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"Fascicular twitch" on muscle ultrasonography in neuromuscular disorders: quantitative video-image analysis

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ABSTRACT

Objective: In contrast to needle electromyography, the diagnostic performance of muscle ultrasound (MUS) for differentiating neurogenic from myopathic disorders remains unclear. We examined the clinical utility of "fascicular twitch," a visible movement of muscle bundles during isometric contraction, and evaluated its ability to differentiate neurogenic from myopathic changes.

Methods: We quantified fascicular twitches on MUS during isometric contraction as normalized twitch pixels (nTP) using an original image analysis method based on background subtraction. We analyzed echogenicity and nTP values in the biceps brachii muscle of 89 patients with neuromuscular disorders and 42 controls.

Results: The neurogenic group demonstrated substantially higher nTP values than the myopathic group and controls, achieving a sensitivity of 90.5% and specificity of 88.1% at an nTP cutoff of 0.04. Although echogenicity distinguished patients with neuromuscular disorders from controls, it failed to discriminate between the neurogenic and myopathic groups.

Conclusions: Quantification of fascicular twitch via nTP effectively differentiates neurogenic from myopathic disorders, thereby demonstrating that MUS is a valuable, noninvasive diagnostic method for assessing neuromuscular diseases.

Significance: This study introduces fascicular twitch quantification as a novel ultrasound biomarker that uniquely discriminates neurogenic from myopathic pathology, offering a high-performance, noninvasive approach to neuromuscular diagnosis.

1. Introduction

Muscle weakness observed in patients with neuromuscular diseases is thought to originate from either a neurogenic or myopathic cause. Pathology can explain the difference between the two groups; however, biopsies are not performed in all patients. Needle electromyography (nEMG) is useful for objectively verifying the presence of nerve and muscular diseases (Warmolts, 1981). However, nEMG has some disadvantages, such as invasiveness (Al-Shekhlee et al., 2003), with analysis of limited or superficial areas (Harding et al., 2016; Misawa et al., 2011).

Muscle ultrasonography (MUS) has been used since 1980 to detect skeletal muscle pathology and determine disease severity in patients with all neuromuscular disorders (Heckmatt et al., 1980). It can noninvasively (Heckmatt et al., 1982), quickly (O'Gorman et al., 2016), and widely (Misawa et al., 2011) assess muscles. Simple visual analysis provides extensive information about the overall muscle echogenicity, texture, and anatomical context (O'Gorman et al., 2016). Compared with normal muscles, denervated muscles exhibit high echogenicity (Gunreben and Bogdahn, 1991). However, the interpretation of visual evaluation of muscle texture and grayscale levels strongly depends on the subjects and the experience of the observer (Wijntjes and van Alfen, 2021). Therefore, quantitative evaluation of MUS is required for an examiner-independent objective assessment.

Quantitative analysis of MUS echogenicity utilizes grayscale analysis, calibration backscatter analysis, and quantitative texture analysis. Grayscale analysis is performed first in children (Heckmatt et al., 1989) and subsequently in adults (Reimers et al., 1996). Muscle echogenicity increases in neurogenic disorders and myopathies (Pillen et al., 2003).

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Chronic progressive myopathy and lower motor neuron disease are sometimes challenging to differentiate (Sogawa et al., 2017) and distinguishing the two based on echogenicity alone is difficult. Calibrated backscatter technique uses the backscatter level in decibels to represent the amount of signal reflected by the tissue into the ultrasound transducer (Zaidman et al., 2008). Quantitative texture analysis utilizes computerized analysis of brightness and darkness patterns in MUS images (Sogawa et al., 2017).

Compared with other imaging modalities, such as computed tomography and magnetic resonance imaging, ultrasound has a notable advantage in real-time dynamic evaluation. However, few previous studies exist that deal with real-time, dynamic MUS evaluation.

When quantitative image analysis is performed via ultrasound, variations in image quality across different facilities and devices can result in differences in the evaluation process. Similarly, when performing visual video analysis, differences in the observer's ability to detect abnormalities can lead to differences in evaluation. Therefore, it would be ideal to have characteristic indicators for objective quantitative evaluation.

