Research Article

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Diagnostic Accuracy of Aneurysmal Bone Cyst (ABC): Immunohistochemical Aspects

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ABSTRACT

The incidence of Aneurismal Bone cyst has increased in worldwide. ABC-like lesions involving Giant (GCTOB) cells, fibrohistiocytic or tumor FD like components can confuse pitfalls of ABC and need to observe and defines. We investigated tissue resources from operative materials of total 28 cases of ABC, selected as primary ABC without any bone pathology (n=17) and ABC-like changes accompanying by Giant cell tumor (n=9) and Fibrous dysplasia (FD) (n=2). Female – 10, male – 18, mean age - 27. Cases were reported according clinical, radiological, histological, localization and recurrence features with special attention to immunohistochemistry (IHC) using different markers: P53, Bcl-2, P63, Ki-67, CD68, Cyclin D1. The results of these assessments were compared for the final histologic diagnosis. Results show that IHC has a positive value in dysplastic and cancerous stroma in GCTOB (P63, Cyclin D1); FD field does not show similar activity, demonstrating improved diagnostic accuracy.

Keywords

Aneurysmal Bone Cyst, Fibrous dysplasia, Giant cell tumor of bone, Immunohistochemistry.

Introduction

Aneurysmal Bone cyst(ABC), according last WHO Classification of Tumors of Bone (2020) [1], is now recognized in nomenclature as "ABC"- a true neoplasme, locally aggressive lesion, which accounts ≈70% of the case, and "ABC-like changes", associated of other bone neoplasmes (formed secondary ABC) accounts for ≈30%. There are many and variable lytic and expansive "fluid-filled cavities" in pediatric population, needing differential diagnosis of an ABC [2-5], to find leading trigger mechanisms of a preexisting processes, initiation intraosseous hemodynamic, inflammatory and reactive changes resulting by formation in well-defined "blood-filled" cavities and fibrous trabeculation. According the data [6-9] and our experiences, ABC termed as locally aggressive tumor. Different treatment tools are described in literature [2,3,6,10]. Therefore, this area presents an opportunity risk aggression (metastasis), risk of recidives (relapses). Considering the above, specific objectives of the current study were formulated and described histologically more similar disease for improve the accuracy of diagnosis: 1. ABC, 2. Fibrous dysplasia of bone (FD), 3) benign osteoclastic giant cell-rich neoplasm (GCT).

We evaluated our cases within in period 2021-2023 years [11] and analyzed the histopathological and immunohistochemical features. On the most updates WHO classification (2020), terms and definitions changed, main histological distinction of solid variant ABC as the lack of blood-filled cystic spaces, a predominantly solid architecture [2,4] were described.

Materials and Methods

We conducted a prospective study using cases, diagnosed as ABC

(n=19), and "ABC-like changes", "Giant cell tumor of bone" (GCT) (n=7) and "Fibrous Dysplasia" (FD) (n=2) between the years 2021-2023 and were evaluated according radiological and clinical features: patients age, gender, tumor localization, recurrence and histopathologicals characteristics using 4 histopathologist. Total number of cases – 28, GCT – 7, FD – 2, ABC – 17. Female – 10 (43%), male – 18 (57%); Mean age – ≈27 (Figure 1). ABC-like changes lesions associated with GCT of bone are epiphyseal or proximal location. In cases of FD, the lesion was diaphyseal.

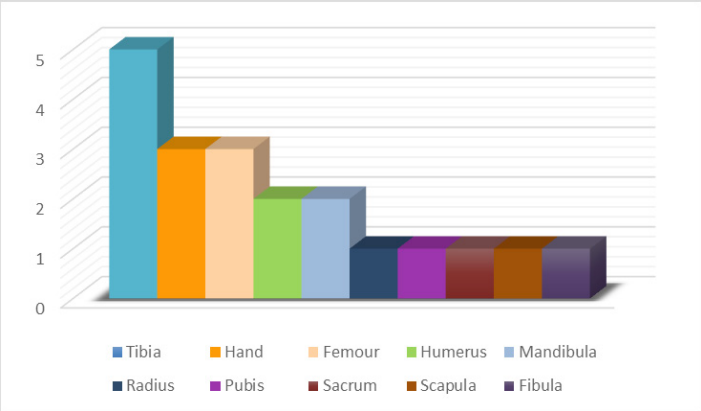

Figure 1: Location of ABC in bone.

