Near Fiber Electromyography in the Diagnosis of Myasthenia Gravis

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Abstract

Background:
Near fiber EMG (NFEMG) focuses on the activity of muscle fibers close to the electrode and offers the ability to semi-automatically assess neuromuscular junction instability using measures conceptually similar to single fiber EMG (SFEMG) jitter. As such, compared to SFEMG, NFEMG measures of instability can be obtained significantly faster, with minimal training and manual editing and no marker positioning. The objective of this retrospective study was to compare the accuracy of using NFEMG and SFEMG measures of instability in diagnosing myasthenia gravis (MG).

Methods:
NFEMG was blindly applied to recordings from 50 patients SFEMG-tested at Surgery, Beth Israel Lahey Hospital and Medical Center (BIDMC) in the prior 18 months (12 with MG, 38 without). Excluding the myopathic and neurogenic patients, diagnosis based on NFEMG and SFEMG results were compared to the clinical diagnosis using cross-validation that involved 10 randomly selected training sets and their corresponding testing sets.

Results:
In patients free of myopathy or neuropathy, NFEMG sensitivity was 100% while specificity ranged from 89% to 95% (mean of 90%). When testing on the entire cohort of patients free of other neuromuscular conditions, NFEMG sensitivity and specificity were 100% and 94%, respectively, while SFEMG sensitivity and specificity were 94% and 97%, respectively.

Conclusion:
NFEMG is a rapid technique, requiring minimal training, which is accessible to any physician trained in basic EMG. The results of this study support its promise as an exciting and practical alternative to SFEMG in diagnosing MG, but prospective studies are needed.

Introduction

Myasthenia Gravis (MG) is an autoimmune neuromuscular disease that is underdiagnosed and likely affects more than 70 thousand people in the United States alone[1,2]. The impact on quality of life can be profound and, beyond the life-threatening nature of the condition. The disease can affect many activities of daily living such as vision, breathing, and swallowing, and it is often associated with significant depression and anxiety[3]. The diagnosis of MG classically relies on a combination of clinical findings, presence of autoantibodies, and neuroelectrophysiological studies including repetitive nerve stimulation (RNS) and SFEMG[4]. Although antibodies can be detected in most patients with MG[5], a subset is seronegative, especially ocular MG[6,7]. This requires neuroelectrophysiologic testing to confirm MG and avoid the significant risks associated with overdiagnosis, including years of unnecessary immune therapy and invasive thymectomy or the risks and quality of life implications of underdiagnosis[8–10]. The potential for seronegative MG leads to its inclusion in the differential diagnosis for many patients with weakness of unclear etiology. At most centers, by far the majority of patients undergoing SFEMG do not have MG[13]. However, without confirmation, this large population of patients is at risk of exposure to unnecessary immune therapies. While smaller in size, an important population of seronegative patients are at risk of undertreatment. This results in a high demand for the limited resource of SFEMG, with a healthcare impact far wider in reach than suggested by the relatively small number of seronegative MG patients. Ever more important is the plethora of therapies now available and in the research pipeline that require high performance, efficient, and practical biomarkers of therapeutic response both clinically and in research.

SFEMG assesses electrophysiological temporal dispersion variability between pairs of muscle fibers belonging to the same motor unit (MU) using high pass filtered potentials recorded using single fiber or concentric needle electrodes, and has been shown highly sensitive and specific in the diagnosis of MG[14,15]; however, sensitivity and specificity varies significantly depending on the level of training and population studied[10,16]. In addition to requiring significant training and time to perform, its poor availability in rural and underserved areas in the United States and worldwide[17] likely results in a consequential healthcare disparity for those suspected of having MG, although this has not been directly quantified to date.

