# Diagnostic Performance of [11C]Methionine Positron Emission Tomography in Newly Diagnosed and Untreated Glioma Based on the Revised World Health Organization 2016 Classification

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# Diagnostic performance of 11C-methionine PET in newly diagnosed and untreated glioma based on the revised WHO 2016 classification

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**Abstract**

BACKGROUND: The relationship between uptake of amino acid tracer with PET and glioma subtypes/gene status is still unclear.

OBJECTIVE: To assess the relationship between uptake of 11C-methionine using PET and pathology, *IDH* mutation, 1p/19q codeletion, and *TERT* promoter status in gliomas.

METHODS: The participants were 68 patients with newly diagnosed and untreated glioma who underwent surgical excision and preoperative 11C-methionine PET examination at Osaka City University Hospital between July 2011 and March 2018. Clinical and imaging studies were reviewed retrospectively based on the medical records at our institution.

RESULTS: The mean L/N ratio of diffuse astrocytomas were significantly lower than those of anaplastic astrocytomas ($p$=.00155), glioblastoma ($p$<.001) and oligodendrogliomas ($p$=.0157). The mean L/N ratio of *IDH* mutant gliomas was significantly lower than that of *IDH* wild-type gliomas (median 1.75 vs 2.61; $p$ = .00162). A mean L/N ratio of 2.05 provided the best sensitivity and specificity for distinguishing between *IDH* mutant and *IDH* wild-type gliomas, 69.2% and 76.2%, respectively. The mean L/N ratio of *TERT* promoter mutant gliomas was significantly higher than that of *TERT* promoter wild-type gliomas ($p$=.0147). Multiple regression analysis revealed that pathological diagnosis was the only influential factor on L/N ratio.

CONCLUSIONS: Distinguishing glioma subtypes based on the revised 2016 WHO classification of the central nervous system tumors on the basis of 11C-methionine PET alone appears to be difficult. However, 11C-methionine PET might be useful for predicting the *IDH* mutation status in newly diagnosed and untreated gliomas

noninvasively prior to tumor resection.

## Introduction

Gliomas are most the common tumors in Japan, accounting for 25.6% of primary brain tumors in the country.[1] In the United States, gliomas are the second most common form of primary brain tumors, with an annual incidence rate of approximately 6 cases per 100,000 people. Nearly 50% of primary malignant brain tumors are glioblastomas, and approximately 17% are other astrocytomas.[2] The revised 2016 World Health Organization (WHO) classification of tumors of the central nervous system requires molecular classification such as isocitrate dehydrogenase (*IDH)* mutation and 1p/19q codeletion for a diagnosis of glioma.[3]

Magnetic resonance imaging (MRI) is the gold standard for diagnosing glioma; however, it can be difficult to distinguish glioma from other non-neoplastic lesion such as radiation necrosis. 11C-methionine positron emission tomography (PET) has been widely used in glioma patients for detecting the tumor, deciding biopsy target location [4-6], predicting the grade [7-11] and prognosis [8, 10, 12-15], evaluating therapeutic response [10, 16], and distinguishing tumor recurrence from radiation necrosis.[17-19] However, the relationship between the uptake of amino acid tracer with PET and glioma subtypes/gene status is still unclear. The aim of this study was to evaluate the association between 11C-methionine uptakes and pathology/gene status in newly diagnosed and untreated gliomas.

## Methods

*Patients*

A total of 68 patients (42 males, 26 females; mean age, 51.8 years; age range, 7-82 years) with newly diagnosed and untreated gliomas underwent surgical resection at Osaka City University Hospital between July 2011 and March 2018. 11C-methionine PET was performed within one month prior to the tumor resection in glioblastoma patients (median, 12.7 days; interquartile range [IQR], 7-15.5 days) and within 6 months in lower grade glioma patients (median, 41.8days; IQR, 10-59.8 days). This study was approved by the Institutional Review Boards of the Graduate School of Medicine of Osaka City University (approval numbers: # 2047, and # 2020-115) and Osaka National Hospital (approval number: # 0713). Genetic analyses were performed with patients' written consent.

*11C-methionine PET*

PET was performed using an Eminence B PET scanner (Shimadzu); a spatial resolution was 4.5mm (full width at half maximum) slice thickness was 5.6mm or a Biograph-16 PET scanner (Siemens); a spatial resolution was 4.6mm (full width at half maximum) and slice thickness was 5.1mm. Scanning was performed parallel to the orbitomeatal line of the patients. During a period of fasting, 6 MBq/ kg of 11C-methionine was injected intravenously over 30 seconds. After obtaining a transmission scan, a 10-minute static scan was begun 20 minutes after injection. PET data were analyzed using the same region of interest (ROI) settings as previously reported.[17] Irregular ROIs were manually placed in the co-registered MRI for each lesion and the contralateral cerebral cortex. ROIs were transferred to the corresponding PET image to calculate the uptake of 11C-methionine. Activity counts were normalized relative to the injected dose per kilogram of patient body weight (standardized uptake value). The

mean and max standardized uptake values were calculated by semi-quantitative analysis of 11C-methionine uptake by each lesion. The mean and max lesion-to-normal contralateral brain tissue (L/N) ratios were determined by dividing the tumor standardized uptake value by the mean standardized uptake value of the normal contralateral region of the brain, as previously reported.[17, 20]

*Gene Analysis*

Genomic tumor DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA) or NucleoSpin Tissue (Machery-Nagel, Duren, Germany), and hot spots mutations of *IDH*1/2 (codon 132 of *IDH*1 and codon 172 of *IDH*2) and telomerase reverse transcriptase (*TERT)* promoter (termed C228 and C250) by Sanger sequencing with a 3130xLGenetic Analyzer (Thermo Fisher Scientific) and Big-Dye® Terminator V1.1 Cycle Sequencing Kit (Thermo Fisher Scientific), as previously reported.[21, 22] The copy number status of 1p19q was determined by multiplex ligation-dependent probe amplification (Oligodendroglioma 1p-19q probemix and EK1 reagent kit, MRC-Holland, Amsterdam, Netherlands).

*Statistical Analysis*

All data were evaluated using EZR software (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, it is a modified version of R commander designed to add statistical functions frequently used in biostatistics.[23] To compare the scores of the mean and max L/N ratios of each group, we performed statistical analysis using Kolmogorov-Smimov test, Kruskal-Wallis test and Mann-

Whitney U test. Receiver operating characteristic (ROC) curves were analyzed to determine the best cut-off value for differentiating gliomas with *IDH* mutant from those with *IDH* wild-type and those with *TERT* promoter mutant from those with *TERT* promoter wild-type. Multiple regression analysis was used to evaluate influential factor except for the patient with anaplastic oligodendroglioma (n=1) on L/N ratio. Statistical significance was defined at the level of $p<.05$.

**Results**

*Patient characteristics* (Table 1,2)

The 68 tumors were classified according to the revised 2016 WHO classification of tumors of the central nervous system. Eleven patients were classified into *IDH* mutant diffuse astrocytoma, 9 patients were classified *IDH* wild-type diffuse astrocytoma, 8 patients were *IDH* mutant and 1p/19q co-deleted oligodendroglioma, 2 patients were *IDH* mutant anaplastic astrocytoma, 9 patients were *IDH* wild-type anaplastic astrocytoma, 1 patient was *IDH* mutant and 1p/19q co-deleted anaplastic oligodendroglioma, 4 patients were *IDH* mutant glioblastoma, and 24 patients were *IDH* wild-type glioblastoma. Patients were further subdivided into four types based on *IDH* and *TERT* promoter mutation status (Table 1). Group A (*IDH* mutant/ *TERT* promoter mutant) comprised 9 patients, with pathological diagnoses of *IDH*-mutant and 1p/19q-codeleted oligodendroglioma (n=7), anaplastic oligodendroglioma (n=1), and glioblastoma (n=1). Group B (*IDH* mutant/ *TERT* promoter wild type) comprised 17 patients, with pathological diagnoses of diffuse astrocytoma (n=11), glioblastoma (n=3), anaplastic astrocytoma (n=2) and *IDH* mutant and 1p/19q co-deleted oligodendroglioma

(n=1). Group C (*IDH* wild-type/ *TERT* promoter mutant) comprised 23 patients, with pathological diagnoses of glioblastoma (n=10), diffuse astrocytoma (n=7), and anaplastic astrocytoma (n=6). Group D (*IDH* wild type/ *TERT* promoter wild-type) comprised 19 patients, with pathological diagnoses of glioblastoma (n=14), anaplastic astrocytoma (n=3), and diffuse astrocytoma (n=2). (Table2)

Table 3 lists the mean and the max L/N ratio of gliomas classified according to WHO grade, pathology, *IDH* status, and *TERT* promoter status.