Tissue samples, taken from operation by curettage including ABC cystic wall and tumor area in GCT, were treated by routine methods: Hematoxylin and Eosin (H&E) staining for diagnosis, make by 2 pathologists (D.M., A.M.). Tissues biopsy material from FD characteristic area, were treated by similar methods before special radiation therapy procedure. Radiological consultation was given for all patients, secondary changes, complicating basic processes, also took into account.

IHC for this study was performed on the "Leica N" using anti-Bcl2, anti-P63, P53, anti-Ki67, CD68 and anti-Cyclin D1 (EP12) primary antibody (Table 1). The protocol was established according "Leica (Novocastra)" recommendations. Immunohistochemical methods used in all discussing pathologies (Table 1).

Table 1: Immunohistochemical markers used in study.

Markers	Clone	Number of Cases
Bcl-2 Oncoprotein	Leica Novocastra Bcl-2/100/D5	28
P53 Protein	Leica Novocastra DO-7	28
P63 Protein	Leica Novocastra 7JUL	28
CD68 Monoclonal Anitody	Leica Novocastra 514H12	28
Cyclin D1 (EP12) Primary Antibody	Leica Novocastra EP12	28
Ki 67 Antigen	Leica Novocastra MM1	28

As basic concepts, we conclude that transcriptional proteinsp53, p63 and bcl-2 are most frequently mutated gene in the human dysplastic processes; high mitotic index (7 and more) recently [12] the histology of the specimens correlated with the risk of recurrence.

Statistical analysis of the reaction results was carried out using SPSS 21.0 (SPSS, US) student's test utilized to compare status differences ($p \leq 0.05$).

Results

ABC. The total cases of "Aneurismal Bone cyst" diagnosed materials include 28 patients, 10 female (≈43%) and 18 males (≈57%). Age ranged between 7 and 49 (mean age of ≈27). Processes were localized most frequently at the epiphysial or proximal part of tibia (5 cases) and hands (3 cases), for each case at distal humerus, sacrum, pubis, mandibula and radius (all 9 cases). Most of cases were as monostotic form, only one patient presents 2 affectation of ABC: in pubic and sacrum region (figure 2). Location of the ABC is demonstrating in figure1.

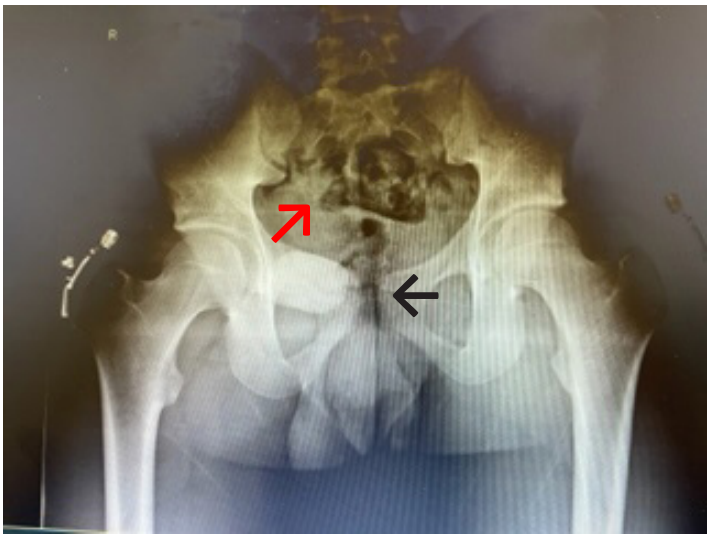

Figure 2: Magnetic resonance image of the 18-year-old male patient: rare location of ABC –Right pubic (black arrow) and sacrum (red arrow) regions.

MRI scanning shows fluid-filled cystic formation as solitary, multicystic structures; well-defined locally destructive plane can be fixed (Figure 2). Our materials show that ABC is presented as blood and interstitial liquid filled cavities of different size [11]. According researchers, since Panoutsakopoulos et al. [13], Oliveira et al. [14], ABC discuss as a recurrent chromosome ablation [15], leads to a fusion gene of the entire Ubiquitin-Specific Protease 6 (USP6), coding sequence at 17p13 and the promoter region of the osteoblast Cadherin 11 gene (CDH11) [15]. Based on this analysed date, ABC, involving USP6, termed as Primary neoplasm and in none- as the secondary ABCs [16].

