An algorithm for automatic detection of repeater F-waves and MUNE studies

N. Tuğrul Artuğ [a],[*], N. Görkem Şirin [b], Emel Oğuz Akarsu [b], M. Baris Baslo [b], A. Emre Öge [b]

[a] Electrical and Electronics Engineering, Istanbul Arel University, Tepekent, Buyukcekmece, Istanbul, Turkey
[b] Istanbul Medical Faculty, Istanbul University, Fatih, Capa, Istanbul, Turkey

ABSTRACT

The present study aims to develop an algorithm and software that automatically detects repeater F-waves which are very difficult to analyze when elicited as high number of recordings in motor unit number estimation studies. The main strategy of the study was to take the repeater F waves discriminated by the neurologist, from limited number of recordings, as the gold standard and to test the conformity of the results of the new automated method.

Ten patients with ALS and ten healthy controls were evaluated. 90 F-waves with supramaximal stimuli and 300 F-waves with submaximal stimuli were recorded. Supramaximal recordings were evaluated both manually by an expert neurologist and automatically by the developed software to test the performance of the algorithm. The results both acquired from the neurologist and from the software were found compatible. Therefore, the main expected impact of the present study is to make the analysis of repeater F waves easier primarily in motor unit number estimation studies, since there is currently a continuing need for such automated programs in clinical neurophysiology.

Submaximal recordings were examined only by the developed software. The extracted features were: maximum M response amplitude, mean power of M response, mean of sMUP values, MUNE value, number of baskets, persistence of F-waves, persistence of repeater F-waves, mean of F-waves' powers, median of F-waves' powers. Feature selection methods were also applied to determine the most valuable features. Various classifiers such as multi-layer perceptron (MLP), radial basis function network (RBF), support vector machines (SVM) and k nearest neighbors (k-NN) were tested to differentiate two classes. Initially all features, then decreased numbers of features after feature selection process were applied to the aforementioned classifiers. The classification performance usually increased when decreased features were applied to intelligent systems. Ulnar recordings under submaximal stimulation showed better performance when compared with supramaximal equivalents or median nerve equivalents. The highest performance was obtained as 90% with k-NN algorithm which was a committee decision based classifier. This result was achieved with only two features, namely mean of sMUP amplitude and MUNE value.

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1. Introduction

F-waves are one of the late responses acquired during routine EMG studies. The letter "F" comes from the initial of "foot" which is the first recording location of that signal [1]. F-waves appear after the M response following the nerve stimulation [2]. F-waves consist of the action potentials which are debounced from the lower motor neuron dendrites and returned to muscle where they are being recorded [3]. The action potentials contributed to F-waves are also present in M response [4–6]. However, all components that form M response can't debounce from spinal cord. F-waves change from stimulus to stimulus because an F-wave may be a produced signal from a motor unit or it can be a combined signal from several motor units [3,7]. However the morphology of the F-wave for a single motor unit is the same [3].

F-waves have latencies after the latency of M response because of the long pathway they have to travel. The length of this pathway determines the latency of F-wave [8]. It is seen a decrement in the number of F-wave producing motor neurons in denervation which is depicted by their decreased persistence while the ratio of repeater F-waves increases [8–11]. F-waves are low amplitude signals in healthy state but it can be observed that some signals may reach up to 700 μV amplitude [12,13]. The ability for F-wave generation changes from muscle to muscle such as there are more

F-waves generated in ulnar nerve innervated hand muscles than those of the median nerve [14].

F-waves are recorded in response to supramaximal stimulation. For this recording method, maximum amplitude value of M response must be acquired first and then the stimulus strength should be increased to a 10–50% higher level [2,15–20]. It is also possible to record F-waves using submaximal stimulus levels [21–23] although supramaximal stimulus produces F-waves with high persistence [2,15,24,25].

Puksa et al. have conducted a study on healthy participants about determining the reference values for F-wave parameters [26]. The parameters were extracted from participants who were aged between 14 and 95. They were minimum F-wave latency, mean F-wave latency, maximum F-wave latency, number of F-waves to 20 stimulus ratio, and F-wave dispersion. They recorded signals by stimulating the median, peroneal, ulnar and tibial nerves. They also questioned the effect of height, age and gender in this study. They found that 10 cm increment in height causes 1.6 to 3 ms prolongation of minimum F-wave latency according to the localization of recording muscle. Age also has an effect on latency prolongation but less pronounced comparing to the height. Gender was found ineffective.

Repeater F-waves are known as the F-waves that have the same latency, same amplitude and same morphology [3]. The ideal population to do a study on repeater F-waves is obtained as the result of at least 90 stimuli [3,27]. In the study that was conducted by Stashuk et al. 300 stimulus were applied [15].

In daily practice the measureable parameters of F-waves are evaluated manually by neurologist. There are studies about doing the investigations automatically. The developed algorithm by Stashuk et al. evaluates F-waves automatically [15] and calculates maximum M response, sum of F-waves, mean of S-MUAP as well as the estimation of the number of motor units. Even if the program does these calculations automatically the selection of S-MUAP is done the by operator manually. For motor unit number estimation (MUNE) 300 F-waves are recorded with supramaximal stimuli. The developed algorithm can be applied to every muscle that has F-wave latency more than 20 ms.

Hachisuka et al. conducted a study [28] about calculating the MUNE value. They recorded F-waves of median and tibial nerves from 43 Polio patients and 20 healthy individuals in response to 100 stimuli. They observed decreased persistence of the F-waves along with an increment in the number of repeater F-waves in the patients. They also have found a negative correlation between the number of repeater F-waves and the MUNE value.

A study conducted by Chroni et al. [29] determined the characteristics of repeater F-waves by exciting low and high threshold motor fibers in healthy individuals. Collision technique and submaximal stimulus were used for this purpose to examine if motor neuron subgroups were responsible of repeater F-waves. Collision technique was preferred to eliminate the F-waves generated from low threshold neurons. The recordings were acquired from ulnar nerves (ADM muscle) of 12 healthy participants in five different sessions by using supramaximal and submaximal stimuli. A new software was developed and introduced for detecting repeater F-waves. Their study showed that the presence of repeater F-waves were more related to high persistence rather than the neuron subgroups generating them.

Kamel et al.'s study [30] was about combining F-wave with single fiber conduction velocity. They included 16 healthy participants and 16 patients with mild neuropathy. F-wave measurement was done from fibular nerve (EDB muscle) with 20 supramaximal stimuli using a surface electrode. The extracted parameters were minimum and maximum F-wave latency, F-wave dispersion, the difference between Fmax and Fmin, F-wave persistence. Single fiber F-wave recordings were done from the same muscle. In

the conventional F-wave study, they found a significant prolongation in patients by means of Fmin and Fmax parameters. In single fiber F-wave recordings, on the other hand, not only Fmin and Fmax parameters but also F-wave dispersion parameter was prolonged significantly in patient group. They found that in early diabetic neuropathy, single fiber F-wave studies were better for finding abnormalities when compared with conventional F-wave recordings.

Certainly, finding the repeater F-waves manually just by looking the screen is too much time consuming. Software that speeds up this process and makes it easier will help physicians to use their time efficiently.

This study aims to:

a Develop an algorithm and software for detecting repeater F-waves automatically,
b Test the performance of the method by comparing the results of F-wave analysis manually done by an expert with the ones of developed software
c Extract some features from F-waves to classify patients and healthy individuals with intelligent systems

The paper continues with materials and methods in Section 2. Theory/Calculation part contains the algorithm and flow chart for detecting F-waves which is in Section 3. Results are presented in Section 4. In Section 5, Discussion part argues the pros and cons for the proposed method and compares them with previous studies. This paper ends with the Conclusion.

