

E-Norms: A Method to Extrapolate Reference Values From a Laboratory Population

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Purpose: Collecting reference values in subpopulations such as infants and children can pose a daunting challenge to gather through epidemiologic studies. The authors propose to evaluate a method the authors refer to as extrapolated norms (e-norms) to derive Stimulated Single Fiber EMG jitter reference values from the laboratory data of pediatric and adult cohorts. Single Fiber EMG studies are considered the gold standard test for evaluation of neuromuscular transmission disorders.

Methods: Data that lie in the plateau part of an inverted S curve derived from sorted jitter data were used to calculate descriptive statistics for pediatric and adult e-norms jitter.

Results: The e-norms derived jitter was $22 \pm 2.83 \mu\text{s}$ for our pediatric and $21 \pm 2.79 \mu\text{s}$ for our adult cohort. Our adult e-norms values compared favorably with the $22 \pm 1.99 \mu\text{s}$ published jitter range derived from a healthy adult cohort.

Conclusions: The e-norms method the authors describe seems to be useful in recovering reference ranges when such values are difficult to obtain, such as in a pediatric subpopulation.

Key Words: Normal Values, Pediatric, Neuromuscular Transmission, Single Fiber EMG, Jitter MCD.

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INTRODUCTION

Normal ranges of many biomedical markers are unavailable for some subpopulations—such as infants and children—due to the extra burden of a measurement that may inflict on this cohort. Since these values are unlikely to be obtained through epidemiologic studies, innovations in estimating reference values may serve to provide some insight in the study of such subpopulations. In this work, we present a scenario to derive a hypothetical reference range of a clinical neurophysiology test with different subsamples using a technique developed by Jabre (Proceedings of the 30th International Congress of Clinical Neurophysiology ICCN 2014, Berlin, Germany, 279) and referred to as extrapolated norms or e-norms. We will describe our efforts in deriving reference values from 2 cohorts; a pediatric subpopulation of infants from 1 week to children younger than 18 years of age, referred to a pediatric academic medical center

for Stimulated single fiber electromyogram (StimSFEMG) for evaluation of a suspected disorder in neuromuscular transmission; and an adult cohort aged 18 to 88 years old referred to an academic medical center for StimSFEMG evaluation, also for a suspected disorder in neuromuscular transmission.

Single Fiber EMG studies, performed either with voluntary contraction of the subject or with stimulation of the nerve or muscle fibers, are the gold standard tests for the evaluation of disorders of neuromuscular transmission. Using a special needle electrode inserted in a muscle, they measure the variability of neuromuscular transmission time referred to as jitter, expressed in microseconds, and measured by calculating the mean consecutive difference (MCD) of successive single muscle fiber action potential discharges (Stålberg et al., 2010).

In our search of the literature, no reference values derived from an age-matched healthy pediatric cohort were found. Only adult reference values existed for these studies and were widely available in the literature (Kouyoumdjian and Stålberg, 2011).

METHODS

All StimSFEMG studies were performed by trained electromyographers (EMGers) who specialize in the SFEMG technique (M.P., J.D.).

Our objectives were as follows:

1. To use the e-norms method to analyze the data submitted to us in the adult population for which the authors had not developed their own normal values but where normal values for such cohort existed in the literature.
2. To compare the e-norms data derived from our adult cohort to healthy individuals reference values from the published studies in the literature (Kouyoumdjian and Stålberg, 2011) for validation purposes.
3. To use the same e-norms method to analyze the data submitted to us in the pediatric population for which the authors had not developed their own normal values and where no normal values for a similar cohort exist in the literature.

Sources of Data

All investigators obtained approval from their local research and development departments to use their collected laboratory data for the purpose of analysis and publication. Two sets of SFEMG data obtained from patients referred for the evaluation of a suspected disorder in neuromuscular transmission were analyzed. In both, only the mean MCD jitter value was made available for analysis.

