Title Page

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Title: **An experimental investigation into staging CTS utilizing portable ultrasound and Doppler as a diagnostic alternative.**

Final Progress Report

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Date report completed: 4/30/13

Table of contents

List of terms

ACR-American College of Radiology

CSA-cross sectional area

CTS-carpal tunnel syndrome

EDX-electrodiagnostic testing

IRB-internal review board

MIPAV- medical image processing, analysis, and visualization

MMN-median mononeuropathy

MRI-magnetic resonance imaging

NIH-national institute of health

PD-power Doppler

ROI-region of interest

SD-spectral Doppler

SNR-signal to noise ratio

T2- MRI time 2 imaging sequence

T1-MRI time 1 imaging sequence

Abstract

This study explored the use of portable ultrasound to detect changes in the median nerve tissue of 11 macaca fascicularis monkeys, which were exposed to a pinch-pinch task. Inducing median mononeuropathy (MMN) otherwise known as carpal tunnel syndrome (CTS), was joined to a study by Sommerich, Buford, et al. This connected our imaging study to their effort and allowed for the longitudinal measure of both vascular and geometric changes within the median nerve tissue at baseline, during task exposure, and during the recovery phase. Portable ultrasound was provided by GE Healthcare using a Logiq i laptop ultrasound unit with a 12 MHz transducer. This was used to image the median nerve on all 11 subjects across the study period. Data was gathered on the size of the median nerve at several anatomical locations. Geometric measures were taken from the images at the forearm, the carpal tunnel inlet, and the carpal tunnel outlet. A measure that has been advocated was also taken at the hook of the hamate, which includes the carpal tunnel bulge. All ultrasound measures were made in the laboratory and meticulously recorded for comparison with electrodiagnostic testing (EDX) and magnetic resonance imaging (MRI).

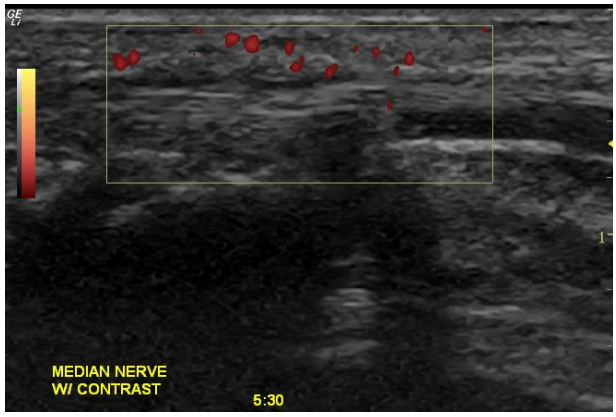
The timing of MRI and EDX was critical in order to compare the results for power Doppler (PD) and spectral Doppler (SD) ultrasound. Temperature of the subject was maintained with heated rice bags and imaging was timed with the schedule of MRI and EDX. The data were examined in accordance with the American College of Radiology's (ACR) Appropriateness guidelines for imaging the painful wrist. The ACR rates MRI as the gold standard therefore our geometric measures were taken as cross-sectional areas (CSAs) of both the outer and inner median nerve at all the anatomical locations indicated. Using Medical Image Processing, Analysis, and Visualization (MIPAV) software, from the National Institute of Health (NIH), we traced CSAs on the ultrasound and MRI images. The results indicated that the ultrasound CSAs at the level of the carpal tunnel inlet were statistically matched for the outermost layer of the nerve. Further image analysis demonstrated that as the outer tissue enlarged, the CSA of the inner layer inversely contracted. The hypothesis is that the residual nerve tissue was enlarging and constricting the fascicles of the median nerve. This has been reported as the "notch sign".

Retrospective analysis was completed on a strict inclusion of SD waveforms taken within the median nerve over the length of the project. Given the geometric change in the outer and inner layers of the nerve, the hypothesis was that inflammation was causing this acute physiologic change. SD waveforms were compared a baseline, during working, and into the recovery phase and minute changes were very difficult to detect statistically. One of the problems was the sensitivity of the SD for low flow changes. Separate examinations were conducted using ultrasound contrast to attempt to amplify the signals for increased detection and analysis. The EDX data was retained by Dr. Sommerich and will be reported as part of her R01 final report.

Section 1 of the final report

Significant (Key) Findings.

