Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Fine-Needle–Based Localization Techniques in Parathyroid Adenomas: A Retrospective Cohort Study of Histopathological Safety and Fibrosis Patterns

Tuğba Günler
Hamidiye School of Medicine, University of Health Sciences, Konya City Hospital
ilker Çordan
dr.ilkercordan@windowslive.com

Hamidiye School of Medicine, University of Health Sciences, Konya City Hospital
Sabri Özden
Hamidiye School of Medicine, University of Health Sciences, Konya City Hospital
Mustafa Çaycı
Hamidiye School of Medicine, University of Health Sciences, Konya City Hospital

Research Article

Keywords: Parathyroid adenoma, PTH washout, ROLL technique, stromal fibrosis, histopathological changes

Posted Date: April 10th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9182117/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Diagnostic Pathology on August 31st, 2026. See the published version at https://doi.org/10.1186/s13000-026-01849-w.

Abstract

Background

This study aimed to evaluate the association between fine-needle-based interventions widely used for preoperative and intraoperative localization of parathyroid adenomas and the development of post-excision stromal fibrosis and other histopathological changes that may mimic malignancy.

Methods

A total of 164 patients who underwent parathyroidectomy for primary hyperparathyroidism were retrospectively evaluated. The patients were divided into three groups according to the number of ultrasound-guided fine-needle interventions: needle-naive (Group 0, *n* = 70), single-needle (Group 1, *n* = 43), and two-needle (Group 2, *n* = 51). All surgical specimens were histopathologically examined for the presence and extent of stromal fibrosis, thick fibrous capsule, fibrous bands, hemorrhage, hemosiderin deposition, capsular pseudoinvasion, tumor implantation, granulation tissue, and necrosis. Intergroup comparisons were performed using Welch's t-test, the chi-square test, Fisher's exact test, and Tukey post hoc analysis.

Results

The mean age of the patients was 54.8 ± 11.9 years, and 83.5% were female. Histopathological examination revealed fibrosis in 28 cases (17.1%), with a median stromal fibrosis percentage of 2.0% (1.0–10.0). The presence of fibrosis differed significantly among the needle-naive (12.9%), single-needle (30.2%), and two-needle (11.8%) groups ($p = 0.028$). Among cases with fibrosis, the stromal fibrosis percentage was higher in the needle-naive group than in the single- and two-needle groups (median 6.5% versus 2.0% and 2.0%, respectively, $p = 0.045$). No significant differences were observed in the remaining histopathological parameters ($p > 0.05$ for all).

Conclusion

In this study, the association between fibrosis and fine-needle interventions in parathyroid adenomas differed according to intervention frequency. Although the presence of fibrosis was more frequent in cases with a single needle intervention, the stromal fibrosis percentage in cases with fibrosis was higher in the needle-naive group. In contrast, the remaining histopathological parameters did not differ significantly among the needle groups. These findings suggest that fine-needle-based interventions have an acceptable histomorphological safety profile in parathyroid adenomas.

Background

Primary hyperparathyroidism (PHPT) is a common endocrine disorder, most frequently caused by parathyroid adenomas, and is characterized by hypercalcemia. The definitive treatment for PHPT is parathyroidectomy. In current practice, bilateral neck exploration has largely been replaced by minimally invasive parathyroidectomy, the success of which depends on accurate preoperative and intraoperative localization [1, 2].

The most commonly used methods for preoperative adenoma localization are ultrasonography and technetium-99m sestamibi scintigraphy. However, both techniques have limited sensitivity and specificity and may yield false results in small adenomas, thyroid nodules, lymph nodes, cystic lesions, and regional malignancies [3, 4]. In cases where localization cannot be achieved with first-line tests, four-dimensional computed tomography offers additional diagnostic value but is used as a second-line modality only in selected centers because of its cost and limited availability [5]. Particularly in ectopic adenomas and in patients with a history of neck surgery, no single imaging modality alone can provide reliable localization [6, 7].

Ultrasound-guided fine-needle aspiration and the parathyroid hormone washout (PTH-WO) test have emerged as valuable diagnostic tools for confirming parathyroid tissue in PHPT cases where imaging findings are inconclusive [8]. Similarly, the radioguided occult lesion localization (ROLL) technique improves surgical success by allowing precise intraoperative identification of small or ectopic adenomas and reduces the need for extensive exploration [9, 10]. Nevertheless, there are still concerns regarding whether fine-needle interventions used for diagnosis and localization of parathyroid adenomas are associated with degenerative histopathological changes, including stromal fibrosis that may mimic malignancy. Although aspiration-related histopathological changes in parathyroid adenomas have been reported, the effect of these findings on the diagnostic process remains unclear [11–14]. Available studies are limited, and comprehensive datasets comparing needle-naive cases with patients exposed to different numbers of interventions at different time points are particularly scarce.

This study aimed to investigate the association between single or multiple fine-needle-based interventions related to PTH washout and ROLL procedures and histopathological changes in excised parathyroid adenomas that may be considered diagnostic pitfalls in terms of stromal fibrosis and suspicion of malignancy.

Methods

Study Design and Patient Selection

This retrospective cohort study was conducted at a tertiary care teaching and research hospital between June 2021 and June 2024. The study was carried out through multidisciplinary collaboration among the departments of pathology, endocrinology, general surgery, and nuclear medicine.

The inclusion criteria were age ≥ 18 years and being scheduled for surgical treatment with a diagnosis of primary hyperparathyroidism caused by a parathyroid adenoma. The exclusion criteria were secondary

or tertiary hyperparathyroidism due to chronic kidney disease, familial hyperparathyroidism syndromes, parathyroid tissues incidentally removed during thyroid surgery, concomitant thyroidectomy and parathyroidectomy, and a history of parathyroidectomy or cervical surgery accompanied by marked fibrotic adhesions. In addition, cases in which a diagnostic PTH washout procedure was attempted but did not yield successful results because of technical or biochemical reasons were excluded to eliminate the potential confounding effect of needle intervention. Patients with a history of malignancy that could affect stromal fibrosis or histopathological evaluation, those who had received radiotherapy to the neck region, and cases with missing preoperative, intraoperative, or histopathological data were also excluded.

A total of 342 parathyroidectomy specimens excised by the general surgery clinic over the study period were reviewed. After applying the defined inclusion and exclusion criteria, 164 patients with histopathologically confirmed parathyroid adenomas were included in the final analysis. To minimize selection bias, all consecutive patients who met the eligibility criteria were included. Demographic, clinical, biochemical, imaging, and surgical data for all patients were retrospectively obtained from the electronic medical record system of the hospital.

The patients were divided into three groups according to the frequency of ultrasound-guided fine-needle interventions performed for diagnostic or localization purposes. This classification was designed to assess potential histopathological risks related to intervention frequency. Table 1 presents the definitions of study groups, applied procedures, number of patients, and clinical implications.

Table 1
Definition of Study Groups and Distribution of Applied Procedures and Clinical Significance by Group

Group	Definition	Applied Procedure	Patients, n (%)	Clinical Significance
Group 0	No fine-needle intervention performed	None	70 (42.7%)	Natural control group independent of needle effect
Group 1	One fine-needle intervention performed	PTH-WO (diagnostic purpose)	43 (26.2%)	Group in which minimal needle effect was evaluated
Group 2	Two fine-needle interventions performed	PTH-WO + ROLL (preoperative-intraoperative localization)	51 (31.1%)	Group in which potential effects of repeated needle interventions were evaluated

[Insert Table 1 here]

Fine-Needle Interventions and Localization Techniques

Ultrasound-guided fine-needle interventions were performed by a single experienced endocrinologist with extensive expertise in ultrasound-guided fine-needle aspiration, using a standard 23-gauge needle under real-time ultrasonographic guidance. The procedures were carried out under sterile conditions using a linear transducer (13–15 MHz; Mindray DC-60 Exp HD, Shenzhen, China), with patients in the supine position and the neck in slight extension.

