Clinical Research Article

Measurement of the AST to LD ratio in parathyroid tissue suspension can precisely differentiate a hyperfunctioning parathyroid

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The authors declare no conflicts of interest.

Abstract

Background: Frozen section of excised tissue is used to confirm removal of the etiology of primary hyperparathyroidism in the current era of intraoperative parathyroid hormone measurement and provides safeguards for surgeons. We recently reported that the aspartate aminotransferase (AST)/lactate dehydrogenase (LD) ratio in tissue suspension can accurately distinguish normal parathyroid tissue from other tissues. Therefore, we hypothesized that this ratio may also be applied to distinguish hyperfunctioning parathyroid tissue (HPT) from other tissues.

Methods: We prospectively analyzed 22 patients who underwent parathyroidectomy for primary hyperparathyroidism (benign, 21; malignant, 1) from July 2018 to October 2019. In total, 27 specimens were examined. Approximately 1 mm^3 of minced HPT as confirmed by frozen sections was suspended in 1 mL of normal saline and AST and LD levels were measured. The AST/LD ratios of other tissues (normal parathyroid tissue, thyroid gland, adipose tissue, and others; n=94) were obtained from our previous report.

Results: The AST/LD ratio of benign HPT was consistently higher than that of other tissues ($P < 0.001$). The optimal cut-off value was 0.36 according to the receiver operating characteristic curve, with 100% sensitivity and specificity. The AST/LD ratio in malignant HPT was also markedly lower than that in benign HPT.

Conclusion: This method might be a new adjunct for intraoperative differentiation of HPT

with an accuracy and turnaround time comparable with those of frozen sections, minimal cost, and no need for dedicated pathological staff. Additionally, this method might increase the treatment success rate in settings with limited medical resources.

Introduction

The success rate of treatment of primary hyperparathyroidism has improved over the last three decades. This improvement has largely resulted from development of the intraoperative parathyroid hormone (IOPTH) assay (1) and improvements in preoperative localization modalities (e.g., ^{99m}technetium sestamibi [^{99m}Tc MIBI] scintigraphy and ultrasound) (2). A focused approach has become the mainstay of the surgical strategy when preoperative localization is concordant among multiple imaging modalities and confirmation of removal of the etiology with IOPTH measurement is recommended (3). However, the sensitivity and specificity of IOPTH measurements for this purpose range from 80%–90% (4) (5). These values suggest that IOPTH measurement occasionally produces false results leading to unnecessary exploration, which may prolong the operation time and cause postoperative complications. Furthermore, IOPTH measurement has the burden of cost and may not be feasible worldwide (6).

In patients with multinodular and/or huge goiter or with scar tissue because of reoperation, even experienced surgeons who perform high-volume thyroid and parathyroid surgeries occasionally have difficulty distinguishing parathyroid tissue (PT) from lymph nodes, fat, or thyroid nodules. Intraoperative pathological confirmation that the removed tissue is PT (i.e., frozen section) is still necessary to confirm removal of the etiology in such cases, as well as in routine surgeries, and provides safeguards for surgeons (6). However, frozen section is not

always feasible in developing countries where there is a shortage of pathologists (7).

PT is rich in mitochondria (8), which harbor Krebs cycle enzymes, such as aspartate aminotransferase (AST). In contrast, lactate dehydrogenase (LD) is ubiquitously expressed, and is thus used as a marker of common injuries and organ damage. These enzyme levels are routinely measured in clinical examinations at low cost. We recently reported a retrospective study, which showed that the AST/LD ratio could be used to accurately distinguish normal PT from other tissues (9).

Therefore, we hypothesized that this ratio can also be applied to distinguish hyperfunctioning PT (HPT) from other tissues. In this study, we undertook a prospective approach to determine whether our previous result could be extrapolated to HPT. If this ratio can reliably distinguish HPT from other tissues, its measurement might be an alternative to frozen sections.

