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Histopathologic features of Spitzoid lesions in different age groups

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Abstract

Spitz nevus is one of the most difficult melanocytic lesions to diagnose in regard of malignancy, even for experienced dermatopathologists. We analyzed 28 tumors with Spitzoid morphology from 15 children (three little children 2–4-year-old, 12 peripubertary children 9–17-year-old) and 13 adults; there were 21 Spitz nevi, five atypical Spitz tumors and two Spitzoid melanomas in order to establish the diagnostic value of several morphologic parameters in different age groups. No significant differences in respect of age and/or tumor type occurred for gender, location, dimension, symmetry, sharp lateral demarcation, junctional nests orientation, adipose tissue extension, side-to-side cytologic symmetry, uniform melanin deposits, nuclear pleomorphism, presence of mitoses, inflammation and epidermal alterations. Pagetoid growth and/or melanin deposits in the keratin layer were significantly higher in little children. In adults, presence of isolated cells within the lateral margins allows differentiating Spitz nevus from atypical Spitz tumor and Spitzoid melanoma. Deep located mitoses were statistically associated with Spitzoid melanoma in adults. Ulceration was statistically more frequent in peripubertary patients than in adults, probably due to trauma. In conclusion, presence of worrisome morphologic features (pagetoid growth, isolated cells within the lateral margins or ulceration) is correlated to patient's age and less to tumor type; there is no unique morphologic feature to relay on when evaluating a Spitzoid tumor, the final diagnosis being the results of interpretation of multiple clinical, morphologic, immunohistochemical and molecular data and not least dermatopathologist's personal experience.

Keywords: Spitz nevus, atypical Spitz tumor, Spitzoid melanoma, morphologic features, age.

Introduction

Spitzoid tumors represents a paradox for histopathology; it is very easy to recognize due to the special appearance of the tumor cells (first report date from the beginning of the last century – 1910 [1]); it is also one of the most difficult melanocytic lesion to diagnose in regard of malignancy, even in the hands of experienced dermatopathologists. The morphologic hallmark of the lesion is the proliferation of unusual large, spindle or epithelioid melanocytic cells with large nuclei, prominent nucleoli and eosinophilic/amphophilic sometimes ground-glass cytoplasm, worrisome features difficult to interpret, especially in incomplete resected lesions [2, 3].

Biological evolution is intriguing. The first series was presented by Sophie Spitz in 1948 (13 patients) as malignant melanoma with unusual favorable prognosis [4]. Current acceptation of this entity recognize a benign (Spitz nevus – SN), atypical Spitz tumor/Spitz tumor with uncertain malignant potential (AST) and malignant counterpart (malignant melanoma with Spitzoid features – malignant Spitz tumor, so-called Spitzoid melanoma – SM). There are debates about the true evidence for Spitzoid melanomas (based on a unique molecular signature) [5–12], about the possibility of proper identification of those few Spitzoid lesions without morphologic anomalies (histopathologically labeled as "benign") but with distant metastases [13] or the true meaning of Spitzoid tumors with regional lymph node implants but no further progression [14–17]. Also, some reports rise the hypothesis of a possible link between a deficient immune status of the patients and a fatal evolution of otherwise benign looking Spitzoid lesions (both in published case reports or anecdotic cases [18, 19]).

Several histopathologic features and/or immunohistochemical markers were identified as significant in establishing the biologic potential of Spitzoid lesion

[20–25] but there are variations with partial agreements between different studies. *In vivo* confocal microscopy offers information for classifying a lesion as Spitzoid but not relevant for biologic nature of the lesion [26, 27].

However, despite the various disagreements in microscopic appearance, all authors conclude that one of the most important parameter to take into consideration when evaluating a Spitzoid lesion is a clinical one, *i.e.* the age of the patient. Most of such lesion in children have much better prognosis than those arising in adulthood and even in children, the younger the age, the better the prognosis [28, 29]; age is included in a relatively simple grading system for atypical Spitzoid tumors in pediatric population [30]. This is why we decide to evaluate several morphologic parameters in patients with Spitzoid lesions in an attempt to establish their diagnostic value in different age groups.

Materials and Methods

We analyzed 28 tumors with Spitzoid morphology diagnosed in Department of Pathology, Colentina University Hospital, Bucharest, Romania, during four years period of time. No ethical approval was necessary since all the lesions were surgically removed and sent to our laboratory for histopathologic diagnosis. All the cases were reevaluated by two dermatopathologist (CS and SZ); consensus was reached in each case, the final diagnosis taking into consideration clinical, dermoscopic, *in vivo* confocal microscopic and histopathologic data.

Clinical information consisted in age, location, history of recent growth. None of the patients was immunodepressed. None of the patients presents either recurrences or metastases to date, follow-up interval varying between 11 months and five years; all the cases diagnosed as AST had follow-up at least 22 months (22 months–5 years); the SM cases had follow-up of 11 and 12 months. No sentinel lymph node excision was performed in any patient.

The histopathologic diagnosis was established based on morphologic features in Hematoxylin–Eosin (HE) and Periodic Acid–Schiff (PAS) slides and immuno-histochemical (IHC) data (S100 protein, melanocytic markers: HMB45, Melan A and Tyrosinase; progression markers: Ki67, Cyclin D1, p16, p21 and p53). Since there are no IHC makers confidently differentiating between benign, atypical and malignant Spitzoid lesions to date, we used the antibodies listed above in order either to enhance the morphologic alterations (pagetoid growth, maturation, side-to-side symmetry, etc.) or to evaluate the pattern of expression of cell cycle and apoptosis regulators.

The patients were separated based on the age as it follows:

- 15 pediatric patients – group A; within this group two separated subgroups were immediately evident: subgroup A1 (little children 2–4-year-old) – three cases and subgroup A2 (peripubertary children 9–17-year-old) – 12 cases;
- 13 adults – group B (22–42-year-old).

We analyzed the clinical data, dermoscopic and/or *in vivo* confocal microscopic appearance available in six cases and morphologic features reported as related with benign or malignant behavior (symmetry, regularity of the nests, isolated cells extension or well demarcated nests in the lateral borders (edges) of the junctional component, junctional proliferation, vertical and/or horizontal junctional nests orientation, maturation, single cell dermal extension and/or nesting pattern in the base of the lesion, adipose tissue extension, pagetoid spread, melanin deposits in the keratin layer, melanin distribution and dimension of the pigment granules, cellularity, cellular pleomorphism, nuclear pleomorphism, highly prominent and/or atypical nucleoli, presence and number of mitoses, mitoses in lower third of the lesion, ulceration, tumor necrosis, epidermal hyperplasia, Kamino bodies, inflammation) (Figures 1 and 2).

