Reviewer Comments:

Reviewer 1

The author is saying there is no difference in CSA between patients and controls, …., with current data we cannot rely and neither color or power Doppler in the diagnosis.

Response: We clearly stated that “CSA of the median nerve in the CTS patients was significantly larger than that in the healthy controls at all three temperatures” in the Abstract and demonstrated the differences in the Results section of the manuscript. Regarding using color or power Doppler in diagnosing CTS, these two methods have been used and validated in several previous studies, which showed significant differences in the intraneural vascularity of the median nerve between CTS patients and healthy controls (Ghasemi-Esfe, Khalilzadeh et al. 2011, Dejaco, Stradner et al. 2013, Kutlar, Bayrak et al. 2017). Therefore, our results not only corroborate previous studies but also add new findings regarding the influence of skin temperature on intraneural blood flow in CTS patients by power Doppler.

Ghasemi-Esfe, A. R., O. Khalilzadeh, M. Mazloumi, S. M. Vaziri-Bozorg, S. G. Niri, H. Kahnouji and M. Rahmani (2011). "Combination of high-resolution and color Doppler ultrasound in diagnosis of carpal tunnel syndrome." Acta Radiologica **52**(2): 191-197.

Dejaco, C., M. Stradner, D. Zauner, W. Seel, N. E. Simmet, A. Klammer, P. Heitzer, K. Brickmann, J. Gretler and F. C. Fürst-Moazedi (2013). "Ultrasound for diagnosis of carpal tunnel syndrome: comparison of different methods to determine median nerve volume and value of power Doppler sonography." Ann Rheum Dis **72**(12): 1934-1939.

Kutlar, N., A. O. Bayrak, I. K. Bayrak, S. Canbaz and H. Turker (2017). "Diagnosing carpal tunnel syndrome with Doppler ultrasonography: a comparison of ultrasonographic measurements and electrophysiological severity." Neurol Res **39**(2): 126-132.

Reviewer 2

General

Carpal tunnel syndrome (CTS) is the most common peripheral neuropathy of the upper extremity. Ultrasonography (US) plays a central role in its imaging diagnosis. This cross-sectional study evaluated the impact of three grades of local skin temperature on median nerve CSA and intraneural blood flow by power Doppler in CTS patients. The sample included 50 CTS and 50 healthy controls. The CTS patients demonstrated increased CSA at all 3 grades of temperature while intraneural vascularity of the median nerve was not seen at the highest skin temperature 34°C. The study findings contribute to the role of US in the CTS diagnosis and may serve to improve its specificity. The manuscript is of appropriate length, well organized and clearly written. A few points of clarification would improve an already high quality manuscript.

Specific

Abstract

The abstract is structured and reflects the content of the main text.

Introduction

The aims of the study and research question are clearly stated and referenced. Local skin temperature increase is known to vary the sensory and motor conduction during NCS examinations. The CSA and intraneural blood flow using US have not been studied as a function of skin temperature variations, a clinically relevant question which may impact testing performance measures.

Methods

The inclusion and exclusion criteria for CTS patients and controls are identified and appropriate for the study aims. The evaluation protocol for CTS reflected the standard of care e.g. clinical, sensory testing, NCV and US with one possible exception, the exclusion of the Boston Carpal Tunnel Questionnaire. This questionnaire is validated and widely utilized in clinical and research settings for CTS.

Under physical examination section, the paragraph addressing Semmes Weinstein monofilament (SWM) sensory test makes use of the reference to a "positive test" when participants could identify which finger is

being tested, this is probably a typo. Please revise (1). In the conventional SWM method a positive test is when the recorded threshold value of a radial digit is higher than 2.83. Please revise (2).

Responses #1 and #2:
Thanks for the reviewer kindly reminding. We have revised the whole paragraph to make it clearer for the readers.

(page 9, line 110)
The Semmes–Weinstein monofilament sensory test was carried out by applying the force-calibrated monofilament perpendicularly to the volar digital surface, and the pressure was increased until the monofilament began to bend. The participants were asked to close their eyes, and a normal response was recorded when they could identify which finger was tested based on one out of three responses to the 2.83 g monofilament, while a positive response to higher than 2.83 g indicated "diminished" sensation. A weighted score from 1 to 5 was given depending on the calculated force of each filament, and a lower score indicated greater force (Bell-Krotoski, Weinstein et al. 1993).

