

# Ultrasonographic diagnosis of carpal tunnel syndrome: introducing a new approach

Stylianos Kolovos<sup>1</sup> · Dimitrios Tsiotas<sup>2</sup>

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## Abstract

**Introduction** CTS, the most common nerve entrapment syndrome of the upper limb, is being diagnosed by clinical criteria, in most cases supported by the electrodiagnosis method, which appears limits regarding its sensitivity and specificity and suggests an intervening and expensive technique. The purpose of this study was to contribute to establishing U/S examination as a method with at least of the same accuracy with electrodiagnosis.

**Material and method** A sample of 60 healthy individuals and 30 patients suffering from CTS was scanned. The diagnosis was conducted by both clinical and electrodiagnostic criteria, or by clinical criteria supported by post-surgical outcome.

**Method** In order to improve the accuracy of measurements, the anteroposterior to transverse diameter of the median nerve inside the canal and in its entrance was scanned and compared, by sonography. The examination conducted three times for each dimension, and the mean value per individual was calculated.

**Results** The mean ratios for the 60 healthy wrists was found to range within the interval 0.49–0.88 (presenting a mean value of 0.66), and the corresponding for the 30 suffering from CTS wrists was within the interval 1.12–1.59 (with a mean value of 1.39).

**Conclusion** The statistical analysis of the examination results clearly demonstrates that the interval of ratios over the value 1.07 can be considered completely safe to diagnose that someone is suffering from CTS. In correspondence, a U/S measurement of ratios in the area up to 0.79 is completely safe to opine that this wrist refers to a healthy individual. The intermediate range of ratios 0.79–1.0 suggests a *grey zone*, which, by the rational of this study, does not include discrete CTS or healthy cases. This “*gap*” may describe subclinical or mild cases of CTS which were not been taken under consideration and for which there is no rational to interfere surgically. In the everyday’s practice clinical point of view, the *grey zone* cases are considered healthy.

**Keywords** Carpal tunnel syndrome · CTS · Ultrasonography · Nerve compression

## Introduction

The carpal tunnel syndrome (CTS) is the most common nerve entrapment syndrome of the upper limb. It is caused by the compression of median nerve in the wrist area. Risk factors may be relevant to anatomic variations (narrow carpal tunnel, an artery accompanying the median nerve, abnormal and secondary muscles and tendons), to sensitivity of the nerve to compression (diabetes mellitus, systemic neuropathies), to systemic and endocrine diseases (pregnancy, hypothyroidism, amyloidosis) or to space occupying lesions inside the tunnel. Clinically, the patient presents a history of tingling, numbness and burning sensation, located on thumb, index, middle finger and the radial side of the ring finger. The symptoms are getting more intense, by continued forceful manual activity. The

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✉ Dimitrios Tsiotas  
tsiotas@uth.gr

Stylianos Kolovos  
stelioskolovos04@yahoo.gr

<sup>1</sup> Orthopedic Department, General Hospital of Larissa, Larissa, Thessaly, Greece

<sup>2</sup> Department of Planning and Regional Development, University of Thessaly, Pedion Areos, 38 334 Volos, Greece

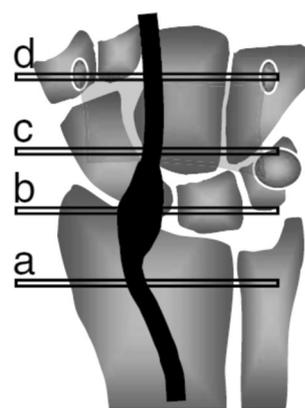
dominant hand is affected more often. The pain during the night could be explained at least partially, from the local oedema and the prolonged flexion of the wrist during sleeping. Generally, the symptoms subside by repetitive movements of the hand.

Three clinical tests help to establish the diagnosis, the *Tinel*, the *Fallen* and the *wrist compression* test. The first is undertaken by percussion of the volar aspect of the wrist by an examination hammer, the second by flexing the wrist for 1 min and the third by pressuring with the finger the carpal tunnel for 30 s. All these tests are considered to be positive, if they reproduce the patient's symptoms. However, the examiner should know that there could be also false-negative results in case of chronicity. It is recorded [1] that these tests are more often positive in tendono-elitritis of the flexor tendons in the area of the wrist rather than in carpal tunnel syndrome.