Set 1 was derived from a pediatric cohort of 657 patients investigated between July 17, 2007 and September 2, 2013, aged 1 week to 18 years (0–18 years), who underwent a StimSFEMG study of the Orbicularis Oculi muscle using a concentric needle electrode

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recording of single fiber action potentials. The skin was anaesthetized in this cohort using topical anesthesia. StimSFEMG was performed on a Dantec Keypoint EMG machine (Alpine Biomed ApS, Skovlunde, Denmark). High and low band pass filter settings were 3 and 10 kHz, respectively.

Set 2 was derived from an adult cohort of 98 patients investigated between June 19, 2010 and October 25, 2013, aged 18 to 88 years, who also underwent a stimulated SFEMG study of the Orbicularis Oculi muscle using a concentric needle electrode recording. No skin anesthesia was used in this cohort. The studies were performed on an XLTEK XCALIBUR v 1.4 EMG machine (Excel-Tech Ltd, Oakville, Canada). High and low band pass filter settings were 2 and 10 kHz, respectively. All the data were collected retrospectively.

Both pediatric and adult studies were performed on the Orbicularis Oculi muscle using a facial concentric needle electrode recording inserted just lateral to the outer canthus. Muscle fiber potentials were first identified at a stimulation rate of 2 to 3 Hz. The stimulation rate was then increased to 10 Hz.

A minimum of 20 sweeps were collected and this constituted a run. Single fiber action potentials or apparent single fiber action potentials (Stålberg and Sanders, 2009) were identified using the algorithm on the EMG machines, which used peak detection methodologies, and also calculated the jitter MCD. In the pediatric population, the mean number of fibers studied was 33, although in 35 of 657 pediatric patients, 10 fibers or less were collected. We decided to include those in our data set as the small number of fibers collected reflected more the fact that the study was quite abnormal early in the course of the examination indicating that there was no longer a need to continue with it. In routine clinical work, the mean jitter and the percentage of the abnormal individual potentials that exceeded 34 μ s were recorded. The mean MCD was considered abnormal if $>26 \mu$ s.

Conceptualization of the E-Norms Method

We will first use a simulation study to describe the e-norms concept we propose. Figure 1 displays 1,000 simulated MCD values

that, for illustration purposes, have a mean of 20 μ s and a SD of 1.5, giving a mean ± 4 SD value of 14 and 26 μ s, respectively.

We set out to use the e-norms method on these data to see whether we can extract the mean ± 2 SD values using only the plot of the MCD data (ranked in ascending order) along with a fitted “tangential” and the MCD first-order derivative.

The MCD data display a graph that reveals an inverted S curve with a steep lower left, a middle “plateau,” and a steep upper right as can be seen in Fig. 1.

Our plateau identification process relies on the concordance of two parameters; one, the tangential inflection points with the inverted S curve; and two, the row of first-order derivatives (e.g., difference of MCD 2 – MCD 1, MCD 3 – MCD 2, etc.) that display the lowest first-order difference. We find that when there is concordance between these two parameters, we tend to get the most reliable results. The MCD data points at the two left and right extremes show higher first-order differences between them, whereas those at the center (between points A and B identified by the inflection points and delineated by the two vertical dotted lines) display smaller first-order differences, with steady increment. Such steady increments correspond to the flattest or plateau part of the curve indicating that when examining the value-to-value differences of ranked continuous measurements, the plateau population can be characterized with the slowest rate of increase.

The graph shows that the values in the lower left and upper right of the curve lay outside of the normal limits we set out for our simulated data. In the steep line at the lower left of the curve, mean MCD values lay in the range of 13 to $<17 \mu$ s, which in this case would include subjects with “supra” normal values, that is, better mean MCD values that would be expected from those derived from a normal age-matched population. The values in the ascending steep portion at the upper right of the curve lay in the range of >23 to 28 μ s, which in this case would be derived from subjects that do reveal the neuromuscular transmission abnormality they were referred to for electrophysiological evaluation.

