Robust, quick and convenient intraoperative method to differentiate parathyroid tissue

Toyone Kikumori, MD, PhD, Takahiro Inaishi, MD, Noriyuki Miyajima, MD, Masahiro Shibata, MD, PhD, Dai Takeuchi, MD

Department of Endocrine and Breast Surgery, Nagoya University Hospital

Address for Correspondence: Toyone Kikumori, Department of Breast and Endocrine Surgery, Nagoya University Hospital, 65 Tsurumaicho, Showaku, Nagoya, Japan, 466-8550. Tel: +81-52-744-2251; Fax: +81-52-744-2252;

E-mail: kikumori@med.nagoya-u.ac.jp

Abstract

Background: Identification of parathyroid tissue (PT) during surgery is necessary for its preservation *in situ* or autotransplantation, to avoid postoperative hypoparathyroidism. Frozen sections are the gold standard for distinguishing PT from other tissues during thyroidectomy. Although frozen sections are very accurate, they are costly and require pathologists and technical staff. PT is rich in mitochondria, which harbor Krebs-cycle enzymes such as aspartate aminotransferase (AST). In contrast, lactate dehydrogenase (LDH) is ubiquitously expressed. These two enzymes are routinely measured as leaked enzymes. We hypothesized that the AST/LDH ratio in suspended tissue could distinguish PT from other tissues.

Methods: We analyzed 94 specimens (43 parathyroid, 19 cancerous, 13 normal lymph nodes, 10 adipose, 6 thyroid and 3 miscellaneous) from 55 patients who underwent thyroid/parathyroid surgery between July 2018 and June 2019 in our institution. Trace amounts of remnant autotransplantation PT were suspended in 1 ml of normal saline and measured for AST and LDH. Approximately 1 mm^3 of apparently distinct tissue minced by scissors (e.g., thyroid gland, metastatic lymph node, etc.) or washouts of needles used for preoperative aspiration biopsy were also measured for comparison.

Results: The AST/LDH ratios in suspended PT specimens were consistently higher than those of other tissues ($P < 0.001$, Mann-Whitney test); 0.27 was the optimal cut-off value, with 100% sensitivity and specificity.

Conclusion: This method quickly and conveniently distinguished PT from other tissues, intraoperatively, with minimum cost and without dedicated pathological staff; and could decrease incidence of postoperative hypoparathyroidism, especially in settings with limited access to pathologists.

Introduction

A persistent worldwide increase in thyroid cancer incidence has increased the number of thyroid surgeries.[1] Postoperative hypoparathyroidism (PH) can complicate outcomes among thyroid surgery patients. Preservation *in situ* or autotransplantation are standard procedures to avoid PH. Identification of parathyroid tissue (PT) is necessary for either technique. Frozen sections are the gold standard for distinguishing PT from lymph nodes, thyroid nodules or fat during thyroidectomy.[2] Although frozen sections are very accurate, they are costly (approximately $200 in Japan) and require pathologists and technical staff. Furthermore, use of frozen sections is often unfeasible in areas with few pathologists.[3] Even Japan, a highly developed country, is facing a shortage of pathologists.[4] An alternative approach—intraoperative parathyroid hormone (PTH) measurement using fine needle aspiration washout fluids—has been developed.[5] However, it also requires a dedicated apparatus and is still costly.

As PT is rich in mitochondria, where the Krebs cycle functions as an energy source,[6] it has abundant Krebs-cycle enzymes, including aspartate aminotransferase (AST). In contrast, lactate dehydrogenase (LDH) is utilized as a marker of common injuries and organ damage because it is ubiquitously expressed. These two enzymes are routinely measured as leaked enzymes in clinical examinations, at low cost. We therefore hypothesized that the ratio of AST to LDH in the tissue suspension could distinguish PT from other tissues. If PT could be confirmed at a lower financial and technical burden, these obstacles to exploration of parathyroid-like tissue could be minimized and unintentional discard of PT could be reduced.

