




Touch imprint cytology is useful for the intraoperative pathological diagnosis of PitNETs' surgical margins

Noriaki Tanabe^{1,2,3} · Naoko Inoshita^{1,2}  · Atsushi Ishida⁶ · Masataka Kato⁶ · Haruko Yoshimoto⁶ · Hideki Shiramizu⁶ · Hidetaka Suga^{1,4} · Toru Tateno⁵ · Kenichi Ohashi² · Shozo Yamada^{1,6}

Received: 5 May 2023 / Accepted: 24 September 2023 / Published online: 6 October 2023
© The Author(s) 2023

Abstract

Touch imprint cytology (TIC) and frozen section (FS) procedures are essential for intraoperative pathological diagnosis (IPD). They are invaluable tools for therapeutic decision-making, helping surgeons avoid under or overtreatment of patients. Pituitary neuroendocrine tumors (PitNETs) are generally small, slow-growing tumors with low-grade malignancy located at the base of the skull where it is impossible to maintain a wide tumor margin. Therefore, transsphenoidal surgery (TSS) should be performed with necessary caution, and with sufficient and minimal resection. Thus, this study aimed to evaluate the diagnostic accuracy of TIC for the diagnosis of PitNET and determine its ability to accurately evaluate the surgical margin compared to the FS procedure. A total of 104 fresh specimens from 28 patients who underwent TSS for PitNETs were examined using TIC and FS. TIC specimens were categorized according to the cell imprinting pattern. All specimens with a large number of neuroendocrine cells diffusely attached to the glass surfaces had PitNET components. Contrarily, no rich or diffuse cell attachments were observed in any non-tumoral endocrine cells. In conclusion, recognizing a pattern of endocrine cell adherence to glass is highly effective in IPD to certify the existence of a PitNET component.

Keywords Pituitary neuroendocrine tumor · PitNET · Intraoperative pathological diagnosis · Surgical margin · Touch imprint cytology

Introduction

Pituitary neuroendocrine tumors (PitNETs), formerly referred to as pituitary adenomas, are clinically classified into two groups: (1) functional PitNETs with hormonal disorders and (2) non-functional tumors with locally

compressive and invasive symptoms, such as visual disturbances and headaches. PitNETs are low-grade malignant neuroendocrine tumors located at the base of the skull, where it is nearly impossible to maintain a wide tumor margin. As a result, transsphenoidal surgery (TSS) must be performed with necessary caution ensuring sufficient but minimal resection.

In general, intraoperative pathological diagnosis (IPD) has two main purposes: (1) to determine tumor existence and its pathological type and (2) to evaluate the tumor boundary for complete resection. Touch imprint cytology (TIC) and frozen section (FS) procedures are crucial for IPD [1]. For pituitary tumors, the FS procedures require pathologists who are highly skilled in specimen processing and diagnosis due to the small size of the specimen (average, 1–2 mm), and occasionally, cell morphology cannot be evaluated due to artifacts caused by the freezing process [1, 2]. Therefore, for pituitary tumors, cytology is sometimes more effective than FS for IPD, especially in determining the histological subtypes and hormone-producing activity [2–4]. The latest atlas from the Armed Forces Institute of Pathology

✉ Naoko Inoshita
inonao3939@gmail.com

¹ Department of Pathology, Moriyama Memorial Hospital, Tokyo 134-0081, Japan

² Department of Human Pathology, Tokyo Medical and Dental University, Tokyo, Japan

³ Department of Neurosurgery, Japan Self Defense Forces Hospital, Tokyo, Japan

⁴ Department of Endocrinology and Diabetes, Nagoya University Graduate School of Medicine, Nagoya, Japan

⁵ Division of Endocrinology and Metabolism, Department of Medicine, University of Alberta, Edmonton, AB, Canada

⁶ Hypothalamic & Pituitary Center, Moriyama Neurological Center Hospital, Tokyo, Japan

(AFIP) mentions that cytology is not useful for surgical margin determination because it does not provide information on tissue architecture [5]. However, our team has been performing marginal evaluation using IPD for PitNETs and has used both FS procedures to examine architecture and TIC techniques to examine cell characteristics and attachment patterns in all routine IPDs for the past several years. Using a combination of these techniques, we achieved higher accuracy rates compared to those achieved using a single technique.

