Cyto-morphological features of parathyroid lesions: Fine-needle aspiration cytology series from an endocrine tumor referral center

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Abstract
Background: Descriptions of parathyroid cell cyto-morphology are limited. Fine-needle aspiration cytology (FNAC) with immunocytochemistry (ICC) or biochemical PTH measurements may help verify the parathyroid origin in extraordinary cases, although these methods are nowadays largely replaced by imaging techniques.

Methods: We collected all available FNAC reports of parathyroid lesions from our department spanning 20 years, to characterize the clinical use of parathyroid FNAC, and to assess morphological correlates between cytology and subsequent histopathology.

Results: Twenty-eight cases with assessable cytological smears were found, of which 21 cases were surgically resected and 20 available for histological review (15 adenomas, 2 carcinomas, 2 atypical tumors and a single case with secondary hyperplasia). FNAC was predominantly performed to localize the diseased gland in cases with inconclusive imaging, in cases with a suspicion of intrathyroidal localization or in cases with persistent hypercalcemia following unsuccessful surgery. The diagnosis was verified using either PTH ICC and/or PTH measurements for most cases, and the procedure affected the clinical decision-making for the majority of patients in this selected cohort. Cytological differences between parathyroid adenomas and carcinomas were found, as only carcinomas showed pleomorphism with irregular nuclei and prominent nucleoli. Morphologically, no correlations to predominant cell types or growth patterns visualized at histological investigations among adenomas were noted, and biopsy artifacts were evident in 40% of cases.

Conclusions: Parathyroid FNAC could be considered as a complementary analysis for a small group of selected patients, but benefits have to be weighed against the risk of biopsy artifacts in histological preparations.

KEYWORDS
cytology, fine-needle aspiration biopsy, morphology, parathyroid, parathyroid adenoma, parathyroid carcinoma
1 | INTRODUCTION

Primary hyperparathyroidism (pHPT) is recognized as symptomatic or asymptomatic hypercalcemia and high to borderline high levels parathyroid hormone (PTH) in the blood. If secondary reasons for hyperparathyroidism have been ruled out (such as renal failure), the cause is one or several parathyroid neoplasms, such as an adenoma (single or multiple) or carcinoma (almost always single nodules).1,2 Traditionally, patients were surgically treated using a bilateral neck exploration approach. However, since most cases are caused by single adenomas, less invasive approaches have replaced bilateral neck exploration, facilitated by preoperative localization of the abnormal gland using high resolution ultrasound and/or technetium sestamibi scintigraphy.3,4 Even so, some of these lesions are still difficult to find by radiological methods. Sestamibi scans exhibit limitations when it comes to small tumors, as well as tumors with water clear cell appearance,5 and thyroid nodules may sometimes create false positive scan results. High-resolution ultrasound may be optimal for the identification of cysts and smaller lesions, but is generally sub-par in distinguishing parathyroid lesions from thyroid nodules and adjacent lymph nodes.6 When the patient exhibits concomitant thyroid disease, radiological modalities may not be entirely sufficient, and ultrasonography guided fine-needle aspiration cytology (FNAC) could in certain cases be used to pinpoint the location.3

FNACs of parathyroid tumors are not recommended in general, and are usually utilized as part of a work-up of a thyroid nodule. Indeed, it is estimated that 0.12% of all thyroid FNACs report intrathyroidal parathyroid glands.7 Normal parathyroid glands as well as adenomas usually contain conventional chief cells, with or without smaller populations of clear-type cell chief cells and oxyphilic cells. Thyroid lesions on the other hand contain follicular epithelial cells—but they are still traditionally difficult to distinguish from parathyroid cells in cytology preparations.2,4,7–13 The literature regarding cytomorphological hallmarks for parathyroid cells is rather limited, and specifically meager in terms of studies that offer clues how to differ parathyroid cells from thyroid epithelium. If the cytopathologist, already while investigating a quick-stained smear, could raise the suspicion of a parathyroid lesion, part of the aspirate can be submitted for biochemical estimation of PTH to establish the diagnosis. Absher et al and Dimashkieh et al made the first attempts to find common hallmarks for parathyroid cells to differ them from thyroid cells using fine-needle aspiration biopsies from 12 to 20 parathyroid lesions, respectively.8,10 Up until these studies were launched, contemporary scientific literature on the subject was restricted to occasional case reports, or were based on small studies performing aspirations or imprints from surgically resected specimens. Since then, several studies have been performed to define cyto-morphological criteria for parathyroid lesions,6,7,11–13 but no clear-cut definitions or recommendations in terms of morphological classification have been proposed. Generally, when listing subjective parameters based on pattern recognition, the interobserver variability is usually high. Moreover, different terminology and definitions for cytological patterns have been used, adding to the heterogeneous results obtained. In this study, we correlated cytological characteristics of parathyroid nodules with postoperative histology in a large series of patients in order to see whether or not the FNAC report had an impact on the clinical handling, and if the morphological aspects observed on FNACs were correlated to specific histological attributes. Of course, if parathyroid lesions could be recognized cyto-morphologically, it would be of direct clinical value, to avoid falsely diagnosing a parathyroid lesion as thyroid-derived.

