

CLINICAL RESEARCH ARTICLE

Orbicularis Oculi Stimulated Jitter Analysis in Children With Autoimmune Myasthenia Gravis

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ABSTRACT

Introduction/Aims: Stimulated jitter analysis (stim-JA) involves analyzing the variation in time intervals between stimulations and action potentials, expressed as the mean consecutive difference (MCD). The MCD upper limits are derived from adult populations and warrant a re-evaluation in children to accurately diagnose neuromuscular junction (NMJ) defects.

Methods: A retrospective chart review was conducted which analyzed orbicularis oculi stim-JA studies performed on children between January 2014 and December 2021. The clinical profile, acetylcholine receptor (AChR), and muscle-specific kinase (MuSK) antibody status as well as stim-JA study results were retrieved. Bootstrapping was applied to the stim-JA studies to derive de novo MCD upper limits.

Results: Twenty-seven stim-JA studies were performed on patients aged 3–19 years with either definite myasthenia gravis presentations and NMJ defects ($n = 19$, 17 AChR+, 2 MuSK+) or those with normal neurological examinations who were seronegative ($n = 8$). Four hundred ninety-nine apparent single fiber action potentials (ASFAPs) were analyzed with the individual and mean MCD significantly higher in children with autoimmune myasthenia ($p < 0.05$). Bootstrapping analysis revealed that MCD upper limits of $39 \mu\text{s}$ for individual MCD and $24 \mu\text{s}$ for mean MCD significantly improved specificity ($p < 0.05$) while maintaining sensitivity of the test in distinguishing definite MG NMJ defect from normal NMJ function.

Discussion: Stim-JA using revised upper limits may help clinicians avoid the over-diagnosis of NMJ disorders in children. Stim-JA is a safe and well-tolerated method to screen for definite MG in children over 2 years.

1 | Introduction

Neuromuscular jitter measurement using concentric needle electrodes (CNE) is widely regarded as a sensitive electrodiagnostic (EDX) method for confirming disorders of the neuromuscular junction (NMJ) such as myasthenia gravis (MG) [1, 2]. Because composite signals may appear to be produced from a single muscle fiber, action potentials recorded using CNE are termed apparent single fiber action potentials (ASFAPs). The variation in time intervals between pairs of consecutive

ASFAPs, expressed as the mean consecutive difference (MCD), is widely used to quantify neuromuscular jitter for the rapid diagnosis of NMJ disorders. Despite its utility in adults, the practicality of jitter measured with CNE in children has been limited due to difficulties in having children maintain constant voluntary activation [3]. Therefore, jitter measurement using electric stimulation and ASFAP recording with CNE, referred to as stimulated jitter analysis (stim-JA), has emerged as an acceptable alternative to the volitional jitter measurement with CNE [4–8].

Abbreviations: ASFAPs, apparent single fiber action potentials; CDF, cumulative distribution factor; CMS, congenital myasthenic syndrome; MCC, Matthews correlation coefficient; MCD, mean consecutive difference; NMJ, neuromuscular junction; Stim-JA, stimulated jitter analysis.

We have previously reported the utility of stim-JA of the orbicularis oculi (OO) muscle for the early diagnosis and serial assessment of NMJ defects in infants and children [9, 10]. However, the jitter or MCD upper limits for stim-JA study are not well-established in pediatric populations [11, 12]. On several occasions using the available jitter reference limits, we erroneously reported stim-JA studies as abnormal despite no clinical or laboratory evidence for a NMJ disorder.

Previously, Jabre et al. developed the E-norm algorithm to derive pediatric MCD upper limits and applied it to a pediatric cohort to derive a mean MCD upper limit of 26 μ s in children between the ages of 2 and 3 years, though an individual MCD upper limit in the same age group was not offered [13]. In 2018, Nandedkar et al. developed the E-ref methodology to derive MCD upper limits, though the cohort under investigation was not comprised exclusively of pediatric patients [14]. Further, a multicenter study by Stålberg et al. only included patients 20–80 years of age [15].

