

## ARTICLE

## Esthesioneuroblastoma – a Clinicopathologic Study and Role of DNA Topoisomerase Alpha

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Esthesioneuroblastoma (ENB) differs from adrenal neuroblastomas in its histopathologic and biologic characteristics. Hyams grading and Kadish staging have shown correlation with survival. Scant data are available on proliferation indices and prognosis. We retrospectively reviewed the clinicopathologic characteristics of ENB. Both Kadish and UCLA staging systems were used. Hyams grading was simplified into low and high grade. DNA topoisomerase II alpha labeling index (T2 $\alpha$  LI) was obtained in 8 cases using immunohistochemistry. Of the 19 cases studied, 14 were males and 5 females. Age range was 2 to 62 years (average 27 years). The mass primarily involved the nose in 12 (63%) and paranasal sinuses in 7 cases (37%). Patients presented with nose block in 19 (100%), epistaxis in 10 (53%), proptosis in 9 (47%) and loss of vision in 6 cases (32%). Bony involvement was seen in 7 cases (37%), and intracranial spread in one case (5%). Thirteen (68%) were low-grade tumors

and 6 were (32%) high-grade. There was no statistically significant difference between the low- and high-grade ENB in age (years) ( $p=0.2882$ ), duration of symptoms (months) ( $p=0.5636$ ), and either in the Kadish ( $p=0.5456$ ) or the UCLA staging system ( $p=0.7771$ ). The difference in DNA topoisomerase alpha labeling index between the low- and high-grade ENB (medians: 10.4 and 22.3, respectively) was not statistically significant ( $p=0.0714$ ), but it was suggestive of a positive association. The results of this study should be interpreted with caution, because of the limited sample size. Three cases recurred locally, one each stage A, B and C, but all low-grade. This preliminary study suggests the need to combine a simplified histologic grading with accurate staging in a reasonable attempt to assess local progression in esthesioneuroblastoma. Larger studies may clarify the role of T2 $\alpha$  LI in improving histologic grading. (Pathology Oncology Research Vol 13, No 2, 123–129)

*Key words:* esthesioneuroblastoma, topoisomerase alpha

### Introduction

Esthesioneuroblastoma (ENB), a rare distinctive malignant tumor of the sinonasal region, deriving from neuroendocrine cells of olfactory epithelium, accounts for about 3-6% of tumors in this region. About 1000 cases have been described in the literature since its first descrip-

tion by Berger and Luc in 1924.<sup>3,9,12,15,20,24,30</sup> The tumor is well known for its rarity, variety of symptoms, rapid progression and delayed detection, making it difficult to reach a consensus on an ideal staging system, prognostic evaluation and best treatment modalities. Although histologically similar, ENB differs from neuroblastomas arising in adrenal or sympathetic nervous system in genetic aspects such as absence of MYCN amplification, 1p deletion or neurotrophin receptor and tyrosine hydroxylase expression.<sup>12</sup> The initial claim of a possible genetic relationship to Ewings/PNET has not been confirmed in recent studies.<sup>1,23</sup> Thus, even the cell of origin and histopathologic classification are issues of continuing debate.<sup>6,10,12,14,18</sup>

Esthesioneuroblastomas can be usefully graded using a scheme developed by Hyams, based on histologic features.<sup>13</sup>

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This system has been found to correlate with prognosis in most studies. Several staging systems have been developed with variable prognostic utility.<sup>4,12</sup> Kadish system has been the most commonly used staging system with strong prognostic correlation.<sup>16</sup> Although proliferation indices have been used successfully in prognostication of primary central nervous system tumors and systemic cancer, very scant data are available on esthesioneuroblastomas.<sup>14,25,32</sup> Topoisomerase II alpha, a critical enzyme in DNA replication and maintenance of genomic stability is required for chromosomal segregation during mitosis, and is thus used as a marker of cellular proliferation in many tumors such as breast carcinoma, glioblastoma and oligodendroglioma.<sup>11,28,29</sup> It is also a marker of chemosensitivity, since it is targeted by many chemotherapeutic agents. This single institutional study reviews the clinicopathologic features of esthesioneuroblastoma, and to the best of our knowledge for the first time attempts to determine the usefulness of DNA topoisomerase II alpha (T2 $\alpha$ ) as a proliferation marker in ENB.

