Utilizing contrast-enhanced ultrasound for the detection of perineural trauma and associated hypervascularity of the median nerve: A small cohort study.

Undergraduate Research Thesis

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2013

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Abstract

This small cohort study was conducted to determine, by way of contrast-enhanced ultrasound (CEUS), if there is a quantifiable vascular difference in patients affected with carpal tunnel syndrome (CTS) symptoms versus patients who are not affected by the disease's symptoms. The clinical translation of this study was to identify CEUS as a novel diagnostic tool for the detection and evaluation of CTS and encourage the FDA approval of CEUS studies in the U.S. medical field.

Ultrasound equipment settings and dosing were optimized to provide consistent CEUS imaging. The contrast dosing amounts were determined at the discretion of the lead echo sonographer and varied in both dosing amount and number of dosing injections. UCA dosing amounts varied from 3 ml (Patient 1) to 5 ml (Patient 5), with a mean dose of 3.33 ml over all patients. The contrast solution for all subjects contained 1.3 ml of Definity® to 8.7 ml of saline. In addition, the equipment parameters were maintained at 4% output power and a mechanical index (MI) of 0.13. The transmit frequency of the linear transducer was held at a constant of 9 MHz throughout the trials.

The use of an image analysis software program was employed to quantiitize the amount of perinueral vascularity. An interclass correlation coefficient (ICC) was calculated between the investigators for manual signal counts and Iavg within the ROI to determine user reliability. Chronbach's alpha for signal counts and Iavg were 0.90 and 0.80 and ICCs were 0.90 ($p< 0.01$) and 0.80 ($p<0.01$), respectively.

This clinical study confirmed successful equipment settings and image analysis that allowed for a valid and reliable demonstration of vascularity surrounding the human median nerve; furthermore, the study found a qualitative difference in the vascularity of asymptomatic versus symptomatic median nerves.

Key Words: contrast enhanced ultrasound, carpal tunnel syndrome, contrast kinetics

Table of Contents

Acknowledgements

The amazing journey of conducting and concluding this research may be effortlessly summed by the words of Ralph Waldo Emerson, "*do not go where the path may lead, go instead where there is no path and leave a trail*." I have been extremely fortunate to conduct research at The Ohio State University and The Wexner Medical Center at The Ohio State University; two institutions that are deeply rooted in providing academic and professional success. It would be irreverent to say that I have blazed this path alone, as I have had many mentors and guidance along the way. I owe an immense amount of my accomplishments to the wonderful faculty and staff at OSU, who took a young and ambitious student under their collective wing and sculpted her into the bright and proficient young woman I am today. For that, I am forever grateful. I could also never forget to mention my cherished family and friends, as they are my greatest supporters and the basis of my personal motivation; they have never faltered in their patience and encouragement as I chase my dreams and blaze my life's trail.

Although this project has been a labor of love, there has been an abundance of blood, sweat, and tears shed along the way. My professor, my advisor, and one of my greatest advocates, Dr. Kevin Evans, has proven invaluable for the success of my research. The patience and dedication that he bestows upon his students is both admirable and unwavering. With true honesty I can write that my successes are a direct reflection of his commitment and passion for our wonderful profession of sonography. I look forward to continuing our endeavors for many years to come.

I am also perpetually grateful to the faculty and staff of the Radiologic Sciences and Therapy Division within the OSU School of Health and Rehabilitation Sciences. I would like to specifically thank Rachel Pargeon, Kevin Volz, Kate Zale, Pamela Foy, and Brian Keller for their contributions and encouragement. Your time and patience has been greatly appreciated and I am thankful for the friendships we have developed along the way.

My family is my everything, and they have provided for me the foundations of personal achievement and self-worth. My mother, Cheryl, the sunshine of my life; who has picked me up and dusted me off more times than I can count, believing in me when I didn't believe in myself. She is the never-ending warmth to my soul that only a

mother's love can provide. My sister, Jennifer, who has continuously watched over me as all older sisters do, yet has (cautiously) given me the space to make my own mistakes to learn from. Because of her I understand the true importance of inner strength and independence; she is my ultimate paradigm of strength and beauty. Also, the love and encouragement from my remaining family and friends has truly inspired me; I could have never achieved my success without any of you, and for that I am forever grateful.