2. Material and methods

In this study a two class dataset is formed that contains neurogenic patients and healthy individuals. F-waves were recorded in response to supramaximal and submaximal stimuli. Interpretation was done both with manually by an expert clinical neurophysiologist and by the developed algorithm. Results of both interpretations were compared.

The study has been approved by the local ethics committee of Istanbul University, Istanbul Medical Faculty (2016/162).

2.1. Subjects

Ten patients with ALS and ten healthy controls were evaluated for preliminary analysis of automated F-wave. The mean age was 53.4 ± 10.2 in patients (ranged 36 to 64) and 51.5 ± 13.6 in healthy controls (ranged 30 to 67). Eight of the patients were classified as definite ALS and two were classified as possible ALS due to Awaji criteria [31]. In the patient group, mean duration of symptoms was 15.2 ± 20.2 months (2–72) and mean ALS-FRS score was 41.4 ± 5.3 (34–47) [32]. Patients who had sensorimotor polyneuropathy, carpal tunnel syndrome, ulnar entrapment neuropathy, diabetes, uremia, chronic alcohol use, or malignancy were excluded from the study. Patients having compound muscle action potential (CMAP) amplitudes less than 1 mV in either of the studied muscles were excluded from the study. Healthy controls had normal neurological examination, normal EMG studies and no clinical symptoms of carpal tunnel syndrome, ulnar entrapment neuropathy or polyneuropathy.

2.2. Electrophysiological evaluation

Compound muscle action potentials and F-waves were recorded from the less affected upper extremity in the patients. If both upper extremities were affected equally, then non-dominant side was chosen for the study. For healthy controls, non-dominant side was

chosen. Median and ulnar CMAPs were elicited by stimulating the nerves at the wrist and recording from abductor pollicis brevis (APB) and abductor digiti minimi (ADM) muscles respectively, with a Medelec Synergy EMG machine. Disposable recording electrodes were placed over muscles according to the "belly-tendon record-ing" principle as described previously [33]. CMAP was recorded in both muscles by supramaximal stimulus. The filter cut-off settings were arranged as 20Hz-10 kHz. Then, cathode of the stimulator was placed proximal to anode for F-wave recording. For the prelimi-nary analysis, 90 F-waves were elicited by supramaximal stimuli at a frequency of 0.5 Hz for each muscle. For F-wave MUNE analysis, stimulus intensity was decreased to a level of 10–50% of the base to peak amplitude of maximum CMAP which is known as submaximal stimulus. Then, 300 F-waves were recorded by submaximal stimuli at a frequency of 0.5 Hz for each muscle [15,17].

2.3. Manual analysis

Ninety F-waves were analyzed visually by the same experienced clinical neurophysiologist (EOA) from print-outs made with the sensitivity of 500μV-1 mV/division and sweep duration of 100 ms without splitting the screen. Repeating F-waves more than once with same latency, amplitude and shape were considered as single motor unit potential (sMUP). Peak to peak amplitudes of repeating F-waves were measured in order to calculate the mean amplitude of sMUPs.

The MUNE value was calculated by dividing the amplitude of the CMAP to that of the mean sMUP for both methods. After calculating sMUP and MUNE values, one of the researchers performed the sta-tistical analysis for preliminary analysis. Calculating MUNE values from 300 F-waves elicited by submaximal stimuli were performed only by automated analysis.

2.4. Automated analysis

An algorithm was developed for extracting F-waves from each signal record and grouping each repeater F-wave in different "bas-kets", calculating the number of repeater F-waves and the number of repeats for each repeater F-wave in the baskets. The software is also able to display the most similar F-wave pairs, calculates the M response maximum amplitude value, F-wave peak to peak amplitude value, power value for each F-wave and MUNE value.

SPSS v21 was used to perform statistical analysis. MUNE and sMUP were calculated visually by the neurophysiologist and auto-matically by the proposed method. The results were compared with Wilcoxon single rank test. Spearman's rho test was used to measure the correlation between the two methods.

3. Theory/calculation

The method for extracting F-waves and determining repeater F-waves are described as follows. First the recorded signals are fil-tered from noise by using wavelet transform based noise reduction method. The Daubechies wavelet is preferred for noise reduction. The threshold function is chosen as hard threshold. In the multi res-olution analysis, decomposition level was selected as 3. The graphic for the 15 recorded sample signals which belongs to a patient is given in Fig. 1.

Two millisecond part from the beginning of every signal is dis-carded. This part contains stimulus artifact so it is unnecessary for calculations. Then the maximum amplitude of M response is determined for each recorded signal and mean of it is calculated (MGloMax).

After the F-waves are cut from the beginning and the end loca-tions, the maximum (Fmax) and the minimum (Fmin) amplitude values are calculated. The locations of Fmax and Fmin are recorded.

The graphic for the F-waves after cutting from the raw signals is shown in Fig. 2.

If a signal's peak to peak amplitude value Vpp <=40μV, it is acknowledged as noise, floored to level 0 and is not evaluated, according to the recommendations for the clinical neurophysiol-ogy studies [3,14]. The signals that have amplitudes greater than 40μV are approved as F-waves. Moreover, if a signal does not goes down 40μV to the left and right in 3 ms from the Fmax location, this signal is evaluated as noise and is floored to level 0 too.

After these processes, all signals are aligned according to their Fmax locations. Because the repeater F-waves must have the same amplitude, same latency and same morphology; the signal pairs that are closer than 0.5 ms up to the Fmax and Fmin locations are determined as repeater F-wave candidates.

If the difference value for Fmax between candidates is lower than 10% and the difference of individual power values between them is lower than 20%, they keep their candidacy. Besides, the correlation coefficient between candidates is inspected. If the coef-ficient is lower than 0.9 that pair is discarded from candidacy.

The difference of amplitudes for candidate signal pairs is calcu-lated and the difference signals are rectified to calculate powers. A "similarity coefficient" is calculated according to these candidates' amplitude difference and power difference. The threshold value for similarity coefficient is determined as 0.6, during the preparation period of the present study after examining a multitude of different signals. If any candidate's similarity coefficient is lower than 0.6 it becomes a repeater F-wave. If any other identical repeater F-wave pair is present, they are combined in the same basket.

The unique signals that are not in any basket are aligned up to the Fmin location this time. If signal pairs are closer than 0.5 ms up to the Fmin locations, they are determined as repeater F-wave candidates.

If the difference value for Fmin between candidates is lower than 5% and the difference of individual power values between them is lower than 10%, they keep their candidacy.

The difference of amplitudes for candidate signal pairs is calcu-lated and the difference signals are rectified to calculate powers. A "similarity coefficient" is calculated up to these candidates' ampli-tude difference and power difference. If any candidate's similarity coefficient is lower than 0.6 it becomes a repeater F-wave. If any other identical repeater F-wave pair is present, they are combined in the same basket.

The mean of the peak to peak amplitudes (sMUP) of all signals in a basket is calculated. The mean value for all baskets' sMUP value is calculated. MUNE value is calculated with the formula given below:

$$MUNE = \frac{MGloMax}{\left(\sum_{k=1}^{j} sMUP_k\right)/j} \tag{1}$$

The number of F-waves is displayed. How many of them are in a basket and how many of them are unique can be seen on the monitor. Lastly MUNE value is displayed. The most similar signal pairs can be plotted one under the other or overlapped with simi-larity coefficient value. The most similar F-wave repeaters among the recorded sample signals can be seen in Fig. 3.

The algorithm for the developed software is given in Fig. 4.