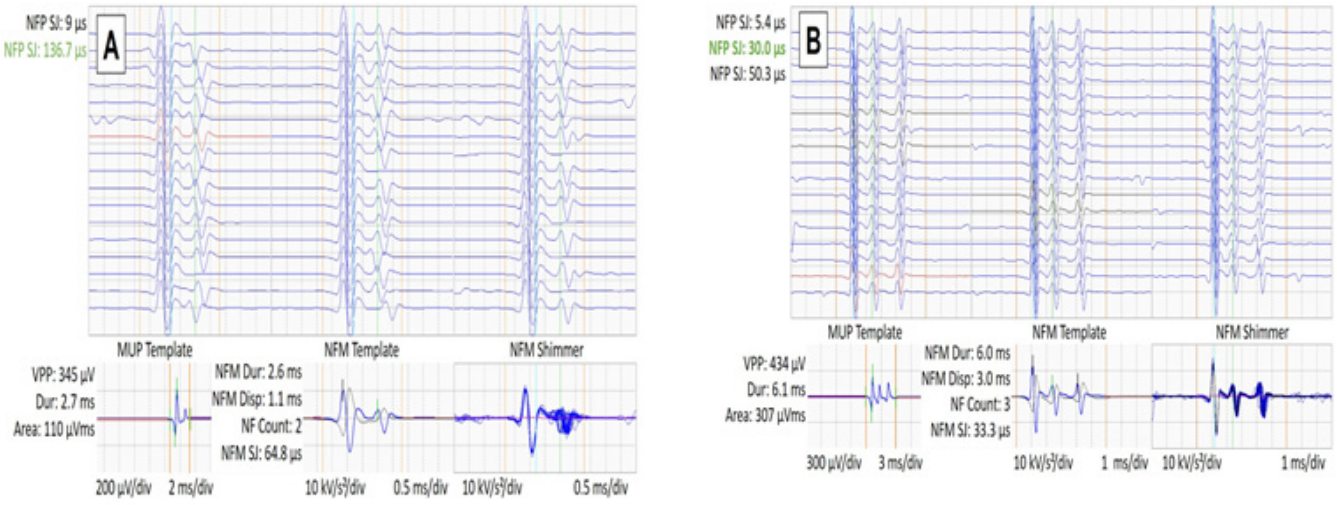

Figure 1. Exemplary NFEMG. The lower panel in each figure shows a raster of NFMs, aligned to the cyan lines, containing 2 NFPks (A) and 3 NFPks (B), respectively. To the left of each raster are the NFPk SJ values associated with each NFPk; these are conceptually similar to SFEMG fiber-pair jitter values. In the lower panels of each figure section, from left to right: a MUP and NFM template and NFM shimmer (overlapped single NFM traces). NFM and MUP feature values are shown: the orange lines demarcate NFM duration. Each short vertical line on the NFM template corresponds to a NFPk. The time interval between the first and last NFPk is the NFM dispersion. NFPk count is the total number of NFPks in the NFM template. NFM duration, dispersion and NFPk count inform about electrophysiological temporal dispersion. NFM SJ and NFPk SJ inform about global and local electrophysiological temporal dispersion variability, respectively.

NFEMG is the study of near fiber MU potentials (NFMs)[18–20], which are motor unit potentials (MUPs) that have been filtered using a low-pass-double differentiation filter. Each symmetric-shaped peak in an NFM, or NF peak (NFPk), represents the contribution of an individual fiber, or a small group of fibers, close to the recording electrode. To assess electrophysiological temporal dispersion variability, NFEMG uses segment jitter (SJ) values based on offset times between matched segments of consecutive NFMs and calculated in a fashion similar to mean consecutive difference (MCD) values. Global NFM instability can be assessed using NFM SJ values based on all NFM segments within the NFM duration. Local NFM instability can be assessed using NFPk SJ values based on the segments of individual NFPks. Example NFEMG data from two MUs are shown in Fig.1. In comparison, SFEMG uses fiber-pair jitter statistic values based on times between individual threshold crossings of pairs of MU fiber potentials. The primary NFEMG parameter of interest in this study is NFPk SJ.