### Materials and Methods

#### Histopathology and grading

In the present study, we retrospectively reviewed the cases of esthesioneuroblastoma diagnosed at the pathology department at the All India Institute of medical Sciences, New Delhi, from 1989 to 2002. H&E-stained sections were studied and reviewed with particular emphasis on the histologic criteria proposed by Hyams<sup>13</sup> in his grading scheme (Table 1). Hyams grading was modified to divide cases into low- and high-grade ones, putting maximum emphasis on necrosis and nuclear atypia.

#### Immunohistochemical staining

Immunohistochemical staining was performed using streptavidin-biotin technique. Main steps included antigen retrieval where applicable, hydrogen peroxide blocking in methanol, overnight incubation with primary antibody, followed by about 30-minute incubations with the biotinylated secondary antibody and streptavidin, followed by developing the chromogen (DAB), light counterstaining with hematoxylin, dehydration, clearing and mounting. Appropriate neuronal (synaptophysin, chromogranin, NSE, MIC2), epithelial (cytokeratin AE1/AE3) and mesenchymal markers (actin, desmin, S100) were studied as applicable to individual cases. In addition, immunostaining for DNA topoisomerase II alpha (M/S DAKO, 1:100), a proliferation-associated nuclear antigen, was obtained in 8 cases in which paraffin blocks were available and contained sufficient representative tissue for evaluation. Microwave antigen retrieval was performed for DNA topoisomerase II alpha using Tris-EDTA buffer at pH 8.9 for 30 minutes prior to incubation with the primary anti-

body. The rest of the method was the same as above. DNA topoisomerase II alpha labeling index (T2 $\alpha$  LI) was obtained by visual counting of the positively stained nuclei in the most densely stained areas (hotspots) and calculating their percentage.

#### Clinical staging

Staging was obtained using data from histopathologic evaluations as well as from clinical records wherever available. Each case was allotted a stage according to both Kadish (modified by Morita)<sup>16,27</sup> and UCLA systems<sup>12</sup> (Table 1). Follow-up data was obtained from the clinical records at our Institute.

#### Statistical analysis

The endpoint of interest in this study was DNA topoisomerase II alpha labeling index (T2 $\alpha$  LI). We analyzed the effect of grade on DNA topoisomerase T2 $\alpha$  LI, age (years), sex, duration of symptoms (months), presence of epistaxis, proptosis (or loss of vision), clinical diagnoses (malignant vs. benign), bone involvement, brain involvement, Kadish stage (4 groups), and UCLA stage (4 groups).

Due to the limited sample size, the exact Wilcoxon non-parametric test was used for comparing the distribution of DNA topoisomerase II alpha labeling indices between the

**Table 1. Summary of criteria for Hyams histologic grading, Morita modification of Kadish staging and UCLA staging systems**

Histologic criteria	Grade I	Grade II	Grade III	Grade IV
Lobular architecture	++	+	-	-
Neuropil (stroma and islands)	++	+	+/-	-
Rosettes	+/-	+/-	+/-	-
Necrosis	-	-	+	++
Nuclear pleomorphism	-	+	++	+++

Stage A – tumor involving nasal cavity

Stage B – tumor involving paranasal sinuses

Stage C – tumor extending beyond nasal and paranasal sinuses to involve cribriform plate, skull base, orbit or intracranial cavity

Stage D (Morita modification) – tumor metastatic to cervical lymph nodes or distant sites

T1 – tumor involving nasal cavity and/or paranasal sinuses (excluding the sphenoid and sparing the most superior ethmoidal cells)

T2 – T1 including sphenoid, extending or eroding the cribriform plate

T3 – tumor extending to orbit or protruding into anterior cranial fossa

T4 – tumor involving the brain

**Table 2. Age and symptom duration in cases of low- and high-grade esthesioneuroblastoma (results of two-sample t-test)**

Variables		Low-grade (N=13)	High-grade (N=6)	p-value
Age (years):	Range	15.0-62.0	2.0-35.0	0.2882
	Mean $\pm$ SD	29.8 $\pm$ 14.3	22.3 $\pm$ 12.3	
Symptom duration (months):	Range	1.0-24.0	2.0-48.0	0.5636
	Mean $\pm$ SD	7.2 $\pm$ 6.4	11.8 $\pm$ 17.8	

low- and high-grade ENB. Fisher's exact test was used to examine differences between low-grade and high-grade ENB for each categorical variable, because the assumptions for the chi-square test were violated. Two-sample t-test was used for continuous variables. Statistical significance was assessed using an alpha level of 0.05. Statistical analyses were performed using SAS 9.1.3.