Lastly, a path always begins somewhere and I know deep in my heart which pioneer first began to carve my way… my stepfather, Tim; my best friend, my role model, and my unsung hero. He is the epitome of all that is good in this world, and I cannot imagine my life without him. Knowing me better than I know myself, he has pushed me to achieve personal goals and dreams that were far beyond an extent that I ever felt possible for myself. He is the one person whom I dedicate the last four years of my life and the culmination of this research to. Tim- I whole-heartedly thank you for your continual love and support; you are my inspiration and my eternal strength, I am only successful because I have you. I love you.

List of Tables

Chapter 1 – Introduction

Background

Carpal tunnel syndrome (CTS) is a collection of characteristic symptoms and signs that occur following entrapment of the median nerve within the carpal tunnel. CTS is the most commonly reported nerve entrapment syndrome, as well as the most common work-related musculoskeletal disorder. Despite the large number of original research studies on CTS, considerable uncertainty exists about its extent and etiology, the contribution of work and non-work risk factors to its development, the criteria used to diagnose it, the outcomes of various treatment methods, and the appropriate strategies for intervention and prevention.[1] The only certainties of the disease are the subsequent pathophysiology of the entrapped median nerve and resultant CTS, as well as the syndrome's alarmingly increased incidence rate across the globe.

The epidemiology of carpal tunnel syndrome has been reported in general populations; mainly in specific regional, national groups, or in working populations related to workers' compensation claims. Studies in the Netherlands, Italy, and in the United States have focused on single cities or regions and have noted incidence rates from 1.3 to 3.5 per 1,000 person-years.[2-3] Korea has been documented to have the highest prevalence, with a rate of 4.96 per 1,000 person-years.[3] Other demographic studies have shown a higher incidence of carpal tunnel syndrome in working populations and employees of at-risk occupations, consistent with data showing that repetitive motion of the wrist may be contributory to the symptoms of CTS.[4] Diagnostic Medical Sonographers (DMS) are model examples of workers in an at-risk occupation, as studies have indicated that approximately 65% of DMS have experienced CTS symptoms throughout their career and over 90% are working in pain.[5]

Frequent or repeated compression and stress on the median nerve creates a constellation of pathological symptoms, with the eventual outcome of CTS. The initial insult of median nerve entrapment is a reduction in epineural blood flow, caused by the elevated pressure and stress. This impaired nerve perfusion triggers ischemia and damage to the nerve-blood barrier within the endoneurial capillaries. Continued or increased pressure eventually causes epineural edema and inflammation, with the inflammation resulting in fibrosis. The effects of

perineural fibrosis lead to scar tissue formation, axonal injury, and localized demyelination.[6-8] This pathological cascade of CTS symptoms result in physiological symptoms surrounding the affected nerve, and increase in severity as the individual's CTS progresses. Early clinical symptoms include intermittent numbness, tingling, burning, or pain in the lateral palm, thumb, index, and middle fingers. The hallmark symptom of CTS is nocturnal pain and numbness, often causing affected individuals to be awaken at night. Clinical symptoms increase in severity as the syndrome progresses; which leads to paresthesia, the inability to grip or pinch, constant numbness, and decreased pain due to nerve demyelination. At the most advanced stage of CTS, the affected individual may experience muscle atrophy and increased weakness.[7-8]

Diagnostic procedures for carpal tunnel syndrome remain largely inconsistent to-date, mainly due to a lack of a "gold standard" test for the syndrome. Diagnoses often rely on an assemblage of testing procedures that vary from clinical evaluations and elecrodiagnostic studies (EDX), to the recently novel sonographic examinations (US). Clinical diagnoses include an extensive examination of symptom specifics: including location, characteristics, provocative factors, and functional status surveys (FSS) or symptom severity surveys (SSS).[9] Physical testing is often utilized for clinical diagnosis with the help of a myriad of subjective testing, including but not limited to Phalen's test, Tinel sign, Durkin's test, carpal tunnel compression test (with and without wrist flexion), and a hand elevation test. Electrodiagnostic studies (EDX) include electromyography (EMG) and nerve conduction studies (NCS), with NCS more commonly used.[7] While EDX has previously been considered a "gold standard" for entrapment neuropathy diagnosis, its true significance has recently been debated within the research literature.[10-13] Imaging studies, including sonography, MRI, and CT, have recently been incorporated as a novel diagnostic tool.