In this article, we first discuss the ABC like changes associated with preexisting osseous pathology, in cases of Giant cell tumor and Fibrous Dysplasia. Next generation of our research, including

CDH11 examination in order to diagnose our cases classified primary ABCs from secondary ABCs. Blackburn et al. [17] and Deventer N et al. [10] have been demonstrated RNA sequencing initial step of US P9X-USP6.

Our results are given in Figures 3-5 and briefly conclude here:

1. The histopathological and comparative IHC profiles of the three most common osseous pathology: GCT, FD with ABC and ABC-like changes, focused on the: cellular component – multinucleated giant cells; fibrillar component – extracellular matrix (neoplastic stroma); and an osteoid organic bone matrix.
2. Histologically, most of ABCs cases, regardless location, appear as large multicystic cavities, well-defined lytic lesion, concerning hemorrhage liquid substances, osteoclastic and mono- and multinuclear cells infiltrates.Rarely, composition of cystic cavities was mixture, or solid (3 cases). Lesion of bone presents thin capillaries vessels, hemosiderin inclusions (Figure 3). It is notable, according our data, that in ABC, developed from GCT, giant cells are smaller size, stroma with collagen fibers, amorphous substance, cells – fibroblast, macrophages, mast cells. Chondroid like materials, immature trabecule neogenesis were shown with prominent fat necrosis. In these cases, trabeculation was lined by active osteoblasts and osteoclasts with resorbtive activity. Microscopically, cystic walls were composed mainly by mature lamellar bone tissue and immature fibrous bone tissue. Osteoclastic resorbtion activities also have been presented at the margin of cystic cavities (Figure 3a).

In the context of diagnostic routine, Ki-67 antibody activities from the affected region samples found to be non-elevated (less than 8%) only in periphery of bone constructions regenerating foci that's way solid bone cyst count determined as neoplastic process.

3. Common preexisting pathology of ABC in reported material was Giant cell Tumors (n=7), mean age – 22,6; Location in proximal tibia (3 cases), femur (2 cases) and sacrum and pubis (2 cases). The appearance on IHC is that of numerous nonneoplastic osteoclastic $CD68^{+}$ positive and remarkable neoplastic p63 positive giant cells, massives of collagen composed fibrous tissue (figure 3,4a).

Activities of bcl2 and p53 were determined as less positive reaction in secondary cystic wall into circulating oriented perimembraneus areas.

Microscopically, pathognomonic for ABC, transforming from Giant cell tumor, was non spongy reactive bone. Hemorragic masses brown hemosiderin and calcification are not detected. Under low-power view, cystic changes with solid areas, resembling in common solid tissue of GCT, were shown. But accurate visualization of ABC and preexisting Giant cell tumor