2 | MATERIAL AND METHODS

The pathology database at the Karolinska University Hospital, Stockholm, Sweden was searched retrospectively for parathyroid lesions diagnosed by cytology. For this purpose, we used the SNOMED nomenclature for “parathyroid” (T97xxx) and investigated the cytology records between January 1, 2000 and May 20, 2020. In total, 37 cytology reports were found, which should be compared to the >1600 thyroid-related FNACs performed annually at our institution. The diagnoses, biochemical tests, ultrasonography-guided FNAC smears and available histological tissue sections (if surgical resection was performed) were retrieved. All FNA smears had been air-dried and stained with May Grunwald-Giemsa (MGG). Unstained smears were also previously acquired for the purpose of PTH ICC which had been performed for the majority of cases. The PTH ICC was conducted in a clinically accredited laboratory setting using the Novocastra anti-PTH antibody (clone NCL-PTH-488, Leica Biosystems, Newcastle, UK) and an automated Ventana Benchmark ULTRA system (Roche, Basel, Switzerland). Additional clinical information, including patient follow-up, was obtained through accessing the medical files of each patient. Ethical approval was granted by the local ethics committee of Karolinska Institutet.

All cytological smears were reviewed by two of the authors (S.S., M.H.), and evaluated in terms of the following predefined parameters; architectural patterns, nuclear features, cytoplasmic appearance (granularity, vacuolation), presence of macrophages, mitoses and colloid. Histological tissue sections for all parathyroids that were subsequently surgically resected were reviewed by three authors (S.S., M.H., C.C.J.) for proportions of cell types, proportions of growth patterns as well as signs of biopsy artifacts. Comparisons were then made between smears and histology.

