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Original research: Early diagnosis of amyotrophic lateral sclerosis based on fasciculations on muscle ultrasonography: A machine learning approach

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List of abbreviations: ALS, amyotrophic lateral sclerosis; ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised; AUC, area under the curve; BB, biceps brachii; CI, confidence interval; FCU, flexor carpi ulnaris; FDI, first dorsal interosseous; FDP, flexor digitorum profundus; FDS, flexor digitorum superficialis; GC, gastrocnemius; MUS, muscle ultrasonography; MUS-FAST, muscle ultrasonography-based fasciculation testing; RA, rectus abdominis; SBMA, spinal and bulbar muscular atrophy; SCM, sternocleidomastoid; TA, tibialis anterior; TB, triceps brachii; VM, vastus medialis.

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HIGHLIGHTS

- Fasciculations were abundantly detected on muscle ultrasonography from the early stage of amyotrophic lateral sclerosis.
- Fasciculation in the brainstem and thoracic regions has high specificity in the diagnosis of amyotrophic lateral sclerosis.
- Via machine learning, we developed a muscle ultrasonography-based diagnostic tool for early-stage amyotrophic lateral sclerosis.

ABSTRACT

Objective: Although fasciculation on muscle ultrasonography (MUS) is useful in diagnosing amyotrophic lateral sclerosis (ALS), its applicability to early diagnosis remains unclear. We aimed to develop and validate diagnostic models especially beneficial to early-stage ALS via machine learning.

Methods: We investigated 100 patients with ALS, including 50 with early-stage ALS within 9 months from onset, and 100 without ALS. Fifteen muscles were bilaterally observed for 10 s each and the presence of fasciculations was recorded. Hierarchical clustering and nominal logistic regression, neural network, or ensemble learning were applied to the training cohort comprising the early-stage ALS to develop MUS-based diagnostic models, and they were tested in the validation cohort comprising the later-stage ALS.

Results: Fasciculations on MUS in the brainstem or thoracic region had high specificity but limited sensitivities and predictive profiles for diagnosis of ALS. A machine learning-based model comprising eight muscles in the four body regions had a high sensitivity (recall), specificity, and positive predictive value (precision) for both early- and later-stage ALS patients.

Conclusions: We developed and validated MUS-fasciculation-based diagnostic models for early- and later-stage ALS.

Significance: Fasciculation detected in relevant muscles on MUS can contribute to the diagnosis of ALS from the early stage.

Keywords:

Amyotrophic lateral sclerosis

Early diagnosis

Fasciculation

Machine learning

Muscle ultrasonography

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease predominantly affecting upper and lower motor neurons, resulting in generalized muscle weakness (Brown and Al-Chalabi, 2017). One of the lower motor neuron signs is fasciculation, which is the random, spontaneous twitching of a group of muscle fibers belonging to a single motor unit (Dengler et al., 2020). Fasciculations can be clinically observed if they produce movement of the overlying skin in limb or trunk muscles or mucous membrane in the tongue (Dengler et al., 2020); if fasciculations occur in a deep part of the muscle, they may be clinically undetectable. The electrical activity associated with fasciculations is termed a fasciculation potential, which has the configuration of a motor unit potential but occurs spontaneously (Dengler et al., 2020). Fasciculation potentials can be detected using needle electromyography even when fasciculations are not clinically observed. Fasciculation potentials are considered equivalent to fibrillation potentials and positive sharp waves in recognizing denervation in the context of a suspected clinical diagnosis of ALS (de Carvalho et al., 2008). That said, needle electromyography is invasive and time-consuming and can evaluate fasciculation potentials only in a small area of a muscle.

Fasciculations can also be detected on muscle ultrasonography (MUS), which has recently attracted greater attention as it is non-invasive and can observe many muscles in a short time. Studies have indicated that MUS is more sensitive for detecting fasciculations than either clinical examination or

needle electromyography (Misawa et al., 2011; Grimm et al., 2015; Johansson et al., 2017). Furthermore, it is revealed that the number of muscles with fasciculations was significantly higher in patients with ALS than that in those without ALS (Grimm et al., 2015; Johansson et al., 2017; Tsuji et al., 2017; Juan et al., 2020). A fasciculation scoring method for ALS diagnosis was also developed using logistic regression analysis of MUS-based fasciculations in muscles in the side of onset, with each muscle observed for 30 s, while the contribution of each muscle to the model remained unclear (Tsuji et al., 2017). We note that previous reports examined relatively small numbers of patients with relatively late stages of ALS, with the average duration of 14–29 months after onset, while early diagnosis and treatment is critical for the effectiveness of ALS therapeutics (Kaji et al., 2019; Oki et al., 2022). In addition, previous studies did not incorporate model validation processes. Thus, the following issues remained unclear: (1) characteristics and diagnostic usefulness of MUS-fasciculations in early-stage ALS; (2) utility of other machine leaning methods such as hierarchical clustering, neural network, and ensemble learning to develop more reliable and interpretable diagnostic models regardless of disease stage; and (3) reproducibility and generalizability of MUS-fasciculation scoring methods. To address these issues, we aimed to first characterize MUS-fasciculations in patients with early-stage ALS, then compare different machine learning-based models using training and validation cohorts that included early- and later-stage ALS, and finally interpret the machine learning results to develop and validate a new MUS-fasciculation scoring system to diagnose ALS at any stage.

2. Methods

2.1. Participants

We retrospectively analysed data of 100 patients with ALS (64 men and 46 women; age, 67.5 ± 11.7 [mean ± standard deviation] years, range 31–88 years) who consecutively underwent MUS examination at Tokushima University Hospital from April 2016 to March 2021. The patients underwent genetic testing of *SOD1*, *ALS2*, *SETX*, *SPG11*, *FUS*, *VAPB*, *ANG*, *TARDBP*, *FIG4*, *OPTN*, *VCP*, *UBQLN2*, *SIGMAR1*, *CHMP2B*, *PFN1*, *ERBB4*, *HNRNPA1*, *MATR3*, *TUBA4A*, *CHCHD10*, *SQSTM1*, and *TBK1*; all coding exons were simultaneously PCR amplified and sequenced. Abnormal repeat expansion in the *C9orf72* gene was assessed using repeat-primed PCR as previously described (Renton et al., 2011). Of them, 98 had sporadic ALS and two had familial ALS with *SOD1* mutation. Twenty-six were bulbar onset, four were neck or truncal onset, 48 were upper limb onset, and 22 were lower limb onset. The diagnosis of ALS was made by board-certified neurologists based on the updated Awaji criteria (Geevasinga et al., 2018), and 86 patients fulfilled the definite (n = 11), probable (n = 30), probable laboratory-supported (n = 27), and possible (n = 18) categories at the initial MUS examination. Although 14 patients did not fulfill the updated Awaji criteria initially, all of them were confirmed to fulfill the criteria during the subsequent courses of the disease; two were categorized as probable, six probable laboratory-supported, and six possible. The disease severity in patients with ALS was evaluated using the Amyotrophic Lateral

Sclerosis Functional Rating Scale–Revised (Cedarbaum et al., 1999). Moreover, 100 consecutive non-ALS patients (78 men and 22 women; age, 64.2 ± 14.8 years, range 19–87 years) who underwent MUS examination from May 2017 to March 2021 were investigated. Of them, 46 were diagnosed with cervical or lumbar spondylosis, seven with multifocal motor neuropathy, five with chronic inflammatory demyelinating polyradiculoneuropathy, 14 with other neuropathies, 16 with myositis or muscular dystrophy, and 12 with Parkinson's disease or parkinsonian syndrome. Additionally, to examine how MUS would work in motor neuron diseases other than ALS, we also investigated five consecutive male patients with spinal and bulbar muscular atrophy (SBMA) (age, 52.4 ± 11.7 years; duration before MUS, 48.6 ± 55.2 months; CAG repeats in the androgen receptor gene, 48.6 ± 3.0) who were examined during the same period.