Specific Aim #1. To document the change in perineural blood flow within the median nerve, as an indicator of inflammation and acute onset of median mononeuropathy (MMN). Given the geometric changes that were detected in the cohort of macaca fascicularis, in the outer and inner layers of the nerve, the hypothesis was that inflammation was causing this acute physiologic change. SD waveforms were compared at baseline, during working, and into the recovery phase and minute changes were very difficult to detect statistically. One of the problems was the sensitivity of the equipment's SD for low flow changes. This was feasible, however, the spectral peak velocity was the only endpoint detected. The end diastolic component was often masked with artifactual noise. We have begun analyzing the waveforms on the final cohort after being injected with ultrasound contrast to assist in amplifying these important physiologic endpoints.



Specific Aim #2. To determine whether changes in the diameter of the median nerve, recorded with ultrasound, will indicate chronic changes due to MMN. The results indicated that the ultrasound CSAs at the carpal tunnel inlet was statistically matched for the outer layer of the nerve. Further image analysis demonstrated that as the outer tissue enlarged the inner CSA inversely contracted. The hypothesis is that the residual nerve tissue was enlarging and constricting the fascicles of the median nerve. This has been reported in the literature in Europe as the diagnostic "notch sign" on a sonogram. We now believe we better understand what is happening within the layers of the nerve tissue and contributes to an overall swelling of the nerve and ultimate entrapment under the transverse carpal ligament.

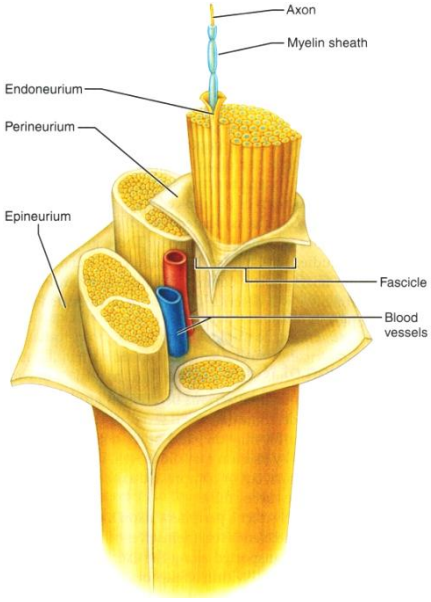
Specific Aim #3. To determine if changes in perineural vascular flow and nerve diameter coincide with changes detected with EDX and MRI. The data was examined in accordance with the American College of Radiology's (ACR) Appropriateness guidelines for imaging the painful wrist. The ACR rates MRI as the gold standard therefore our geometric measures were taken as cross-sectional areas (CSAs) of both the outer and inner median nerve at all the anatomical locations indicated. Using MIPAV software, from the National Institute of Health (NIH), we traced CSAs on the ultrasound and MRI images. We were successful in using MIPAV to complete image analysis on both the MRI and ultrasound images. Image analysis is the hallmark of our lab and we have also mastered a new image software –PixelFlux Scientific from Germany. PixelFlux allows our lab personnel to analyze Doppler and contrast Doppler images. These biomarkers for increased vascular perfusion allowed us to compare T2 weighted images on the macaca fascicularis and the Doppler images. Some difficulty was encountered with comparing data collected with MRI as a volume set compared to ultrasound Doppler captured in two dimensional planes. Dr. Sommerich is analyzing the EDX data and we understand that several of the subjects had appreciable nerve conduction drops that coincided with our geometrical measures.

Translation of Findings.

We are very encouraged that we have mastered the analysis of SD waveforms generated by the median nerve's perineural and intraneural vascularity. We have developed published inclusion

criteria for investigators to use when examining SD waveforms for scientific consideration. Waveform morphology is a clinical skill that most vascular labs use to determine the presence or absence of disease. Our lab has taken this to a much more detailed level of analysis by applying this to vascularity with the nerve. This is the first diagnostic attempt to characterize increased or diminished vascular flow within nerve tissue. We have already begun translating our findings to a set of experiments with ultrasound contrast agents. Definity® is a pharmaceutical agent that we were approved to inject into the subjects and we are the first lab to analyze vascularity that has been amplified using Definity®. In addition, the use of PixelFlux Scientific software proved to assist us in this added exploration of quantifying vascularity captured with ultrasound.

The formulation of a hypothesis that outer epineurium behaves differently than endoneurium tissue and that expansion and contraction of these layers could be the acute response to repetitive injury is a very important step forward. Geometric changes driven by vascular inflammation is a key biomarker and will likely fuel the continued investigation of how entrapment syndromes move from the acute to chronic stage of development. These important preclinical biomarkers have great influence on the human studies that are being staged and for clinicians trying to determine what imaging evidence is indicative of CTS. The affirmation that the sonographic "notch sign" seen when the median nerve swells prior to the carpal tunnel inlet is now much better understood at a physiologic level. This has some formative applications to the workplace with screening of the median nerve at the onset of pain and discomfort. This will allow the clinician to possiblly detect the physiologic changes that may be occurring both at the epineurium, the residual tissue, and the perineurium. At the diagram indicates the vessels that exist within the epineurium have penetrating branches that reach into the perineurium. Inflammation at the earliest stage could have detrimental effects on the median nerve, and if left untreated, could result in the nerve swelling, subsequently causing increased damage within the carpal tunnel.