## Results

### Clinical data

Nineteen cases with adequate clinical and pathologic data were identified. The age range was 2 to 62 years with an average of 27 years (Table 2). There were 14 males and 5 females. The duration of symptoms ranged from 1 to 48 months with an average of 9 months (Table 2). All cases of ENB were clinically thought to be malignant, except one case that was thought to be an inflammatory mass. The mass primarily involved the nose in 12 cases (63%) and paranasal sinuses in 7 cases (37%). Patients commonly presented with nose block in 19 (100%), epistaxis in 10 (53%), proptosis in 9 (47%), loss of vision in 6 (32%) and headache in 8 cases (40%). Bony involvement was seen in 7 cases (37%), and one (5%) had intracranial involvement radiologically (Table 3). The patients were staged using both the conventional Kadish<sup>14</sup> as well as the UCLA<sup>8</sup> staging systems (Table 3).

### Histopathology and grading

Thirteen tumors were low-grade (Hyams grade I and II) (Fig. 1c), and six were high-grade (Hyams grade III and IV). Low-grade cases had a relatively well-formed lobular architecture, fibrillary background, with relatively well-formed neuropil islands both within and at the periphery of the lobules (Fig. 1a). Mitoses were occasional and a few rosettes were seen. No necrosis was identified. All high-grade cases showed tumor necrosis (Fig. 1b), in addition to nuclear atypia and high mitotic rate, along with sparse fibrillary background, rosettes and neuropil islands. A majority of the low-grade cases showed less than 5 mitoses per 10 high-power fields, in contrast to high-grade cases. Expression of neuronal markers was heterogeneous, with neuron-specific enolase and synaptophysin being positive in the

majority of tumor cells. Staining for S100 and chromogranin showed focal reactivity. MIC2 and cytokeratin were negative (except one case each). Strong nuclear immunoreactivity for DNA topoisomerase II alpha was noted in both low-grade (Fig. 2a) and high-grade (Fig. 2b) cases.

### Clinicopathologic correlation

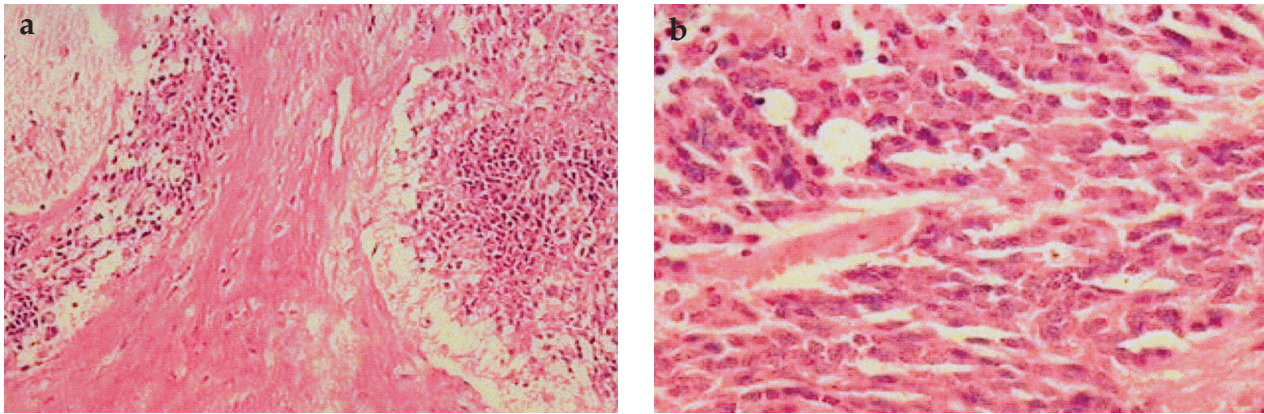
High-grade tumors occurred at a slightly younger age, although the difference was not statistically significant (Table 2). There was no statistically significant difference between the low- and high grade ENB in the duration of symptoms either (Table 2). The common symptoms were proportionately distributed in the two groups (Table 3). The majority of cases in Kadish stage C and UCLA stages T3 and T4 were low-grade cases, however, there was no statistically significant difference between low- and high-grade ENB for either the Kadish or the UCLA staging system (Table 3).

