An Analysis of Giant Cell-Rich Bone Lesions Evaluating p63 Expression as a Diagnostic Biomarker for Giant Cell Tumour of Bone

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Abstract

Objectives: This study explores the expression of p63 in giant cell-rich bone lesions to assess its diagnostic relevance in distinguishing giant cell tumour of bone (GCTB) from similar entities.

Methods: A retrospective diagnostic accuracy analysis was conducted on 128 bone biopsy cases diagnosed as giant cell-rich lesions at Khoula Hospital, over a 12-year period (2009–2021). Immunohistochemical staining for p63 was performed, and results were interpreted independently by two pathologists. Data were analysed using IBM SPSS Statistics, Version 28.0.

Results: The mean age of patients was 23 years, with lesions predominantly found in the femur, tibia, and small bones. Strong nuclear p63 expression was observed in most GCTB cases, while variable positivity was noted in aneurysmal bone cysts, chondroblastomas, brown tumours of hyperparathyroidism, and other giant cell-rich lesions. Although p63 was highly sensitive for GCTB, its specificity was limited due to overlap with other entities.

Conclusions: p63 may aid in identifying GCTB, especially in diagnostically ambiguous cases. However, due to its limited specificity, it should be interpreted in conjunction with clinical and radiological findings rather than used as a sole diagnostic marker.

Keywords: Giant Cell Tumors; Bone; Tumor Protein p63, Human; Immunohistochemistry.

Introduction

Giant cells are found in a broad spectrum of reactive and neoplastic bone conditions, including giant cell tumour of bone (GCTB), aneurysmal bone cysts (ABC), giant cell reparative granuloma (GCRG), brown tumour of hyperparathyroidism (BTH), chondroblastoma, chondromyxoid fibroma (CF), and non-ossifying fibroma (NOF).[1] GCTB is a primary bone neoplasm that predominantly affects young adults, with a slight female predominance.[1] This neoplasm exhibits locally aggressive behaviour and is associated with a high risk of recurrence, typically arising in the epiphyseal region of long bones.[2] Although pulmonary metastases may occur in some cases, transformation into high-grade sarcoma remains an uncommon event.[3]

GCTB accounts for 4–5% of all primary bone tumours and is most frequently observed in skeletally mature individuals, with peak incidence between the ages of 20 and 45 years.[1,2] Histologically, the neoplastic mononuclear stromal cells in GCTB exhibit a range of morphologies, from round or polygonal shapes to spindle-shaped forms

arranged in a storiform pattern. Additional features may include haemorrhage, fibrosis, ossification, necrosis, and xanthomatous change. Secondary aneurysmal bone cyst-like changes are present in approximately 10% of cases.[1] These histological variations can make differentiation of GCTB from other giant cell-rich neoplasms such as ABC, GCRG, chondroblastoma, and NOF challenging.

Although radiological assessment plays a significant role in diagnosing GCTB, some cases remain difficult to interpret, particularly when based on small core needle biopsies, involvement of atypical anatomical sites, or in skeletally immature patients.[2]

The p63 protein, encoded by a member of the p53 gene family, is commonly expressed in the basal layers of epithelial tissues across several organs, such as the skin, cervix, breast, urinary bladder, and prostate.[4] It also plays a significant role in skeletal formation, with mutations linked to congenital syndromes like limb-mammary syndrome in humans, and skeletal defects observed in knockout mouse models.[5]

Owing to its strong reactivity in mammary myoepithelial and prostatic basal cells, p63 has become a routinely used immunohistochemical marker in the diagnostic evaluation of breast and prostate malignancies.[6,7] More recently, both immunohistochemistry and molecular investigations have shown elevated p63 expression in the stromal component of most GCTB, suggesting a potential diagnostic role in this context.[8-13]

The aim of this study is to analyse the spectrum of giant cell rich bone lesions, assess the expression of p63 in GCTB, and evaluate whether p63 can serve as a discriminative biomarker for distinguishing GCTB from other morphologically similar lesions.