The results were statistically analyzed using Microsoft Excel and EpiInfo programs; the level of statistical significance was $p<0.05$.

Results

We identified 21 SN (Figure 3), five AST and two SM. AST were present in subgroup A2 (Figure 4) and group B in similar proportion (A1=0%, A2=16.66%, B=23.07%) and SMs (Figure 5) were identified in two male patients of 27 and 61-year-old (tumor of 3 mm on the anterior abdominal wall, Breslow index 1.08 mm and 6 mm on the thorax, Breslow index 1.2 mm respectively). All the data are summarized in Table 1 (group A – pediatric population) and Table 2 (group B – adults).

One case had mixed cellular population – Spitz/pigmented spindle cell nevus in a 3-year-old girl. Combined lesions (Spitzoid morphology+common nevus) were present in three patients: one 10-year-old boy with overall benign features, one 12-year-old girl with atypical features and one 33-year-old male with overall benign features; one patient (17-year-old girl) had a combined SN and cellular blue nevus lesion. No significant differences occurred in dimension of the lesion, for either age and/or tumor type; it is worth mentioning that all the AST and SM lesions were small tumors, less than 6 mm in diameter. Pure junctional lesions were noted in two patients of subgroup A1 and one SN in adults, the presence of this pattern being statistically significant associated with young age ($p_{A1\ vs.\ A2}=0.002$, $p_{A1\ vs.\ B}=0.018$) but not with tumor type ($p_{SN\ group\ A1\ vs.\ SN\ group\ B}=0.07$).

There was no gender difference irrespective of age and/or type of tumor. Also, there were no differences regarding the location of the lesions – five tumors on trunk in both pediatric patients (group A) and adults (group B), all the remaining lesions being located on the limbs. Differences in diameter were minor but the tumors were larger in pediatric population (6.63 mm) than in adults (4.9 mm). Small children have smaller lesions (medium diameter 3.43 mm in group A) but the largest lesions were noted in subgroup A2 – medium diameter 7.43 mm, benign lesions being much larger than atypical one (8.01 mm in SN, 4.55 mm in AST); minor differences were noted in group B when type of tumor was considered – 5.08 mm in SN, 4.66 mm in AST and 4.5 mm in SM.

We evaluated several morphologic parameters listed as significant when one is evaluating a melanocytic lesion with Spitzoid morphology.

