

SPITZOID MELANOMA-CASE REPORT; POTENTIAL ROLE OF ALK EXPRESSION IN THE DIAGNOSIS OF SPITZOID MELANOCYTIC LESIONS

<https://doi.org/10.26574/rojced.2017.4.4.196>

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Abstract

Keywords:

Spitzoid neoplasm,
ALK,
Spitzoid melanoma.

Spitzoid neoplasms are classified as Spitz naevi, atypical Spitz tumours and spitzoid melanomas. In 10-15% of these lesions, anaplastic lymphoma kinase (ALK) fusions were identified, that can be demonstrated by immunohistochemical tests.

We report a case of spitzoid melanoma in a 15-year-old female patient. The tumor occurred as an un-ulcerated polypoid pigmented nodule on the left thigh; the histopathologic examination revealed a spitzoid melanoma with Breslow index of 13 mm, with lympho-vascular invasion and immunohistochemical phenotype HMB45+, T311+, Melan A+, p16-, p21+, ALK-; sentinel lymph node biopsy identified three of four positive lymph nodes. The patient is well 20 months after the first diagnosis.

Differential diagnosis in spitzoid melanoma is extremely difficult, no reliable biomarkers for treatment response prediction or prognosis being available to date.

<https://doi.org/10.26574/rojced.2017.4.4.196>

Cite this article:

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Spitzoid melanoma-case report; potential role of ALK
expression in the diagnosis of spitzoid melanocytic
lesions. *RojCED* 2017;4(4):196-201.

<https://doi.org/10.26574/rojced.2017.4.4.196>

Introduction

Spitzoid melanoma (SM) belongs to the group of melanocytic lesions with spitzoid features, along with Spitz nevus (SN) and atypical spitzoid tumor (AST). Spitzoid melanocytic tumors have considerable morphologic and molecular differences from the other melanocytic lesions (1, 2). In some cases, prediction of biologic behavior and metastatic risk evaluation may be very difficult.

Recent studies report the presence of kinase fusions within this class of neoplasm, efforts being made to identify new markers with prognostic sig-

nificance in these melanocytic tumors (3-5). Rearrangement of receptor tyrosine kinases like anaplastic lymphoma kinase (ALK), ROS1,

neurotrophic tyrosine kinase receptor type 1 (NTRK1), hepatocyte growth factor receptor (MET), RET, and serine-threonine kinase BRAF are identified in almost half of all Spitzoid tumours (6).

Case report

A 15-year-old girl presented a polypoid pigmented nodule on her left thigh. The lesion was present at least two years prior to excision but grew significantly in the last few months.

Macroscopic appearance showed a fragment of skin of 1.5/1 cm with polypoid grayish-purplish tumor of 1.7/1.5/0.6 cm. Routine histopathologic processing was performed.

Low power **microscopic examination** revealed a melanocytic proliferation arranged in sheets, nests and trabeculi involving mostly the dermis (Figures 1 & 2). At higher magnification, a minor junctional component (consisting in small nests and isolated cells with predominant basal location, without pagetoid spread) was identified. In the deeper areas, tumor proliferation consists mainly in small trabeculi dissecting dermal components with focal arrangement in small nodules in perivas-

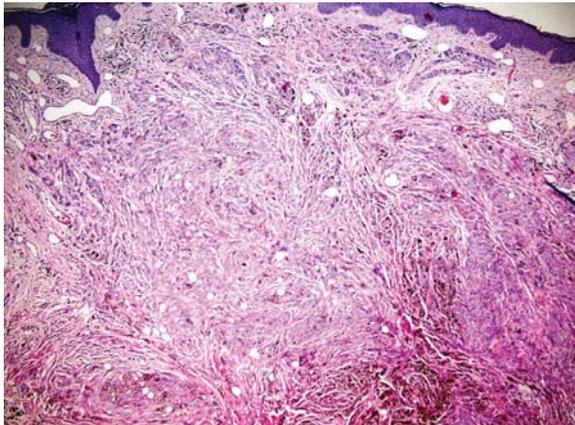


Figure 1. HEx40. Proliferation of irregular large nests of spitzoid cells with confluence of adjacent nests forming solid sheets; presence and uneven distribution of the pigment in the deep portion of the lesion can be noted; no maturation is present (tumoral cells do not become smaller in deep dermis)