*Correlation between L/N ratio and pathological diagnosis*

The medians of the mean L/N ratios of WHO grade II, III, and IV gliomas were 1.60 (IQR 1.00-2.06), 2.26 (1.80-4.20), and 3.02 (2.50-3.42), respectively ($p$<.001). Significant differences were found between grade II and III gliomas ($p$=.0042), and between grade II and IV gliomas ($p$<.001), but not between grade III and IV gliomas (Figure 1A). The mean L/N ratio of high-grade gliomas (median, 2.93; IQR, 2.28-3.49) was significantly higher than that of low-grade gliomas (median, 1.60; IQR, 1.00-2.06; $p$<.001). The mean L/N ratio of glioblastomas (median, 3.02; IQR, 2.50-3.42) was significantly higher than that of diffuse astrocytomas (median, 1.20; IQR, 0.75-1.86, $p$<.001) and oligodendrogliomas (median, 2.03; IQR, 1.82-2.35; $p$<.001). The mean L/N ratio of diffuse astrocytomas was significantly lower than that of anaplastic astrocytomas (median, 2.06; IQR, 1.67-3.32; $p$=.00155) and oligodendrogliomas ($p$=.0157) (Figure 1B).

*Correlation between L/N ratio and pathological diagnosis with IDH status*

Among grade II gliomas, oligodendrogliomas showed a significantly higher mean L/N ratio compared with *IDH* mutant diffuse astrocytomas (median,1.08; IQR, 0.63-1.65; $p<.001$). There was no statistically significant difference between oligodendrogliomas and *IDH* wild-type diffuse astrocytomas (median, 1.58; IQR, 1.02-2.14) (Figure 2A). Among grade III gliomas, there was no significant difference among anaplastic oligodendrogliomas, *IDH* mutant anaplastic astrocytomas, and *IDH* wild-type anaplastic astrocytomas (Figure 2B); there was also no significant difference between *IDH* mutant and *IDH* wild-type glioblastomas (Figure 2C).

*Correlation between L/N ratio and IDH/TERT promoter status*

The medians of the mean L/N ratios of groups A, B, C, and D were 2.08 (IQR, 1.89-2.67), 1.68 (0.96-1.80), 2.49 (1.80-3.21), and 2.92 (2.26-3.14), respectively ($p=.00223$) (Table 3). Statistically significant differences in the mean L/N ratio were found between groups A and B ($p=.0336$), B and C ($p=.009$), and B and D ($p=.000162$) (Figure 3A). The medians of the max L/N ratios of group A, B, C, and D were 3.27 (IQR, 2.55-3.79), 2.49 (1.60-3.08), 4.38 (2.73-5.20), and 4.56 (3.55-4.90), respectively. Statistically significant differences in the max L/N ratio were found between groups B and C ($p=.0295$), and B and D ($p=.00162$) (Figure 3B).

The mean L/N ratio of *IDH* mutant gliomas (n=26) was significantly lower than that of *IDH* wild-type gliomas (n=46) (median 1.75; IQR1.31-2.23 vs. median, 2.61; IQR, 2.05-3.19; $p=.00162$) (Figure 4A). The max L/N ratio of *IDH* mutant gliomas was also significantly lower than that of *IDH* wild-type gliomas (median, 2.56; IQR, 2.20-3.75 vs. median, 4.51; IQR, 2.99-5.12; $p=.00332$) (Figure 4B). The area under the ROC curves of the mean and the max L/N ratio were 0.725 and 0.711, respectively (Figure

4c, d). A mean L/N ratio of 2.05 provided the best sensitivity and specificity for distinguishing between *IDH* mutant and *IDH* wild-type gliomas, 69.2% and 76.2%, respectively (Figure 4C). A max L/N ratio of 3.92 provided the best sensitivity and specificity for distinguishing between *IDH* mutant and *IDH*-wild type gliomas, 76.9% and 64.3%, respectively (Figure 4D).

The mean L/N ratio of *TERT* promoter mutant gliomas (n=28) was significantly higher than that of *TERT* promoter wild-type gliomas (n=40) (median, 2.64; IQR, 2.04-3.09 vs. median, 1.92; IQR, 1.35-2.81; *p*=.0147) (Figure 5A). However, there was no significant difference between *TERT* promoter wild-type patients and *TERT* promoter mutant patients in terms of max L/N ratio (median, 4.11; IQR, 3.05-4.85 vs. median, 3.07; IQR, 2.18-4.62; *p*=.0554) (Figure 5B). The area under the ROC curve was 0.674 for the mean L/N ratio. A mean L/N ratio of 1.88 provided the best sensitivity and specificity for distinguishing between *TERT* promoter wild-type and *TERT* promoter mutant gliomas, 50.0% and 89.3%, respectively (Figure 5C).

*Multiple regression analysis for influential factor on L/N ratio*

Multiple regression analysis revealed that pathological diagnosis was the only influential factor on both mean L/N ratio (*p* value of F test, <.0001; Adjusted R-squared, 0.435) and max L/N ratio (*p* value of F test, <.0001; Adjusted R-squared, 0.445). *IDH* mutation status and contrast enhancement lesion in MRI might influence on both mean and max L/N ratio, although there were no statistically differences. On the other hand, age, sex, and *TERT* promoter mutation might little influence on L/N ratio (Table4).

**Discussion**

11C-methionine PET has recently recommended to use in the management of glioma.[24] 11C-methionine accumulates preferentially in tumor, but also accumulates in normal brain tissue.[25] Thus, we used the mean and max of the L/N ratio, which is the tumor standardized uptake value divided by the mean standardized uptake value of the normal contralateral region of the brain.

*Correlation between L/N ratio and pathological diagnosis with WHO grade*

In the present study, the L/N ratios of high-grade gliomas were significantly higher than those of low-grade gliomas, which is agreement with the findings of previously studies.[7, 9, 11, 15, 26-29] We also found a positive correlation between the WHO grade and the accumulation of 11C-methionine in PET among astrocytomas, although there was no statistically significant difference between anaplastic astrocytomas and glioblastomas. 11C-methionine PET has been widely used to evaluate glioma and several reports have investigated the relationship between the uptake of 11C-methionine using PET and the pathological diagnosis of glioma based on the morphology. Shinozaki et al. reported that L/N ratio increased significantly as tumor grade advanced in astrocytomas[11], whereas Hatakeyama et al. found that there were significant differences only between diffuse astrocytomas and anaplastic astrocytomas.[28] Moreover, Kato et al. also found significantly differences between glioblastomas and anaplastic astrocytomas/diffuse astrocytomas.[30] However, there is still controversy regarding the accumulation of amino tracer in PET in patients with oligodendroglioma. Shinozaki et al. and Takei et al. reported that among grade II gliomas, the mean L/N ratio in oligodendrogliomas was significantly higher than that in astrocytomas[11], in agreement with the findings of the present study. Kebir et al., Kato et al., and Saito et al. also

reported that among grade II and grade III gliomas, the mean L/N ratio was significantly higher in oligodendrogliomas than in astrocytomas.[30-32] Okubo et al. reported that the expression of L-type amino acid transpoter 1 in the tumor endothelial cells which is one of the major routes for the transport of 11C-methionine increased along with glioma grade and was significantly correlated with L/N ratio, probably because of the increased number of microvessels in the tumor.[33] Nojiri et al. reported that an increase in microvessels in oligodendrogliomas correlated with higher 11C-methionine uptake compared with that in astrocytoma.[34] In contrast, Iwadate et al. reported that among grade II and grade III gliomas, L/N ratios in astrocytomas were higher than those in oligodendrogliomas, and Verger et al. concluded there was no difference in L/N ratios between astrocytomas and oligodendroglioma.[34]