In early stages of CTS, it is believed that there are no morphological changes of the median nerve. Later, the nerve become swollen and displays changes in its shape, yet histological changes with progressive demyelination and fibrous sclerosis are also observed. Chronic cases may result in permanent loss of sensibility and motor function in the area of median nerve innervations. At the present, the diagnosis of CTS is based on clinical and EMG findings. Nevertheless, the continuing improvement in the ultrasound probes has allowed the imaging of the median nerve in high resolution and consequently the assessment of CTS by ultrasonography [2]. The diagnostic approaches by U/S are based on the alterations of the shape of median nerve, the alterations in its echotexture, the transverse ligament anomalies and finally the detection of soft tissue exercising press inside the canal. Despite the fact that median nerve displays distinct morphological anomalies in most of the patients suffering from CTS, there are still some early cases in which there are not identified any ultrasonographic alterations [3–9].

### Level of diagnostic accuracy with the up-to-date knowledge

It is noted that on CTS patients, the median nerve presents to be flattened inside the distal carpal tunnel and swollen in the proximal segment of tunnel [8, 10]. However, according to the authors of [11], the point of maximal oedema is located just proximal of the transverse ligament (see Fig. 1). On the axial imaging of the nerve, someone can easily observe an abrupt increase in its diameter in front of the entrance of the carpal tunnel and a flattening inside the later “Notch sign” [6] (see Fig. 2). Among the several worldwide existing techniques, the greatest amount of information concerns the measurement of the area of the transverse section and the flattening ratio of the nerve



**Fig. 1** Possible places of scanning. Incision *b* represents a plane just proximal to transverse carpal ligament. Incision *c* represents a plane just distal to the TCL (this figure appeared in [11, p. 461] ©Springer-Verlag Berlin Heidelberg 2007, reused with permission of Springer, all rights reserved)

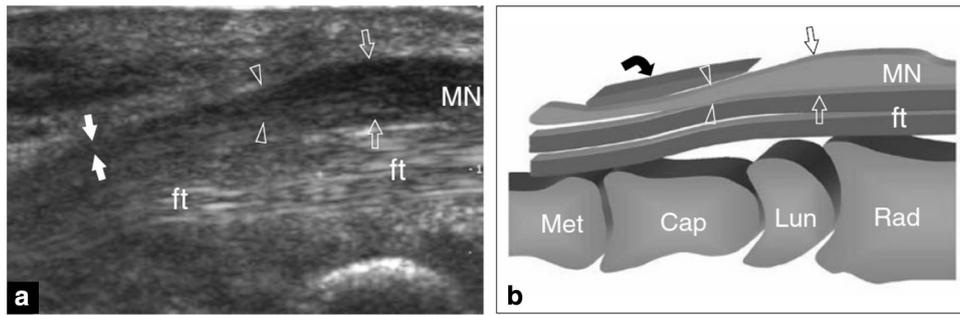
(reference necessary), as is illustrated at Fig. 3. According to the first, it is counted the cross-sectional area of the nerve by using the mathematical formula  $A_{\text{Nerve}} = \frac{a \cdot b \cdot \pi}{4}$ , where *a* stands for maximum anteroposterior diameter and *b* for maximum transverse diameter.

However, there is an open argument among the researchers about the pathologic limit. For some, an area that counts 9 square millimetres [12] is considered pathologic, while others [2, 10] consider it as to be 10, 15 or even 15 square millimetres [6]. On the other hand, some investigators estimate the cross-sectional area, using a continuous line [13] (see Fig. 4). The second method is about the flattening ratio. This ratio is defined as the ratio between the transverse diameter and the anteroposterior, in the distal part of carpal tunnel. A ratio greater than 3 suggests the diagnosis [8, 10]. Others [14] declare that they have not found a relation between that index and the diagnosis of carpal tunnel syndrome. Most of researchers take into account the cross-sectional area in several locations of carpal tunnel, finding results similar or mildly inferior in diagnostic value compared with electrodiagnosis, with a sensitivity range between 48 and 89 % and specificity between 47 and 95 % [3, 4, 6, 8, 9]. Other studies take into account the flattening ratio finding it also useful, with sensitivity 72 % and specificity 90 % [7], or 99 and 100 %, respectively [5].

