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Original Article

Primary malignant ossifying fibromyxoid tumour of the bone. A clinicopathologic and molecular report of two cases

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Summary

Objective. To report the exceptional occurrence of ossifying fibromyxoid tumour (OFMT) as a primary bone lesion. OFMT is a rare soft tissue tumour of uncertain differentiation and variable malignant potential, that occurs in adults with a slight male predominance. It is typically located in the subcutis or in the skeletal muscles of the extremities, followed by trunk or head and neck.

Methods. Two cases of OFMT proven to arise from bone are presented. The first is a 65-year old female with a history of rib "osteosarcoma", presenting with an inferior lobe left lung mass. The second is a man with a lytic lesion of the 5th cervical vertebra that recurred shortly after resection. Following H&E and immunohistochemical examination, tumour samples were analysed by NGS and by break-apart FISH to detect rearrangement of the *PHF1* and *TFE3* genes.

Results. *PHF1* gene-rearrangement was identified by FISH on both the primary and the metastatic lesion of first patient. NGS identified a *PHF1*(intron1) and *EPC1* (exon 10) fusion transcript later confirmed by positive *PHF1* rearrangement on FISH in the second case.

Conclusions. The demonstration of *PHF1* gene rearrangements represents a fundamental ancillary diagnostic test when presented with challenging examples of OFMT.

Key words: OFMT, soft tissue tumours, *PHF1*, bone tumours, rare tumours

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Conflict of interest
The Authors declare no conflict of interest.

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Introduction

Ossifying fibromyxoid tumour (OFMT) represents an extremely rare soft tissue tumour, classified by current WHO in the category of tumour of uncertain differentiation (WHO 2020). First described by Enzinger in 1989 [1], OFMT typically affects adults, with slight male predominance. It occurs most often in the subcutis, although approximately 30% of cases originate intramuscularly. Primary occurrence in bone appears to be exceedingly rare. The most common anatomic sites are represented by the extremities, followed by the trunk and the head and neck region. Histologically conventional OFMT is composed of monomorphic small, round to ovoid cells with a lobular pattern of growth. Tumour cells are embedded in varying amounts of fibromyxoid to collagenous stroma sometimes associated with foci of immature osteoid matrix. Frequently, a variably developed shell of lamellar bone at the periphery of lesion is observed. The concept of malignant OFMT was introduced by Kilpatrick in 1995, based on the presence of increased nuclear atypia, mitotic activity (> 2 mitoses/10 mm^2) and cellularity [2]. In presence of these criteria a higher

rate of local recurrences and occurrence of metastatic spread was reported [2-5]. It also was suggested that those cases deviating from conventional OFMT but not exhibiting all criteria of malignancy could be prudentially labelled as atypical OFMT. It has to be however admitted that there still exist several points of debate: 1. diagnostic criteria for malignant OFMT are not entirely well established; 2. there is no complete agreement on the very existence of pure malignant OFMT (i.e. with no associated conventional component); recurrences and metastases have been occasionally reported in conventional OFMT [6]. Nonetheless, molecular genetics has proved helpful in supporting diagnosis, that, also in consideration of the extreme rarity, is still very challenging. A large percentage of OFMT are in fact characterised by *PHF1* gene rearrangements leading to the fusion with various partners genes such as *EP400*, *MEAF6, EPC1*, and *TFE3* [7-10]. Alternative gene fusions have been reported in approximately 5-10% of cases, including *ZC3H7B-BCOR*, *CREBBP-BCOR-LI* and *KDM2A-WWTR1* [11-13]. The fact that these molecular aberrations are observed also in atypical and malignant variants strongly supports the existence of a morphologic spectrum of OFMT, characterised by distinct clinical behaviours.

Ossifying fibromyxoid tumour occurs primarily in superficial soft tissues,although as with most soft tissue sarcomas, primary origin from bone, albeit rarely, should be expected [14,15]. Even if deep-seated OFMT with secondary erosion of the bone have been reported [16] to date, only one bona fide, molecularly confirmed, primary OFMT of bone has been published [10]. We herein report two cases of molecularly confirmed malignant OFMT arising primarily in bone.

Materials and methods

Patients.

Patient n. 1.

