Innovative Techniques in Surgery Around the World

Development of a rapid intraoperative point-of-care method using tissue suspension to differentiate parathyroid tissue: a possible substitute for frozen sections

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A short running head: Point-of-care testing to differentiate parathyroid

Key words: point-of-care testing, parathyroid, identification

Conflicts of interest

The point-of-care testing device (NX500) was loaned free of charge by Fujifilm

Corporation. The authors declare no conflicts of interest. We have received no funding for this study.

Informed consent was obtained from all individual participants included in the study.

This study was approved by the ethical review board of our institution.

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Abstract

Background: We reported that aspartate aminotransferase (AST)/lactate dehydrogenase (LDH) ratio of a tissue suspension can precisely differentiate normal and hyperfunctioning parathyroid tissue (PT) from other tissues. However, in these studies, LDH and AST were measured using the standard method for blood samples, with a turnaround time of approximately 1 hour, hampering clinical application. Here, we developed a rapid and robust method to differentiate PT instead of using frozen sections.

Methods: Excised specimens from 28 patients (n = 69) who underwent thyroid or parathyroid surgery between October 2019 and April 2020 were analyzed. AST and LDH were measured in suspensions of PT or other tissues, using both the standard method in the in-facility laboratory and a point-of-care testing (POCT) device (NX500, Fujifilm, Japan).

Results and Conclusions: A good correlation was found between the standard method and NX500 for AST and LDH levels >10 IU/L. In the analyses using 52 specimens with ≥10 IU/L of both AST and LDH measured using the NX500, PT was distinguished with 100% sensitivity and specificity using an optimal cut-off AST/LDH ratio of 0.48. The turnaround time was estimated to be less than 10 minutes. This method could be a cost-

and labor-effective alternative to frozen sections to reduce the incidence of postoperative hypoparathyroidism and improve the outcome of primary hyperparathyroidism in low-resource areas.

Background

Permanent hypoparathyroidism is a severe complication of thyroid surgery. Preservation *in situ* and autotransplantation of parathyroid tissue (PT) are standard procedures to avoid permanent hypoparathyroidism. Regarding the treatment of primary hyperparathyroidism (PHP), a focused approach has become predominant for surgical treatment strategies when preoperative localization is definitive by multiple imaging diagnoses and confirmation of the excision of the pathogenic gland(s) using intraoperative parathyroid hormone (IOPTH) measurement is recommended. Frozen section (FS) is necessary for secure autotransplantation of the PT and confirmation of the removal of the pathogenic glands during surgery for thyroid and PHP, respectively. However, FS is not always feasible in areas with a lack of pathologists[1]. Furthermore, IOPTH measurement is a financial burden and may not be feasible worldwide[2]. We have previously shown that the aspartate aminotransferase (AST)/lactate dehydrogenase (LDH) ratio of a tissue suspension can precisely differentiate normal[3] and hyperfunctioning PT[4] from other tissues. In these studies, the suspension was handled similar to routine clinical blood samples and measured using standard methods in the central clinical laboratory of our hospital. Therefore, the results took approximately 1 hour to obtain, which was substantially longer than the time required for FS. To introduce this new method in actual clinical practice, the turnaround time should be reduced such that it is similar to that using FS. Several point-of-care testing (POCT) devices (e.g., NX500, Fujifilm, Japan; Piccolo Xpress, Abaxis, USA, etc.) can analyze these enzymes from blood sample in approximately 10 minutes and be placed in operation theaters. Therefore, we conducted a prospective pilot study using a POCT device to evaluate whether the accuracy of this new method is equivalent to that of FS and estimate the actual turnaround time of this method.

Material and Methods

In total, 20 samples of PT and 20 samples of other tissues were considered sufficient to show a statistically significant difference ($P < 0.1 \times 10^{-6}$) using a POCT device based on the difference in the AST/LDH ratio between them in the previous study[3]. In a previous study, several readings from the samples of PT and other tissues (~25%) were lower than the dynamic range of AST and/or LDH of the NX500 (data not shown). Therefore, in anticipation of unevaluable data, we prospectively analyzed 28 patients who underwent thyroid or parathyroid surgery between October 2019 and April 2020 at our institution (Table 1).

