar robusta, pode representar uma alternativa econômica e eficaz para contribuir no diagnóstico futuro dos RMSs com base em suas alterações moleculares (Rudzinski et al., 2014). Embora alguns estudos sugiram que a positividade intensa e difusa, ou negatividade, de MyoD1 possa indicar a mutação do gene *MYOD1* (Rekhi et al., 2016; Shern et al., 2021), os RMSs de células fusiformes/esclerosantes avaliados na revisão sistemática revelaram expressão intensa e difusa de Myo-D1, independentemente da mutação do gene (Gallagher et al., 2022). Pesquisas adicionais (Ouchi et al., 2020; Rudzinski et al., 2021; Rudzinski et al., 2014), inclusive o estudo multicêntrico desenvolvido neste trabalho, indicam que a expressão imuno-histoquímica de AP2β e HMGA2 pode servir como alternativa para diferenciar os RMSs com base no status de fusão *PAX3/7-FOXO1*. Entretanto, é importante notar que a expressão desses marcadores ainda apresenta resultados heterogêneos entre os estudos. Portanto, torna-se imperativo conduzir pesquisas futuras para estabelecer valores quantitativos, como pontos de corte, e apresentar associações estatísticas para validar a expressão imuno-histoquímica dos marcadores mencionados na identificação dos RMSs com base em alterações genéticas (Rekhi et al., 2016; Shern et al., 2021; Gallagher et al., 2022).

4 CONCLUSSÕES

Os três artigos exploram a complexidade biológica dos RMS de cabeça e pescoço, chegando às seguintes conclusões:

- A mutação *MYOD1* emerge como um possível indicador prognóstico, embora a correlação entre alterações moleculares e prognóstico geral não tenha sido conclusiva.

- O rabdomiossarcoma com rearranjo no gene *TFCP2* é raro e agressivo. O reconhecimento do seu perfil clinicopatológico e imuno-histoquímico pode reduzir o risco de diagnósticos incorretos.

- A série de RMS em pacientes pediátricos revela algumas diferenças clínicopatológicas em comparação com a literatura, embora sem conclusões definitivas. Sugere-se o uso da imuno-histoquímica para determinar o status de fusão, com AP2β e HMGA2 destacados como possíveis marcadores, requerendo validação adicional.

- Reforça-se a necessidade de abordagens multidisciplinares e mais estudos para compreender e diagnosticar melhor esses tumores.

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* De acordo com as normas da UNICAMP/FOP, baseadas na padronização do International Committee of Medical Journal Editors - Vancouver Group. Abreviatura dos periódicos em conformidade com o PubMed

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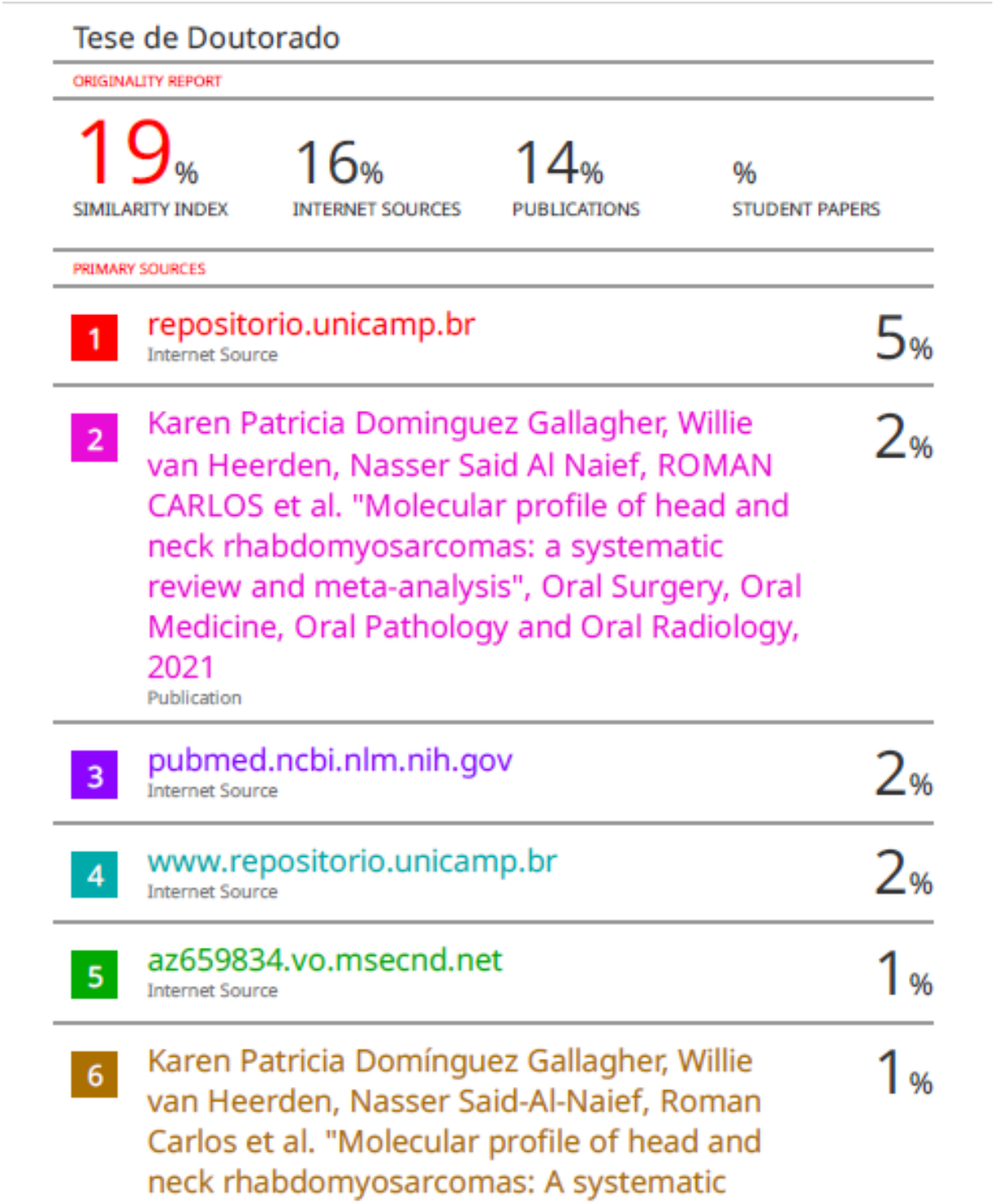
ANEXOS

Anexo 1. Relatório de verificação de originalidade e prevenção de plagio.