In October 2017, a 65-year-old female with history of primary osteoblastic (G2 according to Broders) osteosarcoma of the rib, removed 5 years before presented at our institution with a 3 cm lesion of the inferior lobe of the left lung. The primary tumour had been resected but no systemic treatment or radiotherapy was administered. Clinical chart and histologic materials were retrospectively reviewed.

Patient n. 2.

A 29-year-old male presented at another institution with a lytic lesion localised in the 5th cervical vertebra, and the case was sent at our institution for a second opinion. Nine months from onset the patient underwent a local recurrence that was resected and was also sent to us for pathologic examination.

Histopathology and immunohistochemistry.

Formalin-fixed, paraffin-embedded 4-µm sections were stained with haematoxylin and eosin (H&E). Immunohistochemistry was performed using commercially available antibodies according to the manufacturers' instructions.

Molecular genetics

Total nucleic acid was extracted from formalin-fixed and paraffin-embedded samples using the Agencourt-FormaPure System (Beckman Coulter). The quantity of RNA extracted was measured using the Qubit fluorometric quantification system (Thermo Fisher Scientific). RNA, 50 to 250 ng, was used for library preparation utilising the Archer FusionplexSarcoma Kit (ArcherDx) following the manufacturer's protocol. In brief, reverse transcription of RNA was followed by real-time quantitative PCR to determine the sample quality. Hereafter, end repair, adenylation and universal adapter ligation of double strand DNA were followed by two rounds of PCR with universal primers and gene specific primers, covering 26 target genes. The prepared library was quantified using KAPA Library Quantification Kit for Ion Torrent (KAPA Biosystems), according to the user guide (KAPA Biosystems) and sequenced using an Ion S5 next-generation sequencer (Thermo Fisher Scientific) according to the manufacturer's protocol. Data were analysed by the Archer Data Analysis software Version 6.0. The produced library was analysed for presence of relevant fusions.

FISH analysis was performed on formalin-fixed, paraffin embedded tissues. In the tumour analysed, cytogenetic analysis was performed using commercially available locus-specific dual-color break-apart rearrangement probes mapping *TFE3* gene (ZytoLight® SPEC TFE3s Dual Color Break Apart Probe) and *PHF1* gene (PHF1 Break Apart Probe, Empire Genomics). FISH procedure was developed as previously described [17]. Two hundred nonoverlapping tumour cells were counted, and cases with > 15% of nuclei with break-apart signals were considered positive.

Results

Patient 1.

X-ray and MRI demonstrated a tumour located in the right rib measuring 5 cm (Fig. 1A and B). Histologically the primary lesion was hypercellular, composed of atypical spindle to oval neoplastic cells set in a fibrous