The aim of the study was to investigate stim-JA of the OO muscle in children with autoimmune MG to revise the MCD upper limits. We especially wanted to improve the specificity of the technique in distinguishing between children with and without NMJ defect in our cohort.

2 | Methods

A retrospective chart review of patients who underwent stim-JA in the EMG laboratory at Children's Healthcare of Atlanta from January 2014 to December 2021 was performed. The study was approved by the local institutional review boards (IRB #1066) and met the criteria for waiver of informed consent due to the retrospective nature.

2.1 | Stim-JA Study Procedure

Each stim-JA study was performed with the patient conscious, in an un-sedated state, and lying supine with the examiner (S.V.) approaching the patient from the head end (Video S1). An EDX machine with Synergy software version 21 (Natus Medical Incorporated, Middleton, WI, USA) using a stimulated single-fiber EMG study protocol and a high pass filter setting of 1 kHz was used for ASFAP collection. The stimulation settings were a pulse duration of 0.2 ms and a current range of 2–3 mA. The stimulating monopolar electrode placement was visually confirmed by observing the OO muscle twitch at a frequency of 3 Hz. Thereafter, the ASFAPs were recorded at a stimulating frequency of 10 Hz using a 25 mm CNE (Video S2). Sharp rise time (<300 μ s) and a clear separation from the baseline were used to include ASFAP's for analysis (Figure S1). Composite signals with visible shoulders were excluded. For each study, the examiner confirmed that no myopathy or neuropathy was present while the CNE was in the OO muscle. The recruitment patterns were examined for early recruitment, short-duration, and low-amplitude motor unit potentials (MUPs) indicative of myopathy. When assessing for neuropathy, the examiner looked for reduced recruitment of MUPs with large amplitude and duration.

Based on a limited number of studies in both adults and children, the current putative upper limits for the OO muscle in children over 2 years are 34 μ s for individual MCD and 26 μ s for mean MCD, hereafter referred to as reference upper limits [16].

2.2 | Patients

Electronic medical records of all children who had undergone a stim-JA analysis, neuromuscular examination, anti-acetylcholine receptor (AChR), and/or an anti-muscle specific kinase (MuSK) antibody testing during the study period were screened retrospectively. The inclusion criteria were AChR/MuSK seropositive, autoimmune MG patients' studies with a definite MG diagnosis prior to beginning any immunomodulatory therapy and true normal studies in which the child was seronegative, not in clinical remission, and negative for NMJ disorder despite being referred to the neuromuscular clinic for fatigue, intermittent droopy eyelids, or other symptoms raising concerns for MG. The exclusion criteria were any follow-up studies, stim-JA studies performed on children \leq 2 years with unexplained hypotonia, those with genetically confirmed congenital myasthenic syndrome (CMS), those with infant botulism, and those in whom the sampled muscle was not the OO.

Next, the EDX study reports of the included patients were exported from the EDX machine onto a secure hard drive. The study participant's age, race, sex, clinical diagnosis, muscle sampled, number of ASFAPs, MCD values, and number of blocked ASFAPs for each stim-JA encounter was entered into an Excel sheet (Microsoft, Redmond, WA, USA). Individual MCD values < 5 μ s were considered artifacts and removed, while individual MCD values > 150 μ s were replaced with 150 μ s. Further sorting and calculations were performed using custom scripts written in Python version 3.7.9 (Python Software Foundation, Fredericksburg, VA) [17].