Trace amounts of remnant tissue after autotransplantation of PT (presumably equal to 1 mm^3) or approximately 1 mm^3 of excised hyperfunctioning parathyroid minced using scissors was suspended in normal saline and divided (0.5 and 1 mL each for the NX500 and central clinical laboratory, respectively). Divided samples were measured for AST and LDH using the NX500 according to the manufacturer's instructions (refer to the supplemental video clip.) and using the standard method in the central clinical laboratory of our hospital[3] in parallel. Approximately 1 mm^3 of apparently distinct tissue (e.g., thyroid gland, adipose tissue, etc.) obtained in the same surgery was minced using scissors and measured for comparison. Statistical analysis was performed using JMP 15.1.0 (SAS Institute, Tokyo, Japan).

Results

The value measured using the NX500 had a statistically significant correlation with the measured values using the standard methods for AST (Fig. 1a) and LDH (Fig. 1b), except for AST < 10 IU/L (Fig. 1a inset) and LDH (Fig. 1b inset). Although the dynamic range for LDH of the NX500 is 50–900 IU/L according to the manufacturer's instructions, samples ≥ 10 IU/L of LDH were included in this study considering the good correlation mentioned above. Therefore, further analyses were performed using 52 specimens (≥ 10 IU/L for both AST and LDH with the NX500) of the 69 samples (Table 1). The PT was distinguished with 100% sensitivity and specificity using an optimal cutoff of 0.48, as identified in the receiver operator characteristic curve analysis. (Fig. 2a) The cutoff value for differentiating PT using the standard method in this study (0.24) was almost the same as that of the previous study (0.27) (Fig. 2b)[3].

Discussion

This study demonstrated the feasibility of quantifying AST and LDH in tissue suspensions using a POCT device. PT was successfully differentiated from other tissues based on the AST/LDH ratio using a POCT device as well as using the standard method.

In this study, the POCT device (i.e., NX500) was placed in the laboratory in our department; therefore, the actual turnaround time could not be evaluated in real clinical settings. The overall turnaround time was estimated to be less than 10 minutes (refer to the supplemental video clip). The required time is shorter than the recommended turnaround time of 20 min for FS by The College of American Pathologists[5].

This study has some limitations. Although our results showed that the AST/LDH ratio obtained using a POCT device predicted PT with high accuracy, this finding should be interpreted cautiously because the method was tested only at a single institution. Further large-scale studies in real clinical settings are warranted to confirm the validity of this method.

Another issue with this method is the cost and operation of the POCT device. Market prices for the NX500 vary from country to country, but according to information from the manufacturer's representative, the price of the NX500 in developing countries is less than $ 20,000. This device provides a fully automated procedure for analyzing multiple test parameters in clinical chemistry and does not require a specific license or training. In conclusion, this study suggests that PT can be accurately differentiated based on the AST/LDH ratio measured using a POCT device and that this method could be a cost- and labor-effective alternative to FS in low-resource areas.

Conflicts of interest

The point-of-care testing device (NX500) was loaned free of charge by Fujifilm Corporation. The authors declare no conflict of interest.

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Figure legends

Figure 1

a: Scatter plot showing the correlation of measurement values of AST (≥ 10 IU/L with the NX500) between values measured using the standard method and the NX500. Data are displayed as IU/L. Inset: Scatter plot showing the correlation of measurement values of AST (< 10 IU/L with the NX500) between values measured using the standard method and the NX500.

b: Scatter plot showing the correlation of measurement values of LDH (≥ 10 IU/L with the NX500) between values measured using the standard method and the NX500. The data are displayed as IU/L. Inset: Scatter plot showing the correlation of measurement values of LDH (< 10 IU/L with the NX500) between values measured using the standard method and the NX500

The approximate lines are overlaid. r: the correlation coefficient. ns: not significant.

Figure 2

The AST/LDH ratios of suspensions of parathyroid tissue and other tissues measured using the NX500 (**a**) and using the standard method (**b**) shown as a box-and-whisker plot.