We found a novel observation of a rapid, disorganized movement of muscle fascicular bundles – termed "fascicular twitch"- during minimal contraction on MUS in patients with spinal and bulbar muscular atrophy. This fascicular twitch was also observed in other neurogenic conditions, such as cervical spondylotic amyotrophy, but was not observed in patients with myopathic disorders or healthy individuals (unpublished data). The objective of this study was to determine the feasibility of quantifying this fascicular twitch and its clinical utility in differentiating neurological from myopathic changes.

2. Methods

2.1. Fascicular twitch during isometric contraction

We defined "fascicular twitch" as the movement of muscular bundles on the cross-sectional view of a skeletal muscle ultrasound video (Supplementary Video). We observed fascicular twitch during isometric contraction by properly holding the probe in position. The twitch could not be observed during stronger contraction because the targeted movement of the muscular bundle could not be evaluated due to the contraction of other muscular fibers.

2.2. Ultrasound image analysis

2.2.1. Echogenicity of the muscular ultrasound image

We quantified echogenicity by defining the region of interest (ROI) of the target muscle as a square area to be set into the muscle tissue; subcutaneous tissue or bone was excluded. The size of the ROI was maximized as much as possible under the above conditions. The "0" and "255" values were assigned to the black and white pixels, respectively, and "1-254" was assigned to all gray pixel levels. The average echogenicity value in the ROI was also calculated.

2.2.2. Motion detection algorithm

We developed an original application for motion detection by implementing a background subtraction algorithm using Python 3, which is a common motion detection method (Rakibe and Patil, 2013) that utilizes the difference between current and background images (Fig. 1). A background image was iteratively updated and a pixel within the ROI was flagged as "moving" when the absolute difference between the current frame and the background exceeded a fixed threshold (Sobral and Vacavant, 2014). Detailed algorithmic steps, parameter definitions and the formal mathematical expressions are provided in the Supplementary Data (Supplementary document). To determine the optimal coefficient α, the weighting factor for background image renewal in this algorithm, we first performed visual inspection of the original ultrasound videos to record the number of frames in which fascicular twitch was present. We then ran our motion-detection algorithm on the same videos—varying α in steps of 0.05—and counted the frames flagged as "moving." For each α, we compared the visually observed frame counts with the algorithm's counts. A lower α increased sensitivity but also amplified non-muscle motion artifacts. The mean absolute error between visual and algorithmic counts was minimized at $\alpha = 0.55$ (Supplementary Figure 1); therefore, we selected 0.55 as the optimal coefficient. Finally, we applied this algorithm to the regions of interest in the same manner as for echogenicity analysis.

2.3. Quantifying the amount of fascicular twitch

We defined the twitch area as the mean total number of moving pixels in the ROI divided by the ROI area to quantify the number of fascicular twitches at a fixed period during isometric contraction. The