## Materials and methods

### Study area and data

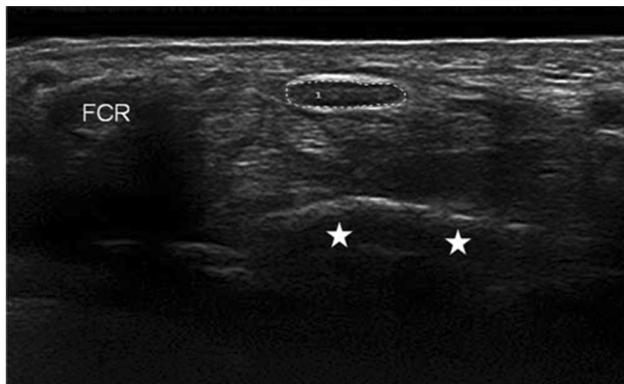
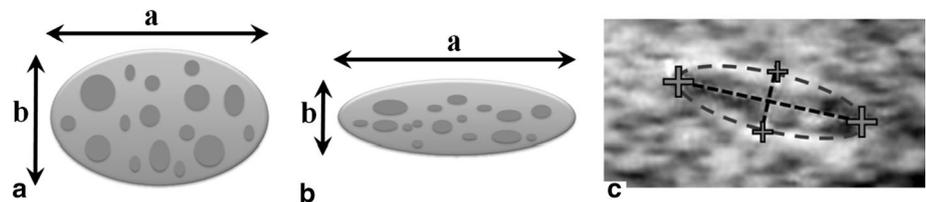
In this study, a sample of 60 healthy individuals between 30 and 60 years old was examined, in order to establish the



**Fig. 2** Longitudinal axis U/S image of the median nerve showing the notch sign and corresponding schematic drawing. Swelling portion (open arrows), proximal to the level of compression (arrowheads) and the nerve flattening (white arrows) deep to the transverse carpal

ligament (curved arrow) (this figure appeared in [11, p. 460] ©Springer-Verlag Berlin Heidelberg 2007, reused with permission of Springer, all rights reserved)

**Fig. 3** Schematic representations of the cross-sectional areas of: **a** normal and **b** suffering nerve. Detail **c** illustrates the procedure of spotting the dimensions *a* and *b*



**Fig. 4** Transverse US image of median nerve (outlined) at level of carpal tunnel in healthy volunteer; the CSAc is 8 mm<sup>2</sup>. FCR flexor carpi radialis tendon, \* carpus, 1 measurement 1 (this figure appeared in [28, p. 173] ©2008 Radiological Society of North America, reused with permission, all rights reserved)

normal values’ thresholds. The definition of the status of *healthy* is set by the criteria of not expressing any clinical symptom of CTS, of presenting negative results to all clinical tests, of presenting negative history for any relative symptom, as also for surgical release for CTS and no symptoms referred from the neck. On the other hand, a sample of 30 patients suffering from CTS was also examined. The exclusion criteria of these patients suggest a history of a wrist surgery or a clinical or EDS indication of a situation which mimics CTS like more central site of compression of the M. nerve, multineuropathy, cervical

**Table 1** Estimated likelihood of carpal tunnel syndrome (CTS) for case definitions of CTS that include electrodiagnostic studies (EDS)

Case	Symptom	EDS	Ordinal likelihood of CTS
1	Classic/probable	Positive	+++
2	Possible	Positive	++
3	Classic/probable	Negative	±
4	Possible	Negative	–
5	Unlikely	Positive	–
6	Unlikely	Negative	–

Source [15]

radiculitis or a history of diabetes mellitus, rheumatoid arthritis, pregnancy, acromegaly or hypothyroidism.

To put at first the diagnosis of CTS, the clinical criteria of American Academy of Neurology (1993) were used, as paresthesia, pain, oedema and weakness or loss of the dexterity of the hand getting worse or triggered by sleep, the hanging of the hand, constant working of the hand and subsiding by a change in the position of the hand or by “jerking” it. In order to confirm the diagnosis of CTS, the “consensus criteria” were applied, as they are described by the authors [15] and summarized in the Tables 1, 2 and 3.

According to the aim of the present study, individuals with the preliminary diagnosis of CTS were also investigated, conducting a confirmation by the clinical improvement after the operation. The groups of patients who were included in the sample regard the cases 1–3 of Table 1 and the cases 1–4 from Table 3.

**Table 2** Classification of symptom quality and location for use with hand diagrams or focused questions

Symptom	Description
Classic/probable	Numbness, tingling, burning, or pain in at least 2 of digits 1, 2, or 3. Palm pain, wrist pain, or radiation proximal to the wrist is allowed
Possible	Tingling, numbness, burning, or pain in at least 1 of digits 1, 2, or 3
Unlikely	No symptoms in digits 1, 2, and 3

Source [15]

**Table 3** Sensitivity, specificity and positive predictive value (PPV) for case definitions of carpal tunnel syndrome that do not include electrodiagnostic studies