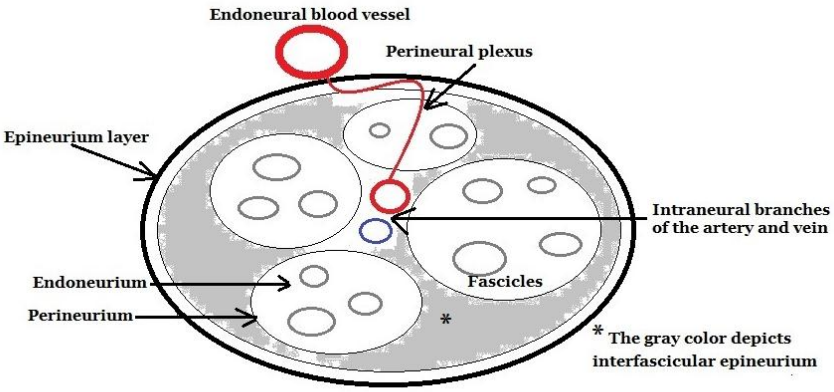
Our scientific work now involves translating all our equipment settings, scanning protocol, imaging analysis, and software prowess to determine the impact for screening a human cohort. We are currently IRB approved to study human subjects who are having a contrast cardiac echogram with Definity® and imaging their median nerve. The patients are segregated into symptomatic and asymptomatic based on provocative testing. We believe our pilot work will lead to greater understanding of this suspected physiologic response to entrapment.

Outcome/Impact.

1. Potential outcomes - Our recommendation is that screening ultrasound is feasible and may aid in the detection of acute physiologic changes within the nerve.
2. Intermediate outcomes - We have already published our early findings and have been speaking nationally and internationally and believe our work is being replicated and applied to higher levels of evidence - cohort studies.
3. End outcomes - It is too early to apply these preclinical results to workplace conditions but we do advocate that these results are indicative of acute changes to the median nerve and need to be seriously considered by employers of workers completing repetitive tasks.

Section 2 of the final progress report

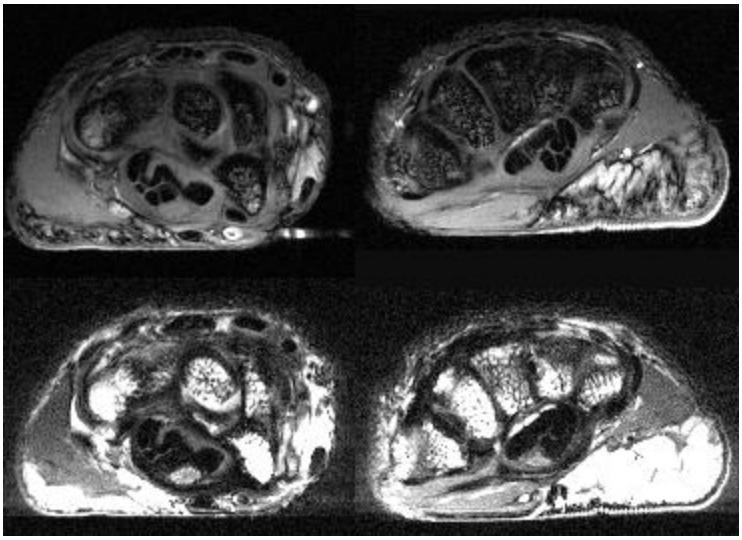
Specific Aim #1. To document the change in perineural blood flow within the median nerve, as an indicator of inflammation and acute onset of median mononeuropathy (MMN). The drawing that we created based on our experiments helps to illustrate our investigation of the residual area between the epineurium and the perineurium, and why an inflammatory response could be the reason that the inverse statistical relationship developed between these layers of nerve tissue. The diagnostic difficulty that occurred was having enough ultrasound equipment sensitivity to detect the vascularity at this micro-tissue level. Frustrated that we could not glean more than the peak systolic velocity from our waveforms, we chose to amplify the detection with the use of Definity® contrast agent which is a microbubble and provides an increased harmonic signal when hit by the ultrasound beam.