The upper and lower quartiles are indicated with a box. The bold line in the box represents the median. The upper and lower whiskers indicate the maximum and minimum, respectively. The dots indicate outliers. The bold horizontal dashed lines indicate the optimal threshold values for differentiating parathyroid tissue from other tissues (0.48 for NX500 and 0.24 for the standard method, respectively). Other tissues: cancerous tissue, normal thyroid, lymph node, adipose tissue, connective tissue

Fig. 1

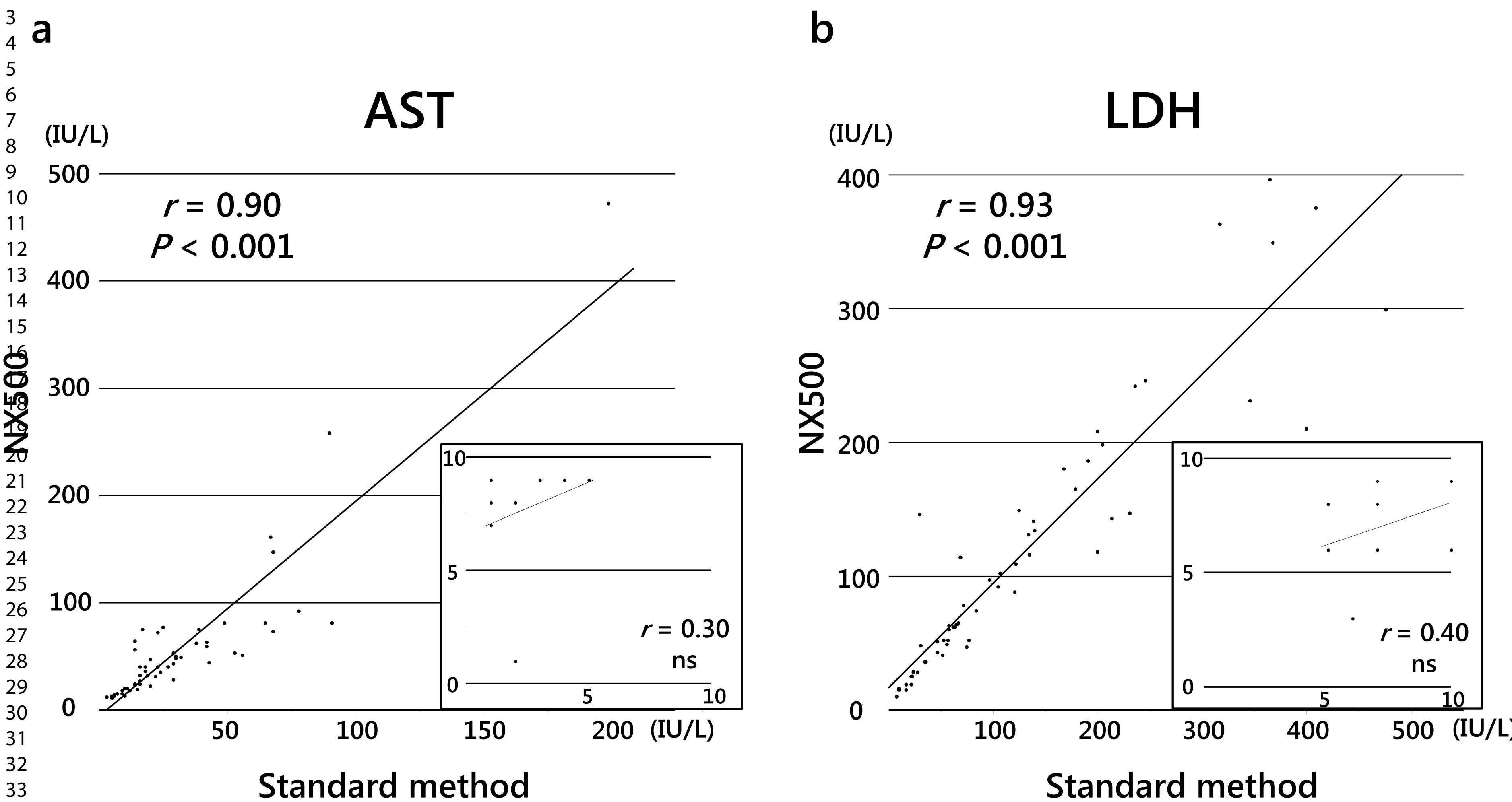
a
AST
(IU/L)
r = 0.90
P < 0.001
NX500
Standard method
(IU/L)
r = 0.30
ns
b
LDH
(IU/L)
r = 0.93
P < 0.001
NX500
Standard method
(IU/L)
r = 0.40
ns