2.3 | Bootstrapping

Bootstrapping is a popular statistical resampling technique that utilizes simulated sample distributions for population inferences [18, 19]. In each of 10,000 bootstrapping iterations, sampling with replacement was performed on the dataset to produce a new, simulated dataset of equal length. In each iteration, studies with \geq (1 or 2) ASFAPs with individual MCD > individual MCD cutoff or mean MCD > mean MCD cutoff were classified as NMJ defect. Additionally, the individual and mean MCD combination resulting in the maximum Matthews correlation coefficient (MCC) was recorded in each iteration to obtain a distribution of individual MCD cutoffs, mean MCD cutoffs, and maximum MCCs over all 10,000 iterations [20, 21]. The MCC was chosen due to its widespread use, strength as a balanced measure, and ease of interpretation: a MCC of -1 indicates perfectly inverse prediction, 0 indicates random prediction, and 1 indicates perfect prediction (Equation 1).

$$MCC = \frac{TP \times TN - FP \times FN}{\sqrt{(TP + FP)(TP + FN)(TN + FP)(TN + FN)}} \quad (1)$$

where TP=True Positive, TN=True Negative, FP=False Positive, FN=False Negative.

2.4 | Statistical Hypothesis Testing

The comparison of continuous variables between definite and normal at-intake studies was performed using a one-tailed Mann–Whitney U test. Statistical comparisons of MCC, sensitivity, and specificity between using reference and revised MCD upper limits were performed using one-tailed bootstrap hypothesis tests each using 10,000 iterations of sampling with replacement. In each iteration, the differences between the MCC/sensitivity/specificity resulting from revised MCD upper limits and those resulting from using reference MCD upper limits were recorded. After all iterations, a distribution of all differences was produced for each metric, namely MCC, sensitivity, or specificity. Then, for each metric, a null hypothesis distribution was formed by subtracting the mean of the differences distribution from each value making up the distribution. To obtain a p value for each metric, the proportion of values in the metric's null distribution greater than or equal to the raw difference between revised/reference for the metric (computed using the original dataset) was reported as the p value. For all tests performed, a p value < 0.05 was considered statistically significant.

3 | Results

3.1 | Stim-JA Distinguishes Definite MG From Normal NMJ Function

Out of 221 stim-JA studies within the study period, a total of 194 studies were excluded, and 27 studies (19 definite NMJ defect, 8 normal NMJ), each representing a unique patient, were included. The age range was 3–19 years. There were 15 African Americans, 5 Caucasians, 6 Hispanics, and 1 Asian. In the definite group, 17 study patients were anti-AChR positive and 2 were anti-MuSK positive (Table 1). Each stim-JA study was well-tolerated without sedation, no pain medication was needed, and no follow-up phone calls for pain were received. Only definite studies contained blocked ASFAPs, with 15 out of 19 having $\geq 10\%$ ASFAPs blocked (Table 1). Most notably, the individual, mean, and median MCDs were all statistically higher in the definite compared to the normal group (Table 1).

Because the prevailing stim-JA protocol considers a study abnormal when ≥ 2 ASFAPs exceed the individual MCD threshold, we analyzed each of the 27 stim-JA studies and plotted the maximum individual MCD cutoff value for which that study would be considered abnormal (Figure 1A). Doing so revealed a distinct clustering of definite and normal studies based on hypothetical individual MCD cutoffs, though a slight overlap between

TABLE 1 | Descriptive statistics for 27 at-intake stim-JA studies of definite and normal patients.

| | Overall | Definite | Normal |
|----------------------------|----------------------------------|----------------------------------|---------------------------------|
| Studies, n | 27 | 19 | 8 |
| Age (years) | 15 [12, 17] 3, 19 | 13 [10, 16] 3, 19 | 17 [15, 17] 6, 18 |
| Sex, n | | | |
| Male | 9 | 6 | 3 |
| Female | 18 | 13 | 5 |
| OO side, n | | | |
| Left | 8 | 3 | 5 |
| Right | 19 | 16 | 3 |
| Total ASFAPs, n | 499 | 339 | 160 |
| Study ASFAPs, n | 20 [18.5, 20] 7, 22 | 20 [17, 20] 7, 22 | 20 [19.8, 20.3] 18, 22 |
| Blocked ASFAPs (%)* | 11.1 [0, 22.5] 0, 100 | 19 [10.6, 27.5] 0, 100 | 0 [0, 0] 0, 0 |
| Individual MCD (μ s)* | 32 [17, 51] 5, 150 | 41 [30, 58] 11, 150 | 13 [11, 20] 5, 59 |
| Mean MCD (μ s)* | 40.6 [21.8, 49.6] 10.4, 133.9 | 46.8 [40.2, 53.8] 25.1, 133.9 | 16.2 [13.7, 17.5] 10.4, 23.5 |
| Median MCD (μ s)* | 37.5 [20.5, 45.5] 11, 150 | 40.5 [37.3, 50.8] 24, 150 | 13.8 [11.8, 15.3] 11, 23.5 |

Note: For continuous variables, the median, IQR, min, and max values are shown and statistically significant differences between definite and normal groups are indicated with an asterisk (*).