To evaluate the characteristics of fasciculations in the early stage of ALS and to develop diagnostic models via machine learning, we classified the participants into the training and validation cohorts (**Fig. 1**). The training cohort consisted of 50 patients with early-stage ALS who underwent initial MUS within 9 months from disease onset (35 men and 15 women; age, 68.0 ± 11.7 years; duration before MUS, 5.5 ± 2.2 months) and 50 non-ALS patients who underwent MUS in the first half of the study period (39 men and 11 women; age, 66.7 ± 12.9 years; duration before MUS, 33.2 ± 41.2 months) (**Table 1**). No significant differences in age and gender were observed between the groups. The validation cohort

consisted of 50 patients with later-stage ALS who underwent initial MUS more than 9 months after disease onset (29 men and 21 women; age, 67.0 ± 11.9 years; duration before MUS, 21.9 ± 14.1 months) and 50 non-ALS patients who underwent MUS in the second half of the study period (39 men and 11 women; age, 61.6 ± 16.2 years; duration before MUS, 40.1 ± 51.8 months). The five cases of SBMA were excluded from either the training or validation cohort because of the insufficient number of cases to draw robust conclusions about the disease. This study was approved by The Ethics Committee of Tokushima University Hospital, and written information was provided to all participants and an option to opt-out of the study was provided; this information was published on the website of Tokushima University Hospital.

2.2. Muscle ultrasonography

LOGIQ e Premium Ultrasound System (GE Healthcare Japan, Tokyo, Japan) with a 12-MHz linear array transducer was used for sonographic analysis. Ultrasonographic delineation was performed with a short-axis image at 90° to the muscle fibers with a depth and width of 3.5–4 cm. MUS was performed with the patient in a supine, resting position by either one of three examiners with expertise in MUS (NT, a board-certified clinical laboratory technician and neurosonologist; HY, a board-certified neurologist, clinical neurophysiologist, and neurosonologist; and KFuku, a board-certified neurologist and clinical neurophysiologist). The examiners were not blinded to the clinical findings and final diagnosis. Fasciculation was defined as the presence of involuntary twitching of small parts of the muscle (Dengler

et al., 2020), and each muscle was observed using MUS for 10 s. Fasciculations were recorded as present or absent in each muscle, and exact numbers of fasciculations were not assigned.

We evaluated the following 15 muscles: the tongue, sternocleidomastoid (SCM), trapezius, deltoid, biceps brachii (BB), triceps brachii (TB), flexor carpi ulnaris (FCU), flexor digitorum profundus (FDP), flexor digitorum superficialis (FDS), first dorsal interosseous (FDI), rectus abdominis (RA), vastus medialis (VM), tibialis anterior (TA), medial head of the gastrocnemius (GC), and soleus. All muscles, except for the tongue, were bilaterally examined, and 29 muscles were evaluated. For patients who underwent multiple MUS studies, we assessed their initial MUS study. The muscles were observed in the axial and longitudinal planes at the standardized anatomical points. The FCU, FDP, and FDS were simultaneously observed using a transducer placed approximately 5 cm distal from the elbow on the ulnar side. Similarly, the GC and soleus were simultaneously observed using the transducer placed at approximately 1/2 the height of the lower leg and approximately 10 cm inside the anterior border of the tibia.

2.3. Machine learning

We explored MUS-fasciculation-based models to diagnose early- and later-stage ALS. Chiefly because the number of muscles in the cervical region was high, it was considered that muscles with similar properties would have multicollinearity and might cause overfitting. To reduce multicollinearity and

overfitting, appropriate muscles were selected in advance via hierarchical clustering with Ward's method in the patients with early-stage ALS using Statistical Package for Social Sciences (SPSS, version 26; IBM Corp., Armonk, New York, USA); From each cluster of muscles, the one with the highest positive rate of fasciculation was selected.

First, nominal logistic regression analysis was performed to model the relationship between the categorial response variable, i.e., ALS, and explanatory variables, i.e., muscles, in the training cohort, and the model was tested in the validation cohort. Second, a network-based model (neural network) was built with three hidden nodes in a single hidden layer using the hyperbolic tangent function, to describe the impact that multiple predictor variables have on the categorical outcome in the training cohort, and to make predictions of the categorical outcome in the validation cohort. The above models were investigated using JMP (version 14; SAS Institute Inc., Cary, North Carolina, USA). Third, an ensemble learning-based model was built using neural networks and gradient boosting trees with internal cross-validation in the training cohort and the model was tested in the validation cohort, using proprietary algorithms of Prediction One (Sony Network Communications Inc., Tokyo, Japan; https://predictionone.sony.biz/).

Via ensemble learning, the muscles selected by the hierarchical clustering were ranked in order of contribution to the diagnosis of ALS. Diagnostic models were developed using the highly ranked muscles,

and each model was evaluated for the ability to diagnose ALS. The muscles were assigned 0 point with no fasciculation, 1 point with fasciculation on one side, and 2 points with fasciculation on the bilateral sides (excluding the tongue, which was assigned 0 or 1 point). Moreover, we compared the number of fasciculation-positive body regions in the models, considering the current diagnostic criteria of ALS, which evaluate motor neuron dysfunction in the four body regions, that is, the brainstem and cervical, thoracic, and lumbosacral spinal regions (Brooks et al., 2000; Geevasinga et al., 2016; Shefner et al., 2020). Subsequently, the diagnostic ability of a candidate model was assessed in the validation cohort.

2.4. Statistical analysis

Differences in patients' age and the number of muscles with fasciculations were tested with Student's t-test. Differences in the proportion of categories were tested with Fisher's exact test. Relationship between disease duration and the number of muscles with fasciculations was tested with Pearson correlation coefficient. All significance tests were two-sided. Differences with *p*-values of less than 0.05 were considered statistically significant. Receiver operating characteristic curves were prepared to calculate the sensitivity (recall), specificity, positive predictive value (precision), F-measure, and area under the curve (AUC) of each model. Data were analysed using SPSS, JMP, Prediction One, and GraphPad Prism (version 8; GraphPad Software, San Diego, California, USA).

3. Results

We investigated 5,945 muscles in 100 patients with ALS, 100 non-ALS patients, and 5 patients with SBMA. The clinical characteristics of the participants, except for those with SBMA, are summarized in **Table 1**. The number of muscles with fasciculations for each patient was significantly higher in patients with ALS than that in non-ALS patients (15.0 ± 6.8 vs. 1.4 ± 2.1, $p < 0.01$, Student's t-test); it was 7.2 ± 3.5 in those with SBMA. In the non-ALS patients, the number did not significantly differ between the first and second half groups (1.4 ± 1.9 vs. 1.5 ± 2.4, $p = 0.78$, Student's t-test). A significant difference in the distribution of fasciculations was observed between patients with ALS and non-ALS patients; fasciculations were detected in two or more muscles with different dominant nerves and nerve roots in 100 patients with ALS (100%); however, they were detected in only 28 non-ALS patients (28%).