3 | RESULTS

3.1 | Clinical aspects of the study cohort

At our institution, the parathyroid lesions diagnosed by FNAC have increased over the past years, with seven cases diagnosed in 2000-2010 compared with 29 cases between 2011 and 2020. Out of 37 FNACs from 36 patients in which parathyroid tissue was acknowledged in the cytology report, two cases were not found in our archives and hence excluded. Of the remaining 35 cases, two cases
| Case no. | Age | Sex | Pre-FNAC pHPT | Indication for FNAC | Histopathological diagnosis | Did FNAC affect the choice of treatment? |
|----------|-----|-----|---------------|---------------------|-----------------------------|----------------------------------------|
| 1        | 76  | F   | Yes (mild)    | Persistent pHPT after resection of an adenoma | No surgery | No, no surgery performed because of mild pHPT only |
| 2        | 74  | M   | No            | Other radiological indication for FNAC | Parathyroid adenoma | Yes, leading to the diagnosis of pHPT |
| 3        | 22  | M   | Yes           | pHPT and previous negative surgical exploration | Parathyroid adenoma | Yes, adenoma localized leading to parathyroidectomy |
| 4        | 16  | M   | Yes           | Persistent pHPT after resection of an adenoma | Parathyroid carcinoma | Yes, additional adenoma localized leading to parathyroidectomy |
| 5        | 70  | F   | Yes           | pHPT and previous surgery in the neck | Parathyroid adenoma | Yes, adenoma localized leading to parathyroidectomy |
| 6        | 63  | F   | No            | Other radiological indication for FNAC | No surgery | Yes, no surgery needed because no malignancy was detected |
| 7        | 33  | F   | Yes           | pHPT and a concomitant thyroid nodule | Parathyroid adenoma | Yes, adenoma localized, no concomitant thyroid neoplasia |
| 8        | 46  | F   | Yes           | pHPT and a concomitant thyroid nodule | Parathyroid adenoma | Yes, adenoma localized, no concomitant thyroid neoplasia |
| 9        | 52  | M   | Yes           | pHPT and previous negative exploration | Parathyroid adenoma | Yes, adenoma localized leading to parathyroidectomy |
| 10       | 65  | F   | No            | Other radiological indication for FNAC | No surgery | Yes, no surgery needed because no malignancy was detected |
| 11       | 26  | M   | No            | Other radiological indication for FNAC | No surgery | Yes, no surgery needed because no malignancy was detected |
| 12       | 43  | F   | Yes           | pHPT and concomitant thyroid nodule | Parathyroid adenoma | Yes, adenoma localized, no concomitant thyroid neoplasia |
| 13       | 49  | F   | No            | Other radiological indication for FNAC | No surgery | Yes, no surgery needed because no malignancy was detected |
| 14       | 65  | M   | No            | Other radiological indication for FNAC | No surgery | Yes, no surgery needed because no malignancy was detected |
| 15       | 64  | M   | No            | Other radiological indication for FNAC | Secondary hyperplasia | Yes, leading to the diagnosis of pHPT |
| 16       | 75  | F   | Yes           | pHPT and previous surgery in the neck | Parathyroid adenoma | Yes, adenoma localized leading to parathyroidectomy |
| 17       | 49  | F   | Yes           | pHPT and previous negative surgical exploration | Parathyroid adenoma | Yes, adenoma localized leading to parathyroidectomy |
| 18       | 37  | F   | Yes           | Recurrent pHPT after resection of an adenoma | Parathyroid carcinoma | Yes, additional tumor localized leading to parathyroidectomy |
| 19       | 66  | F   | Yes           | Persistent pHPT after resection of an adenoma | Parathyroid adenoma | Yes, adenoma localized leading to parathyroidectomy |
| 20       | 53  | F   | Yes           | pHPT with suspicious intrathyroidal adenoma | No surgery | Yes, adenoma localized, surgery not performed due to comorbidity |
| 21       | 60  | F   | Yes           | Persistent pHPT after resection of an adenoma | Atypical parathyroid tumor | Yes, adenoma localized leading to parathyroidectomy |
| 22       | 61  | F   | Yes           | pHPT and a concomitant thyroid nodule | Parathyroid adenoma | Yes, concomitant parathyroid and thyroid lesions localized. |
| 23       | 74  | F   | Yes           | pHPT, localization of adenoma | Parathyroid adenoma | Yes, adenoma localized, no concomitant thyroid neoplasia |
| 24       | 59  | F   | Yes           | Recurrent pHPT after resection of an adenoma | Parathyroid adenoma | Yes, adenoma localized leading to parathyroidectomy |
| 25       | 62  | F   | Yes           | pHPT with suspicion of intrathyroidal adenoma | Parathyroid adenoma | Yes, adenoma localized leading to parathyroidectomy |
| 26       | 50  | F   | Yes           | pHPT with suspicion of intrathyroidal adenoma | Atypical parathyroid tumor | Yes, adenoma localized leading to parathyroidectomy |
| 27       | 68  | F   | Yes           | pHPT and previous negative surgical exploration | Intrathyroidal parathyroid adenoma | Yes, adenoma localized leading to parathyroidectomy |

(Continues)
were excluded based on PTH biochemical estimations under the threshold value. Moreover, an additional five cases contained too few cells for evaluation. In total, 28 FNACs of bona fide parathyroid tissue were assessed cyto-morphologically. Seven of these cases did not proceed to surgical intervention and were therefore not included in the comparative analyses with histology, and for one case the histological sections were not available for evaluation, leaving 20 cases in which comparison with histopathologic characteristics of the subsequently excised tumor could be made.

The clinical parameters of this cohort are summarized in Table 1. In detail, out of the 28 patients investigated, the female: male ratio was 3:1, and the median age at the time of cytology was 55 years (range 16–76). Twenty-one patients (75%) had a pre-FNAC clinical diagnosis of pHPT. Of these, the FNAC investigation was initiated with the aim to verify the assumed location of a parathyroid lesion in 17 patients (81%), of which many exhibited a history of inconclusive tumor localization using radiology and/or negative findings using surgical exploration. Therefore, the reason for performing the FNAC was primarily not to establish a preoperative parathyroid tumor diagnosis, but rather to verify the location in cases in which standard clinical work-up was inconclusive. Cases with an established PHPT and initial negative findings using imaging and/or surgical exploration were followed biochemically and re-investigated using scintigraphy, and if the results were inconclusive, the patients were then assessed with ultrasonography-guided FNAC in order to try to establish the exact location in order to avoid excessive explorative surgery. The majority of patients (15/28; 54%) was somewhat unconventional in its clinical presentation, as patients either exhibited synchronous thyroid nodules upon ultrasonographic investigations (n = 4), presented with an intrathyroidal nodule (n = 4) or had no previous diagnosis of PHPT (n = 7). In these cases, the FNAC was therefore needed to pinpoint the parathyroid origin in order to plan the correct surgical procedure.