3.1. Feature extraction from dataset

Nine features were extracted from this dataset. These were mean of maximum M response amplitude, mean power of M response, mean of sMUP values, MUNE value, number of baskets, persistence of F-waves, persistence of repeater F-waves, mean of F-waves' powers, median of F-waves' powers.
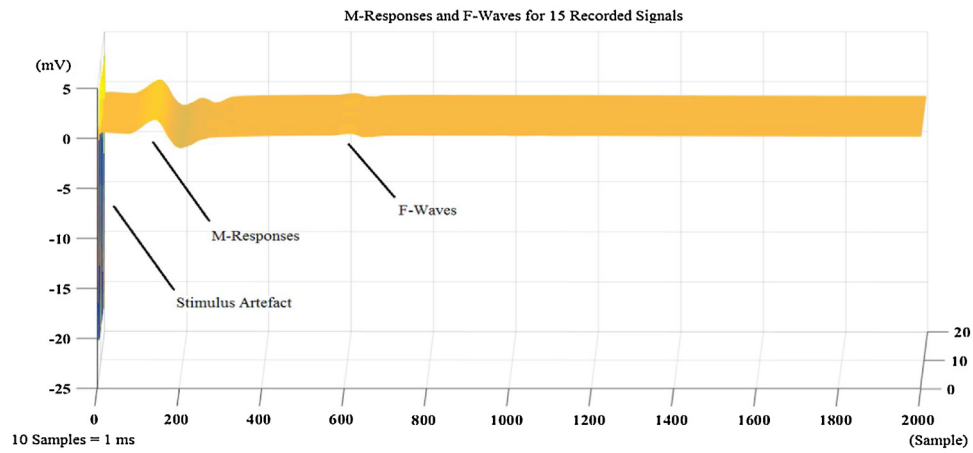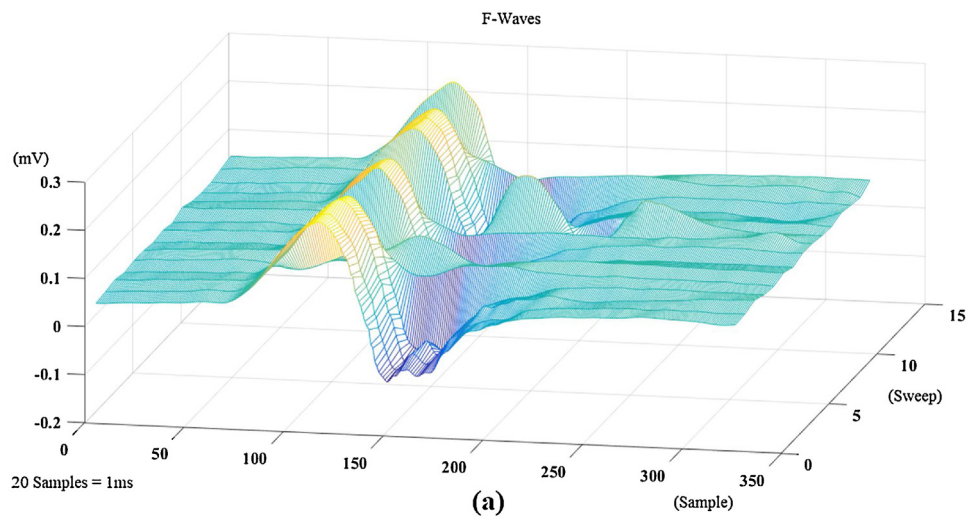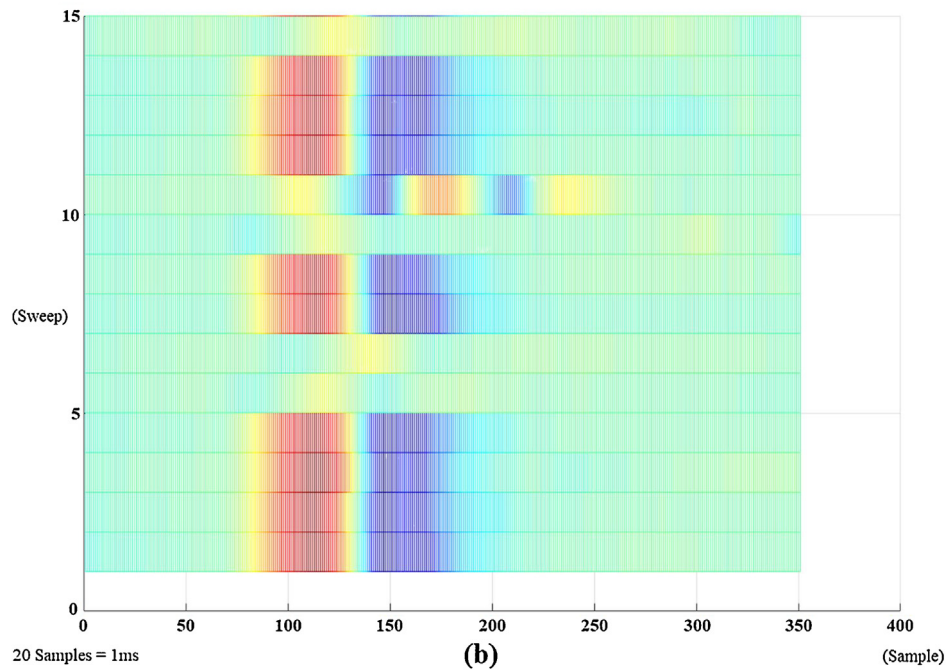
Fig. 1. M-Responses and F-Waves in 15 Recorded Sample Signals.

Fig. 2. F-Waves After the Cutting Operation. a) Isometric view. b) Colormap of top view.
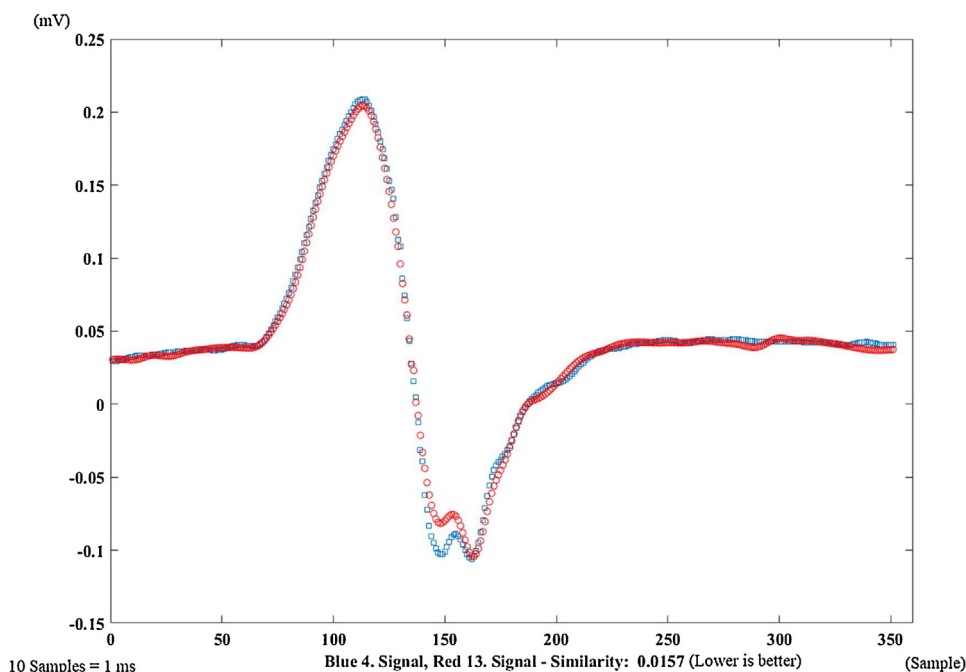
Fig. 3. Most Similar Repeater F-Wave Pair Among the Recorded Sample Signals.