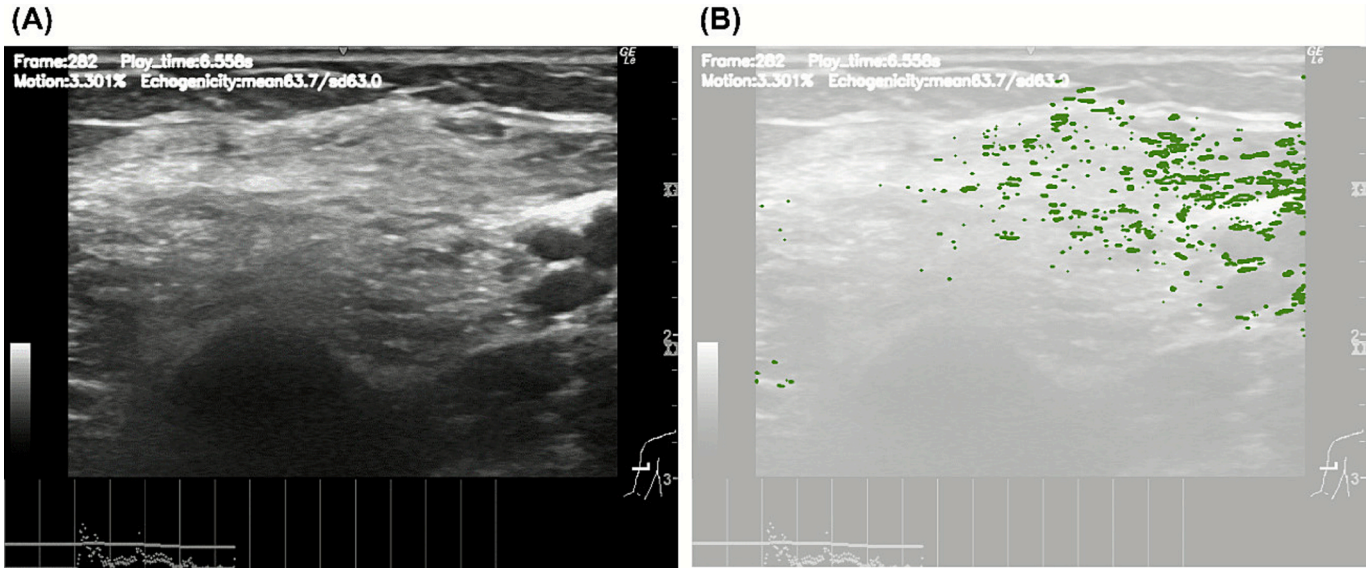

Fig. 1. Motion detection analysis of muscle ultrasound on biceps brachii. A cross-sectional image of the biceps brachii was shown (A). Moving pixels (green dots) detected by the original background subtraction motion analysis application were superimposed on the corresponding image (B). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

twitch area fluctuated in each frame; therefore, to standardize the twitch area, we defined normalized twitch pixels (nTP) as the average of the twitch area during isometric contraction for 2 s.

We compared the number of fascicular twitches detected by the algorithm in the test videos with visual observations conducted by humans to validate the accuracy of the algorithm. These test videos were obtained from anonymized in-house ultrasound archives and included the data from patients with motor neuron disease. Each of the three board-certified neurologists independently counted the number of fasciculations in 20 videos, and the average counts per video were calculated. Thereafter, the algorithm was applied to the same set of videos to identify motion, and the correlation between the average fasciculation count and the detected motion count was examined.

2.4. Participants

All patients who visited the EMG/US section at Kobe University Hospital between September 2021 and March 2024 and inpatients at Hyogo Chuo Hospital in May 2023 were included as candidates. Among them, we registered the patients who matched following condition as participants: 1) suspected having neuromuscular disease and underwent ultrasound examination for diagnosis; 2) above 18 years old; 3) the Medical Research Council (MRC) score of biceps brachii was over grade 3; and 4) confirmed diagnosis by another examination or clinical practice after the examination. The control group included participants with no evidence of abnormality. This study excluded participants who could not indicate their intention to participate in our study or declined to participate on opt-out. Finally, we enrolled 89 patients and 42 controls and divided them into 3 groups according to their clinical diagnosis.

Table 1 presents the demographic and clinical characteristics of the neurogenic, myopathic, and control groups. In total, 42 patients (age: 64.76 ± 14.23 [mean ± standard deviation]) were diagnosed with neurogenic disorders, including 16 patients with cervical spondylotic amyotrophy (CSA), 9 with amyotrophic lateral sclerosis (ALS), 10 with progressive muscular atrophy (PMA), 3 with spinal muscular atrophy (SMA), and 4 with spinal-bulbar muscular atrophy. Furthermore, 47 patients (age, 59.32 ± 16.27) were diagnosed with myopathic disorders, including 9 patients with myositis, 5 patients with muscular dystrophy, 3 patients with immune-mediated necrotizing myopathy (IMNM), 5 patients with inclusion body myositis, 2 with mitochondrial disease, 2 with facioscapulohumeral muscular dystrophy (FSHD), 7 with myotonic dystrophy, and 14 with other myopathies.

Table 1
Mean ± Standard Deviation, median (interquartile range), or proportion of the clinical variables in the three groups of participants.