Please clarify (3) how long, following the hot pad application, did the skin temperature remain at the target temperature?

Response #3**:**
Thanks for the reviewer's comment. In our experience, after applying a hot pack, the skin temperature usually remained at the target temperature for approximately 2 minutes, so we would have sufficient time to finish the ultrasonography.

We have added this statement in the manuscript.
(page 12, lines 160)
After finishing the above ultrasonography at 30°C, we applied a hot pack to each subject's hand and distal part of the forearm until the skin temperature increased to 32°C and performed the same ultrasonography protocol again. The skin temperature usually remained at the target temperature for approximately 2 minutes, so we had sufficient time to finish the ultrasonography.

The use of a customized segmentation algorithm is a novel approach to acquire precise intraneural blood flow and constitutes a major strength of the study design. Please (4) indicate why the area of intraneural blood flow data was not included in the results section of the manuscript.
The statistical model is appropriate to address the study aims.

Response #4**:** We presented the detailed data of the area of intraneural blood flow on power Doppler images measured by the customized segmentation algorithm in Table 3. We have revised the subheading of Table 3 to make it clearer and revised the manuscript to describe the results of statistical analysis of the intraneural vascularity data in detail.

(page 15, line 220)
There were also significant differences between the two studied groups in the CSA of the median nerve measured at 30, 32 and 34°C. The area of intraneural vascularity of the median nerve on power Doppler images was also significantly larger in the CTS patients than the healthy volunteers at 30 and 32°C but not at 34°C (1.13 vs 0.65 mm2, p = 0.07) (Table 3). In comparison, among the within-group results measured at 30, 32 and 34°C in both studied groups, no significant differences were found in the sonographic findings of the median nerve, including the CSA and the power Doppler signal (Table 3).

Results
The study results are clearly described in the text and are accompanied by 3 tables clearly displaying the relevant data.

Discussion
There is a useful and informative discussion of vascular pathological mechanisms and thermal effects to explain the study findings.
Please comment (5) in the discussion on the role of maladaptive cortical neuroplasticity in the pathophysiology of CTS.

Response #5**:**
Thank you for your comments. We have added the following statements to the second paragraph of the Discussion section.

(page 18, lines 265)

Moreover, CTS may not only be a peripheral nerve disorder but also might be accompanied by maladaptive cortical neuroplasticity. For example, Maeda, Y., et al. demonstrated significant gray matter reductions in the contralesional primary somatosensory cortex (hand), pulvinar and frontal pole on magnetic resonance imaging (MRI) scans in CTS patients compared to healthy controls [27]. Event-related functional MRI also revealed that CTS patients had a reduced second/third interdigit cortical separation distance in the contralateral primary somatosensory cortex [28]. Therefore, chronic repetitive painful peripheral stimuli might induce plastic changes in the central nervous system that could lead to central sensitization. This might be the reason why some patients with CTS show symptoms beyond the median nerve innervated dermatome, such as the forearm [29].

Please discuss (6) the future directions of this research.

Response #6**:** Since microangiopathy and dysfunctional vasomotor response might influence intraneural vascularity of the median nerve, we recommend studying CTS patients with comorbidities, such as diabetes and chronic renal failure, in the future. We have added this statement to the Discussion section.

(page 20, lines 318)

Second, we only recruited patients with idiopathic CTS, which might not be reflective of the reality of daily clinical practice, where patients may have median neuropathy at the wrist level that may be secondary to various types of underlying conditions. Thus, future study of CTS patients with comorbidities, such as diabetes and chronic renal failure, is recommended.

Conclusion

The stated conclusion is commensurate with the study data.

References/Tables/Figures/Appendix

The references are relevant and appropriate in number. There are two high resolution US figures displaying short and long axis images of the median nerve in B-mode and power Doppler. The appendix displays two figures, the HSV color space model and color segmentation results.

Summary

The study question is clinically relevant and the design appropriate to address the aims. The methods employed a novel algorithm to quantitatively establish the intraneural blood flow, a major strength of the study. The study results have translational value.