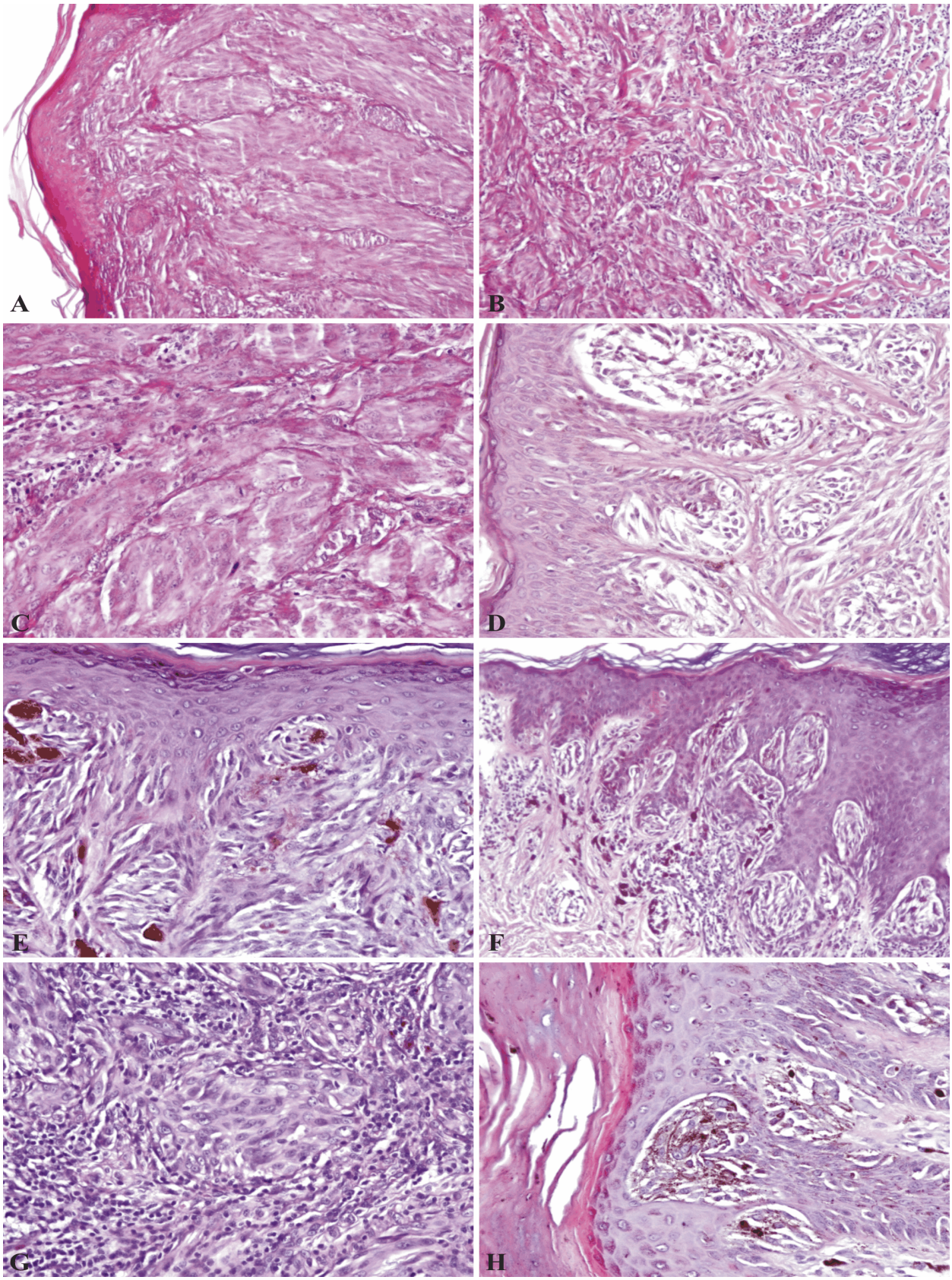

Figure 1 – ***Morphologic findings in Spitzoid tumors: (A) Proliferation of large spindle melanocytes with eosinophilic homogeneous cytoplasm (HE stain, ×100); (B) Diminishing in size of the tumor cells in deep dermis (HE stain, ×100); (C) Large mainly spindle melanocytes with eosinophilic ground glass cytoplasm, mild nuclear pleomorphism, prominent nucleoli and one mitosis (HE stain, ×200); (D) Junctional component consisting in spindle cells with long axis perpendicular on dermo-epidermal junction ("bunches of bananas") (HE stain, ×200); (E) Junctional nests with various orientations – both perpendicular and horizontal to the dermo-epidermal junction (HE stain, ×200); (F) Sharp demarcation of the lateral border (HE stain, ×100); (G) Marked inflammatory infiltrate (HE stain, ×200); (H) Pagetoid ascension with nests of tumor cells (HE stain, ×200).***

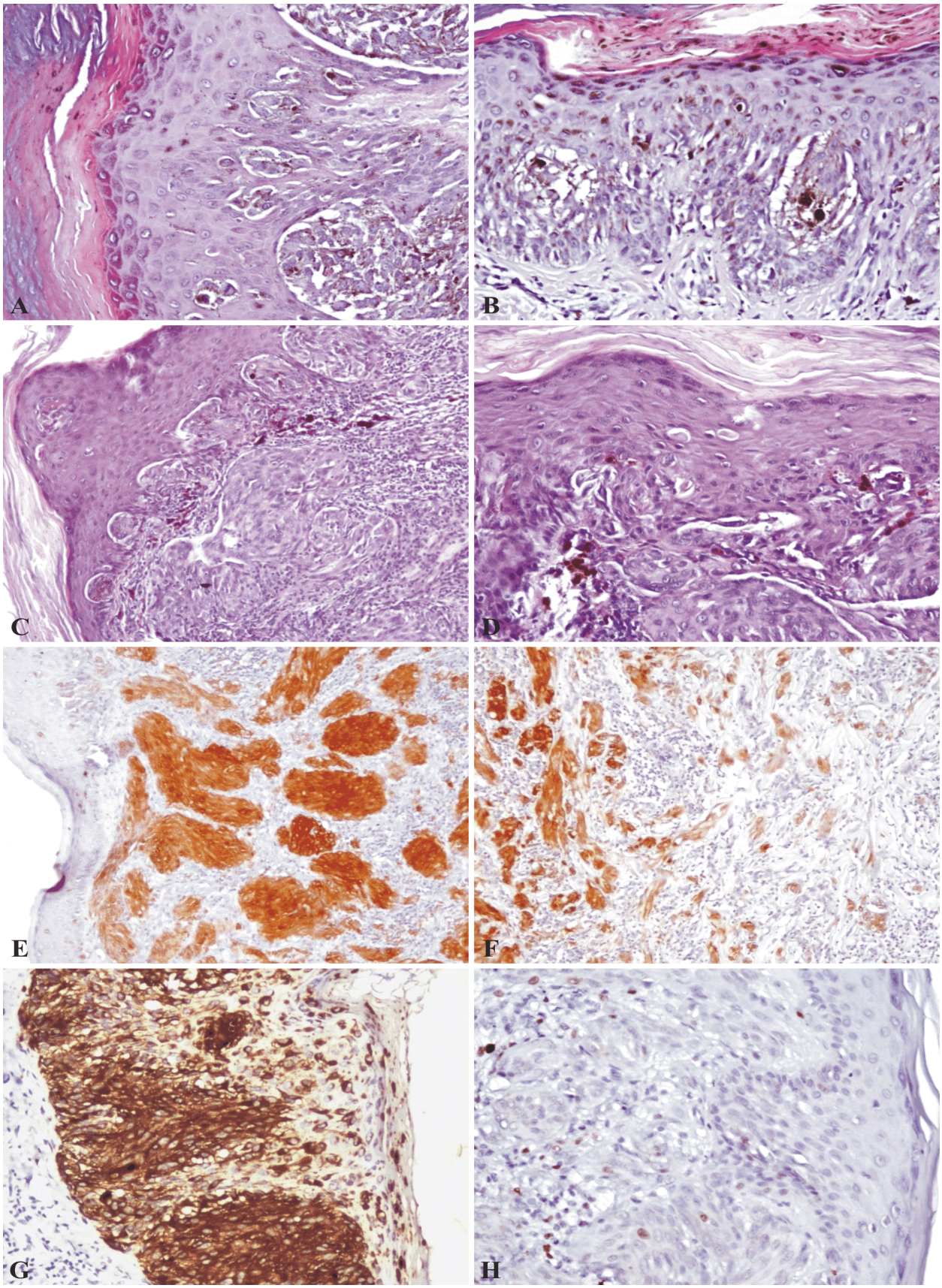

Figure 2 – ***Morphologic findings in Spitzoid tumors: (A) Isolated cells in pagetoid growth (HE stain, ×200); (B) Abundant melanin deposits within the corneous layer (HE stain, ×200); (C) Relatively large Kamino bodies within the epidermis (HE stain, ×100); (D) Two small pale Kamino bodies (HE stain, ×200); (E) Intense positivity for HMB45 of the tumor cells in the vicinity of epidermis (HMB45 stain, ×100); (F) Decreasing of the HMB45 positivity with dermal descending of the tumor cells (HMB45 stain, ×100); (G) Positivity for S100 protein of the tumor cells with revealing of numerous cells in pagetoid ascension (S100 stain, ×200); (H) Few tumor nuclei positive for Ki67 (Ki67 stain, ×200).***