Abbreviations: ASFAP: apparent single fiber action potential, MCD: mean consecutive difference, OO: orbicularis oculi.

the definite (min = 37 μ s) and the normal group (max = 38 μ s) was observed (Figure 1A). For mean MCD, we observed a non-overlapping separation between the definite (min = 25.1 μ s) and normal (max = 23.5 μ s) groups (Figure 1B). Overall, our findings highlight that stim-JA values differed between patients who were determined to have either definite or normal NMJ function based on non-EDX measures.

3.2 | Revised MCD Upper Limits

We next applied bootstrapping to derive de novo MCD upper limits. When requiring ≥ 2 ASFAPs with elevated MCD for a study to be considered NMJ-defect, a perfect MCC of 1.0 was achieved in 100% of iterations and the most frequent individual MCD cutoff maximizing the MCC was 39 μ s (IQR: 39–39 μ s) (Figure 2A). For mean MCD, a cutoff of 24 μ s (IQR: 21–24 μ s) most frequently resulted in a perfect MCC (Figure 2B).

To see if requiring only one elevated ASFAP was comparable to requiring two, we repeated the bootstrapping procedure and considered a study NMJ-defect when ≥ 1 ASFAP with elevated MCD was present. Using ≥ 1 ASFAP, the optimal individual MCD cutoff was 45 μ s, the mean maximum MCC was 0.816 (95% CI [0.56, 1.0]), and a perfect MCC was achieved in only 12.95% of iterations. These findings suggest that at least two ASFAPs with elevated MCD are necessary when evaluating individual ASFAPs.

Because a study is commonly considered NMJ-defect when blocking is observed in $\geq 10\%$ ASFAPs regardless of individual or mean MCD, we further repeated the bootstrapping procedure (using ≥ 2 ASFAPs for individual MCD) after removing 15 definite studies with $\geq 10\%$ blocked ASFAPs from the dataset. An identical individual MCD cutoff of 39 μ s and mean MCD cutoff of 24 μ s was obtained, with perfect MCC achieved in 99.26% of iterations, highlighting the utility of stim-JA in distinguishing definite from normal studies even without considerable blocking.