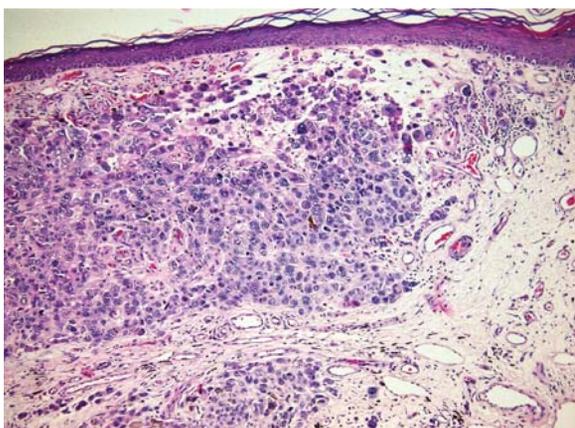


Figure 2. HEx100. Thin epidermis with rare atypical solitary melanocytes at the junction; compact dermal proliferation of highly atypical epithelioid melanocytes, with focal intercellular discohesivity; some cells with monstrous nucleus are intermingled

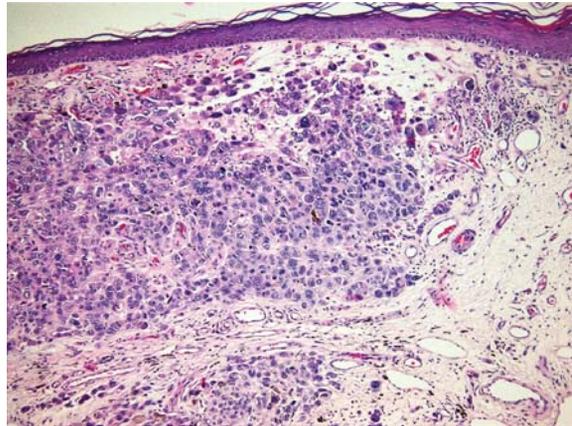


Figure 3. HEx100. The deep portion of the lesion shows pleomorphic melanocytes with no tendency to maturation – tumor cells keep almost the same size in the whole thickness of the lesion; uneven distribution of the pigment

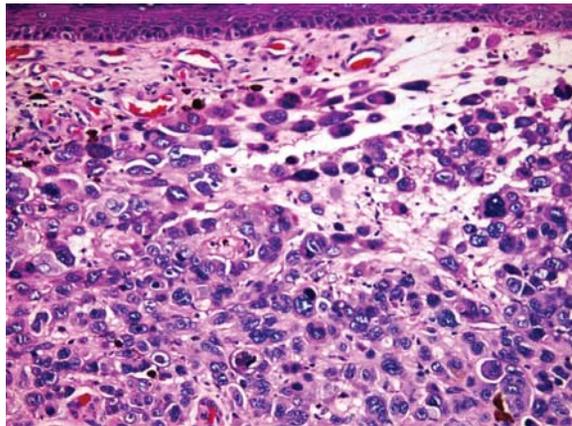


Figure 4. HEx200. Highly atypical melanocytes in the superior part of the tumor, some of them with bi-/multinucleation or nuclear monstruosity; some apoptotic cells can be identified

cular and/or periadnexal location in deep dermis. The maximum thickness of the tumor was 13 mm. Tumor growth extended up to 1.8 mm from the deepest resection margin and 0.5 mm from the closest lateral resection margin. Most of the tumor cells were large, epithelioid, with abundant cytoplasm, vesicular nuclei, prominent eosinophilic nucleoli and occasional intranuclear inclusions (Figures 3 & 4) (spitzoid phenotype); a second population of smaller cells with hyperchromatic nuclei and/or clear almost foamy cytoplasm was identified; prominent nuclear pleomorphism with numerous giant tumor cells dispersed in the whole thickness of the tumor was present (Fig. 5); no obvious decrease of the dimensions of tumor cells in the deeper parts of the tumor was recorded (absence of maturation); variable pigmentation