Then, we evaluated the fasciculation detection rate in each muscle and found that it was significantly higher in patients with ALS than that in non-ALS patients in all muscles (**Fig. 2A**). It was high in BB (91%) and TB (87%) and low in SCM (23%) and RA (42%) in ALS; it was 23%–52% in ALS and only 1% in non-ALS in muscles in the brainstem or thoracic regions. Thus, fasciculations in at least one muscle in the brainstem or thoracic region could distinguish ALS from non-ALS with a sensitivity of 81% and specificity of 99% in the whole cohort consisting of the 200 cases, and with a sensitivity of 78% and specificity of 98% in the early cohort of 100 cases; as reference information, all five patients with SBMA

showed fasciculations in the brainstem region. The relatively low sensitivity, especially in early-stage ALS, and unknown reproducibility remained an issue.

To explore more sensitive and reliable models for MUS-based diagnosis, we applied machine learning algorithms to the training cohort consisting of 100 patients. Before the further analyses, we performed hierarchical clustering of the muscles, considering the presence/absence of fasciculation, in the 50 patients with early-stage ALS. From each cluster that comprised multiple muscles, the muscle with the highest fasciculation positivity rate (**Fig. 2B**) was selected: the BB among the deltoid, FCU, BB, and FDS; the tongue among SCM and tongue; and the VM among the VM and TA. Eventually, 10 muscles were selected.

First, nominal logistic regression analysis was performed, and the whole model test showed χ^2 of 103.78 ($P(\text{Prob}>\chi^2) < 0.0001$), McFadden's pseudo R^2 of 0.749, and root mean squared error of 0.230. The lack of fit test showed χ^2 of 19.18 ($P(\text{Prob}>\chi^2) = 0.9999$), thus we did not need to add more terms to the model. The parameter estimate was significant only for FDI (1.51; 95%CI, 0.05–2.97; $P = 0.043$) (**Table 2**). The model achieved a sensitivity (recall) of 90%, specificity of 96%, precision of 93.9%, F-measure of 0.919, and AUC of 0.975 (95%CI, 0.948–1) in the training cohort, and a sensitivity of 90%, specificity of 94%, precision of 93.8%, F-measure of 0.919, and AUC of 0.969 (95%CI, 0.937–1) in the validation cohort

(**Fig. 3**). Second, a neural network model (**Fig. 4A**) showed that generalized R^2 and root mean squared error were 0.856 and 0.229 respectively in the training cohort and 0.835 and 0.249 respectively in the validation cohort. Prediction profiles of each muscle were as follows: BB 0.72, TB 0.66, FDI 0.60, VM 0.55, soleus 0.52, FDP 0.43, trapezius 0.34, GC 0.32, RA 0.26, and tongue 0.16. The model achieved a sensitivity of 88%, specificity of 98%, precision of 97.8%, F-measure of 0.926, and AUC of 0.977 in the training cohort, and a sensitivity of 88%, specificity of 96%, precision of 95.7%, F-measure of 0.917, and AUC of 0.970 in the validation cohort (**Fig. 4B–C**). Third, an ensemble learning-based model was developed with the training cohort dataset, which achieved a sensitivity of 90%, specificity of 90%, precision of 90%, F-measure of 0.9, and AUC of 0.955 (95%CI, 0.918–0.993) (**Fig. 5A**). The model had a sensitivity of 92%, specificity of 94%, precision of 93.9%, F-measure of 0.929, and AUC of 0.968 (95%CI, 0.936–1) in the validation cohort (**Fig. 5B**).

Additionally, we explored models that would be more readily applied to clinical practice. The 10 muscles were ranked in order of ALS diagnostic contribution via ensemble learning (**Table 3**). Ten models comprising the muscles in order of diagnostic contribution were created and evaluated in terms of their performance in diagnosing ALS; three models comprising 7–9 muscles had comparably high sensitivity, specificity, precision, and AUC (**Table 3**). In contrast, the number of fasciculation-positive body regions was larger in the 8-muscle model than the 7-muscle model and did not differ between the 8- and 9-muscle

models (**Fig. 6**). Thus, the model consisting of the following eight muscles—trapezius, tongue, BB, TB, FDI, RA, VM, and soleus—was determined to be the most appropriate; they also included the five muscles that had relatively high prediction profiles in the above-mentioned neural network model. The optimal cut-off value was 3, with a sensitivity of 86%, specificity of 96%, precision of 95.6%, F-measure of 0.905, and AUC of 0.948 (95%CI, 0.903–0.993) in the training cohort (**Fig. 7A**). The model had a sensitivity of 90%, specificity of 92%, precision of 91.8%, F-measure of 0.909, and AUC of 0.970 (95%CI, 0.941–1) in the validation cohort (**Fig. 7B)**. The diagnostic profiles were comparable to those of the logistic regression, neural network, and ensemble learning models. The determined model was termed "muscle ultrasonography-based fasciculation testing" (MUS-FAST). As expected, no significant correlation was observed between disease duration and the number of fasciculation-positive muscles in the MUS-FAST system in the overall cohort of ALS (r = 0.10, p = 0.31; Pearson correlation coefficient) (**Fig. 8**). Additionally, the MUS-FAST system was positive in three of the five patients with SBMA.

4. Discussion

We investigated the ability of MUS in detecting fasciculations in 100 patients with ALS, including 50 patients evaluated to have early-stage ALS (within 9 months from onset). We demonstrated the following. First, we confirmed that on MUS, fasciculations were extremely rare in the brainstem and thoracic regions in neurological diseases other than motor neuron diseases. Second, the MUS-FAST system had

high sensitivity and specificity to distinguish ALS from other neurological diseases in the training (early) and validation (later) cohorts. Finally, fasciculations were abundantly detected on MUS from the early stage of ALS, and no disease duration-related change in the detection rate was observed.

We observed each muscle for 10 s, but not 30 s or more, to evaluate many muscles in a short time. Fasciculations were easy to distinguish from other movements, such as unifocal movements, rhythmic arterial pulsations, or voluntary movements with a longer duration and involving the entire muscle (Arts et al., 2011; Scheel et al., 1997). Thus, even a single detection in 10 s would be adequate to accurately identify fasciculations on MUS. In support of this idea, a meta-analysis has reported that the detection rate of fasciculations in patients with ALS did not differ between 10-s and 30-s observations (Duarte et al., 2020). In the current study, there were 10 patients with ALS in whom the initial MUS was performed within 3 months after disease onset; still, fasciculations were observed in multiple muscles with different dominant nerves and nerve roots in all patients. In the early stages of ALS, fasciculations may be present in multiple muscles not necessarily associated with weakness, so broad observation would be warranted.

This study showed that fasciculations in the brainstem and thoracic regions were highly specific of ALS or possibly motor neuron diseases, including SBMA, a finding that largely accords with those observed in previous reports. No fasciculations were detected on MUS in the brainstem (Juan et al., 2018; Liu et al.,

2021) or thoracic (Ma et al., 2021) regions in disease and healthy controls. It was also reported that fasciculations were absent in the tongue and truncal muscles in patients with multifocal motor neuropathy (Tsuji et al., 2020). Fasciculation potentials on needle electromyography in patients with ALS were compared with those in patients with cervical spondylosis, and the detection rates of fasciculation potentials were 7% in the SCM and 39% in the trapezius in patients with ALS but 0% in both muscles in patients with cervical spondylosis (Sonoo et al., 2009). Our study similarly showed high specificity in detecting fasciculations in the brainstem or thoracic region in patients with ALS. In these regions, however, the detection rate of fasciculations was less than 50% in each muscle, and the overall sensitivity was only 78% in the patients with ALS within 9 months from disease onset. To effectively diagnose early-stage ALS using MUS, developing models that also incorporated muscles in other regions were necessary.