Materials and Methods

We analyzed 94 specimens from 55 randomly selected patients who underwent thyroid or parathyroid surgery between July 2018 and June 2019 in our institution; their characteristics, surgical indications and information about their specimens are shown in Table 1. All measurements obtained from submitted samples were analyzed. This study was approved by the institutional ethical review board, which waived consent in view of the study's retrospective design.

Tissues that appeared macroscopically to be PT were isolated from the excised tissue. Prior to autotransplantation, confirmation of the nature of the tissue was made by a frozen section of a small portion (approximately 1 mm^3) of the excised tissue. The remaining tissue was soaked in sterile normal saline and stored until the identity of PT was confirmed. PT that was confirmed by frozen section was then minced into pieces to obtain a gel-like consistency by scissors onto culture dishes and transplanted in the patient's sternocleidomastoid muscle. Trace amount of remnant tissue on the dish (presumably equal to 1 mm^3) was suspended in 1 ml of normal saline, and the suspension was sent to the biochemical laboratory in our hospital. AST and LDH were measured by a standard automatic analyzer (Labospect 800, Hitachi High-Technologies Corporation, Tokyo, Japan) using commercially available reagents for automatic analyzers (Quick Auto Neo AST JS-HLS for AST, Shino-test Corporation, Tokyo, Japan and Quick Auto Neo LDH JS-HLS for LDH, Shino-test Corporation, Tokyo, Japan) in the same manner as in a normal clinical blood sample. Approximately 1 mm^3 of apparently distinct tissue (e.g., thyroid gland, metastatic lymph node, etc.) minced by scissors or washouts of needles used for preoperative aspiration biopsy were also analyzed for comparison in the same manner. The AST/LDH ratio was calculated using raw measurement values (IU/L). If the concentration was below the measurement limit (< 1 IU/L for AST, < 5 IU/L for LDH), 1 or 5 was assigned to AST or LDH, respectively. Statistical analysis was performed using JMP 14.2.0 (SAS institute, Japan, Tokyo). $P < 0.05$ was considered significant.

Results

Levels of AST and LDH were readily measurable in most specimens (Table 2). Most notably, the AST/LDH ratio of PT was consistently and significantly higher than those of other tissue types (PT vs other tissues, $P < 0.001$, Mann–Whitney test; Figure 1). The

AST/LDH ratios did not significantly differ among other tissue types. Receiver operating characteristic curve analysis indicated that 0.27 was the optimal cut-off ratio, (Figure 1), and predicted PT with 100% sensitivity and specificity.

Discussion

Identification of PT is major requirement for its preservation *in situ* or autotransplantation, for avoiding PH. Several methods are currently used to confirm PT, but they are all expensive and depend on access to pathological staff or dedicated apparatus. In contrast, AST and LDH are measured in routine laboratory tests all over the world. Medical facilities where thyroid or parathyroid surgeries are performed would be expected to have in-facility laboratories to measure these enzymes.

Our study shows that the simple ratio of these routine clinical test results in the tissue suspensions could be a robust tool in identifying PT. Additionally, using the AST/LDH ratio eliminates the influence of dilution for each specimen. Results will be highly reproducible in common clinical settings.

In this study, suspended minced tissue was handled similarly to routine clinical blood samples. Therefore, results took 30 to 40 minutes. However, clotting can be omitted to obtain measurable specimen, which might allow the AST and LDH levels to be ascertained in approximately 15 min after mincing the suspected tissue. Some analyzers (e.g. FUJIFILM NX500) can analyze these enzymes of blood sample in less than 10 minutes according to the product catalog. Thus, the turnaround time for this method could be equivalent to a frozen section and intraoperative PTH assay—but at the cost of less than $3 in Japan. Approximately 1 mm^3 of minced PT or parathyroid-like tissue was enough to measure AST and LDH, and is equivalent to that used in a frozen section. Therefore, measurement of AST and LDH in a suspension of minced tissue is unlikely to hamper successful PT autotransplantation or pathological diagnosis of the resected tissues.