First the maximum amplitude for all M responses from baseline was determined and their mean was calculated. Then power value for all M responses from baseline to positive peak value in each record was calculated. Mean of power values for M-responses was calculated. Third feature was calculated as the mean of each F-wave repeater basket sMUP values. MUNE value was calculated as the fourth feature as described before. Number of different F-wave repeaters was the number of baskets. Persistence was the ratio of F-wave signals to the all signal records. Seventh feature was persistence of repeater F-waves. It can be calculated as given below.

$$Persistance \ of \ repeater \ F-waves$$
$$= \frac{(All \ Records - Noise \ Signals) - Unique \ Signals}{All \ Records - Noise \ Signals} \quad (2)$$

Next feature was mean of all individual F-wave power values and the last feature was the median value of all individual F-wave power values.

3.2. Feature selection

In feature selection process ReliefF algorithm was preferred. Relief algorithm was developed for multi-featured data sets on feature selection process. In the algorithm two nearest neighbors are determined from each class for each sample. Neighbor in the same class with the sample is marked as nearest hit (H), other neighbor is marked as nearest miss (M) [34]. For each feature a weight is calculated and features are evaluated to be selected by their importance (weights). ReliefF algorithm was developed to overcome the drawback of generic Relief algorithm which can only separate two classes [35]. In ReliefF algorithm k nearest neighbors are determined instead of two neighbors for each sample.

3.3. Classification of data

After the feature extraction process supramaximal recordings had 10 instances in median nerve and 8 instances in ulnar nerve for each class which were healthy individuals and ALS patients.

Table 1
Specifications for Data Set.

Stimulation Type	#instances		#features	
	Median	Ulnar	Median	Ulnar
Supramaximal	10 Healthy 10 ALS	8 Healthy 8 ALS	9	
Submaximal	5 Healthy 5 ALS		8	

The reason for lower number of instances in ulnar nerve was no repeater F-wave was detected by the software, and so 2 instances from each class were discarded. After this process 5-fold cross validation for median nerve and 4-fold cross validation for ulnar nerve could be applied. Submaximal recordings had 5 instances for each class but they had 8 features by excluding M response mean power. A summary for data specifications were given in Table 1.

Four different classifiers were tested for separation of the two classes. First one is multi-layer perceptron (MLP). It has two hidden layers and there are 18 and 9 neurons for supramaximal records, 16 and 8 neurons for submaximal records in those layers. Levenberg–Marquardt was selected as the training algorithm for MLP network.

Second classifier was radial basis function network (RBF). Spread parameter was 0.2 for median nerve recordings and 0.6 for ulnar nerve recordings. RBF networks have only one hidden layer and the algorithm adds neurons to it for meeting the performance goal. The algorithm was added 25 neurons to its hidden layer for each trial in this classification task.

Support vector machines (SVM) were suitable for this classification problem because it was sufficient for recognition of the two classes.

Last classification algorithm was k-NN and neighborhood value was selected as 1, 3 and 5.

4. Results

If no repeater F-wave is observed in a nerve by the expert neurologist or the software, this data were discarded from the statistical

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Automated F-Wave Repeater Detection Algorithm

First step

1:	Select EMG Signal
2:	Apply Wavelet Transform Based Noise Reduction to EMG Signal
3:	Cut The First 2 ms Part From The Signal
4:	Calculate The MGloMax Value
5:	Extract The F-waves by Cutting From Start to End
6:	Calculate The Fmax and Fmin Values with Their Locations
7:	Determine The Noise Signals and Floor Them to 0
7:	Align All Signals According to The Fmax Locations
8:	**If** a Signal Pair is Closer Than 0.5ms According to The Fmin and Fmax Location
	They Become Repeater Candidates
	end
9:	**If** (Fmax Difference Value of Pairs <= 10% &&
	The Power Difference Value of Pairs <= 20% &&
	The Correlation Coefficient of Pairs >= 0.9)
	They Keep Their Candidacy
	end
10:	Calculate The Amplitude Difference and Power Difference of Pairs
11:	Similarity Coefficient = Amplitude Difference + Power Difference
12:	**If** Similarity Coefficient <= 0.6
	Mark This Pair As Repeater F-Waves
	end

Second step

13:	Align The Single Signals According to The Fmin Locations
14:	**If** a Signal Pair is Closer Than 0.5ms According to The Fmin Location
	They Become Repeater Candidates
	end
15:	**If** (Fmin Difference Value of Pairs <= 5% &&
	The Power Difference Value of Pairs <= 10%)
	They Keep Their Candidacy
	end
16:	Calculate The Amplitude Difference and Power Difference of Pairs
17:	Similarity Coefficient = Amplitude Difference + Power Difference
18:	**If** Similarity Coefficient <= 0.6
	Mark This Pair As Repeater F-Waves
	end
19:	Calculate Mean Vpp Value for All Repeater F-waves (sMUP)
20:	Calculate Mean Value for All sMUP Values
21:	Calculate The MUNE Value

$$MUNE = MGloMax / \left[\left(\sum_{k=1}^{j} sMUP_k \right) / j \right]$$

22:	Print The Number of F-Waves
23:	Print The Number of Signals in Each Repeater F-Wave
24:	Print The MUNE Value

Fig. 4. Automated F-Wave Repeater Detection Algorithm.

analysis. After this process, a total of 18 median and 16 ulnar nerve recordings are evaluated for supramaximal stimuli. For submaximal stimulation, 5 recordings for each nerve acquired both from healthy participants and ALS patients were included.

4.1. Statistical analysis

Main interest for the recordings with supramaximal stimulation was to test the performance of the developed software. Therefore,

Table 2
Mean sMUP and MUNE Values Acquired by Supramaximal Stimulation for Healthy Participants.

Healthy Controls 8 Median, 7 Ulnar	Software [mean ± SD] (min-max)	Neurologist [mean ± SD] (min-max)
Mean sMUP Median [μV]	347.45 ± 158.21 (145.9-568)	437.34 ± 296.99 (132.5–903.6)
Mean sMUP Ulnar [μV]	283.26 ± 149.37 (148.3–552)	257.82 ± 282 (78.3–873.5)
MUNE Median	28.08 ± 14.1 (16.3–53.4)	40.43 ± 35.28 (12.1–110.2)
MUNE Ulnar	31.26 ± 12.74 (15.6–50.2)	70.29 ± 40.95 (13.6–141.8)

Table 3
Mean sMUP and MUNE Values Acquired by Supramaximal Stimulation for ALS Patients.

Patient (ALS) 10 Median, 9 Ulnar	Software [mean ± SD] (min-max)	Neurologist [mean ± SD] (min-max)
Mean sMUP Median [μV]	466.37 ± 292.84 (184.4–1083)	509.26 ± 321.85 (186.8–1120.5)
Mean sMUP Ulnar [μV]	373.38 ± 165.14 (157.5–619.5)	486.14 ± 220.52 (238–849.4)
MUNE Median	17.34 ± 11.14 (3.2–40.9)	19.81 ± 10.5 (4.4–35.2)
MUNE Ulnar	15.62 ± 7.96 (5.8–28.4)	17.49 ± 8.77 (5.1–30.3)

comparisons were done between the results of the software and the expert neurologist. However, for the recordings of submaximal stimulation, it was aimed to test the performance of the developed software in differentiation between the healthy ones and ALS patients.

For supramaximal stimulation, 8 median and 7 ulnar nerve recordings were evaluated for healthy participants. sMUP values for median nerve are calculated as 437.34μV and 347.45μV by an expert neurologist and software, respectively. The same values for ulnar nerve are 257.82μV and 283.26μV. Mean MUNE value for median nerve is calculated as 40.43 and 28.08 by the expert neurologist and the software respectively, this value is obtained as 70.29 and 31.26 for the ulnar nerve. Descriptive statistical values for healthy participants which were acquired from median and ulnar nerves are given in Table 2.

sMUP value of ulnar nerve in healthy controls was the only parameter which has a significant correlation between the expert neurophysiologist and the software (p < 0.05).

