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REVIEW AND PERSPECTIVE

Current practices in performing frozen sections for thyroid and parathyroid pathology

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Abstract In this review article, current trends in thyroid and parathyroid frozen sections are discussed. In Japan and other countries, the numbers of thyroid frozen sections have been dramatically decreasing over the past decade. The decline in the number of thyroid frozen sections has been attributed to two major factors: highly diagnostic preoperative fine needle aspiration for papillary carcinomas, the most frequent type of thyroid cancers, and the acknowledgment in the literature of the disadvantageous frozen sections for follicular tumors. Several authors have argued that the frozen section of thyroid nodules should be limited only to cases that have a preoperative cytology diagnosis as "atypical" or "suspicious". In contrast, frozen sections for parathyroid glands have been increasing in numbers. This increase is thought to be largely due to the high number of parathyroidectomies for secondary hyperplasia in dialysis patients. Frozen sections are usually performed to confirm the removal of parathyroid tissue for either cyropreservation or auto-transplantation. It is concluded that thyroid and parathyroid frozen section examination is restricted to selected situations.

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Introduction

Frozen sections are performed with the aim to provide a rapid diagnosis that has immediate impact on surgical decision making [1, 2]. Frozen section examinations in endocrine pathology have been most commonly used for intraoperative assessment of thyroid and parathyroid tumors and cervical lymph node metastases. In recent years, however, the number of frozen sections in thyroid surgery has been steadily decreasing worldwide [3–5]. This decline has been attributed to two major factors: the high diagnostic accuracy of fine needle aspiration (FNA) cytology for papillary thyroid carcinoma (PTC) and the low sensitivity of frozen section diagnosis for follicular lesions of the thyroid. Many institutions have therefore restricted the use of thyroid frozen sections to thyroid nodules with a preoperative cytology diagnosis of "atypical" or "suspicious."

In contrast, frozen sections for parathyroid diseases have been increasing in number. This is mainly due to an increase in the number of parathyroidectomies for secondary hyperplasia in chronic dialysis patients, while surgery for parathyroid adenomas has remained fairly constant. However, in some institutions, the use of rapid intraoperative determination of the serum level of parathyroid hormone has decreased the need to confirm the removal of a parathyroid adenoma by frozen section. Some authors believe that this serum test may eventually eliminate the need for frozen section examination in surgical treatment of parathyroid adenoma [6–8].

The arguments for and against performing frozen sections in thyroid and parathyroid surgery are not just economic and not just based on specificity and sensitivity rates. Artifacts induced by freezing can substantially alter the nuclear morphology, and the tissue can be largely wasted in the course of tissue sectioning. These factors are particularly important in small lesions, from which only one or two sections may be obtained.

This review will describe recent trends in frozen section diagnosis for thyroid and parathyroid lesions and provide some practical tips and techniques for approaching thyroid and parathyroid frozen section pathology.

Thyroid frozen sections

Incidence of frozen sections on thyroid glands

Several recent studies have reported a decrease in the number of frozen section diagnosis for thyroid lesions [3–5, 9]. To some degree, the number and type of thyroid frozen sections are likely to depend on the experience of the surgical team, the case volume, and on the prevalent types of surgeries being performed. To illustrate the trends in frozen section usage with reference to these variables, the incidence and practices around thyroid frozen sections at four different Japanese hospitals were analyzed.

Tokai University Hospital

Tokai University Hospital is a large teaching hospital. In 2006, 55 surgeries were performed for thyroid-related diseases, and no frozen sections were done. When analyzed as a trend over time, there was a decrease in frozen sections noted over the past 15 years (Fig. 1), despite a near-constant number of surgeries for a similar disease spectrum.

Itoh Hospital

Itoh Hospital is a specialized hospital that specializes almost entirely on thyroid management and surgery. In 2006, for a total of 1,358 cases of thyroid-related surgery performed (Fig. 2), no frozen sections were done.

Kuma Hospital

At Kuma Hospital, another thyroid-specialized facility in Japan, around 1,800 cases of thyroid-related surgery were performed in 2006. Approximately 10% of these cases did have frozen sections. The primary reason for these frozen sections was to evaluate for direct tracheal invasion by cancer or to evaluate potential parathyroid tissue for auto-transplantation during total thryoidectomy.