All interventional procedures were undertaken after obtaining written informed consent from the patients. Appropriate techniques and safety precautions were applied to minimize hematoma formation and tissue trauma. After the procedure, the patients were observed for approximately 30 minutes to monitor for potential complications.

Parathyroid Hormone Washout (PTH-WO) Test

The PTH-WO test was performed to confirm the parathyroid origin of lesions with suspicious imaging findings. Lesion contents were aspirated under negative pressure using a 23-gauge needle, then rinsed with 1 mL of isotonic saline and collected into an Eppendorf tube. Intact PTH levels in the samples were measured using Cobas e411 (Roche Diagnostics, Germany) and Immulite XPi (Siemens Healthineers, USA) analyzers (3–5,000 pg/mL). A washout PTH level higher than the simultaneous serum PTH level was considered diagnostic in favor of parathyroid tissue.

Radioguided Occult Lesion Localization (ROLL) Procedure

The ROLL technique was applied in small or ectopic adenomas that were confirmed by PTH-WO and were difficult to localize intraoperatively. Approximately 1 hour before surgery, 3.7–5.55 MBq (0.1–0.15 mCi) of 99mTc-macroaggregated albumin was injected intralesionally using a 23-gauge needle, in collaboration between endocrinology and nuclear medicine specialists. The accuracy of the injection was confirmed preoperatively using a gamma probe (Crystal Photonics GmbH, Germany).

Histopathological Evaluation

All surgically excised parathyroid adenoma specimens were fixed in formalin, entirely sampled, embedded in paraffin, and re-evaluated on 4 µm-thick hematoxylin and eosin-stained sections.

All cases were examined under light microscopy (Nikon Eclipse E200, Japan) by two experienced pathologists in the same laboratory. Histopathological evaluation included the assessment of hemorrhagic changes, hemosiderin deposition, edema, granulation tissue formation, tumor implantation, capsular pseudoinvasion, thick fibrous capsule, fibrous bands, and fibrosis (Fig. 1). In cases with fibrosis, the percentage was calculated by evaluating the entire largest sectional area of the fibrotic region at ×20 magnification (Figs. 2 and 3).

All assessments were performed in a blinded manner, independent of clinical, radiological, and fine-needle intervention data.

Statistical Analysis

All statistical analyses were performed using R software (version 4.3.2; R Foundation for Statistical Computing, Vienna, Austria). The distribution of continuous variables was assessed using the Shapiro-Wilk test. Variables with a normal distribution were expressed as mean ± standard deviation, whereas non-normally distributed variables were expressed as median (Q1–Q3). Categorical variables were summarized as number (percentage).

For intergroup comparisons, Welch's t-test was used for continuous variables under the assumption of unequal variances, and Welch's analysis of variance test was used for three-group comparisons. When a significant difference was detected, Tukey post hoc testing was applied to identify intergroup differences. Categorical variables were compared using the chi-square test, and Fisher's exact test was used when expected frequencies were < 5.

For the analysis of stromal fibrosis, only adenomas demonstrating histological fibrosis were included, and the fibrosis percentage was evaluated as a continuous dependent variable. All tests were two-tailed, and a p-value < 0.05 was considered statistically significant.

Results

Descriptive Characteristics

Of the 164 patients included, 83.5% (n = 137) were female and 16.5% (n = 27) were male, with a mean age of 54.8 ± 11.9 years. The demographic, clinical, biochemical, and histopathological findings of the study cohort are summarized in Table 2.

Table 2
Demographic, Clinical, Biochemical, and Histopathological Characteristics of the Study Cohort

Variable	(n = 164[1])
Patient Groups	
Group 0	70 (42.6)
Group 1	43 (26.22)
Group 2	51 (31.10)
Demographic Characteristics	
Sex	
Male	27 (16.46)
Female	137 (83.54)
Age (years)	54.79 ± 11.95
Biochemical Parameters	
Parathyroid hormone (pg/mL)	143.50 (110.00–196.50)
Calcium (mg/dL)	11.31 ± 1.35
Phosphorus (mg/dL)	2.69 ± 0.64
Vitamin D (ng/mL)	16.32 ± 8.75
24-hour urinary calcium (mg/24 h)	334.81 ± 173.51
Clinical Characteristics	
Interval between PTH-WO and surgery (days)[2]	60.00 (27.00–126.00)
Tumor Morphology	
Tumor size (mm)	15.18 ± 5.66
Histopathological Findings	
Presence of fibrosis	28 (17.07)
Stromal fibrosis percentage[3]	2.00 (1.00–10.00)
Thick fibrous capsule	12 (7.32)
Fibrous bands	28 (17.07)
Hemorrhage	87 (53.05)
Hemosiderin deposition	10 (6.10)

Variable		(n = 164[1])
Patient Groups		
	Capsular pseudoinvasion	8 (4.88)
	Tumor implantation	3 (1.83)
	Granulation tissue	1 (0.61)
	Edema	28 (17.07)
	Necrosis	1 (0.61)

[1]Continuous variables are presented as mean ± standard deviation or median (Q1–Q3), and categorical variables are presented as n (%), according to data distribution.

[2]The analysis was performed in a subcohort of 94 patients from Groups 1 and 2 with available PTH-WO data.

[3]The percentage of stromal fibrosis was calculated only in the 28 adenomas presenting with fibrosis.

Group 0: needle-naive group (PTH-WO not performed), **Group 1**: single-needle group (PTH-WO performed once), **Group 2**: two-needle group (two needle interventions performed for diagnostic and preoperative radioguided localization purposes), PTH-WO: parathyroid hormone washout

[Insert Table 2 here]

Comparison of Clinical and Histopathological Findings Among Groups

Comparisons among the three groups revealed statistically significant differences in tumor size (p = 0.011) and serum PTH levels (p = 0.006). Both parameters showed a decreasing trend with an increasing number of needle interventions.

On histopathological evaluation, the frequency of fibrosis was highest in Group 1 (30.2%). Among patients with fibrosis, the percentage of stromal fibrosis was significantly higher in Group 0 (Group 0: 6.5%; Group 1: 2.0%; Group 2: 2.0%; p = 0.045). Among the remaining histopathological findings, only the presence of fibrous bands differed significantly among the groups (p = 0.009). Comparison of data among the study groups is presented in Table 3.

Table 3
Comparison of Clinical, Biochemical, and Histopathological Parameters Among Study Groups

Variable	Group 0 (n = 70)[1]	Group 1 (n = 43)[1]	Group 2 (n = 51)[1]	p[2]
Tumor size (mm)	16.6 ± 6.4	14.9 ± 5.1	13.5 ± 4.5	**0.011**
Parathyroid hormone (pg/mL)	150.5 (111–237)	158 (125–245)	123 (89.6–153)	**0.006**
Calcium (mg/dL)	11.33 ± 0.78	11.51 ± 2.35	11.11 ± 0.60	0.16
Vitamin D (ng/mL)	13.7 ± 9.6	17.9 ± 9.2	17.5 ± 6.9	0.076
Presence of fibrosis	9 (12.9)	13 (30.2)	6 (11.8)	**0.028**
Fibrosis percentage[3]	6.5 (1.00–25.00)	2.0 (2.00–2.00)	2.0 (1.00–10.00)	**0.045**
Thick fibrous capsule	4 (5.7)	4 (9.3)	4 (7.8)	0.75
Fibrous bands	15 (21.4)	11 (25.6)	2 (3.9)	**0.009**
Hemorrhage	34 (48.6)	22 (51.2)	31 (60.8)	0.40
Hemosiderin deposition	3 (4.3)	4 (9.3)	3 (5.9)	0.55
Capsular pseudoinvasion	3 (4.3)	3 (7.0)	2 (3.9)	0.81
Tumor implantation	0 (0.0)	2 (4.7)	1 (2.0)	0.11
Granulation tissue	0 (0.0)	0 (0.0)	1 (2.0)	0.57
Edema	16 (22.9)	8 (18.6)	4 (7.8)	0.091
Necrosis	0 (0.0)	1 (2.3)	0 (0.0)	0.26

[1]Continuous variables are presented as mean ± standard deviation or median (Q1–Q3), and categorical variables are presented as n (%), according to data distribution.