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Anexo 2. Certificado do Comitê de Ética em Pesquisa

UNICAMP - FACULDADE DE ODONTOLOGIA DE PIRACICABA DA UNIVERSIDADE DE CAMPINAS - FOP/UNICAMP

PARECER CONSUBSTANCIADO DO CEP

DADOS DA EMENDA

Título da Pesquisa: ANÁLISE CLINICOPATOLÓGICA, MORFOLÓGICA, IMUNOISTOQUÍMICA E MOLECULAR DOS LINFOMAS NÃO-HODGKIN E SARCOMAS DA REGIÃO DE CABEÇA E PESCOÇO EM PACIENTES PEDIÁTRICOS

Pesquisador: Lady Paola Aristizabal Arboleda

Área Temática: Genética Humana:
(Trata-se de pesquisa envolvendo Genética Humana que não necessita de análise ética por parte da CONEP;);

Versão: 4

CAAE: 12469119.6.0000.5418

Instituição Proponente: Faculdade de Odontologia de Piracicaba - Unicamp

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 5.644.936

Apresentação do Projeto:
O parecer inicial é elaborado com base na transcrição editada do conteúdo do registro do protocolo na Plataforma Brasil e dos arquivos anexados à Plataforma Brasil. Os pareceres de retorno, emendas e notificações são elaborados a partir do último parecer e dos dados e arquivos da última versão apresentada. Trata-se de SOLICITAÇÃO DE EMENDA (E1) AO PROTOCOLO originalmente aprovado em 03/06/2019 para inclusão de instituição coparticipante, para inclusão de novos pesquisadores, para atualizar os dados da pesquisadora Karen Patricia e para extender o cronograma de realização da pesquisa. O parecer foi atualizado de acordo com a documentação apresentada. A solicitação está detalhadamente descrita ao final do parecer.

A EQUIPE DE PESQUISADORES citada na capa do projeto de pesquisa, em ordem alfabética, exceto pesquisadora responsável, inclui LADY PAOLA ARISTIZABAL ARBOLEDA (Cirurgiã Dentista, Doutoranda no PPG em Estomatopatologia da FOP/UNICAMP, Pesquisadora Responsável), ALAN ROGER DOS SANTOS SILVA (Cirurgião Dentista, Docente da Área de Semiologia da FOP-UNICAMP),

Endereço: Av.Limeira 901 Caixa Postal 52
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UF: SP **Município:** PIRACICABA
Telefone: (19)2106-5349 **Fax:** (19)2106-5349 **E-mail:** cep@fop.unicamp.br

IVA LOUREIRO HOFFMANN (Médica, Médica Oncologista Pediátrica do Centro Infantil Boldrini), IZILDA APARECIDA CARDINALLI(Médica, Médica Patologista do Centro Infantil Boldrini), JOSÉ ANDRÉS YUNES (Engenheiro Agrônomo, pesquisador do Centro Infantil de Investigações Hematológicas Dr Domingos A Boldrini), KAREN PATRÍCIA DOMINGUEZ GALLAGHER (Cirurgiã Dentista, Doutoranda no PPG em Estomatopatologia da FOP/UNICAMP), LARA MARIA ALENCAR RAMOS INNOCENTINI (Cirurgiã-dentista do Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto-USP, Incluída em E1), LEANDRO DORIGAN DE MACEDO (Cirurgião-dentista do Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto-USP, Incluído em E1), REGINA MARIA HOLANDA DE MENDONÇA (Cirurgiã Dentista, Cirurgiã Dentista do Centro Infantil Boldrini, Pesquisadora Colaboradora do Departamento de Diagnóstico Bucal da FOP-UNICAMP), o que é confirmado na declaração dos pesquisadores e na PB.

DELINEAMENTO DA PESQUISA: Trata-se de estudo laboratorial, observacional, comparativo, retrospectivo, com base em arquivo, que envolverá 174 casos de indivíduos, com idades até 19 anos, presumidamente em sua maioria menores de idade, de ambos os sexos, pacientes diagnosticados e tratados no Centro Infantil Boldrini e no Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto – USP, no período de 1986 a 2018, que receberam diagnóstico de dos linfomas não Hodgkin (LNH) e sarcoma de cabeça e pescoço. As amostras serão submetidas a uma variedade de técnicas imunoistoquímicas e moleculares. O objetivo deste estudo é avaliar a morfologia, investigar translocações cromossômicas, determinar o imunofenótipo, e marcadores prognósticos dos linfomas não Hodgkin (LNH) e sarcomas de cabeça e pescoço tratados no Centro Infantil Boldrini durante o período de 1986-2018. Serão avaliados os casos com disponibilidade de bloco de parafina, para a realização de novos cortes histológicos, utilizando-se colorações de hematoxilina e eosina (H&E), assim como reações imunoistoquímicas (realizadas pelo programa de pós-graduação em Estomatopatologia da Faculdade de Odontologia de Piracicaba), utilizando os seguintes anticorpos: Vimentina, Desmina, Miogenina (MyoD1), Actina Músculo Específica (HHF35), EMA, CD99, LCA, CD20, CD79a, CD3, CD45RO, CD30CD10, Bcl6, Proteína p53, Ki67, IRF4/MUM-1 e hibridização in situ contra o vírus Epstein-Barr (EBV)(EBER). Para a investigação de translocações cromossômicas será realizado técnica de PCR (Polymerase Chain Reaction), FISH (hibridização in situ por fluorescência) e Illumina TruSight RNA Pan-Cancer Panel. Os dados demográficos e clinicopatológicos serão revisados. Por meio deste estudo espera-se gerar conhecimento sobre o

perfil clinicopatológico, morfológico, molecular e o imunofenótipo de LNH e sarcomas que acometem exclusivamente a região de cabeça e pescoço de pacientes pediátricos. Além disso, determinar se existe correlação entre os resultados desse estudo e critérios prognósticos oncológicos.

Critérios de Inclusão: •Pacientes pediátricos até 19 anos de idade com diagnóstico de LNH ou sarcomas na região de cabeça e pescoço; •Disponibilidade dos blocos de parafina dos casos de LNH e sarcomas que permitam pelo menos 10 cortes para cada caso.