NFEMG is a semi-automated process, requiring significantly less time to perform than SFEMG. Potential benefits of NFEMG over SFEMG stem from: a) there being no need to focus/trigger on a specific fiber-pair, saving significant time in searching for a fiber-pair inherent in standard SFEMG and requiring significant training, b) multiple fiber-pairs are extracted for each contraction, in contrast to SFEMG that triggers on a single fiber-pair, c) a significant degree of post-processing/signal cleaning is automated, reducing time spent selecting signals to include in the jitter analysis, d) multiple other metrics are obtained simultaneously, including MU characteristics, that may aid in diagnosis and improve specificity. The training requirement for NFEMG compared to SFEMG is minimal and includes basic signal cleaning, likely requiring less than an hour of training (from our experience); although further development and automation promises to minimize this aspect further still.

A complementary and important feature of NFEMG is the additional information provided. This includes a wide range of additional quantitative morphological and firing frequency metrics that have the potential to improve diagnostic accuracy beyond just a measure of electrophysiological temporal dispersion variability; this may especially be of significance to specificity.

In an initial study into the diagnostic potential of NFEMG for MG[19], NFPk SJ values were directly compared to the jitter value of the exact corresponding fiber-pair as measured by conventional SFEMG to assess correlation and diagnostic concordance between the two techniques when measuring the jitter from the same fiber pair. Correlation (Spearman) between SFEMG and NFPk jitter values was 0.76. The mean difference between SFEMG and NFPk jitter values was 16 µs, without a trend towards over or underestimation. Using a dichotomous classifier, only 12.8% of SFEMG fiber-pairs with increased jitter values showed normal NFPk SJ values (false negative indications), and 9.6% of SFEMG fiber-pairs with normal jitter showed increased NFPk SJ (false positive indications). SFEMG thresholds used for classification were obtained from a multicenter study using concentric needles[21] whereas NFEMG thresholds were obtained

applying the extrapolated reference values procedure[22]. This study formed the basis for the current study and the decision to evaluate SFEMG versus NFEMG decisions at the muscle level.

Given the conceptual similarity between information provided by SFEMG and NFEMG, coupled with the outcomes of initial investigations, this current study was designed to further evaluate the potential utility of NFEMG in the diagnosis of MG by assessing its performance in a setting that reflects its clinical application, i.e. the population with diagnostic uncertainty that comprises those referred for SFEMG testing. The objective of this single-center, retrospective observational study was to assess the performance of NFEMG in diagnosing MG through comparison to both a patient's clinical diagnosis and to SFEMG. NFEMG can be applied with minimal training in a fraction of the time taken to perform SFEMG, reducing patient discomfort, increasing reliability due to minimizing user variability through automation, and potentially addressing an important health service gap.

Methods

NFEMG was applied to EMG signals recorded under a SFEMG protocol from 50 adult patient studies completed over an 18-month interval. IRB approval was acquired and requirement for informed consent was waived. A patient study was included if raw (i.e. 10Hz-10kHz bandpass filtered, removing the SFEMG high-pass filter) EMG signals were available, the study was performed to assess for MG, and the study contributed to a formal clinical diagnosis. Studies were excluded if the EMG signals were corrupted or contaminated with significant artifacts, fewer than nine contractions were obtained, or the diagnosis remained unconfirmed at the time of data analysis. The raw EMG signals were recorded for a minimum of 5 s using Natus Synergy EDX systems and 30-gauge disposable CNE electrodes (Natus Teca Elite; uptake area of 0.03 mm^2) and filtered with a 1–10 kHz bandpass filter for SFEMG analysis (exported with standard CNE filter settings of 10Hz-10KHz). Recordings were conducted by five different but experienced and fellowship trained electromyographers.