The AST/LDH ratio of parathyroid showed a wide distribution in our study. This could be attributable to various proportions of oxyphilic cells (with abundant mitochondria) and adipocytes.[6] Other than PT, the AST/LDH ratios did not significantly differ between cancerous tissues and other tissue types; this seems to contradict the Warburg effect (Aerobic glycolysis is dominant in cancerous tissues, where LDH functions as a key enzyme.) [7, 8]. This could be attributable to the fact that LDH activity in differentiated thyroid cancer cells is comparable to that of follicular cells,[9] and to the dormant nature of differentiated thyroid cancer.[10]

This study has some limitations. Although our result showed that the AST/LDH ratio predicts PT with neither false positivity nor false negativity, we need to interpret deliberately because the method was only validated in a relatively small number of cases at a single institution. Thyroid tissue may undergo oxyphilic cell change; oxyphilic cell tumors may also arise. These tissues should be rich in mitochondria,[11, 12] and might give false positive results. On the other hand, increased proportion of adipocytes in parathyroid with age has been reported,[6] and might give false negative results. Therefore, further studies with large numbers of cases are warranted to validate this method. Although AST and LDH measurements are performed in a relatively standardized manner worldwide, the optimal threshold for AST/LDH ratio to distinguish PT may also require adjustment in each institution. Another limitation is that this method is only applicable for autotransplantation because it requires a small piece of resected tissue. However, the AST/LDH ratio could be determined using fine-needle aspiration washout fluids in place of measurement of PTH.

This method will be a cost- and labor-effective solution to reducing PH incidence, both in low-resource countries with pathologist shortages and in developed countries, by providing an alternative to frozen sections, at minimal cost.

Conflicts of Interest:

The authors declare no conflicts of interest.

References

1. Jegerlehner S, Bulliard JL, Aujesky D, Rodondi N, Germann S, Konzelmann I, et al. Overdiagnosis and overtreatment of thyroid cancer: A population-based temporal trend study. PloS one. 2017;12:e0179387.
2. Shaha AR, Jaffe BM. Parathyroid preservation during thyroid surgery. American journal of otolaryngology. 1998;19:113-7.
3. Hitchcock CL. The future of telepathology for the developing world. Archives of pathology & laboratory medicine. 2011;135:211-4.
4. Aozasa K. Current status of the Japanese society of pathology with its 100th anniversary. Japan Medical Association journal : JMAJ. 2012;55:416-8.
5. Pelizzo MR, Losi A, Boschin IM, Toniato A, Pennelli G, Sorgato N, et al. Rapid intraoperative parathyroid hormone assay in fine needle aspiration for differential diagnosis in thyroid and parathyroid surgery. Clinical chemistry and laboratory medicine. 2010;48:1313-7.
6. Isono H, Shoumura S, Emura S. Ultrastructure of the parathyroid gland. Histology and histopathology. 1990;5:95-112.

7. Koppenol WH, Bounds PL, Dang CV. Otto Warburg's contributions to current concepts of cancer metabolism. Nature reviews Cancer. 2011;11:325-37.

8. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science. 2009;324:1029-33.

9. Mirebeau-Prunier D, Le Pennec S, Jacques C, Fontaine JF, Gueguen N, Boutet-Bouzamondo N, et al. Estrogen-related receptor alpha modulates lactate dehydrogenase activity in thyroid tumors. PloS one. 2013;8:e58683.

10. Ringel MD. Metastatic dormancy and progression in thyroid cancer: targeting cells in the metastatic frontier. Thyroid. 2011;21:487-92.

11. Kendall CH, McCluskey E, Meagles JN. Oxyphil cells in thyroid disease: a uniform change? J Clin Pathol. 1986;39:908-12.

12. Katoh R, Harach HR, Williams ED. Solitary, multiple, and familial oxyphil tumours of the thyroid gland. The Journal of pathology. 1998;186:292-9.

Figure legend

Scatterplot shows AST/LDH ratios in suspensions of various tissues, logarithmically plotted on the graph. The median and standard deviation (SD) for each of the tissue types are indicated at the bottom of the figure. Thyroid cancer: local recurrences, metastatic lymph nodes and primary tumor; miscellaneous tissues: median cervical cyst, connective tissue and thymus. Bold horizontal line: optimal threshold value to distinguish parathyroid from other tissues (0.27). NA: not applicalble

Acknowledgment

We thank Marla Brunker, from Edanz Group (www.edanzediting.com/ac), for editing a draft of this manuscript. This study has no funding to declare.