### DNA topoisomerase

Nuclear positivity was detected in all the limited subset of cases studied, with appropriate controls (Fig. 2a,b). The difference between the low-grade (median=10.4) and high-grade ENB (median=22.3) for DNA topoisomerase alpha labeling index was not statistically significant (p=0.0714) (Table 4), but was suggestive of a positive

**Table 3. Sex, epistaxis, proptosis (or loss of vision), bone or brain involvement, Kadish and UCLA stage in cases of low- and high-grade esthesioneuroblastoma (results of Fisher's exact test)**

Variables	Low-grade (N= 13)	High-grade (N=6)	p-value
Sex: male (vs. female)	10 (3)	4 (2)	1.0000
Epistaxis (yes)	5	5	0.1409
Proptosis (yes) or <vision	7	2	0.6285
Bone involvement (yes)	6	1	0.3331
Brain involvement (yes)	1	0	1.0000
Kadish stage: A	3	3	0.5456
B	2	1	
C	8	2	
D	0	0	
UCLA stage: T1	3	3	0.7771
T2	2	1	
T3	7	2	
T4	1	0	



**Figure 1.** (a) Low-grade ENB; two lobules of tumor separated by a thin fibrovascular septum. Well-formed neuropil-like fine fibrillary islands are seen within the tumor, both inside the lobules as well as at the periphery of the lobules (H&E, x100). (b) High-grade ENB; tumor showing greater cellularity, moderately pleomorphic focally overlapping nuclei with coarse chromatin. The fine fibrillary background is seen in focal areas. Necrosis is noted within the lobules (H&E, x200)

association. It should be pointed out that the results in this study should be interpreted with caution, because of the limited sample size.

#### Follow-up

Limited follow-up data are available on 6 patients for up to 5 years. Three cases showed recurrence at 3, 6 and 6 months following initial diagnosis and treatment. The case with recurrence at 3 months had involvement of both orbits and left frontal lobe at presentation. One case with first recurrence at 6 months showed a second recurrence at 18 months interval. All the cases that have recurred so far have been low-grade on histology but one each in Kadish stages A, B and C.

#### Discussion

The prognosis of esthesioneuroblastomas is generally determined by a combination of clinical and pathologic factors. The factors that have a negatively impact on prognosis include advanced stage, younger age, high grade of differentiation, positivity of resection margins, regional nodal and distant metastases, high proliferation indices, p53 overexpression, and a recently described deletion of chromosome 11 and gain on 1p.<sup>2,6,12,19,22,26</sup> The present study reviews the clinicopathologic features of esthesioneuroblastoma and attempts to determine the usefulness of DNA topoisomerase II alpha (T2 $\alpha$ ) as a proliferation marker in ENB.

#### Histologic grading

Hyams developed a histologic grading system of esthesioneuroblastoma,<sup>13</sup> using 4 grades based upon lobular architecture, fibrillary matrix, rosettes, calcification,

mitoses and necrosis. It was found that grade I patients had a uniformly good outcome whereas grade IV behaved considerably worse.<sup>13</sup> Grade I and II are well-differentiated tumors and are relatively easily identified, whereas grade III and IV are sometimes difficult to differentiate from other high-grade tumors of the sinonasal tract such as sinonasal undifferentiated carcinoma (SNUC) or sinonasal neuroendocrine carcinoma (SNEC), thereby affecting the number of patients in different grades.<sup>10,18,30</sup> It is, however, important to differentiate ENB from non-ENB neuroendocrine tumors of the sinonasal region because of better overall survival and local control of the former.<sup>30</sup> About half of the cases of ENB are generally high-grade.<sup>5</sup> In the series by Levine et al from the University of Virginia, 80% of tumors were low-grade, possibly because some of the grade-IV esthesioneuroblastomas were classified as SNUC.<sup>18</sup> They did not advocate the Hyams grading, but preferred Kadish staging system, an observation also noted in other studies.<sup>2,7</sup> In another series, only 28% of cases were low-grade.<sup>26</sup>