Critérios de exclusão:•Casos de LNH e sarcomas em cabeça e pescoço sem presença de bloco de parafina ou com material insuficiente para realizar as colorações, técnicas moleculares e as reações imunoistoquímicas.

MATERIAIS E MÉTODOS:

Foi realizado um levantamento para identificar a casuística do câncer de cabeça e pescoço nos pacientes pediátricos sub aprovação do comitê de ética em pesquisa da mesma instituição com o CAAE: 64034217.5.0000.5376, e foram identificados 104 casos de LNH e 70 sarcomas no Centro Infantil Boldrini no período de 1986 até o ano de 2016 (Arboleda et al., 2018). Assim, baseados neste primeiro estudo, a faixa etária será caracterizada nos seguintes grupos etários: menores de 0 anos, de 1-4 anos, de 5-9 anos, de 10-14 anos e de 15-19 anos, onde a estimativa vai ser maior para o grupo dos pacientes de 5-9 anos e os pacientes de 10-14 anos, e os pacientes do gênero masculinos provavelmente terão uma maior incidência Desta maneira serão coletados os casos com disponibilidade dos blocos de parafina e levantamento complementar dos casos de tumores malignos em região de cabeça e pescoço, diagnosticados nos anos de 2017 e 2018. Os prontuários médicos serão avaliados nas áreas previamente autorizadas do Centro Infantil Boldrini, para confirmação dos dados previamente coletados e também serão revisados os prontuários médicos do Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto – USP para coletar os dados clínicos dos casos a serem incluídos. As seguintes variáveis clinicopatológicas serão analisadas: gênero, idade, localização do tumor primário, sintomatologia, tratamento, ocorrência de segundos tumores primários, recidivas, follow-up (paciente vivo ou morto), tempo de sobrevida livre de doença, e o tempo de sobrevida global.

Uma vez coletados os blocos de parafina, novos cortes histológicos de 5 µm de espessura, corados em hematoxilina e eosina, serão confeccionados no laboratório de patologia do departamento de

Diagnóstico Oral da FOP-UNICAMP para avaliação e analise histopatológico, que será realizada em conjunto com os médicos patologistas do Centro Infantil Boldrini e do Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto – USP coautores deste projeto.

Reações Imunoistoquímicas desenvolvidas no laboratório de patologia do Departamento de Diagnóstico Oral da Faculdade de Odontologia de Piracicaba (UNICAMP) serão realizadas para determinar o perfil de marcações e correlacionar com fatores prognósticos. Os anticorpos a ser utilizados são: Vimentina, Desmina, Miogenina (MyoD1), Actina Músculo Específica (HHF35), EMA, CD99, LCA, CD20, CD79a, CD3, CD45RO,CD30 CD10, Bcl6. Para avaliar a importância do prognóstico, serão testados os seguintes anticorpos: Proteína p53, KI67, IRF4/MUM-1 (Andrade et al., 2012).

As reações de hibridização in situ (HIS) também serão realizadas no laboratório de Imunoistoquímica da FOP/UNICAMP para identificação do vírus EBV ((EBER) PNA Probe/FluoresceinCode Y5200) nos casos de LNH, assim como nos demais casos onde a identificação possua valor diagnóstico (Rumayor et al., 2017).

Os casos submetidos às reações Imunoistoquímicas supracitadas serão classificados por patologistas como sendo negativos (5% de células neoplásicas positivas) ou positivos (> 5% de células neoplásicas positivas) para cada marcador (adaptações nestes valores poderão ser feitas sempre que sugerido na literatura pertinente). Para a determinação do potencial de proliferação celular mensurado pelas reações direcionadas contra KI67 será realizada a quantificação digital das marcações por meio do uso do escaneador de lâminas Aperio ScanScope CS® (Aperio Technology, Vista, CA, USA) disponível no Departamento de Diagnóstico Oral (Patologia) da Faculdade de Odontologia de Piracicaba – UNICAMP e obtido por meio de auxílio FAPESP processo 2009/53839-2. Para realização desta quantificação digital serão utilizados o software ImageScope (Aperio Technology, Vista, CA, USA) e o algoritmo Nuclear V9 (Aperio Technology, Vista, CA, USA) seguindo-se os parâmetros determinados no momento da padronização do algoritmo digital. Serão avaliadas as 10 áreas mais marcadas para determinação do índice de proliferação de cada caso

Os resultados obtidos serão então submetidos a testes estatísticos de associação, correlação e de análise de sobrevida (testes univariados e multivariados) que deverão ser selecionados no momento mais apropriado e serão desenvolvidos por um especialista em análises bioestatísticas.

As técnicas moleculares serão realizadas no laboratório de biologia molecular do Centro Infantil

Boldrini em conjunto com o Doutor José Andrés Yunes, membro da equipe em Genética e Biologia Molecular do Centro Infantil Boldrini. PCR e FISH serão realizadas com a finalidade de encontrar as translocações cromossômicas que são reportadas na literatura para certos subtipos de LNH e sarcomas, porém, para os tipos de LNH e sarcomas sem translocações cromossômicas conhecidas, será realizado uma técnica de TruSight RNA Pan-Cancer Panel, obtendo uma compreensão mais profunda dos padrões de expressão gênica e detecção de genes de fusão (Mariano et al., 2016).

Desta maneira, a importância da avaliação genética deste estudo se concentra no conhecimento de novas translocações cromossômicas e na confirmação das já existentes, para realizar um perfil mais definido destas malignidades em pacientes pediátricos com câncer, ajudando no conhecimento da patogênese, e consequentemente para o desenvolvimento e futuros estudos sobre de novos tipos de tratamentos, avaliações de prognostico, e diagnóstico precoce destas malignidades em pacientes pediátricos.

A análise dos resultados será realizada nas três instituições, Faculdade de Odontologia de Piracicaba, Centro Infantil Boldrini e o Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto – USP.