Isehara Kyodo Hospital

Isehara Kyodo Hospital is a community hospital in Japan with a limited number of thyroid resections. Between 2002 and 2006, 74 thyroid resections were performed, and of these, 53% had frozen sections. This series included 22 cases with follicular lesions, eight cases of PTC and seven cases with miscellaneous diagnoses. This represented 5% of all frozen sections done in that institution.

Aside from the differences in utilization of thyroid frozen sections because of experience level and case volume, there is likely also a contribution from better education of both surgeons and pathologists around the standard of care of preoperative FNA and the utility of thyroid frozen sections, especially regarding diagnostic sensitivity and specificity.

First, it has become the standard of care to approach most thyroid nodules with a preoperative FNA [10]. It is recognized that thyroid FNA cytology is highly sensitive and specific for a diagnosis of PTC, with diagnostic

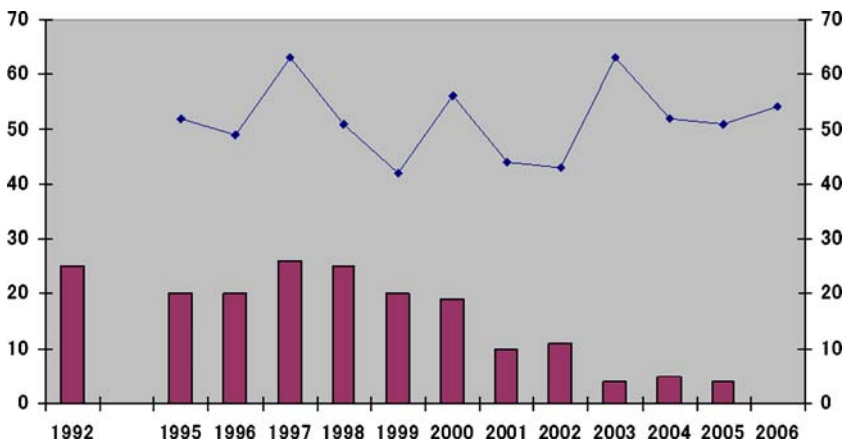

Fig. 1 Trend over 14-year period for the use of frozen section diagnosis for thyroid surgeries at Tokai University Hospital. The *red bars* demonstrate the number of frozen sections performed for thyroids each year, and the *blue line* indicates the number of surgeries performed for thyroid disease in each year. The number of frozen sections decreased dramatically, while the number of overall thyroid surgeries remained relatively constant

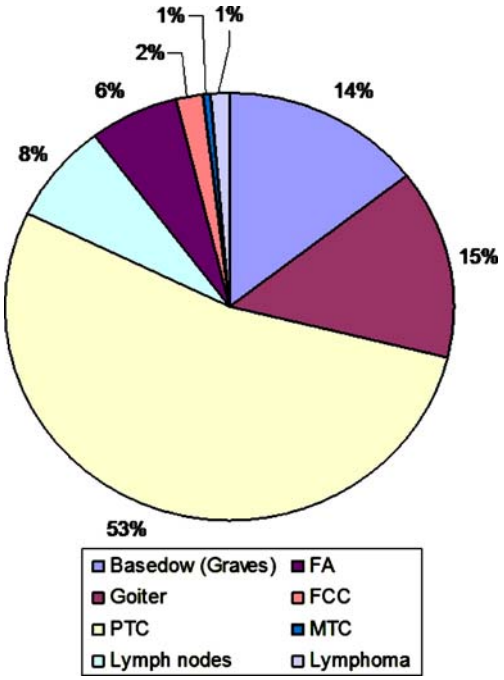

Fig. 2 Incidence of different diseases and diagnoses in surgical thyroid lesions at Itoh Hospital for Thyroid Diseases in 2006. None of these cases underwent frozen section

features including specific cytologic abnormalities such as intranuclear inclusions and nuclear grooves (Fig. 3) [11]. For example, between 2002 and 2006 at Tokai University Hospital, a diagnosis of PTC was made in 105 patients in which both FNA and histology diagnoses were performed. In these patients, the preoperative FNA diagnosis was "PTC" in 91 cases and "suggestive of PTC" in another six cases. This represents a sensitivity of 92.4% (97/105). Because of the high sensitivity and specificity of FNA, LiVolsi and Baloch [12] emphasized that the intraoperative frozen sections should no longer be performed on thyroid nodules with a definitive preoperative FNA diagnosis of PTC.