Adrenocorticotrophic hormone (ACTH) producing PitNETs, which cause Cushing disease, may be difficult to detect using imaging. Kurosaki et al. reported that intraoperative cytology is highly accurate in detecting the presence of PitNETs [6]. However, in previous reports, while cytology was used to evaluate tumor presence, it has rarely been used to evaluate surgical margins. In other organs, TIC performed alone replaces the FS process to evaluate the surgical margins. For example, the evaluation of TIC for sentinel lymph nodes in breast cancer has been shown to be effective [7]. During partial nephrectomy for renal tumors, marginal evaluation with TIC is highly accurate in determining whether partial resection is acceptable [8].

Although we already use a combination of TIC and FS for IPD in our clinical practice routinely, in this study, we aimed to determine the accuracy of TIC compared to FS for PitNET diagnosis and the evaluation of surgical margins.

Materials and methods

A total of 104 fresh specimens from 28 patients who underwent TSS for PitNETs between April and June 2022 at Moriyama Memorial Hospital were examined for intraoperative pathological consultation. Our clinical team always uses IPD to decide regarding clinically effective resection. All cases had one specimen obtained from the clinically central part of the tumor (cIT) to identify the histological variation of the tumors and subsequently, other specimens from clinically surgical margins (cISM), which were defined as pseudo-membranous tissue composed of PitNET and compressed anterior lobe tissue, or from sites where the surgeons could not certify whether tumor components existed, sometimes with irregular fibrosis.

Coated glass slides (MAS-01; Matsunami Glass Industry, Osaka, Japan) were used for both the TIC and the FS procedure. At first, small and fresh tissue samples retrieved during TSS were touched onto dry microscopic glass slides for imprint preparation, and the slides were immediately fixed with 95% alcohol. During the imprinting procedure, at least two surfaces were attached and rolled to examine circumferentially (Fig. 1). After cytology processing, the specimens were mounted in a compound (Tissue Tek

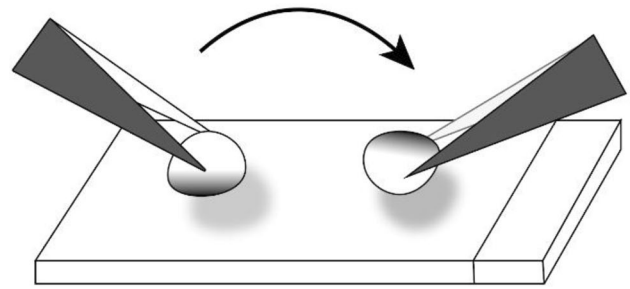


Fig. 1 In our touch imprint cytology method, we rolled the specimens and touched two or more surfaces with the microscope glass slides

O.C.T Compound; Sakura Finetek, Tokyo, Japan), rapidly frozen in isopentane chilled by dry ice, and sectioned at 5 μ m. The specimens from both the TIC and FS procedures were stained using the rapid hematoxylin and eosin procedure, dehydrated, permeabilized, and sealed with a glass cover. After the FS procedure, the specimens, excluding the pure tumors for deep freeze storage, were re-fixed with formalin and processed into paraffin-embedded sections for re-diagnosis.

All slides were independently examined and discussed by a pathological trainee (NT) and a specialized pathologist (NI) with experience in diagnosing over 3,000 PitNET cases.

The cytological findings were classified into the following three categories according to the cell imprint pattern (Fig. 2): pattern cyA, rich and diffusely spread neuroendocrine cells (Fig. 2b); pattern cyB, a small number of scattered endocrine cells (Fig. 2d); and pattern C, no apparent endocrine cells (Fig. 2f). Patterns cyA and cyB were further classified into three subcategories based on the morphology of the endocrine cells: 1; PitNETs, 2; adenohypophyseal cells, and 3; difficult to determine (Fig. 3).

Histologically, with FS on the site of IPD, each specimen was divided into one of the three groups: “histologically tumor (hT)” (tumor component only), “histologically boundary (hB)” (both PitNET and a normal component), and “histologically normal (hN)” (no tumor component). Subsequently, they were evaluated histologically using the formalin re-fixed specimens after FS. The diagnostic accuracy of TIC was validated based on histological findings using both frozen and formalin re-fixed specimens for the final diagnosis.