Ten median and 9 ulnar nerve records were evaluated for patients. sMUP values for median nerve are calculated as 509.26μV and 466.37μV. The same values for ulnar nerve are calculated as 486.14μV and 373.38μV. Mean MUNE value for median nerve is calculated as 19.81 and 17.34; this value is obtained as 17.49 and 15.62 for the ulnar nerve.

Descriptive statistical values for ALS patients' data which were acquired from median and ulnar nerves are given in Table 3.

In ALS patients' data, there was significant correlations (p < 0.05) between the calculated sMUP and MUNE values which were done by the expert neurologist and the software.

Fig. 5 shows the correlation of the values by the software and the expert neurologist which depicts the sMUP and MUNE values from median and ulnar nerves of the ALS patients.

The features other than mean sMUP and MUNE values were unnecessary to be presented in this paper because there was no significant correlation between the values provided by the expert neurologist and the developed software.

Five median and ulnar nerve recordings in response to submaximal stimulation in healthy participants and ALS patients were evaluated by the developed software.

Descriptive statistical values for both healthy controls and ALS patient data which were acquired from median and ulnar nerves are given in Table 4.

Mean of sMUP values for healthy controls and ALS patients in median nerve are calculated as 184.74μV and 200.12μV respectively. The same values for the ulnar nerve are calculated as 154.17μV and 241.73μV. Mean MUNE value for median nerve in the healthy controls and ALS patients are calculated as 56.2 and 33.09; this value is obtained as 63.87 and 32.62 for the ulnar nerve.

The MUNE values acquired from median and ulnar nerves were significantly lower in patients with ALS comparing to those acquired from healthy participants (p < 0.05). The intergroup difference between the mean sMUP amplitudes was only significant for the ulnar nerve recordings (p < 0.05). For the other features calculated, there was no significant difference between the healthy participants and ALS patients.

4.2. Power spectral density of F-wave signals

Signals from a patient and a healthy volunteer were presented as superimposed traces in Fig. 6.

The persistence of F-waves in healthy volunteer was 81.11% while it was low in the patient (21.11%). Sum of the rectified traces were calculated and the power spectral density (PSD) was obtained. The points used in the discrete Fourier transform were same as those of the input signal. The sampling frequency was 20 KHz for the recordings. The PSD graph for signals from a healthy volunteer and a patient was given in Fig. 7.

In the healthy volunteer, the power of the signal was high like the persistence of the F-waves. Frequency spectrum yielded a wider band comparing to patient's data, probably related to the higher variability of F-wave in healthy situation. Conversely, patient's spectrum revealed less power that was in accordance with the low persistence of the F-waves. Likewise patient's data showed a narrow band reflecting the presence of repeater F-waves.

4.3. Classification results

The classification accuracies for all four different classifiers are given in Table 5.

The highest performance (90%) was obtained in the ulnar nerve with submaximal stimulation by using 1-NN algorithm. Both of the median nerve with supramaximal stimulation by using 5-NN and the ulnar nerve with submaximal stimulation by using MLP and RBF gave 80% classification accuracy.

After feature selection with ReliefF algorithm the classification accuracy that was acquired for all previous classifiers is given in Table 6.

The highest performance (90%) was obtained in the ulnar nerve with submaximal stimulation by using k-NN algorithm for all k values relying on meansMUP amplitude and MUNE values. Median and ulnar nerves with submaximal stimulation by MLP network performed 80% accuracy.

5. Discussion

Under the term of "late responses", F-waves are produced by the backfiring of lower motor neurons in response to peripheral stim-
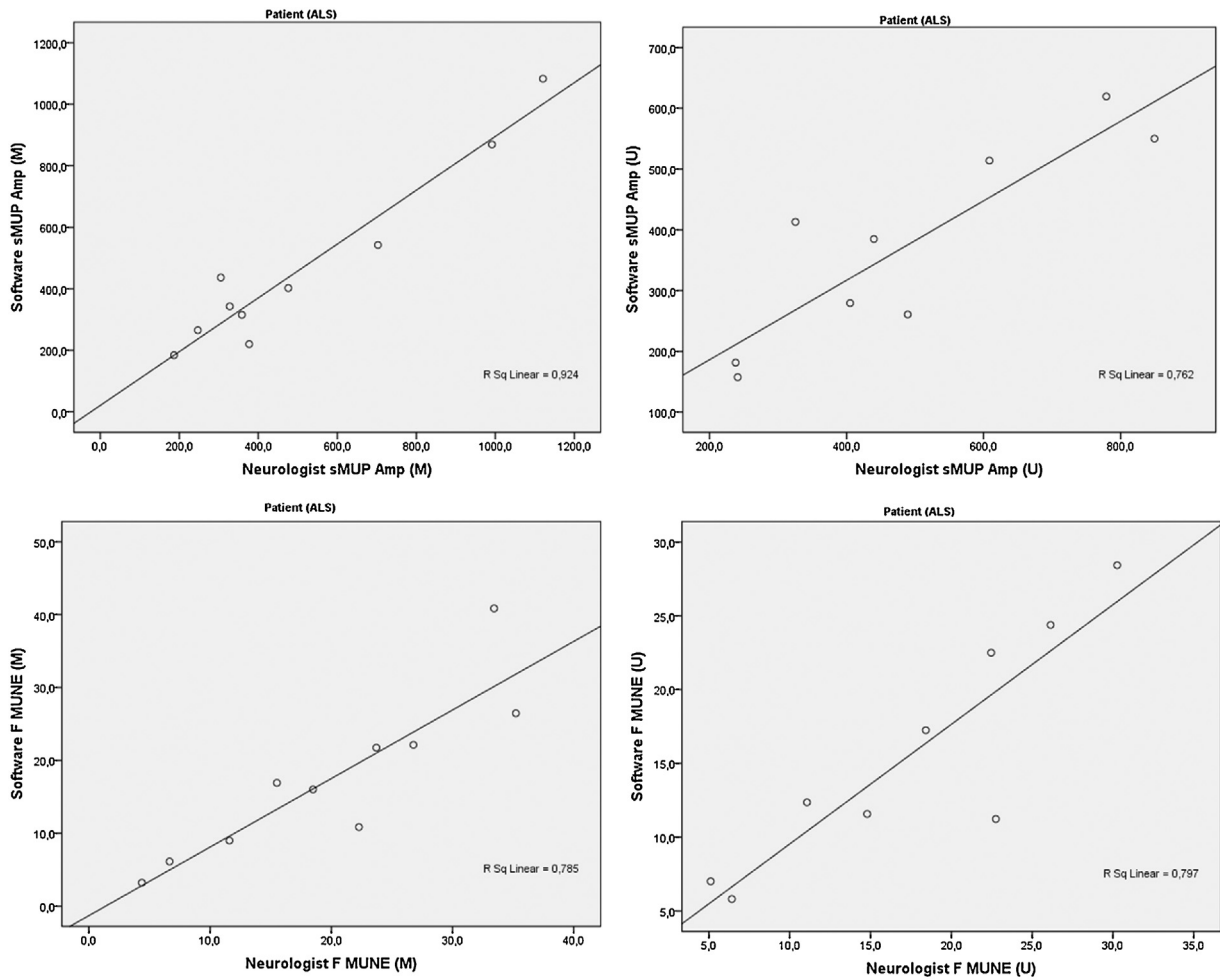
Fig. 5. Correlation Graphs of sMUP Amplitudes and MUNE Values of Median (M) and Ulnar (U) Nerves for ALS Patients.
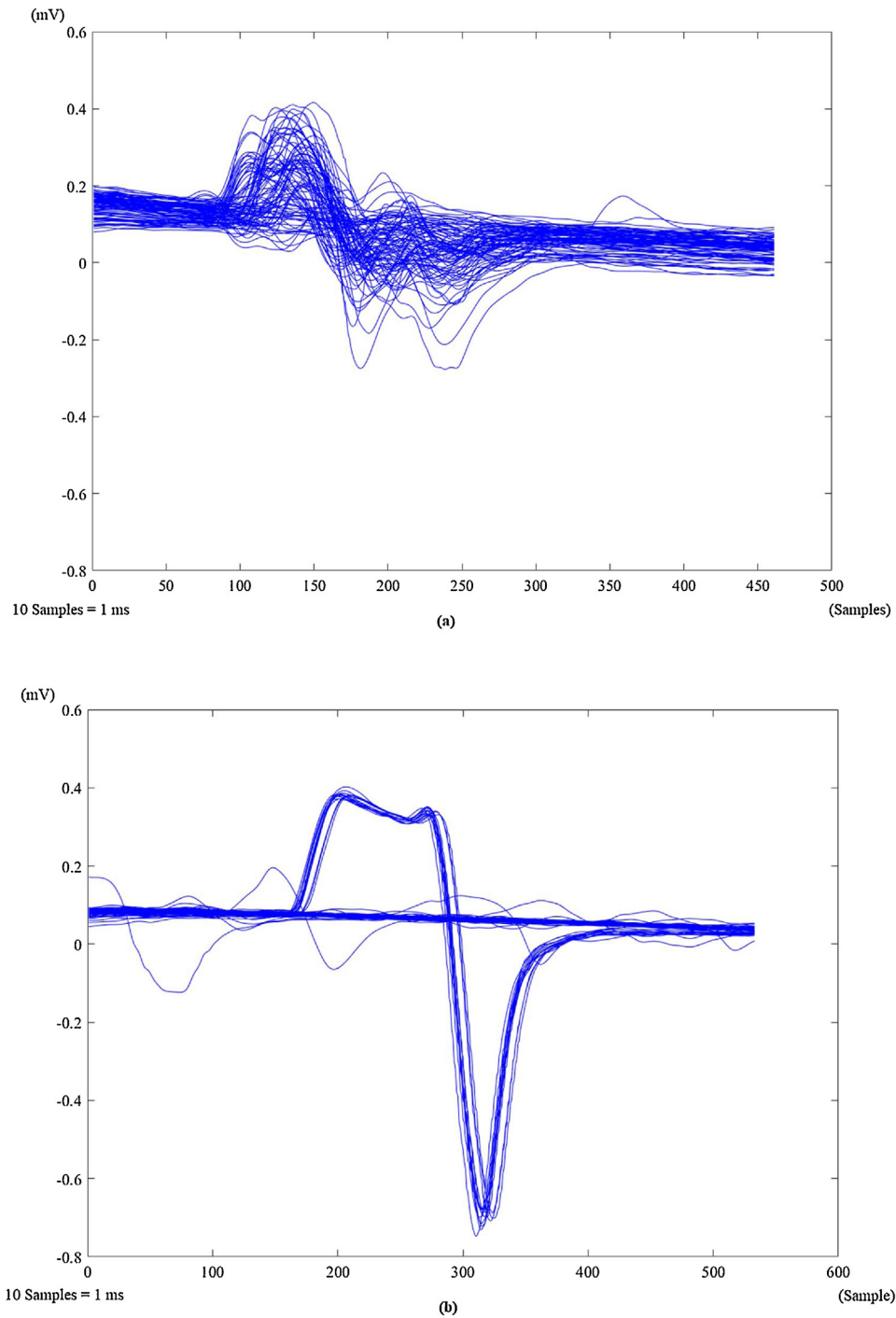
Table 4
Calculated Features Acquired by Submaximal Stimulation for Healthy Participants and ALS Patients.