Several authors have found that Hyams grading correlates with survival and may even predict the utility of chemotherapy.<sup>4,9,26,27,31</sup> In one study, the degree of histopathologic differentiation was the most important and the only significant risk factor for development of recurrence in esthesioneuroblastoma, with high-grade tumors

**Table 4.** DNA topoisomerase alpha labeling indices in cases of low- and high-grade esthesioneuroblastoma (results of exact Wilcoxon non-parametric test)

	N	Range	Median	p-value
Low-grade	6	6.5-16.2	10.4	0.0714
High-grade	2	20.2-24.3	22.3	

exhibiting a significantly reduced 10-year survival (40%) compared to patients with low-grade tumors (100%).<sup>4</sup> Morita et al described a significantly higher 5-year survival of 80% for low-grade tumors as opposed to 40% for high-grade ones.<sup>27</sup> In other studies the difference was not so striking, such as in a study from Brazil that reported 5-year disease-free survival rate of 64% and 43% for the low- and high-grade tumors, respectively.<sup>7</sup> In the present series, using a slight modification of the Hyams grading by dividing the cases into 2 categories of low- and high-grade, we found that the grade distribution in our study was similar to that of the University of Virginia series.<sup>18</sup>

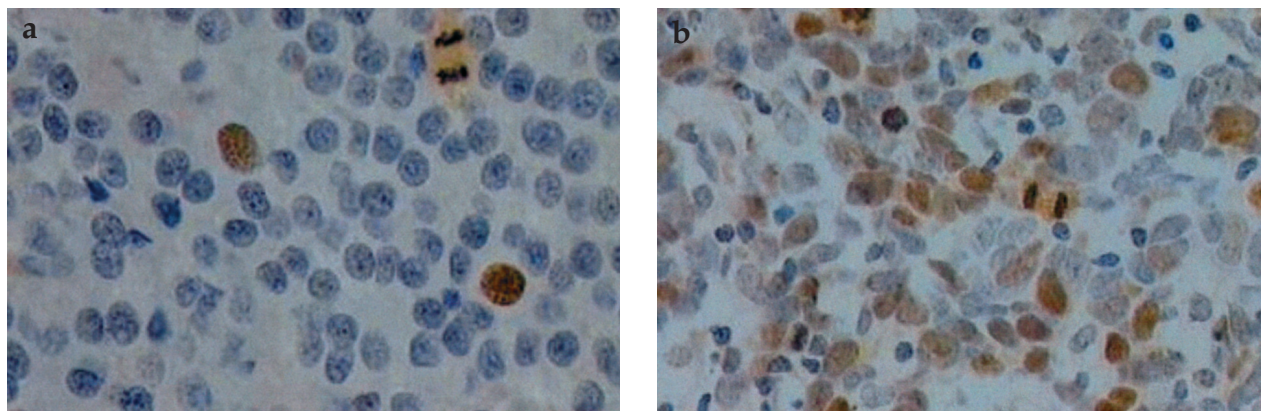
### Staging

The best known and most widely used method is the clinically oriented staging system developed by Kadish,<sup>16</sup> and modified by Morita,<sup>27</sup> in order to separately identify cases with tumor metastases to cervical lymph nodes or distant organs as stage D.<sup>24</sup> A recent study found the modified Kadish staging system, lymph node status, treatment modality and age useful predictors of survival in patients with esthesioneuroblastoma.<sup>15</sup> Among classifications associated with UICC staging, a system proposed by Dulguerov at UCLA is based on invasion of the ethmoid plate, orbit, anterior cranial fossa and dura to facilitate early identification of cases.<sup>6</sup> The present study utilizes both Kadish and the UCLA staging systems. In the present study, cases that showed recurrence included advanced stage, but were all low-grade. Moreover, no case with regional or distant metastasis was noted. This can be explained in part with the long duration of symptoms from onset to first management (average 9 months, range 1-48 months). Similar findings have been noted in the literature. In one study, average duration from symptom onset to management was 6 months with a range of 0-18 months.<sup>12</sup> It was also noted that 13 of 21 patients

presented in advanced stages (T3 and T4),<sup>12</sup> quite similar to the present study in which 10 of 19 cases belonged to stages T3 and T4. In the reported study, 5-year survival for early stage (T1, T2) was 38.1% as opposed to 9.1% for advanced stage (T3,T4) cases,<sup>12</sup> which is worse than in many other published studies,<sup>6,9,18,27</sup> probably because the majority of patients presented in advanced stages.<sup>12</sup> Levine et al managed to obtain a 80.4% 8-year survival rate using aggressive combined modality therapy (surgery, radiotherapy and/or chemotherapy), even though 62.9% of their patients presented with advanced stage C disease and 37.1% developed metastatic disease.<sup>18</sup> Staging is particularly important since the completeness of primary tumor excision has repeatedly been shown to be the single best measure for treatment effectiveness.<sup>17</sup>