We employed machine learning methods of hierarchical clustering and logistic regression, neural network, or ensemble learning to develop diagnostic models for ALS. Overall, the models had high diagnostic (predictive) performance. Going through the process, we were able to estimate which muscles were more relevant to the diagnosis (prediction) of ALS; the results suggest that multiple muscles of the cervical and lumbosacral regions should also be incorporated in modelling. We also noted that models relying on specific software would not be widely used and tested in clinical practice. Thus, we aimed to develop a more practical diagnostic method, using the machine learning results as a guide. To this end, the

MUS-FAST system has been developed and validated, which achieved a predictive performance comparable to those of the above-mentioned models. Although previous studies have evaluated fasciculation scoring systems to distinguish patients with ALS from those with ALS mimics or healthy adults (Tsuji et al., 2017; Juan et al., 2018; Ma et al., 2021), they examined only few cases and did not focus on the early stages of the disease. Ours is the first study, to our knowledge, that has investigated the characteristics of fasciculations on MUS in patients with early-stage ALS. We created a diagnostic model via machine learning in the training cohort comprising 50 patients with ALS within 9 months after disease onset. Using the MUS-FAST system, we could diagnose early ALS with a sensitivity of 86%, specificity of 96%, and precision of 95.6%. Moreover, the MUS-FAST system showed comparable diagnostic ability in the validation cohort comprising 50 patients with ALS over 9 months after disease onset. Therefore, the MUS-FAST system can be used as a simple and minimally invasive test when diagnosing ALS at any stage.

The number of fasciculation-positive muscles in the MUS-FAST showed no correlation with disease duration in ALS. When the results are extrapolated to an earlier stage, it is suggested that MUS-based fasciculations could also be observed before symptom onset, which accords with an electromyography study showing that fasciculation potentials are early abnormality in muscles of normal strength and motor unit potentials in ALS (de Carvalho and Swash, 2013). If this is the case, MUS-based fasciculations

would be a useful prodromal biomarker in those who have a family history of ALS or a disease-associated gene variant such as *SOD1* and *C9orf72*. The hypothesis could be tested by incorporating MUS-based fasciculations in future natural history studies of familial ALS.

This study has some limitations. First, it was performed in a single center and followed a retrospective design. Second, the detection of fasciculations on MUS depended on single operators, and interrater agreement was not evaluated. Nonetheless, the number of fasciculation-positive muscles and the sensitivity of the MUS-FAST system in patients with ALS did not differ between the operators. Third, the fasciculations observed on MUS were not compared with fasciculation potentials on needle electromyography. Finally, the differentiation between ALS and other motor neuron diseases was outside the scope of this study; the investigation of a few patients with SBMA suggests that the MUS-FAST system also detects some motor neuron diseases other than ALS.

5. Conclusions

We confirmed that the detection rate of fasciculations using MUS was significantly higher in patients with ALS than that in non-ALS patients. MUS-detected fasciculations in the brainstem and thoracic regions had high specificity, albeit low sensitivities and predictive profiles, in diagnosing ALS. We have developed and validated a novel diagnostic model called MUS-FAST, which can be implemented easily

and in a short time and has high diagnostic performance not only in early but also in later stages of ALS.

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Authors contributions

KFuku and NT conceptualized the project. KFuku acquired, analyzed, and interpreted the data and drafted and revised the manuscript. NT, HY, TY, YO, and SH acquired and analyzed the data and critically revised the manuscript. YY analyzed the data using statistical methods and drafted and critically revised the manuscript. KFuji designed the work, analyzed and interpreted the data using statistical methods, and

critically revised the manuscript. KS and YI supervised the study, interpreted the data, and critically revised the manuscript. All authors provided the final approval of the version to be published.

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Figure legends

Fig. 1. Study flowchart. Abbreviations: ALS, amyotrophic lateral sclerosis.

Fig. 2. Fasciculation detection rate in each muscle. The overall cohort (A) and training cohort (B). In each muscle, fasciculations were regarded as positive if they were present at least on one side. The detection rate of fasciculations is significantly higher in ALS (black bars) than non-ALS (gray bars) in all examined muscles. Abbreviations: ALS, amyotrophic lateral sclerosis; BB, biceps brachii; FCU, flexor carpi ulnaris; FDI, first dorsal interosseous; FDP, flexor digitorum profundus; FDS, flexor digitorum superficialis; GC, gastrocnemius; MUS, muscle ultrasonography; RA, rectus abdominis; SCM, sternocleidomastoid; TA, tibialis anterior; TB, triceps brachii; VM, vastus medialis.

Fig. 3. Performance of the nominal logistic regression model. The area under the curve is 0.975 (95% confidence interval, 0.948–1) in the training cohort (A) and 0.969 (95% confidence interval, 0.937–1) in the validation cohort (B).

Fig. 4. Development and validation of the neural network model. The diagram (A) shows the single-hidden-layer neural network with 10 variables (muscles) and the categorical outcome (ALS). The hidden layer has three nodes, and all nodes are a function of the 10 variables. The predicted outcome is a function of all nodes in the layer. Area under the curve is 0.977 in the training cohort (B) and 0.970 in the validation cohort (C). ALS, amyotrophic lateral sclerosis; BB, biceps brachii; FDI, first dorsal interosseous; FDP, flexor digitorum profundus; GC, gastrocnemius; RA, rectus abdominis; TB, triceps brachii; VM, vastus medialis.

Fig. 5. Performance of the ensemble learning model. The area under the curve is 0.955 (95% confidence interval, 0.918–0.993) in the training cohort (A) and 0.968 (95% confidence interval, 0.936–1) in the validation cohort (B).

Fig. 6. The number of fasciculation-positive body regions in each model. The proportions significantly differ between the 7- and 8-muscle models (1–3 vs. 4 body regions, $p < 0.001$, Fisher's exact test) but not between the 8- and 9-muscle models in patients with early amyotrophic lateral sclerosis.

Fig. 7. Performance of the 8-muscle model. The area under the curve is 0.948 (95% confidence interval, 0.903–0.993) in the training cohort (A) and 0.970 (95% confidence interval, 0.941–1) in the validation cohort (B).

Fig. 8. Correlation between the disease duration and number of fasciculation-positive muscles. The disease duration and the number of fasciculation-positive muscles in the 8-muscle model are not statistically correlated in patients with amyotrophic lateral sclerosis (r = 0.10, p = 0.31; Pearson correlation coefficient).

Table 1: Clinical and laboratory profiles.