Based on these definitions, the positivity, sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of TIC were evaluated. The Kappa coefficient was used for diagnostic concordance between TIC and FS histology. Statistical analyses were performed using EZR ver. 1.54 [9]. The Kappa coefficient was assessed per Koch et al.’s definition [10]. Although cyA1 and cyB1 were cytological PitNET-positive specimens, the possibility that a small number of PitNET cells in the surgical field were attached to the surface

Fig. 2 a–f Microscopic images of hematoxylin and eosin staining of touch imprint cytology preparation and frozen section. All specimens were obtained with $\times 10$ magnification. Both **a** and **b** were from the same specimen from hT. **c** and **d** were from hB. **e** and **f** were from hN. **a**, **c**, and **e** were from frozen section, and **b**, **d**, and **f** were touch imprint cytology and classified as follows; **b** as cyA1, **d** as cyB1, and **f** as cyC

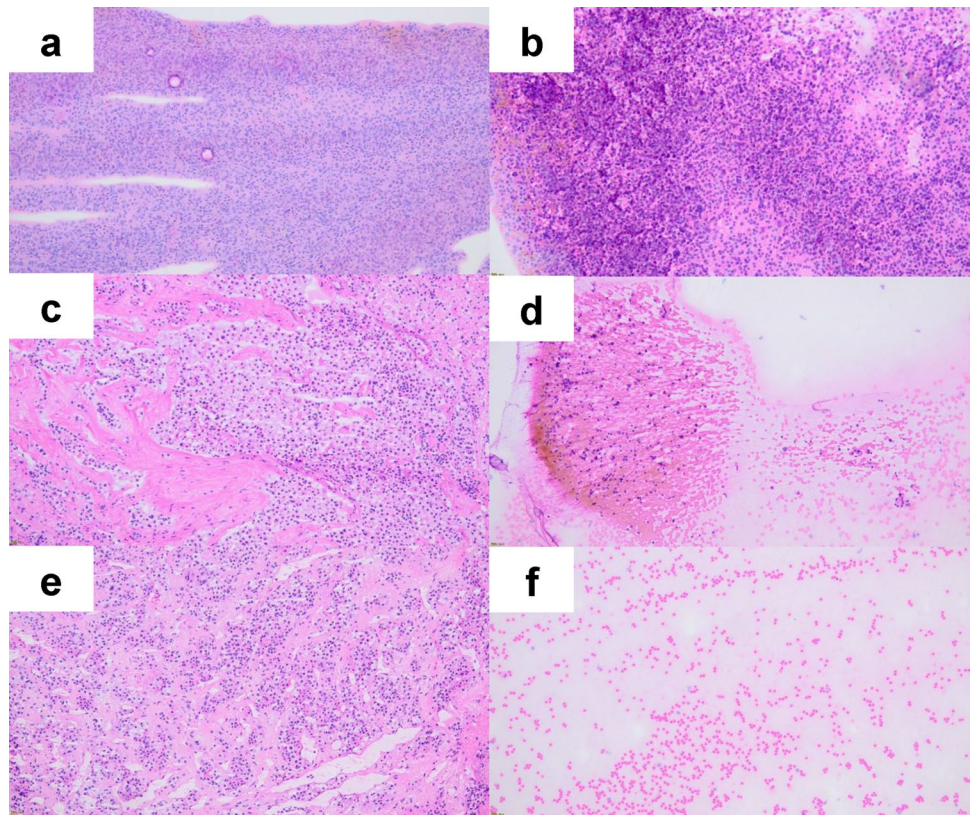
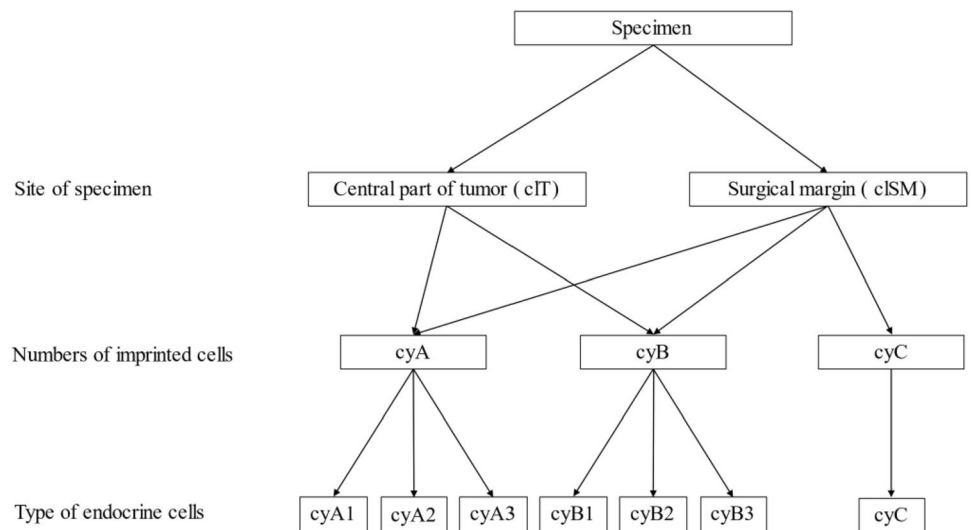