Análise dos dados: Neste estudo, as variáveis demográficas (idade, gênero, etnia), fatores de risco, aspectos topográficos (área acometida pelo tumor e estadiamento clínico), comorbidades, informações do exame físico, modalidades terapêuticas e marcadores prognósticos serão analisadas e apresentadas por meio de estatística descritiva, lançando mão de números absolutos, frequências, porcentagens, média (± desvio-padrão) ou mediana, conforme apropriado. Serão utilizados testes de associação e os desfechos clínicos serão expressos por meio do tempo para ocorrência de óbito segundo método de Kaplan-Meier por meio de curvas de sobrevida. Os dados serão analisados com auxílio do pacote software SAS (version 9.3; SAS Institute Inc., Cary, NC, USA).

RESULTADOS ESPERADOS: A consecução do presente projeto de pesquisa testará as diferenças morfológicas e imunoistoquímicas que existem nos diferentes subtipos de LNH e sarcomas localizados na região de cabeça e pescoço dos pacientes pediátricos. Além disso, provara que marcações positivas para MUM-1 e EBV nos LNH afetam o prognostico oncológico. Finalmente, com as técnicas moleculares, novas translocações cromossômicas dos sarcomas e LNH serão encontradas, o que gera um conhecimento da biologia tumoral e possíveis correlações clínicas.

Local da pesquisa: A análise dos resultados será realizada nas três instituições, Faculdade de

Odontologia de Piracicaba, Centro Infantil Boldrini e o Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto – USP.

Foram apresentados em anexo ao projeto de pesquisa os seguintes anexos: Anexo I (Linfoma não Hodgkin em cabeça e pescoço de pacientes pediátricos do Centro Infantil Boldrini), Anexo II (Sarcomas relacionados às anomalias genéticas) e Anexo III (Sarcomas em cabeça e pescoço de pacientes pediátricos do Centro Infantil Boldrini).

Pendência 1 (atendida em 20/05/19)- Os pesquisadores informam que "Foi inserida a faixa etária e distribuição por sexo dos pacientes que farão parte da pesquisa, baseados na publicação prévia sobre a distribuição demográfica e clinicopatológica do câncer de cabeça e pescoço nos pacientes pediátricos do Centro Infantil Boldrini".

Pendência 2 (atendida em 20/05/19)- Os pesquisadores informaram que "Foi modificada a lista das metodologias, associando cada uma delas no respectivo local de realização, sendo que as atividades da pesquisa serão desenvolvidas em ambas instituições (Faculdade de Odontologia de Piracicaba e Centro Infantil Boldrini)".

Pendência 3 (atendida em 20/05/19)- Quanto à publicação prévia, os pesquisadores informaram que "Foi inserido a aprovação do CEP do Centro Infantil de Investigações Hematológicas Dr.Domingos A Boldrini da publicação previa dos pesquisadores, com o CAAE: 64034217.5.0000.5376, Número do Parecer: 1.947.205".

Pendência 4 (atendida em 20/05/19)- Quanto à possível utilidade da avaliação genética para os participantes da pesquisa, os pesquisadores informaram que "O estudo abrange desde o ano 1986 até 2016, razão pela qual, a maioria absoluta dos pacientes que participarão da nossa pesquisa, é de pacientes que concluíram tratamento oncológico. Desta maneira, a importância da avaliação genética deste estudo se concentra no conhecimento de novas translocações cromossômicas e na confirmação das já existentes, para realizar um perfil mais definido destas malignidades em pacientes pediátricos com câncer, ajudando no conhecimento da patogênese, e consequentemente para o desenvolvimento e futuros estudos sobre de novos tipos de tratamentos, avaliações de prognostico, e diagnóstico precoce destas malignidades em pacientes pediátricos. Portanto não está prevista utilidade direta dos resultados para os pacientes deste estudo retrospectivo".

Pendência 5 (atendida em 20/05/19)- O cronograma proposto para a pesquisa no projeto informa que serão necessários 20 meses para conclusão do estudo. O cronograma descrito na PB indica que a pesquisa será iniciada em 01/07/2019 e será concluída em 01/03/2021, em cerca de 20

meses.

O arquivo ajustado do projeto de pesquisa, com as áreas modificadas marcadas em amarelo foi apresentado.

Pendência 1 de emenda 1 (atendida em 07/09/22): O cronograma do protocolo na E1 informa que serão necessários 48 meses para a conclusão do estudo. O cronograma descrito na PB em E1 indica que a pesquisa foi iniciada em 01/07/2019 e será concluída em 27/12/2024, em cerca de 42 meses, cerca de 22 meses de extensão de cronograma.

Objetivo da Pesquisa:

JUSTIFICATIVA: Em virtude dos LNH e Sarcomas serem os tipos de CCPPP mais comuns mundialmente, especialmente no Brasil, torna-se fundamental ter um conhecimento amplo sobre o comportamento biológico e clínico destas malignidades, principalmente porque pacientes nessa faixa etária apresentam notórias diferenças em termos prognósticos, gerando um verdadeiro desafio no diagnóstico oportuno e manejo satisfatório, causando um impacto negativo na qualidade de vida dos pacientes afetados. Grandes séries de casos investigando as características clinicopatológicas, morfológicos e imunoistoquímicos dos LNH e sarcomas acometendo a região de cabeça e pescoço nos pacientes pediátricos são raras, o que dificulta a compreensão das caraterísticas clinicopatológicas destas condições.

HIPÓTESE: A consecução do presente projeto de pesquisa testará a hipótese de que existem diferenças morfológicas e imunoistoquímicas nos diferentes subtipos de LNH e sarcomas localizados na região de cabeça e pescoço dos pacientes pediátricos. Além disso, provara que marcações positivas para MUM-1 e EBV nos LNH afetam o prognostico oncológico. Finalmente, com as técnicas moleculares, novas translocações cromossômicas dos sarcomas e LNH serão encontradas, o que gera um conhecimento da biologia tumoral e possíveis correlações clínicas.

OBJETIVO PRIMÁRIO: Este estudo tem como objetivo determinar a morfologia, as características moleculares e o perfil imunoistoquímico dos LNH e sarcomas localizados na região de cabeça e pescoço de pacientes pediátricos, criando padrões que facilitem o diagnóstico precoce e, consequentemente, o prognóstico e a qualidade de vida. Além disso, propõe-se a analisar os marcadores prognósticos correlacionados com as características do estadiamento oncológico e de sobrevida dos pacientes estudados.