Figure 1 Cytological features of parathyroid adenomas. (A) May Grunwald-Giemsa (MGG) stain showing a complex, branched, three-dimensional epithelial group with partly centrally located capillaries (×200 magnification). (B) MGG stain showing a mixture of architectural patterns, including tight clusters, loose aggregates and some dispersed cells in the background (×100 magnification). A combination of different patterns was the most frequent feature in our series. (C) MGG stain showing small group of parathyroid tumor cells arranged in microacinar formations (×400 magnification). (D) Parathyroid hormone (PTH) immunocytochemistry was used for most cases, proving the parathyroid origin of the cells via diffuse cytoplasmic staining (×400 magnification) [Color figure can be viewed at wileyonlinelibrary.com]

Table 1 (Continued)

| Case no. | Age | Sex | Pre-FNAC pHPT | Indication for FNAC | Histopathological diagnosis | Did FNAC affect the choice of treatment?* |
|----------|-----|-----|--------------|---------------------|----------------------------|-----------------------------------------|
| 28       | 49  | F   | Yes          | Persistent pHPT after resection of an adenoma | Histological sections not available | Yes, concomitant parathyroid and thyroid lesions localized. |

Abbreviations: FNAC, fine-needle aspiration cytology; pHPT, primary hyperparathyroidism.

*Defined as a change of planned procedures.
| Case no. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 |
|---------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| **Histology** |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| **Growth patterns** (% |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Microacinar | 30 | 20 | 40 | 100 | 60 | 30 | 10 |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Solid | 70 | 60 | 100 | 80 | 50 | 60 | 100 | 100 | 100 | 100 | 50 | 80 | 100 | 40 | 80 | 70 | 90 |   |    |    |    |    |    |    |    |
| Trabecular | 40 |   | 50 |   |   |   |   |   |   | 20 | 20 |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |
| Other (cystic/ sinusoidal) |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| **Cell proportions** (%) |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Conventional chief cells |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Clear cells | 20 | 20 | 100 | 100 | 60 | 60 | 90 | 5 | 100 | 100 | 100 | 100 | 70 | 80 | 100 | 40 | 80 | 70 | 90 | 20 |   |    |    |    |    |
| Oxyphilic cells | 20 | 60 | 80 | 70 | 30 | 60 | 50 | 50 | 20 | 100 | 60 | 40 | 70 | 80 | 30 | 10 | 70 |   |    |    |    |    |    |    |    |
| **Cytology** |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| **Architectural patterns** |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Dispersed | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Loose aggregates | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Microacinar formations | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Tight clusters | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| **Cellular features** |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Nuclear size | 1.2 | 1.5 | 1.1 | 2.2 | 2 | 1.2 | 1.1 | 1.1 | 1.2 | 1.3 | 1.5 | 1.5 | 2 | 2 | 4 | 1.5 | 1.2 | 2.2 | 1.2 | 2.2 | 1.5 | 1.5 | 1.2 | 1.5 | 2 |
| Anisokaryosis | L | L | M/H | M | L | L | H | L | L | L | L | L | H | L | L | M | M | M | L | M | M | M | M |
| Stippled chromatin | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Nuclei present | X | N.A. | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Nuclear/ cytoplasmic ratio | H | H | M | L | L | H | M | H | M | M | L | M | M | M | M | L | M | M | H | M | L | M | M |
| Cytoplasmic granularity | X | X | X | X | N.A. | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Intracytoplasmic vacuolation | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |

**Note:** Gray squares indicate that the data was unavailable for histology. Blank squares indicate an absence of the specific parameter on cytology. Nuclear size reported as a factor in comparison with the diameter of an erythrocyte, and reported as the largest nucleus observed. **X** = present, **L** = low, **M** = moderate, **H** = high, **N.A.** = not assessable.

**Abbreviations:** AT, atypical parathyroid tumor; ND, not determined; PA, parathyroid adenoma; PC, parathyroid carcinoma; SH, secondary hyperplasia.
biopsy-associated changes was weighted against the potential morbidity of additional neck surgery in a scar tissue area (recurrent laryngeal nerve damage and/or hypoparathyroidism).