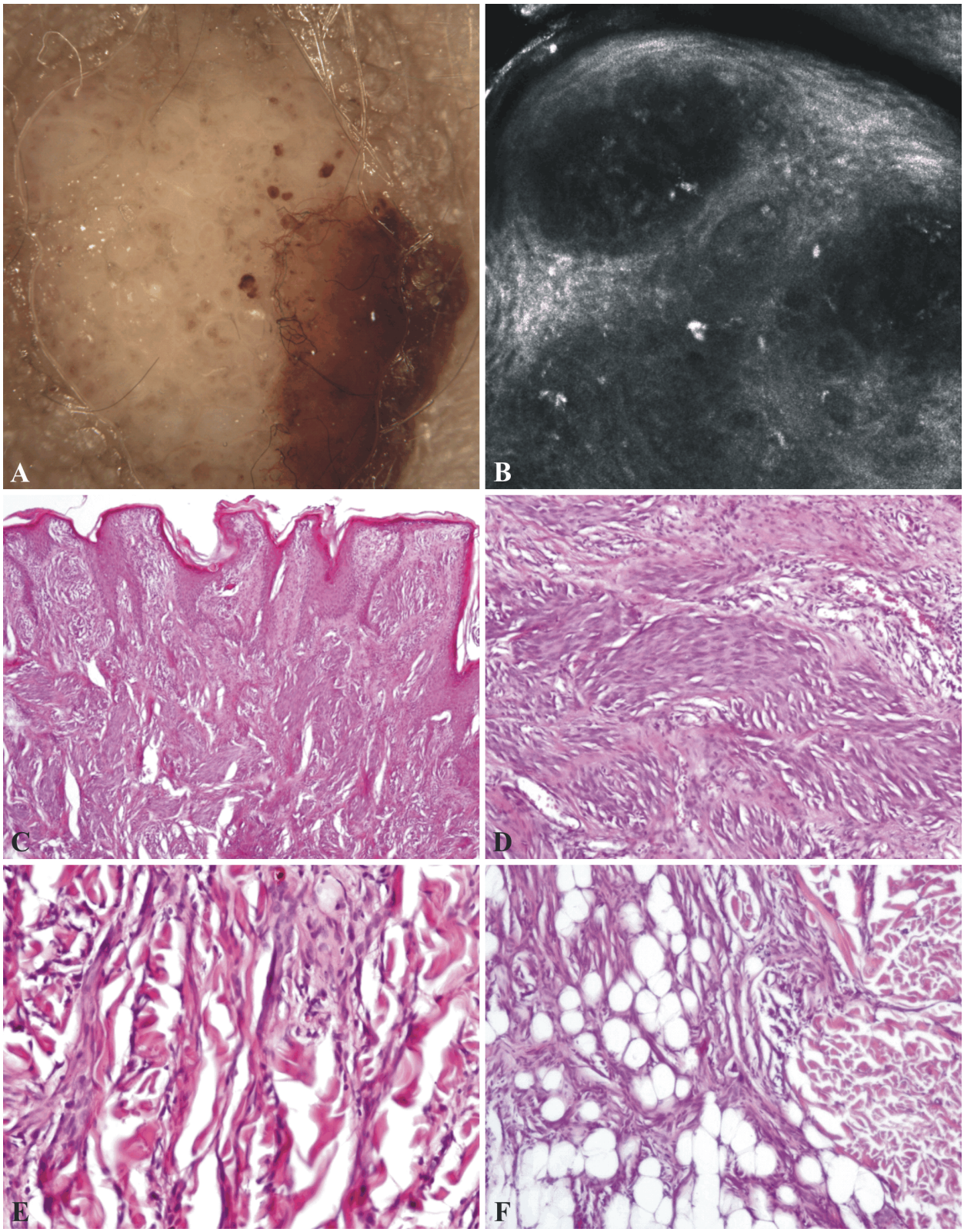

Figure 3 – ***Spitz nevus: (A) Dermoscopic appearance; (B) In vivo confocal microscopy: relatively irregular nests with inhomogeneous pigmentation; isolated large cells within the epidermis, few of them with long dendrite (pseudo-pagetoid infiltration); (C) Large predominantly spindle melanocytes arranged in nests and fascicles; prominent epidermal hyperplasia (HE stain, ×100); (D) Mild pleomorphism of the tumor cells (HE stain, ×200); (E) Infiltration of the adjacent dermis with isolated cells, smaller in size than those adjacent to the epidermis (HE stain, ×200); (F) Adipose extension of the tumor (HE stain, ×200) with smaller cells, without nuclear pleomorphism, mainly isolated or in short fascicles; no nest pattern in the base of the lesion (HE stain, ×200).***