[2]Pearson's chi-square test, one-way analysis of means not assuming equal variances, and Fisher's exact test

[3]The percentage of stromal fibrosis was calculated only in the 28 adenomas presenting with fibrosis.

Bold p-values indicate statistical significance ($p < 0.05$).

Group 0: needle-naive group (PTH-WO not performed), **Group 1**: single-needle group (PTH-WO performed once), **Group 2**: two-needle group (two needle interventions performed for diagnostic and preoperative radioguided localization purposes), PTH-WO: parathyroid hormone washout

[Insert Table 3 here]

Comparison Between Needle-Naive and Needle Intervention Groups (Groups 0 vs. 1 and 2)

When the needle-naive group (Group 0) was compared with the needle intervention groups (Groups 1 and 2), tumor size was larger in the former (16.6 ± 6.4 mm vs. 14.2 ± 4.8 mm; p = 0.010), whereas vitamin D levels were significantly higher in the latter (p = 0.023).

Histopathological examination showed a higher frequency of fibrosis in needle intervention groups, although this difference was not statistically significant (12.9% vs. 20.2%; p = 0.22). In contrast, the stromal fibrosis percentage was significantly higher in the needle-naive group (6.5% vs. 2.0%; p = 0.049). No significant differences were observed between the two groups with respect to the remaining histopathological findings (p > 0.05 for all).

Table 4 presents the histopathological comparison between needle-naive adenomas and adenomas subjected to fine-needle intervention.

Table 4
Histopathological comparison between needle-naive and needle intervention groups

Variable	Group 0 (n = 70)[1]	Groups 1 and 2 (n = 94)[1]	p[2]
Tumor size (mm)	16.56 ± 6.42	14.16 ± 4.82	**0.010**
Parathyroid hormone (pg/mL)	150.50 (111.00–237.00)	138.00 (102.00–177.00)	0.33
Serum calcium (mg/dL)	11.33 ± 0.78	11.29 ± 1.65	0.87
Vitamin D (ng/mL)	13.65 ± 9.60	17.65 ± 8.02	**0.023**
Presence of fibrosis	9 (12.86%)	19 (20.21%)	0.22
Fibrosis percentage[3]	6.50 (1.00–25.00)	2.00 (1.00–3.00)	**0.049**
Thick fibrous capsule	4 (5.71%)	8 (8.51%)	0.50
Fibrous bands	15 (21.43%)	13 (13.83%)	0.20
Hemorrhage	34 (48.57%)	53 (56.38%)	0.32
Hemosiderin deposition	3 (4.29%)	7 (7.45%)	0.52
Capsular pseudoinvasion	3 (4.29%)	5 (5.32%)	> 0.99
Tumor implantation	0 (0.00%)	3 (3.19%)	0.26
Granulation tissue	0 (0.00%)	1 (1.06%)	> 0.99
Edema	16 (22.86%)	12 (12.77%)	0.089
Necrosis	0 (0.00%)	1 (1.06%)	> 0.99

[1]Continuous variables are presented as mean ± standard deviation or median (Q1–Q3), and categorical variables are presented as n (%), according to data distribution.

[2]Pearson's chi-square test, Welch's two-sample t-test, and Fisher's exact test

[3]The percentage of stromal fibrosis was calculated only in the 28 adenomas presenting with fibrosis.

Bold p-values indicate statistical significance ($p < 0.05$).

Group 0: needle-naive group (PTH-WO not performed), **Group 1**: single-needle group (PTH-WO performed once), **Group 2**: two-needle group (two needle interventions performed for diagnostic and preoperative radioguided localization purposes), PTH-WO: parathyroid hormone washout

[Insert Table 4 here]

Comparison of Needle Intervention Groups (Groups 1 and 2)

After exclusion of needle-naive cases, comparisons between single-needle (Group 1) and two-needle (Group 2) intervention groups were performed. Histopathological examination showed that the frequency of fibrosis was significantly higher in Group 1 (30.2% vs. 11.8%; p = 0.026). However, no significant difference was detected between the two groups in terms of stromal fibrosis percentage (p = 0.29). The presence of fibrous bands was also significantly more frequent in the single-needle group (25.6% vs. 3.9%; p = 0.002). In contrast, no statistically significant differences were observed between the two groups with respect to thick fibrous capsule, hemorrhage, hemosiderin deposition, capsular pseudoinvasion, tumor implantation, granulation tissue, edema, or necrosis (p > 0.05 for all) (**Supplementary Table 1**).

Post Hoc Analysis for Intergroup Differences in Stromal Fibrosis Percentage

Tukey post hoc analysis was performed to evaluate differences in stromal fibrosis percentage among the three groups. Although the overall model was not significant (p > 0.05), a borderline difference was observed between Group 0 and Group 1, indicating a tendency toward a higher fibrosis percentage in Group 0 (mean difference = 10.19; 95% confidence interval: − 5.84 to 19.40; p = 0.055). The difference between Group 0 and Group 2 was not significant (mean difference = 5.58; p = 0.474), and no significant difference was observed between Groups 1 and 2 (mean difference = − 4.61; p = 0.628).

Correlation Between Stromal Fibrosis and Clinical or Biochemical Parameters

The correlations between stromal fibrosis percentage and clinical and biochemical variables were evaluated using Spearman correlation analysis. The fibrosis percentage showed weak correlations with the interval between PTH-WO and surgery (r = − 0.24, p > 0.05) and plasma PTH levels (r = 0.24, p > 0.05). Although these associations did not reach statistical significance, they were interpreted as trend-level associations. Similarly, no statistically significant correlations were detected between stromal fibrosis percentage and age (r = − 0.18), vitamin D (r = − 0.16), serum calcium (r = 0.06), urinary calcium (r = − 0.21), serum phosphorus (r = − 0.09), or tumor size (r = − 0.14) (p > 0.05 for all).

Multivariable Regression Analysis for Independent Predictors of Stromal Fibrosis

In the linear regression analysis performed to identify factors affecting fibrosis percentage, only the group variable was independently associated with fibrosis percentage. The fibrosis percentage was significantly lower in Group 1 than in Group 0 (β = − 10; 95% confidence interval: − 19 to − 1.6; p = 0.022). A similar decreasing trend was observed in Group 2, although it did not reach statistical significance (p = 0.25). No significant associations were identified between fibrosis percentage and other clinical or biochemical variables, including age, tumor size, timing of PTH-WO, PTH levels, vitamin D, serum calcium, urinary calcium, and related parameters (p > 0.05 for all).

Discussion

Accurate localization of parathyroid adenomas is a fundamental determinant of the success of minimally invasive parathyroidectomy. PTH-WO and ROLL are complementary minimally invasive techniques that are increasingly used for this purpose, particularly in cases with inconclusive imaging findings or ectopically located lesions [8, 10]. The present study evaluated the potential effects of these interventions and the frequency of needle use on the presence and extent of fibrosis and on histopathological findings that may mimic malignancy based on 164 surgically excised parathyroid adenoma specimens.