*Correlation between L/N ratio and IDH status*

Since the revision of the WHO classification of central nervous system tumors in 2016, genetic analysis has become essential for the diagnosis of glioma.[3] In several recent reports regarding the relationship between molecular analysis of gliomas and the PET findings.[8, 9, 21, 29, 31, 35-38], all but one[21] concluded *IDH* wild-type gliomas were significantly higher accumulation of amino tracer than those of *IDH* mutant gliomas.[8, 9, 29, 31, 35-38] In the present study, the mean and max L/N ratios were significantly higher in the *IDH* wild-type gliomas than in the *IDH* mutant gliomas. In addition, a mean L/N ratio of 2.05 provided the best sensitivity and specificity for distinguishing between *IDH* mutant and *IDH* wild-type gliomas, 69.2% and 76.2%, respectively (area under the curve [AUC], 0.725). Takei et al. reported that when the cutoff value of the mean L/N ratio of 11C-methionine was set at 2.69, the sensitivity and specificity were 71.8% and

92.2%, respectively, and the AUC was 0.877. [38] They also reported that 11C-choline PET provided more precise diagnosis and could distinguish between the *IDH* mutant gliomas and the *IDH* wild-type gliomas, with AUC of 0.906. [38] Verger et al. also reported that the usefulness of 18F-fluoroethyl-L-tyrosine PET. They concluded that the combined the mean L/N ratio and time from the beginning of the dynamic acquisition up to the maximum uptake of amino tracer in the lesion achieved an accuracy of 73% in predicting *IDH* status. [35] The max L/N ratio of 11C-methionine PET has also been considered useful for distinguishing between the *IDH* mutant gliomas and the *IDH* wild-type gliomas. Ogawa et al. reported that a cutoff value of 3.724 provided the best sensitivity and specificity (51.7% and 88.5%, respectively; AUC, 0.727). [36] They also reported that the AUC of the L/N ratio using the 18F-fluoroethyl-L-tyrosine PET was significantly higher than that for 11C-methionine PET. [36] In the present study, a max L/N ratio of 3.92 provided the best sensitivity and specificity for distinguishing between *IDH* mutant and *IDH* wild-type gliomas (76.9% and 64.3%, respectively; AUC, 0.711). However, the mean L/N ratio provided a more precise diagnosis than max L/N ratio for predicting *IDH* status. Although multiple regression analysis revealed that pathological diagnosis was the only influential factor on both mean L/N ratio and max L/N ratio in the current study, predicting the *IDH* mutation status in newly diagnosed gliomas noninvasively prior to tumor resection was meaningful to decide surgical strategy.

*TERT promoter mutation and ATRX alteration in gliomas*

In the revised 2016 WHO classification of central nervous system tumors, "*IDH* wild-type gliomas" are still fuzzy because they contain diffuse astrocytoma, anaplastic astrocytoma, and glioblastoma. Astrocytomas are defined only by the presence of *IDH*

mutation, whereas oligodendrogliomas are defined by the presence of *IDH* mutation and 1p/19q codeletion; thus, further molecular markers are necessary to assess the precise prognosis, particularly in categorizing *IDH* wild-type astrocytomas. Oligodendroglioma is frequently accompanied by *IDH* mutation, *TERT* promoter mutation, and 1p/19q codeletion. Alpha thalassemia/mental retardation syndrome X-linked gene (*ATRX*) alteration is common in diffuse astrocytoma and secondary glioblastoma, and *TERT* promoter mutation is frequently seen in oligodendroglioma and primary glioblastoma. [39, 40] Eckel-Passow et al. categorized gliomas based on 1p/19q codeletion, *IDH* mutation, and *TERT* promoter mutation[41] and Pekmezci et al. categorized gliomas based on 1p/19q codeletion, *IDH* mutation, *ATRX* alteration, and *TERT* promoter mutation. [42] Arita et al. also classified glioma patients into four groups according to the *IDH* and *TERT* promoter status. [43] The common result of these previous studies is that gliomas with *TERT* promoter mutation with *IDH* mutation have a better prognosis, whereas those with *TERT* promoter mutation without *IDH* mutation have a worse prognosis. [42, 43] Therefore, *TERT* promoter status may add prognostic value in the management of glioma.

*Correlation between L/N ratio and TERT promoter status*

Regarding *TERT* promoter, only one study has investigated the relationship between the uptake of the amino tracer and *TERT* promoter mutation.[9] Unterrainer et al. used 18F-GE-180 PET in both newly diagnosed and recurrent gliomas and stated that there was no association between uptake intensity and *TERT* promoter mutation.[9] To the best of our knowledge, the present study is first report of L/N ratio of 11C-methionine and the status of *TERT* promoter mutation in the newly diagnosed and untreated gliomas. In

the present study, the mean L/N ratios of *TERT* promoter wild-type gliomas were significantly lower than those of *TERT* promoter mutant gliomas, but there was no statistically significant difference in terms of max L/N ratio. This is probably because *TERT* promoter mutation is seen frequently in oligodendroglioma and primary glioblastoma, in which accumulation of amino tracer in the tumor is greater compared with that in lower-grade astrocytomas.[15, 28, 30, 31]

*Correlation between L/N ratio and IDH/TERT promoter status*

In the present study, there were statistically significant differences for both the mean and max L/N ratios between group B (*IDH* mutant/ *TERT* promoter wild-type) and C (*IDH* wild-type/ *TERT* promoter wild-type), and also between group B (*IDH* mutant/ *TERT* promoter wild-type) and group D (*IDH* wild-type/ *TERT* promoter mutant). This is probably because group B comprised mostly diffuse astrocytoma patients, and the proportion of high-grade glioma patients increased in the order of group B, group C, and group D. There were also statistically significant differences in the mean L/N ratio between group A (*IDH* mutant/ *TERT* promoter mutant) and group B (*IDH* mutant/ *TERT* promoter wild-type). In Group A, about 90% of tumors were oligodendrogliomas, whereas about 70% of tumors in Group B were low-grade gliomas (mainly diffuse astrocytomas). As shown in Figure 2, the mean L/N ratio was higher in oligodendrogliomas than in diffuse astrocytomas. In the present study, multiple regression analysis revealed that *IDH* status had more impact on L/N ratio than *TERT* promoter status.

*Limitations*

There are some limitations in this study. First, the relatively small number of patients might influence the analysis. Second, there was inconsistency in the timing between evaluation with 11C-methionine PET and tumor resection, which could possibly have influenced the L/N ratio.

## Conclusion

Distinguishing glioma subtypes based on the revised 2016 WHO classification of the central nervous system tumors on the basis of 11C-methionine PET alone appears to be difficult. Although multiple regression analysis revealed that pathological diagnosis was the only influential factor on L/N ratio, the present finding that L/N ratio of 11C-methionine was significantly higher in *IDH* wild-type gliomas than *IDH* mutant gliomas indicates that 11C-methionine PET may be a useful and noninvasive technique for predicting *IDH* mutation status in newly diagnosed and untreated gliomas prior to tumor resection.

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**Figure legends**

Fig. 1

a: Box plots showing the mean L/N ratio of WHO grade II gliomas, grade III gliomas, and grade IV gliomas. There was a significant difference between grade II gliomas and grade III gliomas, and grade II gliomas and grade IV gliomas.

b: Box plots showing the mean L/N ratio in relation to histopathological classification. Mean L/N ratio of glioblastomas was significantly higher than those of diffuse astrocytomas and oligodendrogliomas. Mean L/N ratio of diffuse astrocytomas was significantly lower than those of anaplastic astrocytomas and oligodendrogliomas.