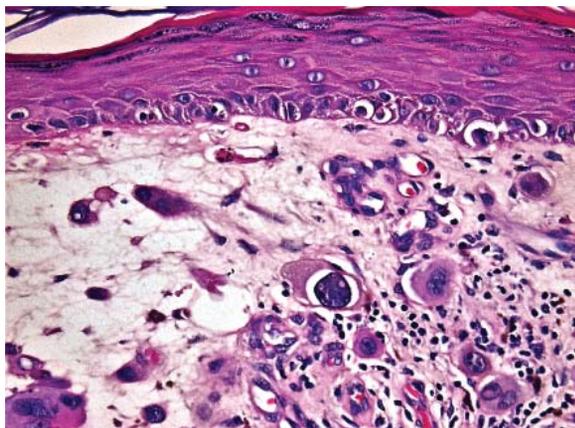


Figure 5. HEx400. Marked cyto-nuclear pleomorphism with frequent multinucleated and monstrous cells

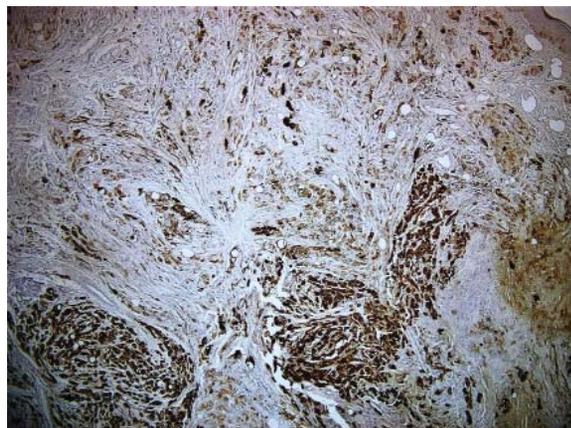


Figure 7. HMB45x200. HMB45 shows positivity in the melanocytic proliferation without gradual diminuation of the staining in the deep part of the tumor (absence of maturation)

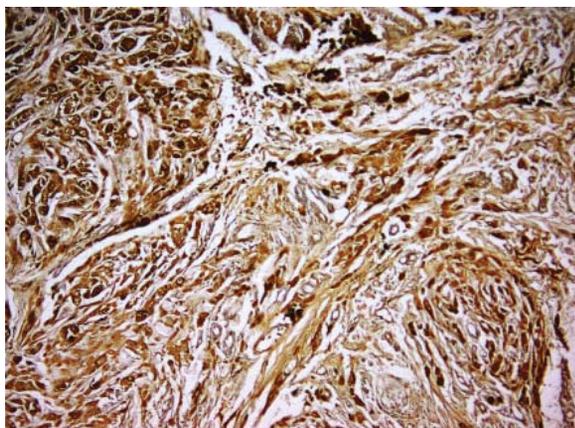


Figure 6. Melan Ax200. Positivity for Melan A in the tumor cells

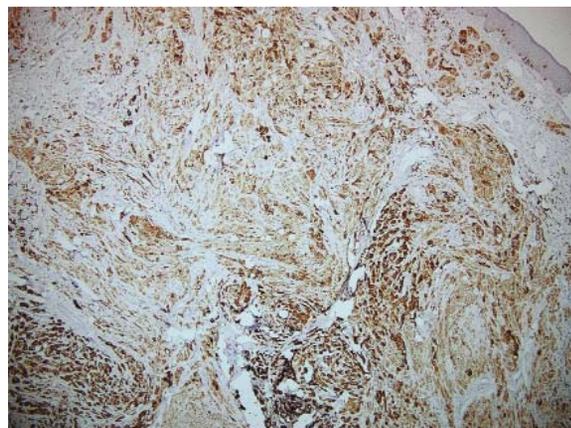


Figure 8. T311x200. T311 positivity in the melanocytic proliferation without maturation

of the tumor cells - areas with numerous deposits of melanin within the cytoplasm as large and/or fine granular/dusty appearance, alternating with areas without pigmentation, were identified. There were rare mitotic figures - one mitosis/1 mm². No extension into subcutaneous fat was present. No

ulceration, perineural invasion or tumor regression were identified. Few tumor emboli (lympho-vascular invasion) and tumor satellites were present.