In this study, tumor size was smaller in the needle intervention groups (Groups 1 and 2) compared with the needle-naive group (Group 0). Serum PTH levels were higher and vitamin D levels were lower in Group 0. Certain histopathological differences were observed among Groups 0, 1, and 2. Although the frequency of fibrosis was more pronounced in Group 1, the intensity of stromal fibrosis was higher in Group 0, and this finding was also supported by regression models. When Groups 1 and 2 were compared, the frequency of fibrosis and the presence of fibrous bands were more prominent in Group 1, whereas the stromal fibrosis percentage was similar between the two groups. In analyses comparing Groups 1 and 2 together with Group 0, only the stromal fibrosis percentage differed, being higher in Group 0. In contrast, histopathological features suggestive of malignancy, such as fibrous bands, capsular pseudoinvasion, tumor implantation, and necrosis, were very rarely observed and were detected at similar frequencies across all groups.

Taken together, these findings indicate that fine-needle-based interventions do not appear to produce a marked alteration in the overall morphology of parathyroid adenomas. The similar frequency of malignancy-mimicking histopathological patterns across groups suggests that diagnostic and localization techniques such as PTH-WO and ROLL have an acceptable histopathological safety profile when applied with appropriate indications. On the other hand, the modest increase in fibrosis frequency in needle intervention groups and the higher stromal fibrosis percentage observed in needle-naive patients suggest that the observed fibrotic changes may be related not only to the interventions themselves but also to intrinsic structural characteristics of the tumor.

Although fine-needle-based interventions have been used for many years, data in the literature regarding their histopathological effects in parathyroid adenomas remain limited and heterogeneous [15]. Early studies reported that these procedures could lead to marked fibrotic reactions and surgical difficulties, with Norman et al. describing fibrosis and prolonged operative times in the majority of cases undergoing fine-needle aspiration [11, 12, 16]. Similarly, Hirokawa et al. emphasized that thick fibrous capsules and fibrous bands developing after fine-needle aspiration could mimic atypical adenoma or parathyroid carcinoma [14]. In contrast, a more recent study by Ahmad et al. demonstrated low fibrosis percentages that were comparable to those observed in needle-naive cases [17].

In the present study, stromal fibrosis was identified in only 17.1% of cases, a rate that is markedly lower than those reported in early series and is consistent with the contemporary literature [17]. The higher

frequency of fibrosis observed particularly in Group 1 suggests that needle-related mechanical trauma may act as a trigger for fibrosis development. However, the finding that the highest percentage of stromal fibrosis was observed in Group 0, together with multivariable analyses indicating an association between the group variable and stromal fibrosis percentage in favor of needle-naive cases, suggests that the fibrotic response cannot be attributed solely to interventional trauma. These observations imply that, in addition to mechanical effects, the biological and structural characteristics of the tumor may also play a role in the development of stromal fibrosis.

It has been proposed that fibrosis development may be affected by biological and structural characteristics such as tumor size, intratumoral hemorrhage, and degeneration [18]. Stromal fibrosis has also been reported to be associated with microvascular remodeling and stromal remodeling processes related to chronic PTH excess [19]. In the current study, despite larger tumor sizes in needle-naive cases and higher PTH levels in Groups 0 and 1, fibrosis and fibrous bands were observed at lower rates in Group 2, which was characterized by smaller tumor size and lower PTH levels. This pattern suggests that the fibrotic response may be more closely related to the biological properties of the tumor than to the mechanical effect of needle intervention. Furthermore, the relatively higher fibrosis percentage observed in Group 0, which had the lowest vitamin D levels, may be interpreted as indirect evidence of a potential association between chronic vitamin D deficiency and stromal fibrosis, a concept that is consistent with fibrotic remodeling processes described in secondary hyperparathyroidism [20].

Aspiration technique-related factors may also have an impact on fibrotic responses in parathyroid tissue. Needle gauge, number of needle passes, operator experience, and the interval between needle intervention and surgery may affect the frequency and severity of fibrotic responses in parathyroid tissue [11, 12, 14, 16, 17]. Norman et al. reported that marked fibrotic reactions and surgical difficulties were particularly associated with multiple needle passes (3–10 passes) and the use of larger-gauge needles (18–22 G) and noted no fibrosis with 3 passes using a 27-G needle, whereas fibrosis percentages reached 85% with 8 or more passes. However, because the procedures were performed by different operators and methodological standardization was poorly reported, the generalizability of these high fibrosis percentages is limited [11].

In this study, all parathyroid fine-needle aspirations were performed using a standardized approach, with a 23-G needle, by a single endocrinology specialist, with a limited number of passes (mean 2–3), and under high-resolution real-time ultrasonographic guidance. This standardized methodology is considered to have led to the limited fibrotic responses observed. Consistent with this interpretation, Ahmad et al. reported that interventions performed with 25–27 G needles and a limited number of passes did not result in clinically significant fibrosis and that histopathological findings were similar to those in needle-naive cases [17]. In contrast, the use of larger-gauge needles and the longer median interval between aspiration and surgery in the study by Hirokawa et al. may have contributed to more pronounced needle-related tissue trauma and a more evident secondary fibrotic response over time [14].

When the findings of the present study are evaluated alongside the existing literature, fine-needle aspiration using 23-G or thinner needles, a limited number of passes, experienced operators, and a short interval between intervention and surgery may represent an appropriate approach in PHPT cases with a clear surgical indication. Considering the limited evidence regarding long-term potential risks such as parathyromatosis [21, 22], the use of fine-needle aspiration in parathyroid lesions for differential diagnosis and localization purposes should be carefully considered in clinically necessary and selected cases.

Early literature reported that perilesional fibrotic adhesions were more frequently observed in parathyroid adenomas subjected to fine-needle aspiration, leading to more difficult and prolonged surgery and, in some cases, a need for microdissection. On this basis, it was suggested that fine-needle aspiration should be avoided unless strictly necessary [11, 23, 24]. In the current study, the primary focus was on stromal fibrosis, and perilesional fibrotic adhesions were not systematically analyzed. However, the fact that all surgeries were performed by the same experienced surgeon provides a degree of observational consistency. Notably, in cases undergoing PTH-WO and particularly ROLL, shorter operative times were observed, and advanced microdissection due to fibrotic adhesions was not required, despite smaller lesion size. These findings are consistent with recent studies using similar methodologies [9, 10]. Despite repeated needle interventions required for PTH-WO and intralesional marking, the ROLL technique has been reported to facilitate the surgical process and to be associated only with limited reactive histopathological changes [9]. In our study, the absence of histomorphologically significant changes and the lower fibrosis percentages observed in Group 2 (ROLL-treated cases) further support the acceptable histopathological safety profile of this technique.

Another major concern regarding fine-needle interventions is whether these procedures induce histopathological changes that may constitute diagnostic pitfalls with respect to malignancy. Parathyroid lesions encompass a broad histopathological spectrum ranging from adenoma to atypical parathyroid tumor and parathyroid carcinoma. Although long-term follow-up studies of atypical parathyroid tumors have reported low recurrence rates, these lesions are still regarded as borderline entities because of their uncertain malignant potential [18, 25, 26]. In the present study, histopathological patterns defined within the spectrum of atypical parathyroid tumors, including thick fibrous capsule, fibrous bands, capsular pseudoinvasion, and tumor implantation, were rarely observed and did not differ significantly among groups according to the presence or frequency of needle interventions. Despite the warnings by Hirokawa et al. that these patterns may develop secondary to fine-needle aspiration [14], the similar rates observed in needle-naive cases and different needle intervention frequencies suggest that it may not be sufficient to interpret these changes solely as iatrogenic artifacts. The rate of atypical parathyroid tumors detected in the needle-naive group (1.4%) was also lower than those reported in the literature, further supporting the notion that these findings may represent part of the natural histological overlap between parathyroid adenomas and the atypical spectrum [25, 27].