### Proliferation indices

Limited data are available on proliferation indices in esthesioneuroblastoma. Ki67 labeling indices were generally high, varying between 3-42% with an average of 16% in one study<sup>31</sup> and between 20-40% in another.<sup>25</sup> Ki67 labeling showed a correlation with postoperative outcome, although this was not statistically significant in one study.<sup>32</sup> However, other authors have proposed that a high proliferation index along with the presence of necrosis and diffuse growth pattern were equally significant prognostic factors.<sup>14</sup> Ours is the first study in the literature that tests the role of DNA topoisomerase II alpha in esthesioneuroblastoma. The difference between the low-grade and high-grade ENB for DNA topoisomerase alpha labeling index was not statistically significant, but was suggestive of a positive association. Because of the limited sample size, the results in this study should be interpreted with caution. Larger studies are needed to clarify the role of T2 $\alpha$  LI in improving histologic grading.



**Figure 2.** (a) Low-grade ENB; intense nuclear immunopositivity for DNA topoisomerase II alpha (x400). (b) High-grade ENB; intense nuclear immunopositivity for DNA topoisomerase II alpha (x400)

Future studies may also address its role as a potential marker of chemosensitivity and chemoresponse in unresectable esthesioneuroblastomas.

#### *Clinicopathologic correlation*

Esthesioneuroblastomas are typically located intranasally, and in advanced stages, extend to the ethmoid plate and sinus, sphenoid sinus, orbit and anterior cranial fossa. In keeping with this pattern of progression, the most common symptoms in the present study were nasal block (100%), epistaxis (53%), proptosis and reduced vision (47%) and headache (40%), similarly to the observations of Hwang et al.<sup>12</sup> The age range was 2 to 62 years with an average of 27 years, similar to that in the literature. However, the patients were fairly well distributed across all age groups, and the bimodal peak (teens and sixth decade) observed in the literature<sup>3,6,22,27</sup> was not seen in this study. The male to female ratio was 14:5 in our study unlike the literature where roughly equal distribution is observed, with one study describing a 13:8 ratio<sup>12</sup>.

This study also analyzed the distribution of clinical parameters in patients with modified Hyams low- and high-grade tumors. High-grade tumors occurred at a slightly younger age, but the difference was not statistically significant. There was no statistically significant difference between low- and high-grade tumors in duration of symptoms either. Younger patients have previously been shown to have higher distant metastasis rate and poorer prognosis.<sup>19</sup> The majority of cases in Kadish stage C and UCLA stages T3 and T4 were low-grade cases, however, there was no statistically significant difference between the low- and high-grade ENB for either the Kadish or the UCLA staging system.

The absence of MIC2 immunoreexpression in this study is in keeping with the recent literature that ENB does not belong to the Ewing's/PNET group.<sup>1,23</sup>

#### *Recurrence*

Tumor recurrences are seen in 27% to 62% of ENB cases and are mostly loco-regional.<sup>4,8,20</sup> As many as 70% to 80% of these recurrences occur within the first 2 years of diagnosis, however, delayed recurrences are also known, necessitating long-term follow-up.<sup>4,8</sup> In a recent study by Loy et al, mean time to recurrence was 6 years, with distant relapses occurring sooner.<sup>20</sup> In the study by Levine et al, the average time for first metastasis after therapy was 74.8 months, which is longer than that for most head and neck malignancies.<sup>18</sup> In a recent study, ENB showed a superior overall 5-year survival and local control at 5 years as compared to non-ENB sinonasal malignancies,<sup>30</sup> thereby emphasizing the need for an early and accurate histopathologic diagnosis. Unlike most sinonasal malignancies,

surgical salvage is possible.<sup>12,17,20,30</sup> Based on a very limited follow-up, the few cases in this study that recurred were all low-grade but included high stage cases as well.