Case	Criteria evaluated in workplace studies	Sensitivity	Specificity	PPV
1	Classic/probable and PE and night symptoms <sup>a</sup>	0.07	0.99	0.44
2	Classic/probable and PE	0.12	0.97	0.31
3	Classic/probable and night symptoms <sup>a</sup>	0.12	0.96	0.25
4	Classic/probable <sup>a</sup>	0.22	0.90	0.20
5	Possible <sup>b</sup>	0.34	0.84	0.19
6	PE <sup>c</sup>	0.41	0.76	0.16

Source [15]

Electrodiagnostic study findings alone were used as the gold standard

PPV was calculated assuming a disease prevalence of 0.10

PE positive physical examination (Tinel's test, Phalen's test, two-point discrimination, or carpal compression test)

<sup>a</sup> Data from 822 workers (Homan et al. unpublished data, 1998)

<sup>b</sup> Study of 408 workers [18]

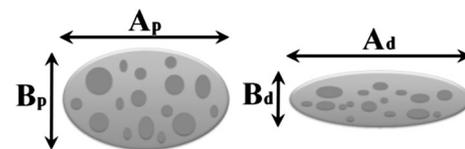
<sup>c</sup> Study of 130 workers [10]

Also, in order to investigate CTS without conducting the EDS examination, we use the data provided in Table 3. The confirmation of the clinical improvement conducted 3 months after the operation date was performed, by using *pre-* and *postoperative*, as is described at the *Levin Boston questionnaire*. According to [16, 17], in order to study mild cases of CTS, only five valid studies until 2008 used clinical criteria for the diagnosis of CTS, and they are the only studies that have got the capability to compare U/S test with EDS.

In this study, patients with positive and negative EDS, as also patients with no EDS, were included to the sample. According to the research of [19], each patient is holding two fixed groups of questions according (a) the severity of his symptoms and (b) his capability for certain simple everyday works. The findings of the research reveal that the score for the (a) is improving from the range of  $3.1 \pm 0.9$  preoperatively, to  $2.0 \pm -1$  postoperatively. Also, the score for the (b) improved from the range of  $2.7 \pm -1.0$  preoperatively to  $2.1 \pm -1.0$  postoperatively. This method has been used from several investigators, and its reliability has been verified by research [20].

### Methodological framework

The polyphony regarding the scanning approaches for the CTS diagnosis and the different threshold values for



**Fig. 5**  $A_p$  the transverse diameter of median nerve in front of canal entrance ( $p =$  proximal)  $B_p$  the anteroposterior diameter, respectively ( $p =$  proximal).  $A_d$  and  $B_d$  transverse diameter and the anteroposterior diameter inside the canal ( $d =$  distal)

considering an individual as CTS patient motivated the medical author personnel of this study to seek for more disciplinarian criteria over the CTS diagnosis. This motivation inspired the authors to proceed to a synthetic diagnostic approach, which introduces the simultaneous examination of both parameters, first the flattening inside the canal and second the swelling of the nerve in the canal entrance. That was accomplished by a comparison of the flattening ratios inside the canal and in its entrance, assuming that inside the canal the nerve increases mainly the transverse diameter, while in the canal entrance it increases mainly the anteroposterior diameter, as it is being schematically described at Fig. 5.

The individuals were examined by using a General Electric Logicbook (XP), which has a linear transducer that ranges between 6 and 10 MHz. The examinee sited in front of the examiner, who was holding his hand with the wrist in

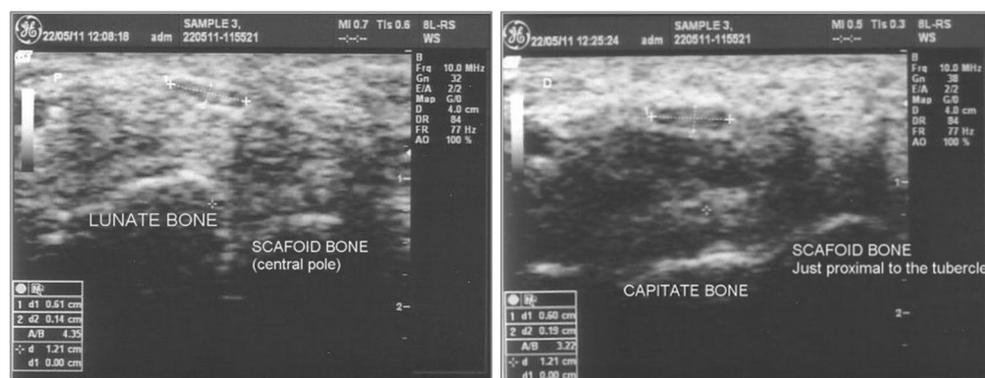
a neutral position or 15° of extension, so as to facilitate the scanning with the other hand. The position of the wrist with the minimal pressure to the nerve is the neutral with some cases were the values of +15° or -15° are considered advantageous [21]. The fingers were placed in a rest position, without the permission of movements, except if it was instructed to do so. The applied position was causing a horizontal movement of the nerve, like floating above the flexor tendons. By this way the position of the nerve became more obvious and clearer to depict its border, and a slight differentiation of the nerve's shape to be more oval was achieved [14]. The position and the borders of the median nerve can be outlined more clear also by tilting the transducer, because of the different anisotropism of the tendons compared to the nerve. This examination technique targeted to locate the site of maximum compression (flattening) of the nerve inside the canal and to locate the site of maximum swelling of the nerve near the entry of the canal (Figs. 6, 7).