Fig. 3 A breakdown of cytological classification of specimens



of the specimen could not be ruled out in cyB1. Therefore, we raised two statistical questions that defined only cyA1 as

cytologically positive and both cyA1 and cyB1 as cytologically positive.

Table 1 Distribution of the cytological pattern according to the histological evaluation

	Cytological pattern* ¹						
	cyA1	cyA2	cyA3	cyB1	cyB2	cyB3	cyC
Histological diagnosis* ²							
cl T* ³							
hT* ⁴ (n=28)	26	0	0	2	0	0	0
cl SM* ³							
hT* ⁴ (n=19)	11	0	0	6	0	1	1
hB* ⁴ (n=25)	5	0	0	7	0	8	5
hN* ⁴ (n=32)	0	0	0	2	3	8	19

PitNET pituitary neuroendocrine tumor, *cy* cytological, *cl* clinical, *h* histological, *T* tumor, *SM* surgical margin, *B* boundary, *N* normal

*1 Cytological pattern: cyA1, rich monomorphous tumoral cells; cyA2, rich polymorphous endocrine cells; cyA3, rich undeterminable cells; cyB1, sparse monomorphous tumoral cells; cyB2, sparse polymorphous endocrine cells; cyB3, sparse undeterminable cells; cyC, no apparent endocrine cells

*2 Performed using frozen sections and FFPE sections of the same specimens, except the pure tumor sample for deep freeze storage

*3 clT, specimens from the center of the tumor; cl SM, specimens for surgical margin evaluation

*4 hT, histologically containing tumor only; hB, histologically containing both tumor and anterior lobe tissue; hN, histologically containing no tumor component

Results

The results of the cytological evaluations are presented in Table 1. Tumor types of these cases were classified as follows: thirteen gonadotroph PitNETs, four somatotroph PitNETs, four lactotroph PitNETs, three corticotroph PitNETs, two thyrotroph PitNETs, an immature PIT1- lineage tumor, and a mammosomatotroph tumor. All 28 cytological specimens from clT comprised 26 specimens of cyA1 and 2 specimens of cyB1. Regarding clSM, the final histological diagnoses by FS and formalin re-fixed sections were 19 hT, 25 hB, and 32 hN, among a total of 76 specimens. Among the hN specimens, 13 cases (40.6%) had neuroendocrine cells and 2 cases (6.2%) were determined to be cyB1; positive cytologically. However, none of the TIC specimens of the hN type were classified as pattern cyA (Fig. 4a).

cyA1 + cyB1, defined as cytologically positive, had a sensitivity of 61.4%, specificity of 93.8%, and positive predictive value of 93.1%. Assuming that only cyA1 was positive, the sensitivity was 36.4%; however, the specificity and positive predictive values were 100%. When comparing the accuracy between the two groups, cyA1 + cyB1 was significantly more accurate ($P < 0.001$). The Kappa coefficient for A1 as positive was 0.32 and that for cyA1 + cyB1 as positive was 0.52 (Table 2).

