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Additional Cytomorphological Criteria in Diagnosis of Pilomatricoma – Benign Tumor with Bad Reputation

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A B S T R A C T

Pilomatricomas (PM) are benign skin appendageal tumors, with differentiation towards hair-forming cells, usually found in children. They are frequently misdiagnosed by clinicians, and there are also many reports of false positive diagnoses made on fine needle aspiration (FNA) cytology. PM are often mistaken for »small round blue cell« tumors in children, or for Merkel cell carcinoma, basalioma and metastatic small cell carcinoma in adults, with possible over-aggressive therapeutic approach. We present 6 cases of PM, correctly diagnosed preoperatively by FNA. Clinical, cytomorphologic and basic morphometric features were analyzed, and compared with 4 cases of malignant tumors with similar clinical presentation. Morphometric data (longest nuclear diameter) did not prove to be helpful, while basophilic cytoplasmatic protrusions, observed in all 6 analyzed cases, could be useful additional cytomorphologic feature of PM. We concluded that cytomorphologic characteristics of PM are reliable enough for correct preoperative diagnosis in adequate specimens, however the best results are achieved when FNA is performed by an experienced cytologist, and when all relevant clinical data are obtained.

Key words: *pilomatricoma, fine needle aspiration, cytomorphology, morphometry*

Introduction

A pilomatricoma (PM) is a benign skin appendageal tumor with differentiation towards hair cells. It was first described as »epithelioma cutis necroticum calcificatum Malherbe« back in 1880[1]. The most common localizations are head, neck and upper extremities. However, it can occur on the lower extremities and the trunk[2]. It is more common in children, but it can arise in adults as well. It usually manifests as a relatively small, solitary, asymptomatic, firm nodule. It has long been considered a rare tumor, but it may be more common than previously realized. It is usually asymptomatic, and sometimes presents with bluish discoloration of the skin[3]. Pirouzmanesh and al. noted appearance of PM after injury and infection of the involved area[4]. The treatment of choice is surgical excision[5].

Histologically, the lesions are located in the dermis. They contain islands of epithelial cells that are embedded in a connective tissue stroma[6]. The epithelial cells with scant cytoplasm are located in the peripheral parts of the tumor. In the centre there are anucleated »ghost« cells. Usually there are calcium deposits in the tumor[2]. Often there is granulomatous inflammation in areas of keratinization[4].

Tumor cells are positive for proto-oncogene bcl-2, that suppresses apoptosis, marker for hair matrix LEF-1, and for S100 proteins, that shows some differentiation towards hair-forming cells[6,7]. Mutations in the β-catenin gene have been found in PM in some studies, and have been considered responsible for the tumor growth[8].

Diagnostic criteria for PM in fine-needle aspirates (FNA) are quite consistent, and include: dense clusters of small epithelial cells with high nucleo/cytoplasmatic ratio; pink fibrillary substance surrounding small cells; »ghost« cells, often in sheets; multinucleated giant cells (foreign body giant cells); calcificated debris[2,9]. Still, there are many reports on false positive diagnoses made on FNA of PM, which may have adverse effects on thera-

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peutic approach[9,10]. Pulvermacker describes PM as »usually misdiagnosed and yet the most common hair follicle tumor«[11]. It is not a pitfall only in FNAs, but clinical diagnosis is also frequently missed[1,12].

The aim of this study was to compare 6 cases of pilomatricoma with 4 malignant »small round cell tumors«, presented as subcutaneous masses, by cytomorphologic and morphometric analysis, in order to reveal possible differences which could be used as additional diagnostic criteria in FNA.

Patients and Methods

We analyzed 6 patients with pilomatricoma diagnosed on FNA in our laboratory, and subsequently confirmed on pathological slides, in 5-year period (2003–2008). All 6 tumors were located superficially, readily palpable, and FNA was performed without ultrasonic guidance. Aspirates were stained according to May-Gruenwald-Giemsa (MGG) method.

Several characteristics were analyzed in FNA smears: cellularity, presence of large cell aggregates, nuclear moulding, visible nucleoli, isolated cells, presence of pink fibrillary substance between the cells, presence of »basophilic protrusions« of cytoplasm, mitoses, atypical squamous cells, calcifications, »ghost-cells«, multinuclear giant cells (foreign body giant cells), granulocytes, background debris, background basophilic amorphous substance and phagocytes.