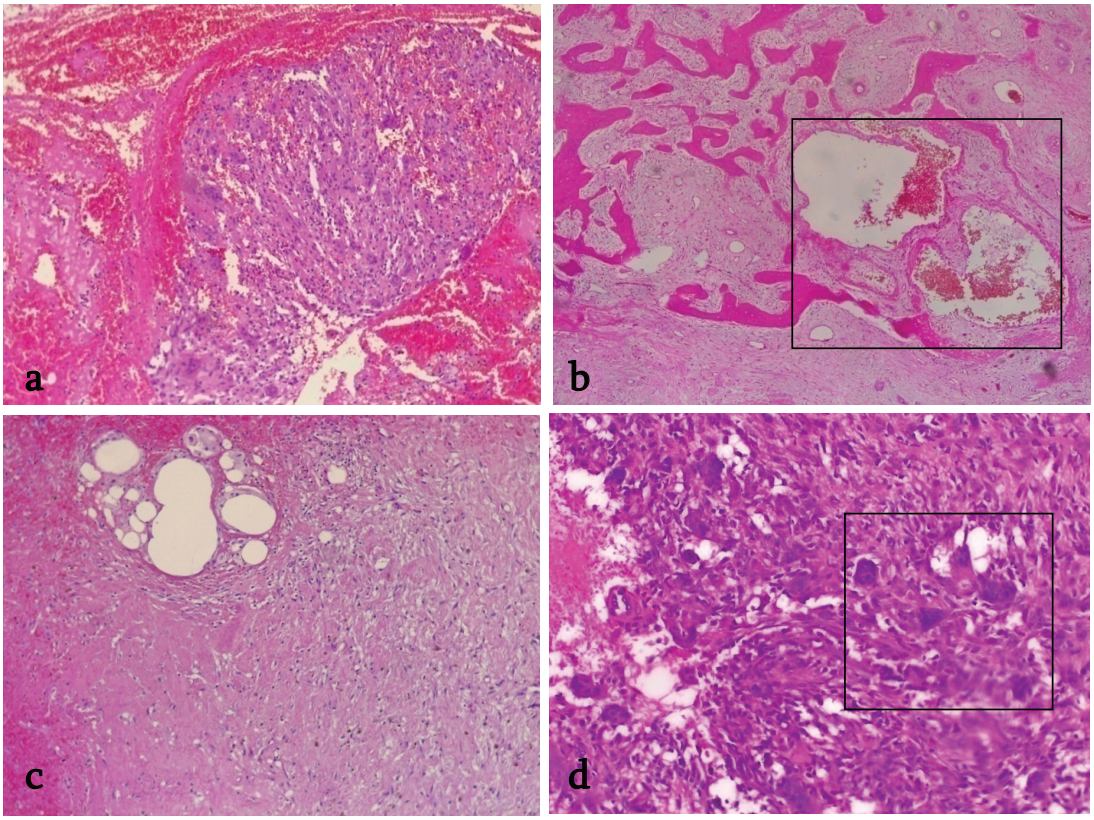

Figure 3: ABC microscopical features. H&E staining revealed marked differences and similarity between two pathological processes: a) in cystic cavities wall giant cells, haemorrage cystic transformation after GCT degeneration forming ABC-like changes, X100; b) presence of Aneurysmal Bone cyst like structure with blood mass, X100; c) fibrous connective tissue with granulomatosis (inflammation), fat necrosis, X200; d) multinuclear giant cells in GCT, X400.

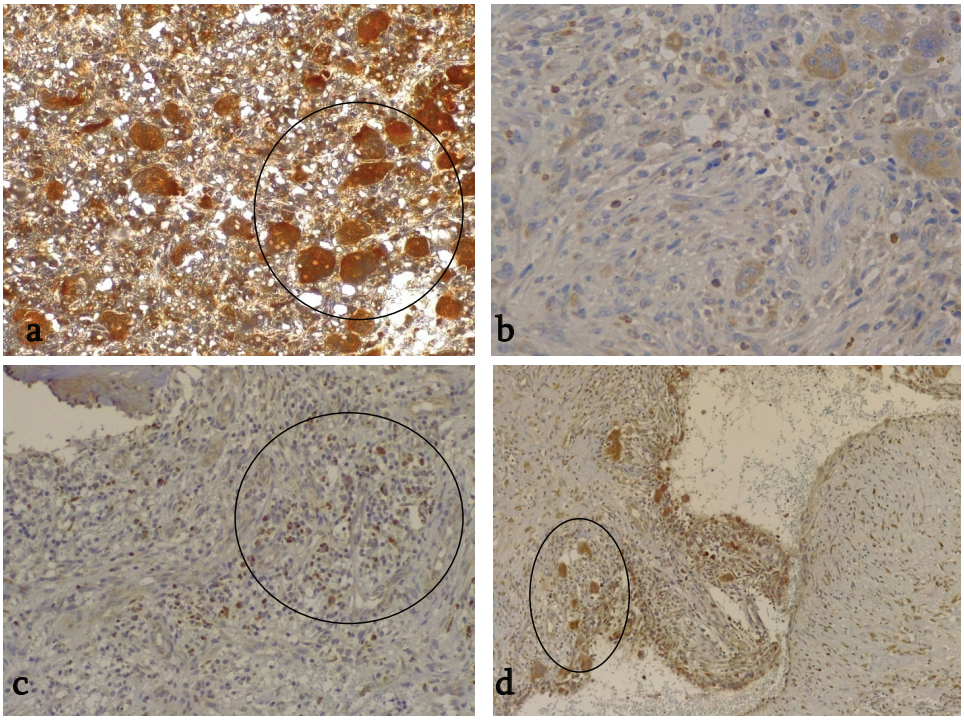

Figure 4: Immunomarkers validation in Aneurysmatic Bone cyst: a) Giant cells tumor background staining CD68, X200; b) Fibrous dysplasia – hypercellular fibrous stroma background revealed marked reduction of bcl2, X400; c) same - p63 high positive reaction, X200; d) Cyclin D1 positive cell infiltration of bone cysts tissue, X100.