In all specimens, pattern cyA were determined to be from samples that contained the tumor, and notably, no cyA2 or cyA3 were present (Fig. 4b). In addition, 17 of the 19 hT specimens in clSM appeared as cyA1 and cyB1, suggesting that we possibly only touched the normal surface of the

boundary to the glass and that touching the glass slides with many sides of the specimen is imperative.

Interesting findings were observed in some cases. In one case of corticotroph PitNET, TIC of the hN specimens had relatively many non-tumoral cohesive endocrine cells. We found scattered bizarre large cells with Crooke's hyaline change, a well-known morphological change of non-tumoral corticotroph cells under the systemic condition of hypercortisolism, and then, we could classify it as cyB2 (Fig. 5a, b). In other cases, the ciliated columnar epithelial cells of the Rathke's cleft cyst and small retention cysts surrounding PitNETs were difficult to distinguish from neuroendocrine cells. Columnar cells were similar to endocrine cells, especially in nuclear size, and were difficult to distinguish without cilia (Fig. 5c, d).

Discussion

TIC is easier to prepare for intraoperative consultation than the FS procedure, which requires highly trained and skilled pathological technicians to prepare sufficient glass slides for accurate diagnosis [11]. Our study demonstrated that the diagnostic accuracy of TIC was equivalent to that of the FS procedure in IPD for PitNETs' surgical margins.

Generally, TIC is considered acceptable for the rapid diagnosis of brain tumors when compared to histological diagnosis [1, 12]. Although a previous report examined the diagnostic concordance rate from the tumor, the present study showed 100% concordance at the macroscopical tumor level, with 92.9% of specimens being determined to