The treatment of carpal tunnel syndrome varies and is determined by the severity of the disease. Nonoperative management includes splinting the wrist, oral medications, or corticosteroid injection into the carpal canal. These conservative treatments help to reduce associated pain and may completely relieve CTS symptoms. However, when nonoperative management fails, surgical treatment is indicated. Carpal tunnel surgery involves the division of the transverse carpal tunnel ligament to alleviate compression of the median nerve, and may be

performed openly or endoscopically. The most common surgery performed is endoscopic carpal tunnel release, which has provided excellent success rates.[7] In rare cases, some patients may continue to have persistent CTS symptoms even after operative procedures, and may require additional evaluation.[6,7]

Significance of the Problem & Clinical Translation

Carpel tunnel syndrome risk and occurrence are ever-increasing in today's population. Diabetes, obesity, and occupational injuries are several of the contributing factors expected to increase the prevalence rate of CTS.[7,9] Consequently, the current trends of carpal tunnel syndrome identify the need for a reliable, non-invasive method for evaluating and diagnosing CTS. The lack of a "gold standard" testing procedure for such a common syndrome is becoming increasingly elusive in medicine. While clinical diagnosis and confirmatory EDX provides some form of CTS identification, this combination of testing procedures lacks both patient comfort and consistency. Electrodiagnostic studies are invasive, uncomfortable, time-consuming, and costly. Most significantly, the results of EDX studies are highly questionable, often producing false-positives and a wide range of sensitivities.[14-15]

Fortunately, sonography has presented itself as an exciting, novel diagnostic test for the diagnosis of carpal tunnel syndrome. This modality is widely available throughout the world and provides a cost-efficient, portable, and easily tolerable testing alternative. Additionally, the non-invasive nature and lack of radiation makes sonography an ideal testing procedure for repeat examinations. Current research literature has reported the sonographic evaluation of the cross sectional area (CSA) of the median nerve as beneficial for assessing CTS, and a recently published study concluded that there was a significant agreement between US and NCS when using a combination of sonographic and clinical parameters.[9] To-date, researchers at The Ohio State University have begun to establish a reliable and valid imaging protocol for identifying and measuring the hypervascularity of median nerves in symptomatic CTS patients by use of contrast-enhanced ultrasound.[13] It is plausible that, with increasingly supportive data from current and upcoming research trials, sonography may replace EDX as a first-line test to confirm clinically diagnosed CTS, and may potentially provide for a "gold standard" in carpel tunnel syndrome diagnosis.

Research Objective

Recent research has not only provided exciting new evidence that sonography may be used in the identification and evaluation of CTS, but also supports the idea that sonography may become the "gold standard" for the detection and diagnosis for carpal tunnel syndrome. The modality has already provided *definite evidence* for the subjective and objective detection of the pathological effects observed in CTS patients.[9] Furthermore, clinical studies have shown that contrast-enhanced ultrasound (CEUS) plays an important role in providing a quantifiable CTS diagnosis.[13] Ultrasonic contrast, coupled with perfusion software evaluation, has the logical potential to detect the perinueral trauma and associated hypervascularity of the symptomatic entrapped median nerve.

Before implementing the use of sonography as a first-line diagnostic tool for carpel tunnel syndrome, and confidently deeming the modality as a "gold standard", the use of CEUS for evaluating CTS must be further explored. Specifically, appropriate sonography equipment settings and a definite protocol must be identified to provide consistent and replicable CEUS images of the median nerve. Additionally, a quantifiable and irrefutable vascular difference in the median nerve must be determined for asymptomatic CTS individuals versus symptomatic CTS individuals. By assessing and determining these central variables, sonography may prove its ability to provide a definitive and preferred testing procedure for the diagnosis of CTS.