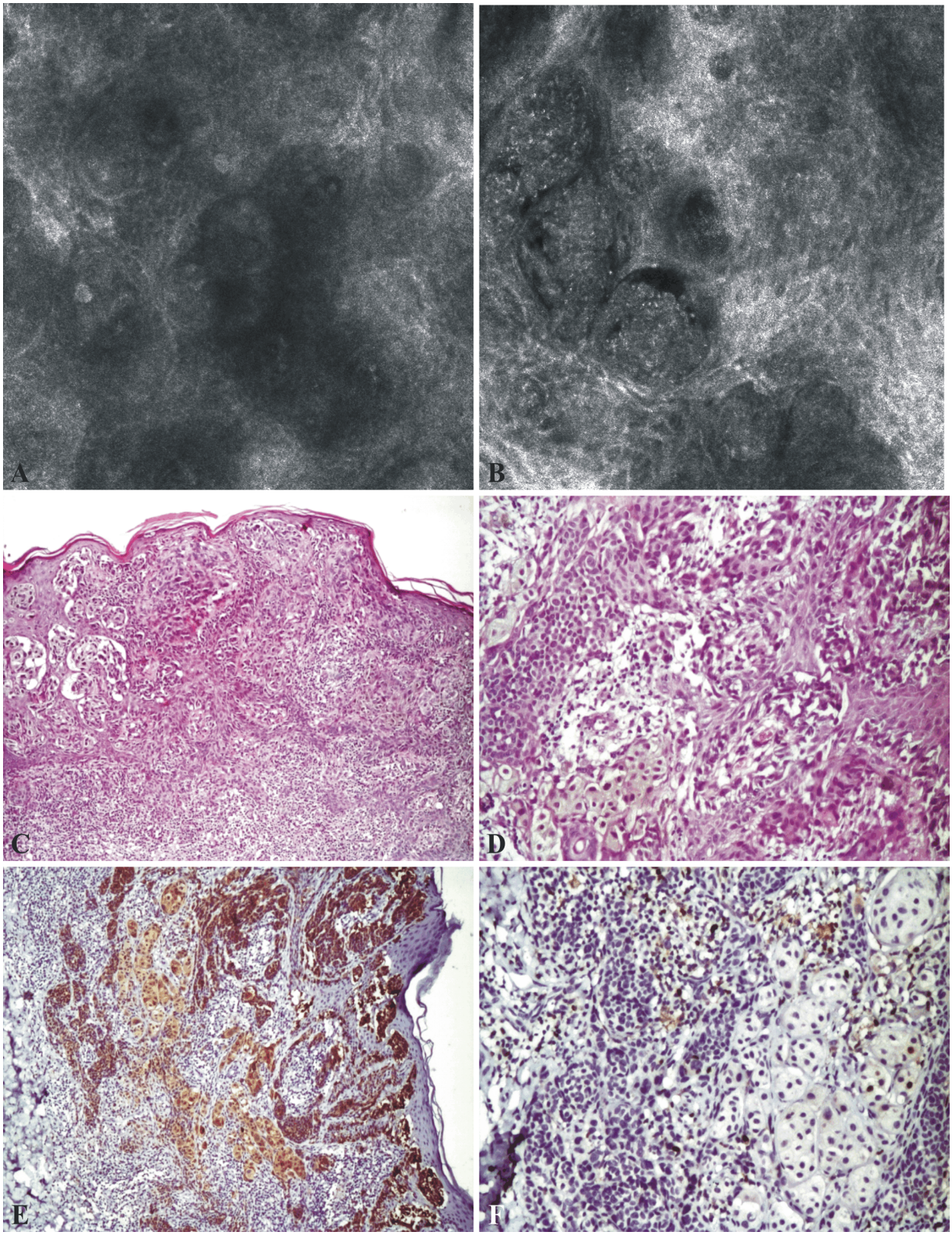

Figure 4 – ***Atypical Spitz tumor: (A) In vivo confocal microscopy: large confluating tumor nests with isolated epithelioid pleomorphic cells within the spinous layer; (B) Inhomogeneous nests; (C) Combined melanocytic tumor – nests of large spindle and epithelioid cells with nuclear pleomorphism and nests of smaller typical melanocytes (HE stain, ×100); (D) Melanocytic proliferation interspersed with elongated rete ridges (HE stain, ×200); (E) Positivity for Melan A of the spindle and epithelioid cells while smaller nevoid cells are negative (Melan A stain, ×200); (F) Low Ki67 index – few melanocytic nuclei are positive (Ki67 stain, ×200).***

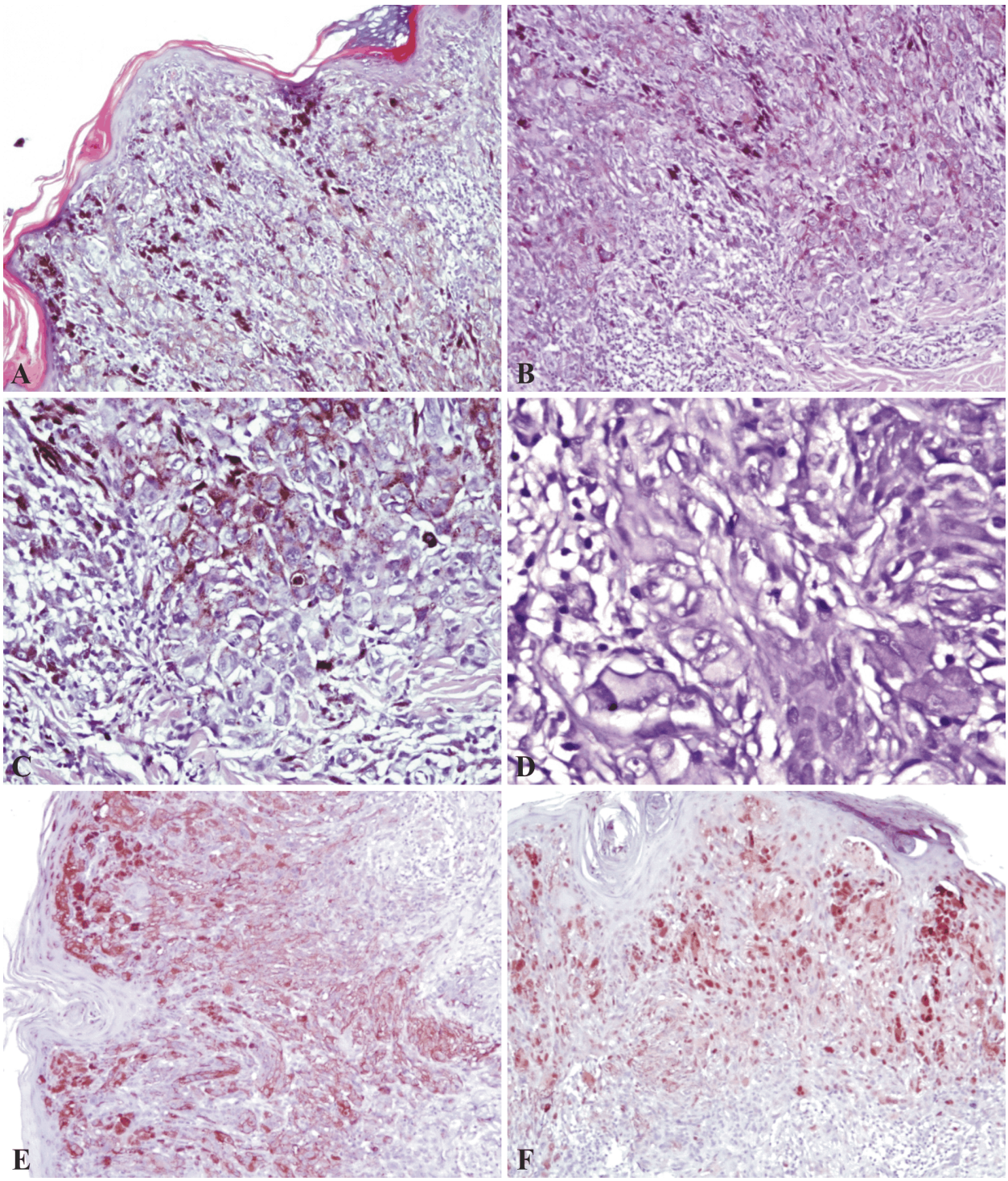

Figure 5 – ***Spitzoid melanoma: (A) Thinning of the epidermis (HE stain, ×100); (B) Proliferation of large epithelioid and spindle melanocytes with pleomorphic nuclei and large nucleoli; marked inflammatory infiltrate (HE stain, ×100); (C) Lack of maturation in the deepest part of the tumor (HE stain, ×200); (D) Atypical mitosis in deep location (HE stain, ×400); (E) Positivity for HMB45 revealing lack of site to site symmetry and maturation (HMB45 stain, ×200); (F) Nuclear positivity for Cyclin D1 in the whole thickness of the tumor (Cyclin D1 stain, ×200).***

Symmetry was present in all but six cases (two SN and one AST of subgroup A2; one AST and the two SM cases of group B); no statistical significance was evident regarding symmetry for age-related groups or histopathologic type except when comparing presence of symmetry in SN and SM (p=0.002). No nodular growth or large sheets of cells in deeper parts of tumor were present in either case.