	ALS (n = 100)	Non-ALS (n = 100)	*P*-value	Early-stage ALS (n = 50)	Non-ALS in the first half period (n = 50)	*P*-value
Age, years	67.5 ± 11.7	64.2 ± 14.8	0.08	68.0 ± 11.7	66.7 ± 12.9	0.61
Sex, male	64	78	0.04	35	39	0.49
Disease duration, months	13.7 ± 13.0	36.7 ± 46.7	<0.01	5.5 ± 2.2	33.2 ± 41.2	<0.01
Total ALSFRS-R score	39.5 ± 6.8	N/A	N/A	40.6 ± 5.8	N/A	N/A
Region of onset	Bulbar, 26 Arm right, 27; left, 21 Leg right, 11; left, 11 Neck or trunk, 4	N/A	N/A	Bulbar, 13 Arm right, 14; left, 11 Leg right, 6; left, 3 Neck or trunk, 3	N/A	N/A
Background disease	ALS, 100	Cervical or lumbar spondylosis, 46 Neuropathy, 26 Myositis or muscular dystrophy, 16 Parkinson's disease or Parkinsonian syndrome, 12	N/A	ALS, 50	Cervical or lumbar spondylosis, 25 Neuropathy, 10 Myositis or muscular dystrophy, 4 Parkinson's disease or Parkinsonian syndrome, 11	N/A
The number of muscles with fasciculations	15.0 ± 6.8	1.4 ± 2.1	<0.01	14.1 ± 7.0	1.4 ± 1.9	<0.01

Values are means ± standard deviations or numbers. Abbreviations: ALS, amyotrophic lateral sclerosis; ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised; N/A, not applicable.

Table 2: Parameter estimates of the nominal logistic regression model.

		Estimate (95% confidence intervals)	Standard error	χ^2	*P*-value (Prob>χ^2)
Intercept		-3.25 (-4.69–-1.81)	0.74	19.56	<.0001
Trapezius	Unstable	19.24 (-5196.31–5234.78)	2661.04	0.00	0.994
Tongue	Unstable	19.92 (-7517.88–7557.72)	3845.89	0.00	0.996
Biceps brachii		0.83 (-0.62–2.29)	0.74	1.26	0.262
Triceps brachii		0.75 (-0.89–2.39)	0.84	0.81	0.368
Flexor digitorum profundus		1.83 (-0.01–3.66)	0.94	3.81	0.051
First dorsal interosseous		1.51 (0.05–2.97)	0.75	4.11	0.043
Rectus abdominis	Unstable	6.34 (-3925.33–3938.01)	2005.99	0.00	0.998
Vastus medialis		-0.47 (-3.59–2.65)	1.59	0.09	0.769
Gastrocnemius		2.15 (-0.97–5.26)	1.59	1.83	0.177
Soleus		1.17 (-0.42–2.76)	0.81	2.07	0.150

Estimates are for log odds of ALS/non-ALS. The confidence intervals were calculated with Wald method.

Table 3: Profiles of candidate models.

Muscles in order of contribution to the diagnosis	Number of muscles in the model (bilaterally)	Cut-off value	Sensitivity, specificity (%)	Precision (%)	Area under the curve (95% confidence interval)
1. Vastus medialis	1 (2)	1	70, 96	94.6	0.837 (0.769–0.905)
2. Trapezius	2 (4)	1	78, 96	95.1	0.881 (0.820–0.942)
3. Soleus	3 (6)	1	84, 90	89.4	0.899 (0.841–0.958)
4. Tongue	4 (7)	1	88, 90	89.8	0.922 (0.870–0.974)
5. Biceps brachii	5 (9)	2	86, 92	91.5	0.940 (0.893–0.986)
6. Triceps brachii	6 (11)	2	90, 92	91.8	0.949 (0.907–0.992)
7. First dorsal interosseous	7 (13)	3	84, 96	95.5	0.947 (0.902–0.992)
8. Rectus abdominis	8 (15)	3	86, 96	95.6	0.948 (0.903–0.993)
9. Gastrocnemius	9 (17)	3	86, 96	95.6	0.955 (0.915–0.995)
10. Flexor digitorum profundus	10 (19)	2	92, 88	88.5	0.965 (0.932–0.997)