Fig. 2

Box plots showing the mean L/N ratio in relation to histopathological and gene status classification according to the revised 2016 WHO classification among grade II (a), grade III (b), and grade Ⅳ(c). There was a statistically difference between oligodendrogliomas and *IDH* mutant diffuse astrocytomas.

Fig.3

Box plots showing the mean (a) and the max (b) L/N ratios in relation to gliomas subgrouping *IDH/TERT* promoter mutation. There were statistically differences in the mean and the max L/N ratios between groups B and group C, and groups B and group D. There was a statistically difference in the mean L/N ratio groups A and group B.

Fig.4

Box plots showing the mean (a) and the max (b) L/N ratios in relation to gliomas subgrouping *IDH* mutation. There were statistically differences in the mean and the max L/N ratios *IDH* mutant gliomas and *IDH* wild-type gliomas. ROC curve of the mean (c) and the max (d) L/N ratios for differentiating *IDH* mutant gliomas and *IDH* wild-type gliomas. The area under the curve of the mean L/N ratio was larger than that of the max L/N ratio with the value of 0.725 vs 0.711.

Fig.5

Box plots showing the mean (a) and the max (b) L/N ratios in relation to gliomas subgrouping TERT promoter mutation. There were statistically differences in the mean L/N ratio between *TERT* promoter mutant gliomas and wild-type gliomas. ROC curve of the mean L/N ratio for differentiating *TERT* promoter mutant gliomas and *TERT* promoter wild-type gliomas. The area under the curve of the mean L/N ratio was 0.674 (c).

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Table(s)

| Characteristics | Value | % |
|---|---|---|
| Age(years), median (range) | 55.5 (7-82) | |
| Sex | | |
| Male | 42 | 61.8 |
| Female | 26 | 38.2 |
| Contrast enhancement in MRI | | |
| Yes | 46 | 67.6 |
| No | 22 | 32.4 |
| Histology | | |
| DA | 20 | 29.4 |
| *IDH* mutant | 11 | 16.2 |
| *IDH* wild-type | 9 | 13.2 |
| OD, *IDH* mutant and 1p19q-codeleted | 8 | 11.8 |
| AA | 11 | 16.2 |
| *IDH* mutant | 2 | 2.9 |
| *IDH* wild-type | 9 | 13.2 |
| AO, *IDH* mutant and 1p19q-codeleted | 1 | 1.5 |
| GBM | 28 | 41.2 |
| *IDH* mutant | 3 | 4.4 |
| *IDH* wild-type | 25 | 36.8 |
| *IDH*/*TERT* promoter status | | |
| Group A: *IDH* mutant/ *TERT* promoter mutant | 9 | 13.2 |
| Group B: *IDH* Mutant/ *TERT* promoter wild-type | 17 | 25.0 |
| Group C: *IDH* wild-type/ *TERT* promoter wild-type | 23 | 33.8 |
| Group D: *IDH* wild-type/ *TERT* promoter mutant | 19 | 27.9 |

**TABLE 1.** Patients characteristics and pathology based on the revised 2016 WHO classification.

DA, Diffuse Astrocytoma; *IDH*, Isocitrate Dehydrogenase; OD, Oligodendroglioma; AA, Anaplastic Astrocytoma; AO, Anaplastic Oligodendroglioma; GBM, Glioblastoma; *TERT*, Telomerase Reverse Transcriptase.

Table(s)

| | *TERT* promoter mutant (n=28) | *TERT* promoter wild-type (n=40) |
|---|---|---|
| *IDH* mutant (n=26) | Oligodendroglioma 7 (77.8%)<br>Anaplastic oligodendroglioma 1 (11.1%)<br>GBM 1 (11.1%) | Diffuse astrocytoma 11(64.7%)<br>GBM 3 (17.6%)<br>Anaplastic astrocytoma 2 (11.8%)<br>Oligodendroglioma 1 (5.9%) |
| *IDH* wild-type (n=42) | GBM 14 (73.7%)<br>Anaplastic astrocytoma 3 (15.8%)<br>Diffuse astrocytoma 2 (10.5%) | GBM 10 (43.5%)<br>Diffuse astrocytoma 7 (30.4%)<br>Anaplastic astrocytoma 6 (26.1%) |

**TABLE 2.** Subgroups based on *IDH*/*TERT* promoter status.
*IDH*, Isocitrate Dehydrogenase; *TERT*, Telomerase Reverse Transcriptase; GBM, Glioblastoma.

Table(s)

| | Mean L/N, median (IQR) | *p* value | Max L/N, median (IQR) | *p* value |
|---|---|---|---|---|
| Age | | **.0321** | | **.0112** |
| ≥65 | 2.82(2.35-3.15) | | 4.74(3.33-5.27) | |
| <65 | 2.04(1.60-2.81) | | 3.13(2.43-4.57) | |
| Sex | | .426 | | .594 |
| Male | 2.37(1.67-3.00) | | 3.73(2.33-4.79) | |
| Female | 2.26(1.89-3.44) | | 3.77(2.66-4.83) | |
| Contrast enhancement in MRI | | **<.0001** | | **<.0001** |
| Yes | 2.67(2.05-3.38) | | 4.56(3.25-5.26) | |
| No | 1.60(0.97-1.94) | | 2.34(1.52-2.78) | |
| WHO grade | | **<.0001** | | **<.0001** |
| Ⅱ (n=28) | 1.60 (1.00-2.06) | | 2.49 (1.59-3.06) | |
| Ⅲ (n=12) | 2.26 (1.80-4.20) | | 3.83 (3.01-5.94) | |
| Ⅳ (n=28) | 3.02 (2.50-3.42) | | 4.77 (4.35-5.29) | |
| Histology | | **<.0001** | | **<.0001** |
| DA (n=20) | 1.20 (0.75-1.86) | | 2.09 (1.48-2.63) | |
| *IDH* mutant (n=11) | 1.08 (0.63-1.65) | | 2.14 (1.34-2.52) | |
| *IDH* wild-type (n=9) | 1.58 (1.02-2.14) | | 2.04 (1.56-2.83) | |
| OD, *IDH* mutant and 1p19q-codeleted (n=8) | 2.03 (1.82-2.35) | | 3.17 (2.54-3.65) | |
| AA (n=11) | 2.06 (1.67-3.32) | | 3.67 (2.99-5.18) | |
| *IDH* mutant (n=2) | 2.85 (2.27-3.44) | | 4.74 (3.91-5.57) | |
| *IDH* wild-type (n=9) | 2.06 (1.81-2.61) | | 3.67 (2.97-4.58) | |
| AO, *IDH* mutant and 1p19q-codeleted (n=1) | 6.05 (NA) | | 8.86 (NA) | |
| GBM (n=28) | 3.02 (2.50-3.42) | | 4.77 (4.35-5.29) | |
| *IDH* mutant (n=3) | 2.61 (2.25-2.82) | | 3.18 (3.08-3.87) | |
| *IDH* wild-type (n=25) | 3.02 (2.50-3.46) | | 4.84 (4.46-5.30) | |
| *IDH* status | | **.00162** | | **.00332** |
| mutant (n=26) | 1.75 (1.31-2.23) | | 2.56 (2.2-3.75) | |
| Wild-type (n=42) | 2.61 (2.05-3.19) | | 4.51 (2.99-5.12) | |
| *TERT* promoter status | | **.0147** | | .0554 |
| mutant (n=28) | 2.64 (2.04-3.09) | | 4.11 (3.05-4.85) | |
| Wild-type (n=40) | 1.92 (1.35-2.81) | | 3.07 (2.18-4.62) | |

| | | | | |
|---|---|---|---|---|
| *IDH/TERT* promoter status | | **.00223** | | **.0110** |
| Group A: *IDH* mutant/ *TERT* mutant (n=9) | 2.08 (1.89-2.67) | | 3.27 (2.55-3.79) | |
| Group B: *IDH* mutant/ *TERT* wild-type (n=17) | 1.68 (0.96-1.80) | | 2.49 (1.60-3.08) | |
| Group C: *IDH* wild-type/ *TERT* wild-type (n=23) | 2.49 (1.80-3.21) | | 4.38 (2.73-5.20) | |
| Group D: *IDH* wild-type/ *TERT* mutant (n=19) | 2.92 (2.26-3.14) | | 4.56 (3.55-4.90) | |

**TABLE 3.** The mean and the max L/N ratio of 68 patients. *P* values in bold font are statistically significant.

MRI, Magnetic Resonance Imaging; WHO, World Health Organization; DA, Diffuse Astrocytoma; *IDH*, Isocitrate Dehydrogenase; OD, Oligodendroglioma; AA, Anaplastic Astrocytoma; AO, Anaplastic Oligodendroglioma; GBM, Glioblastoma; *TERT*, Telomerase Reverse Transcriptase.