There is a relative discrepancy among the investigators about the site of maximum swelling. Nevertheless, most of them believe that this site is in the entrance of the carpal tunnel just proximal to the transverse carpal ligament above the radioulnar joint. This study followed this general consideration but performed a slight forward-backward movement to locate in more fine way the site of maximum swelling. In order to locate the site of maximum flattening, the general consideration that it is found when the scaphoid bone is visible was followed. Ought to technical restrictions, the scanning was stopped when the distal pole of the scaphoid bone could be distinguished. The same bone landmarks were used also for the healthy wrists (Figs. 6, 7). In order to confirm that the landmarks were used properly, the examination in the wrist also held with an MRI. Before applying this introducing method to the sample, its reproducibility was inspected in a pilot procedure. Initially the scanning of the proximal and distal site

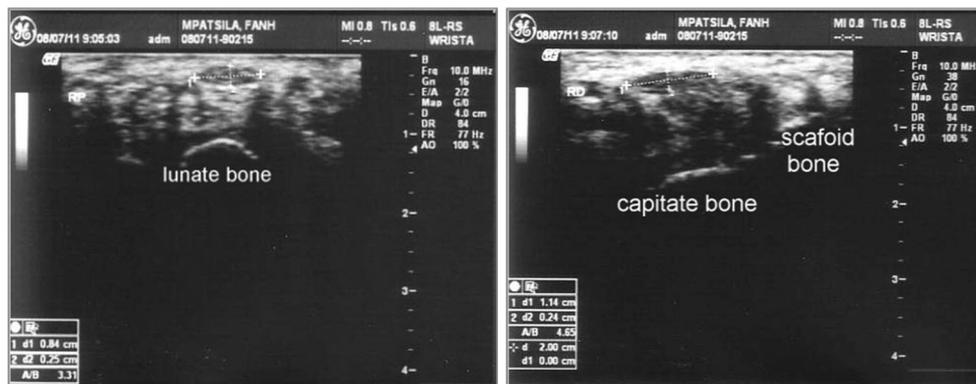
of a wrist was checked 10 times in each pilot sample unit. The pilot results indicated that there was insignificant variation (less than 10 %), between the extremes in the range, and through a trial-and-error process, the diagnostic measurement applications were reduced to three scans per individual.

After the individuals' examination, the results were submitted to a statistical analysis. The analysis included the calculation of descriptive statistic measures (graphical and quantitative) [22, 23], the construction of confidence intervals for the mean and limit values of the ranges [25] and the application of the nonparametrical one-sample Kolmogorov-Smirnov test (K-S) [23–26]. The descriptive statistic measures as the sample's mean value, median, range, variance, standard deviation, coefficient of variation, skewness and kurtosis help to determine the shape and the general form of the distribution [22, 23]. The sample volumes of both healthy and individuals suffering from CTS are greater than the central limit theorem's threshold of 30 units [22], so confidence interval estimators under the normal distribution's projection are constructed (95 % by default). Despite the fact that the data of the examinations are turned into ratios, these ratios cannot be considered as proportions, because both their components (numerator and denominator) do not refer to frequencies, but to measured quantities. This remark rejects constructing confidence intervals for proportions [22, 25]. The normality assumption for constructing confidence intervals is examined through the nonparametric K-S test. The one-sample K-S test is used to validate the null hypothesis that a sample comes from the theoretical normal distribution  $N(\mu, \sigma^2)$ . This is formulated by calculating the largest difference (in absolute value) between the empirical and the theoretical normal cumulative distribution functions.