Figure 1

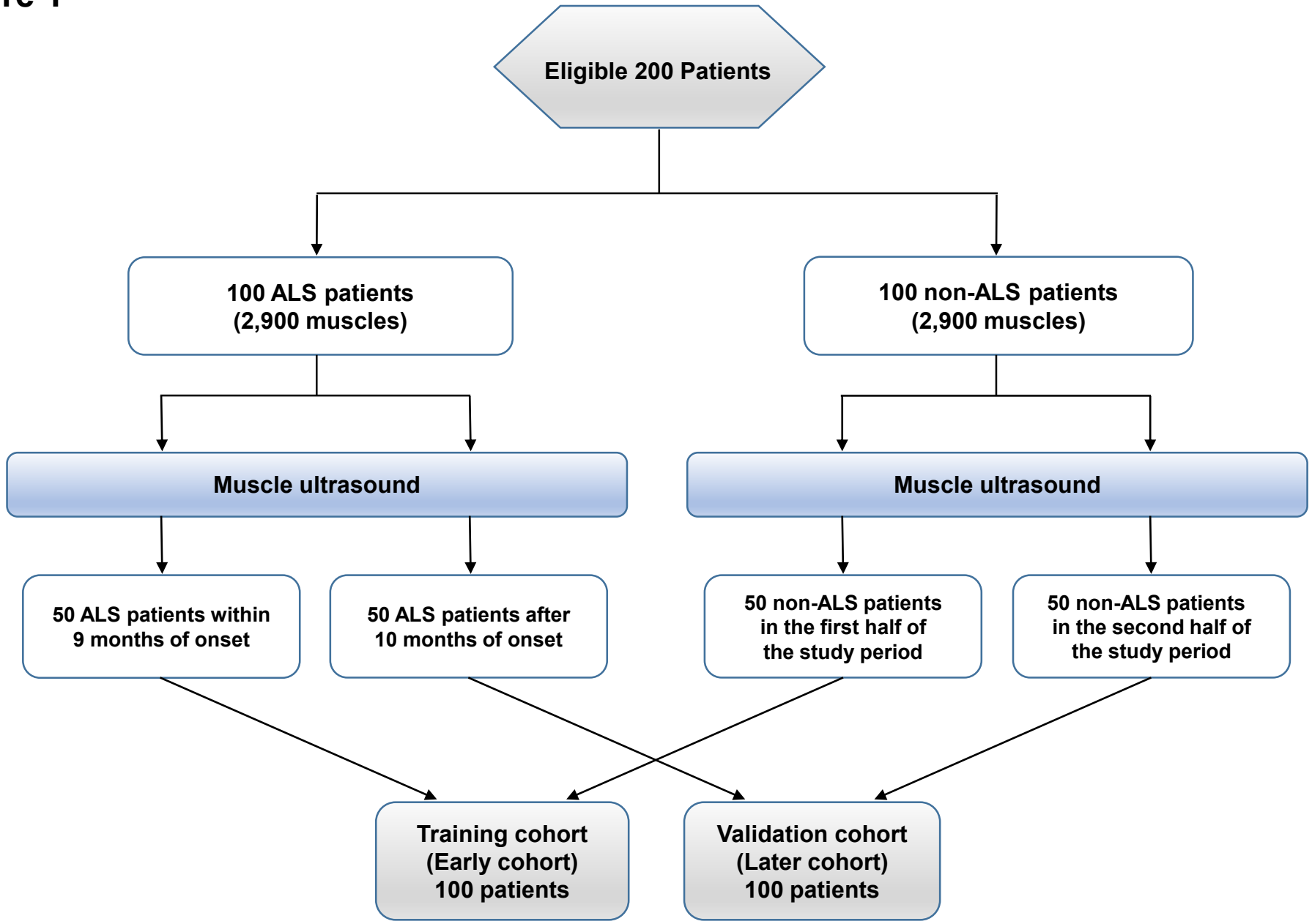


Figure 2

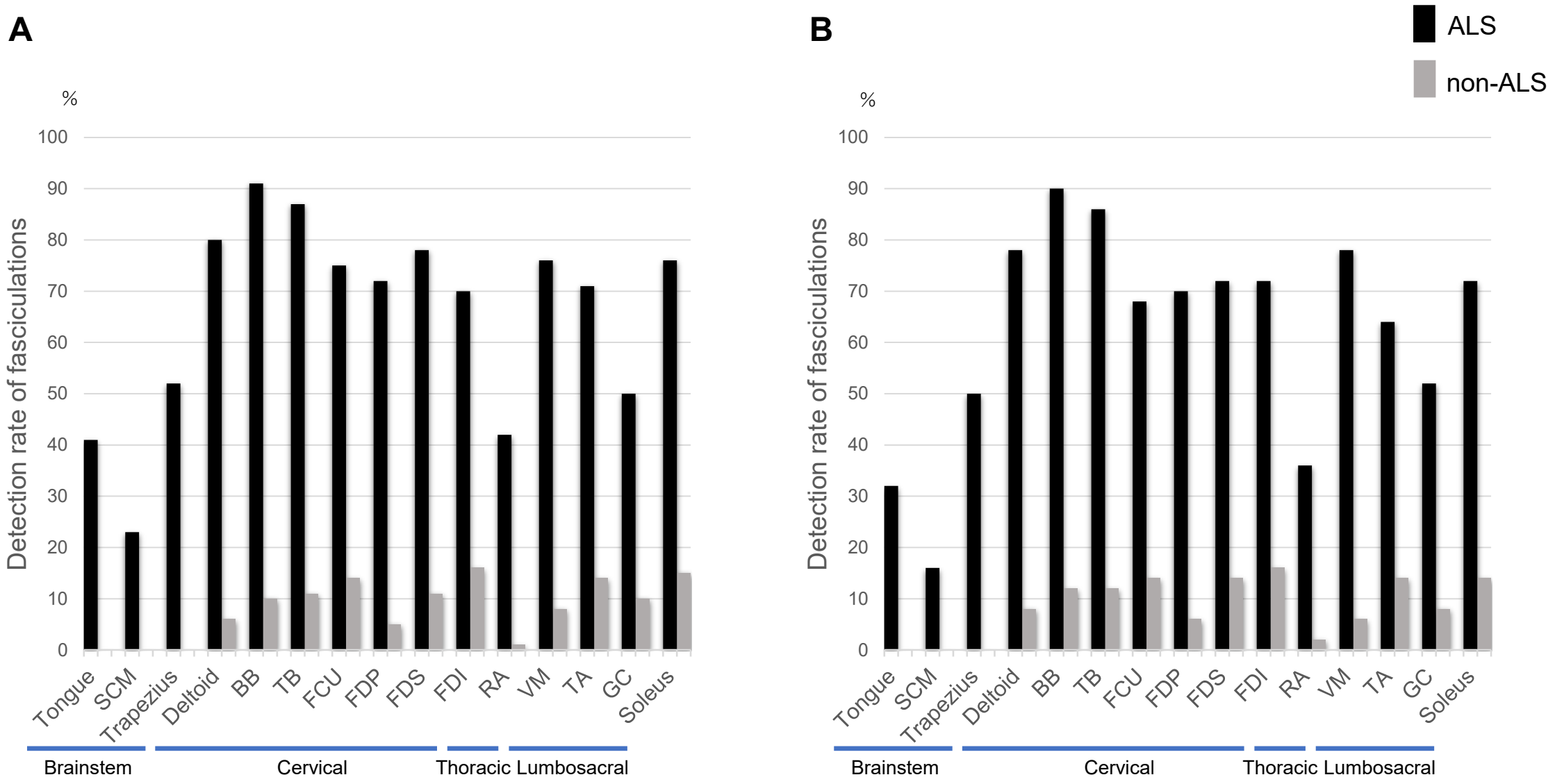
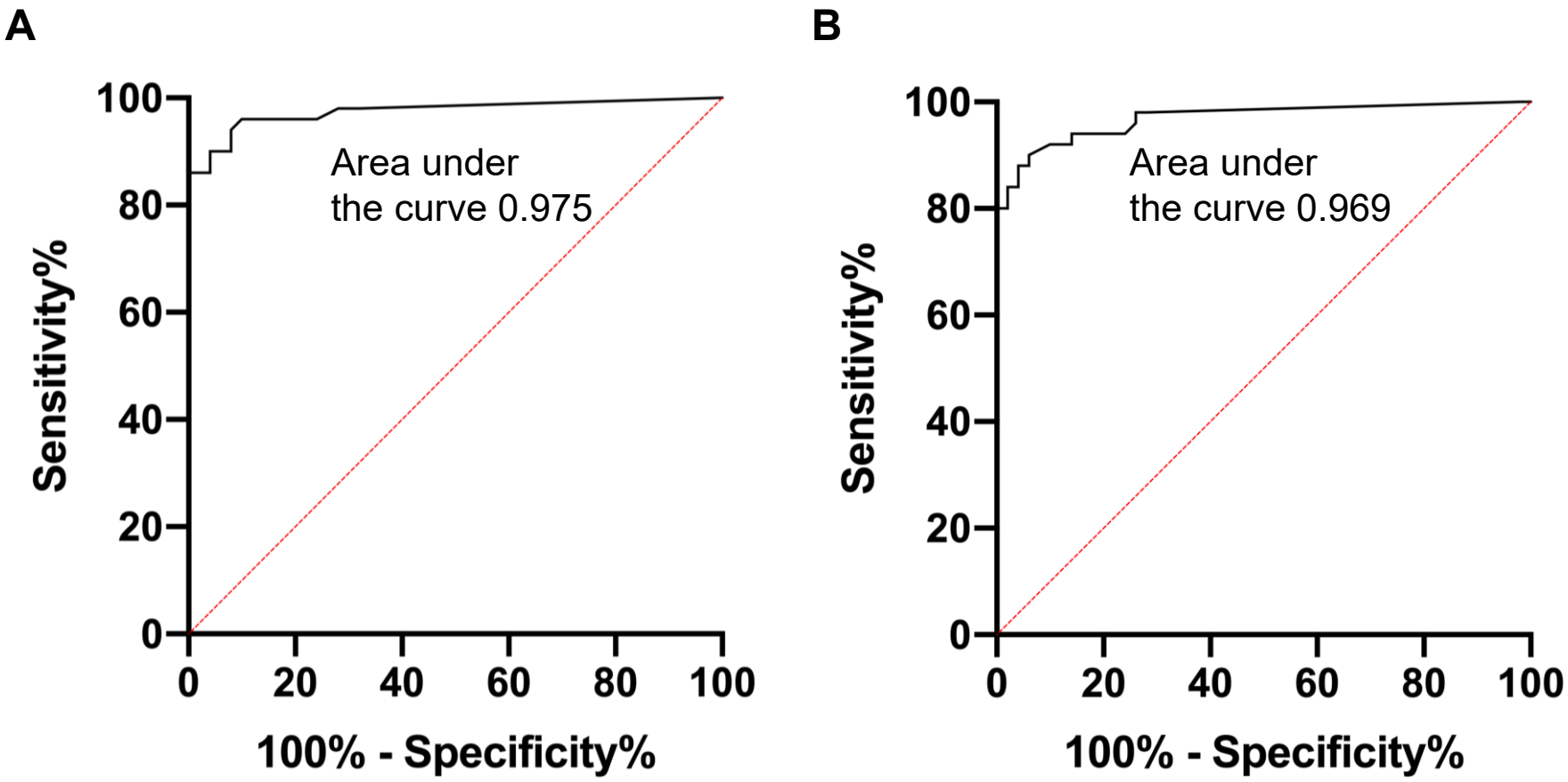