Fig. 2

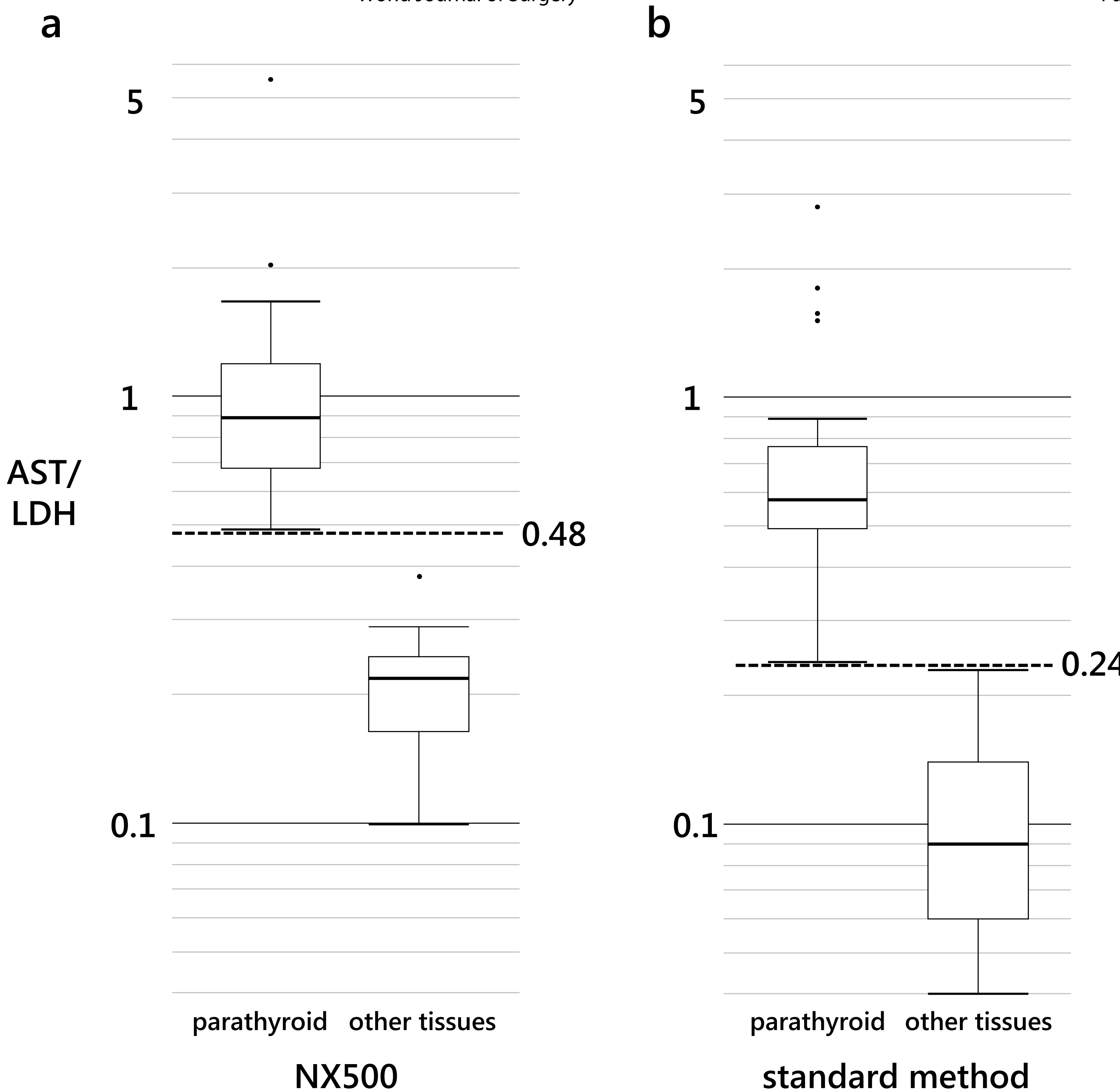
a
b
5
1
0.1
AST/
LDH
0.48
0.24
parathyroid
other tissues
parathyroid
other tissues
NX500
standard method

Table 1

Table 1

Patients' characteristics, surgical indications, and specimen information.

Sex (M : F)	5:23
Age (mean, (range))	54y, (17-83)
Surgical indication	
papillary carcinoma	19
primary hyperparathyroidism	5
follicular adenoma	3
medullary carcinoma	1
Total	28
Analyzed specimens	
parathyroid	34 (28)
adipose tissue	17 (9)
normal thyroid	10 (8)
lymph node	5 (4)
carcinoma	2 (2)
connective tissue	1 (1)
Total	69 (52)

The numbers in parentheses indicate the number of evaluable samples（≥ 10 IU/L for both AST and LDH with NX500）.