Among the histopathological patterns that raise the greatest suspicion for malignancy, capsular pseudoinvasion and tumor implantation were rarely observed in the overall study cohort (4.9% and 1.8%,

respectively) and did not differ significantly between needle-naive and needle intervention groups. Although capsular pseudoinvasion can be difficult to distinguish from true capsular invasion and tumor implantation from direct invasion into adjacent soft tissues [14], these patterns did not provide definitive evidence of malignancy on their own in our study. In some cases, these findings may necessitate more detailed histopathological evaluation, immunohistochemical analysis (when appropriate), and clinical follow-up similar to that applied to atypical parathyroid tumors.

Parathyroid carcinomas represent the rarest lesions within this spectrum and are distinguished by definitive malignant criteria such as invasion, vascular or perineural spread, metastasis, or overtly atypical mitotic activity [18]. No cases fulfilling these criteria were identified in our series, and therefore fine-needle-based interventions are not expected to induce histopathological changes that mimic these invasive features. In lesions suspected of parathyroid carcinoma, the principal concern is the potential risk of tumor seeding related to the intervention. Because these malignancies usually present as large lesions with marked hypercalcemia, very high PTH levels, and imaging findings that readily raise suspicion, fine-needle-based procedures such as PTH-WO or ROLL, which are intended for selected cases with localization difficulties, are generally not required in this patient group [18, 23, 28].

Parathyroid adenomas are known to be highly vascular lesions, and hemorrhagic areas adjacent to the capsule may create peliosis-like appearances that raise suspicion for invasion [29]. However, the absence of a true endothelial lining in these patterns allows differential diagnosis through careful histopathological assessment and, when necessary, immunohistochemical evaluation. Consistent with this knowledge, hemorrhage was the most frequently observed histopathological finding in our series (53%). Together with the prominent vascular structure of parathyroid tissue and its sensitivity to surgical manipulation, this finding suggests an intrinsic tendency of these lesions toward bleeding in response to interventional procedures. Although a relative increase in hemorrhage rate was observed in the group undergoing a second needle intervention on the morning of surgery (Group 2), the lack of statistical significance and the similar rates detected across all groups, including needle-naive cases, indicate that hemorrhage cannot be attributed solely to needle intervention. This observation suggests that, in addition to iatrogenic mechanical effects, lesion vascularity and individual stromal characteristics may also play a determining role in the development of hemorrhage. From a clinical perspective, limited hematoma areas did not negatively affect the surgical procedure and sometimes even facilitated intraoperative localization. These observations indicate that the hemorrhagic changes do not pose a significant surgical risk and should rather be considered nonspecific, reactive phenomena.

Hemosiderin deposition is among the reactive and iatrogenic changes described after fine-needle aspiration in parathyroid adenomas and is not considered to be directly associated with malignant transformation because it does not provide definitive evidence of invasion [14]. In our series, despite the frequent occurrence of hemorrhage, hemosiderin deposition was detected in only a limited number of cases (7.4%) and was observed at similar rates across groups. Nevertheless, it should be remembered that, particularly when limited tissue samples are evaluated, these changes may create appearances that mimic malignancy. The combined consideration of clinical and biochemical data, intraoperative

macroscopic findings, and a comprehensive histopathological evaluation including the entire parathyroid tissue supports the interpretation that these changes belong to a nonspecific, reactive spectrum. Therefore, awareness of a history of fine-needle-based intervention by the evaluating pathologist may contribute to reducing the risk of diagnostic misinterpretation.

Fine-needle aspiration and PTH-WO provide important clinical advantages in parathyroid adenomas. They support differential diagnosis, reduce the need for additional investigations, and optimize surgical planning, regardless of whether ROLL is performed. These approaches also have the potential to reduce additional costs and comorbidity risks associated with repeat surgeries. Furthermore, PTH-WO represents an important complementary tool for biochemical confirmation of parathyroid origin before the application of methods such as thermal ablation in patients who are not suitable candidates for surgery or who refuse surgical intervention. In our study, PTH-WO and fine-needle-based interventions were not associated with prominent histopathological changes mimicking malignancy or with surgically significant technical difficulties in parathyroid adenomas. These findings may provide additional and supportive evidence in the literature regarding the use of these methods under appropriate indications and with standardized techniques. Nevertheless, potential long-term effects of parathyroid aspiration procedures and rare but clinically significant conditions such as parathyromatosis were beyond the scope of the present study. Prospective, multicenter studies with longer follow-up are needed to evaluate these risks in detail. In addition, a more comprehensive investigation of the mechanical and biological factors that may be involved in the development of parathyroid fibrosis would help more clearly define the complementary role and safety boundaries of fine-needle-based interventions in clinical decision-making.

The main strength of this study lies in its systematic evaluation of the histopathological effects of fine-needle-based localization methods used for parathyroid adenomas in a relatively large patient cohort, as well as a comparative analysis of fibrosis and diagnostic pitfalls that may mimic malignancy. In addition, the fact that all interventions were performed by the same endocrinologist and endocrine surgeon strengthens procedural standardization and methodological consistency. However, the study also has several limitations. Due to the retrospective and observational design, causality between fine-needle-based interventions and histopathological changes cannot be established. It is also unclear whether the observed fibrosis reflects needle-related mechanical effects or intrinsic tumor biology. Potential selection bias related to intervention indications and patient selection, as well as limited generalizability associated with the single-center and registry-based data structure, should also be acknowledged. Although the overall case number was high, the relatively small number of cases with detected fibrosis and the low frequency of other histopathological features limited the ability to robustly and comprehensively evaluate factors associated with these variables through statistical modeling. Although single- and two-needle intervention groups were defined, the lack of quantitative recording of the number of needle passes during procedures limited the assessment of mechanical effects. Lastly, observations regarding the association between stromal fibrosis and surgical difficulty were based on observational data, and long-term clinical outcomes such as parathyromatosis and recurrent laryngeal nerve injury were not evaluated within the scope of this study. For these reasons, there is a need for prospective

studies incorporating standardized needle gauge and pass protocols and providing long-term follow-up data to clearly delineate the potential effects of parathyroid fine-needle aspiration and PTH-WO on histopathological changes and surgical and clinical outcomes.

Conclusion

This study quantitatively evaluated stromal fibrosis and histopathological patterns that may mimic malignancy in parathyroid adenomas undergoing fine-needle-based localization interventions such as PTH-WO and ROLL. Although the frequency of fibrosis was moderately higher in patients undergoing needle interventions, the higher intensity of stromal fibrosis observed in needle-naive patients suggests that needle-related mechanical effects may be limited and that the observed changes may be related not only to the interventions themselves but also to tumor biology. With respect to malignancy-suspicious patterns, PTH-WO and ROLL appear to be generally histopathologically safe when applied with appropriate indications and standardized techniques, although some findings may occasionally overlap with the histology of atypical parathyroid tumors. Prospective studies with standardized protocols and larger sample sizes are required to elucidate these associations.