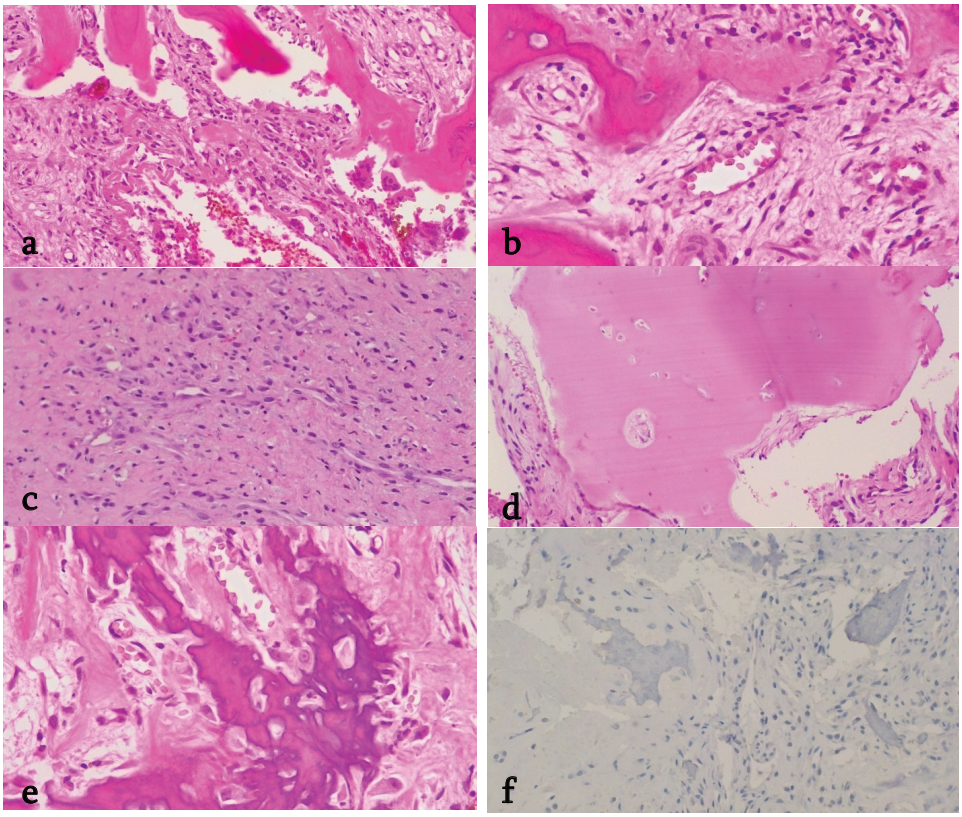

Figure 5: Histological characteristics of ABC areas in FD background. H&E. a) Neoangiogenesis in trabecules, X200; b) Blood full cavities, X400; c) Cellular polymorphismus, X200; d) Atypical ossification, X200; e) Trabecules immaturity, osteoclasts activities, X400; f) P53 negative reaction within damage area, X200.

background changes is important for differential diagnoses from benign and malignant lesions.

In this point of view, marked positivity of p63 cells are important. Under high-power microscopical and immunohistochemical evaluation: characteristic appearance is the degenerative cystic part of the lesion [6,15,18].

4. "Aneurysmal bone cyst" associated to Bone neoplasm (FD).

ABC like changes would be termed as a secondary cystic bone change as opposed to the septal proliferations of fibroblast in case of ABC [19]. Less common precursor of ABC is Fibrous Dysplasia transforming after specific pathophysiologic change [20].We obtained 2 cases of FD in humerus dyaphisis (patients average age ≈ 36 years). Sampling areas, showing FD with "nonspecific cyst": a small space, separated by a narrow septum, does not have the ABC appearance, but composed of wide bands of solid fibrous structures. Additionally, cystic areas were lined by granulation (inflammation) tissue. High mitotic activity and marked cell polymorphism are not characteristic for this preexisting lesion. According IHC in inflammatory areas and granulation tissue Ki-67 is positive in 15% of cells, P63 – was negative (Figure 4c). Cyclin D1 reaction revealed middle positivity in mono- and multinuclear infiltration area of bone cyst tissue (Figure 4d).

ABC, transforming for FD, demonstrates large cavity with fully blood, new cavities formation (Figure 5b), neoangiogenesis in trabecules, hemosiderine accumulations (Figure 5a), chronic inflammatory infiltrates, active osteoclasts. It's notable incomplete ossification processes and at this time, trabecules immaturity lysis, osteoclastic activity in more areas (Figure 5e). We have noted that ABCs with increased cellularity containing predominantly stromal and Giant cells, rearrangements of trabecular immaturity, had a higher risk of recurrence.