For 21 FNAC cases (75%), the parathyroid nature of the cells could be anticipated based upon previous clinical information, but for seven cases (25%), the parathyroid origin was not expected. A total of 13 cases (46%) were verified by laboratory estimation of PTH from cytology aspirates, and in 18 patients (64%), a PTH immunocytochemical staining was performed (Figure 1). In eight patients (29%), the diagnosis was verified by both immunocytochemistry and laboratory estimation of PTH. In two patients (7%), no ancillary methods were used, thus the diagnosis was based on morphology alone. For 27 patients (96%), the result of the FNAC investigation affected clinical decisions concerning treatment, defined as a change of planned procedures (Table 1). More specifically, 20 pHPT patients exhibited parathyroid tumors without a clear localization that were successfully identified using FNAC, thereby guiding the surgical approach from extensive explorations to focused surgery. Seven patients without any suspicion of pHPT were investigated for lesions suspicious for neck malignancies, and the subsequent FNAC reports indicating parathyroid tissue thus saved the patients from unnecessary surgery, while in two patients the FNAC and subsequent biochemical evaluation diagnosed them with pHPT. For the remaining patient, the decision to ignore the FNAC result was based on morphology alone. For 27 patients (96%), the result of the FNAC investigation affected clinical decisions concerning treatment, defined as a change of planned procedures (Table 1). More specifically, 20 pHPT patients exhibited parathyroid tumors without a clear localization that were successfully identified using FNAC, thereby guiding the surgical approach from extensive explorations to focused surgery. Seven patients without any suspicion of pHPT were investigated for lesions suspicious for neck malignancies, and the subsequent FNAC reports indicating parathyroid tissue thus saved the patients from unnecessary surgery, while in two patients the FNAC and subsequent biochemical evaluation diagnosed them with pHPT. For the remaining patient, the decision to ignore the FNAC result was based on morphology alone. For 27 patients (96%), the result of the FNAC investigation affected clinical decisions concerning treatment, defined as a change of planned procedures (Table 1). More specifically, 20 pHPT patients exhibited parathyroid tumors without a clear localization that were successfully identified using FNAC, thereby guiding the surgical approach from extensive explorations to focused surgery. Seven patients without any suspicion of pHPT were investigated for lesions suspicious for neck malignancies, and the subsequent FNAC reports indicating parathyroid tissue thus saved the patients from unnecessary surgery, while in two patients the FNAC and subsequent biochemical evaluation diagnosed them with pHPT. For the remaining patient, the decision to ignore the FNAC result was based on morphology alone. For 27 patients (96%), the result of the FNAC investigation affected clinical decisions concerning treatment, defined as a change of planned procedures (Table 1). More specifically, 20 pHPT patients exhibited parathyroid tumors without a clear localization that were successfully identified using FNAC, thereby guiding the surgical approach from extensive explorations to focused surgery. Seven patients without any suspicion of pHPT were investigated for lesions suspicious for neck malignancies, and the subsequent FNAC reports indicating parathyroid tissue thus saved the patients from unnecessary surgery, while in two patients the FNAC and subsequent biochemical evaluation diagnosed them with pHPT. For the remaining patient, the decision to ignore the FNAC result was based on morphology alone. For 27 patients (96%), the result of the FNAC investigation affected clinical decisions concerning treatment, defined as a change of planned procedures (Table 1). More specifically, 20 pHPT patients exhibited parathyroid tumors without a clear localization that were successfully identified using FNAC, thereby guiding the surgical approach from extensive explorations to focused surgery. Seven patients without any suspicion of pHPT were investigated for lesions suspicious for neck malignancies, and the subsequent FNAC reports indicating parathyroid tissue thus saved the patients from unnecessary surgery, while in two patients the FNAC and subsequent biochemical evaluation diagnosed them with pHPT. For the remaining patient, the decision to ignore the FNAC result was based on morphology alone.