Materials and Methods

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

On the basis of the difference in the AST/LD ratio between PT and other tissues in our previous report (9), we determined that 20 samples would be sufficient to demonstrate a statistically significant difference between HPT and other tissues ($P = 3.9 \times 10^{-9}$). Therefore, we prospectively analyzed 22 patients who underwent parathyroidectomy for primary hyperparathyroidism from July 2018 to October 2019 in our institution. In total, 27 specimens were examined. The patients' characteristics, surgical indications, and specimen-related data are shown in Table 1. One case of parathyroid carcinoma was preoperatively diagnosed on the basis of the appearance of local recurrence. Preoperative localization was performed with ^{99m}Tc MIBI scintigraphy and ultrasonography and was concordant in all cases. For patients with multiple endocrine neoplasia type 1 (MEN1), we adopted total parathyroidectomy followed by autotransplantation to the muscle of non-dominant forearm. Macroscopically, non-hypertrophic parathyroid glands were selected for autotransplantation. Therefore, only one hyperfunctioning parathyroid gland was suitable for use in evaluating our new method in four of six patients with (MEN1). In the remaining two patients, multiple specimens per case were submitted for analysis. One patient underwent subtotal parathyroidectomy because of multiple liver metastases of a pancreatic neuroendocrine tumor and the other patient had multiple enlarged parathyroid glands.

Therefore, a sufficient amount of PT was available for analysis after autotransplantation. Cases of secondary hyperparathyroidism (SHP) were not included in this study. Preoperative renal function was within the normal range in all cases. AST and LD values in other tissues (e.g., normal PT, thyroid gland, and adipose tissue) were collected from our previous report (n = 94) (9).

Adenomatous or hyperplastic PT was intraoperatively confirmed by frozen sections of a small portion (approximately 1–2 mm^3) of the excised tissue. Approximately 1 mm^3 of excised HPT as confirmed by frozen sections was minced with fine scissors onto culture dishes and suspended in 1–1.5 mL of normal saline. This suspension was sent to the biochemical laboratory in our hospital (refer to supplemental video (10)). AST and LD levels were measured with a standard automatic analyzer (Labospect 008; Hitachi High-Technologies Corporation, Tokyo, Japan) using commercially available reagents for automatic analyzers (Quick Auto Neo AST JS-HLS for AST and Quick Auto Neo LDH JS-HLS for LD; Shino-Test Corporation, Tokyo, Japan) in the same manner as that for a normal clinical blood sample. Intact parathyroid hormone (PTH) concentrations in the tissue suspension were measured with a Cobas 8000 (Roche Diagnostics K.K., Tokyo, Japan) using an ECLusys PTH kit (Roche Diagnostics K.K.) in the same manner as that for a normal clinical blood samples. Specimens for the IOPTH assay were handled in the same manner as routine clinical blood samples in our hospital. Therefore, the turnaround time was usually

122 approximately 1 hour. Because the IOPTH assay is not performed in our hospital if
123 preoperative localization is concordant and reliable, IOPTH assays were not performed in this
124 study. The AST/LD ratio was calculated by using raw measurement values (IU/L). Statistical
125 analysis was performed with JMP 14.2.0 (SAS Institute, Tokyo, Japan). $P < 0.05$ was
126 considered significant.
127

Results

AST and LD in HPT levels were measurable in all specimens (Table 2). Notably, the AST/LD ratio of benign HPT was consistently and significantly higher than that of other tissue types (benign HPT vs. other tissues, $P < 0.001$, Mann–Whitney test) (Figure 1). The ratio of malignant HPT (i.e., parathyroid carcinoma) was markedly lower than that of benign HPT (Figure 1). Receiver operating characteristic curve analysis indicated that 0.36 was the optimal cut-off ratio for differentiating benign HPT from other tissues (Figure 1). This cut-off predicted benign HPT with 100% sensitivity and specificity. The median of this ratio in benign HPT (0.80) was significantly higher than that in normal PT (0.43; $P < 0.001$, Mann–Whitney test) (9).