Methods

This study is a retrospective, cross-sectional diagnostic accuracy study. The target population included patients of all age groups who were diagnosed with GCTB via biopsy at Khoula hospital between 2009 and 2021. Only cases with adequate material (paraffin blocks and haematoxylin & eosin-stained slides) and available clinical details were included. All cases were reviewed to confirm the diagnosis; those with diagnostic discrepancies or insufficient tissue for immunohistochemical analysis were excluded. The final sample comprised 128 cases, which included 52 cases of giant cell tumour of bone, 26 aneurysmal bone cysts, one chondromyxoid fibroma, 23 non-ossifying fibromas, six brown tumour of hyperparathyroidism, 12 chondroblastomas, and eight giant cell reparative granulomas.

Expression of p63 was evaluated using immunohistochemistry. A case was considered positive when nuclear staining was observed in at least one lesional cell. The stained slides were independently reviewed by two pathologists who were blinded to the original histological diagnoses. In the absence of a universally accepted scoring system, staining intensity was categorised as weak (1+), moderate (2+), and strong (3+), while the proportion of positive cells was recorded as either ≤10%, between 11% and 50%, or >50%.

Staining was carried out on three-micrometre-thick, formalin-fixed, paraffin-embedded tissue sections. All samples were processed alongside appropriate positive and negative controls. The tissue sections were pre-treated with EnVision FLEX Target Retrieval Solution of High pH (Tris/EDTA buffer, pH 9) using Dako PT Link system (Agilent Technologies, Santa Clara, USA), and were subsequently transferred to the Daka Autostainer Link48 (Agilent) for proper staining. The staining protocol involved the use of EnVision FLEX peroxidase-blocking reagent, which contains phosphate buffer with hydrogen peroxide, sodium azide at a concentration of 15 mmol/L, and detergent. Monoclonal mouse anti-human p63 protein, clone DAK-p63, was used in ready-to-use form. This was followed by EnVision FLEX/HRP, which comprises dextran-coupled peroxidase and goat-derived secondary antibody molecules targeting both rabbit and mouse immunoglobulins in a buffered solution containing stabilising protein and preservatives. Additional reagents included Envision FLEX wash buffer (Tris-buffered saline with Tween 20, pH 7.6), EnVision FLEX DAB+ Chromogen (3,3’-diaminobenzidine tetrahydrochloride in organic solvent), and Envision FLEX haematoxylin used as a counterstain.

Analysis was conducted using MedCalc software Version 19.1.6 to calculate the median and mean age of the cohort, the sex distribution, and the overall expression rate of p63. Sensitivity, Specificity, positive predictive value

and negative predictive value were computed for p63 expression in distinguishing GCTB from other lesions. Comparative statistical assessments between GCTB and non-GCTB, as well as p63-positive and p63-negative groups, were performed using the Statistical Package for the Social Sciences (SPSS) Version 28.0 (IBM Corp., Armonk, New York, USA).

The study was approved by the research ethics committee of Khoula Hospital, Ministry of Health (XXXXXX) in Oman.

Results

In this study, the mean age of patients with giant cell-rich lesions was 23 years, with a range from 4 to 78 years. Of these patients, 45% were male and 55% were female. Lesions were most frequently located in the femur, tibia, and small bones (Figure 1).