3.2 Cytological hallmarks of parathyroid lesions

The cyto-morphological aspects of the parathyroid FNACs are listed in Table 2, and illustrated in Figures 1–3. A few common denominators were found among parathyroid FNACs. In terms of cellular characteristics, the smears were generally monomorphic to slightly pleomorphic. Cells were commonly small, ranging from sizes slightly larger than an erythrocyte up to twice the size of an erythrocyte in the majority of cases (24/28 cases, 86%). In most smears, the cells showed a low to moderate nuclear-to-cytoplasm ratio (seen in 23/28 cases, 82%), a round to oval (seen in 25/28 cases, 89%) decentralized nucleus (seen in 21/28 cases, 75%) with stippled chromatin (seen in 25/28 cases, 89%). Cytoplasmic granularity was seen in 14 cases (50%) and intracytoplasmic vacuolization was noted in seven cases (25%; Figure 3). The background characteristics were generally without macrophages (only seen in four cases, 14%) and colloid (only seen in four cases, 14%). No mitotic activity was identified in any smear. Outliers in many parameters were two cases (7%) in which the corresponding histological sections were diagnosed as parathyroid carcinoma. Those smears showed size-varied cells (from twice up to four times the size of an erythrocyte) with round to irregular nuclei and prominent nucleoli, a feature not seen in the adenoma pool (Figures 2 and 3).

FIGURE 2 Cytological smears and corresponding histological sections from a parathyroid carcinoma. (A) May Grunwald-Giemsa (MGG) stain of a cytological smear containing a tight, three-dimensional cluster. Note the nuclei showing anisokaryosis and a size substantially bigger than erythrocytes (×200 magnification). (B) Corresponding histology section stained with hematoxylin–eosin displaying brisk pleomorphism and conspicuous nucleoli (×200 magnification). (C) An intravascular tumor thrombus was found, thereby fulfilling the histopathological criteria for parathyroid carcinoma (×40 magnification) [Color figure can be viewed at wileyonlinelibrary.com]

Architectural patterns of the smears were generally mixed (Figure 1). Six cases (21%) contained all four patterns; dispersed cells, loose aggregates, microacinar formations and tight clusters. Eleven cases (39%) contained three of the patterns and nine cases (32%) contained two patterns. The remaining two cases (7%) contained only one pattern (tight clusters). The presence of central capillaries within tight clusters was noted in nine smears (32%).

3.3 Histological correlations

Out of the 20 cases with available histological sections, the dominating growth pattern was solid formations, which was present in 18 cases (90%; Figure 4). Of these, seven cases exhibited this growth pattern exclusively. The corresponding smears to the seven cases with sections containing solid growth only included two to four cytological patterns and thus did not pinpoint any architectural correlation. The remaining two histological cases without solid growth contained solely microacinar growth and sinusoidal patterns, respectively. The
smear of the case with exclusive acinar growth on histology exhibited a mixture of four cytological patterns, whereas the case with sinusoidal growth on histology contained both dispersed and loose aggregates of cells on FNAC. Moreover, the presence of a microacinar growth pattern on histology was noted in seven cases (35%) but no matching correlation to cytology was found. Trabecular growth pattern was seen in four cases (20%) on histology but no such architectural feature was found in the corresponding cytological smears.

By histological examination, the most common cell type was the conventional chief cell and in 10 cases this cell type constituted >70% of the total amount of tumor cells (Figure 3). Cytoplasmic granularity, suggestive to be a cytological hallmark for conventional chief cells, was seen in 14 cases, and only 6 of these exhibited a high percentage of conventional chief cells on histology. Clear cell variant chief cells were present on histology in 11 cases, though in quite small percentages ranging from 10% to 70% of the total amount of tumor cells and with only two cases with more than 40% (containing 60% and 70%, respectively). Intracytoplasmic vacuolization, suggestive to be a cytological feature of clear chief cells as they have a rich content of lipid and glycogen, was seen in smears from seven cases. Two of the 11 cases with clear chief cells on histology showed intracytoplasmic vacuolization in corresponding cytological smears. The presence of oxyphilic cells were noted on histology in 13 cases. In the cytological smears though, oxyphilic cells were only seen in two cases.

In terms of biopsy artifacts (fibrosis, hemorrhage, calcifications, inflammatory infiltrates), these parameters were evident in histological preparations from 8 out of 20 cases (40%), including 6 adenomas and 2 atypical tumors.