There was no significant correlation between the AST/LD ratio and intact PTH concentrations in the tissue suspension of HPT (Figure 2). Intact PTH concentrations in the suspension of malignant HPT were comparable with those of benign HPT (30,850 vs. 55,224 ± 98,461 pg/mL, respectively) (Figure 1 and Table 2). Among benign HPT samples, there was no significant difference in the AST/LD ratio between patients with adenoma and those with hyperplasia (data not shown). Postoperative normalization of hypercalcemia was achieved in all patients.

Discussion

This study proposes a new method of expeditious and robust confirmation of HPT using routine clinical biochemical analysis. Measurement of AST and LD levels in supernatant of a suspension of a trace amount of suspected HPT could become a rapid diagnostic tool that could be used as an alternative to frozen section.

The findings that the cut-off ratio of AST/LD for differentiating benign HPT (0.36) from other tissues was higher than that for normal PT (0.27) (9) and that benign HPT had a larger margin to other tissues suggest that this ratio is more reliable for benign HPT than for normal PT. These results could be attributable to the presence of adipocytes in normal PT, which are expected to lower this ratio (11).

Because only one patient had malignant HPT in our study, no definitive conclusions could be drawn. However, the finding that the AST/LD ratio of malignant HPT was markedly lower than that of benign HPT is noteworthy. Intact PTH concentration in the tissue suspension of malignant HPT was comparable with those of benign HPT. Therefore, a standard biochemical method, such as a PTH assay using washout fluids from fine-needle aspiration (12), would have led to erroneous results in this case.

In the present study, suspended minced tissue was handled similarly to routine clinical blood samples. Therefore, obtaining the results took 30–40 minutes. However, some analyzers (e.g., NX500 system; Fujifilm, Tokyo, Japan) can analyze these enzymes in a blood sample

(equivalent to tissue suspension) in less than 10 minutes, according to the manufacturer's information. Therefore, the turnaround time for this method could be equivalent to that of frozen section and the IOPTH assay, but it has a cost of less than $3 in Japan. However, to apply this method to actual clinical practice, discussing specimen handling in the laboratory with clinical laboratory technicians is necessary.

This study has some limitations. Although our results showed that the AST/LD ratio predicted HPT with perfect accuracy, this finding must be interpreted with caution because the method was only validated in a relatively small number of cases at a single institution. However, the finding that the AST/LD ratio was consistently higher in HPT and normal PT (9) than in other tissues indicates that this method is universally reproducible. Furthermore, because measurement of AST and LD levels is a relatively standardized technique internationally and because use of the AST/LD ratio eliminates the influence of dilution of each specimen, this method could be applied in any clinical setting. However, the content of oxyphilic cells in thyroid tumor tissue and HPT may affect the decision threshold. Therefore, the threshold of the optimal AST/LD ratio to distinguish HPT may require adjustment in each institution. SHP was not included in this study. Hyperfunctioning parathyroid glands in SHP are usually multinodular with heterogeneity. This feature may affect the results of our method. We speculate that validation of this method is not compatible with randomized, controlled trials. Further studies with large numbers of cases including SHP are warranted to validate this

method. Another limitation is that this method is only applicable for excised HPT because it requires a small piece of excised tissue. However, the AST/LD ratio could be determined using washout fluids from fine-needle aspiration instead of PTH measurement (13). This method is a cost- and labor-effective solution for reducing the incidence of treatment failure in patients with primary hyperparathyroidism. Our method can be used in low-resource countries with a shortage of pathologists and in developed countries by providing an alternative to frozen sections at minimal cost.

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240 ***Figure legends***

241 **Figure 1**

242 Box plot showing logarithmically transformed AST/LD ratios in suspensions of various
243 tissues. The box plot shows upper and lower quartiles. The median is represented by a bold
244 line within the boxes. The whiskers extend from each quartile to the minimum or maximum,
245 and outliers are indicated by dots. The median for each of the tissue types is indicated at the
246 bottom of the figure. Thyroid cancer: local recurrence, metastatic lymph nodes, and primary
247 tumor; miscellaneous tissues: median cervical cyst, connective tissue, and thymus. The bold
248 horizontal broken line indicates the optimal threshold value for distinguishing HPT from
249 other tissues (0.36).