Declarations

Acknowledgments

The authors sincerely thank Associate Professor Yaşar Ünlü for his valuable contributions to the evaluation and interpretation of the histopathological specimens and Assistant Professor Mehmet Sinan İyisoy for his contributions to the biostatistical analyses. The authors also thank Elif Denizaslanı for her professional support in English language editing of the manuscript. In addition, ChatGPT (OpenAI, GPT-4; accessed May 2025) was used to improve the language of the text and enhance clarity of expression. All scientific content, interpretations, and conclusions of the study are the sole responsibility of the authors.

Author Contributions

İlker Çordan, Tuğba Günler designed the study, performed the investigation, collected and analyzed the data, and drafted the manuscript. **Sabri Özden** performed the surgical procedures and contributed to the methodology and manuscript revision. **Mustafa Çaycı** performed the nuclear medicine procedures, including radiotracer localization (ROLL), and contributed to the methodology and manuscript revision. All authors reviewed and approved the final manuscript.

Funding

The authors declare that no financial support was received for the research and/or publication of this article.

Availability of data and materials

The datasets generated and/or analysed during the current study are available in the **Zenodo** repository, https://doi.org/10.5281/zenodo.18201811 [30].

Ethics approval and consent to participate

This study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the KTO Karatay University Faculty of Medicine Ethics Committee for Non-Interventional Drug and Non-Medical Device Research (meeting date: January 30, 2025; decision number: 2025/040). In accordance with institutional protocols, written informed consent was obtained from all patients before all interventional procedures, including surgery and the parathyroid hormone washout procedure.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

References

1. Bilezikian JP, Khan AA, Silverberg SJ, Fuleihan GE, Marcocci C, Minisola S et al. Evaluation and management of primary hyperparathyroidism: summary statement and guidelines from the Fifth International Workshop. J Bone Miner Res. 2022;37(11):2293–2314. 10.1002/jbmr.4677
2. Udelsman R, Åkerström G, Biagini C, Duh QY, Miccoli P, Niederle B et al. The surgical management of asymptomatic primary hyperparathyroidism: proceedings of the Fourth International Workshop. J Clin Endocrinol Metab. 2014;99(10):3595–3606. 10.1210/jc.2014-2000
3. Morris MA, Saboury B, Ahlman M, Malayeri AA, Jones EC, Chen CC, et al. Parathyroid imaging: past, present, and future. Front Endocrinol (Lausanne). 2022;12:760419. 10.3389/fendo.2021.760419.
4. Centello R, Sesti F, Feola T, Sada V, Pandozzi C, Di Serafino M, et al. The dark side of ultrasound imaging in parathyroid disease. J Clin Med. 2023;12(7):2487. 10.3390/jcm12072487.
5. Barranquero AG, Pastor P, Ortega A, Corral S, Gómez Ramírez J, Luengo P, et al. 4D-CT as a second line preoperative localization test for the evaluation of primary hyperparathyroidism. Cir Esp (Engl Ed). 2023;101(8):530–7. 10.1016/j.cireng.2022.07.015.
6. Lee SW, Shim SR, Jeong SY, Kim SJ. Direct comparison of preoperative imaging modalities for localization of primary hyperparathyroidism: a systematic review and network meta-analysis. JAMA Otolaryngol Head Neck Surg. 2021;147(8):692–706. 10.1001/jamaoto.2021.0915.
7. Philippon M, Guerin C, Taieb D, Vaillant J, Morange I, Brue T, et al. Bilateral neck exploration in patients with primary hyperparathyroidism and discordant imaging results: a single-centre study. Eur J Endocrinol. 2014;170(5):719–25. 10.1530/EJE-13-0796.

8. Çordan İ, Aksu O. Lesion size and cystic morphology are key determinants of parathyroid hormone washout in primary hyperparathyroidism. Front Endocrinol (Lausanne). 2025;16:1654110. 10.3389/fendo.2025.1654110.
9. Ilgan S, Ozbas S, Bilezikci B, Sengezer T, Aydin OU, Gursoy A, et al. Radioguided occult lesion localization for minimally invasive parathyroidectomy: technical consideration and feasibility. Nucl Med Commun. 2014;35(11):1167–74. 10.1097/MNM.0000000000000188.
10. Soylu L, Aydın OU, Ilgan S, Özbaş S, Bilezikçi B, Gürsoy A, et al. Radioguided occult lesion localization for minimally-invasive parathyroidectomy without quick PTH monitoring and frozen section: impact of the learning curve. Turk J Surg. 2020;36(3):297–302. 10.47717/turkjsurg.2020.4470.
11. Norman J, Politz D, Browarsky I. Diagnostic aspiration of parathyroid adenomas causes severe fibrosis complicating surgery and final histologic diagnosis. Thyroid. 2007;17(12):1251–5. 10.1089/thy.2007.0081.
12. Bancos I, Grant CS, Nadeem S, Stan MN, Reading CC, Sebo TJ, et al. Risks and benefits of parathyroid fine-needle aspiration with parathyroid hormone washout. Endocr Pract. 2012;18(4):441–9. 10.4158/EP11148.OR.
13. Suzuki A, Hirokawa M, Kanematsu R, Tanaka A, Yamao N, Higuchi M, et al. Fine-needle aspiration of parathyroid adenomas: indications as a diagnostic approach. Diagn Cytopathol. 2021;49(1):70–6. 10.1002/dc.24595.
14. Hirokawa M, Suzuki A, Higuchi M, Hayashi T, Kuma S, Miya A, et al. Histological alterations following fine-needle aspiration for parathyroid adenoma: incidence and diagnostic problems. Pathol Int. 2021;71(6):400–5. 10.1111/pin.13091.
15. Doppman JL, Krudy AG, Marx SJ, Saxe A, Schneider P, Norton JA, et al. Aspiration of enlarged parathyroid glands for parathyroid hormone assay. Radiology. 1983;148(1):31–5. 10.1148/radiology.148.1.6856859.
16. Alwaheeb S, Rambaldini G, Boerner S, Coiré C, Fiser J, Asa SL, et al. Worrisome histologic alterations following fine-needle aspiration of the parathyroid. J Clin Pathol. 2006;59(10):1094–6. 10.1136/jcp.2005.029017.
17. Ahmad S, Lilla E, Miller DV, Austin K, Hazel M, Ansari I, et al. Ultrasound-guided fine-needle aspiration biopsy of parathyroid adenomas in patients undergoing parathyroidectomy does not lead to clinically significant fibrosis. Gland Surg. 2025;14(6):974–82. 10.21037/gs-2025-26.
18. Erickson LA, Mete O, Juhlin CC, Perren A, Gill AJ. Overview of the 2022 WHO Classification of Parathyroid Tumors. Endocr Pathol. 2022;33(1):64–89. 10.1007/s12022-022-09709-1.
19. Khan AA, Hanley DA, Rizzoli R, Bollerslev J, Young JE, Rejnmark L, et al. Primary hyperparathyroidism: review and recommendations on evaluation, diagnosis, and management. A Canadian and international consensus. Osteoporos Int. 2017;28(1):1–19. 10.1007/s00198-016-3716-2.
20. Wada Y, Kunimura T, Sato S, Hisayuki T, Sato M, Imataka H, et al. Proliferating potential and apoptosis in the development of secondary hyperparathyroidism: a study based on Ki-67

immunohistochemical staining and the terminal dUTP nick-end labeling assay. Ther Apher Dial. 2008;12(4):319–28. 10.1111/j.1744-9987.2008.00594.x.