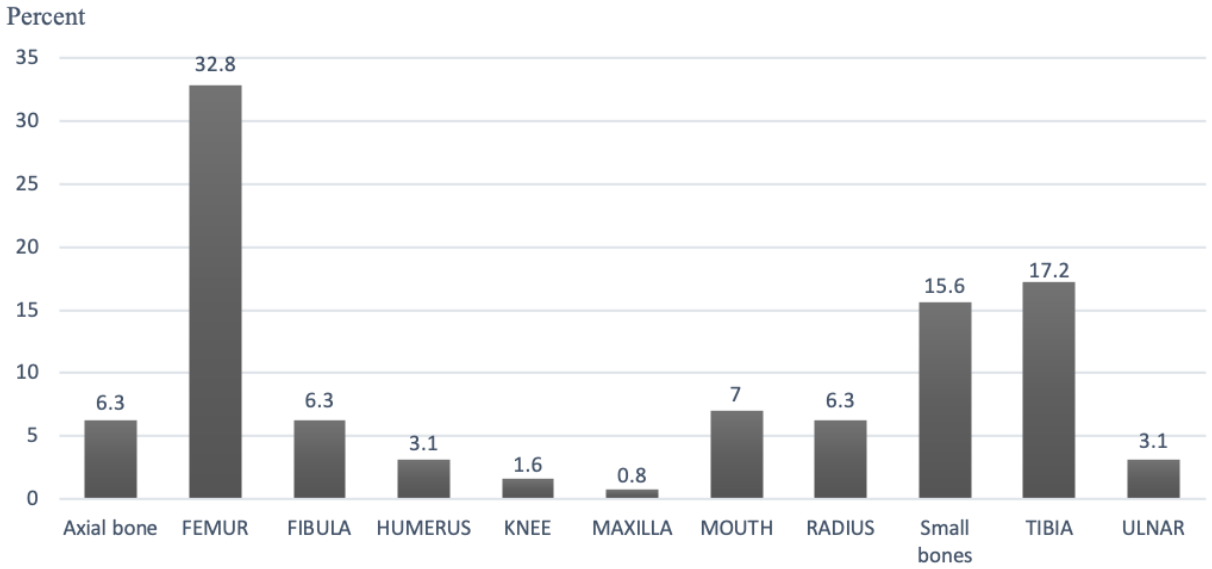

Figure 1: Distribution of Giant cell rich lesions in different bone locations.

Immunohistochemical analysis demonstrated nuclear p63 expression in 48 out of 52 cases (92.3%) of GCTB. Expression was also observed in 11 of 26 cases (42.3%) of ABC, one of one case (100%) of CF, three of 23 cases (13.0%) of NOF, four of six cases (66.7%) of BTH, nine of 12 cases (75%) of chondroblastoma, and two of eight cases (25%) of GCRG (Table 1). Immunoreactivity was predominantly restricted to mononuclear stromal cells. Although the intensity and percentage of positive cells varied among cases, strong to moderate staining was seen in most cases of GCTB and in a limited subset of other giant cell-rich lesions.

Table 1: p63 immunohistochemical findings in different types of giant cell-rich lesions.

Diagnosis	No. of cases	p63 positive cases	Staining intensity			p63 positive cells		
		n (%)	1+	2+	3+	≤10%	11–50%	>50%
Giant cell tumour of bone	52	48 (92.3)	9	13	26	6	9	33
Aneurysmal bone cyst	26	11 (42.3)	6	5	0	4	5	2
Brown tumour of hyperparathyroidism	6	4 (66.7)	3	1	0	1	1	2
Chondromyxoid fibroma	1	1 (100)	1	0	0	1	0	0
Chondroblastoma	12	9 (75)	1	3	5	2	1	6
Giant cell reparative granuloma	8	2 (25)	2	0	0	1	1	0

Non-ossifying fibroma	23	3 (13)	2	0	1	0	2	1

Strong nuclear p63 expression was detected in 26 of 48 cases (54%) of GCTB, five of nine cases (55.6%) of chondroblastoma, and one of three cases (33.3%) of NOF (Figure 2). No strong staining was observed in ABC, BTH, CF, or GCRG. Moderate staining was present in 23 cases of GCTB, three cases of chondroblastoma, five cases of ABC, one case of BTH. No moderate p63 expression was observed in CF, GCRG, or NOF.