The raw EMG signals were exported and reformatted for DQEMG[23,24], which automatically extracted one or more MUPTs per contraction and performed the NFEMG analysis. One experienced electromyographer (RM) reviewed extracted MUPTs in all recordings using the DQEMG interface, with secondary review performed on several recordings to ensure consistency (DS) (both reviewers were blinded to the diagnosis). MUPTs were excluded if there was significant artifact or needle movement in the recordings that could not be rapidly accounted for using manual editing, in a similar fashion to cleaning of SFEMG traces. NFPk SJ values less than 10 µS and those associated with a NFM with only one NFPk were excluded.

Numerous different approaches on varying patient populations have been taken for determining thresholds for SFEMG jitter over the years[21,25,26]. All of these involve defining a threshold value for a specific SFEMG jitter statistic (i.e. mean or number of individual outliers) calculated using a control-data training set. A positive indication of MG is provided if the SFEMG jitter statistic, calculated using values sampled from an examined muscle, exceeds the defined threshold. To define similar diagnostic criteria to be applied to NFPk SJ values, threshold values for specific NFPk SJ statistics (i.e. mean or number of individual outliers) calculated using a control-data training set were defined. Two NFPk SJ statistics were considered: mean-NFPk SJ and percentage of high-NFPk SJ values.

The mean-NFPk SJ is simply the mean of the NFPk SJ values measured in a muscle/patient. A high-NFPk SJ value is above a high-NFPk SJ threshold and is not expected to be measured frequently in a control muscle/patient. A high-NFPk SJ value suggests some level of abnormality (a possibility of disease). The high-NFPk SJ threshold value was set as 2 standard deviations above the mean of all individual NFPk SJ values across all the 34 control studies considered. The percentage of high-NFPk SJ values is the percentage of high-NFPk SJ values measured in a muscle/patient.

A control-data training set included 15 healthy controls, randomly selected from the total pool of 34 controls (i.e. patients without MG, neuropathy, or myopathy). Given a specific randomly selected control-data training set, the corresponding 31-member test set contained the remaining 19 unselected controls and the 12 MG patients (and excluding 4 cases with myopathy and/or neuropathy). Using the threshold defined for each statistic, two diagnostic criteria were applied to the data in the corresponding test set and evaluated. For the mean-NFPk SJ and percentage of high-NFPk SJ values statistics, a positive indication of MG was assumed if the corresponding NFPk SJ statistic, calculated using values from a muscle/patient in the test set, exceeded the defined threshold associated with the corresponding NFPk SJ statistic.

Ten-fold cross validation was completed to assess the generalizability of each of these diagnostic criteria (i.e. to provide a range for sensitivity and specificity). Across the ten selected training sets, for each training set, the mean of the mean-NFPk SJ values was calculated, and this mean plus 2 SD of the mean-NFPk SJ training set values was calculated as a training set mean-NFPk SJ threshold. The mean of the ten mean-NFPk SJ thresholds was then used as the mean-NFPk SJ threshold for all testing sets. The threshold value for the percentage of high-NFPk SJ values used for all testing sets was empirically determined as the value that provided the highest sensitivity-specificity performance across the testing sets, with a bias toward sensitivity given the potential for NFEMG to act as a screening test prior to SFEMG. The calculated mean-NFPk SJ threshold and determined percentage of high-NFPk SJ

values threshold criteria were then applied to the entire cohort of patients free of other neuromuscular conditions.

SFEMG jitter values were reported as the mean absolute value of consecutive differences (MCD). Diagnosis, age, gender, and presence of a condition that might affect jitter results, as well as mean jitter and percentage of individual pairs above published age-adjusted SFE thresholds[27] were recorded. Internal parameters of the automatic DQEMG technique were set to the same values as described in prior articles[28]. We report descriptive statistics, including mean and standard deviations, and 95% confidence intervals. We created two-by-two tables to assess the occurrence of abnormal jitter values in patients with and without MG. From these tables, we calculated sensitivity and specificity for NFEMG and SFEMG. All P values were two-sided with a significance of 0.05. Results were analyzed using SPSS version 26.