21. Kim J, Horowitz G, Hong M, Orsini M, Asa SL, Higgins K. The dangers of parathyroid biopsy. J Otolaryngol Head Neck Surg. 2017;46(1):4. 10.1186/s40463-016-0178-7.
22. Balbaloglu H, Deniz O, Ozaydin RY, Tasdoven I, Karadeniz Cakmak G. Parathyroid fine needle aspiration with PTH washout: can it lead to parathyroid cell seeding in primary hyperparathyroidism? Med (Baltim). 2024;103(15):e37754. 10.1097/MD.0000000000037754.
23. Patel KN, Yip L, Lubitz CC, Grubbs EG, Miller BS, Shen W, et al. The American Association of Endocrine Surgeons guidelines for the definitive surgical management of thyroid disease in adults. Ann Surg. 2020;271(3):e21–93. 10.1097/SLA.0000000000003580.
24. Wilhelm SM, Wang TS, Ruan DT, Lee JA, Asa SL, Duh QY, et al. The American Association of Endocrine Surgeons guidelines for definitive management of primary hyperparathyroidism. JAMA Surg. 2016;151(10):959–68. 10.1001/jamasurg.2016.2310.
25. Maconi C, Saibene AM, Castellani L, Lozza P, Pescia C, Falleni M et al. Is atypical parathyroid tumor a different clinical entity than parathyroid adenoma and carcinoma? A retrospective review of a large single-center case series. Updates Surg. 2025 Oct 29 [Epub ahead of print]. 10.1007/s13304-025-02445-1
26. Barale M, Nervo A, Craparo A, Pusterla A, Retta F, Maiorino F, et al. Recurrence and mortality rate in an Italian multi-center case series of parathyroid atypical adenomas and carcinomas. Front Endocrinol (Lausanne). 2023;14:1158474. 10.3389/fendo.2023.1158474.
27. Galani A, Morandi R, Dimko M, Molfino S, Baronchelli C, Lai S, et al. Atypical parathyroid adenoma: clinical and anatomical pathologic features. World J Surg Oncol. 2021;19(1):19. 10.1186/s12957-021-02123-7.
28. Duan K, Mete O. Parathyroid carcinoma: diagnosis and clinical implications. Turk Patoloji Derg. 2015;31(Suppl 1):80–97. 10.5146/tjpath.2015.01316.
29. Juhlin CC, Mete O. Avoiding overdiagnosis of parathyroid carcinoma. Virchows Arch. 2025 Nov 14 [Epub ahead of print]. 10.1007/s00428-025-04274-4
30. Günler T, Çordan İ, Özden S, Çaycı M. Ultrasound-guided PTH washout and ROLL technique are not associated with clinically significant stromal fibrosis or malignancy-mimicking histopathologic changes in parathyroid adenomas [dataset]. Zenodo; 2026. 10.5281/zenodo.18201811

Figures

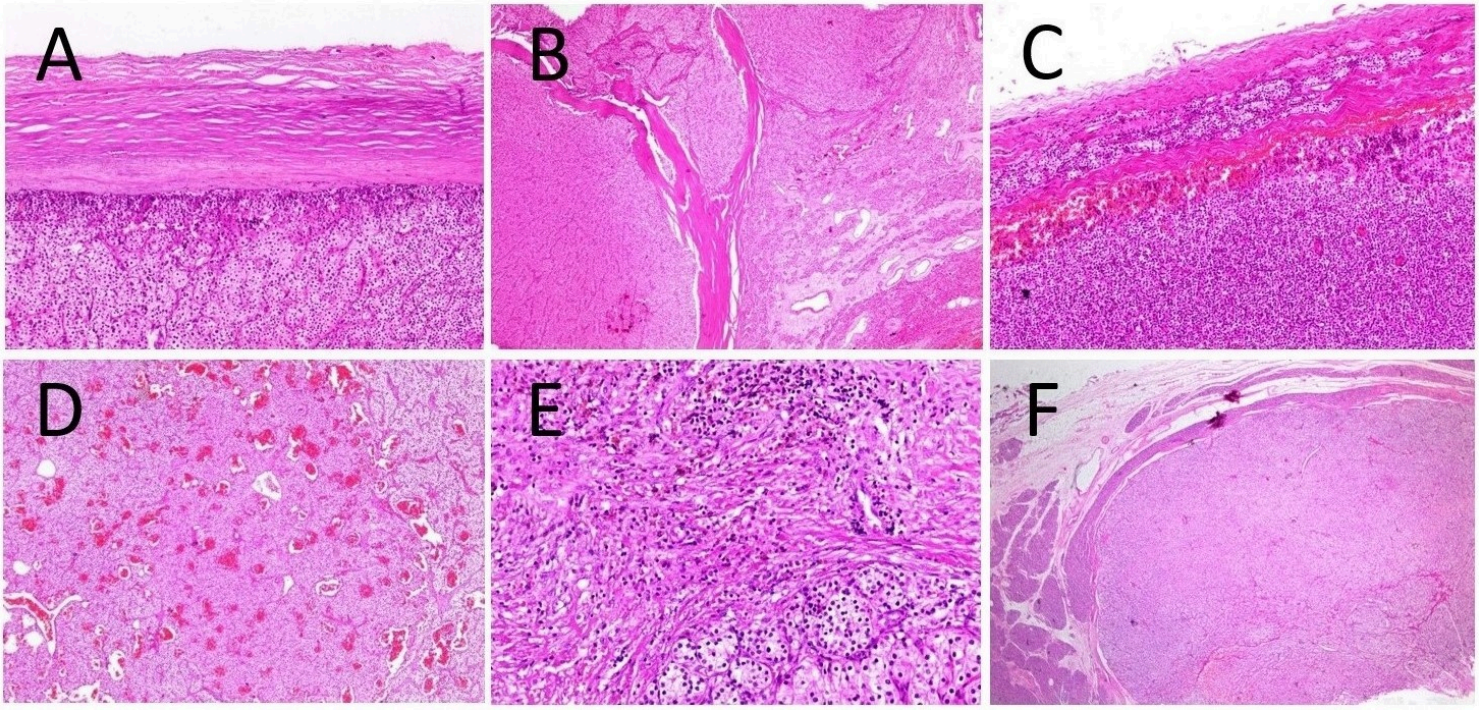


Figure 1

Histopathological patterns in parathyroid adenomas after fine-needle aspiration, which overlap with atypical parathyroid tumors and may represent iatrogenic artifacts.

(A) A fibrous capsule showing marked thickening around a parathyroid adenoma *(H&E; ×100)*.
(B) Fibrous bands in the stroma that may create appearances mimicking malignancy *(H&E; ×40)*.
(C) Areas of capsular pseudoinvasion giving the impression of disrupted capsular integrity *(H&E; ×100)*.
(D) Foci of intratumoral hemorrhage that may have developed secondary to previous needle intervention *(H&E; ×100)*.
(E) Hemosiderin deposits secondary to previous hemorrhagic areas *(H&E; ×200)*.
(F) A rarely observed area of tumor implantation located as small foci around the tumor tissue *(H&E; ×20)*. (H&E: hematoxylin and eosin)