	Myopathic disorders (n = 47)	Neurogenic disorders (n = 42)	Controls (n = 42)	p value disease group comparison (three group comparison)
Age	62.00 (50.50–73.00)	66.50 (58.00–73.00)	53.17 ± 18.09	0.1536 (0.0122)
Gender, male (n, %)	27, 53.2 %	32, 76.2 %	31, 76.2 %	0.1004 (0.0824)
MRC score	5 (4–5)	5 (4–5)	5	0.8734 (0.0001)
Duration, month	36.0 (5.5–156.0)	24.5 (9.0–72.8)	N/A	0.9901 (N/A)
disease (number of patient)	myopathy(11), myositis(16), MD (5), IMNM(7), IBM(4), FSHD(3), myotonic dystrophy(1)	CSA(16), SMA (3), ALS(9), PMA(10), SBMA(4)		

Abbreviations: ALS, amyotrophic lateral sclerosis; IBM, inclusion body myositis; IMNM, immune-mediated necrotizing myopathy; MD, muscular dystrophy; MRC, Medical Research Council; PMA, progressive muscular atrophy; SBMA, spinal and bulbar muscular atrophy; SMA, spinal muscular atrophy; FSHD, facioscapulohumeral muscular dystrophy; N/A, not applicable.

2.5. Ultrasound examination and data processing

MUS was performed using the GE LOGIQ e system with a 12-MHz linear-array probe (GE Healthcare Japan, Tokyo, Japan) in the biceps brachii by one of two examiners with expertise in MUS (SM and YN, board-certified neurologists and clinical neurophysiologists). The ultrasound image sequence was acquired at 43 frames per second.

Ultrasonographic delineation was performed using a short-axis image at 90 degrees to the brachial bone, and the probe was set at the mid-portion of the biceps brachii. First, the recordings included patients extending their arms in the supine position. They were subsequently asked to flex their elbow and hold it for several seconds with an ultrasound probe on their arms. The resulting videos were saved in Audio Video Interleave (AVI) format and transferred to an external device for analysis. In offline analysis, we examined echogenicity and mean twitch area.

2.6. Statistical analysis

Continuous variables (age, MRC score of the biceps brachii, and disease duration) were compared across neurogenic, myopathic, and control groups by the Kruskal–Wallis test, with pairwise comparisons between neurogenic and myopathic groups by the Mann–Whitney *U* test. Categorical variables (sex) were analyzed by chi-square test. The correlation between fascicular twitch and nTP values was assessed by the Spearman's rank correlation. Receiver operating characteristic curves were generated to determine the sensitivity, specificity, and area under the curve (AUC) for nTP cutoff differentiating the neurogenic group from the myopathic group. All tests were two-tailed, with $p < 0.05$ denoting statistical significance. Analyses were performed in GraphPad Prism (version 8; GraphPad Software, San Diego, California, USA).

2.7. Ethics

The Ethics Committee of Kobe University Hospital approved this study (approval number: B220238). All participants were provided an option to opt out of the study, which was published on the website of Kobe University Hospital.

3. Results

3.1. Validation of an automatic detect algorithm of the twitch pixels

The average number of twitches by visual count and the number of motions detected by the background subtraction algorithm were highly correlated in the test video ($r = 0.97$, $p < 0.0001$; Supplementary Figure 2).

The simple linear regression equation used to detect motion was 1.337 × visual count – 1.174, with $p < 0.0001$ considered statistically significant. The model was significant, and the coefficient of the visual count was 1.337.

3.2. Participants

Table 1 presents the demographic and clinical characteristics of the neurogenic, myopathic, and control groups. No significant difference was observed in age ($p = 0.08$), sex ($p > 0.99$), MRC score ($p = 0.37$), and disease duration ($p = 0.78$) between the neurogenic and myopathic groups.

3.3. Ultrasound image analysis

3.3.1. Echogenicity of the muscular ultrasound images in each group

Echogenicity was significantly higher in the neurogenic ($p < 0.0001$) and myopathic groups ($p < 0.0001$) than in the control group (Fig. 2). However, no significant differences were observed between the neurogenic and myopathic groups.