(5 Median, 5 Ulnar)	Healthy Controls [mean ± SD] (min-max)	Patient (ALS) [mean ± SD] (min-max)
Mean sMUP (M) [μV]	184.74 ± 45.22 (138.8–238.18)	200.12 ± 57.36 (120.02–278.57)
Mean sMUP (U) [μV]	154.17 ± 37.29 (91.87–181.18)	241.73 ± 55.43 (151.92–288.86)
MUNE (M)	56.2 ± 11.02 (47.74–75.37)	33.09 ± 17.99 (9.83–53.60)
MUNE (U)	63.87 ± 18.53 (49.12–94.7)	32.62 ± 8.25 (21.46–44.76)
# baskets (M)	24 ± 13,27 (14–47)	19.6 ± 10.43 (9–35)
# baskets (U)	14.2 ± 4.27 (10–19)	17 ± 8.46 (7–30)
Mean of Max M Amp (M) [μV]	10,200 ± 2185.18 (7600-12500)	6920 ± 4613.24 (2100-11700)
Mean of Max M Amp (U) [μV]	9360 ± 1388.52 (8100–11700)	7560 ± 1180.25 (6200–9100)
Persistence (M)	0.54 ± 0.17 (0.3–0.75)	0.52 ± 0.15 (0.3-0.7)
Persistence (U)	0.4 ± 0.15 (0.24–0.62)	0.45 ± 0.16 (0.21–0.64)
Persistence F-Rep (M)	0.52 ± 0.11 (0.34–0.65)	0.51 ± 0.16 (0.27–0.67)
Persistence F-Rep (U)	0.53 ± 0.23 (0.26–0.78)	0.62 ± 0.22 (0.25–0.78)
Mean of F-waves' Powers (M)	3.92 ± 2.51 (1.68–7.91)	2.6 ± 1.24 (0.84–3.91)
Mean of F-waves' Powers (U)	3.91 ± 2.18 (1.1–6.13)	4.82 ± 3.4 (1.9-8.91)
Median of F-waves' Powers (M)	3.55 ± 2.28 (1.45–7.17)	1.88 ± 1.26 (0.47–3.75)
Median of F-waves' Powers (U)	3.62 ± 2.03 (1.03-5.96)	3.85 ± 3.43 (0.72-8.23)

Table 5
Classification Accuracy for Classifiers.

All Features		MLP	RBF	SVM	1-NN	3-NN	5-NN
Supramaximal	**Median**	55	50	45	50	70	80
	Ulnar	75	56.25	50	50	50	68.75
Submaximal	**Median**	60	50	20	40	20	50
	Ulnar	80	80	60	**90**	60	30

Fig. 6. Superimposed F-Wave Traces of a Healthy Volunteer (a) and an ALS Patient (b).

Table 6
Classification Accuracy After Feature Selection for Classifiers.

ReliefF		MLP	RBF	SVM	1-NN	3-NN	5-NN
Supramaximal	**Median**	55 (4)	35 (3)	70 (3)	60 (4)	75 (4)	60 (4)
	Ulnar	75 (5)	56.25 (4)	56.25 (5)	56.25 (5)	50 (5)	50 (5)
Submaximal	**Median**	80 (3)	50 (3)	60 (2)	50 (3)	60 (3)	50 (3)
	Ulnar	80 (4)	70 (4)	70 (4)	**90 (2)**	**90 (2)**	**90 (2)**