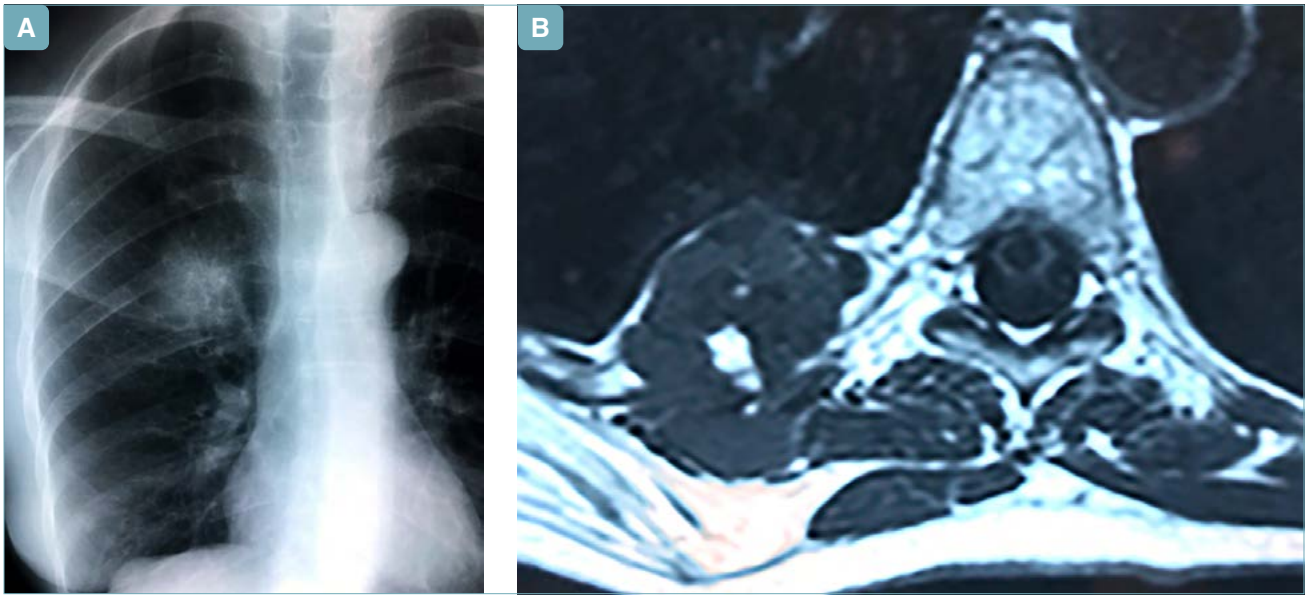

Figure 1. (A) Plain radiographs showing a well circumscribed mass with calcifications. (B) MRI illustrating a lytic lesion centred on the rib.

stroma (Fig. 2A) with multifocal production of immature bone matrix (Fig. 2B). A mitotic index of 5/10 mm^2 was present. Subsequently, the patient underwent atypical lung resection. The surgical specimen showed a diffuse proliferation of uniform spindle to oval cells organised in lobules, morphologically similar to the primary lesion, but without bone production (Fig. 2C-D). A mitotic index of 7/10 mm^2 was observed. No foci of necrosis and vascular invasion were present. Both the primary and the metastatic lesions were immunostained for desmin, EMA, SATB2, MDM2 and MUC4. SATB2 and EMA were focally positive whereas MUC4 and MDM2 were negative. Desmin picked up isolated neoplastic cells. The presence of *PHF1* gene-rearrangement was identified by FISH on both the primary and the metastatic lesion (Fig. 3). No *TFE3* gene rearrangement was detected. The morphological and molecular features were in keeping with the diagnosis of a primary malignant ossifying fibromyxoid tumour of the bone, metastatic to the lungs. No systemic treat-

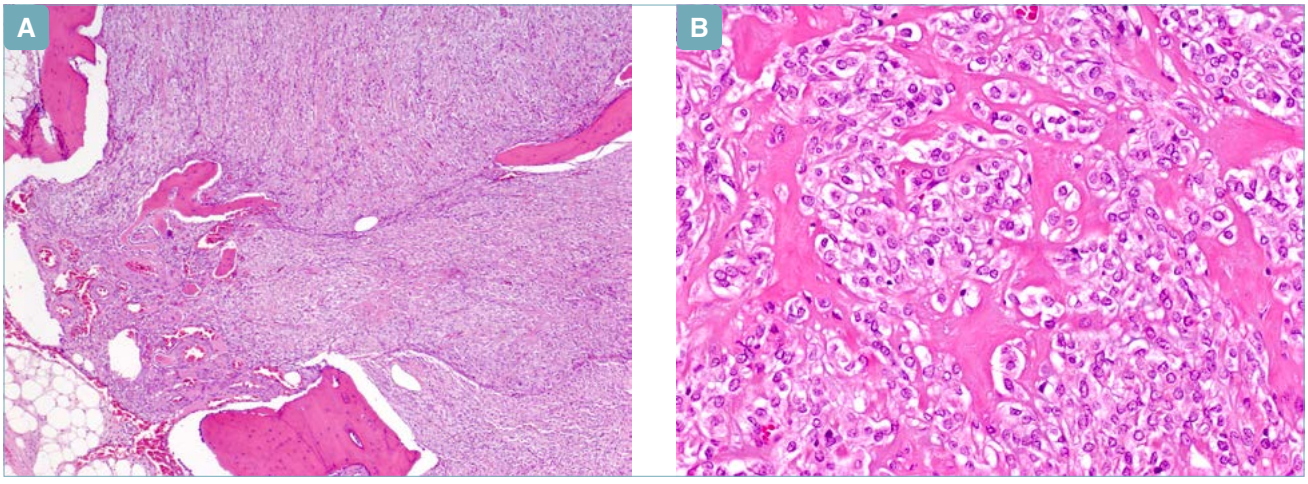

Figure 2. (A). The neoplastic proliferation infiltrates the host bone of the rib. (B) Neoplastic cells are embedded in immature osteoid matrix.