Four malignant small cell subcutaneous tumors were also included in analysis (2 Ewing sarcomas, one Merkel-cell carcinoma and one basal cell carcinoma). FNA was performed without ultrasonic guidance as well. Preoperative cytological diagnoses for all 4 tumors were malignant, Ewing sarcoma as »small round cell tumor, probably Ewing sarcoma with the aid of PAS staining, Merkel-cell carcinoma was correctly diagnosed using immunocytochemistry, Ewing-sarcoma on the upper arm was diagnosed as malignant, probably neuroendocrine tumor, and basalioma was diagnosed as such on the morphologic basis alone.

All cytomorphologic characteristics mentioned above were also assessed on the smears of 4 malignant tumors.

Morphometric analysis of the largest nuclear diameter, on available slides, was performed with image-analysis software AnalySIS (Olympus).

Results

Clinical and morphological characteristics of all analyzed patients are presented in Table 1.

In all 6 cases of PM in our series samples were adequate, and contained more diagnostic elements of PMs, in addition to small basaloid cells.

As it can be seen in the group with PM there were 4 female (66.6%) and 2 male patients (33.3%), aged 7–76 years (mean 34.3 years). Lesions were predominantly localized in head and neck region, only in one case tumor was located on the right shoulder. All except one were less than 1 cm in diameter; the largest one was 2 cm in longest diameter, located in the left cheek of 11 year old girl. All 4 patients with the diagnosis of malignant tumors were females, aged 36–80 years. One Ewing sarcoma was located on the upper arm, the other in the femoral region. Merkel-cell carcinoma was located on the forearm and basalioma in the left retroauricular region. These lesions were 2–4 centimeters in diameter.

All cytomorphological characteristics, as described in literature, and one more, to our knowledge not yet mentioned in the literature – »basophilic cytoplasmatic protrusions», are presented in Table1. Cellularity of PM varied from low to medium. The most constant features encountered in smears of PM were: presence of large aggregates of small cells with high nuclear/cytoplasmatic (N/C) ratio (Figure 1a), »basophilic cytoplasmatic protrusions» (Figure 1b), background granulocytes, no single tumor cells and no mitoses (all in 100% of cases). Some of »hallmarks» in cytological diagnosis of PM were relatively rarely observed such as: pink fibrillary substance (50% of cases), calcifications (50% of cases), »ghost cells» and multinucleated giant cells (MGC, 33.3 % of cases).

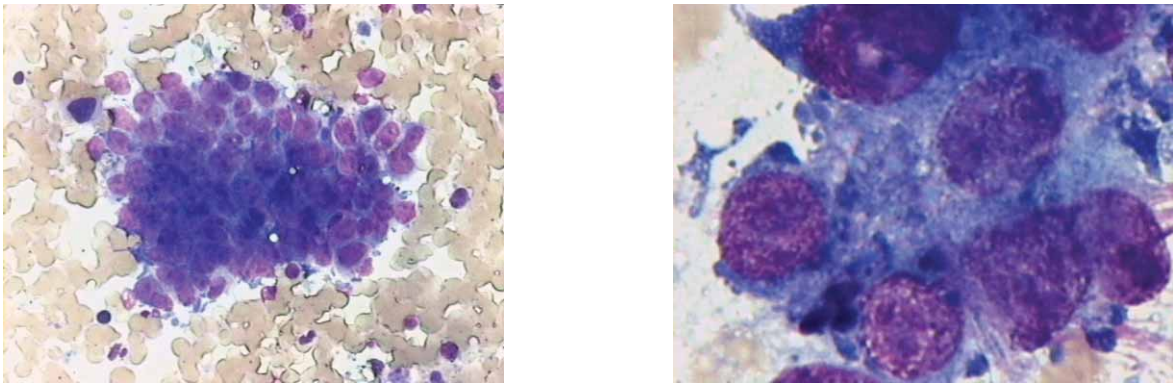

Fig. 1. FNA of pilomatricoma. May-Gruenwald-Giemsa (MGG) stain. (a) Tight cluster of small cells with high N/C ratio (x200) (b) »basophilic cytoplasmatic protrusions«, visible at the cluster margins (x1000).