OBJETIVOS SECUNDÁRIOS: •Descrever as características morfológicas dos LNH e dos sarcomas em

cabeça e pescoço de pacientes pediátricos; •Determinar a existência de translocações cromossômicas por meio de técnicas moleculares dos LNH e dos sarcomas em cabeça e pescoço de pacientes pediátricos; •Determinar o imunofenótipo de cada subtipo histopatológico de LNH e sarcomas em cabeça e pescoço de pacientes pediátricos; •Avaliar o impacto das características morfológicas e imunoistoquímicas na sobrevida de pacientes pediátricos com LNH e sarcomas de cabeça e pescoço; •Determinar se os marcadores MUM1, Ki-67 e p-53 influenciam na sobrevida dos pacientes afetados por LNH em cabeça e pescoço de pacientes pediátricos; •Avaliar os padrões de marcação de EBV nos LNH em cabeça e pescoço de pacientes pediátricos por meio da hibridização in situ.

Avaliação dos Riscos e Benefícios:

Pendência 6 (atendida em 20/05/19)-Quanto aos riscos e desconfortos previstos para os participantes,os pesquisadores informaram que "Como o presente projeto é retrospectivo, as informações serão obtidas a partir de registros de prontuários, e as análises serão realizadas nos blocos histopatológicos sem expor o paciente, consideramos esse estudo como de baixo risco".

Pendência 7 (atendida em 20/05/19)-Quanto aos benefícios diretos previstos para os participantes, os pesquisadores informaram que "A importância da avaliação genética deste estudo se concentra no conhecimento de novas translocações cromossômicas e na confirmação das já existentes, para realizar um perfil mais definido destas malignidades em pacientes pediátricos com câncer, ajudando no conhecimento da patogênese, e consequentemente para o desenvolvimento e futuros estudos sobre de novos tipos de tratamentos, avaliações de prognostico, e diagnóstico precoce destas malignidades em pacientes pediátricos. Portanto não está prevista utilidade direta dos resultados para os pacientes deste estudo retrospectivo".

O arquivo com os comentários éticos ajustados, com as áreas modificadas marcadas em amarelo foi apresentado.

Comentários e Considerações sobre a Pesquisa:

COMENTÁRIO-Quanto ao modo de abordagem dos participantes da pesquisa para a obtenção do TCLE os pesquisadores informaram no arquivo "comentários" que "O presente projeto utilizará todas as informações já coletadas nos prontuários médicos, zelando-se por preservar a identidade dos pacientes elegíveis por meio das iniciais do nome dos pacientes e um número de registro, que

será vinculado ao número do prontuário. Serão utilizados dados secundários a partir do estudo de material já coletado para fins diagnósticos (blocos histopatológicos); portanto, não haverá participação direta de pacientes que possa gerar desconforto ou risco aos mesmos. Desta maneira, solicitaremos ao Comitê de Ética em Pesquisa a dispensa do Termo de Consentimento Livre e Esclarecido". Já no registro do protocolo na PB informaram que "Propõe dispensa do TCLE? Sim. Justificativa: Todas as informações coletadas dos prontuários médicos provenientes do Centro Infantil Boldrini serão colhidas, analisados e publicados zelando -se por preservar a identidade dos pacientes elegíveis por meio das iniciais do nome dos pacientes e um número de registro, que será vinculado ao número do prontuário. Serão rigorosamente cumpridos todos os aspectos éticos, especificamente determinados pelas diretrizes e normas da Resolução 466/12, do Conselho Nacional de Saúde. Como as análises do presente estudo retrospectivo serão realizadas nos blocos histopatológicos, e as informações necessárias serão obtidas a partir de registros de prontuários, sem expor o paciente a riscos, solicitaremos ao Comitê de Ética em Pesquisa do Centro Infantil Boldrini a dispensa do Termo de Consentimento Livre e Esclarecido". Considerando que os pacientes são em sua totalidade do Centro Infantil Boldrini, que tem um CEP associado, que avaliará este protocolo na sequência da aprovação pelo CEP-FOP, considera-se mais adequado que a decisão de permitir o uso dos dados e das amostras com ou sem a aplicação do Consentimento aos pacientes e seus responsáveis esteja a cargo do CEP do Centro Infantil Boldrini, que assumirá a responsabilidade presente e futura da decisão. Destaca-se que o posicionamento corrente do CEP-FOP sobre a dispensa da aplicação do Consentimento em pesquisas que utilizam amostras e dados de arquivo só é favorável com a justificativa ética fundamentada na proteção do participante e não fundamentada na conveniência dos pesquisadores.

Quanto à justificativa para participação de grupos vulneráveis os pesquisadores informaram que "O presente estudo utilizará o material proveniente do Centro Infantil Boldrini que é especializado no atendimento de crianças e adolescentes, desta maneira, os pacientes menores de idade serão vulneráveis ao estudo; no entanto, como as análises do presente projeto serão realizadas nos blocos histopatológicos, e as informações necessárias serão obtidas a partir de registros de prontuários, sem expor o paciente a riscos, solicitaremos ao Comitê de Ética em Pesquisa do Centro Infantil Boldrini a dispensa do Termo de Consentimento Livre e Esclarecido".

Quanto às medidas de proteção à confidencialidade os pesquisadores informaram que "Serão rigorosamente cumpridos todos os aspectos éticos, especificamente determinados pelas diretrizes

e normas da Resolução 466/12, do Conselho Nacional de Saúde. Todas as informações coletadas dos prontuários médicos serão colhidas, analisados e publicados zelando-se por preservar a identidade dos pacientes elegíveis por meio das iniciais do nome dos pacientes e um número de registro, que será vinculado ao número do prontuário".

Quanto à previsão de ressarcimento de gastos os pesquisadores informaram que "Não haverá previsão de ressarcimento, pois a participação na pesquisa não causará despesas adicionais aos voluntários. Todas as etapas que serão realizadas no estudo e as despesas desta pesquisa será financiada por agências de fomento em pesquisa".

Quanto à previsão de indenização e/ou reparação de danosos pesquisadores informaramque "Não haverá previsão de indenização e/ou reparação de danos, pois não há risco previsível pela participação na pesquisa".

Quanto aos critérios para suspender ou encerrar a pesquisa os pesquisadores informaram que "Não há previsão de suspensão da pesquisa e que a mesma será encerrada quando as informações desejadas forem obtidas, ou seja, ao final dos experimentos. Sob nenhum pretexto haverá suspensão ou encerramento da pesquisa sem a apresentação de resultados que retroalimentem a pesquisa".