Maturation was present in all cases of mixed Spitzoid tumors except one SM; no statistical significance was evident when comparing presence of maturation in groups A and B; in group B, when comparing presence maturation in SN, AST and/or SM we also obtained statistically not significant values for SN *vs.* AST+SM ($p_{SN\ vs.\ AST+SM}$=0.077) or AST *vs.* SM ($p_{AST\ vs.\ SM}$=0.087); however, statistically significant values were identified for maturation in SN *vs.* SM ($p_{SN\ vs.\ SM}$=0.0009), irrespective of age ($p_{SN\ group\ A\ vs.\ SM}$=0.0083; $p_{SN\ group\ B\ vs.\ SM}$=0.035).

Table 1 – ***Morphologic characteristics of the Spitzoid tumors in pediatric patients (group A)***

Case No.	*1.*	*2.*	*3.*	*4.*	*5.*	*6.*	*7.*	*8.*	*9.*	*10.*	*11.*	*12.*	*13.*	*14.*	*15.*
Sex/age [years]	M/2	F/3	F/4	M/9	F/9	M/10	M/10	M/10	M/11	M/12	F/12	F/13	F/17	F/12	M/16
Location*	Forearm	Finger, foot	Thigh	Arm	Thorax, P	Foot	Inguinal	Calf	Thigh	Thorax	Lumbar	Thorax, A	Thigh	Calf	Ankle
Tumor type**	SN, J	Reed/ SN, J	SN, M	SN, M	SN, M	SN, M	SN, C, M	SN, M	SN, M	SN, M	SN, M	SN, M	SN, C, M	AST, C, M	AST, M
Diameter [mm]	2	4.1	4.2	5.5	9	8	9.5	8	3	14	2.4	4.7	16	5.1	4
Symmetry	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	–	Yes	–	–	Yes
Isolated cells in lateral	–	–	–	–	–	–	–	–	–	–	Yes	–	–	–	–
Ulceration	–	–	–	Yes	Yes	–	Yes	–	–	–	–	–	–	–	Yes
Epidermis***	H	H	H	H	T	H	H	H	H	H	H	H	H	H	H
Kamino	–	–	–	–	Yes	–	–	–	–	–	–	Yes	–	–	–
Parallel nests	–	–	Yes	–	–	–	–	–	–	–	–	–	–	–	–
Pagetoid growth	Yes	Yes	Yes	–	–	Yes	Yes	–	–	–	–	Yes	–	Yes	–
Adipose extension	–	–	–	–	Yes	–	–	–	–	–	–	–	–	–	–
Melanin in keratin	Yes	Yes	Yes	–	–	Yes	–	–	–	–	–	Yes	–	–	–
Coarse melanin	Yes	Yes	Yes	–	–	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Dusty melanin	–	–	–	–	–	–	Yes	–	–	–	–	–	–	Yes	–
Uniform pigmentation	Yes	Yes	Yes	–	–	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Mitoses	–	Yes	–	Yes	Yes	Yes	Yes	Yes	–	Yes	–	Yes	Yes	Yes	Yes
Deep mitoses	–	–	–	–	–	–	–	Yes	–	–	–	–	Yes	Yes	–
Maturation	n/a	n/a	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Inflammation	–	Yes	–	–	Yes	Yes	–	Yes	Yes	Yes	Yes	Yes	Yes	Yes	–

*P – Posterior, A – Anterior; **J – Junctional, M – Mixed, C – Combined; ***H – Hyperplastic, T – Thinned, N – Normal.

Table 2 – ***Morphologic characteristics of the Spitzoid tumors in adults (group B)***

Case No.	*16.*	*17.*	*18.*	*19.*	*20.*	*21.*	*22.*	*23.*	*24.*	*25.*	*26.*	*27.*	*28.*
Sex/age [years]	F/22	F/26	M/33	M/33	M/33	F/36	F/36	M/42	F/28	M/30	M/39	M/27	M/61
Location*	Calf	Arm	Thorax, A	Arm	Thorax, P	Arm	Abdomen	Arm	Thigh	Calf	Knee	Abdomen	Thorax
Tumor type**	SN, M	SN, J	SN, M	SN, M	SN, C, M	SN, D	SN, M	SN, M	AST, M	AST, M	AST	SM, M	SM, M
Diameter [mm]	8	1.8	5.4	3.4	3.2	9	5.4	4.5	4.5	4.5	5	3	6
Symmetry	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	–	Yes	–	–
Isolated cells in lateral	–	–	–	–	–	–	–	–	Yes	–	–	Yes	Yes
Ulceration	–	–	–	–	–	–	–	–	–	–	–	–	–
Epidermis***	H	H	H	H	N	H	T	N	H	N	H	T	T
Kamino	–	–	–	Yes	–	–	–	–	–	–	–	–	–
Parallel nests	Yes	–	–	–	–	–	Yes	–	–	–	–	–	–
Pagetoid growth	–	Yes	Yes	–	–	–	–	–	–	–	–	–	Yes
Adipose extension	–	–	–	–	–	–	–	–	–	–	–	–	–
Melanin in keratin	–	–	Yes	–	–	–	–	–	–	–	–	–	–
Coarse melanin	–	Yes	–	–	–	–	Yes	Yes	Yes	–	Yes	Yes	Yes
Dusty melanin	–	–	–	–	–	–	Yes	–	–	–	–	Yes	–
Uniform pigmentation	–	Yes	–	–	–	–	Yes	Yes	Yes	–	–	Yes	–
Mitoses	–	–	Yes	Yes	–	Yes	–	Yes	Yes	Yes	Yes	Yes	Yes
deep mitoses	–	–	Yes	–	–	–	–	–	–	Yes	–	Yes	Yes
Maturation	Yes	n/a	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
Inflammation	–	Yes	Yes	Yes	–	Yes	Yes	–	Yes	Yes	Yes	Yes	Yes

*P – Posterior, A – Anterior; **J – Junctional, M – Mixed, C – Combined, D – Dermal; ***H – Hyperplastic, T – Thinned, N – Normal.