TABLE 1
CYTOMORPHOLOGIC AND CLINICAL DATA OF 6 PATIENTS WITH DIAGNOSIS OF PILOMATRICOMA AND 4 WITH MALIGNANT TUMORS

	Pilomatricomas						Malignant Tumors			
	1	2	3	4	5	6	Merkel cell Ca[1]	Ewing Sa[2] 1	Ewing Sa[2] 2	Basalioma
Gender	F	F	M	F	F	M	F	F	F	F
Age (year)	51	7	13	11	48	76	71	37	36	80
Localization	neck	preauricular	shoulder	cheek	neck	forehead	forearm	femoral	upper arm	retroauricular
Tumor size (cm)	0.8	0.5	1.0	2x1	1.0	1.0	4	3	4	2x1
Cellularity*	++	++	++	+	+	++	+++	++	++	++
Large aggregates	+	+	+	+	+	+	0	0	+	+
Single cells	0	0	0	0	0	0	+	+	+	0
Nuclear moulding	+	+	+	+	0	+	+	0	+	0
Mitoses	0	0	0	0	0	0	+	+	0	0
Pink fibrillary substance	+	+	0	0	0	+	0	0	0	+
Basophilic cytoplasmatic protrusions	+	+	+	+	+	+	0	0	0	0
Atypical squamous cells	0	+	0	0	0	+	0	0	0	0
Calcifications	+	0	+	+	0	0	0	0	0	0
Ghost cells	0	+	0	+	0	0	0	0	0	0
MGC**	0	+	0	+	0	0	0	0	0	0
Granulocytes***	++	++	++	+	+	+	0	0	0	0
Debris	+	+	+	0	0	0	0	0	0	0
Basophilic amorphous substance	0	+	0	0	+	+	0	0	0	0
Phagocytes	+	+	+	0	+	0	0	0	+	0

Cellullarity*: + low, ++ medium, +++ high, Ca[1] =carcinoma, MGC**= multinucleated giant cells, Sa[2] = sarcoma, Granulocytes***: 0 none, + some, ++ many

In malignant tumors cellularity was medium to high, and we didn't observe any of well known elements of PM. Mitoses were seen only in malignant tumors (66.6%).

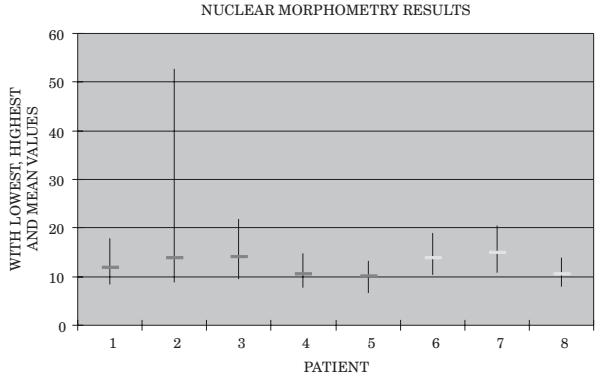

Fig. 2. Results of morphometric analysis: longest – maximal (max) nuclear diameter (lowest, highest and mean values in micrometers) in 5 cases of pilomatricomas (pink), and 3 cases of malignant tumors (yellow).

Smears of both malignant tumors and PM contained aggregates of cells with high N/C ratio. Nuclei of malignant tumors, when observed in smears, seemed bigger than in PM, but this observation could not be confirmed by morphometric analysis (Table 2). Mean nuclear diameter in PMs was 10.19–14.11 μm, and in malignant tumors 10.65–15.11 μm. There was no obvious difference between two groups of tumors, PM-group (patients 1 to 5), and malignant group (patients 6 to 8) in the mean nuclear diameter as shown on the chart (Figure 2). Readily observed is marked anisonucleosis in one case of PM, but that was isolated case. The largest nuclei were observed in one case of Ewing sarcoma. Because of the small number of cases, the statistical analysis was not performed.