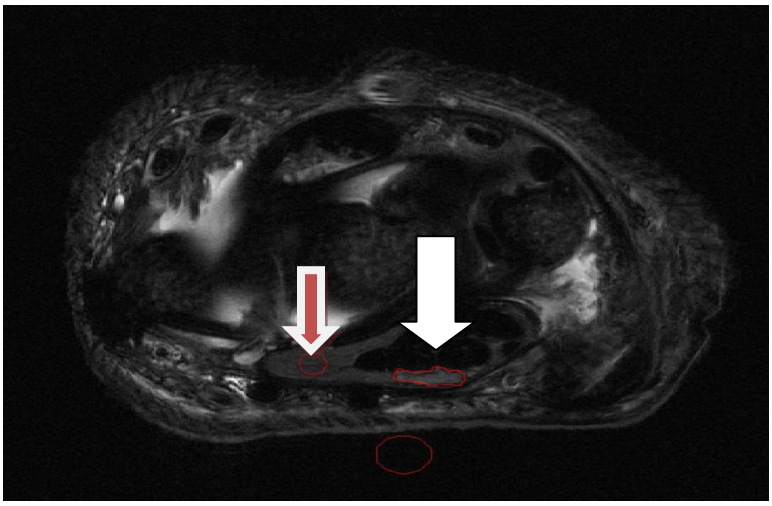


Cross-section of a peripheral nerve and associated vascularity

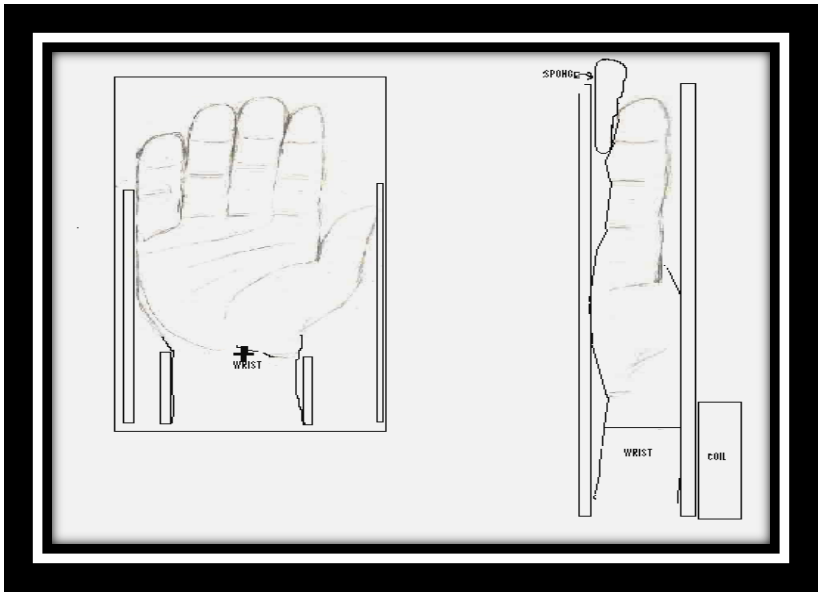
Specific Aim #2. To determine whether changes in the diameter of the median nerve, recorded with ultrasound, will indicate chronic changes due to MMN. Not all of the macaca fascicularis subjects demonstrated this relationship. Controlling for sustained work and using subjects from other studies proved to be a bad decision. We are encouraged by the overall group measures and realized that staging a preclinical study of this nature is much more difficult that was imagined. We appreciated Dr. Buford's efforts to train the subjects and monitor their working efforts.

Specific Aim #3. To determine if changes in perineural vascular flow and nerve diameter coincide with changes detected with EDX and MRI. Dr. Buford gave us the MRI images of the 11 subjects which were reviewed by an ARRT certified MRI technologist in our masters (MS) program in an attempt to analyze the images at both the carpal tunnel inlet and outlet. We asked that he analyze the T1 images for geometry and the T2 images for signal intensity. It became clear that the MRI images were difficult to analyze due to differences in subject positioning in the MRI bore. A splint was used but the subject had to have the working extremity over their





head to fit into the magnet. This induced the possibility of turning of the arm and perhaps a twisting of the median nerve for imaging. Our MRI technologist did an extra set of image analysis using the coronal views of the arm to determine the possibility of ulnar or radial deviation of the nerve due to positioning. This had the possibility of affecting the CSA outcomes which were derived from tracing around the median. The T2 images were also slightly affected by the positioning as the Signal to Noise Ratio (SNR) data was to be quality control checked by taking signal in the muscle and often that image wasn't stable for muscle on repeated measures. The image provided above, has a white arrow indicating the red region of interest (ROI) on the nerve and the second red ROI on the muscle, indicated by the smaller red arrow.