FIGURE 3 Cytological characteristics of parathyroid tumors. All images are seen with May Grunwald-Giemsa (MGG) stain. (A). Fine-needle aspiration cytology of a parathyroid carcinoma displaying nuclear pleomorphism and prominent nucleoli, the latter phenomenon marked with arrowheads (×600 magnification). (B). Same case displaying tumor cells with cytoplasmatic granularity (×400 magnification). (C). Focal areas with cytoplasmic vacuolization were also noted (arrowheads, ×600 magnification) [Color figure can be viewed at wileyonlinelibrary.com]

FIGURE 4 Histological representation of the three main cell types of a parathyroid adenoma visualized via a hematoxylin–eosin staining at ×200 magnification, displaying variable proportions of oxyphilic cells (left), clear cell variant cells (center) and conventional chief cells (right) arranged in solid and cord-like formations. There were no evident correlations between the FNAC smear morphology and architecture and the corresponding tumor cell proportions and growth patterns on histology [Color figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

At our institution, the proportion of parathyroid lesions diagnosed by FNAC has increased in the last years, and the reason for this surge is most likely a combination of an increased demand for preoperative cytology in general, and localization of parathyroid lesions in particular but most probably also an increased alertness by the cytologists as the diagnosis is no longer as rare in the FNAC setting. Confirmation of the diagnosis by chemical PTH analyses at our department started in 2013, and has been performed in 13 out of 18 FNAC diagnosed parathyroid lesions cases since then. Especially for acellular aspirates
from cystic lesions (not included in this cohort), it may be invaluable since morphological and immunocytochemical methods may be of little use.⁶,⁸

In this study, we have collected one of the largest series of parathyroid lesions investigated by FNAC and describe the cytomorphic aspects with the aim to correlate various aspects to the final histopathology report. The cytological parameters in our study include many of the parameters previously described by Kumari et al, Domingo et al and Absher et al, and we were able to reproduce several of them (cytoplasmic vacuolization, granular cytoplasm and dispersed cellular patterns).⁷,⁸,¹² Indeed, dispersed cells were noted in 79% of cases, cytoplasmic granularity was also a recurrent theme (seen in 50% of cases), and intracytoplasmic vacuolization was noted in seven cases (25%). When combining these cytological parameters, 25/28 cases (89%) displayed at least one of these three criteria. Moreover, although based on few cases, we found cytological differences between parathyroid adenomas and carcinomas using FNAC, worthy of follow-up studies. Specifically, carcinomas displayed larger nuclei with more prominent atypia than conventional adenomas using FNAC. For the adenoma group, smears showed a mix of architectural patterns and cellular features, with small, relatively monomorphic cells with round to oval nuclei with stippled chromatin and a background generally free from macrophages and colloid. No obvious correlations to predominant cell types and growth patterns visualized at histological investigations were noted.

As thyrocytes and most thyroid tumor cells usually present with cytological features consistent with a fragile, pale-blue cytoplasm with indistinct cell borders and small and round nuclei with inconspicuous nucleoli or as naked nuclei, we consider these general features to overlap substantially with those of parathyroid cells, making a distinction between thyroid and parathyroid lesions based on cytology alone difficult.¹⁴ At our department, we use ICC using antibodies against TTF1 and PTH, but previous work indicate that also GATA3 may facilitate the discrimination between these two tissue types.¹⁵ Moreover, in terms of diagnostic pitfalls when dealing with FNAC on parathyroid lesions, previous studies highlight the risk of falsely diagnosing a papillary thyroid carcinoma since the presence of tight, three-dimensional clusters with a central capillary can be seen in smears from a parathyroid lesion—a pattern that may be interpreted as papillary formations. Previous studies have suggested the phenomenon to be correlated to a hypervascular lesion detected on color Doppler sonogram.¹¹ Another possible diagnostic pitfall is the risk of misinterpreting an oncocytic parathyroid tumor as a Hürthle cell thyroid neoplasm. In this study though, oxyphilic cells were not easily identified in the smears even though several histological sections contained a substantial percentage of oxyphilic cells.

Parathyroid adenomas are histomorphologically heterogeneous tumors often presenting with several growth patterns and cell types in varying proportions within the same tumor. Even though most parathyroid tumors are quite small compared to other epithelial neoplasms, the FNAC will only cover a small proportion of the tumor area. Therefore, it is not entirely surprising to find that the FNACs were not completely representative of the subsequent histology in terms of cellular composition and growth patterns. In normal parathyroid tissues, it has been described that the chief cells, responsible for the production and secretion of PTH, undergo a secretory cycle with a resting, synthetic, transporting, packaging, secretion and involuting phase. The resting phase correspond to the inactive chief cell and is characterized by the accumulation of glycogen and lipid droplets and thus have a bright appearance by light microscopy (clear chief cells). Similarly, intracellular fat tissue content has been proven to inversely correlate with the endocrine activity in normal and neoplastic parathyroid glands.¹⁶ As most pathological parathyroid glands contain various amount of conventional chief cells and clear chief cells, this correlation between metabolic activity and cellular phenotypes opens a window for the cell proportions and characteristics to differ from the time of the FNAC performed and the subsequent surgical removal. Moreover, some cytological hallmarks may be less pronounced in histological preparations and vice versa, which may be due to the different methods employed in terms of fixation.