	Host	Clone	Source	Antigen retrieval	Dilution	Incubation (min)
S100 protein	rabbit	-	Leica	no	1:400	60
HMB45	mouse	HMB45	Leica	proteinase K*	1:200	30
Melan A	mouse	A103	Leica	HIER, pH 6**	1:200	30
T311	mouse	T311	Cell-Marque	HIER, pH 8***	1:200	60
p16	mouse	E6H4	Roche	HIER, pH 8***	RTU	30
p21	mouse	DCS-60.2	Cell-Marque	HIER, pH 8***	2:200	60
Ki67	rabbit	SP6	Cell-Marque	HIER, pH 8***	1,5:200	60
ALK	mouse	5A4	Leica	HIER, pH 6**	1:200	60

*proteinase K digestion of paraffin section 37o C for 10 min

**high temperature antigen retrieval using 0.01 M citrate retrieval solution pH 6 for 15 min

***high temperature antigen retrieval using EDTA retrieval solution pH 8 for 15 min

Table 1. Specifications of primary antibodies

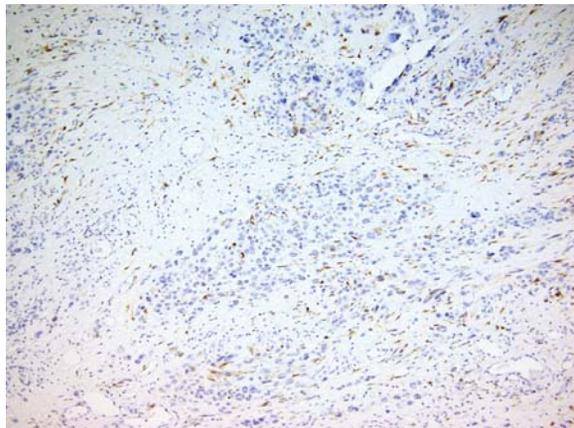


Figure 9. p16x200. p16 negativity in the melanocytic proliferation

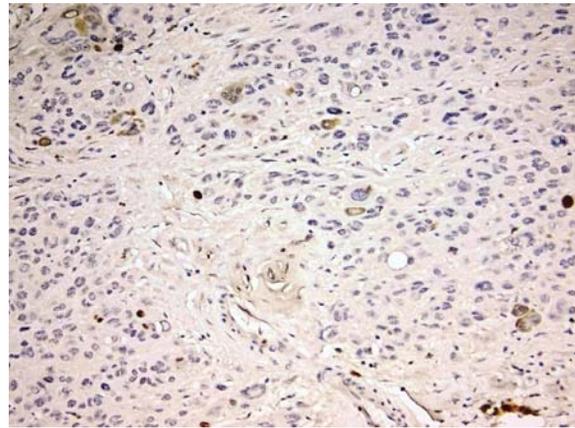


Figure 11. Ki67x200. Ki 67 positive in few tumor nuclei

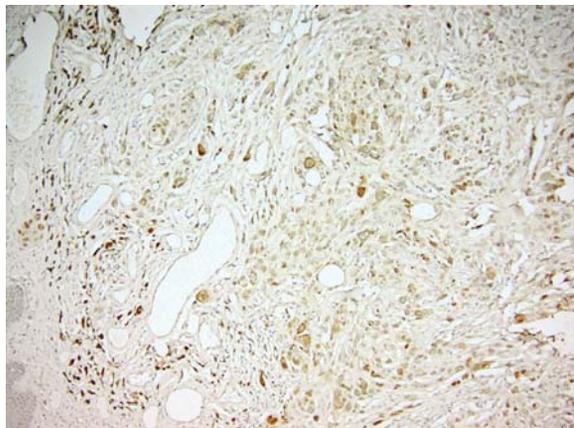


Figure 10. p21x200. p21 positivity in the nuclei of the melanocytic proliferation

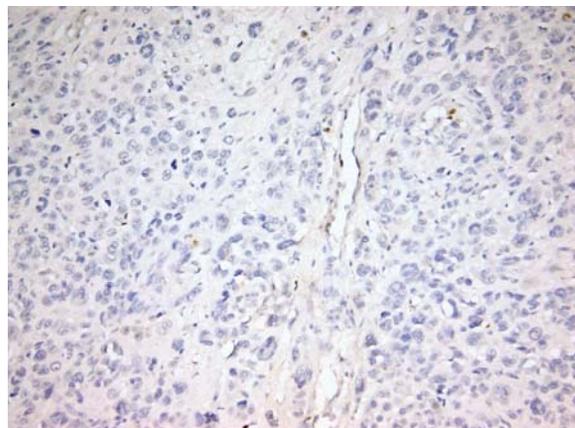


Figure 12. ALKx200. ALK negativity in the melanocytic proliferation

The tumor showed mild to moderate lymphocytic inflammatory infiltrate with relatively frequent melanophages and focal desmoplasia.