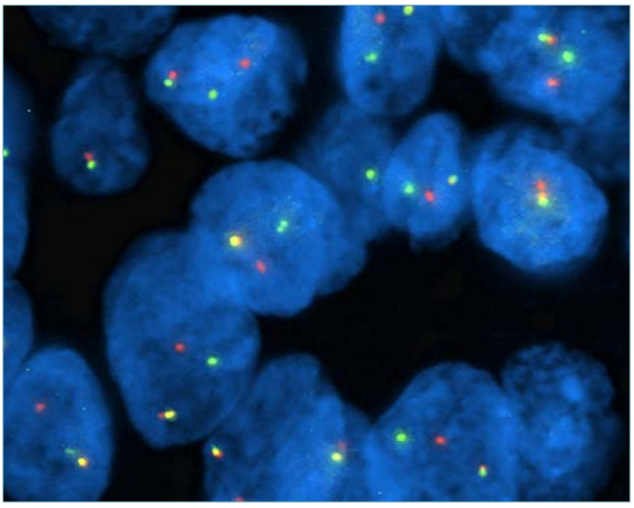

Figure 3. FISH analysis showing rearrangement of the *PHF1* gene.

ment was administered, and the patient is disease free at last follow-up.

Patient 2.

The vertebral lesion was treated with curettage. Morphologically the lesion was composed of a highly cellular ovoid cell population, often organised in chords and small clusters surrounded by eosinophilic, dense collagenous stroma (Fig. 4A). A mitotic index of 8 mitoses/10 mm^2 was observed. No atypical mitotic figures were present. Immunohistochemically the neoplasm was unequivocally positive for MUC4 whereas desmin, EMA, S100, MDM2 and SATB2 were all negative. MUC4 immunopositivity in context with morphology seemed to be compatible with a diagnosis of sclerosing epithelioid fibrosarcoma (Fig. 4B). However, Next Generation Sequencing (ARCHER™ FusionPlex Sarcoma) identified the presence of a fusion transcript between *EPC1* (exon 10) and *PHF1* (intron 1) with a number of reads (#/%) of 180/35 supporting the event (Fig. 5). FISH analysis confirmed the presence of *PHF1* gene rearrangement. Based on morphologic, immunohistochemical and molecular finding a diagnosis of malignant OFMT was made. Nine months from onset the patient underwent a local recurrence that was resected. Histomorphology showed increased cellularity associated with higher mitotic activity (Fig. 6). The patient is now disease free at last follow-up.

Discussion

Ossifying fibromyxoid tumour (OFMT) is a rare neoplasm, classified by 2020 WHO classification among the group of tumours of uncertain differentiation, typically involving the subcutis or deep sites of the extremities. Primary occurrence in bone seems to be exceedingly rare. Conventional OFMT tend to behave indolently, metastatic spread being reported only occasionally. However, since the publication of the initial series several single cases and small series of morphologically atypical lesions were reported, featuring a more aggressive clinical behaviour [2-5]. The presence of high cellularity, high nuclear grade and mitotic activity exceeding 2 mitoses/10 HPF correlated with a higher risk of both local recurrence and metastatic spread (mostly to bone and lungs). The true existence of a malignant variant of OFMT has been source of controversy. It has,