Table 1

Patient characteristics, surgical indications and specimen information.

Sex (M : F)	11:44
Age (mean, (range))	54y, (13-86)
Pathology of surgical indication	
Papillary thyroid cancer	40
Follicular adenoma or hyperplasia	7
Follicular thyroid cancer	4
C-cell hyperplasia	1
Medullary thyroid cancer	1
Parathyroid cancer*	1
Parathyroid adenoma†	1
Total	55
Analyzed Specimens	
Parathyroid	43
Papillary thyroid cancer	
Primary tumor	1
Local recurrence	2
Metastatic lymph node	14
Follicular thyroid cancer	
Local recurrence	2
Normal lymph node	13
Normal thyroid	6
Adipose tissue	10
Miscellaneous	
Median cervical cyst	1
Connective tissue	1
Thymus	1
Total	94

[*]Thyroid was measured.

[†]Adipose tissue was measured.

Table 2.

Patients' age, sex, tissue types, AST and LDH values (IU/L) and AST/LDH ratios in the tissue suspension

parathyroid

age sex	**62 F**	**34 F**	**40 F**	**13 M**	**42 F**	**42 F**	**42 F**	**67 F**	**33 F**	**47 F**	**31 F**	**24 M**	**56 F**	**14 F**	**34 M**	**25 F**
Surgical Indication	FTC	PTC	PTC	CCH	PTC	PTC	PTC	PTC	PTC	PTC	PTC	FA	PTC	PTC	PTC	FTC
AST (IU/L)	31	16	6	13	52	56	26	3	9	15	13	3	3	19	22	28
LDH (IU/L)	46	< 5†	20	25	129	157	94	5	10	43	46	< 5†	7	31	68	38
AST/LDH	**0.67**	**3.20**	**0.30**	**0.52**	**0.40**	**0.36**	**0.28**	**0.60**	**0.90**	**0.35**	**0.28**	**0.60**	**0.43**	**0.61**	**0.32**	**0.74**

parathyroid

age sex	**67 F**	**60 M**	**60 M**	**78 F**	**70 F**	**70 F**	**75 F**	**43 F**	**78 M**	**37 F**	**53 M**	**53 M**	**66 F**	**72 F**	**83 F**	**50 M**
Surgical Indication	PTC	FA	FA	PTC	PTC	PTC	PTC	PTC	FA	PTC	PTC	PTC	PTC	PTC	PTC	PTC
AST (IU/L)	42	59	35	15	72	49	54	23	21	19	25	23	30	4	32	32
LDH (IU/L)	57	133	78	35	159	134	142	70	46	48	68	66	92	15	< 5†	99
AST/LDH	**0.74**	**0.44**	**0.45**	**0.43**	**0.45**	**0.37**	**0.38**	**0.33**	**0.46**	**0.40**	**0.37**	**0.35**	**0.33**	**0.27**	**6.40**	**0.32**

parathyroid

age sex	**38 F**	**14 M**	**65 F**	**65 F**	**77 F**	**77 F**	**47 F**	**47 F**	**47 F**	**47 F**	**56 F**
Surgical Indication	PTC	MTC	PTC	PTC	PTC	PTC	FA	FA	FA	FA	PTC
AST (IU/L)	41	12	15	18	59	78	24	29	34	8	17
LDH (IU/L)	70	11	42	38	216	153	52	79	73	25	47
AST/LDH	**0.59**	**1.09**	**0.36**	**0.47**	**0.27**	**0.51**	**0.46**	**0.37**	**0.47**	**0.32**	**0.36**