Immunohistochemical tests for S100 protein, HMB45, T311, Melan A, p16, p21, Ki67, ALK were performed using specific autoantibodies and Novolink Max DAB (Polymer, Leica, Nussloch, Germany) (for specific details of primary antibodies, see Table 1).

S100 protein was diffuse positive. Melan A immunohistochemical stain confirmed melanocytic origin (Fig. 6); HMB45 (Fig. 7) and T311 (Fig. 8) markers stained numerous cells unevenly distributed within the whole tumor mass, including the deepest parts, without signs of maturation. p16 was negative (Fig. 9) and p21 positive in tumor cells (Fig. 10). Ki67 was positive in few tumor cell nuclei (Ki67 index 1-5%) (Fig. 11). The tumor cells were negative for ALK (Fig. 12).

The lesion was diagnosed as nodular melanoma with spitzoid features, with reticular dermis invasion (Clark IV), with a 13 mm Breslow index and with one mitotic figure/1 mm².

Sentinel lymph node biopsy was performed in the same surgical session with local re-excision, two months after primary tumor resection; no recurrent tumor was identified within the surgical scar; four lymph nodes were resected, three of them showing metastases. No other metastases were identified by CT scan elsewhere.

The patient is alive and well, free of disease, 20 months after primary tumor resection.

Discussion

Spitzoid melanoma (SM) is a rare tumor. It represents a melanoma characterized by some traits specific for Spitz nevus. To emphasize this matter, some authors prefer the term *melanoma with Spitz nevus-like features* (7-9). Practically, SM represents a melanocytic proliferation that at first sight appears much like a Spitz nevus, but at closer view shows traits of malignancy. The majority of SM are diagnosed in adult age, although some occur in adolescents and prepubertal children (9, 10). Head, neck and limbs represent the most common location, but SM can be considered to have a ubiquitous occurrence (11). No strong predilection

for one gender is noticed. There are no specific macroscopic features of SM (12); most often, it appears as an amelanotic nodule, less often pigmented with variegated color; it may reach over 1 cm in size and sometimes ulcerates (9, 13, 14). There are evidences that SMs have a better prognosis, with a lower risk of tumor recurrence and metastasis than other types of melanomas (14).

Efforts were made to formulate some histopathological diagnostic criteria for SM; unfortunately, in some cases, they turn out to be insufficient (15-17):

1. Thick/ deep lesion – A spitzoid lesion broader than 10 mm or a lesion that reaches hypodermis should be suspected of malignancy.

2. Dermal architecture in solid sheets – SM shows a dermal arrangement in large nests, with a tendency to confluence and sheets formation; identification of large nests in the base of the tumor also supports the diagnosis of melanoma.

3. Tumoral nodules with expansive growth pattern – There are often nodules of tumoral melanocytes that are considerably larger than the adjoining nests; these nodules often show a more marked cytonuclear atypia.

4. High mitotic rate pleads for malignancy, but the statement is debatable. In a specific morphological background, with architectural and cytologic marked atypia, even the finding of an isolated mitotic figure favors the diagnosis melanoma. Pediatric patients with Spitz nevi, especially those younger than 10 years old, can have some mitoses, even near the deepest part of the lesion; this finding in this specific group of patients will not be considered an unconditional malignancy trait; on the other hand, in adult patients, mitoses in the profound tumoral front provide a hint for SM diagnosis. The presence of clusters of mitotic figures also favour the diagnosis of melanoma.

5. Cellular pleomorphism – The cellular morphology of a SM resembles that of a Spitz nevus, but the nuclei have higher pleomorphism. An important clue for diagnosis of SM is the abrupt change of cells morphology at the same level of the tumor (absence of side-to side maturation).

6. Individual cell necrosis – The presence of individual necrotic cells or nuclear debris favors malignancy. Necrosis can be seen in a Spitz nevus in the context of posttraumatic changes.

7. High cellular density – The presence of a high cellularity on a high-power field points towards a diagnosis of melanoma.