3.3.2. nTP of the muscular ultrasound image of biceps brachii in each group

Values of normalized twitch pixels (nTP) was significantly higher in the neurogenic group than the myopathic and control groups (both $p < 0.0001$; Fig. 2). We established a cutoff value of nTP > 0.04 using Youden's index (Youden, 1950) for differentiating the neurogenic group from the myopathic group. Using an nTP cutoff of 0.04, fascicular twitch quantification discriminated the neurogenic group from the myopathic group with 90.5 % sensitivity, 88.1 % specificity, and an area under the ROC curve of 0.924 (95 % CI, 0.852–0.978) (Fig. 3). A high positive rate was achieved using this cutoff value even at 6 months after the disease onset, which is considered early stage, in the neurogenic group (Table 2).

4. Discussion

In this study, irregular, disorganized muscle bundle movement – "fascicular twitch" – was noted during isometric contractions of the biceps brachii in patients with neurogenic disorders. We analyzed the muscular ultrasound videos of different patients to quantify these movements. Quantitative analysis application was developed to examine fascicular twitching. Our findings indicate that fascicular twitches during contraction can differentiate between neurogenic, myopathic, and control groups.

The fascicular twitch observed in MUS during isometric contraction may resemble to phenomena previously described as "contraction fasciculation" (Denny-Brown and Pennybacker, 1938) or "contraction pseudotremor" (Riggs et al., 1983), which are thought to result from an enlarged neurogenic motor unit on visually observation. Historically, these phenomena have been observed mainly in the distal periphery. The extent to which fascicular twitches on ultrasound in proximal muscle correspond to these previously documented visually "contraction fasciculation" remain unclear. To the best of our knowledge, neither fascicular twitch nor "contraction fasciculation" have not been previously reported in patients with myopathies.

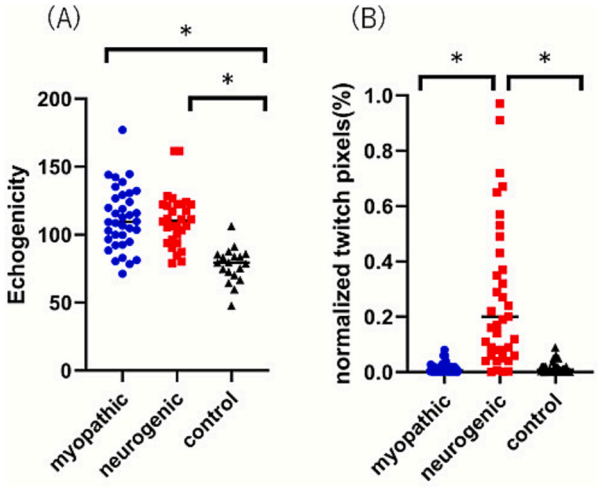

Fig. 2. Echogenicity and normalized twitch pixels (nTP) were compared among the neurogenic, myopathic, and control groups. A: Echogenicity was significantly higher in both the neurogenic and myopathic groups compared with the control group (both $p < 0.0001$), with no significant difference between the neurogenic and myopathic groups. B: nTP was significantly higher in the neurogenic group than in the myopathic and control groups ($p < 0.0001$). *: $p < 0.05$.

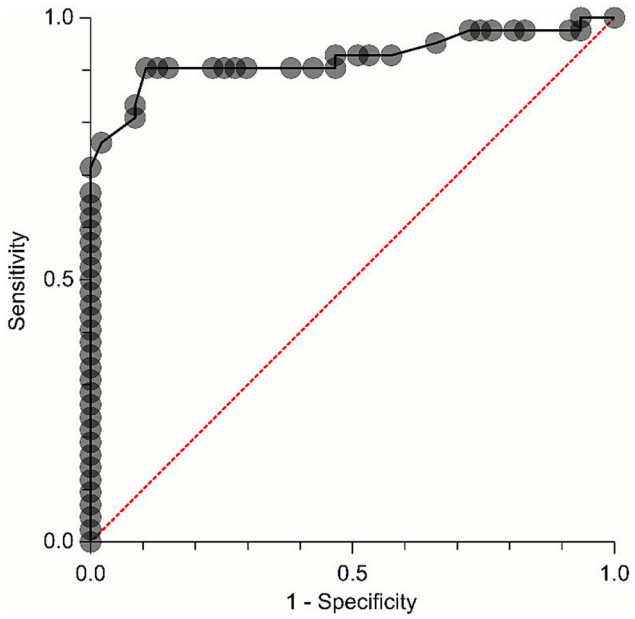

Fig. 3. The performance of normalized twitch pixels(nTP) in diagnosis of neurogenic disorders. The area under the receiver operating characteristic (ROC) curve for differentiating the neurogenic group from myopathic group was 0.924 (95% confidence interval, 0.852–0.978). At a cutoff value of 0.04, sensitivity and specificity for diagnosing neurogenic disorders were 90.5% and 88.1%, respectively.