Presence of isolated cells within the lateral margins of the tumor was identified in four cases: one SN in 12-year-old girl, one adult AST and both SM cases. No difference where recorded between groups A or B, also no differences were present between SN and AST in subgroup A2. However, in group B, presence of isolated cells in at least one lateral margin was statistically associated with either AST or SM ($p_{SN\ vs.\ AST+SM}$=0.012, $p_{SN\ vs.\ AST}$=0.0046, $p_{SN\ vs.\ SM}$=0.0015, statistically significant). All the tumors presented *nests of tumor cells in lateral intraepidermal extension* in at least one margin.

The *junctional component* was present in all cases but one (dermal SN occurring in 36-year-old female); all these cases presented nests of spindle or epithelioid cells arranged *perpendicularly* on the dermo–epidermal junction; however few cases (one SN in subgroup A1 and two SN in group B) had also *parallel* arranged nests. *Pagetoid growth* was present in all cases in subgroup A1 (all these cases had significant and, occasional worrisome, pagetoid ascension of the tumor cells within the epidermis, either in small groups or in isolated cells); pagetoid growth was also present in three SN cases and the AST case in subgroup A2 and two SN cases in adults (group B); none of the AST cases in adults and only one case of SM had pagetoid growth. Pagetoid growth occurs more frequent in little children irrespective of tumor type (subgroup A1 *vs.* subgroup A2 – $p_{A1\ vs.\ A2}$=0.017 and subgroup A1 *vs.* group B – $P_{A1\ vs.\ B}$=0.013); also, SN presented significantly more prominent pagetoid growth in little children than in older patients ($p_{SN\ subgroup\ A1\ vs.\ SN\ subgroup\ A2}$=0.032 and $p_{SN\ subgroup\ A1\ vs.\ SN\ group\ B}$=0.026). Also, significant difference occurred when the presence of *melanin deposits* within the corneous layer was evaluated; this morphologic feature occurred exclusively in cases with pagetoid growth, except three cases (one SN case from both subgroup A2 and group B and one SM). Statistically significant differences occurred in subgroup A1 when compared with both subgroup A2 ($p_{A1\ vs.\ A2}$= 0.0061) and group B ($p_{A1\ vs.\ B}$=0.0008). Also, when comparing presence of melanin within keratin in SN in different age groups, the differences where significant in favor of presence of melanin in the youngest patients ($p_{SN\ subgroup\ A1\ vs.\ SN\ subgroup\ A2}$=0.012 and $p_{SN\ subgroup\ A1\ vs.\ SN\ group\ B}$=0.007).

Adipose tissue extension was present in one case of SN in a 9-year-old child; we considered this appearance as a feature in favor for a deep penetrating nevus and not invasion.

All the cases presented *cytologic symmetry* from side to side. *Nuclear pleomorfism* was mild or mild to moderate in all cases, irrespective of histological type. No major differences in size, shape or color of nucleoli was identified. No *necrosis* was present; no *vascular or neural/perineural invasion* was evident.

Melanin was present in most of the cases, in various amounts, most often in coarse granules (100% subgroup A1, 80% subgroup A2 and 53.84% group B). Dusty-looking pigment was present in four cases (one SN and one AST in subgroup A2, one SN and one SM in group B); there were not significant differences in respect of age of the patients or type of the lesion. The pigment was more or less uniformly distributed within the tumor mass, two SN in subgroup A2, five SN and two ASTs in group B and one SM having obvious uneven distribution of the melanin deposits but the results were statistically not significant (p>0.05 for each category).

Mitoses were present in both pediatric and adult group: 33% in subgroup A1, 83.33% in subgroup A2 and 69.23% in group B. Only one of our cases had mitotic indexes higher than one mitosis/mm^2 (SN in a 10-year-old boy, 8 mm lesion on the calf). No associations were found with either patients' age and/or type of tumor. Deep located mitoses were present in five cases (the AST case from subgroup A2, one SN, one AST and both SM cases from group B). No significant differences in respect of age of the patients were noted. When comparing the presence of this morphologic feature according the tumor type we found some differences in group B – $p_{SN\ vs.\ SM}$=0.015 (significant); in children, the presence of deep-seated mitoses was not associated with a specific tumor type (p>0.05). However, when comparing the presence of deep-seated mitoses in SN and atypical Spitzoid lesions (either AST or SM), p was close to the level of significance but still higher than the limit ($p_{SN\ vs.\ AST+SM}$=0.071 not significant).

Inflammation was less frequent in the youngest patients (33% subgroup A1, 75% subgroup A2 and 76.92% group B) but no specific associations were present between inflammation type of the tumor and/or age of the patients.

Ulceration was present only in subgroup A2 patients (four cases) with positive statistic association for age between subgroups A2 and B: $p_{A2\ vs.\ B}$=0.023. Epidermal hyperplasia was present in most of the cases; normal thickness epidermis was identified in three cases of adult patients (two SN, one AST) and thinned epidermis in three cases (AST case from subgroup A2 and two SM cases from group B) but no significant correlations were present. There were no differences in regard of the presence of Kamino bodies in respect of both age and type of the lesion.

Discussion

Our analysis revealed several quite surprising results. First of all, several morphologic parameters typically considered as associated with malignancy (such as asymmetry, lack of maturation or ulceration) have not the expected distribution. Symmetry was lacking in the SM cases, as expected; two of the AST cases, one in children and one in adults, also lacked symmetry. Moreover, two typical benign looking SNs lacked symmetry, one of them possibly inflammation causing the disturbance of the microscopic appearance, the other most likely due to the presence of an associated blue nevus component. Not surprisingly all SN but also all our cases of AST, irrespective the age of the patients, presented maturation (decreasing in size of the tumor cells when descending into dermis); maturation was lacking in SM and also in junctional SNs for obvious reasons (lack of dermal component). Lack of maturation is a parameter strong enough to differentiate between SN and SM but not between AST and SM, possibly related to the small number of cases.

Ulceration was statistically more frequent in peri-pubertary patients than in adults. In all cases it occurred in rather large tumors and was caused by prior trauma, children being more prone to scratch a small recent grew papule while the adults applies the rules of common knowledge about moles – "do not traumatize". Also, none of the lesion in little children was ulcerated, maybe due to the fact that at this age kids are less interested of the lesions covered by clothes or shoes.