Results

Clinical diagnosis of MG was confirmed in 12 out of 50 patients based on clinician judgement following CNE SFEMG (Table 1). Four patients had alternative neuromuscular conditions. Ages ranged from 25 to 86 years (mean 60.7 years), with 57% male and 43% female. For the NFEMG analysis, the mean number of NFPk SJ values included per subject was 61. For the SFEMG analysis the mean number of SFEMG jitter values included per subject was 16. The mean of mean-NFPk SJ and mean-SFEMG jitter values were slightly different for the 34 healthy patients (25.53 µs vs 28.57 µs, p=0.032). The mean of the meanNFPk SJ values for the 12 MG patients was lower compared to the mean of the mean-SFEMG jitter values for MG patients (47.42 µs vs 64.83 µs, P<0.14). Looking at NFPk SJ values in aggregate, without regard for patient association, the mean NFPk SJ value of those under 60 years old was significantly different to those over 60 years old (25.3 µs and 29.5 µs, respectively; p<0.001).

Table 1. Sample size, gender distribution, and mean NFPk SJ and SFEMG jitter values for MG and healthy patients. **Excluding 4 patients with myopathy or neuropathy.*

	Count (%)	Mean (Range) (µs)	SD (Var) (µs)
Age	50 (100%)	60.7 (25-86)	16.27 (265.0)
Males	29 (58%)	-	-
Females	21 (42%)	-	-
mean-NFPk SJ (healthy)*	34 (68%)	25.5 (17.8-34.2)	3.77 (14.2)
mean-NFPk SJ (MG)	12 (24%)	47.4 (33.7-81.5)	15.38 (236.5)
mean-SFEMG Jitter (healthy)*	34 (68%)	28.6 (15.0-42.0)	6.58 (43.4)
mean-SFEMG Jitter (MG)	12 (24%)	64.8 (35.0-146.0)	36.73 (1349)

Correlation statistics between mean SFEMG jitter and mean NFPk SJ for all patients are plotted in Figure 2. All three statistics demonstrate strong correlation between the two metrics, including the intraclass correlation coefficient (ICC).

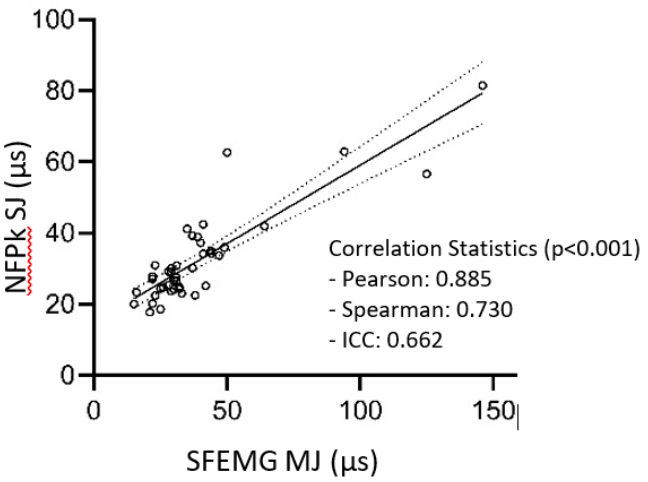

Figure 2: Correlation between patient mean NFPk-SJ and SFEMG jitter across all patients. ICC: intraclass correlation coefficient.

Across the ten-fold cross-validation completed, the mean-NFPk SJ threshold value was calculated to be 33.3 µs. Table 2 (columns 2 and 3) displays the sensitivity and specificity results across the 10 test sets and for the entire cohort using the mean-NFPk SJ statistic and a mean-NFPk SJ threshold value of 33 µs. Across the 10 testing sets, sensitivity ranged from 75%-100% with a mean of 98%, while specificity ranged from 75%-87% with a mean of 82%. For the entire cohort of patients free of other neuromuscular conditions, sensitivity and specificity were 100% and 88%, respectively.