Figure 3



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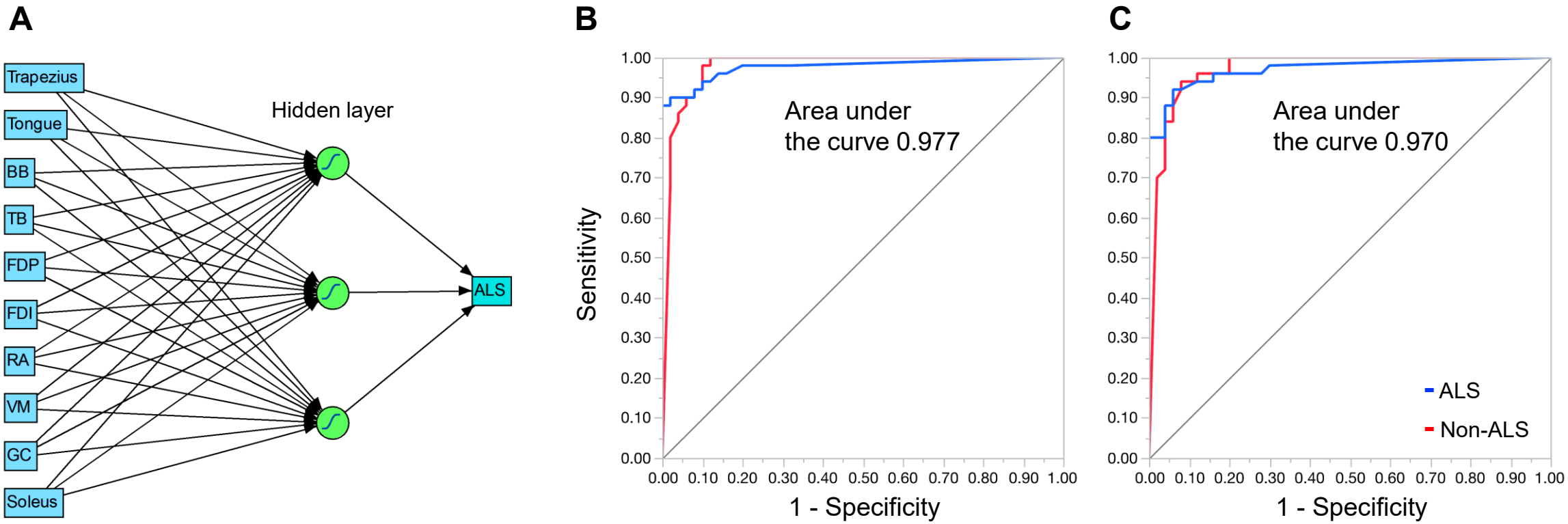

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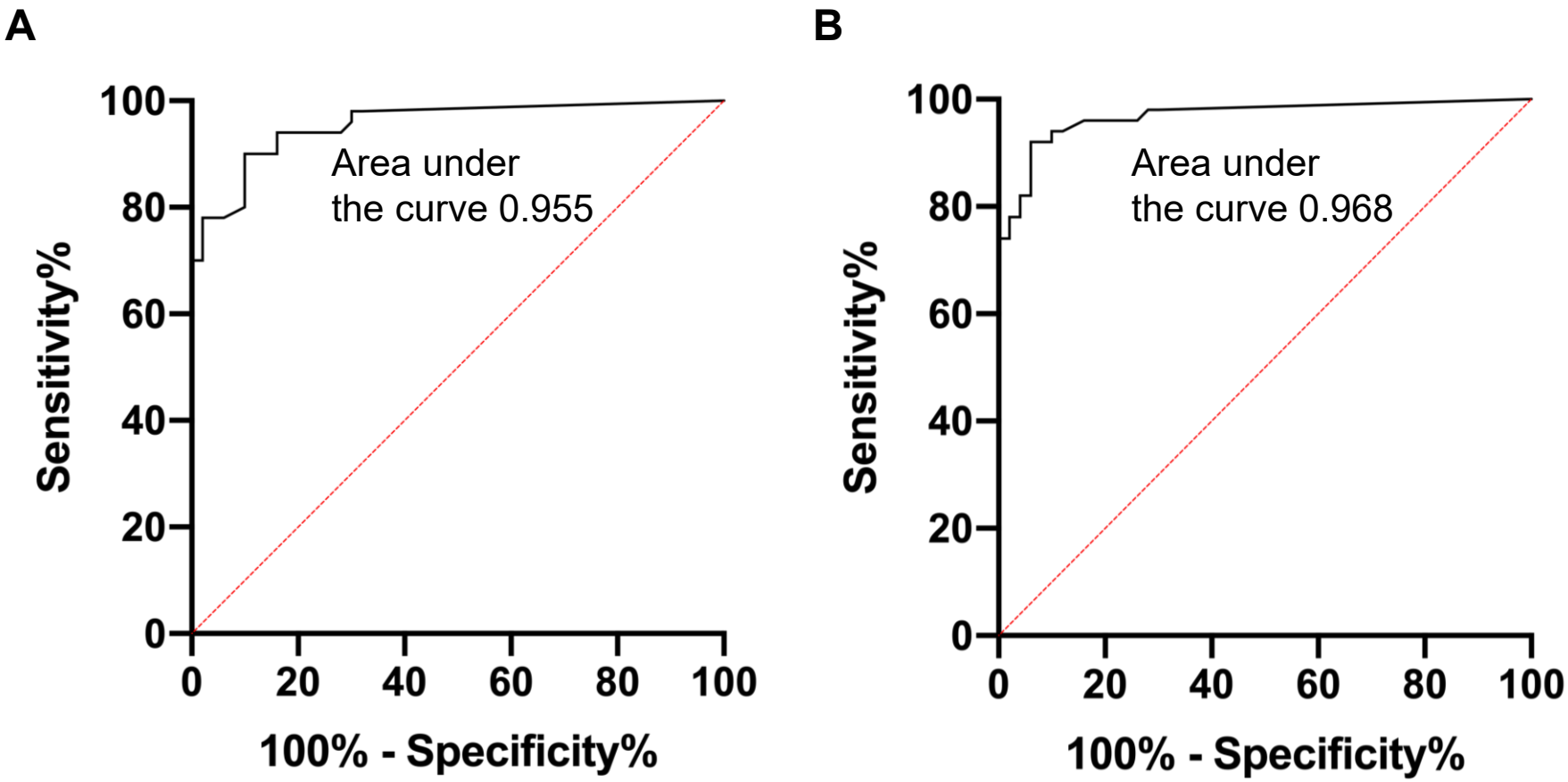