The trial-and-error pilot examination opined to obtain three (ratio) measurements from each individual of the sample, without a significant loss of information. In order



**Fig. 6** *Left* healthy wrist, proximal. The lunate bone and the proximal pole of the scaphoid, indicate the correct site, just proximal to the carpal tunnel. *Right* healthy wrist, distal. The capitate bone and the scaphoid indicate the correct site under the transverse carpal ligament



**Fig. 7** *Left* CTS proximal. Same landmarks as in Fig. 6. Increase of the anteroposterior diameter relative to the transverse. *Right* CTS distal. Same landmarks as in Fig. 7. Increase of the transverse diameter relative to the anteroposterior

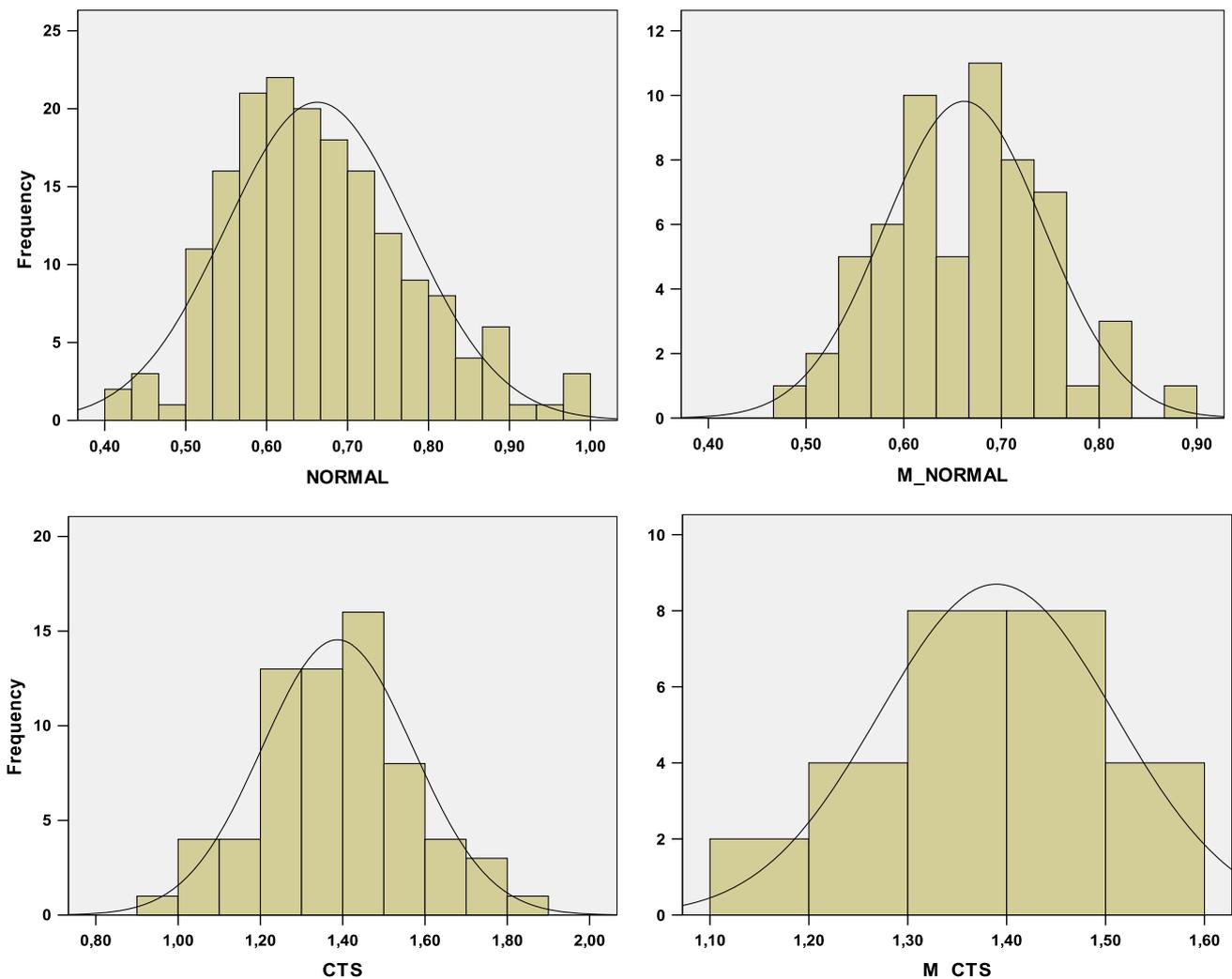
to compare and further validate this examination procedure, all measurements (of ratios) were considered initially as different cases (variables: CTS with  $30 \times 3 = 90$  cases and NORMAL with  $60 \times 3 = 180$ ) and afterwards as cases of each individual, where every set of three (ratio) measurements per individual used to provide a mean value (variables M\_CTS with 30 and M\_NORMAL with 60 cases). The goodness of fit (K–S test) to the normal distribution of the extended variables (CTS, NORMAL) in comparison with the corresponding mean variables (M\_CTS, M\_NORMAL) verified the assumption that the three (ratio) measurements are considered necessary to provide an efficient CTS diagnosis.