The flattest portion of the curve that lay between points A and B is formed by a mean MCD range that lay between 17 and 23 μ s. This range represents the normal limits of data we set out to represent our normal population. In this simulated subgroup of subjects, values

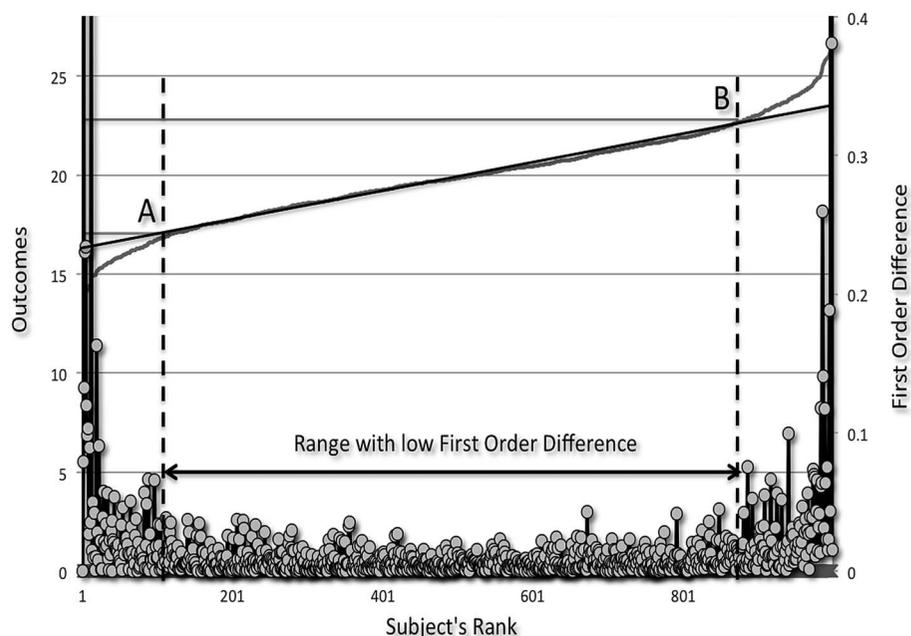


FIG. 1. First-order difference (dots) and cumulative density (curve) of a simulated variable with 1,000 data points, mean of 20 μ s and SD of 1.5. Overlaying a “tangential” straight line onto the inverted S curve, along with a plot of the first-order difference to reveal the data points range with low first-order difference, help in identifying inflection points A and B.

in this range reveal the steadiest increments in MCD values and can be used to derive descriptive statistics for the mean MCD.

E-Norms Analysis of Adult and Pediatric MCD Data

Using the simulation concept we proposed above, we applied the e-norms method to analyze the adult and pediatric MCD data to derive reference values for these two cohorts. Both sets of data were collected and entered into a Microsoft Excel spreadsheet. Variables included patient’s age and mean MCD measures. To compile the plot of the MCD values first-order difference, we sorted the MCD data in ascending order and then calculated a new variable representing their first-order difference. We then plotted the first-order difference against the subjects MCD rank. This resulted in an inverted S-shaped curve that, like in our simulated data, showed three distinguishable parts: a relatively steep line at the lower left, transitioning into a relatively flat portion resembling a plateau at the middle, and ascending into another steep portion at the upper right.

RESULTS

Adult Data Set

Figure 2 shows how our set 2 adult e-norms MCD reference values were derived using the ranked mean MCD (curve) and the first-order difference (dots) of our data. The segment with the lowest first-order differences is easily identified between the two arrows. The corresponding range in MCD data is determined by locating the

cross-sections of the arrows and the curve (points A and B) and the inflection points where the inverted S curve starts to separate from a fitted “tangent.”

The range of our e-norms MCD reference values for our set 2 adult cohort is 16 to 26 μ s. The number of MCD data points within the plateau is 61 of 98 total mean MCD data points in this adult cohort, or 62% of the total.