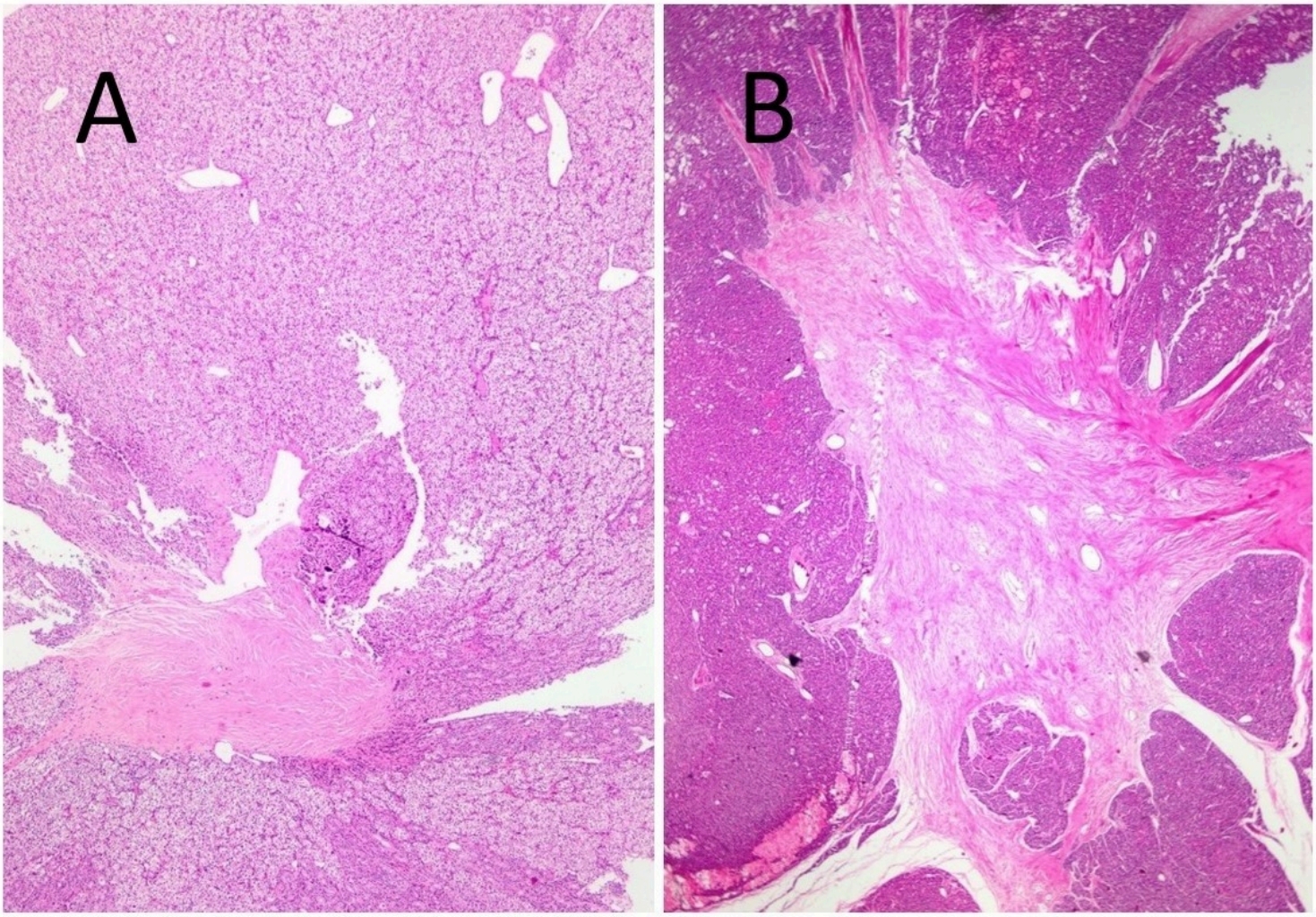


Figure 2

Areas of stromal fibrosis in hematoxylin and eosin-stained sections of a parathyroid adenoma.

(A) Stromal fibrosis area comprising approximately 5% of the total tissue surface area (*H&E; ×40)*. **(B)** A stromal fibrosis area comprising approximately 25% of the total tissue surface area, centrally located within the lesion and containing dense collagen *(H&E; ×20)*. (H&E: hematoxylin and eosin)

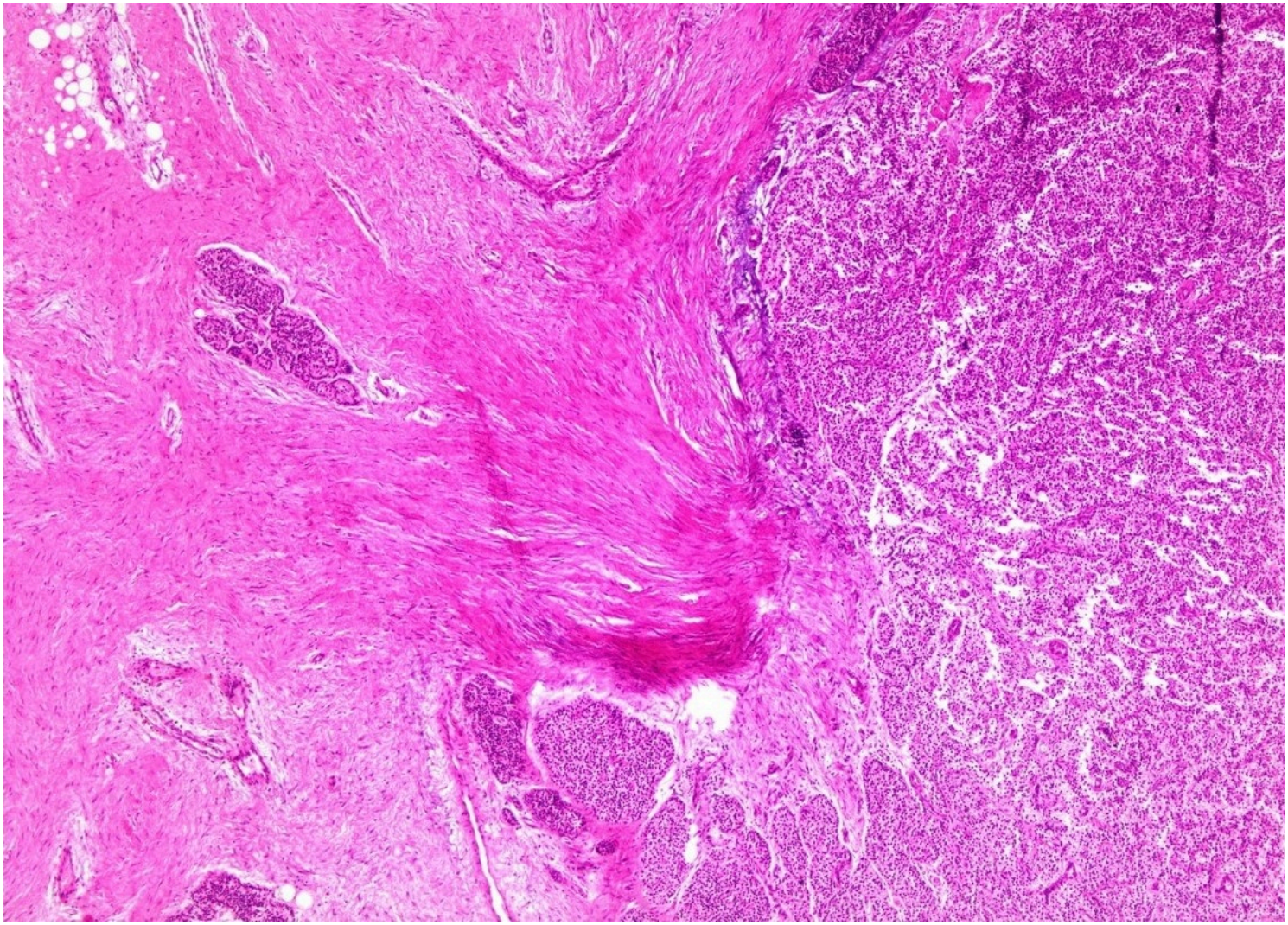


Figure 3

Collagenized stromal fibrosis adjacent to the needle tract. A hematoxylin and eosin stained section (×40) shows collagenized stromal fibrosis with associated parenchymal distortion in the area adjacent to the needle tract, involving approximately 10% of the total sectional area.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable1.docx