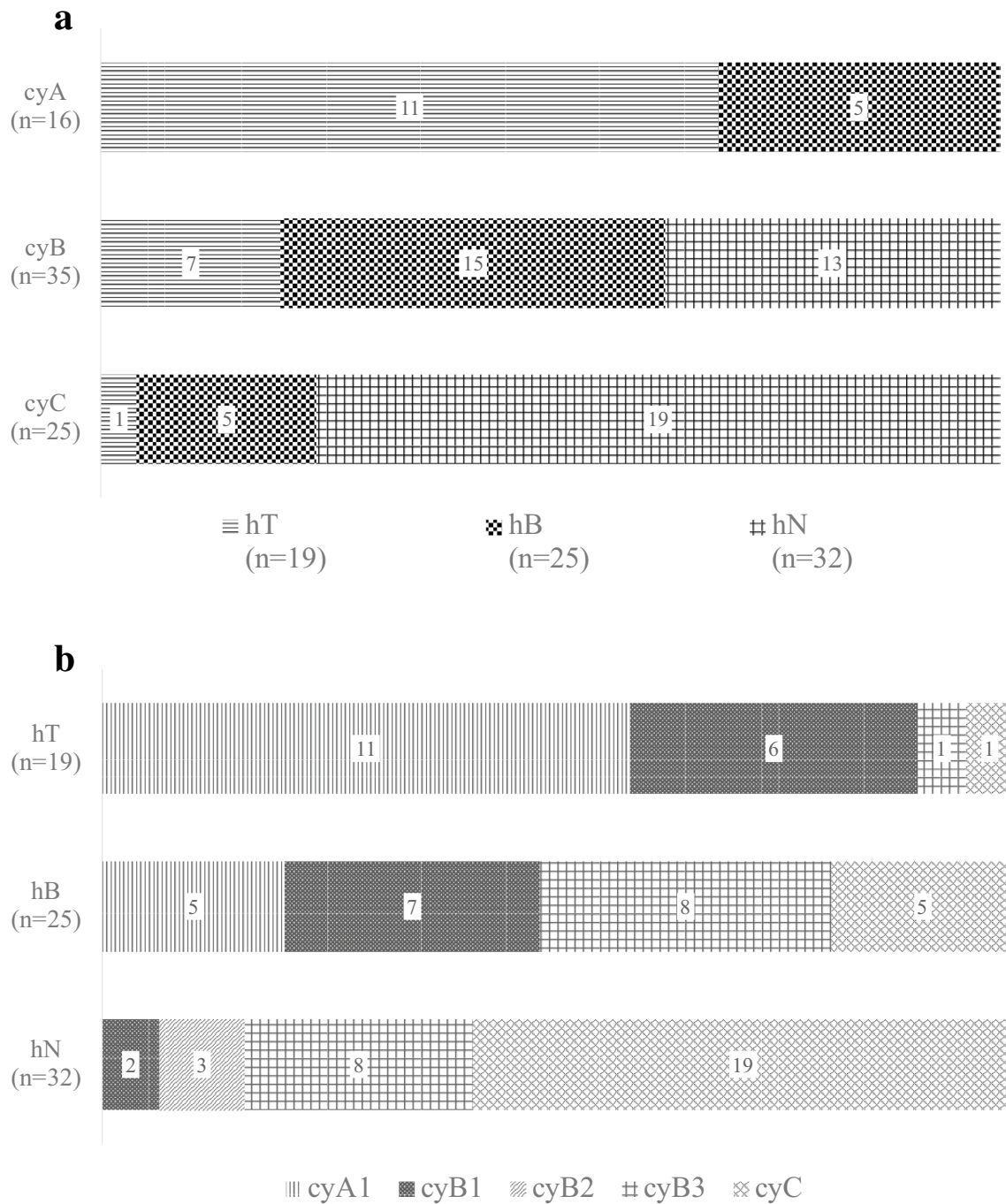


Fig. 4 **a** Histological diagnosis distributions of the each touch imprint cytological pattern from surgical margins. **b** Composition of touch imprint cytology pattern varied from sample site

be cyA1. A previous report that compared the positive diagnosis rate between touch and squash preparation, reported that TIC was useful for small round cell tumors, such as PitNETs and lymphomas [12]. This study also indicated that TIC was accurate.

Therefore, it is important to clearly define “cytological positivity.” In this study, diagnosing “cyA1 + cyB1” as

positive had a significantly higher accuracy than diagnosing only “cyA1” as positive. In contrast, there was 100% specificity and positive predictive value when only cyA1 was defined as positive. cyA1 was positive for many imprinted endocrine cells without considering whether these cells were tumoral because cyA2 and cyA3 were not present. Although, the reason for the difference in the number of imprinted cells

Table 2 Test accuracy with two different definitions of positive cytological diagnosis

	cyA1 as positive	cyA1 + cyB1 as positive
Sensitivity	36.4 (22.4–52.2)	61.4 (45.5–75.6)
Specificity	100 (84.2–100)	93.8 (79.2–99.2)
PPV	100 (71.3–100)	93.1 (77.2–99.2)
NPV	53.3 (40.0–66.3)	63.8 (48.5–77.3)
Accuracy	63.2 (51.3–73.9)	75.0 (63.7–84.2)
κ coefficient	0.32 (0.13–0.52)	0.52 (0.33–0.71)

NPV negative predictive value, PPV positive predictive value

Data are presented as n (%) otherwise indicated

on the TIC glasses is unclear, we estimate that PitNET cells tend to be easily imprinted. The histological distinguishing point of PitNETs from anterior lobe tissue is the breaking of anterior lobe trabecular structure. In fact, during surgery, when the PitNET's capsule breaks, a whitish tumor component flow out (Fig. 6). If specimens are soaked in saline, PitNET cells may wash out. Therefore, we recommend surgeons place specimens on water-repellent sheets without soaking them in a saline solution and submit them immediately after removal. Then, it can be concluded that the specimens are PitNETs when many small round cells are observed on the glass slides.

Although two cases of cyB1 appeared to be over-diagnosed with a negative judgment by frozen and re-formalin-fixed sections, it was estimated that true PitNET cells on

Fig. 5 **a** Frozen section of the Crooke hyaline change $\times 40$, **b** its touch imprint cytology of (a) $\times 60$. **c** FS of the cyst covered by columnar epithelial cells near PitNET $\times 20$, and **d** its TIC of columnar cells with cilia $\times 60$