## Results and discussion

In this study 60 healthy wrists and 30 CTS-suffering wrists were scanned. The results of the measurements are illustrated at the histograms of Fig. 8. As it is shown in Fig. 8, the distribution of the variable of mean values (M\_NORMAL) is more concentrated than the distribution of the corresponding NORMAL, so as to refer to a better homogeneity distribution. This fact is also validated by the comparison of the coefficients of variation (CV) and verifies the correctness of obtaining three (ratio) measurements for the U/S CTS diagnosis. The inequality of the means  $\bar{x}_{M\_NORMAL} < \bar{x}_{NORMAL}$  demonstrates more obvious U/S healthy individual diagnosis intervals for the three measurements examination and the fact that  $Var(M\_NORMAL) < Var(NORMAL)$  indicates better concentration and dispersion of the M\_NORMAL distribution. The range ( $l_s, u_s$ ) of the sample distribution is 0.42–0.98 in the NORMAL and 0.49–0.88 in the M\_NORMAL case. The 95 % confidence intervals for the boundary values of these ranges turn to 0.39–1.01 and 0.47–0.90, respectively.

Regarding the CTS results, the M\_CTS case seems to present better concentration and homogeneity than CTS. The relation  $\bar{x}_{CTS} < \bar{x}_{M\_CTS}$  states more obvious CTS diagnosis intervals for the three measurements examination. The range of the sample distribution is 0.93–1.84 for the CTS and 1.12–1.59 for M\_CTS case. A 95 % confidence interval for each distribution ranges turn to 0.86–1.91 and 1.07–1.64, respectively.

Proceeding to a further nonparametrical analysis [22–26], a K–S is applied. This treatment is expected to reveal the grade, under which the empirical data of the examinations are confronted to the *rules of nature*, since the normal distribution is considered to describe the module that the physical characteristics are distributed. In other words, the examination data are expected to behave normally, by the time they refer to a physical asthenia. Table 4 presents the results of the one-sample Kolmogorov–Smirnov Test. Unlike much statistical testing, a significant result in the K–S test does not indicate goodness of fit to the theoretical distribution [22, 23, 25, 26]. As it can be observed in the data of Table 4, all values of significance for the study variables indicate a significant *goodness of fit* of the empirical cases to the normal distribution. Although this conclusion seems to be a trivial verification of the central limit theorem [22, 24], the comparison between the significances of the variables produces interesting results. The variable NORMAL fits better than the M\_NORMAL to the normal distribution, apparently due to the greater number of available data. The interesting contrast occurs to the CTS-suffering case. In opposition to the number of available data, the M\_CTS distribution fits more significant to the normal distribution than the corresponding CTS variable. This observation indicates that the act of obtaining three (ratio) U/S measurements of a patient consist a better state of diagnosis of CTS than getting a single measurement (of ratios).



**Fig. 8** Histograms of the variables of healthy (NORMAL, M\_NORMAL) and of the CTS-suffering (CTS, M\_CTS) individuals

**Table 4** One-sample Kolmogorov–Smirnov test

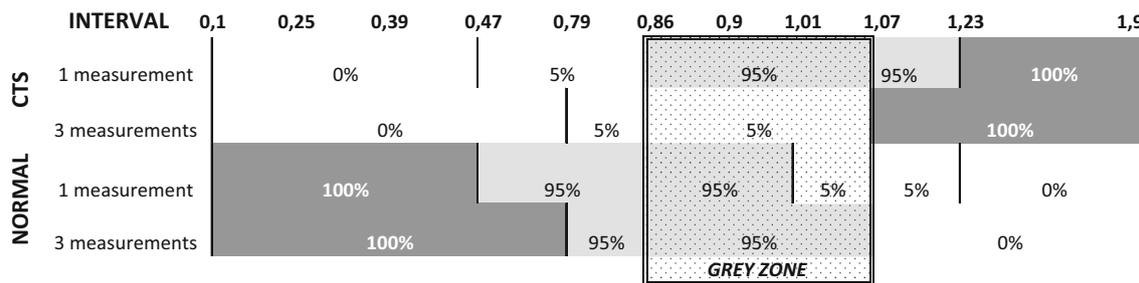
	CTS	M_CTS	NORMAL	M_NORMAL
<i>N</i>	90	30	180	60
Normal parameters <sup>a,b</sup>				
Mean	1.3874	1.3901	0.6652	0.6641
SD	0.18379	0.11922	0.11381	0.08191
Most extreme differences				
Absolute	0.059	0.122	0.059	0.067
Positive	0.059	0.070	0.059	0.067
Negative	−0.051	−0.122	−0.040	−0.049
Kolmogorov–Smirnov <i>Z</i>	0.482	0.622	0.776	0.522
Asymp. Sig. (two-tailed)	0.974	0.834	0.584	0.948