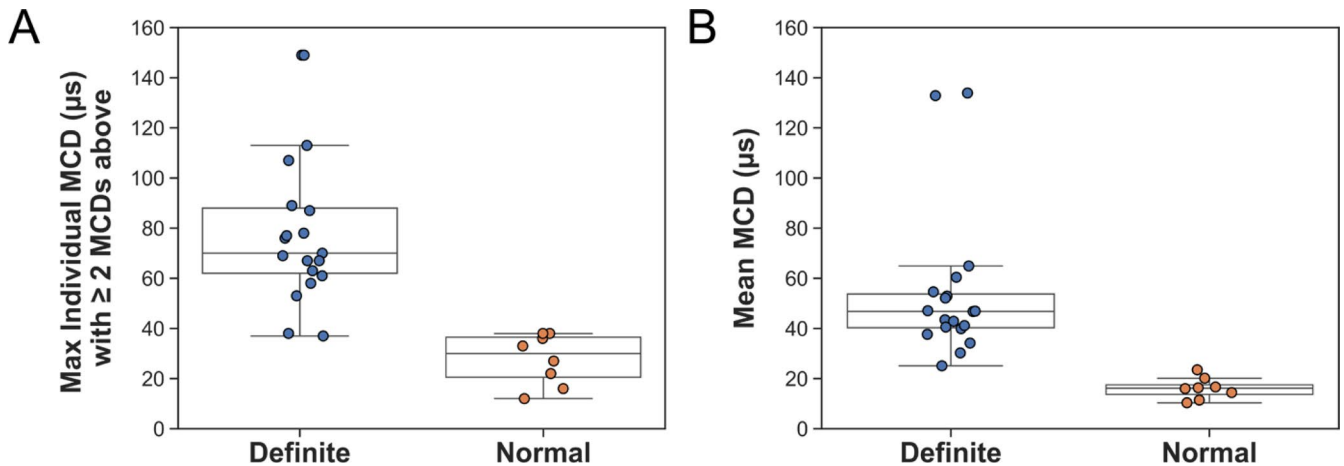


FIGURE 1 | Box-and-whisker plots of all 27 studies consisting of 19 definite (blue) and 8 normal (orange) studies. For definite and normal groups, (A) the maximum individual MCD cutoff value for which each study would be considered NMJ-defect due to having ≥ 2 ASFAPs above the cutoff value is shown. (B) The mean MCD computed from all individual MCDs in the study is also shown.

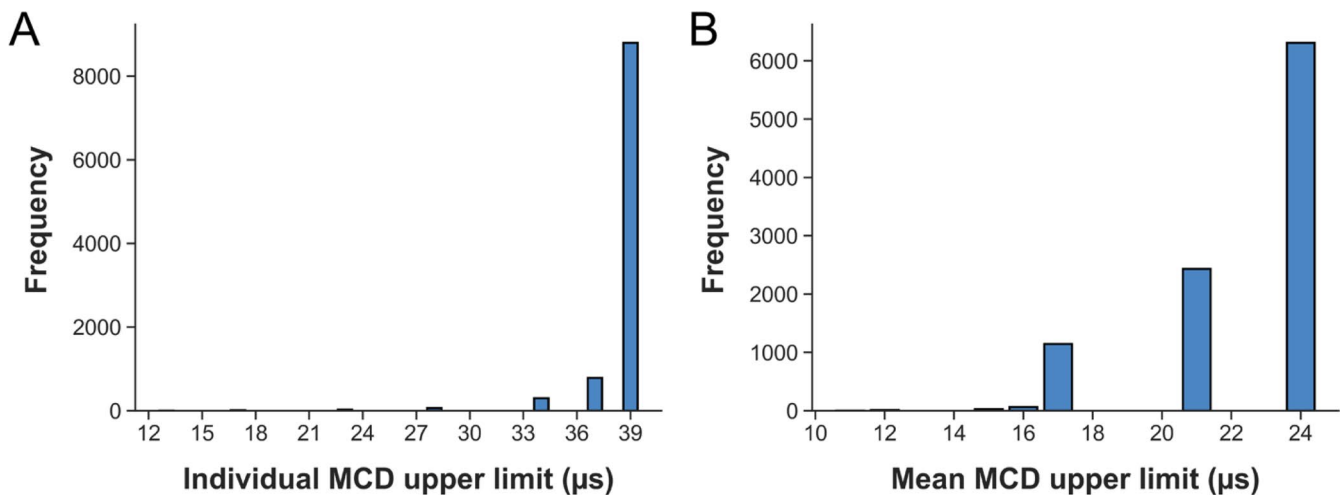


FIGURE 2 | Bootstrapping distributions used to derive revised individual and mean MCD upper limits. Bootstrapping histograms of (A) individual MCD upper limit when requiring ≥ 2 ASFAPs with elevated MCD to be considered NMJ-defect (μ s) and (B) mean MCD upper limit (μ s).

3.3 | Revised MCD Upper Limits Increase Specificity of Stim-JA Study

Compared to reference limits, revised upper limits significantly improved the specificity from 0.625 to 1.0 and the MCC from 0.735 to 1.0 ($p < 0.05$), a change driven by the correction of 3 normal studies that were falsely predicted by reference upper limits to be definite NMJ-defect.