To summarize, this preliminary study suggests the need to combine a simplified histologic grading with accurate staging in a reasonable attempt to assess local progression in esthesioneuroblastomas. Larger studies may clarify the role of T2 $\alpha$  LI in improving histologic grading. The results in this study, however, should be interpreted with caution, because of the limited sample size.

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#### *References*

1. Argani P, Perez-Ordóñez B, Xiao H, Cariana SM, Huvos AG, Ladanyi M: Olfactory neuroblastoma is not related to the Ewing's family of tumors: absence of EWS/FLI1 gene fusion and MIC2 expression. *Am J Surg Pathol* 22: 391-398, 1998
2. Bockmuhl U, You X, Pacyna-Gengelbach M, Arps H, Draf W, Petersen I: CGH pattern of esthesioneuroblastoma and their metastases. *Brain Pathol* 14: 158-163, 2004
3. Broich G, Pagliari A, Ottaviani F: Esthesioneuroblastoma: a general review of the cases published since the discovery of the tumor in 1924. *Anticancer Res* 17: 2683-2706, 1996
4. Constantinidis J, Steinhart H, Koch M, Buchfelder M, Schaenzer A, Weidenbacher M, Iro H: Olfactory neuroblastoma: The University of Erlangen-Nuremberg experience 1975-2000. *Otolaryngol Head Neck Surg* 130:567-574, 2004
5. Dulguerov P, Allal AS, Calcaterra TC: Esthesioneuroblastoma: a meta-analysis and review. *Lancet Oncol* 2: 683-690, 2001
6. Dulguerov P, Calcaterra T: Esthesioneuroblastoma: The UCLA experience 1970-1990. *Laryngoscope* 102: 843-849, 1992
7. Dias FL, Sa GM, Lima RA, Kligerman J, Leoncio MP, Freitas EQ, Soares JR, Arcuri RA: Patterns of failure and outcome in esthesioneuroblastoma. *Arch Otolaryngol Head Neck Surg* 129: 1186-1192, 2003
8. Eden BV, Debo RF, Larner JM, Kelly MD, Levine PA, Stewart FM, Cantrell RW, Constable WC: Esthesioneuroblastoma. Long-term outcome and patterns of failure – the University of Virginia experience. *Cancer* 73: 2556-2562, 1994
9. Eriksen JG, Bastholt L, Krogdahl AS, Hansen O, Joergensen KE: Esthesioneuroblastoma – what is the optimal treatment? *Acta Oncol* 39: 231-235, 2000
10. Haas I, Ganzer U: Does sophisticated diagnostic workup on neuroectodermal tumors have an impact on the treatment of esthesioneuroblastoma? *Onkologie* 26: 261-267, 2003
11. Ho DM, Hsu CY, Ting LT, Chiang H: MIB-1 and DNA topoisomerase II alpha could be helpful for predicting long-term survival of patients with glioblastoma. *Am J Clin Pathol* 119: 715-722, 2003