250 **Figure 2**

251 Scatter plot showing the correlation between the AST/LD ratio and intact PTH in suspensions
252 of HPT. The data were logarithmically transformed. The closed square indicates a malignant
253 case. The closed circle in parentheses indicates a case that was off the scale. The bold vertical
254 line indicates the optimal threshold value for distinguishing HPT from other tissues (0.36).
255 The correlation coefficient (r) is indicated in the graph. ns: not significant.

Table 1

Table 1
Patients' characteristics, surgical indications, and specimen information.

Sex (M : F)		6:16
Age (mean, (range))		57y, (32-83)
Surgical indication		
Primary hyperparathyroidism due to single adenoma		14
Primary hyperparathyroidism due to double adenomas		1
Multiple endocrine neoplasia type 1		6
Parathyroid carcinoma		1
Total		22
Analyzed specimens of hyperfunctioning parathyroid tissue		
Adenoma		16
Hyperplasia		10
Carcinoma		1
Total		27
Previously reported specimens (for comparison)		
Normal parathyroid gland		43
Thyroid cancer		19
Normal lymph node		13
Normal thyroid		6
Adipose tissue		10
Miscellaneous		
	Median cervical cyst	1
	Connective tissue	1
	Thymus	1
Total		94

Table 2

Hyperfuctioning parathyroid tissue (hyperplasia)

age sex	**37 F**	**66 F**	**43 F**	**44 M**	**54 F***			**32 M****		
Surgical Indication	MEN1	MEN1	MEN1	MEN1	MEN1			MEN1		
Surgical procedure	total	total	total	total	total			subtotal		
AST (IU/L)	99	360	138	81	566	168	559	236	107	107
LD (IU/L)	238	342	239	155	511	251	675	250	168	97
intact PTH (pg/mL)	15,395	320,625	152,125	33,975	49,550	130,300	53,460	120,975	43,725	19,090
AST/LD	**0.42**	**1.05**	**0.58**	**0.52**	**1.11**	**0.67**	**0.83**	**0.94**	**0.64**	**1.1**

Hyperfuctioning parathyroid tissue (adenoma)

age sex	**63 F**	**63 M**	**53 M**	**67 F**	**40 M**	**39 F**	**50 F**	**66 F**	**73 F**	**50 F**	
Surgical Indication	SA	SA	SA	SA	SA	SA	SA	SA	SA	DA	
AST (IU/L)	114	704	95	71	223	42	243	25	198	215	425
LD (IU/L)	180	1,982	78	5	375	108	221	48	299	273	366
intact PTH (pg/mL)	23,190	***	13,760	13,125	102,825	56,988	160,875	38,412	78,850	205,875	390,500
AST/LD	**0.63**	**0.36**	**1.22**	**14.2**	**0.59**	**0.39**	**1.1**	**0.52**	**0.66**	**0.79**	**1.16**

Hyperfuctioning parathyroid tissue (adenoma)

age sex	**75 F**	**60 M**	**72 F**	**75 F**	**83F**
Surgical Indication	SA	SA	SA	SA	SA
AST (IU/L)	69	215	139	41	21
LD (IU/L)	81	265	105	49	46
intact PTH (pg/mL)	60,875	14,975	157,500	61,013	25,700
AST/LD	**0.85**	**0.81**	**1.32**	**0.84**	**0.46**

Hyperfuctioning parathyroid tissue (carcinoma)

age sex	**40 F**
Surgical Indication	ParaCa
AST (IU/L)	126
LD (IU/L)	583
intact PTH (pg/mL)	30,850
AST/LD	**0.22**

Table 2

Table 2.

Patients' age, sex, surgical indications, surgical procedures, AST levels, LD levels, intact PTH values, and the AST/LD ratio.

Data are shown for each final pathological diagnosis.

MEN1: multiple endocrine neoplasia type 1; SA: single adenoma; DA: double adenoma; ParaCa: parathyroid cancer.

Total: total parathyroidectomy followed by autotransplantation; subtotal: subtotal parathyroidectomy.

*This patient had multiple enlarged parathyroid glands. Therefore, multiple specimens were available for analysis using a remnant of autotransplantation.