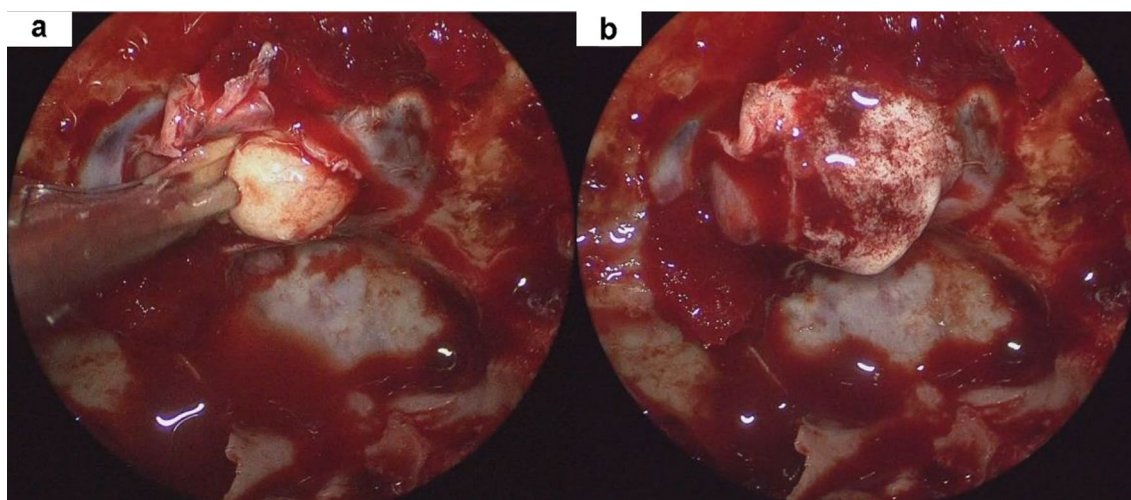
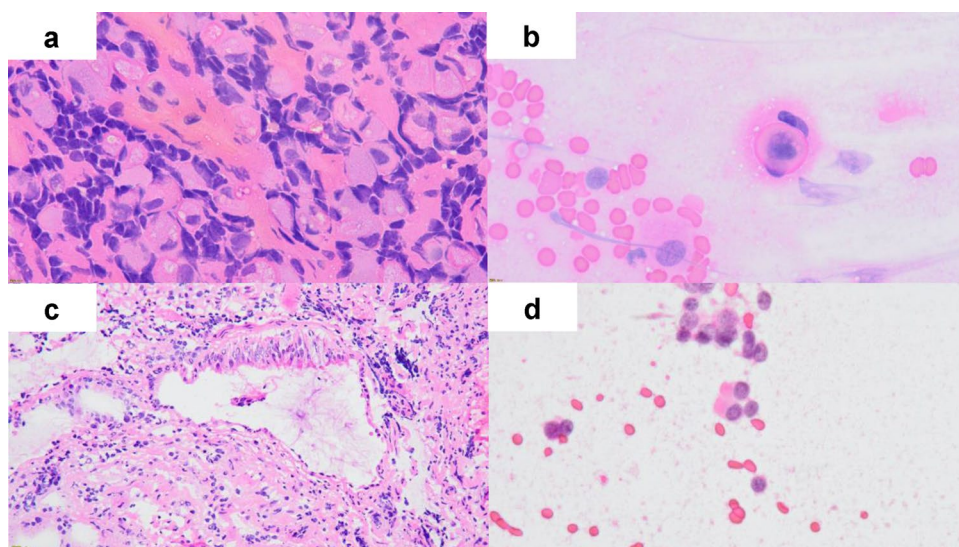


Fig. 6 Intraoperative endoscopic findings in representative cases. **a** encapsulated tumor is exposed. **b** tumor fragments floating in the blood during tumor resection

the surface of hN specimens, which could contaminate the operation area, were observed.

Crooke's hyaline change is a reversible degeneration of corticotroph cells that occurs when exposed to sustained high cortisol levels [13]. In the present study, we observed many imprinted non-tumoral Crooke cells. Thus, the presence of Crooke cells may have loosened the trabecular structure of the anterior lobe. The combination of TIC and FS for IPD for TSS has been routinely performed by our team for several years, and data were partly obtained from many serial cases.

When the frozen and re-formalin fixed sections provided the absolute correct diagnosis, TIC had an accuracy of 75% in the margin evaluation and a Kappa coefficient of 0.52. FS is difficult to evaluate because of the presence of freezing artifacts. In fact, in this study, 10.5% of specimens that were evaluated using the FS procedure were difficult to diagnose. TIC has shown significant promise and avoids artifacts produced by freezing; thus, TIC could be a reliable intraoperative consultation aid.