12. Hwang SK, Paek SH, Kim DG, Jeon YK, Chi JG, Jung HW: Olfactory neuroblastomas: survival rate and prognostic factors. *J Neurooncol* 59: 217-226, 2002
13. Hyams VJ: Tumors of upper respiratory tract and ear. In: Atlas of Tumor Pathology (Eds: Hyams VJ, Batsakis JG, Michaels L, eds.). 2<sup>nd</sup> series, Fascicle 25. Washington, DC, Armed Forces Institute of Pathology, 1988, pp 240-248
14. Ingeholm P, Theilgaard SA, Buchwald C, Hansen HS, Francis D: Esthesioneuroblastoma: a Danish clinicopathological study of 40 consecutive cases. *APMIS* 110: 639-645, 2002
15. Jethanamest D, Morris LG, Sikora AG, Kutler DI: Esthesioneuroblastoma: a population-based analysis of survival and prognostic factors. *Arch Otolaryngol Head Neck Surg* 133: 276-280, 2007
16. Kadish S, Goodman M, Wang CC: Olfactory neuroblastoma: a clinical analysis of 17 cases. *Cancer* 37: 1571-1576, 1976
17. Koka VN, Julieron M, Bourhis J, Janot F, Le Ridant AM, Marandas P, Luboinski B, Schwaab G: Aesthesioneuroblastoma. *J Laryngol Otol* 112: 628-633, 1998
18. Levine PA, Gallagher R, Cantrell RW: Esthesioneuroblastoma: reflections of a 21-year experience. *Laryngoscope* 109: 1539-1543, 1999
19. Liu WS, Tang PZ, Xu GZ: Clinical analysis of 34 cases of esthesioneuroblastoma. *Zhonghua Er Bi Yan Hou Ke Za Zhi* 39: 328-332, 2004
20. Loy AH, Reibel JF, Read PW, Thomas CY, Newman SA, Jane JA, Levine PA: Esthesioneuroblastoma: continued follow-up of a single institution's experience. *Arch Otolaryngol Head Neck Surg* 132: 134-138, 2006
21. McElroy EA, Buckner JC, Lewis JE: Chemotherapy for advanced esthesioneuroblastoma: The Mayo Clinic experience. *Neurosurgery* 42: 1023-1028, 1998
22. Meneses MS, Thurel C, Mikol J, Ramina R, Maniglia JJ, Arruda WO, Cophignon J: Esthesioneuroblastoma with intracranial extension. *Neurosurgery* 27: 813-819, 1990
23. Mezzelani A, Tornielli S, Minoletti F, Pierotti MA, Sozzi G, Pilotti S: Esthesioneuroblastoma is not a member of the primitive peripheral neuroectodermal tumor – Ewing's group. *Br J Cancer* 81: 586-591, 1999
24. Mishima Y, Nagasaki E, Terui Y, Irie T, Takahashi S, Ito Y, Oguchi M, Kawabata K, Kamata S, Hatake K: Combination chemotherapy (cyclophosphamide, doxorubicin, and vincristine with continuous-infusion cisplatin and etoposide) and radiotherapy with stem cell support can be beneficial for adolescents and adults with esthesioneuroblastoma. *Cancer* 101: 1437-1444, 2004
25. Miyagami M, Katayama Y, Kinukawa N, Sawada T: An ultrastructural and immunohistochemical study of olfactory neuroepithelioma with rhabdomyoblasts. *Med Electron Microsc* 35: 160-166, 2002
26. Miyamoto RC, Gleich LL, Biddinger PW, Gluckana JL: Esthesioneuroblastoma and sinonasal undifferentiated carcinoma: impact of histologic grading and clinical staging on survival and prognosis. *Laryngoscope* 110: 1262-1265, 2000
27. Morita A, Ebersold MJ, Olsen KD, Foote RL, Lewis JE, Quast LM: Esthesioneuroblastoma: prognosis and management. *Neurosurgery* 32: 706-715, 1993
28. Olsen KE, Knudsen H, Rasmussen BB, Balslev E, Knoop A, Ejlersen B, Nielsen KV, Schonau A, Overgaard J: Amplification of HER2 and TOP2A and deletion of TOP2A genes in breast cancer investigated by new FISH probes. *Acta Oncol* 43: 35-42, 2004
29. Park SH, Suh YL: Expression of cyclin A and topoisomerase II alpha of oligodendrogliomas is correlated with tumor grade, MIB-1 labelling index and survival. *Histopathology* 42: 395-402, 2003
30. Rosenthal DI, Barker JL Jr, El-Naggar AK, Glisson BS, Kies MS, Diaz EM Jr, Clayman GL, Demonte F, Selek U, Morrison WH, Ang KK, Chao KS, Garden AS: Sinonasal malignancies with neuroendocrine differentiation: patterns of failure according to histologic phenotype. *Cancer* 101: 2567-2573, 2004
31. Sakata K, Aoki Y, Karasawa K, Nakagawa K, Hasezawa K, Muta N, Terahara A, Onogi Y, Sasaki Y, Akanuma A, et al: Esthesioneuroblastoma: a report of seven cases. *Acta Oncol* 32: 399-402, 1993
32. Tatagiba M, Samii M, Dankoweit-Timpe E, Aguiar PH, Osterwald L, Babu R, Ostertag H: Esthesioneuroblastomas with intracranial extension. Proliferative potential and management. *Arq Neuropsiquiatr* 53: 577-586, 1995