8. Cytological change – Cytologic alterations such as marked pleomorphism, coarse chromatin, and prominent eosinophilic nucleoli are not characteristic to the majority of cells in most Spitz nevi and definitely should not be identified in the lower part of any Spitz nevus. Nucleoli as large as half of the nucleus or multiple large nucleoli are typically found in melanoma. Cytoplasm of SM cells

is scant, vacuolated, with dusty melanin. Cell contours are irregular, vague.

9. Uneven distribution of the pigment or presence of pigment in the deep portion of the lesion is also a trait that suggests malignancy.

10. Signs of solar elastosis – The diagnosis of Spitz nevus must be made with caution in sun-damaged skin. However, many SM developed without signs of solar elastosis.

11. Absence of zonation – Absence of homogeneous cytological and architectural traits at the distinct horizontal levels is a suggestion of melanoma; also, nuclear pleomorphism in restricted areas of the lesion should be interpreted with caution.

12. Absence of cell maturation – Cells become smaller in profound dermis in a Spitz nevus, whereas in melanoma this aspect can be absent. The identification of large nests in the base of the tumor supports melanoma.

13. Consumption of the epidermis – Thinning of the epidermis can be seen in both SM and Spitz nevus. However, in Spitz nevus, epidermis consumption is restricted to limited areas; in SM, this phenomenon is more frequent.

14. High Ki-67 rate – Ki-67-positive cells comprise about 2-3% of the population in Spitz nevus, and more than 5-10% in melanoma. Moreover, Ki-67-positive cells are usually present in the base of the melanocytic lesion in melanoma, while in Spitz nevus they are found in the superior half of the lesion.

In our case, the diagnosis was made on morphologic and immunohistochemical features. The peculiar aspect of the tumor, with spitzoid areas and limited areas vaguely resembling blue nevus, raised problems of differential diagnosis. Also, patient location and age represented arguments in favor of a less aggressive biologic behaviour. The dimension of the tumor, the prominent pleomorphism, the presence of satellites and vascular emboli oriented the diagnosis towards malignancy.

Tumor presence in regional lymph nodes (further established by sentinel lymph node biopsy) favors the diagnosis of malignancy; however, tumor implants in regional lymph nodes in spitzoid tumor is still a matter of debate, its biologic significance being unclear – all patients with such a lesion (spitzoid tumors and tumor “metastases” in regional lymph nodes) being alive, free of evidence of disease, with a medium follow-up period of 57-72 months (15, 17). In our case, the patient’s evolution – two years free of disease, despite the involvement of several lymph nodes – speaks in favor of tumor implant within lymph node, and not metastasis.

Patients with fusion-positive Spitzoid neoplasm are younger than those diagnosed with tumors without translocations (5), a trait also shared by individuals with kinase fusions in other tumors –

lung cancers, thyroid cancers, or central nervous system tumors (18-20). ALK was first identified as the protein product of an abnormal fusion gene produced by a specific chromosomal translocation in anaplastic large cell lymphoma (21). Later, it was identified in other neoplasms, either benign or malignant, such as lung adenocarcinoma, Ewing sarcoma, inflammatory myofibroblastic tumor, epithelioid fibrous histiocytomas (22). ALK was also detected in approximately 10-15% of all spitzoid tumors; ALK fusions are found in benign Spitz naevi, atypical Spitz tumors, and less commonly in spitzoid melanoma (23-26). Immunohistochemistry is widely considered a good surrogate for ALK rearrangement (5, 27). There are evidences that spitzoid melanocytic tumors with ALK immunoreactivity have specific histopathological features (25, 26, 28). In a previous study, we analyzed several spitzoid tumors for ALK expression. None of our cases of melanoma (3 SM - present case included - from a total of 33 spitzoid tumors) was positive; moreover, a phenotype of the ALK positive spitzoid tumor patient emerged: young female with large tumor (over 8 mm in the largest diameter) on

trunk with both junctional and dermal component and plexiform dermal architecture (29).

To date, no histopathological diagnostic criteria have been formulated and proved to establish the diagnostic with certainty to anticipate an aggressive course for a spitzoid tumor. Consequently, there is an imperative need to study these tumors, to refine the existing diagnostic criteria and find new ones to facilitate a better diagnosis.

Conflicts of interest: none declared.

Acknowledgements: All the authors equally contributed to this work. This paper is partially supported by Executive Agency for Higher Education, Research, Development and Innovation (UEFISCDI) under the contract number PN-III-P4-ID-PCE-2016-0641 (Project No 183/2017).

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