The high NFPk SJ value threshold calculated across the 34 control studies was 60.3 µs. A high NFPk SJ value threshold of 60 µs was applied to each training set and the mean percentage plus 2 SD of high-NFPk SJ values was determined for each training set. The range of the percentage of high-NFPk SJ values across the 10 training sets (3.2%-9.2%) was then used as the range over which the percentage of high-NFPk SJ values threshold was varied to search for the best sensitivity-specificity performance across the 10 testing sets which resulted in a selected percentage of high-NFPk SJ values threshold value of 8% (threshold values in the 7-9% range provided similar results). The percentage of high-NFPk SJ values statistic and this selected percentage of high-NFPk SJ values threshold value was then applied to the 10 test sets (to estimate a range for sensitivity and specificity) as well as to the entire cohort of patients free of other neuromuscular conditions (Table 2, columns 4 and 5). Across the 10 testing sets, sensitivity was consistently

Table 2: Results using a mean-NFPk SJ threshold of 33 μs for each testing set as well as the entire cohort (columns 2 and 3). Columns 4 and 5 show results when using the combination of a high-NFPk SJ threshold of 60 μs with a percentage of high-NFPk SJ values threshold of 8% for each testing set as well as the entire cohort.

	Mean > 33 μs		**8% > 60 μs**	
Testing Set	**Sensitivity**	**Specificity**	**Sensitivity**	**Specificity**
1	100%	85%	100%	90%
2	100%	-85%	100%	90%
3	100%	84%	100%	89%
4	100%	84%	100%	95%
5	100%	87%	100%	93%
6	100%	75%	100%	90%
7	100%	78%	100%	89%
8	75%	84%	100%	89%
9	100%	83%	100%	89%
10	100%	78%	100%	89%
Mean	**98%**	**82%**	**100%**	**90%**
Min	75%	75%	100%	89%
Max	100%	87%	100%	95%
Entire Cohort	**100%**	**88%**	**100%**	**94%**

100%, while specificity ranged from 89%-95% with a mean of 90%. For the entire cohort of patients free of other neuromuscular conditions, sensitivity and specificity were 100% and 94%, respectively.

When the mean-NFPk SJ threshold and the percentage of high-NFPk-SJ values threshold were used in combination, the results did not surpass the performance achieved by using the percentage of high-NFPk SJ values threshold alone (sensitivity and specificity of 100% and 94%, respectively).

We separately re-analyzed the diagnostic performance of NFMEG using a protocol that more closely matched the intended application of NFEMG, i.e., within the context of a standard EMG protocol. As such, we only included the first 20 NFPk SJ values, which usually occurred within the first 5 or 6 contractions (exported SFEMG recordings). Reassuringly, the performance of NFEMG remained high in the face of this reduced amount of data (sensitivity 92%, specificity 88%).

Across the entire cohort of patients free of other neuromuscular conditions, the SFEMG sensitivity and specificity were 94% and 97 respectively.

Discussion

SFEMG is the most accurate neurophysiological test for assessing the neuromuscular junction instability that occurs in MG, and the most widespread application of this method during the last two decades has been using concentric needle electrodes (CNE). This method requires extensive training, time, and patient tolerance to complete. The low availability outside of major academic centers in many countries potentially exposes seronegative MG patients to the risks of undertreatment and likely far more to overtreatment[8–10]. The significant variability in the application of SFEMG between centers will also likely result in substantial variable diagnostic performance. Using NFPk SJ values has the potential to overcome many of the issues associated with SFEMG including, the time burden of the study, patient discomfort, variability due to user, and significant training requirements. The results of this preliminary investigation into the diagnostic accuracy of using NFEMG, specifically, NFPk SJ values, in MG suggest they perform similarly to SFEMG jitter values.