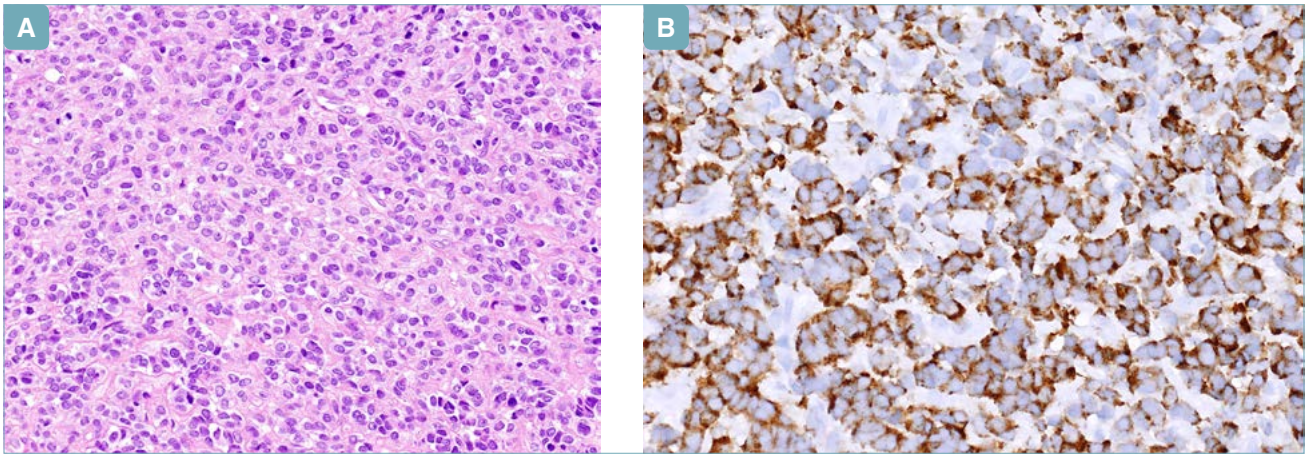

Figure 4. (A) The lesion is highly cellular, composed of ovoid cell, often organised in small clusters surrounded by collagenous stroma. (B) Unequivocal MUC4 positivity is seen.

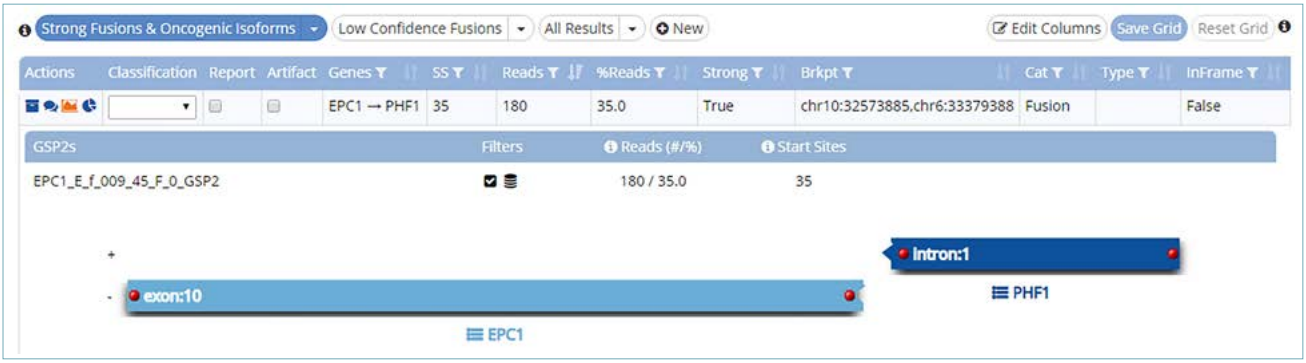

Figure 5. Next Generation Sequencing analysis illustrating fusion between the *PHF1* and the *EPC1* genes.

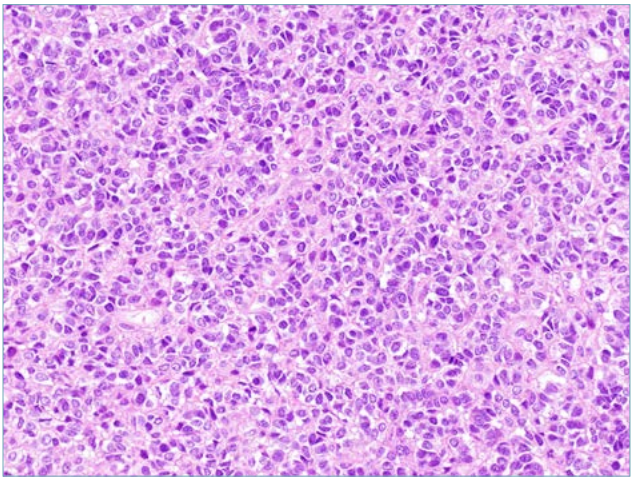

Figure 6. The recurrent lesion featured increased cellularity.

in fact, suggested that metastatic spread never occur [6]. However, in that series only conventional forms of OFMT were analysed. The relatively recent identification of rearrangement of *PHF1* gene in about 80% of OFMT including atypical and malignant variants however strongly supports the existence of a clinicopathologic spectrum of disease encompassing benign and aggressive forms. Rearrangements of the *PHF1* gene was first identified in both benign endometrial stromal nodules and low-grade endometrial stromal sarcoma [18]. *PHF1* in these tumours rearranged with *JAZF1* and *EPC1 PHF1* interacts with polycomb group proteins in particular with polycomb-repressive complex 2 (PRC2), which is essential for silencing of Hox genes and other genes regulating development. The PRC2 consists of several proteins, including SUZ12, EZH1, EZH2 the latter two responsible for the demethylation of lysine 27 on histone H3.