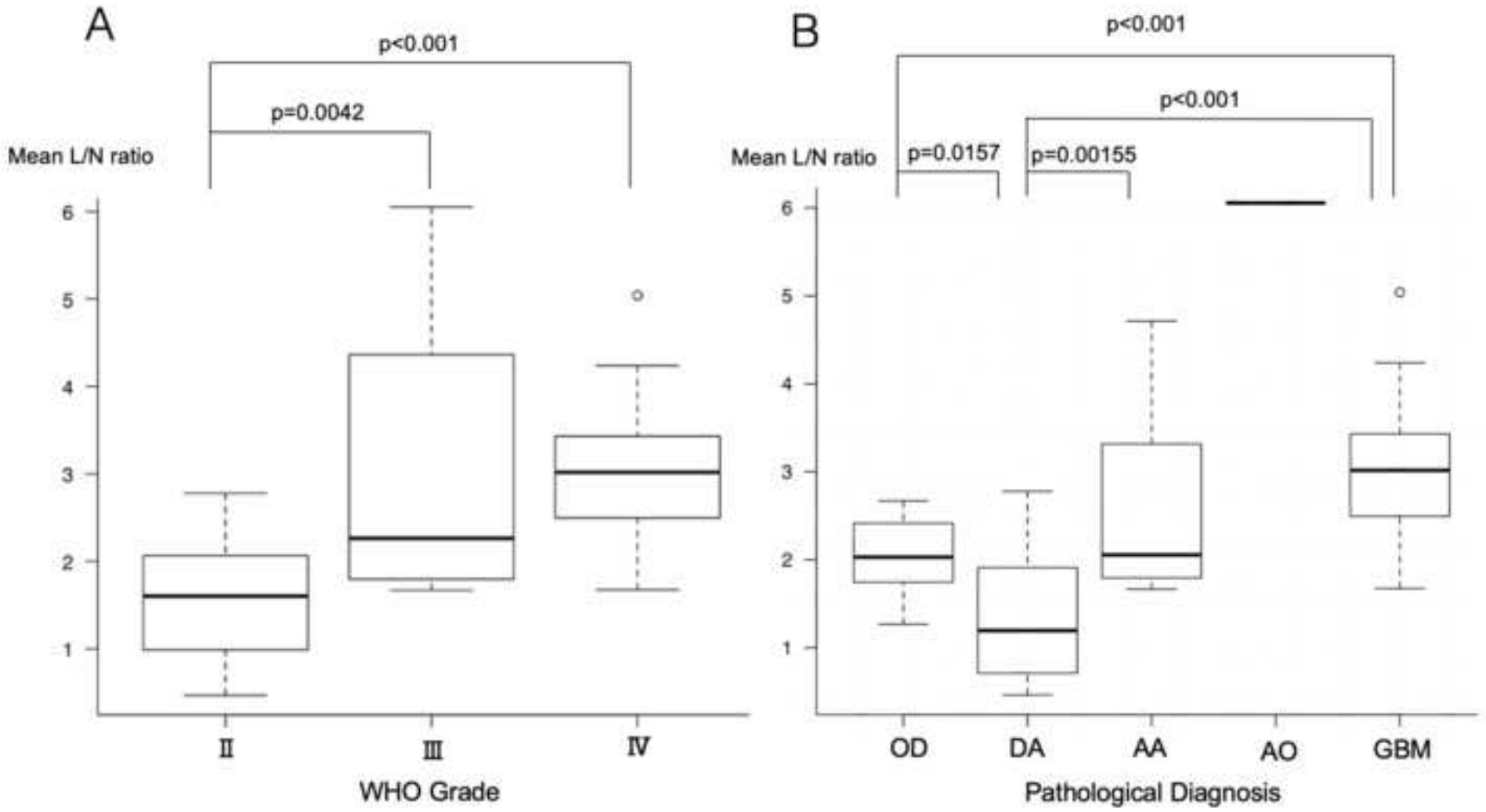
Table(s)

| | Mean L/N | | | | | Max L/N | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Partial Regression Coefficient | 95%CI | VIF | t value | *p* value | Partial Regression Coefficient | 95%CI | VIF | t value | *p* value |
| Age<br><65 vs ≥65 | -0.147 | -0.663, 0.369 | 1.206 | -0.571 | .570 | 0.020 | -0.802, 0.842 | 1.206 | 0.049 | .961 |
| Sex<br>Male vs Female | 0.122 | -0.306, 0.551 | 1.059 | 0.571 | .570 | 0.089 | -0.594, 0.772 | 1.059 | 0.261 | .795 |
| Contrast enhancement in MRI<br>No vs Yes | 0.442 | -0.179, 1.063 | 1.491 | 1.425 | .160 | 0.871 | -0.119, 1.861 | 1.491 | 1.762 | .083 |
| Histology | | | 1.270 | | | | | 1.270 | | |
| DA | Reference | | | | | | | | | |
| OD | 0.874 | 0.024, 1.723 | | 2.059 | **.044** | 1.369 | 0.015, 2.723 | | 2.023 | **.048** |
| AA | 0.929 | 0.225, 1.632 | | 2.643 | **.011** | 1.318 | 0.197, 2.440 | | 2.353 | **.022** |
| GBM | 1.236 | 0.569, 1.904 | | 3.710 | **.0005** | 1.944 | 0.881, 3.008 | | 3.661 | **.0005** |
| *IDH* status<br>Mutant vs Wild-type | 0.448 | -0.124, 1.020 | 1.415 | 1.567 | .123 | 0.538 | -0.374, 1.450 | 1.415 | 1.181 | .243 |
| *TERT* promoter status<br>Mutant vs Wild-type | 0.0317 | -0.471, 0.534 | 1.259 | 0.126 | .900 | 0.134 | -0.666, 0.935 | 1.259 | 0.336 | .738 |