In ABC like changes, developing from Fibrous Dysplasia transformation, neoplastic area presents most important peculiarities: cellular polymorphismus associated (Figure 5c) with osteoclasts massive accumulations, cartilage in immature bone trabecules and incomplete atypical ossification (Figure 5d).

Immunohistochemical data have shown negative reaction of transcriptional proteins p53 (figure 5f) and bcl-2.

Discussion

Our research showed that within bone cyst area there is always an intense osteoclastic bone resorption. In addition, despite the fact that osteogenesis is manifested, the maturation of newly formed bone trabeculae through osteoblasts is disturbed, simultaneously with their differentiation, active resorption events are taking place. The data suggest that the basis of the bone cyst clinical progression is the incomplete (pathological and/or perverted) insufficient remodeling process in its wall, which confirmed by p63 transcriptional factors high expression in stroma and support the opinion that ABC pathogenesis is based on the GCTOB stroma malignant tendency, in parallel with the pathological regeneration.

Instead to the newly formed trabeculation, absorption is also manifested in the ridges represented by already formed osteons -bone defect formed result. In addition, both osteons andimmature bone foci structures are well vascularized, as a result of stretching of bony structures; blood vessels are "undressed" from the normal organic and inorganic environment. This event is followed by damage to the blood vessels, increased permeability of their walls, and blood spilling into the bone defect. That is why the cavity of the bone cyst often contains bloody contents, which many authors point out [11,13,21].

The present study showed that bone cyst consists of bone and connective tissue layers, the thickness of which is variable, as a sign of residual cyst activity, immature trabeculation, which seems to play a large role as characteristic of GCTOB developed ABC. The present study confirms the efficacy of p63 and Cyclin D1 detection guided diagnosis of ABCs, mainly localized in sacro-pubic areas. Intense cellular absorption is usually observed, due to which it is rich in osteoclasts and granulation connective tissue [15,16,18,19,22].

The immunohistochemical examination result of the present study support our concept about connective tissue septae formation in the bone cyst chambers, which develop as a result of bone connective tissue transformation– metaplasia, demonstrating Cyclin D1 expressionincreasing synchronously against the background of giant cell activity: the fibrotic septum surrounding the cyst contains fibroblasts and a stroma rich in Cyclin D1 and P63 positive cells (Figure 4c,d, Figure 5).

P63 protein expression increase in dysplastic and cancerous stroma of GCTOB transforming into secondary ABCs. Comparing the IHC data, it can be suggested that bone neoplasia (true Aneurysmal cysts) is a pathology, characterized by the development of a cystic defect in the process of forming the definite shape of the skeleton in the postnatal ontogeny, but in ABCs, resulting on GCTOB development is a accommodation, taking place in the bone after many metastatic steps of transformation. We confirmed different structural and immunohistochemical activities of ABC in the background of fibrous dysplasia.

On the one hand, Immunohistochemical analysis shows the simultaneous, equall expression of Cyclin D1 and P63 in the secondary ABCs cells developed from GCTOB, by degeneration and transformation, namely (Figure 4a,d) in the nuclei of both mononuclear and multinuclear giant cells, it should be noted here that Giant cells activity corresponding to tumor stroma in terms of CD68 and P53 activities.

On the other hand, the tumor field resulting from FD and containing its background features does not show similar activities, P63 is negative in FD cases. Staining showed 30-68% positive reaction

for GCTOB and up to 50% for tumor cells in ABC cases. Hammas and co-authors reported [22] on P63 activity during ABCs. Our study results of thyroid papillary microcarcinoma and follicular epithelial dysplasia in Hashimoto's thyroiditis (2021, 2022) suggests that P63 - a component of the P53-P63-P73 family of transcriptional proteins [23] is a reliable differential diagnostic biomarker for morphologically similar lesions, especially with the participation of Giant cells.

Conclusion

The immunohistochemical study result was shown demonstrating Cyclin D1 expression in ABC with Giant cell tumor activity. Fibrous Dysplasia as a background process of ABC, is rich in Cyclin D1 and P63 positive cell. Important for diagnostic efficacy of ABC variants is P63 transcriptional protein activity increasing in dysplastic and cancerous stroma of GCTOB transforming into secondary ABC.

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