In contrast, for tumors that do not meet the criteria for a diagnosis of PTC on FNA but show some suspicious or worrisome features, there may be a distinct role for frozen section examination. Mittendorf et al. [13] refined the conditions in which frozen section can be the most useful. They reported 45 patients (7%) with preoperative FNA that was suspicious but not diagnostic of PTC. Eighteen of these patients (40%) later had histologically proven carcinoma, whereas the remainder had benign disease. Prominent nuclear inclusions and/or grooves, papillary formation, and the absence of colloid were features that were most highly associated with the final diagnosis of PTC ($P<0.05$). Rare intranuclear inclusions and/or grooves alone in an otherwise benign-appearing specimen were uniformly associated with benign disease. Importantly, no clinical features could reliably identify the patients that had true malignant disease. Frozen section examination was performed in 27 of the surgical patients, and the results of it altered treatment decision in 15 patients (56%). Therefore, frozen section examination was thought to be of value for

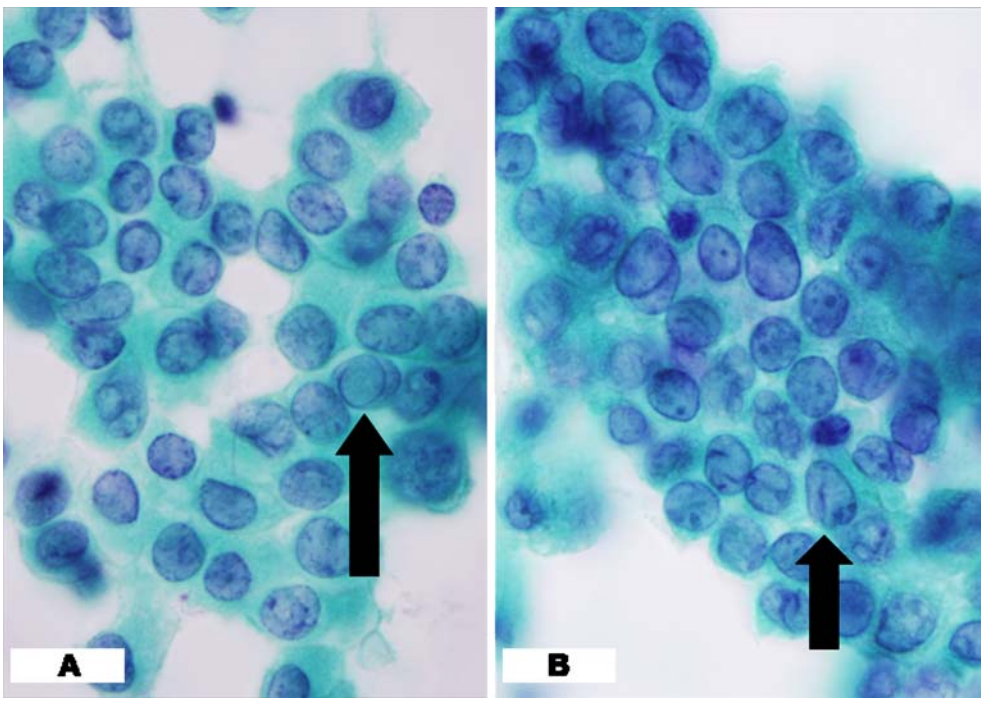

Fig. 3 Papanicolaou stain: Cytology smear preparations from a preoperative fine needle aspiration of papillary carcinoma. **a** An intra-nuclear inclusion (*arrow*); **b** nuclear grooves (*arrow*)

determining the extent of the thyroidectomy in these patients. The usefulness of thyroid frozen sections may also be extended to the cases with other malignancies, including anaplastic or medullary carcinomas or atypical lymphoid cells in the aspirated materials.

The second contributing factor to the decreasing rate of thyroid frozen sections is that both surgeons and pathologists have recognized that frozen section diagnosis has a very low sensitivity and specificity in most follicular tumors of the thyroid [3]. These tumors obviously require careful and complete sampling for an accurate diagnosis of invasion, and this is not a practical approach for frozen section examination in most routine pathology laboratories [12]. In addition to problems induced by incomplete sampling in frozen sections, another issue is the fact that nuclear details are obscured by artifacts in frozen sections (Table 1). Therefore, if only a frozen section is performed on follicular variant of PTC, it is very unlikely that the nuclear features of PTC will be recognized (Fig. 4). Performing intraoperative cytology, which is discussed below, will help to overcome this limitation (Fig. 5).