<sup>a</sup> Test distribution is normal

<sup>b</sup> Calculated from data

In order to provide estimation for the U/S CTS diagnosis of *grey zones*, the composite table of Fig. 9 was constructed. This table unifies the information regarding the

ranges, the confidence intervals for the ranges and the frequencies concentrations’ for the normal distribution  $N(\mu, \sigma^2)$ . In a  $N(\mu, \sigma^2)$  an amount of almost 67 % of data is



**Fig. 9** Interval determination of grey zone of CTS diagnosis

concentrated within the interval of one standard deviation radius ( $\mu \pm \sigma$ ), the 99 % within the interval  $\mu \pm 4\sigma$  and practically the 100 % within the interval  $\mu \pm 5\sigma$  [22, 25]. In order to determine the grey zone allocation in a more detailed way, an analogical scaling technique that uses a proportional calculation of the standard deviations of the variables was applied and opined that the grey subinterval 0.86–0.94 is more probable to describe cases of healthy individuals and the respective 0.94–1.07 of introducing CTS cases. However, for these cases, there is no rationale to interfere surgically. Finally, an amount of 25 individuals were assessed preoperatively by electrodiagnosis and was found to have moderate to severe CTS. Two individuals had got a negative EDS but were assessed clinically preoperatively according to the criteria we have already mentioned and considered to have the diagnosis of CTS. Three individuals had not undergone any preoperative EDS. All the individuals who had got negative EDS or had not undergone an EDS were assessed by the Levin–Boston questionnaire and found to have clinical improvement after operation.

## Conclusions

In order to establish the diagnosis of CTS, in every day practice, the orthopaedic surgeon relies in clinical criteria and to the electrodiagnosis. In the official report of the “American Association of Electrodiagnostic Medicine, American Academy of Neurology, and the American Academy of Physical Medicine and Rehabilitation” in 2002, it is provided the protocol of electrodiagnostic study in which even the study with the maximum diagnostic accuracy has got sensitivity up to 86 % and specificity up to 98 %. That means even with most strict protocols, these methods do not detect all the existing cases. However, except very few studies, the ultrasonographic examination is evaluated in comparison with them. The same is supported by the authors of [27] where it is claimed that by the official data is not essential the use of electrodiagnosis because at any time there are cases with false-negative and false-positive studies.

From the previous data and those of U/S studies, it is concluded that for the diagnosis of CTS in clinical praxis, there is no ultimate diagnostic tool, and it is based in a summation of clinical findings and electrodiagnosis, while later ultrasonography plays a more serious role. In this study, the examination was including simultaneously two parameters: the flattening inside the canal and the swelling of the nerve in the canal entrance. By this way, we enhanced the sensitivity of the method. That was accomplished by a comparison of the flattening ratios inside the canal and in its entrance, assuming that inside the canal the nerve increases mainly the transverse diameter, while in the canal entrance increases mainly the anteroposterior diameter. We found that this site was generally rather above the proximal part of the lunate bone which was demonstrating a constant pattern of a parabolic shadow. Using only ratios there is not a need for measurement units and the initial diameter of the nerve does not play a role, which it could be affected by the body type of the patient and to produce overlapping between pathological and normal values. On the other hand, even the slightest changes compared to the normal were detected. Our results clearly demonstrate that there is a safe range of ratios, of more than 1.07 for which someone could be sure about the diagnosis of CTS. Also that there is a range under the value of 0.79 for which someone is sure that is confronting a healthy wrist. However, there is a range of ratios between 0.79 and 1.07 which belongs to a grey zone.

Our criteria for the diagnosis of CTS were so clear that we are almost sure that this range is belonging to sub-clinical or very mild cases of CTS, in which may be there is not a point to interfere surgically. It is also the reason for which we did not measure any wrist in that range (we scanned only sure CTS cases). So we recommend that every orthopaedic surgeon could rely in our criteria, after he has been familiar with this technique. We recommend to check his/her sonographic results with the EDS and when he is trained enough to rely in his/her own measurements. This study confirms that now it is offered to the orthopaedic surgeon a renewed U/S tool with easy handling and an increased diagnostic accuracy, bringing a step closer to the more accurate diagnosis.

### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

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