Pediatric Data Set

We then applied the same analysis method to the mean MCD data collected from 657 pediatric patients aged 1 week to 18 years (set 1) to derive e-norms reference values for this pediatric cohort. Figure 3 shows how our e-norms reference values were derived using the ranked MCD (curve) and its inflection points from a fitted “tangent” and the first-order difference (dots) of our data.

The range of the e-norms reference values in the plateau part of the curve is 16 to 26 μ s. The number of MCD data points within the plateau is 298 of 657 total mean MCD data points in this pediatric cohort, or 45% of the total.

Table 1 shows the descriptive statistics of our pediatric and adult e-norms MCD reference ranges and a comparison of our age-matched adult e-norms reference range to those published in the literature.

Figure 4 shows a bar graph comparison of our sets 1 and pediatric and adult datasets compared with the published adult reference values in the literature.

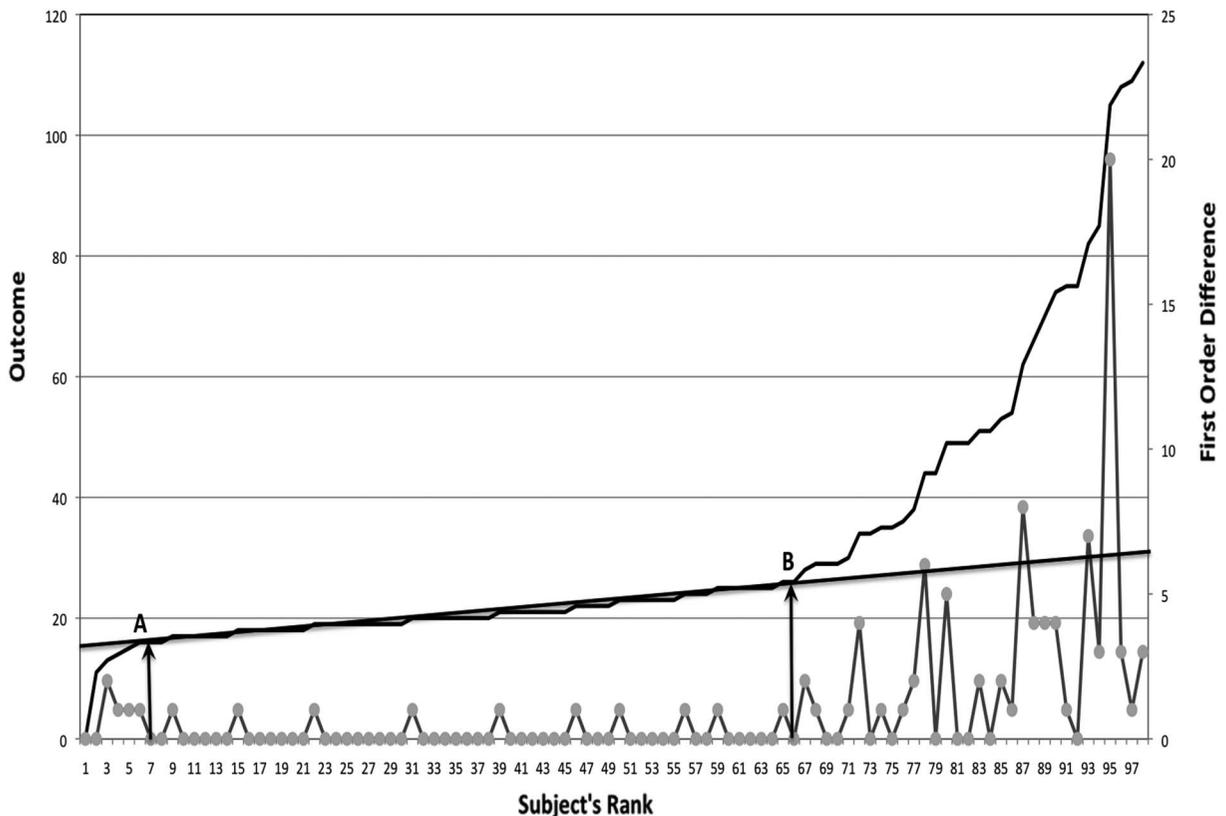


FIG. 2. First-order difference (dots) and cumulative density (curve) mean consecutive difference of a 98 adult cohort (set 2) aged 18 to 88 years referred for a stimulated SFEMG study. The left vertical axis was truncated to avoid compression of the inverted S curve.