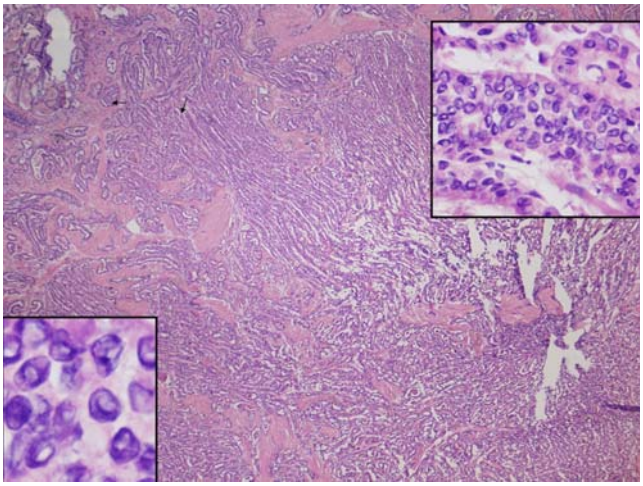

Fig. 4 H&E stained frozen sections: The background image shows a frozen section from a clearly invasive tumor with papillary growth. The *insets* demonstrate the nuclear artifacts from frozen section, which both show obscuring of classic nuclear features of papillary carcinoma

Handling of thyroid tissue for frozen sections

In general, we recommend the following procedure for handling a fresh thyroidectomy specimen. The specimen should be inspected for an intact capsule and then be inked along the external surface. Subsequently, the thyroid can be serially sectioned, usually from superior to inferior.

Recommendations for specific circumstances

Preoperative FNA was "suspicious" or "atypical"

The only way to adequately visualize the nuclear features of PTC in the intraoperative setting is to perform cytologic smears. We prefer the use of scrape preparations over touch preparations. Touch preparations can be heavily contaminated with colloid, and the cellular elements may be quite sparse. In contrast, by carefully blotting away excess colloid from the surface of a lesion and then gently scraping and smearing onto a blank slide, abundant cellular elements can usually be obtained for cytologic interpretation. Cellularity does not need to be assessed, as the purpose of the cytologic intraoperative preparation is only to look for the nuclear features of PTC (Fig. 5; Table 2).

Table 1 Advantages and disadvantages of frozen section examination

Advantages	Disadvantages
Diagnosis available for immediate decision making	Tissue wasting secondary to sectioning Preliminary nature of the diagnosis Sampling errors

Encapsulated nodules

When an encapsulated lesion is discovered, it is recommended that no frozen section is to be performed. The only diagnostic findings in encapsulated follicular lesions will come from assessing the nuclear features and from assessing the entire capsule. In some circumstances, scrape preparations can be performed on an encapsulated lesion. This would be most helpful for ruling out a well-defined follicular variant of PTC. It is important to recognize, however, that the follicular variant of PTC remains a difficult diagnosis intraoperatively even with good cytologic preparations.

Multinodular goiter

In the case of a multinodular goiter, frozen sections should not be performed unless one nodule is worrisome or suspicious based on the gross appearance.

Small lesions (<1 cm)

Lesions smaller than 1 cm should not be examined by frozen section. It is especially critical to absolutely avoid freezing an entire lesion. The resulting tissue alterations may make a diagnosis on permanent sections nearly impossible.