Neither dimension was associated with a certain pattern – almost all our lesions were less than 1 cm in diameter and all the dubious lesions (ASTs and SM)

were less than 6 mm, despite the tendency towards greater size in evolving lesions [3]. We had two lesions more than 1 cm and five lesions of 8–9 mm, all otherwise interpreted as SN; two of them were combined lesion (SN and blue nevus or common nevus; it is possible that the relatively small number of cases may cause this discrepancy.

Of course, error may occur in interpretation. We tried to minimize them by involving several experts in histopathologic slides examinations but there is stillroom for personal interpretation. However, while some alterations are more prone to subjective evaluation (grade of pleomorphism for instance), the presence of the above-mentioned findings cannot be matter of debate (a tumor is either ulcerated or not, the dimension is a measurable parameter) so an explanation related to a possible inter-/intra-observer variation cannot be properly sustained.

One interesting finding is related to the presence of pure junctional lesions; two-third of the cases in subgroup A1 were junctional, while all but one of the other patients had mixed lesions. This may be related with the biologic evolution of these lesions, arising as junctional and latter on descending and maturating in the dermis.

Isolated cells within the intraepidermal lateral margins of the tumor were present in all types of tumors (SN, AST and SM). The only statistically significant result allows differentiating between ST and nonST cases in adults but we have to mention that one child presented such a feature in an otherwise not worrisome SN.

Remarkably, pagetoid ascension of the tumor cells within the epidermis was present more frequently in small children irrespective of tumor type; also, for SN tumor type the differences remain statistically significant between small children and older children and/or adults. Our data are similar to those of another study showing more intraepidermal involvement in SN occurring in younger patients [31]; however, this study have not included atypical and malignant counterpart; it is interesting that our cases included only one dermal Spitzoid lesion (3.57%) while the other study included 15.73% pure dermal lesions [31].

Also, all of these cases associated epidermal hyperplasia, the few patients with normal-looking or even thinned epidermis showing no pagetoid growth (except one of SM cases where pagetoid growth and thinned epidermis were simultaneously present). Pagetoid invasion by tumor cells is an alteration strongly suggestive for malignancy in melanocytic tumors [32]. However, SN may have to a certain degree pagetoid growth; there is also a peculiar pagetoid SN variant described [33]. Our findings underline the necessity of evaluation with caution of certain microscopic alterations in children; the pagetoid melanocytic proliferation was extensive (comprising almost the whole length of the lesion) and involved the whole thickness of the epidermis (focally reaching the surface) in small children; also, melanin deposits within the keratin were present in these cases, their presence being characteristic for SN in small children comparing with older children or adults. Sometimes, the pathologist is receiving specimens without any elementary data except the name of the patient; this practice should be of course discouraged in any case but no pathologist should accept to provide a diagnosis in such conditions in a Spitzoid lesion. At least in theory, the diagnosis of Spitzoid lesion is easy to establish, considering the special morphology of the tumor cells; however, due to the cytologic worrisome appearance of the cells, combined with the propensity towards intra-epidermal ascension, an overdiagnosis of malignancy may easily occurred, the pathologist being falsely drive toward this conclusion by a superficial examination. In our experience, no pathologist will serenely label a melanocytic tumor as malignant in a child, sometimes up to the point of refusing to sign out a melanoma in pediatric patients; this is also an exaggeration which should be avoided since children, even rarely, do have melanomas, but nevertheless offer the possibility of thinking twice before making such an error.

Generally speaking, SNs are slightly pigmented/ unpigmented lesions. However, since Reed nevus (pigmented spindle cell nevus) is by some authors considered pigmented variant of SN and some cases (such as our Case No. 2) cannot be confidently classified as belonging to one or another category, it is not surprisingly how often one can find pigment in Spitzoid lesions. No significant differences were present either for age or tumor type not even for SM cases; practically, it is no use for this parameter in differentiating benign and atypical or malign in Spitzoid lesions. Maybe, the negative issue should be kept in mind – dusty melanin should not be taken as sign of malignancy or even atypia in Spitzoid tumors, characteristic different from ordinary looking melanocytic tumors.

Last but not least, we have to address the issue of mitoses. SNs usually do not have mitoses [3] or, if they have, they are rare, less than one mitoses/mm^2 [23]. SNs may also present atypical mitoses [34–36], irrefutable sign of malignancy for other lesions. The location of the mitoses is also important, since deep-seated mitoses, especially in the lower third of the tumor, is also worrisome [23, 35]. In our cases, mitotic index, when present, was low and no differences were noted between pediatric patients and adults, both for their presence or location. Only one case had more numerous mitoses but all the other characteristics were in favor of an ordinary SN. To further complicate the algorithm, deep-seated mitoses were present in AST in both children and adults and in SM cases. We recorded a SN in a 33-year-old male and an AST in a 30-year-old male with several mitoses (but no more than one mitosis/mm^2), few of them in the deeper 1/3 of the tumor but both lesions associated quite heavily inflammation. In fact, in a careful scrutinizing of the lesions, most of the cases with mitoses associated inflammation; indeed, not all the cases presenting mitoses showed inflammation and not all the cases with inflammation had mitoses but the frequency of this association was high enough to suspect a possible etiologic link. Moreover, a special note should be kept in mind when evaluating such a lesion that, sometimes, the hyperplastic epidermis may show mitoses, features generally associated with malignancy in common malignant melanomas [32];

in any case, the observer should discriminate between mitoses in squamous cells and melanocytes not to artificially increase the mitotic index of the Spitzoid tumor.

The list of the morphologic criteria taking into consideration when diagnosing a Spitzoid tumor is exhaustive to the point of confusing a less experienced pathologist; immunohistochemical tests or even ancillary methods are helpful but not diagnostic either. Probably a semi-quantitative score combining several data (clinical, morphological, immunohistochemical and/or genetic) designed by analysis of a large series of cases and latter on validated in a prospective study might be the answer for a much accurate diagnosis of Spitzoid lesions.

Conclusions

Our study stresses two ideas:

▪ Presence of some worrisome morphologic features (such as pagetoid growth, isolated cells within the lateral margins or ulceration) is correlated to the age of the patients and less to the type of the tumor.

▪ No matter how important, there is no unique morphologic feature to relay on when evaluating a Spitzoid tumor, the final diagnosis being the results of interpretation of a multitude parameters, including clinical, morphologic, immunohistochemical and molecular (when available) information but not least personal experience of the dermatopathologist.

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