In conclusion, the diagnostic accuracy of TIC was equivalent to that of the FS procedure for intraoperative pathological consultation of PitNETs' surgical margins.

Acknowledgements We thank Ms. Kyoko Okamoto, Ms. Kumiko Ishibashi, and Ms. Naoko Okita for preparing pathological specimens and Editage for English-language editing.

Author contributions All authors contributed to the conception and design of this study. Material preparation, data collection, and analysis were performed using instruments by NT, NI, AI, MK, HY, HS, and SY. The draft of the manuscript was written by NT and NI. HS, TT, and KO designed analyses and supervised the study. All authors have commented on previous versions of the manuscript and have read and approved the final manuscript.

Data availability The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study was conducted in full accordance with the ethical standards of the 1964 Declaration of Helsinki. All clinical samples were collected after obtaining approval from the Ethics Committee of the Moriyama Memorial Hospital, Tokyo (Approval No.22002). Informed consent was obtained from all patients prior to the surgery.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated

otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

1. Afroz N, Khan N, Hassan J, Huda MF (2011) Role of imprint cytology in the intraoperative diagnosis of pituitary adenomas. *Diagn Cytopathol* 39:138–140. <https://doi.org/10.1002/dc.21394>
2. Fujita H, Tajiri T, Machida T et al (2020) Intraoperative squash cytology provides a qualitative intraoperative diagnosis for cases in which frozen section yields a diagnosis of equivocal brain tumour. *Cytopathology* 31:106–114. <https://doi.org/10.1111/cyt.12798>
3. Lang HO, Saeger W, Lüdecke DK et al (1990) Rapid frozen section diagnosis of pituitary tumors. *Endocr Pathol* 1:116–122. <https://doi.org/10.1007/BF02915627>
4. Sharifabadi AH, Haeri H, Zeinalizadeh M et al (2016) Intraoperative consultation of central nervous system lesions. frozen section, cytology or both? *Pathol Res Pract* 212:179–184. <https://doi.org/10.1016/j.prp.2015.08.009>
5. Asa SL, Perry A (2020) Tumors of the pituitary gland. AFIP atlas of tumor and non-tumor pathology, ARP Press, Arlington VA: ARP Press
6. Kurosaki M, Luedecke DK, Knappe UJ et al (2000) The value of intraoperative cytology during transsphenoidal surgery for ACTH-secreting microadenoma. *Acta Neurochir (Wien)* 142:865–870. <https://doi.org/10.1007/s007010070071>
7. Motomura K, Nagumo S, Komoike Y et al (2007) Intraoperative imprint cytology for the diagnosis of sentinel node metastases in breast cancer. *Breast Cancer* 14:350–353. <https://doi.org/10.2325/jbcs.14.350>
8. Özsoy M, Klatte T, Wiener H et al (2015) Intraoperative imprint cytology for real-time assessment of surgical margins during partial nephrectomy: a comparison with frozen section. *Urol Oncol* 33:67.e25–67.e29. <https://doi.org/10.1016/j.urolonc.2014.07.016>
9. Kanda Y (2013) Investigation of the freely available easy-to-use software “EZR” for medical statistics. *Bone Marrow Transplant* 48:452–458. <https://doi.org/10.1038/bmt.2012.244>
10. Koch GG, Landis JR, Freeman JL et al (1977) A general methodology for the analysis of experiments with repeated measurement of categorical data. *Biometrics* 33:133–158. <https://doi.org/10.2307/2529309>
11. Satturwar S, Rekhman N, Lin O et al (2020) An update on touch preparations of small biopsies. *J Am Soc Cytopathol* 9:322–331. <https://doi.org/10.1016/j.jasc.2020.04.004>
12. Hamasaki M, Chang KHF, Nabeshima K et al (2018) Intraoperative squash and touch preparation cytology of brain lesions stained with H+E and Diff-Quik™: a 20-year retrospective analysis and comparative literature review. *Acta Cytol* 62:44–53. <https://doi.org/10.1159/000480063>
13. Neumann PE, Horoupian DS, Goldman JE et al (1984) Cytoplasmic filaments of Crooke's hyaline change belong to the cytokeratin class. an immunocytochemical and ultrastructural study. *Am J Pathol* 116:214–222

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.