Table 2
Positive rate of nTP in neurogenic group.

	Duration from disease onset(month)				
	0–6	7–12	13–18	19–24	25-
Positive patient	6	7	5	1	5
Total patient	7	8	5	1	6
Positive rate (%)	85.7	87.5	100	100	83.3

Chronic progressive myopathies and lower motor neuron diseases are often challenging to differentiate (Sogawa et al., 2017), and echogenicity by itself cannot reliably separate these conditions. Additionally, Wijntjes et al. noted that echogenicity analysis is less sensitive than visual assessment for detecting abnormalities in neurogenic disorders because neurogenic pathologies usually present with patchy abnormalities (Wijntjes et al., 2024). Our study quantified visually identified fascicular twitches, which may be a more effective diagnostic marker than echogenicity for differentiating between neurological and myopathic conditions. We used a background subtraction algorithm for quantification. This is the first study which applied this algorithm in muscle ultrasound; however, this technique is well-established in the engineering domain for detecting moving objects (Kalsotra and Arora, 2022).

The detailed pathophysiology of fascicular twitch remains unclear. Multiple factors, including cortical excitability, spinal excitability, maximum motor unit discharge rate, nerve conduction, muscle architecture, muscle mass, myocellular liquid content, and excitation–contraction coupling regulate muscle contraction (Clark and Manini, 2008). However, it remains uncertain which of these factors most significantly influences fascicular twitch occurrence. Older individuals have been observed to have impaired force control ability (an increase in force fluctuation) during precise contractions of index finger abduction, and it is assumed to be a contributing factor that a low-threshold motor unit force – which refers to the initially recruited

motor units based on the "size principle" (Henneman, 1968) – become larger (Galganski et al., 1993). This adaptation is considered that age-related loss of motor units and the increase in the innervation ratio of surviving motor units leads to the earlier activation of larger motor units in elderly individuals (Galganski et al., 1993). Ranganathan et al. reported that force control ability improved when the motor unit force was increased by training (Ranganathan et al., 2001). One reason fascicular twitches may be less likely to occur in normal individuals may be that the biceps brachii muscle has a larger motor unit force than the distal muscle, which contributes to preservation of the force control ability. The fascicular twitch of the biceps brachii may indicate truly abnormal neurogenic alterations and may be useful for estimating the presence of neurogenic alterations.

This study has several limitations inherent to its design and methodology. First, data were obtained from a limited number of institutions, and owing to insufficient data, limitations existed in examining disease duration. These factors may have influenced the accuracy and comprehensiveness of our results. Then, the data used for the analysis were not derived from longitudinal assessments and were biased toward chronic diseases. This may have implications for the applicability of these findings to other diseases. Finally, not all muscles examined using ultrasound were assessed using needle electromyography, which may have influenced the accuracy and reliability of the assessments. However, considering that the study included examinations of neurogenic and myopathic disorders, along with other clinical findings, it played a considerable role in estimating the underlying pathology. Future studies should address these limitations to increase the robustness of these results.

The detailed mechanisms underlying fascicular twitches in neurogenic alterations remain unclear. Moreover, clarification of the clinical importance of this phenomenon necessitates further investigation in the future.

5. Conclusion

We described fascicular twitch, a novel ultrasonographic finding during isometric contraction, and demonstrated that quantifying fascicular twitch effectively differentiates neurogenic disorders from myopathic disorders and controls. Unlike conventional ultrasonographic metrics such as echogenicity, fascicular twitch provides a definitive neurogenic signature, elevating muscle ultrasound to a high-performance, noninvasive diagnostic tool for neuromuscular disorders.

Declaration of generative AI in scientific writing

During preparation for this study, the authors used Chat GPT to edit the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinph.2025.2111447.

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