In addition to the combination of $39\mu\text{s}$ for individual and $24\mu\text{s}$ for mean MCD previously highlighted by bootstrapping, we further interrogated the performance of 36 other possible individual/mean MCD combinations with individual MCD upper limit ranging from reference ($34\mu\text{s}$) to bootstrap-revised ($39\mu\text{s}$) and mean MCD upper limit ranging from the bootstrap-revised 2nd quartile ($21\mu\text{s}$) to the reference ($26\mu\text{s}$).

For all combinations tested, sensitivity was not statistically different between revised and reference MCD cutoffs. We observed statistically significant higher MCC and specificity only when using an individual MCD cutoff of $39\mu\text{s}$ in combination with a mean MCD upper limit of either 24 or $25\mu\text{s}$ (Figure 3). While the MCD cutoff of $26\mu\text{s}$ (reference) yielded a statistically higher specificity in combination with $39\mu\text{s}$ for individual MCD, the increase in MCC was not significant, suggesting that an increase in the individual MCD limit must be accompanied by a decrease in the mean MCD limit to optimize stim-JA performance (Figure 3).

4 | Discussion

4.1 | Benefits of Stim-JA Procedure

The stim-JA procedure was feasible, sensitive, and well-tolerated in children with suspected and confirmed MG NMJ-defect. The electromyographer (S.V.) performing the stim-JA study was also managing the patients in clinics, resulting in not only the control

of inter-operator inconsistencies but also a significant depth of clinical, lab, and genetic data collected alongside the ASFAPs. Stim-JA enabled quick diagnosis of NMJ disorder that facilitated prompt treatment ahead of confirmatory laboratory results. The overdiagnosis of patients as NMJ-defect using current reference MCD cutoffs in the clinic warranted a re-look into the upper limits to improve the specificity of stim-JA in children [20].

4.2 | Stim-JA With Revised MCD Upper Limits Is Both Highly Sensitive and Specific

Compared to an ROC curve approach for deriving upper limits, bootstrapping utilized random sampling over multiple iterations to somewhat alleviate the limitations of the relatively small cohort size. Our bootstrapping procedure revealed $39\mu\text{s}$ as the individual MCD upper limit, in agreement with published work by Pitt and colleagues in which $39\mu\text{s}$ maximized diagnostic accuracy in a pediatric cohort [22]. Importantly, we found that regardless of the total number of ASFAPs collected, two abnormal ASFAPs are necessary for diagnosing NMJ disorder with high sensitivity, an implication that may decrease the examination time and improve the tolerability of the procedure for children.

We found that decreasing the mean MCD upper limit from $26\mu\text{s}$ to either 24 or $25\mu\text{s}$ was necessary to improve both the specificity and MCC of stim-JA (Figure 3). While individual/mean MCD upper limits of $34/26\mu\text{s}$ (reference) already had perfect sensitivity in our cohort, they lacked specificity due to resulting in three false positives, that is, cases in which a patient with normal NMJ function was classified as definite MG NMJ-defect. In contrast, upper limits of $39/24\mu\text{s}$ (revised) correctly classified only all the definite MG NMJ-defect patients as abnormal (perfect sensitivity), while simultaneously classifying only all the normal studies as normal NMJ-function (perfect specificity), representing an ideal diagnostic test. Our revised upper limits of $39/24\mu\text{s}$ were somewhat similar to the $36/27\mu\text{s}$ derived by Stålberg et al. but in an adult cohort [15].