The diagnosis in typical forms is based on morphology. The typical OFMT is well circumscribed, lobular and composed of monomorphic bland ovoid to spindle cells set in a fibromyxoid matrix and in half of cases surrounded by a peripheral shell of mature bone. Importantly in atypical/malignant forms a well-formed bony shell is often absent, and the presence of osteoid matrix deposition scattered through the lesion is observed instead. In a minority of cases bone production is totally absent. This potentially may lead to mislabelling OFMT as osteosarcoma as happened in our first case. Distinction of OFMT from osteosarcoma is of paramount importance, as it would potentially expose patients to cytotoxic systemic treatments, that in case of OFMT may not be justified [19,20].

Immunohistochemically, approximately 50% of tumours show variable immunopositivity for S100, desmin and neurofilaments. MUC4 expression represent a major diagnostic pitfall because OFMT (as exemplified our second case) may exhibit some morphologic overlap with sclerosing epithelioid fibrosarcoma (SEF), an aggressive mesenchymal malignancy consistently featuring MUC4 expression as well the presence of gene rearrangements fusing *EWSR1* or *FUS* genes with *CREB3L1* or *CRB3L2* [21]. Moreover, low grade fibromyxoid sarcoma (LG-FMS), a rare sarcoma that share with SEF the same molecular alteration and the expression of MUC4, may rarely feature a peripheric bony shell, that may contribute to the diagnostic confusion [22,23]. However, LG-FMS is a purely spindle cell tumour characterise by bland cytology as well as distinctive alternation of collagenous and myxoid areas. The demonstration of *PHF1* gene rearrangements represents a key diagnostic support whenever dealing with challenging examples of OFMT, even more in bone whereas the neoplastic nature of the osteogenic component is more difficult to appreciate. Most recently in a small subgroup of atypical/malignant OFMT a novel *PHF1-TFE3* fusion has been identified that was

associated with up regulation of TFE3 mRNA, and consequent immunohistochemically detectable nuclear expression of TFE3 in all cases [10]. It is worth noting that the genetic landscape of mesenchymal tumours appears to be increasingly complex. In our experience the use of NGS-based techniques seems currently to represent the easier way to detect tumour specific fusion genes [24].

Both the occurrence of soft tissue sarcomas in bone [14,25] and of bone sarcomas in soft tissues [26] represent an exceedingly rare event that increases the intrinsic diagnostic difficulty that characterises mesenchymal tumours [27]. To date only three bona fide primary bone OFMTS (including ours) have reported in the English literature. All cases affected adult patients, with involvement of short bones of trunk (scapula, vertebra and rib). All cases were locally aggressive and in one case a single lung metastasis was detected. However, in all cases a prolonged disease-free survival was achieved.

Conclusions

Primary malignant OFMT of bone is exceptionally rare. Occurrence in bone represents a further challenge as it may lead to diagnostic confusion with osteosarcoma. On the other hand, the expression of MUC4, in consideration of potential morphologic overlap with OFMT, make the differential diagnosis with sclerosing epithelioid fibrosarcoma extremely challenging. In both situations, molecular genetics represents an extremely helpful diagnostic clue. Even if FISH analysis certainly represents an effective approach, in consideration of the exponential increase of newly reported fusion genes in sarcoma, NGS-based diagnostics appears to offer superior diagnostic accuracy.

Compliance with ethical standards

This manuscript meets the ethical standards. Specific informed consent to molecular analysis is routinely obtained.

Authors' contributions

APDT and MS reviewed the pathologic diagnosis. MS and EB drafted the manuscript and reviewed the literature. LZ and LT performed the molecular analysis. APTD edited the final version of the manuscript. All authors have approved the manuscript.

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