#Number of features remaining after feature selection with ReliefF is given between parentheses.
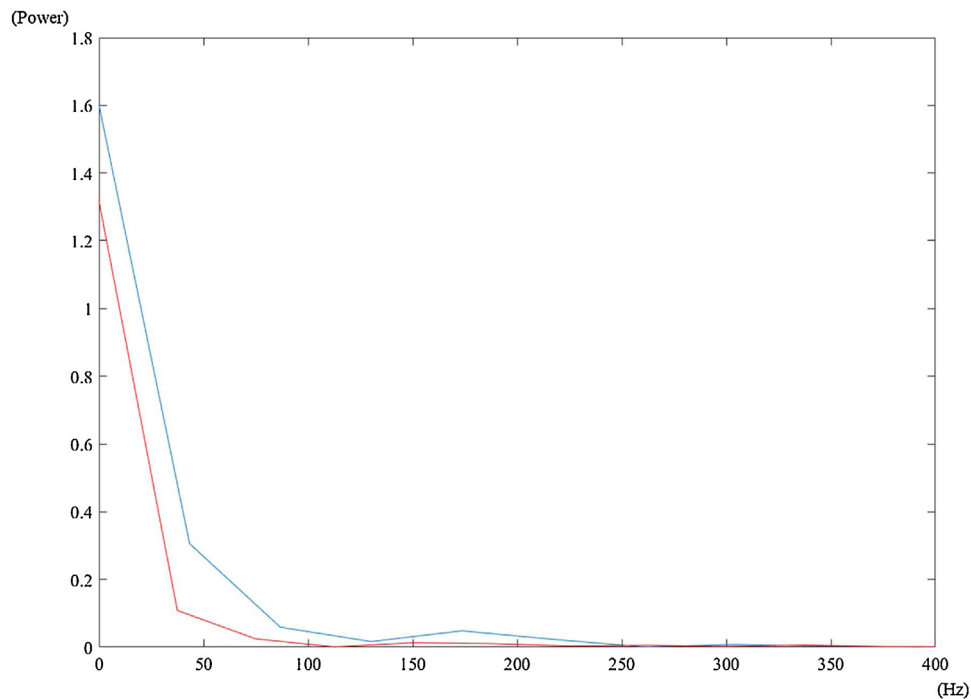
Fig. 7. Power Spectral Density of F-waves Calculated from a Healthy Volunteer (Blue Line) and a Patient (Red Line).

ulation. Although supramaximal stimulation recruits more than one lower motor neuron, it is also possible to record single axon's response with submaximal stimulation. The shape, latency and the persistence of F-waves are the indicators of disease states affecting the lower motor neuron and its axon [2,3,19]. F-waves are useful for the estimation of functioning lower motor neuron count as well [15,17].

In order to get meaningful results in F-wave studies, it is essential to deliver at least 20 stimuli for supramaximal and sometimes hundreds of stimuli for submaximal stimulation [16,36]. Manuel analysis of the traces harboring F-waves is cumbersome and there is always a possibility of misrecognition and faulty interpretation. Automated analysis software, such as the one which is developed in the present study, is a need to overcome the troubled side of naked-eye evaluation.

Scientists were curious about the automated analysis of F-wave parameters since 1990's. In their study, Stashuk et al [15] reported an algorithm in 1994, which was developed for calculating maximum M response amplitude, summing F-waves, calculating mean of S-MUAP and estimating the number of motor units in a muscle. They demonstrated that the estimated number of motor units in thenar muscles calculated by their developed software which selects S-MUAPs from F-responses automatically was almost identical to the one calculated by manually. The estimated number of motor units by using F-waves yielded similar values with another validated MUNE method, namely "multiple point stimulation". Although the algorithm performs perfectly well, some operator control might be needed for recruiting the S-MUAPs in case they could not be selected by the software for the calculation of mean S-MUAP value.

Four years later, Felice, KJ [17] calculated MUNE value of thenar muscles in patients with ALS by using the software developed by Stashuk et al [15] and demonstrated that the patients had significantly low number of motor units comparing to healthy participants. So they carried a step forward by showing that the Stashuk's algorithm works for the patients suffering from motor neuron disease.

Morris A. Fisher questioned the accuracy of an automated method for the measurement of F-wave latency. In his paper [37] published in 2005, he studied the F-waves of 80 peroneal nerves and showed that the computer software (NEUROMetrix) picks the correct sites for latency measurement of F-waves which were in concordance with the ones selected manually.

In a study where the reproducibility of nerve conduction studies was questioned, Kong et al. [38] performed serial measurements of conduction parameters and developed software which calculates F-wave latency automatically in combination with various other features. Among them, automatically calculated mean F-wave latency was the most reproducible one.

In another study of Kong et al. [39], the authors compared the F-wave parameters of peroneal nerve both acquired in response to supra and submaximal stimulation for the sake of patient's comfort. They used automated algorithms for the calculation of F-wave parameters such as the amplitude, persistence, latency, duration and chronodispersion. It is clear that the submaximal stimulation is well tolerated. Although the amplitude and persistence were high with supramaximal stimulation, the latency, duration and chronodispersion of F-waves were almost similar.

Chroni et al. [14] conducted a retrospective study dealing with the quantified parameters of F-waves in 2012. They formed a dataset with 50 healthy participants and patients with diabetic polyneuropathy, ALS, carpal tunnel syndrome, ulnar mononeuropathy and L5 root lesion. Each group of patients consisted of 50 participants. They stimulated median, ulnar and fibular nerves supramaximally 20 times and recorded M and F-waves from abductor pollicis brevis, abductor digiti minimi and extensor digitorum brevis muscles respectively. Among the parameters calculated from their dataset, those dealing with the F-waves were mean latency, maximum amplitude, persistence, repeater neuron index, total F-repeater index, total F-repeater persistence, non-repeater F-wave persistence, repeater neuron mean latency and repeater neuron maximum amplitude. Comparing to healthy participants, patients' data revealed significantly higher percentages of repeater neuron index and total F-repeater index which might be an indicator of

decreased numbers of motor neurons contributing to F-waves. The maximum amplitude of repeater neurons was lower than the F-wave maximum amplitude for median and fibular nerves in healthy participants and for all three nerves in patients. This finding was in contrast to the study conducted by Guiloff and Modarres-Sadeghi in 1991 [40]. The authors discussed this finding as the smaller amplitude of repeater neurons reflects the backfiring of single neurons rather than being a combined activity.

In 2017 Chroni et al. [29] developed a custom designed software. The abilities of this software were plotting the recordings, detecting F-waves, determining the repeaters and extracting their features. It also allowed setting the points manually in which the signal leaves and returns to baseline. However, their algorithm has some differences from ours. They used low pass filtering to suppress high frequency components of the signal. Moreover, they used derivative with a low pass filter to remove the spikes which were caused by the outliers of the signals. On the other hand, our algorithm uses wavelet based noise reduction to smooth the recordings and to discard noisy components. Their algorithm for determining repeater F-waves is stricter than ours. They determined a tolerance band of ± 0.05 ms for all negative and positive peaks, onset and return to baseline of the signals. In addition to those rules, their algorithm had an area based criteria. They also set a $25\mu V$ difference limit to minimum and maximum amplitudes for signal pairs. In our study, we aimed to develop an algorithm that decides by considering minor variability among the subjects into account. First of all, our algorithm calculates the signal's peak to peak amplitude. If this value is lower than $40\mu V$, the signal is marked as noise and is floored to level 0. Then it checks the Fmin and Fmax locations if they are close to each other in a 0.5 ms window. After this step, if the amplitude difference for Fmax value between signal pairs is lower than 10% and difference of individual power values between signal pairs is lower than 20% and lastly the correlation coefficient between them is greater than 0.9 they are marked as candidates. We determined a parameter named as similarity coefficient which consists of amplitude difference and power difference of signal pairs. If their sum is below the empirically determined threshold, the signals are marked as repeaters. After that, a second inspection is done and it is given elaborately in Part 3 "Theory/Calculation" and in summarized form at Fig. 4. The algorithm of Chroni et al. and our study group repeaters similar to each other. Their algorithm additionally assigns a color for each repeater F-wave.