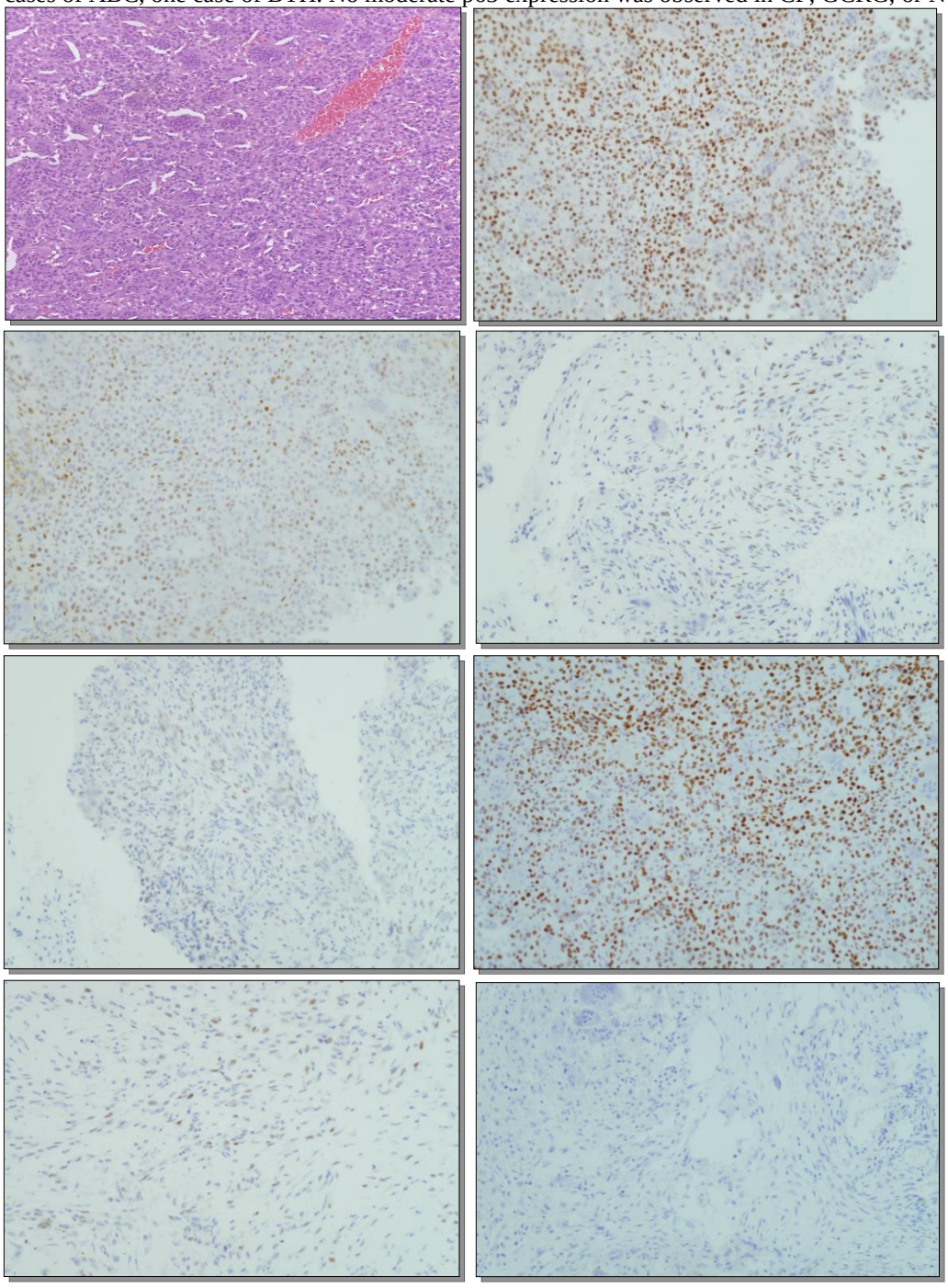

Figure 2: Immunohistochemistry findings of p63 in the mononuclear stromal cells in different diseases. **A:** Histological findings of giant cell tumour of bone (haematoxylin and eosin). **B:** GCTB, 3+ positive staining. **C:**

ABC, 2+ staining. **D:** BTH, 1+ staining. **E:** CF, 1+ staining. **F:** Chondroblastoma, 3+ staining. **G:** GCRG, 1+ staining. **H:** NOF, negative staining.

With regard to the extent of staining, more than 50% of tumour cells were positive in 33 of 48 cases (68.8%) of GCTB, two of 11 cases (18.2%) of ABC, two of four cases (50%) of BTH, six of nine cases (66%) of chondroblastoma, and one of three cases (33.3%) of NOF. In CF and GCRG, p63 expression was limited to less than 50% of tumour cells.

The sensitivity and negative predictive value of p63 immunohistochemistry for GCTB were found to be 92.31% and 92.0%, respectively, whereas the specificity and positive predictive value were lower, calculated at 60.53% and 61.54%, respectively.