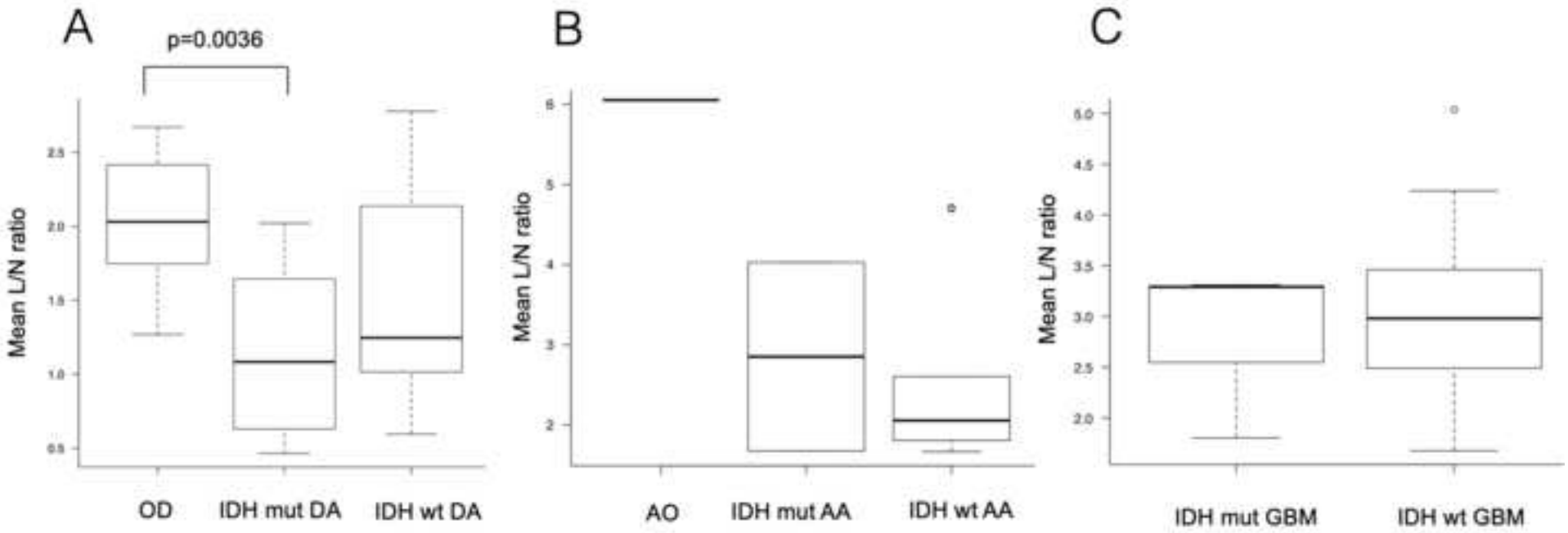
**TABLE 4.** Multivariate regression analysis of 67 patients. *P* values in bold font are statistically significant.

CI, Coefficient Interval; VIF, Variance Inflation Factor; MRI, Magnetic Resonance Imaging; DA, Diffuse Astrocytoma; OD, Oligodendroglioma; AA, Anaplastic Astrocytoma; AO, Anaplastic Oligodendroglioma; GBM, Glioblastoma; *IDH*, Isocitrate Dehydrogenase; *TERT*, Telomerase Reverse Transcriptase.

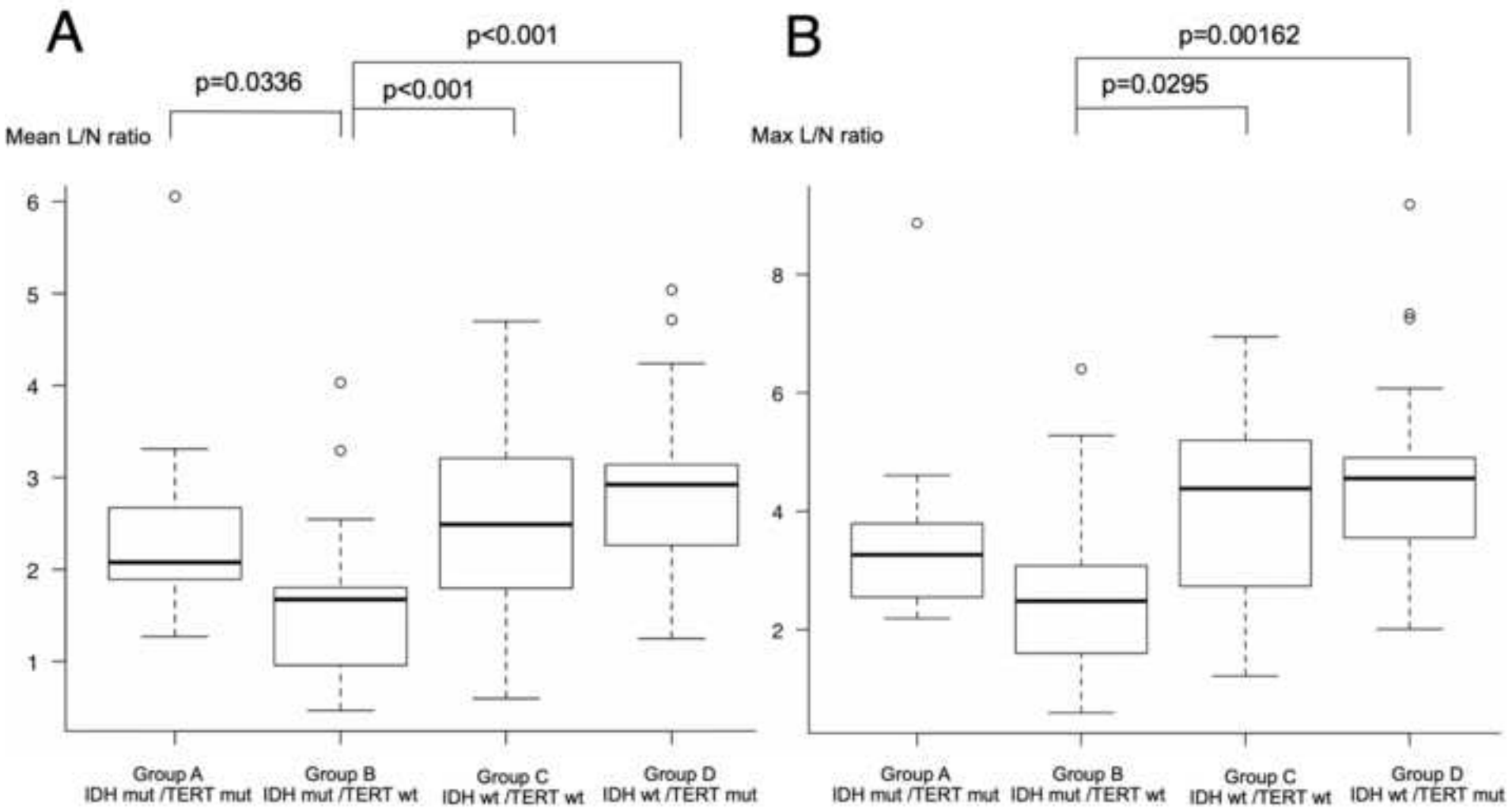
Figure(s)


A
p<0.001
p=0.0042
Mean L/N ratio
6
5
4
3
2
1
Ⅱ
Ⅲ
Ⅳ
WHO Grade
B
p<0.001
p<0.001
p=0.0157
p=0.00155
Mean L/N ratio
6
5
4
3
2
1
OD
DA
AA
AO
GBM
Pathological Diagnosis