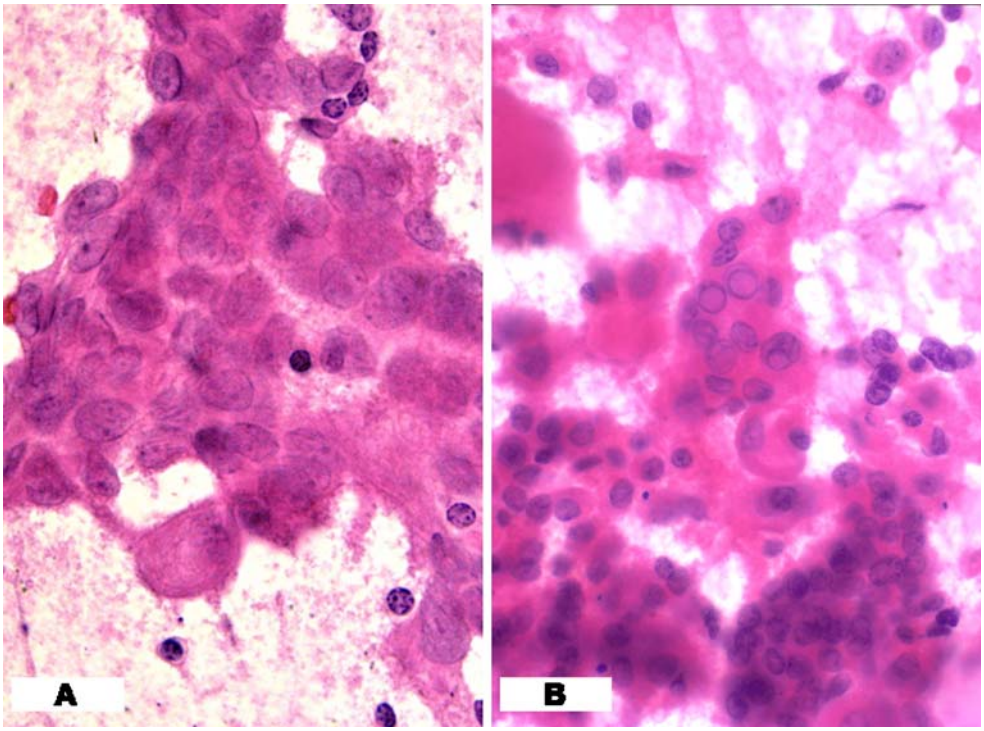

Fig. 5 H&E stain: Cytology scrape preparations from thyroid nodules obtained intraoperatively during frozen section. Note the easily identified features of papillary carcinoma, including grooves (**a**) and inclusions (**b**)

Pitfalls of thyroid frozen section

The most significant pitfall for frozen section in thyroid nodules is a papillary hyperplastic nodule. These lesions have papillary architecture, but the nuclear features are quite bland. Again, using a combination of frozen section and scrape preparation with cytology will enable the pathologist to recognize these lesions.

Parathyroid frozen sections

In contrast to the marked decrease in thyroid frozen sections, there appears to be an increase in the number of parathyroid frozen sections. Many of these cases are related to secondary hyperparathyroidism, where multiple glands are involved (Fig. 6).

At Tokai University Hospital, for example, the numbers of frozen sections for parathyroid adenomas have not changed, but the cases of secondary hyperparathyroidism have increased (Fig. 7). Surgery for secondary hyperparathyroidism, which is often done in the setting of long-standing dialysis for renal disease, includes a total parathyroidectomy with removal of all four glands as the standard of care. Generally, one gland is used for autotransplantation, with intramuscular reimplantation of minced parathyroid fragments of about 1 mm in size into the arm.

Parathyroid frozen sections are typically performed for several scenarios, including tissue identification in normal-sized glands for differentiating parathyroid from fat, thymus, thyroid, lymph node, and other tissue and identification of intra-thyroidal parathyroid gland.

Even in today's surgical practice, preoperative localization of enlarged parathyroid glands remains difficult. It has been estimated that the accuracy of preoperative imaging diagnosis for parathyroids is only about 70% by ultrasound or 75% by methoxyisobutyl isonitrile and CT [14]. The overall insensitivity of these imaging studies is partially responsible for the need for frozen section confirmation of parathyroid tissue during surgery. Frozen section for parathyroids remains an important diagnostic approach when parathyroid tissue is suspected, but the gross appearance is not diagnostic. Frozen sections of parathyroid suffer from

Table 2 Situations in which a thyroid frozen examination is likely or unlikely to be useful

Cytology diagnosis	Gross impression	Frozen potentially useful	Frozen unlikely to be useful
Any	Nodular goiter		X
Follicular or Hurthle cell lesion	Encapsulated nodule		X
Papillary carcinoma	Any		X
Atypical or suspicious for papillary carcinoma	Nodule or lesion	X	
Any	Nodule under 1 cm		X