Discussion

This study demonstrated that a significant number of GCTB cases (92.3%) exhibited nuclear expression of p63, a figure that is significantly higher than that observed in any other giant cell-rich lesions evaluated. However, p63 positivity is not specific to GCTB and may be seen in other entities, including ABC, BTH, CF, chondroblastoma, GCRG and NOF. This non-specificity significantly lowers its utility as a standalone diagnostic marker. Similar observations were reported in several studies, all of which assessed p63 expression by immunohistochemistry.[9,10,12] In the study conducted by De la roza's *et al*., p63 staining was positive in 20 out of 23 GCTB cases (86.9%).[10] Lee *et al*. reported p63 positivity in 81% of GCTB cases, with strong staining in 69%.[9] Shooshtarizadeh *et al*. recorded p63 expression in 96.8% of GCTB cases.[12]

In contrast, findings reported by Dickson *et al*. and Hammas *et al*. differed from those described above.[8,11] Dickson *et al*. identified p63 mRNA expression through real-time polymerase chain reaction and observed p63 protein overexpression by immunohistochemistry in all cases of GCTB, with only limited expression detected in other types of giant cell-rich lesions.[8] Similarly, Hammas *et al*. observed p63 positivity in all GCTB cases.[11] Both studies concluded that p63 may be useful as a diagnostic biomarker for differentiating GCTB from other morphologically similar lesions, particularly GCRG, as none of the GCRG cases in their cohort expressed p63.

In the current study, eight cases of GCRG were evaluated, two of which (25%) demonstrated p63 positivity, though with weak intensity and staining in less than 50% of tumour cells. De la roza *et al.* reported p63 positivity in all four of their recorded GCRG cases, a finding that contradicts the results of Lee *et al,* Dickson *et al*., and Hammas *et al*., who each documented complete absence of p63 expression in GCRG.[8-11]

With regard to ABC, the current study demonstrated a higher rate of p63 expression compared to several previous studies. Dickson *et al*., Lee e*t al*., and Shooshtarizadeh *et al*. reported positivity rates of 28.6%, 20%, and 22.2% respectively.[8,9,12] De La Roza reported a higher rate of 62.5%, while Hammas *et al*. documented a similar result to our own, with p63 positivity in 40% of ABC cases.[10,11]

Our study included one case of CF, which demonstrated very weak p63 staining, limited to 10% or fewer of tumour cells (Table 1). This aligns partially with Hammas *et al*., who reported one p63-positive case among three CF cases.[11] In contrast, none of the 12 CF cases in the study bt Lee *et al*. were p63 positive. CF was not included in the studies conducted by Dickson *et al* or De La Roza *et al*.[8,10]

We recorded 12 cases of chondroblastoma, of which nine cases (75%) demonstrated p63 expression. Among these, five cases showed strong intensity with staining in more than 50% of tumour cells (Table 1). Other studies have reported variable rates of expression. Hammas *et al*. documented a single case with low-intensity staining in fewer than 10% of tumour cells.[11] Dickson *et al*. found p63 positivity in three out of ten chondroblastomas (30%), with mild to moderate staining intensity and a staining range of 7–75% of tumour cells.[8] De La Roza *et al*. reported a higher expression rate, with ten of 12 chondroblastomas (83.3%) testing positive for p63. Of these, one case showed strong staining, six showed moderate staining, and three showed weak staining.[10] Lee *et al*. evaluated 15 chondroblastomas and found p63 expression in six cases (40%). This group employed S-100 immunostaining to differentiate between chondroblastoma and GCTB, noting that chondroblastoma cases were S-100 positive, whereas

GCTB showed negative or only occasional weak immunostaining for S-100.[9] Shooshtarizadeh *et al*. found 68.7% of chondroblastoma cases to be positive for p63.

In our study, p63 expression was observed in 13% of NOF cases (three out of 13), with two cases showing weak staining and one case demonstrating strong intensity (Table 1). De la Rosa *et* al. reported a comparable result, with p63 positivity in one of six NOF cases (16.6%).[10] Hammas *at al*. and Shooshtarizadeh *et al*. found higher positivity rates of 25% and 50%, respectively.[11,12]

We recorded six cases of BTH, four of which (66.7%) demonstrated weak to moderate nuclear p63 staining. These findings are similar to those of Shooshtarizadeh *et al*., who reported 75% p63 positivity in BTH.[12] However, this contrasts with Lee *et al*., who identified no p63 expression in any of the four BTH cases in their series.[9] BTH was not evaluated in the studies conducted by Hammas *et al*., De la Roza *et al*., or Dickson *et al*.[8,10,11]