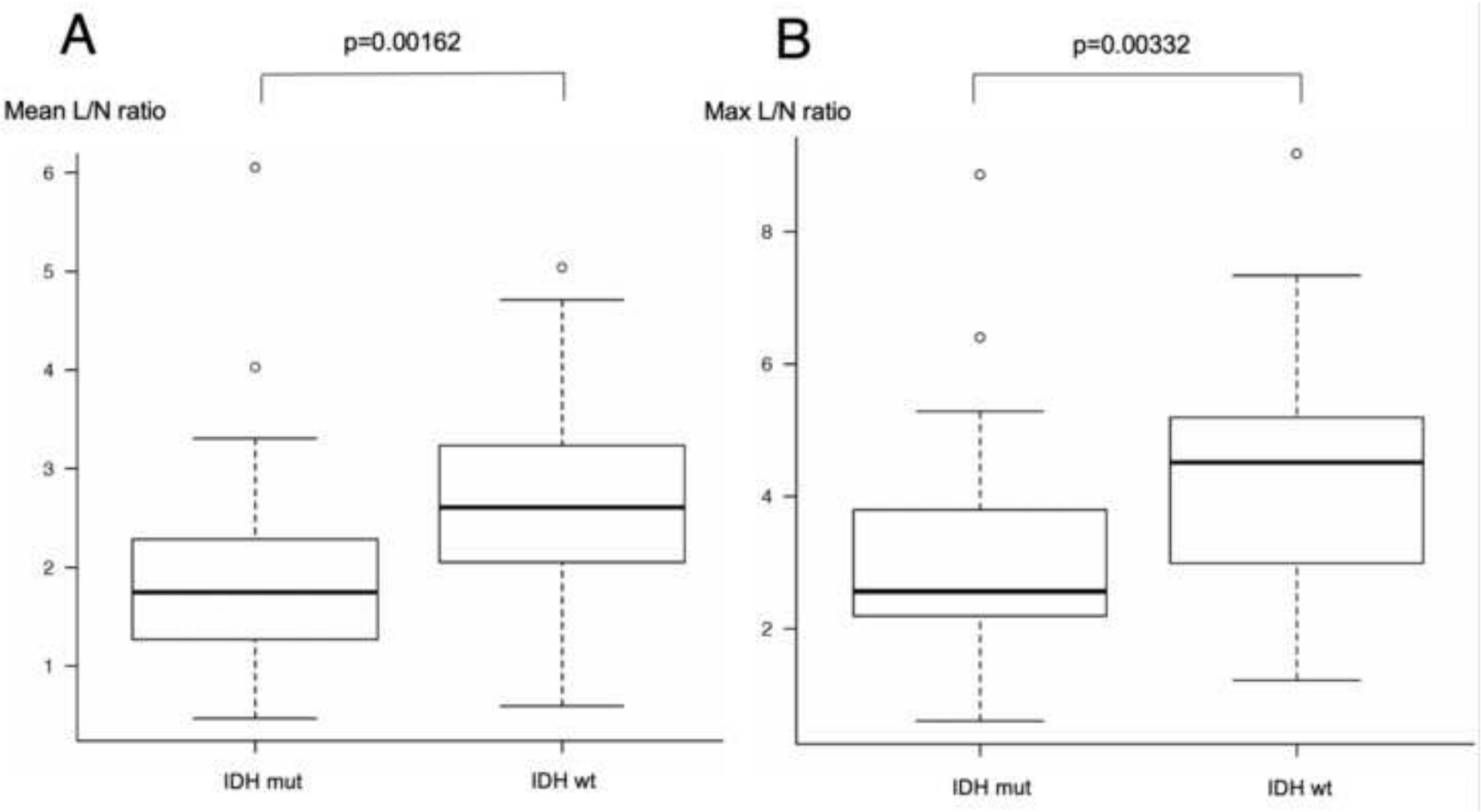
Figure(s)


A
p=0.0036
Mean L/N ratio
OD
IDH mut DA
IDH wt DA
B
Mean L/N ratio
AO
IDH mut AA
IDH wt AA
C
Mean L/N ratio
IDH mut GBM
IDH wt GBM

Figure(s)


A
p=0.0336
p<0.001
p<0.001
Mean L/N ratio
6
5
4
3
2
1
Group A
IDH mut /TERT mut
Group B
IDH mut /TERT wt
Group C
IDH wt /TERT wt
Group D
IDH wt /TERT mut
B
p=0.00162
p=0.0295
Max L/N ratio
8
6
4
2
Group A
IDH mut /TERT mut
Group B
IDH mut /TERT wt
Group C
IDH wt /TERT wt
Group D
IDH wt /TERT mut

Figure(s)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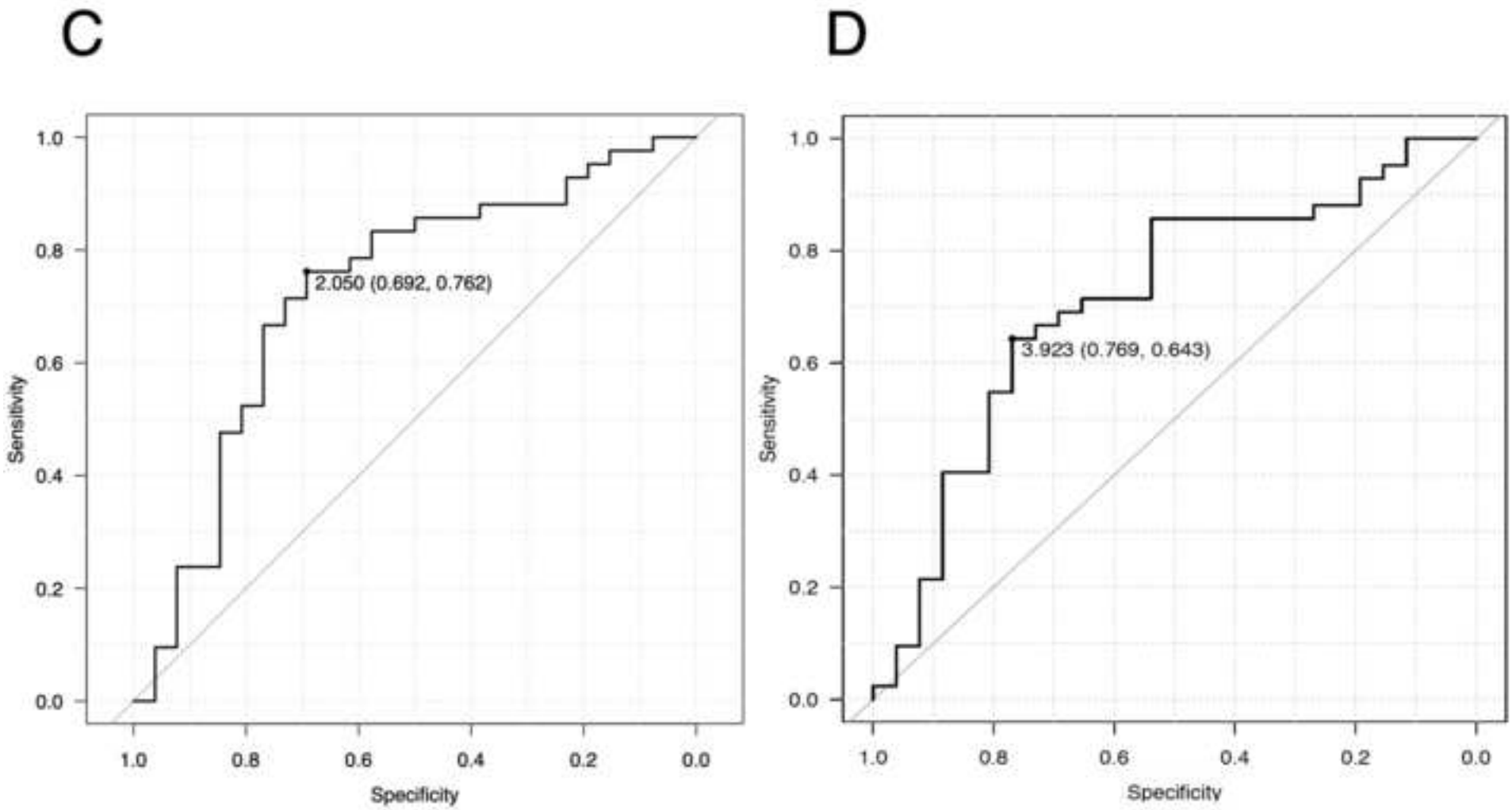
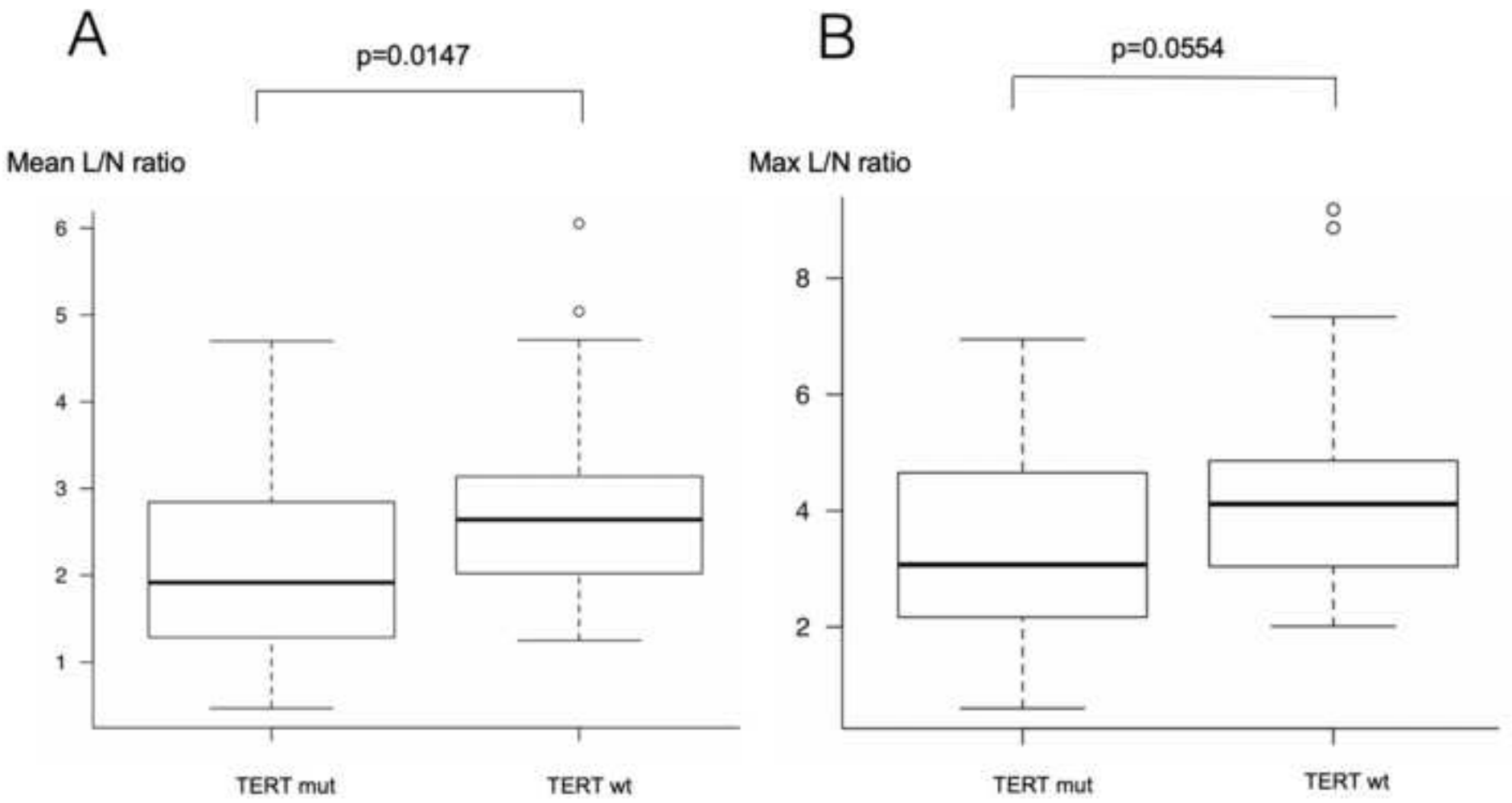
p=0.00162
Mean L/N ratio
6
5
4
3
2
1
IDH mut
IDH wt
B
p=0.00332
Max L/N ratio
8
6
4
2
IDH mut
IDH wt