We found that indeed many of the subjects in the final cohort did have some slight degree of radial or ulnar deviation on the coronal images. Our MS student designed a prototype MRI arm positioned that could be implemented to control for this type of positioning error. See the image provided. The prototype would allow a shim to be placed between the Plexiglas to insure that the fingers are not flexed. Shims are also provided to insure that radial and ulnar deviations are not a problem. We feel strongly that a replicated study of this kind would need to use a MRI wrist positioned to insure that all T1 and T2 images are properly produced and ready for analysis. The coil for picking up the RF signal would be placed on top of the Plexiglas pane that covers the top of the hand.

Dr. Sommerich has processed the EDX data that was taken throughout the imaging sessions and she has told us that several of the macaca fascicularis had nerve conduction drops that likely corresponded to changes in size of the nerve. Dr. Sommerich is still completing her analysis and the drops in nerve conduction will need to be matched to the dates which we also detected changes in the size of the nerve and increased perfusion of the nerve (measured with T2 MRI and contrast ultrasound).

Publications.

Our research is having impact in the area of developing this specific line of scientific inquiry. Both my MS students (MRI technologist and vascular technologist) have compiled their theses on this set of studies and will graduate this spring for The Ohio State University's graduate program:
1. MRI MS thesis #1: *The use of MRI to longitudinally monitor the progression of MMN in an animal model: A preclinical study.-to be submitted to AJR in May.*
2. MRI MS thesis #2:*Retrospective Analysis of Signal Intensity of MRI T2 Weighted Images Compared to Contrast Enhanced Ultrasound Imaging of CTS in an Animal Model.*
1. RVT MS thesis #1: *Comparison of sonography to magnetic resonance imaging in determining the cross-sectional area of the median nerve, in a sample of working macaca fascicularis: A preclinical study.-Published in JDMS.*
2. RVT MS thesis#2: *Longitudinal Analysis of Median Nerve Perfusion Utilizing Spectral Doppler in a Working Animal Model-[under review] with JDMS.*

My personal contributions to distributing our research findings are as follows:
1. Musculoskeletal sonography: Evaluation of the carpal tunnel and median nerve. This **lecture** was presented at the CUDA meeting in Beijing, China 9/2012.
2. **Published**: Utilization of sonography to magnetic resonance imaging in determining the cross-sectional area of the median nerve, in a sample of working macaca fascicularis: A preclinical study. Volz, Evans, Fout, et al. *Journal of Diagnostic Medical Sonography*. 2012; 28:279-288.
3. Translating CEUS Intraneural vascularity from bench to bedside", was presented at the 2013 AIUM Annual Convention, April 6-10. The abstract was published in the *Journal of Ultrasound in Medicine*. ***Impact* factor - 1.25**
4. Evaluating an imaging protocol, utilizing contrast enhanced ultrasound, in the detection of vascularity surrounding the median nerve. **Manuscript** [under review] by the *Journal of Diagnostic Medical Sonography* journal.
5. Retrospective analysis of CEUS images in a cohort of macaca fascicularis with potential MMN. Journal **manuscript** [in press] to *Journal of Ultrasound in Medicine.* **Impact factor - 1.25**

Abstracts published
1. **Evans KD**, Volz KR. "Translating contrast-enhanced ultrasound intraneural vascularity from bench to bedside". *Journal of Ultrasound in Medicine*. 2013; 32:Vol. 51, S24. [Peer-Review] (Published) **Impact factor - 1.25**
2. Roll SC, **Evans KD**, Volz KR, Sommerich CM.. "Longitudinal analysis of grayscale imaging and electromyography in an animal model of carpal tunnel syndrome". *Journal of Ultrasound in Medicine*. 2013; 32 :Vol. 51 S49. [Peer-Review] (Published) **Impact factor - 1.25**
3. **Evans, KD**, Roll, SC, Volz, KR, Buford, JA, Sommerich, CM. "Ultrasound contrast enhanced interrogation of the median nerve to document perineural vascular flow in an animal model". *Ultrasound in Medicine and Biology*. 2011; 37:56. (Published) **Impact factor - 2.493**

Previously I reported on an abstract that was accepted and presented at the 13th annual World Congress of the World Federation of Ultrasound in Medicine and Biology, which was held in Vienna, Austria in August. The title of the abstract is: *Contrast enhanced evaluation of the perineural vascular flow within the median nerve in an animal model.* This scientific presentation was an oral presentation as well as a printed abstract in the journal, Ultrasound in Medicine and Biology.