Several factors may account for the discrepancy in the findings between this study and those of previously reported by Dickson *et al*., Shooshtarizadeh *et al*., Lee *et al*., and Hammas *et a*l.[8,9,11,12] One potential explanation involves differences in antigen retrieval protocols. In our study, immunohistochemistry was performed using EnVision FLEX Target Retrieval Solution, High pH (Tris/EDTA buffer, pH 9) with the Dako PT Link system (Agilent), followed by staining on the Daka Autostainer Link 48 (Agilent). By contrast, the other studies used citrate-based buffers with various heat retrieval methods: microwave-based retrieval in (Dickson *et al.*'s study, pressure cooker in Lee *et al.*'s, and Ventana Benchmark LT automated immunostainer in Hammas *et al*.'s study.[8,9,11]

Another possible source of variation is the use of tissue microarrays in Lee *at al*.'s study,[9] which may have limited their ability to detect heterogeneity in p63 staining. While differences in positivity thresholds across studies may also have contributed,[8,9] they do not fully explain the observed inconsistencies. In this study, nuclear staining for p63 was interpreted as positive regardless of the extent, with further categorisation based on staining intensity—classified as weak, moderate, or strong—and the proportion of tumour cells involved, described as focal (10% or less), intermediate (11–50%), or extensive (greater than 50%). Hammas et al. applied a similar scoring system.[11] However, Dickson et al. defined p63 positivity as staining in more than 5% of cells, whereas Lee *et al*. used a 10% threshold.[8,9] According to Lee *et al*.'s criteria, six GCTB cases in our study that exhibited nuclear p63 staining in fewer than 10% of cells would have been classified as negative.[9] Nonetheless, differences in cut-off thresholds do not account for all discrepancies, as other cases fall outside these margins. Additionally, variability in antibody clones and their respective sources may have influenced staining sensitivity and interpretation across studies.

In routine diagnostic practice, GCTB is typically identified on standard histological examination, and diagnosis is usually straightforward. However, in certain contexts such as limited sampling from core needle biopsies, atypical locations, or lesions occurring in children immunohistochemistry may be useful.

Despite variability in reported findings, some researchers have proposed the utility of p63 in distinguishing GCTB from other morphologically similar giant cell rich lesions. For example, the absence of p63 expression in GCRG in the studies by Dickson *et al*. and Hammas *et al*. supported the use of p63 in this differential context.[8,11] However, De La Rosa *et al*. reported a specificity of only 53.36% for p63, though its negative predictive value was high (91.17%), limiting its practical application as a highly specific immunohistochemical marker.[10]

Although our study also demonstrated a high negative predictive value for p63 (92%) in GCTB, its low specificity (60.53%) means it cannot be relied upon as a definitive diagnostic tool in isolation. In clinical scenarios where sampling is limited, or the lesion presents in an unusual site or in a paediatric patient, caution should be taken when interpreting the absence of p63 expression.

RANKL is highly expressed in the neoplastic mononuclear stromal cells of GCTB, where it drives osteoclast differentiation through RANK signaling, making it a key therapeutic target and diagnostic marker.[14] GCTB harbour pathogenic H3.3A gene mutation in 95% of cases and hence H3.3 p.Gly34Trp (G34W) is a reliable surrogate marker for molecular analysis.[15-17] However we were unable to use neither G34W nor RANKL in our study due to non-availability at our center or regional referral centers.

These findings should be considered in light of certain limitations. This study is limited by its retrospective design and reliance on archival tissue, which may introduce selection bias and restrict control over pre-analytical variables. The use of a single antibody clone and staining platform may affect the reproducibility of immunohistochemical results across different laboratories. Additionally, the relatively small number of cases for certain lesion subtypes, such as chondromyxoid fibroma and brown tumour of hyperparathyroidism, limits the generalisability of findings related to these entities.

Conclusion

The immunohistochemical expression of p63 can be a useful adjunt in supporting the diagnosis of giant cell tumour of bone, particularly in diagnostically challenging cases involving limited biopsy material or atypical presentation. As its expression is not entirely specific, it should not be used in isolation. A multidisciplinary approach that incorporates histological assessment, clinical context, and imaging findings is essential for accurate diagnosis. In light of the histological resemblance between brown tumour of hyperparathyroidism and other giant cell-rich lesions, routine exclusion of hyperparathyroidism through appropriate biochemical testing is recommended.

Disclosure

The authors declared no conflicts of interest. No funding was received for this study.

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