Chapter 2 – Literature Review

Introduction

As stated previously, the use of sonography as a diagnostic tool for carpel tunnel syndrome has become an increasingly researched proposal. Thus far, numerous clinical studies have published conclusions surrounding the potential of sonography as a diagnostic tool for the symptomatic median nerve.[9-15] Subjective sonographic differences between affected and unaffected CTS patients have been definitively established, and a significant agreement between sonography and NCS parameters has been readily identified in the literature.[9,14-15] In addition, the use of CEUS is possibly the most novel and promising verification of the diagnostic tool, providing sonographically quantifiable measurements of pathological CTS symptoms. The only limitation currently restricting the further advancement of CEUS' diagnostic ability for carpel tunnel syndrome within the United States medical field is the lack of the Food and Drug Administration (FDA) approval for the use of ultrasonic contrast agents in the United States. This challenging drawback and its associated implications are further explained in the literature below.

`Contrast-Enhanced Ultrasound (CEUS)

Throughout the past several years there have been extraordinary advancements regarding contrast-enhanced ultrasound (CEUS) and its potential abilities. The use of CEUS has provided the remarkable ability to improve sonographic image quality and increase the transmission of the ultrasonic beam.[16] Numerous animal and human-model studies have been conducted to explore and determine the benefits of CEUS, with Europe and Asia undoubtedly pioneering its use.

Nearly all of the 900 original investigative articles found in PubMed under the search term "Contrast Enhanced Ultrasound" are studies from European or Eastern hemisphere countries.[16] In fact, the first four articles examined within this chapter have been written outside the United States – Switzerland, Canada, Japan, and The Netherlands, respectively. Limited studies have been conducted in the United States due to the lack of the U.S. Food and Drug Administration (FDA) approval of CEUS, with the exception of cardiac echo studies.[17] Europe's

extensive lead in the study of CEUS has led to the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) to release the first guideline on the use of contrast-enhanced ultrasound.[17] There have been two subsequent guidelines written- with the most recent update of the guideline published online in 2011.

The purpose of this review was to explore the published results of CEUS nationally and internationally – and to highlight any gaps that may exist in our understanding of how contrast media could increase diagnostic detection in the U.S., especially in the case of carpal tunnel syndrome. The following articles were reviewed to provide evidence for the exciting prospect of ultrasonic contrast agents in diagnostic sonography, and to accelerate the FDA's approval of CEUS outside of echocardiography studies – ultimately leading to the approval of CEUS as a supported diagnostic tool for the detection of carpal tunnel syndrome.

Literature

Myocardial perfusion imaging, a new advancement in echocardiography, is one new procedure in the field of contrast-enhanced ultrasound.[18] In myocardial contrast echography, minute microbubbles are injected intravenously and travel through the coronary microcirculation to produce an opacification of the myocardium.[18] Researchers from Bracco Research SA in Geneva, Switzerland developed a study to evaluate the potential of SonoVue, a new echo contrast agent, for the detection of myocardial perfusion abnormalities in minipigs.

Five animal-model experiments were performed in closed-chest minipigs and two were performed in open-chest minipigs. The animals were placed under general anesthesia and injected intravenously with the ultrasonic contrast agent (UCA) SonoVue (2.5 μm). Dose range of the UCA varied between 0.01-0.05 mL/kg. Myocardial contrast echography was performed in all experiments using an HDI 3000 ultrasonongraphy machine with intermittent harmonic power Doppler. All machine settings were kept constant throughout each experiment except for the pulse repetition frequency (PRF); which ranged between 500 to 6000 Hz.