**Subtotal parathyroidectomy was performed because of multiple liver metastases of a pancreatic neuroendocrine tumor.

*** off-scale high.

figure 1

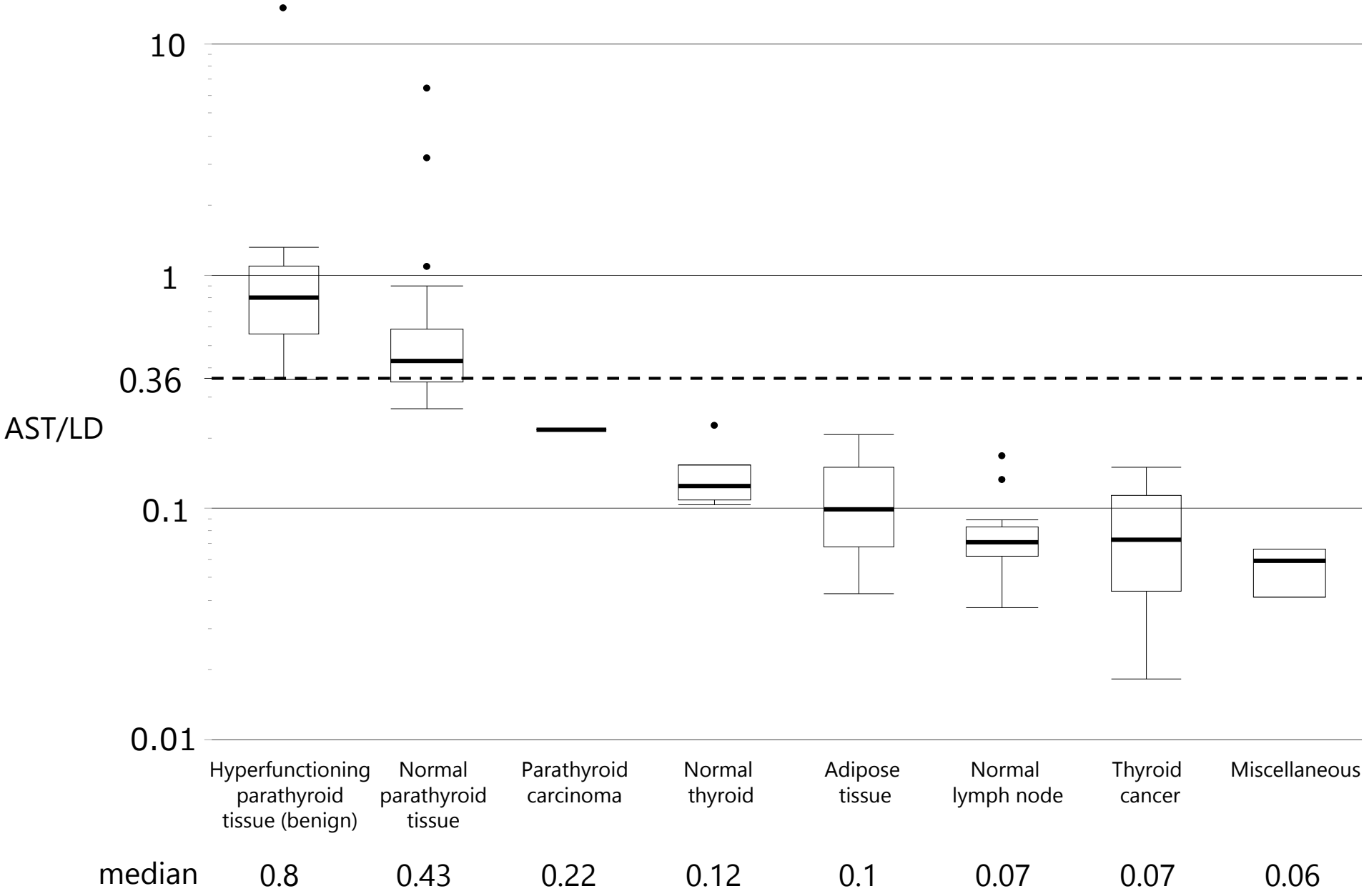

Figure 1

figure 2

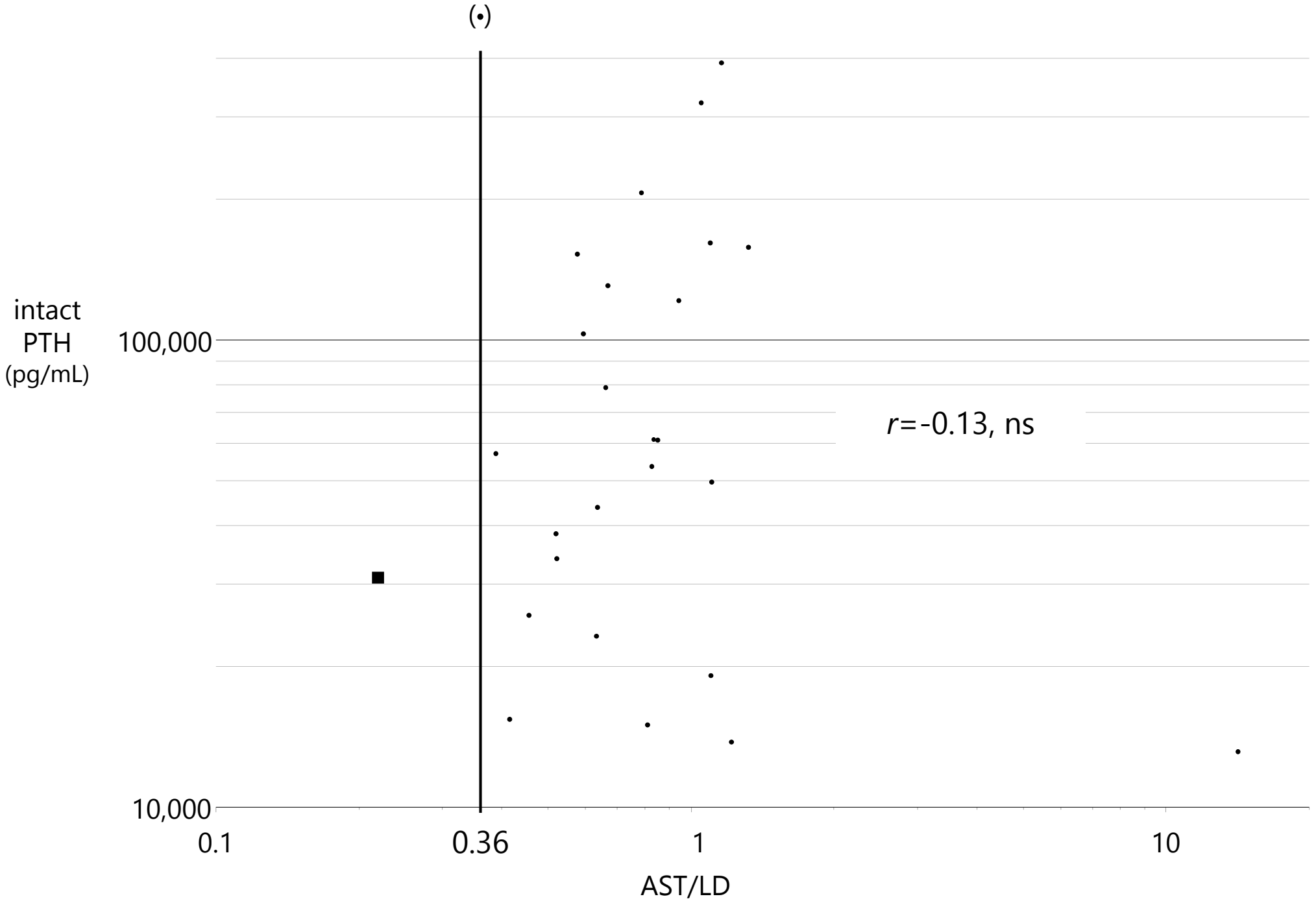

Figure 2