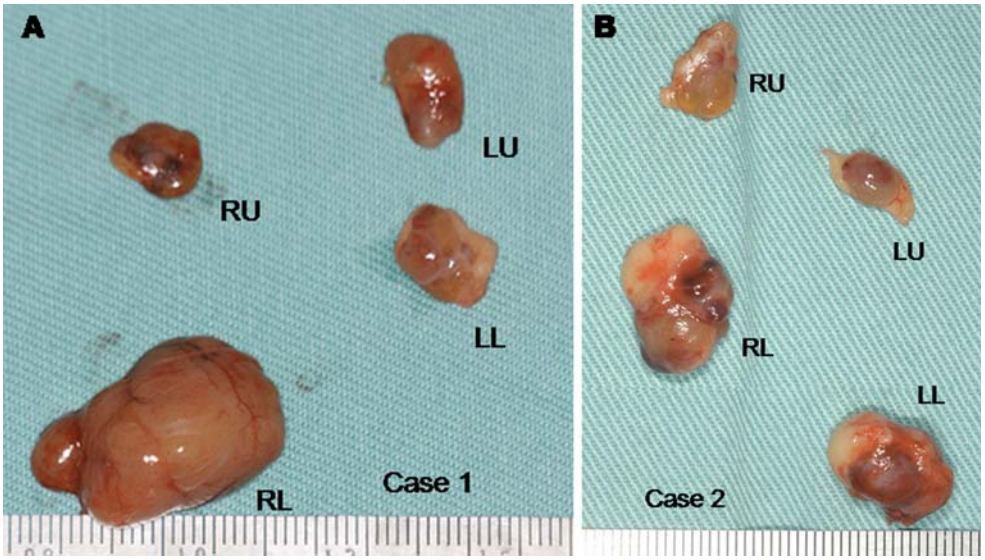

Fig. 6 Two separate cases of parathyroid gland hyperplasia. **a** A case where one gland is significantly more enlarged than the other three glands, which are still larger than normal. **b** A case of parathyroid hyperplasia in which the glands are equally enlarged

similar artifacts as do thyroid frozens (Fig. 8). However, the nuclear features are not used diagnostically for parathyroids, and therefore, the sensitivity and specificity of parathyroid frozens remain quite high.

Despite the increase in surgical interventions for hyperparathyroidism, one qualifying influence on the number of parathyroid frozen sections has been the incorporation of rapid intraoperative parathyroid hormone levels (RI-PTH) into surgical management. In parathyroid adenoma surgeries that are coupled with RI-PTH, frozen section confirmation of the hypercellular gland may therefore no longer be necessary [15–17]. Guarda et al. [16] studied 141 patients who underwent parathyroid surgery, 125 for adenoma, and 16 for secondary hyperplasia. Their results suggested that RI-PTH can be an essential guide for the surgeon performing focused parathyroid surgery. Frozen sections are no longer needed because a significant drop of PTH levels after resection of the largest gland is a far better indicator of adenoma removal and subsequent cure than histologic confirmation.

Handling of parathyroid tissue for parathyroid frozen sections

Parathyroids are characterized by assessing the size and the weight of the glands. Both of these measurements can be important for identifying enlarged and hypercellular glands. Representative sections can be frozen from larger glands,

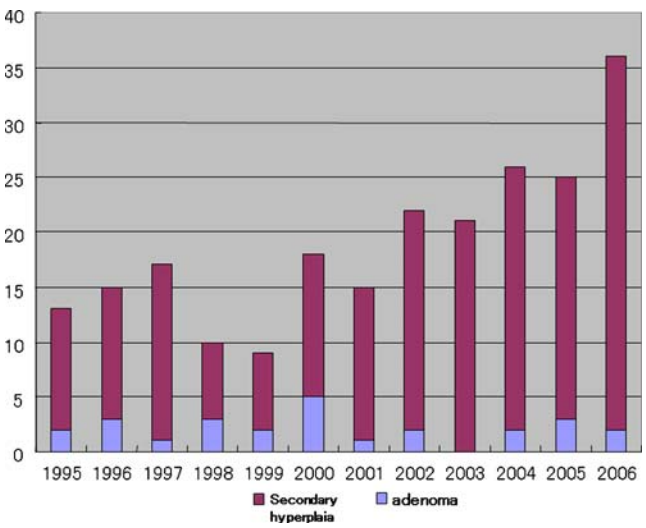

Fig. 7 This graph demonstrates the incidence of parathyroidectomy frozen sections requested for secondary hyperplasia and for parathyroid adenoma over an 11-year period at Tokai University Hospital