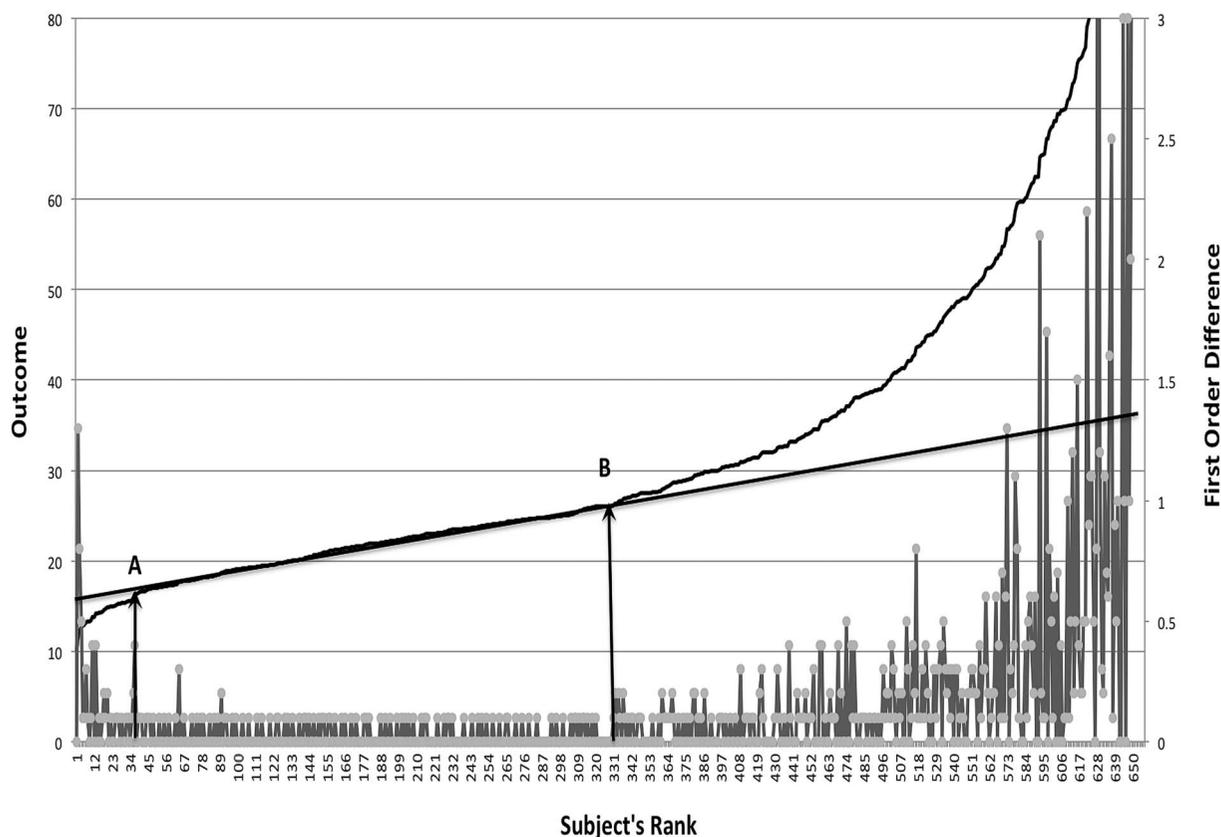


FIG. 3. First-order difference (dots) and cumulative density (curve) mean consecutive difference of a 657 pediatric cohort (set 1) aged 1-week to 18-year old referred for a stimulated SFEMG study. The left vertical axis was truncated to avoid compression of the inverted S curve.