Pendência 8 (atendida em 20/05/19)- Quanto às medidas para proteção ou minimização dos desconfortos e riscos previsíveis os pesquisadores informaram que "Não existe propriamente desconfortos ou riscos para os participantes".

O arquivo com os comentários éticos ajustados, com as áreas modificadas marcadas em amarelo foi apresentado.

Considerações sobre os Termos de apresentação obrigatória:

A FR foi apresentada preenchida (174 participantes, sem patrocinador principal) e assinada pela pesquisadora responsável (Dra Lady Paola Aristizabal Arboleda) e pelo Diretor da FOP-UNICAMP (Dr. Francisco Haiter Neto).

A capa do projeto cita os dados solicitados pelo CEP-FOP.

Foi apresentada a declaração dos pesquisadores, adequadamente preenchida e assinada.

Em 03/08/2022 (E1) foi apresentada a declaração dos pesquisadores, adequadamente preenchida e assinada por todos os pesquisadores.

COMENTÁRIO -Foi apresentada a declaração da instituição, adequadamente preenchida e assinada.

Destaca-se que não foram listados todos os nomes dos pesquisadores no documento, mas o mesmo mantém seu aspecto legal e ético no contexto do protocolo.

COMENTÁRIO -Foi apresentada a anuência do Centro Infantil Boldrini para a realização da pesquisa, assinada pela Dra Silvia Regina Brandalise (Presidente do Centro Infantil Boldrini) e pela Dra Regina Maria Holanda de Mendonça (Departamento de Odontologia do Centro Infantil Boldrini). Destaca-se que não foram listados todos os nomes dos pesquisadores no documento, mas o mesmo mantém seu aspecto legal e ético no contexto do protocolo. Caso o CEP do Centro Infantil de Investigações Hematológicas Dr. Domingos A Boldrini considere necessário, obviamente solicitará o ajuste do documento.

Foi apresentada a Declaração da Instituição Coparticipante assinada pela Dra Silvia Regina Brandalise (Presidente do Centro Infantil Boldrini).

Foi apresentada a justificativa para a não aplicação do consentimento aos pacientes/participantes e seus responsáveis, como descrito acima.

O orçamento descrito na PB informa que a pesquisa terá custo de R$ 49.800,00,para aquisição de material de laboratório e transporte,e que será bancada pelos pesquisadores.

A pesquisa foi classificada nas Grandes Áreas 2 e 4 (Ciências Biológicas e Ciências da Saúde) e tem como título público "ANÁLISE CLINICOPATOLÓGICA, MORFOLÓGICA, IMUNOISTOQUÍMICA E MOLECULAR DOS LINFOMAS NÃO-HODGKIN E SARCOMAS DA REGIÃO DE CABEÇA E PESCOÇO EM PACIENTES PEDIÁTRICOS".

A Instituição proponente da pesquisa é a Faculdade de Odontologia de Piracicaba – Unicamp.

O Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto da USP – HCFMRP e o Centro Infantil de Investigações Hematológicas Dr.Domingos A Boldrini foram listados Instituições Coparticipantes. Os CEPs das Instituições Coparticipantes avaliarão o protocolo após a aprovação do pelo CEP-FOP e a pesquisa só deve ser iniciada após a aprovação daqueles CEPs.

Pendência 9 (atendida em 20/05/19)-Foi apresentado o regulamento de biorrepositório para as amostras. O texto informa que as amostras serão todas oriundas do Centro Boldrini e que serão retornadas aos arquivos daquele centro ao final da pesquisa.

Pendência 10 (atendida em 20/05/19)- A pesquisa foi classificada na área temática especial " "Genética Humana: (Trata-se de pesquisa envolvendo Genética Humana que não necessita de análise ética por parte da CONEP;".

Em 03/08/2022 os PESQUISADORES SOLICITARAM EMENDA E1 AO PROTOCOLO para as seguintes

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alterações: "1- Incluir como Instituição coparticipante do projeto ao Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto – USP, pois contribuirão com casos de rabdomiossarcoma de cabeça e pescoço em crianças que serão incluídos na amostra do estudo. 2- Incluir à equipe do projeto como pesquisadores colaboradores aos seguintes profissionais: a Dra. Lara Maria Alencar Ramos Innocentini - CPF 011.023.501-09 e o Dr. Leandro Dorigan de Macedo – CPF 01276493606, pois eles, selecionarão os casos de sarcomas da região de cabeça e pescoço em crianças, e ajudarão com a coleta de dados clínico patológicos dos prontuários médicos dos pacientes pediátricos com sarcomas de cabeça e pescoço do Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto – USP. Também serão os responsáveis de encaminhar as lâminas em branco dos casos selecionados para o laboratório de Patologia Oral da FOP-UNICAMP. 3- Atualizar os dados da pesquisadora Karen Patricia Dominguez Gallagher de nacionalidade Paraguaia com RNE: F087222-1".

Os pesquisadores informaram ainda que "4- O relatório de atividades parciais já foi incluído na Plataforma Brasil. 5- Não há outras modificações no protocolo, exceto as acima listadas".

Foi apresentado relatório parcial de atividades da pesquisa, que informa estar a pesquisa em andamento, com previsão de término para 22/12/2024, que já foram incluídos 36 casos de rabdomiossarcomas e 52 linfomas não-Hodgkin, que não houve intercorrência com os participantes e que os resultados parciais não foram publicados nem apresentados em Congressos Científicos.

Na resposta de 07/09/2022 os pesquisadores solicitaram a extensão do cronograma como se segue "Devido à pandemia, o Cronograma do projeto teve que ser modificado porque no ano 2020 e inícios de 2021 o acesso aos arquivos era restrito o que dificultou a coleta dos casos e revisão dos prontuários para incluir na pesquisa. Consequentemente, tivemos que postergar os trabalhos de laboratório como colorações histoquímicas e imuno-histoquímicas e também a análise microscópica dos casos. Considerando o mencionado anteriormente o Cronograma foi alterado no projeto e na PB".

Pendência 2 de emenda 1 (atendida em 07/09/22): Foi apresentado o regulamento de biorepositório ajustado para a nova instituição.

Pendência 3 de emenda 1 (atendida em 07/09/22): Foi apresentada a autorização de acesso e uso aos arquivos do Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto – USP, assinada pelo Dr. Hilton Marcos Alves Rics.