Discussion

In this small series of PM there was female preponderance of 2:1, that matches most of the literature data[1,5,13–15] as do the localizations of tumors, predominantly in head and neck regions. The average age of patients in

TABLE 2
NUMBER OF NUCLEI (NO) AND THEIR DIAMETER (D) AND STANDARD DEVIATION (SD) MEASURED BY MORPHOMETRIC ANALYSIS IN FNA OF 5 PATIENTS WITH PILOMATRICOMAS AND 3 WITH MALIGNANT TUMORS

	Pilomatricomas					Malignant tumors		
	1	2	4	5	6	Merkel (1)	Ewing (2)	Basalioma (1)
No	53	54	63	107	81	114	89	92
Mean D*	11.9	13.8	14.1	10.6	10.2	13.9	15.1	10.6
Shortest D	8.5	8.9	9.5	7.7	6.6	10.4	10.7	8.0
Longest D	17.9	52.8	21.8	14.8	13.3	18.9	20.5	13.9
SD**	2.3	7.3	2.3	1.5	1.4	1.7	2.3	1.2

our series was 34.3 years, although many authors consider PM as a predominantly childhood tumor[1,16,17]. We noted some bimodal age distribution, with two peaks, at 10.3 years, and 58.3 years. Similar observation has been mentioned in literature[18].

Only one of our PM patients had multiple tumors (a boy aged 13 when 1st PM has been diagnosed, had two more during following 3 years). That rare condition is described in association with myotonic dystrophy[1], Gardner syndrome and Turner syndrome[19,20]. In our case we can not confirm or exclude any of these conditions, since further clinical data on our patient are not currently available.

PMs are well recognized as diagnostic pitfalls for clinicians. In our series clinical diagnoses for patients referred for FNA were: *tumor (tm) regionis*... in 3 cases, *lipoma colli* in one case, suspicious for relapse of NHL in one case, and in only one case surgeon's diagnosis was *pilomatricoma in obs*. In literature, there is a long list of entities listed as clinical differential diagnoses and the most common misinterpretations are: basal cell carcinoma, cutaneous lymphoma, cutaneous tuberculosis, folliculitis, insect bites, malignant melanoma, Merkel cell carcinoma, metastatic carcinoma of the skin, squamous cell carcinoma[5], small round cell tumor, rhabdomyosarcoma[10]. In our pathology database we found 73 PM diagnosed during last 6 years. In only 6 of them preoperative FNA was performed, and all were correctly diagnosed, but cytological diagnosis was not stated in the referral sheet submitted to pathology department. Among them, we did not find any false positive diagnosis made on FNA. The most often clinical diagnoses were that of atheroma (22 cases), *tm brachii*/*antebrachii* (10 cases), *tm faciei* (7 cases), *tm cutis capitis* (5 cases), and less frequent: *haematoma, cystis supraorbitalis*, *corpus alienum, cystis calcificata* and fibroma. As it can be observed, there were no clinical suspicions of malignancy and clinical diagnoses were quite different from that mentioned in literature.

In all six cases of PM, FNA was performed without any imaging studies. All were palpable, and superficial, so the ultrasonographic guidance was not necessary. There are some reports in literature about using various imaging techniques in preoperative management of PM, like ultrasonography, computed tomography and even magnetic resonance, but none proved conclusive[3,4,21–23]. Rational approach seems that of Pirouzmanesh et al., who find ultrasonography beneficial in cases of PM located for example in parotid and breast regions, where PM could be mistaken for primary tumors of that organs[4].

Cytomorphologic analysis in our series revealed that some of the findings characteristic for PM were not present in all cases. For example, »ghost-cells» are considered characteristic for PM by some authors[2,10], but we found them only in 1/3 of cases, and calcifications in 1/2 of cases. Constant finding in our 6 cases were »basophilic protrusions of cytoplasm», which were never observed in any of malignant tumors analyzed. They are visible as small blue droplets, often somewhat darker than adjacent cytoplasm, on the edge of cell clusters in MGG stained smears of PM (Figure 1a and b). Other main characteristics of PM that were never seen in malignant tumors in our series are: pink fibrillary substance, »ghost--cells«, squamae, calcifications, MGCs, basophilic extracellular substance, debris and granulocytes.