DISCUSSION

Deriving reference values from a laboratory population is a counterintuitive and intellectually challenging proposition. It may nonetheless have some base in reality. In an article entitled “Computing normative ranges without recruiting healthy subjects” Yaar (Yaar, 1997) has argued that “if the existence or nonexistence

of symptoms is the only criterion on which normative data are based, then the latter is not needed at all; the diagnosis would have higher accuracy if based on the symptoms themselves.” But, collecting reference values from a healthy population, let alone in some subpopulation such as infants and children, is a daunting challenge in its own right because the validity of reference values depends on several critical steps (Solberg, 1987). This, along with ethical, informed consent (from a parent or a guardian in a pediatric subpopulation) and institutional review board requirements for approvals, place an undue burden on most laboratories to be able to collect their own reference values. In fact in an Ovid Medline search for published works between 1948 and October 2012 on SFEMG in children, Kosac et al. (Kosac et al., 2013) found only 1 article published in 1975 by Stålberg and Thiele in the investigation of 10- to 19-year old normal subjects by voluntary, not stimulated, SFEMG. The authors of the study concluded that “this is clearly an uncomfortable position given that this technique is currently being used in routine assessment of patients.” To that end, looking for speculative scenarios to derive hypothetical reference values from subpopulations where no such values exist in the literature can certainly be considered an alternative, although nevertheless raises two issues that are worth exploring.

One is that, as producers and consumers of laboratory data, we cannot deny the fact that not every single patient referred for a particular laboratory procedure comes back with an abnormal study. In many instances, these results are variously labeled as

TABLE 1. E-Norms Derived MCD Reference Values for Sets 1 (Pediatric) and 2 (Adult) and Comparison of Adult Mean MCD E-Norms Reference Ranges With Adult Mean MCD Reference Values Published in the Literature

Dataset	Pediatric E-Norms	Adult E-Norms	Published Adult Norms (Kouyoumdjian and Stålberg, 2011)
Age, years	0–18	18–88	21–56
Mean MCD	22	21	22
SD	2.83	2.79	1.99
Mean – 2 SD	16	15	18
Mean + 2 SD	27	26	25
Min MCD	16	16	18
Max MCD	26	26	26

Mean, Min, and Max MCD values are in microseconds.
MCD, Mean Consecutive Difference.

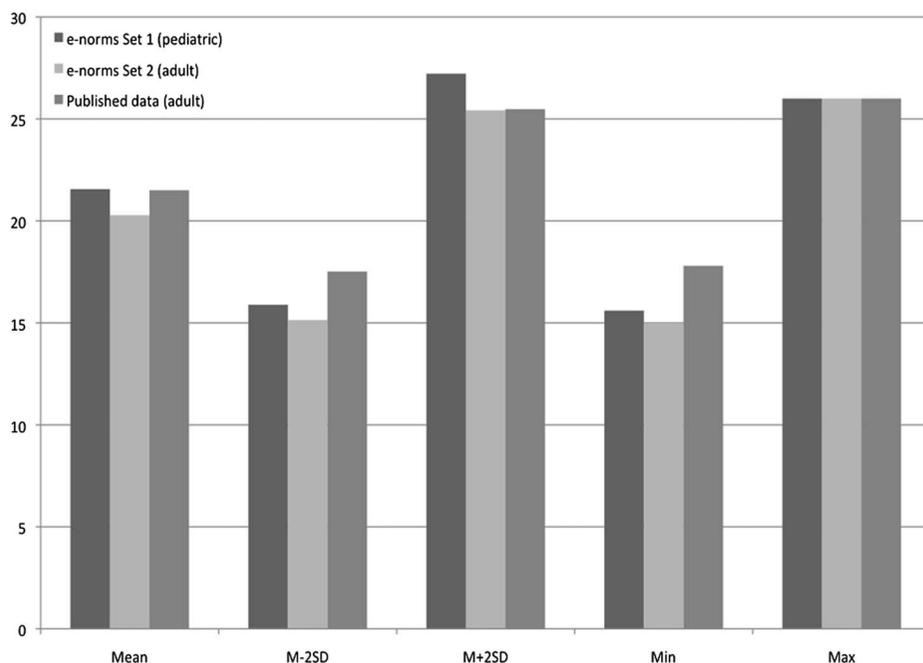


FIG. 4. Bar graph of the e-norms derived mean MCD reference values for sets 1 and 2 and comparison of adult mean MCD e-norms reference values published in the literature. Mean, min and Max MCD values are in microseconds. MCD, mean consecutive difference.