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Recomendações:

As recomendações a seguir não são pendências e podem ou não ser aplicáveis ao protocolo em tela. Não há necessidade de resposta às mesmas. RECOMENDAÇÃO 1- É obrigação do pesquisador desenvolver o projeto de pesquisa em completa conformidade com a proposta apresentada ao CEP. Mudanças que venham a ser necessárias após a aprovação pelo CEP devem ser comunicadas na forma de emendas ao protocolo por meio da PB. RECOMENDAÇÃO 2- Após a aprovação do protocolo de pesquisa os pesquisadores devem atentar para a necessidade de envio de relatórios parciais de atividade (no mínimo um a cada 12 meses) e do relatório final de atividade (ao término da pesquisa). Os pesquisadores devem informar e justificar ao CEP a eventual necessidade de interrupção ou interrupção total ou parcial da pesquisa. RECOMENDAÇÃO 3- Reforça-se a necessidade do registro de Biorepositórios para as amostras biológicas coletadas e que não sejam de uso imediato. A intenção deve ser registrada no projeto, no Regulamento do Biorepositório e no TCLE que será assinado pelo participante. RECOMENDAÇÃO 4- Os pesquisadores devem atentar para a necessidade de aplicação de TCLE para coleta de amostras a serem estocadas em Biobancos e Biorepositórios e para a necessidade de aplicação de novo TCLE quando da realização de novas pesquisas com o material estocado. RECOMENDAÇÃO 5- Pesquisas com dentes doados por profissionais de saúde ainda são toleradas em hipótese pelo CEP-FOP, mas os pesquisadores devem estar cientes de que esta solução dista do ideal ético de consulta direta ao participante por meio de TCLE específico da pesquisa ou da obtenção dos dentes a partir de um Biobanco de dentes e que estas últimas situações deveriam ser escolhidas em substituição à primeira. RECOMENDAÇÃO 6- Os pesquisadores devem manter os arquivos de fichas, termos, dados e amostras sob sua guarda por pelo menos 5 anos após o término da pesquisa. RECOMENDAÇÃO 7- Destaca-se que o parecer consubstanciado é o documento oficial de aprovação do sistema CEP/CONEP e os certificados emitidos pela secretaria do CEP-FOP, a pedido, após a aprovação final do protocolo, só têm valor simbólico e devem ser evitados. RECOMENDAÇÃO 8- Intercorrências e eventos adversos devem ser relatados ao CEP-FOP por meio da PB. RECOMENDAÇÃO 9- Os pesquisadores devem encaminhar os resultados da pesquisa para publicação e divulgação, com devido crédito a todos que tenham colaborado com a realização da pesquisa. RECOMENDAÇÃO 10- O parecer do CEP-FOP é fortemente baseado nos textos do protocolo encaminhado pelos pesquisadores e pode conter inclusive trechos transcritos literalmente do projeto ou de outras partes do protocolo. Trata-se, ainda assim, de uma interpretação do protocolo. Caso algum trecho do parecer não corresponda ao que efetivamente foi proposto no protocolo, os pesquisadores

devem se manifestar sobre esta discrepância. A não manifestação dos pesquisadores será interpretada como concordância com a fidedignidade do texto do parecer no tocante à proposta do protocolo.

Conclusões ou Pendências e Lista de Inadequações:

Não há mais pendências por resolver em relação à solicitação de emenda E1 (vide texto acima).

Considerações Finais a critério do CEP:

Parecer de aprovação de Emenda a protocolo emitido "ad referendum" conforme autorização do Colegiado na reunião de 02/02/2022. O parecer será submetido para homologação na reunião de 05/10/2022.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_1913124_E1.pdf	07/09/2022 19:30:05		Aceito
Declaração de Instituição e Infraestrutura	acesso_arquivo_HCUSP_emenda.pdf	07/09/2022 19:27:56	Karen Patricia Dominguez Gallagher	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_modificado_emenda.pdf	07/09/2022 19:27:37	Karen Patricia Dominguez Gallagher	Aceito
Cronograma	cronograma_modificado_emenda.pdf	07/09/2022 19:24:52	Karen Patricia Dominguez Gallagher	Aceito
Declaração de Manuseio Material Biológico / Biorepositório / Biobanco	Biorrepositorio_modificado_emenda.pdf	07/09/2022 19:24:39	Karen Patricia Dominguez Gallagher	Aceito
Outros	carta_resposta_emenda.pdf	07/09/2022 19:24:17	Karen Patricia Dominguez Gallagher	Aceito
Outros	Justificativa_Emenda.pdf	03/08/2022 18:44:12	Lady Paola Aristizabal Arboleda	Aceito
Outros	Relatorio_Parcial.pdf	03/08/2022 18:42:04	Lady Paola Aristizabal	Aceito

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Continuação do Parecer: 5.644.928

Outros	Relatorio_Parcial.pdf	03/06/2022 18:42:04	Arboleda	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_Emenda.pdf	03/06/2022 18:39:32	Lady Paola Arbildzabal Arboleda	Aceito
Declaração de Pesquisadores	51DeclaraPesquisadores_Emenda.pdf	03/06/2022 18:39:06	Lady Paola Arbildzabal Arboleda	Aceito
Outros	carta_resposta_parecer.pdf	20/05/2019 23:27:38	Lady Paola Arbildzabal Arboleda	Aceito
Declaração de Manuseio Material Biológico / Biorepositório / Biobanco	Regulamento_biorrepositorio.pdf	20/05/2019 23:25:49	Lady Paola Arbildzabal Arboleda	Aceito
Outros	3Comentarios.pdf	20/05/2019 23:25:04	Lady Paola Arbildzabal Arboleda	Aceito
Outros	CEPcompleto.pdf	24/04/2019 11:20:17	Leny Cecilia Faro Pereira	Aceito
Outros	61AnexoDeclara.pdf	23/04/2019 19:32:35	Lady Paola Arbildzabal Arboleda	Aceito
Outros	61AnexoCarta.pdf	23/04/2019 19:31:39	Lady Paola Arbildzabal Arboleda	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	4TCLE.pdf	23/04/2019 19:17:26	Lady Paola Arbildzabal Arboleda	Aceito
Declaração de Instituição e Infraestrutura	52DeclaraInstituicao.pdf	23/04/2019 19:15:51	Lady Paola Arbildzabal Arboleda	Aceito
Folha de Rosto	1Folhaderosto.pdf	23/04/2019 19:13:14	Lady Paola Arbildzabal Arboleda	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