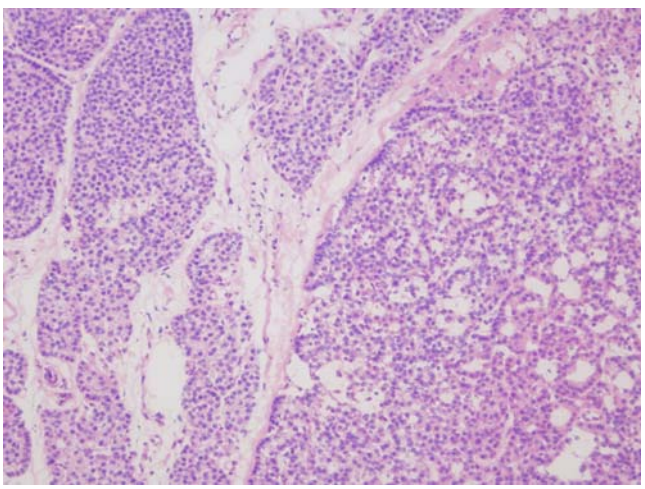

Fig. 8 H&E, frozen section: This image demonstrates the features of an enlarged hypercellular parathyroid gland at the time of frozen section. The chief cells (*left half of image*) and oxyphilic cells (*right half of image*) cannot be well seen because they are obscured by frozen section artifacts

particularly when there are no worrisome features. Particular attention should be paid if there are grossly visible broad fibrous bands or if the gland is found to be adherent to adjacent structures intraoperatively. These features should alert the pathologist to the possibility of a parathyroid carcinoma.

Recommendations for specific circumstances

Parathyroid surgery with the use of rapid intraoperative parathyroid hormone assays

In the setting of parathyroid adenoma surgery, frozen sections should be performed if rapid intraoperative testing of serum PTH level is not available or if the surgeon needs typing of the removed tissue. Frozen section does not need to be performed in conjunction with *RI-PTH* serum measurement, but it is recommended to keep fresh tissue until the results of the RI-PTH are available. If a significant drop in the PTH level is achieved, no freezing is necessary. If there is no significant drop, a frozen section should be performed in order to guide the surgeon.

Very small fragments

It used to be a standard practice for a surgeon to biopsy an unaffected parathyroid gland to assess for cellularity. With the advent of RI-PTH, this practice has become less important. When very small fragments are sent for freezing, the major pitfall is cutting through the tissue while sectioning. Only careful frozen section technique can reduce this risk.

Thymus sent to rule-out intrathymic parathyroid

There is no good solution to assess the cervical thymus intraoperatively because the fat tissue in the specimen will not freeze well. The best approach will be a careful gross inspection of serially sectioned tissue to identify any potential enlarged parathyroid glands.

Parathyroids with worrisome clinical or histologic features

It is particularly important to recognize the potential signs of malignancy in a parathyroid gland intraoperatively. These would include adherence to local structures, broad fibrous bands, increased mitotic activity, or increased pleomorphism. It is not essential to make a diagnosis of parathyroid carcinoma on frozen section, but it is important to alert the clinician to this possibility so that this gland is not used for autotransplantation. Obviously, it is devastating to re-implant a gland only to discover that the permanent sections reveal a carcinoma [18]. Furthermore, identification of the features of a potential carcinoma can enable the surgeon to perform an en bloc resection of the gland, which is considered to be the treatment of choice for parathyroid carcinoma [18].

Conclusions

The number of cases of thyroid frozen sections has decreased remarkably in recent years. This is, on the one hand, due to the excellent results of the preoperative diagnosis by FNA cytology, and on the other hand, the recognition of the low sensitivity of frozen section examination in case of a follicular thyroid lesion. However, it is particularly important to note that thyroid frozen sections are recommended in the cases "suspicious" for PTC or "atypical" by FNA. The number of cases of parathyroid frozen sections may be increasing, likely due to an increasing incidence of parathyroid hyperplasia in dialysis patients. The use of rapid intraoperative PTH testing has the potential to decrease the need for frozen sections for single gland disease. In order to obtain the full benefit of thyroid and parathyroid frozen sections, clear understanding between the surgeon and the pathologist of the pitfalls and risks of frozen section should be a priority in discussions.

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Conflict of interest statement We declare that we have no conflict of interest.

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