Although this study is only an initial assessment of the ability of using NFPk SJ values for diagnosing MG, based on the relatively modest retrospective sample, the sensitivity and specificity of using the combination of a high-NFPk SJ value threshold in conjunction with a percentage of high-NFPk SJ values threshold (100% and 89-95%, respectively, across all 10 testing sets, and 100% and 94% for the entire cohort of patients free of other neuromuscular conditions) for the diagnosis of MG compared well with using SFEMG jitter values (94% and 97% for the entire cohort of patients free of other neuromuscular conditions). In most of the patients that were determined falsely positive using NFPk

SJ values, SFEMG jitter values were also high but did not meet the associated SFEMG lab thresholds or levels of clinical suspicion for a diagnosis. Given the characteristics of the tests and sampling error, it is inevitable that discrepancies will occur in borderline cases between tests (concordance) and, indeed, in the same test at different time-points (reliability). In borderline cases such as these, whether just above or just below a given threshold, numbers should not be relied upon concretely but rather the clinical picture and additional clinical data will always dictate the eventual diagnosis[29]. A borderline range may be clinically more useful than concrete thresholds with a dichotomous result. Whether a lab uses SFE thresholds for SFEMG or one of the newer CNE based thresholds may impact the precise sensitivity and specificity of the test (as do many other factors related to testing, patient characteristics, pretest probabilities, and clinical context) but should not alter clinical management because borderline results are only an indicator of post-test probability, similar to any other clinical data point, and should be used as such within the paradigm of inductive reasoning applied in clinical diagnosis.

SFEMG was used as a secondary comparator in this study, with clinical diagnosis being used for the primary comparison. It is important to note that SFE thresholds[30] were used to determine normal and abnormal SFEMG jitter results in this study, as opposed to the increasingly used CNE thresholds[21] published more recently. As mentioned, using higher thresholds (SFE thresholds) likely reduces false positives. However, this has not necessarily been borne out in studies using CNE to date, which have suggested little effect on diagnostic results between using SFE or CNE electrode thresholds in the few studies to have examined this directly. Several groups have compared CNE to SFE SFEMG jitter values[15,16,21,25,31,31–37]. Erta et al.[38] found no significant difference between CNE and SFE mean jitter values, number of abnormal pairs, or ability to identify patients with unstable neuromuscular junctions when recorded in the same patients. In a slightly later study, Farrugia et al.[36] similarly found no significant difference between mean CNE and SFE jitter values, while Papathanasiou and Zamba-Papanicolaou[39] noted no significant difference when applied to stimulation SFEMG. However, although most studies initially seemed to report no difference in jitter values when directly comparing recording techniques, suggested thresholds for CNE are frequently lower than those published for SFE[21,30], and usually without accounting for age. Kouyoumdjian[35] surmise that summated signal jitter may be more or less than that measured from individual fiber potentials, depending on which analysis method is used (earliest part of the signal or signal peak).

Kokubun et al.[26] report numerous potential cut-off values for voluntary Frontalis CNE SFEMG jitter values; possible thresholds reported for mean jitter values were between 27.7 µs and 53.4 µs, and for individual pair values between 43.8 µs and 56.8 µs. The multicenter study with perhaps the strictest criteria to date[21] recommends mean MCD jitter thresholds (based on 2 SDs above the mean of the mean MCD jitter values) of 31 µs for Orbicularis oculi and 28 µs for Frontalis, with individual jitter value thresholds at 45 µs and 38 µs respectively (using 2 SDs above the mean value of a patient's 18th highest individual jitter value), which is similar among the majority of CNE SFEMG studies. As with any test, effectiveness is dependent on its application in practice, and the level of adherence to published guidelines is dependent on numerous factors, including availability and application of published guidelines[15], quality and quantity of training, patient population, and physician characteristics among others[25]. Few if any studies have assessed the diagnostic accuracy of SFEMG jitter values as generally practiced, where less strict and varied criteria are usually applied. An ability to standardize a diagnostic test as much as possible is essential, and removing examiner and threshold variability through automation is one such way to improve applicability and minimize disparities in testing as widely applied in clinical settings. NFEMG represents one such way to achieve this.