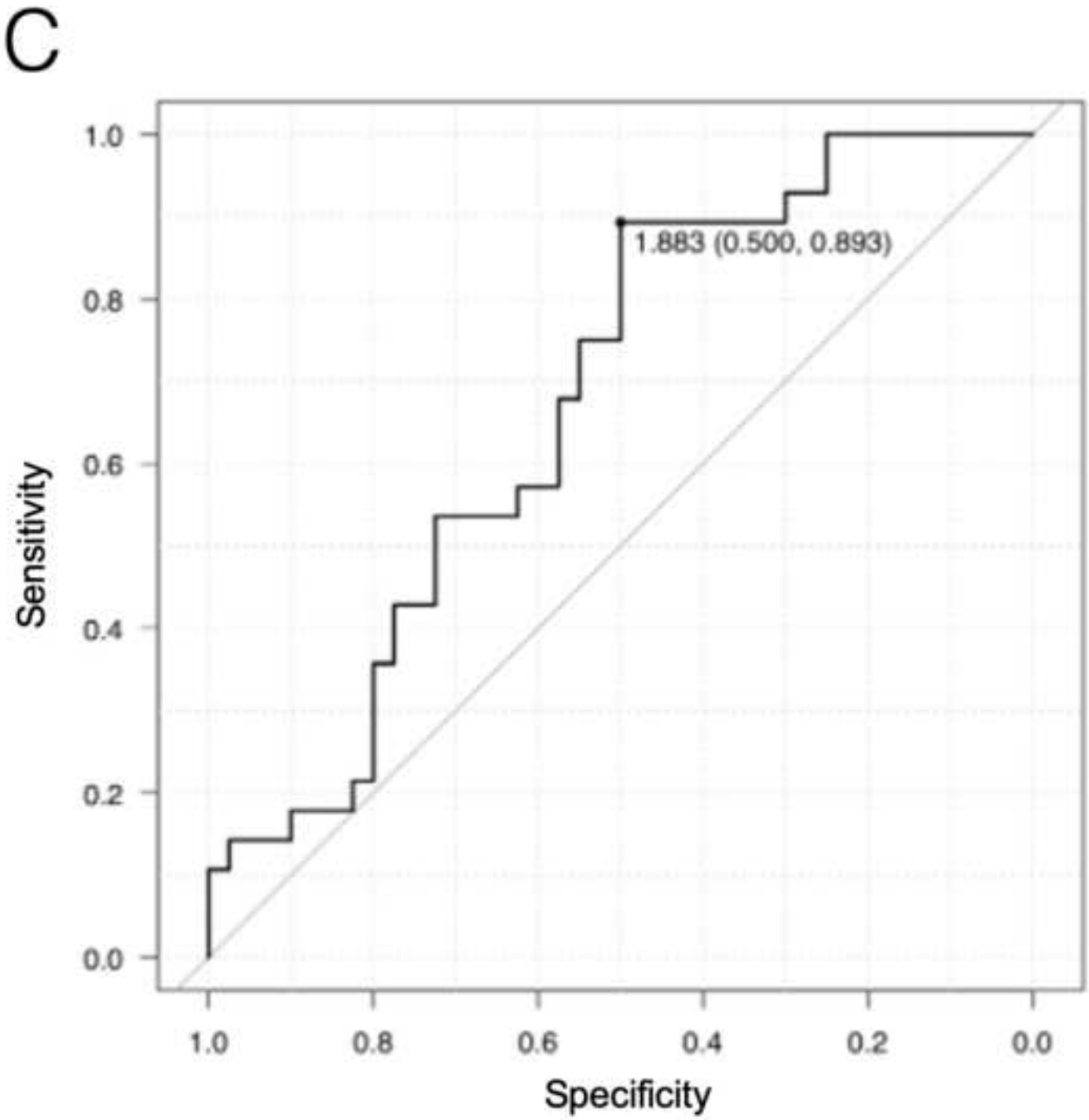
Figure(s)


C
D
Sensitivity
Specificity
1.0
0.8
0.6
0.4
0.2
0.0
2.050 (0.692, 0.762)
3.923 (0.769, 0.643)

Figure(s)


A
p=0.0147
Mean L/N ratio
6
5
4
3
2
1
TERT mut
TERT wt
B
p=0.0554
Max L/N ratio
8
6
4
2
TERT mut
TERT wt

Figure(s)


C
1.0
0.8
0.6
0.4
0.2
0.0
Sensitivity
1.883 (0.500, 0.893)
1.0
0.8
0.6
0.4
0.2
0.0
Specificity