In the present study, the results of the developed software concerning sMUP and MUNE values acquired by supramaximal stimuli were found to be compatible with the observations of the expert neurologist. Interestingly, this concordance was more prominent in ALS patients that can be explained by reduced available numbers of lower motor neurons for F-wave generation. Supramaximal stimulation might also contribute to lower numbers of MUNE values both in healthy volunteers and the patients, by increasing the sMUP amplitude as well. Nevertheless, mean sMUP amplitudes for median and ulnar nerves were close to the values of Chroni's study [14]. They calculated the maximum amplitude of repeater neurons as 0.4 and 0.2 mV for median and ulnar nerves in healthy individuals, respectively. These values were found 0.35 and 0.28 mV by the developed software for median and ulnar nerves in the present study. For patients with ALS, Chroni at al [14]. measured the maximum amplitude of the repeater neurons as 0.5 mV for median and 0.4 mV for ulnar nerve, while we automatically calculated mean sMUP amplitudes as 0.47 mV and 0.37 mV for median and ulnar nerves respectively.

The MUNE values calculated from submaximal stimulus dataset revealed that the ALS patients had significantly decreased numbers of motor units in both of their muscles, namely abductor pollicis brevis and abductor digiti minimi. F-waves which are produced in response to submaximal stimulus are thought that they are coming from a single motor neuron if they repeat at the same latency and in the same shape [15]. Mean sMUP amplitude was calculated from these repeater F-waves which were automatically chosen by the developed software. The calculated mean sMUP amplitude was higher for both muscles in ALS patients although the difference between healthy volunteers and patients was only statistically significant for ulnar nerve. The increase in mean sMUP amplitude might be a reflection of reinnervation by collateral sprouting in affected muscles. MUNE value is simply calculated by dividing the M-response amplitude by mean sMUP amplitude, so that a low M-response amplitude in combination with high sMUP amplitude yielded decreased MUNE value in patients' group a finding which might be expected.

Findings of lower MUNE value, decreased M-response amplitude and high sMUP amplitude in patients with ALS is not surprising because of ALS is characterized by progressive loss of lower motor neurons and reinnervation by collateral sprouting from the surviving ones. However, comparing to previous studies, MUNE values calculated in the present study were also low for both muscles of the healthy subjects as well, a finding that is noteworthy to discuss. Previous studies reported the estimated numbers of motor units are more than two hundred in hand muscles [15,17,41]. The estimated values of motor units in the present study were within 48 to 75 for the median nerve and 49 to 95 for the ulnar nerve. The underlying mechanism of low MUNE values in this study might be related to the recording technique of the M-response and/or preferentially selecting the high-amplitude sMUPs for calculation [42,43]. Nevertheless, techniques dealing with motor neuron counting are indirect and they give only estimated values [44–47]. MUNE is valuable for differentiating patients from healthy individuals but also it makes a great contribution for patient's follow up by tracking the changes in the MUNE values over the time [48–51]. Following the patient by using the same method allows for accurate prediction of the decline in lower motor neuron count, although it is just estimation rather than revealing the real number.

A classification study was also done to determine the most effective features for differentiating ALS patients from healthy volunteers. Extracted features were applied to various intelligent systems such as MLP, RBF, SVM and k-NN. First, all of the extracted features and then, decreased numbers of features were applied which were depicted by feature selection algorithms. The ulnar nerve recordings with submaximal stimulation yielded highest performance. The dataset composed by supramaximal stimulation did not show a great accuracy (maximum 75%). However for submaximal stimulation, MLP network revealed 80% accuracy when 3 or 4 features selected for median and ulnar recordings respectively. Feature selection increased the classification performance nearly for all classifiers. A committee decision based classifier k-NN was superior to all others after feature selection at ulnar nerve recordings under submaximal stimulation. The highest performance was obtained as 90% with only two features. The most valuable features which help differentiation of two classes were mean of sMUP amplitude and MUNE value.

The superior aspects of the present study are (1) algorithm was tested on both healthy individuals and patients, (2) comparison with the results of a neurologist (as for the gold standard) was done to test the performance of the developed algorithm, (3) original filter settings from the EMG instrument were kept as the same so the frequency components of the recorded signals were not distorted. On the other hand there are some drawbacks such as (1) numbers of the cases and the variety of disease groups are needed to be increased, (2) the algorithm is planned to be fully automatic for F-wave analysis but there is an algorithm in a study [15] which allows user to select repeater F-waves manually as well. The developed algorithm has a comprehensive viewer so an option may be added to the algorithm for selecting individual F-waves to be put in to

repeater F-wave baskets. (3) If only a comparison between the software and the results coming from the neurologist had been made for submaximal recordings which were consisted of 300 traces. However, it would be very difficult and extremely time consuming for the neurologist.

It is planned to compare the developed automated F-wave analysis method for MUNE estimation with other recently developed MUNE methods in analyzing the data of patients with ALS and other anterior cell (e.g. post-polio syndrome) as well as peripheral nerve disorders as future work.

6. Conclusion

An algorithm and software was developed for detecting repeater F-waves automatically and MUNE analysis. The reliability of the software was tested with supramaximal recordings. The results were in concordance with the expert neurologist for mean sMUP and MUNE value. A dataset was formed with supramaximal and submaximal recordings. Some features were extracted from F-waves to classify patients and healthy individuals with intelligent systems. Feature selection algorithms were applied to determine the most effective features. The classification performance was increased in many classifiers when the features were decreased. The ulnar nerve recordings showed highest performance as 90% with k-NN algorithm when the stimulus strength was submaximal. The most valuable features were mean sMUP amplitude and MUNE value.

Acknowledgements

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Tuğrul Artuğ is an assistant professor in the Department of Electrical and Electronics Engineering at Istanbul Arel University. He has eight years of academic experience at different universities. Artuğ holds a BSc in Electronics and Communication Engineering from Kocaeli University. He received his MSc and PhD degrees in Electronics from Yildiz Technical University in 2010 and 2015 respectively. His research interests are biomedical signal processing, image processing and neural networks. He is also experienced in microcontrollers. He has participated in international symposiums and submitted conference papers to several national congresses.

Nermin Gorkem Sirin is working as a fellow in clinical neurophysiology department in Marmara University since 2017. She was graduated from medical school in 2006 and started Neurology residency in the same year. After finishing her residency, she worked in Cizre State Hospital as a neurologist for her obligatory service between 2011 and 2013. After finishing her service, she worked in several hospitals in Istanbul as a neurologist. She had her Master of Science degree in clinical neurophysiology in Istanbul University in June 2018. Her academic interests are clinical neurophysiology, neuromuscular diseases and electromyography.

Emel Oguz Akarsu was born in Silifke/ Mersin on April 1982. She graduated from Istanbul University Cerrahpasa School of Medicine (English Program) in 2007. She completed her residency program in Haseki Training and Research Hospital in 2011 and she completed Istanbul University Istanbul Medical Faculty Clinical Neurophysiology fellowship program in 2017. She has been working as a clinical neurophysiologist in Sakarya University Training and Research Hospital since 2017. She is interested in epilepsy, neuromuscular disorders and electrophysiology.

M. Baris Baslo was born in October 1970 in Istanbul. He graduated from Istanbul University Medical Faculty in 1993. He completed his residency program in the Neurology Department of the same faculty in 1998. He studied neuroscience at Mayo Clinic between August 1996 and July 1997. He worked as a consultant neurologist until 2003 when he became an associate professor. In 2009, he was promoted to Professor of Neurology at the same department and two years later he got his degree in "Clinical Neurophysiology" as his subspecialty. His research mainly focuses on electrophysiology of neuromuscular disorders.

A. Emre Öge is a professor in the departments of Neurology and Clinical Neurophysiology, Istanbul University, Istanbul Faculty of Medicine. His main areas of interest and research are peripheral nerve diseases, facial nerve physiology, quantitative electromyography including motor unit number estimation studies and magnetic stimulation methods intended for proximal peripheral nerve disorders, cortical excitability and sensory-motor integration.