CNE SFEMG Jitter values of patients referred for SFEMG but free of neuromuscular conditions in this study ("healthy controls") were similar to Kokubun et al.[26], but higher than most other studies. This may in part be due to the retrospective nature of the study, the variety of examiners, a non-research-based setting, and the age and other characteristics of the participants. The "healthy controls" were not specifically selected, were symptomatic (referred for SFEMG and thereby representative of the target population for the diagnostic), and may have had underlying conditions resulting in increased jitter that were not documented and may also have skewed the values. Furthermore, if reference values are used that have a higher cut-off, there may be a tendency to cut short the cleaning of data once the study is deemed negative and accept lower signal quality, which may artificially inflate the mean jitter values reported.

Although original SFE reference values are based on age brackets[27,30], some studies have reported little difference in mean jitter with age[21], while most reports have noted a trend, perhaps depending on the numbers of elderly included in the studies and muscles tested (limb versus cranial). In this study, the mean-NFPk SJ values of healthy controls over and under 60 years of age differed significantly (25.3 µs vs 29.5 µs; $p<0.05$; see Fig. 5).

There are a number of additional investigations required to fully explore the potential of using NFPk SJ values for the diagnosis of MG. Large prospective studies across centers would allow for, 1) rigorous interrogation of the reliability of the values across the breadth of relevant practitioner characteristics, 2) assessment of

the benefit of the additional quantitative data provided by NFEMG including, NFM duration, NFM dispersion and NFPk count as well as MU firing rates, which may aid substantially in test specificity and patient care[13], and 3) further improvement to algorithms and incorporation of the technique into current workflows and machines.

Limitations

In addition to a relatively small sample size, the retrospective and observational nature of this study was a main limitation. As such, recordings were not obtained or cleaned for SFEMG in as controlled a manner as can be achieved during a prospective study. In addition, in this study the NFEMG analyzed signals were recorded during SFEMG (i.e. using SFEMG needle positioning techniques but with standard EMG filter settings). In general, signals acquired for NFEMG analysis are expected to be acquired during standard needle EMG examinations and as such may not be as "focused" as SFEMG recordings. It is unclear if this will have an impact on the diagnostic performance or efficiency of NFEMG but the majority of NFPk SJ values were not based on the fiber pair targeted by SFEMG, therefore the impact of how the NFEMG analyzed signals were recorded is likely to be minimal. As such, it is anticipated that NFEMG results would likely not differ significantly when standard CNE EMG recordings are used.

Incorporation bias is often present in studies assessing the diagnostic accuracy of SFEMG in MG[40]. This bias is avoided in the primary aim of this study because the diagnostic accuracy of using NFPk SJ values was the main objective, and the results of using NFPk SJ values were not utilized in diagnostic decision making. However, when comparing the performance of NFPk SJ values to SFEMG jitter values, incorporation bias needs to be considered, although this would favor the performance of using SFEMG jitter values over NFPk SJ values. Spectrum bias[40] was also reduced in this study (MG was not confirmed in any patient prior to testing), although pretest probabilities for a diagnosis of MG varied greatly between included patients.

This retrospective observational study was primarily designed to provide preliminary data on the feasibility of using NFEMG to detect and diagnose MG. Increased numbers of MG and control patients need to be studied across multiple sites and users. In addition, whether patients are in clinical remission, the severity of their MG or MGFA class, distinction between ocular and generalized, the AChR antibody titer, presence of prior myasthenic crises, thymoma or thymectomy, or medications should also be considered.

Conclusion

This initial study suggests that NFEMG could be effectively used to diagnose MG with similar accuracy but in a more practical manner compared to SFEMG. However, prospective studies are needed. Characteristics including greater yield of jitter values per recording, significantly reduced acquisition time, minimal training requirement compared to SFEMG, and potential to apply the technique to signals acquired during routine EMG suggest NFEMG may be able to serve as an efficient screen prior to referring for SFEMG or as an effective alternative diagnostic test. The low threshold to widespread clinical uptake offers the potential to cost-effectively address a significant national and global healthcare disparity for the large population of patients with weakness and the potential for seronegative MG.

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