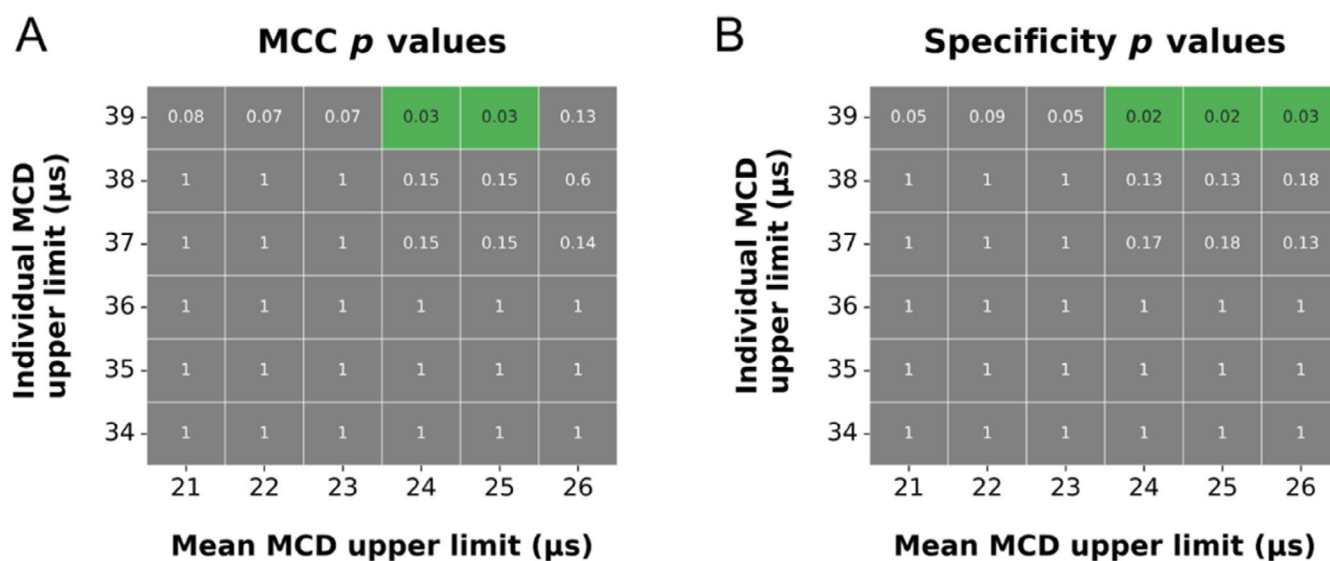


FIGURE 3 | Grids showing bootstrapped p values of (A) MCC and (B) specificity associated with revised versus reference values using various combinations of individual and mean MCD upper limits. Cells with p values ≥ 0.05 are colored gray while those with p values < 0.05 are colored green.

4.3 | Study Limitations, Caveats, and Future Directions

Our study highlights a single center experience, and the stim-JA studies were performed by the treating physician who also knew each patient's clinical diagnosis. Stim-JA is not routinely practiced in EDX laboratories within the United States and there is a relative paucity of trainers to teach this technique to clinicians, which may represent a potential bias. In addition to the data collection methodology, a limitation of the stim-JA procedure is that it may be difficult to interpret when co-morbid neuropathy and/or myopathy is present, given both conditions are associated with increased jitter.

Finally, the reported MCC, sensitivity, and specificity of stim-JA enabled a comparison of revised to reference upper limits in our cohort, but they are not generalizable to the population level due to the small sample size. We have already seen improvements in diagnostic specificity for distinguishing definite MG-defect from normal NMJ function using 39/24 μ s in our center, and plan to monitor the performance of stim-JA with revised upper limits on a larger cohort. Future studies should additionally investigate MCD upper limits for children \leq 2 years and those with either CMS or infant botulism who were excluded from the present work.

4.4 | Conclusion

For distinguishing definite MG NMJ-defect from normal NMJ function in children $>$ 2 years, the stim-JA procedure using revised upper limits of 39 μ s for individual MCD (with \geq 2 ASFAPs above the threshold) and 24 μ s for mean MCD demonstrated increased specificity compared to current reference limits. Our findings may help to reduce the over-diagnosis of MG NMJ defect based on stim-JA evaluation if the results are interpreted carefully in the appropriate clinical setting.

Author Contributions

Vishva Natarajan: conceptualization, methodology, software, data curation, writing – original draft. **Sumit Verma:** conceptualization, methodology, writing – review and editing, investigation, project administration.

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Ethics Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.