“normal” or “nondiagnostic.” So one could ask, under what diagnostic criterion should these data be classified? Indeed, in an unpublished review of diagnostic outcomes of 10,290 patients referred to his academic EMG laboratory for various neuromuscular conditions, one of the authors (J.F.J.) found that 14% or 1440 patients studied were interpreted as normal or abnormal by electrophysiological criteria (unpublished observation, J. F. Jabre, MD, October 2006). These numbers needless to say can vary a great deal depending on practice settings whether private or academic, in or outpatient, rural, or urban etc. The question under those circumstances is when a biomarker value such as the jitter measured as MCD does not reveal abnormal neuromuscular transmission in a subgroup of patients, where would the values of such a cohort fit in the inverted S curve of the sorted data?

We argue in this work that these data are likely to fit in a part of the curve that displays little differences between sorted values from one to the next, in this case, the plateau part of the curve.

And two, the other issue we need to consider is that when laboratories use published reference values collected at other academic medical centers, one needs to keep in mind that such reference values were likely collected from different subpopulations, using different equipment, by differently trained EMGers, all of which in and by themselves are factors that pose limitations on the appropriateness of their use by other laboratories.

Our method addresses these shortcomings using the assumption that, at least from a statistical point of view, it appears promising in recovering reference ranges when compared with available published reference values as shown in our adult cohort.

But the e-norms method has limitations as well. One is that we are still looking for a good answer as to what would constitute a “good” number of data points, or cases, to derive e-norms from in a given cohort. Our experience to date suggests that the higher the number the better. In most instances, we have obtained good results when the number of data points is ≥ 100 . Recently, although we have used the e-norms method to calculate Jitter MCD e-norms for a data set that consisted of only 38 stimulated single fiber EMG.

Despite our reluctance to study it, when we did, we were surprised to find out that we were easily able to identify the inverted S curve inflection points using the “tangent” and first-order derivatives, and the results of our analysis yielded e-norms data that were close to the MCD e-norms we derived from the much larger data set in our sets 1 and 2 described here. Needless to say, this is not a number we would recommend to work with.

Another question is whether the number of patients with disease in the cohort used for data analysis would ultimately affect the shape of the curve and the point of upslope inflection (B) to the right of the inverted S curve. One would wonder then, what effect would this have on the determination of the upper limit of normal. We believe that the e-norms method addresses this point appropriately because although we do not claim that the data points that lie between points A and B (the plateau part of the curve) do not come from patients, we do posit that any abnormalities they have are not in this particular instance in the specific area being tested, namely the neuromuscular junction.

Taken in this context, studies that contain a higher number of patients with abnormal neuromuscular transmission will necessarily have a proportionately “shorter” length plateau than studies that contain a lower number of patients with abnormal neuromuscular transmission. Indeed, in our pediatric cohort that contained a higher number of patients with abnormal neuromuscular transmission, the ratio of plateau data points/total was 45% compared with 62% for the adult cohort.

CONCLUSIONS

The e-norms method we describe to derive reference values from a laboratory population seems to be useful in recovering reference ranges when such values do not exist in the literature or are difficult to obtain in a particular cohort, such as a pediatric subpopulation. Although there is a great potential for this approach, more validations in different biomedical markers as well clinical validation of results is needed to confirm our hypothesis. In this study, without access to reference data from age-matched control

subjects for our pediatric cohort, the adult reference ranges have been used, an approach our results appear to endorse.

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