thyroid cancer

age sex	**58 F**	**74 M***	**74 F***	**42 F**	**74 F**	**67 F***	**77 F***	**74 M**	**56 F**	**67 F**	**85 F***	**60 F***	**75 F**	**68 F***	**38 F***	**38 F***
source	**L (FTC)**	**L**	**M**	**M**	**M**	**M**	**L**	**M**	**M**	**M**	**M**	**M**	**P**	**M**	**M**	**M**
AST (IU/L)	30	1	1	40	56	< 1‡	2	8	34	95	14	29	87	23	9	13
LDH (IU/L)	254	19	23	293	774	10	21	174	268	1806	209	1593	583	204	234	378
AST/LDH	**0.12**	**0.05**	**0.04**	**0.14**	**0.07**	**0.10**	**0.10**	**0.05**	**0.13**	**0.05**	**0.07**	**0.02**	**0.15**	**0.11**	**0.04**	**0.03**

thyroid cancer

age sex	**56 F***	**86 F***	**60 M***
source	**M (FTC)**	**M**	**M**
AST (IU/L)	5	11	2
LDH (IU/L)	46	142	88
AST/LDH	**0.11**	**0.08**	**0.02**

normal lymph node

age sex	**42 F**	**14 F***	**14 F**	**14 F**	**14 F**	**70 F**	**75 F**	**43 F**	**43 F**	**73 F***	**14 M**	**77 F**	**47 F**
Surgical Indication	PTC	PTC	PTC	PTC	PTC	PTC	PTC	PTC	PTC	PTC	MTC	PTC	FA
AST (IU/L)	15	1	4	4	5	19	111	15	7	5	21	9	20
LDH (IU/L)	239	6	52	69	38	266	1836	218	103	135	236	126	274
AST/LDH	**0.06**	**0.17**	**0.08**	**0.06**	**0.13**	**0.07**	**0.06**	**0.07**	**0.07**	**0.04**	**0.09**	**0.07**	**0.07**

adipose tissue

age sex	**74 F**	**48 F**	**63 F**	**74 M**	**56 F**	**67F**	**78 F**	**53 F**	**50 M**	**38 F**
Surgical Indication	PTC	FA	ParaA[#]	PTC	PTC	PTC	FA	PTC	PTC	PTC
AST (IU/L)	6	4	3	3	9	48	7	7	2	17
LDH (IU/L)	140	32	39	58	75	231	95	49	27	101
AST/LDH	**0.04**	**0.13**	**0.08**	**0.05**	**0.12**	**0.21**	**0.07**	**0.14**	**0.07**	**0.17**

normal thyroid

age sex	**40 F**	**42 F**	**56 F**	**47 F**	**78 F**	**47 F**
Surgical Indication	ParaC[**]	PTC	PTC	FA	PTC	FA
AST (IU/L)	33	16	31	23	81	23
LDH (IU/L)	321	125	261	211	359	178
AST/LDH	**0.10**	**0.13**	**0.12**	**0.11**	**0.23**	**0.13**

miscellaneous

	50 F*[§]	**50 M[‖]**	**14 M[¶]**
	PTC	PTC	MTC
	10	1	36
	242	17	546
	0.04	**0.06**	**0.07**

All specimens of thyroid cancer were obtained from papillary thyroid cancers except for those indicated as follicular thyroid cancer (FTC).

CCH: C-cell hyperplasia; FA: follicular adenoma or hyperplasia; L: local recurrence; M: metastatic lymph node; MTC: medullary thyroid cancer; P: primary tumor; ParaA: parathyroid adenoma; ParaC: parathyroid cancer; PTC: papillary thyroid cancer.

* Specimen was obtained by preoperative fine needle aspiration biopsy.

† As concentration was below measurement limit (< 5 IU/L), 5 was used to calculate AST/LDH ratio.

‡ As concentration was below measurement limit (< 1 IU/L), 1 was used to calculate AST/LDH ratio.