FNAC is generally a good modality in differentiating benign from malignant tumors when the diagnosis is based on cellular morphology. However, the histological criteria for parathyroid carcinoma do not rely on cellular characteristics, but rather on tumor behavior (such as capsular and vascular invasion).¹⁶,¹⁷ As of this, one cannot rule in a parathyroid carcinoma diagnosis from FNAC of a primary parathyroid tumor. Even so, certain cellular attributes are overrepresented in parathyroid carcinoma compared with adenoma, including macronucleoli and brisk pleomorphism.¹⁶,¹⁷ Although our study only included two carcinomas, we still saw cytological characteristics (lager nuclei with more prominent atypia) that clearly set them apart from the adenomas, indicating possible features to be recognized using FNAC that potentially could alert the surgeons preoperatively.

In our series, pHPT patients with inconclusive imaging and/or negative surgical exploration were followed biochemically, and assessed using ultrasonography-guided FNAC at a later stage in order to verify locations of the culprit lesions and to avoid excessive explorative surgery. In all, the result of the FNAC investigation affected clinical decisions concerning treatment options in 27 patients (96%), most notably correct localization leading to a more focused surgical approach rather than bilateral exploration. Therefore, parathyroid FNAC may be an aiding tool for subsets of cases when imaging analyses or previous surgery fail to localize the culprit lesion. However, it must be stressed that the cohort described in this manuscript is a highly selected group of extraordinary cases in which the FNAC was deemed necessary during the clinical work-up to guide the surgical approach. This is by no means standard care in our department, and given the fact that we have performed >4000 parathyroid surgeries spanning the study period, the use of FNAC in localizing parathyroid tissue at the Karolinska University Hospital is exceedingly rare. Moreover, the usage of FNAC in the clinical work-up of parathyroid tumors is not without disadvantages. FNAs can complicate parathyroid surgeries due to fibrosis developing after a biopsy procedure, which potentially could lead to increased patient morbidity (for example, damage to adjacent nerves).¹⁸ However, surgical difficulties or complications attributable to the FNAC procedure were not noticed in this
series. Additionally, the appearance of FNA-induced fibrosis on subsequent histology may confuse the responsible pathologist, as this feature might be worrisome for an atypical parathyroid tumor or parathyroid carcinoma.\textsuperscript{16} In our series, biopsy artifacts were noted in 40\% of cases, including six adenomas and two atypical tumors. In the two atypical cases, the fibrosis was accompanied by several other risk features (such as trabecular growth, mitoses and nuclear pleomorphism), thereby arguing against misdiagnosis due to previous manipulation. Even so, the high proportion of cases with biopsy artifacts in our series advocate careful histological assessment of excised parathyroid tumors if a previous FNA procedure was performed, and the clinical team must be aware of the potential risks involved if planning for a parathyroid FNAC.

To conclude, parathyroid FNAC may be considered in a highly selected group of patients when localization of the diseased gland is imperative for the surgical procedure and not easily established using conventional imaging analysis. Although several morphological features seem recurrent on cytology, diagnosis should be confirmed with PTH measurements or PTH ICC. In this cohort, the FNAC investigation contributed with valuable information and affected clinical decisions for the majority of patients, but also induced biopsy artifacts in nearly half of the cases, which may cause diagnostic predicaments on the histological level. Also, although based on few cases, we also found cyto-morphological differences between parathyroid adenomas and carcinomas using FNAC worthy of follow-up studies.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS
Conception or design of the work: C. Christofer Juhlin. Data collection: Sanna Steen, Martin Hysek, Henrik Falhammar, C. Christofer Juhlin. Data analysis and interpretation: Sanna Steen, Martin Hysek, C. Christofer Juhlin. Drafting the article: Sanna Steen, C. Christofer Juhlin. Critical revision of the article: Sanna Steen, Martin Hysek, Jan Zedenius, Henrik Falhammar, C. Christofer Juhlin. Final approval of the version to be published: Sanna Steen, Martin Hysek, Jan Zedenius, Henrik Falhammar, C. Christofer Juhlin.

DATA AVAILABILITY STATEMENT
The data that supports the findings of this study are available within this article.

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