Comparing PM to malignant subcutaneous tumors, we found that malignant tumors were larger, smears more cellular, with many single tumor cells and bare single tumor nuclei, that were never seen in aspirates from PM.

Simple morphometric analysis that we performed (measurement of largest nuclear diameter), did not give any useful information regarding recognizing malignant tumors. There are other, more complex parameters that could be analyzed in smears, such as N/C ratio, nuclear form factor, and overall cell diameter that could give more information[24]. But, it should be pointed out that tight clusters, and practically no single cells with clearly visible nuclear outlines in PM make morphometric measurements very difficult.

Most reports on FNA of PM emphasize the possibility of false positive cytological diagnosis[25–27]. Diagnostic criteria for PM in FNA are well established, but still there are some overlapping features between PM, and other malignant and benign conditions. Diagnostic errors in FNA of PM could be induced by inadequate sampling. When there are only small epithelial cells with high N/C ratio present in the smears, erroneous diagnosis of small round blue cell tumor can be made, especially in children[25]. In adult patients the diagnosis of Merkel cell car-

cinoma, basalioma or metastatic small cell carcinoma can be made in absence of other elements characteristic for PM in the smears.

Conclusions

PMs are rare tumors that can affect any age group, with somewhat bimodal age distribution, one peak beeing about the age of 10, and other at the age of 50–60 years. Very rarely clinicians make the initial diagnosis of PM, and often malignant tumors are suspected. Maybe that is the reason why, at least in our region, in very few of them preoperative FNA is performed. FNA is rapid and cost-effective diagnostic method, well tolerated by patients, even children, and has virtually no complications when superficial lesions are concerned.

We conclude that FNA is very reliable method for preoperative diagnosis of PM. Morphometric data in our study did not prove to be helpful. Instead, pure cytomorphologic analysis proved reliable. We found an additional morphologic feature to be quite characteristic for PM – the »basophilic protrusions of cytoplasm«.

Although there are many reports on false positive FNA findings in PM, we did not share that experience. Very important element in interpretation of FNA of PM, as with any other lesion, is full knowledge of relevant clinical data. The best results are achieved when the cytologist who interprets the smears, performs FNA by himself, because it could be that most of the false positive diagnoses in FNA of PM are made when the sample is not optimal.

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DODATNI CITOMORFOLOŠKI KRITERIJ U DIJAGNOZI PILOMATRIKOMA – DOBROĆUDNOG TUMORA NA LOŠEM GLASU

SAŽETAK

Pilomatrikomi (PM) su dobroćudne novotvorine kožnih adneksa, sa diferencijacijom prema stanicama dlačnog folikula, koji se najčešće susreću u dječjoj dobi. Često su klinički neprepoznati, a u literaturi se nalaze brojni primjeri lažno pozitivnih citoloških dijagnoza postavljenih na punktatima pilomatrikoma. Kod djece, često se zamijene sa tumorima »malih plavih stanica«, a kod odraslih sa karcinomom Merkelovih stanica, bazaliomom ili metastatskim mikrocelularnim karcinomom, što za posljedicu može imati nepotreban agresivni terapijski pristup. U ovom radu prikazali smo 6 slučajeva PM, koji su citološki ispravno preoperativno dijagnosticirani. Analizirani su klinički, citomorfološki i osnovni morfometrijski parametri, u usporedbi sa 4 maligna tumora sa sličnom kliničkom prezentacijom. Rezultati morfome-

trijskih mjerenja (najdužeg promjera jezgre), nisu se pokazali korisnima za dijagnozu. Uz već poznate citomorfološke karakteristike PM-a, u svih 6 analiziranih slučajeva našli smo i, koliko je nama poznato, do sada neopisane »bazofilne protruzije citoplazme«. Zaključujemo da su citomorfološke karakteristike PM-a u adekvatnim uzorcima punktata tumora dovoljno pouzdane za točnu preoperativnu dijagnozu. Najbolji rezultati postižu se kada citolog koji analizira punktat sam i obavlja punkciju, te raspolaže sa svim relevantnim kliničkim podacima.