§ Median cervical cyst

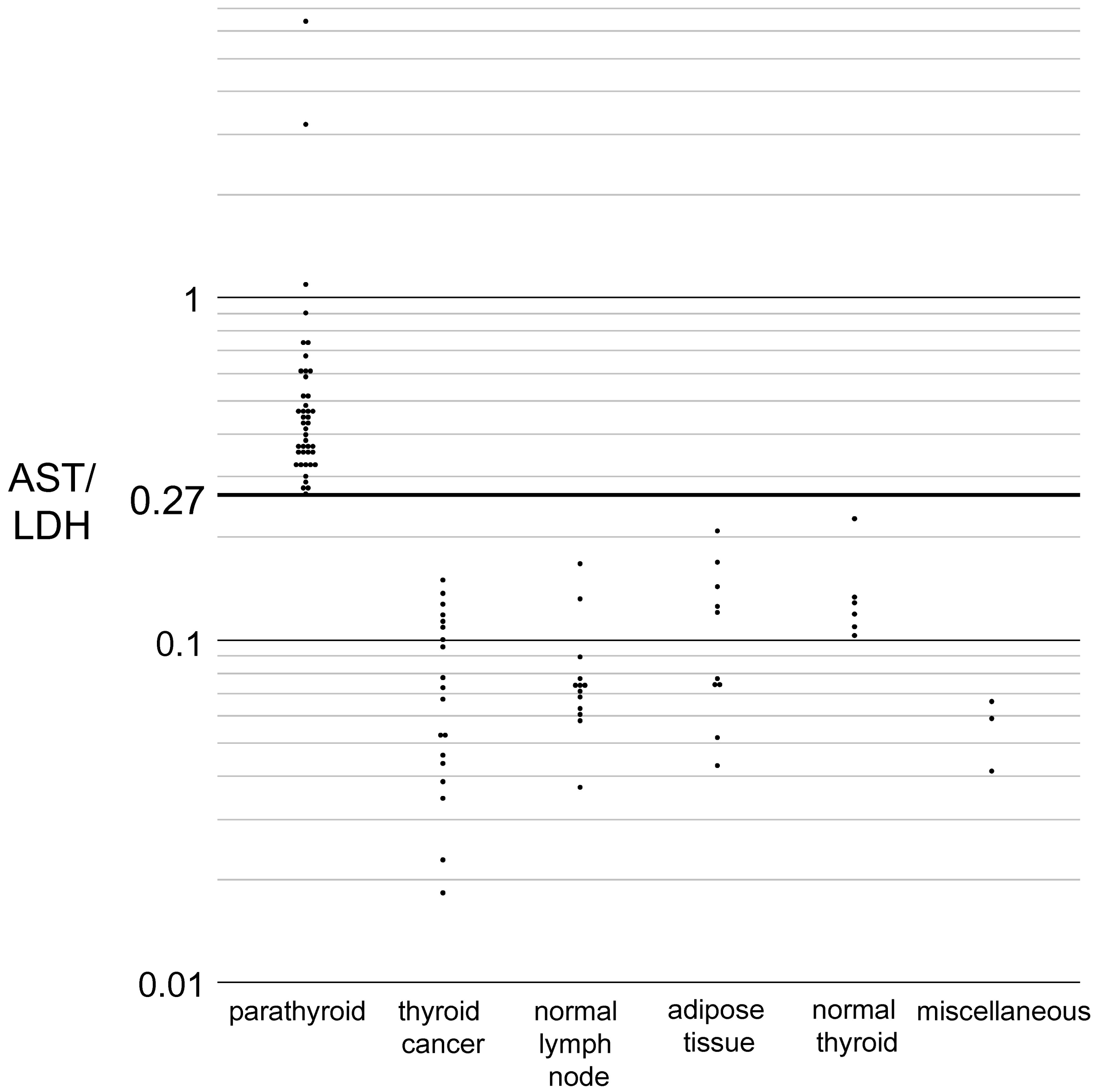
‖ Connective tissue

¶ Thymus

The AST/LDH ratio of this parathyroid adenoma was 0.63.

** The AST/LDH ratio of this parathyroid carcinoma was 0.21.

Figure 1



	parathyroid	thyroid cancer	normal lymph node	adipose tissue	normal thyroid	miscellaneous
median	0.43	0.07	0.07	0.10	0.12	0.06
SD	1.19	0.04	0.03	0.05	0.04	0.01