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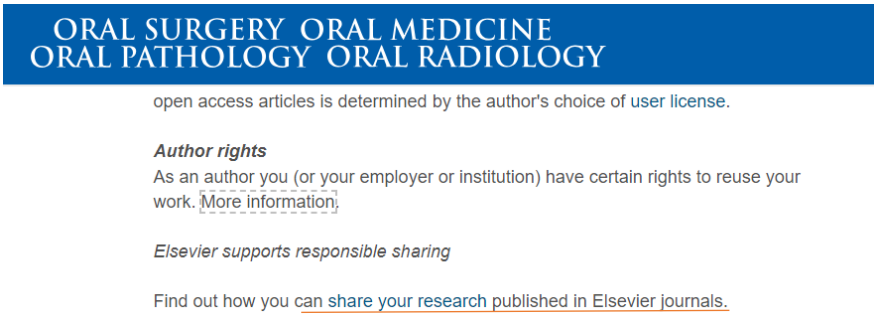
Continuação do Parecer: 5.644.936

PIRACICABA, 15 de Setembro de 2022

Assinado por:
Jacks Jorge Junior
(Coordenador(a))

Anexo 3. Autorização da Editora - Artigo 1.



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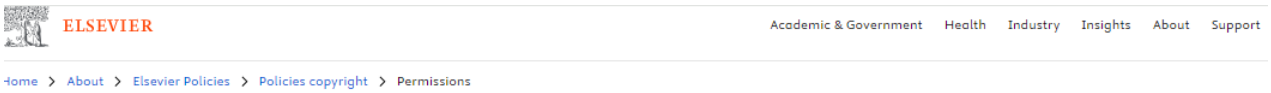

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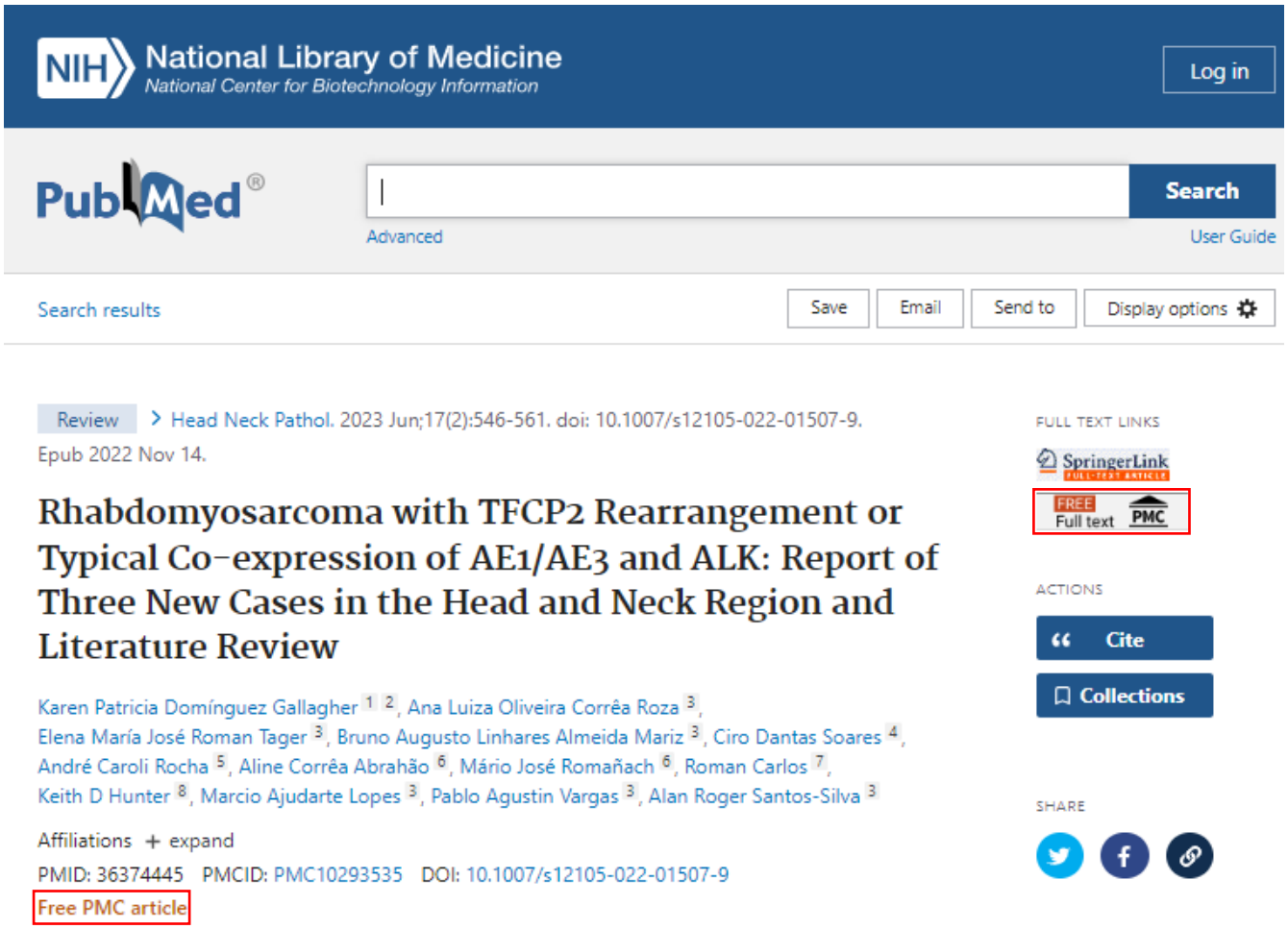
Anexo 4. Free full text - Artigo 2



Anexo 5. Comrpovante de submissão - Artigo 3

Journal of Oral Pathology & Medicine
Original Article

Head and neck rhabdomyosarcoma in pediatric patients: an international collaborative study

Submission Status	Submitted
Submitted On	15 January 2024 by Karen Gallagher
Submission Started	13 January 2024 by Karen Gallagher

This submission has been sent to the editorial office and cannot be edited. Further information will be emailed to you by the journal editorial office.

Submission overview →