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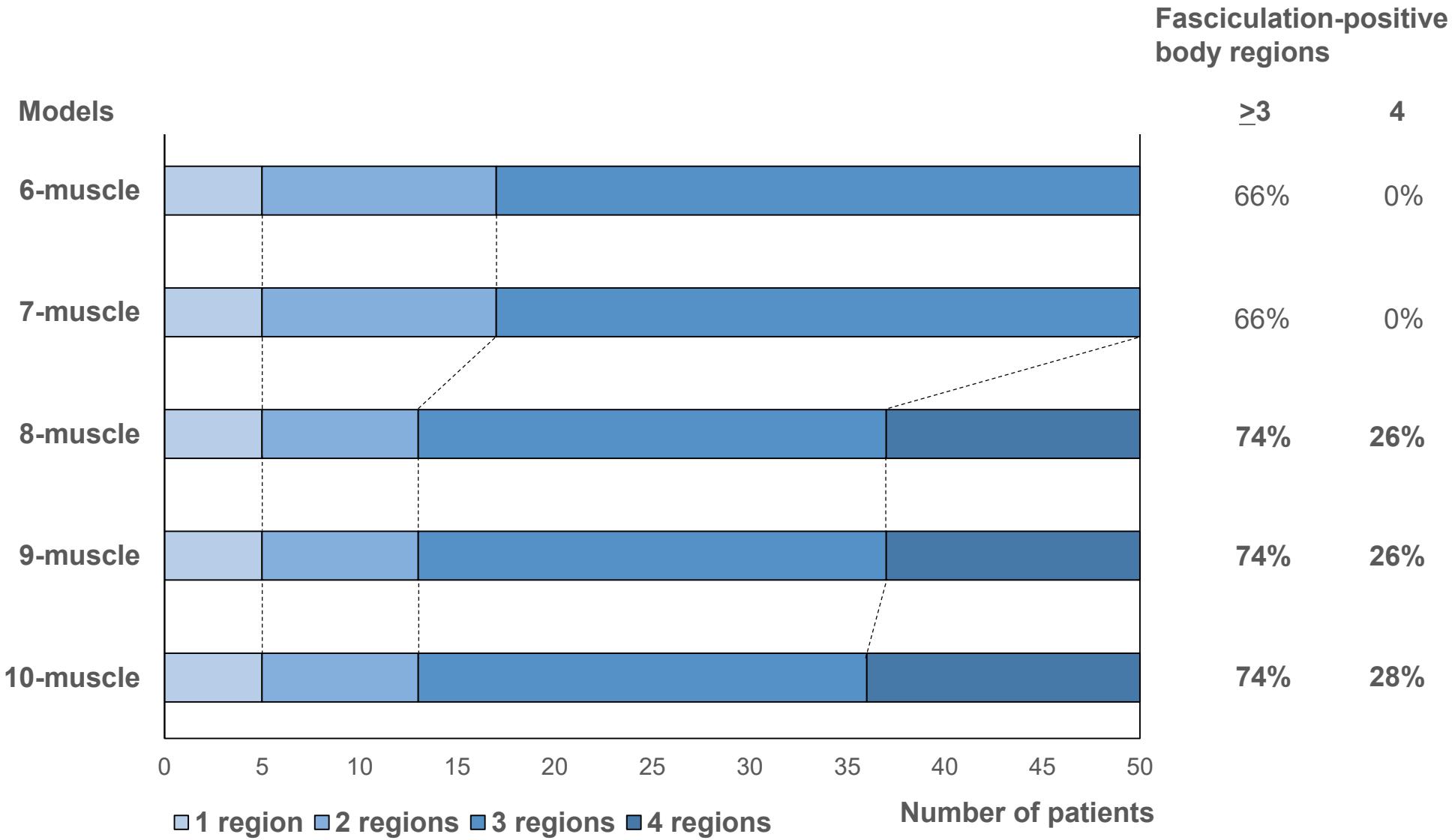

Figure 7

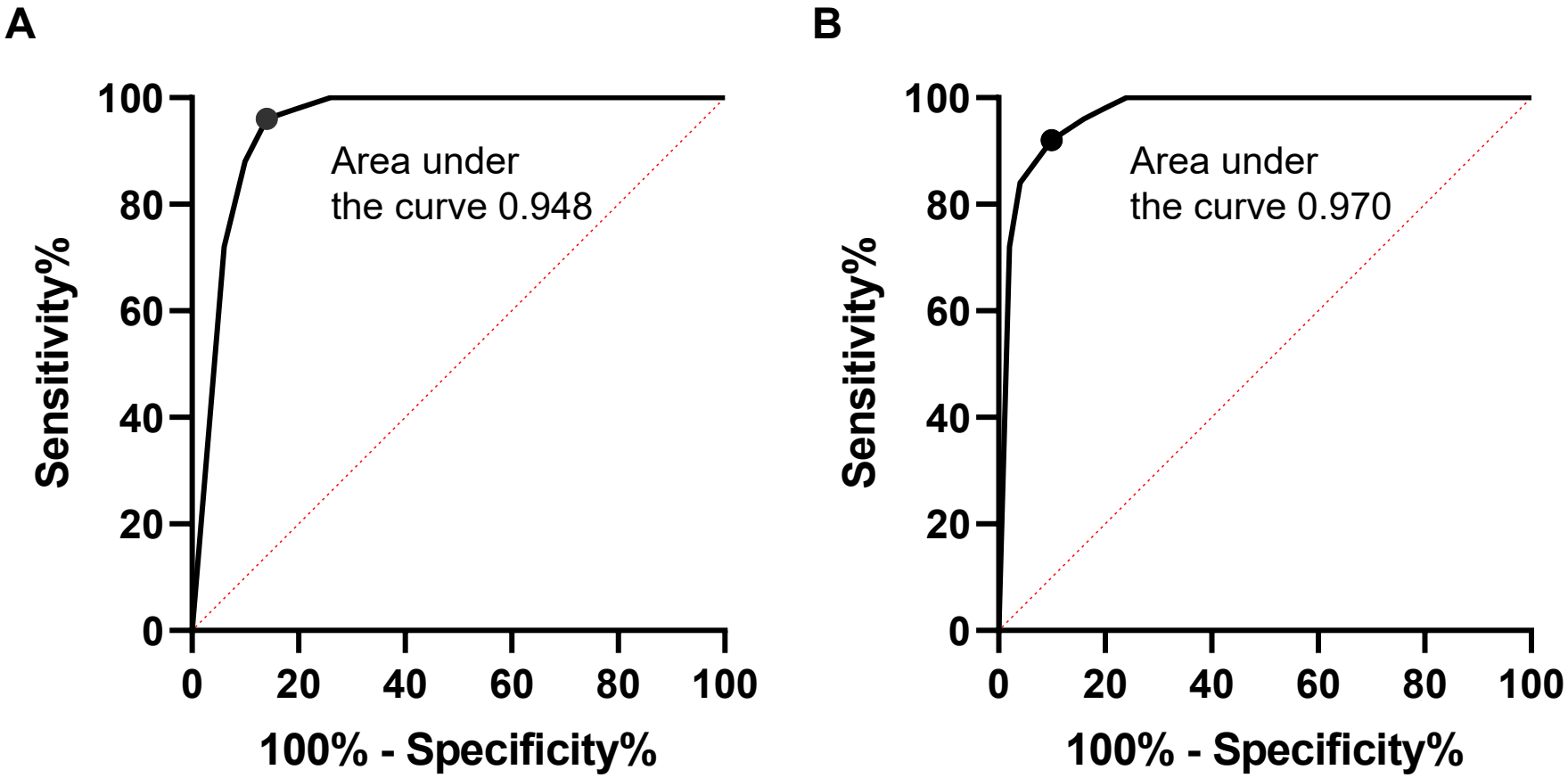

Figure 8