Results confirmed that injection of SonoVue caused reproducible and homogenous myocardial opacification at 0.01 mL/kg.[18] As the dose of echo contrast increased, a slight increase in peak intensity and a prolongation of the myocardial contrast effect was noted[18]. Specifically, the duration improved from 80 seconds at 0.01 mL/kg to

more than 2 minutes at 0.05 mL/kg. However, doses of 0.03 mL/kg and higher resulted with shadowing within the left ventricular cavity.[18] SonoVue provided differentiation of the ischemic zone from normally perfused zones in the left anterior descending aspect of the coronary artery.[18] The mean myocardial contrast intensity was also found to increase when amplifying the pulse repetition frequencies from 500 Hz to 1500 Hz, and reached plateau at 1500 Hz.[18]

Researchers concluded that echo contrast agents, specifically the use of SonoVue, are capable of consistent and prolonged enhancement of the myocardium after intravenous injection.[18] Harmonic Doppler imaging and varying PRFs were also determined by the research to enhance the effects of UCAs.[18]

Previous placental studies detailed the development of the intervillous space- a blood-filled compartment which enables effective exchange between fetal and maternal circulations.[19] Researchers at St. James's University Hospital in Leeds, UK, developed an animal-model series of case studies to evaluate the intervillous flow in early pregnancies with the use of echo-enhancement agents.[19] Using a high-resolution ultrasound scanner, the uteroplacental circulation of the cynomolgus monkey (*Macaca fascicularis*) was examined. This study similarly followed the potential of using UCAs. The contrast agent, Albunex, was injected intravenously and used to enhance the color Doppler images acquired. Intervillous flow in the macaque was explored to decide whether the use of echo-contrast agents would assist in determining placental circulation in human pregnancy at a correspondingly early stage.[18]

Nine cynomolgus monkeys were selected for the study and divided into two groups. The first group consisted of four animals with gestational ages of 18, 27, 31, and 52 days post-conception, respectively.[18] The second group had five animals between 37 and 69 days of gestation.[18] All studies were performed with an Acuson 128/XP10 high-resolution ultrasound scanner and completed using a 7-MHz linear array probe with color and pulsed-wave Doppler capabilities. Albunex was the chosen UCA due to its small diameter characteristic.[18] For a closer examination of various microbubble characteristics, please refer to Table 1.

Each animal was restrained without sedation during the beginning of the sonographic examination. Fetal viability and biometry were first observed, imaging the uterus in both transverse and sagittal planes. After sedation, placental location was confirmed and uteroplacental vasculature was assessed. Albunex (3.8 µm 0.5 ml/kg) was then injected intravenously and all gray-scale, color, and pulsed-wave Doppler images were recorded.[19]

Results determined that the passage of the spiral arteries at the decidualtrophoblastic interface, and their point of discharge into the intracotyledonary space, was clearly identified with the use of color Doppler in the macaque at 52 days gestation.[19] Blood flow analysis of the pulsed-wave Doppler images also revealed the characteristic features of flow velocity in the intracotyldonary space.[19] Venous drainage of the intervillous compartment was also visualized in the macaque at 31 days post-conception.[19]

The authors concluded that intervillous flow in early primate pregnancy can be determined with the use of an echo-enhancement agent and pulsed-wave Doppler strategies.[19] Similar approaches may assist analysis of the early intervillous compartment in human pregnancy.[19]

Sonography has been used as a routine procedure for the detection of focal splenic lesions.[20] Determinations of benignity or malignancy in canine splenic lesions have traditionally been difficult when using conventional sonography methods.[20] Researchers at the Laboratory of Veterinary Internal Medicine replicated previous research by exploring whether contrast-enhanced U/S could assist in differentiation between benign and malignant focal splenic lesions in dogs.[20]

Twenty-nine focal splenic lesions were examined in 29 dogs. Focal splenic lesions in the animals were determined by conventional sonography and the final diagnosis was confirmed by histology or cytology.[20] All examinations were performed with an ultrasound machine and a 5-11 MHz broadband linear probe or a 3.75 MHz convex probe.

Perflubutane microbubbles (0.12 µL microbubbles/kg) were injected intravenously within the cephalic vein.[20] Real-time imaging was performed from preinjection to 1 minute after injection of the contrast during the vascular

phases. Images during the parenchymal phase were obtained from a previous study, with duration of 7-10 minutes after injection of the contrast.

Qualitative assessment of vessel appearance in the lesion was performed immediately after injection. Vessel appearance was divided into 3 groups to compare the vessel with surrounding parenchyma: similar, different, and invisible. Qualitative assessment of the enhancement pattern was performed in the early vascular phase (5-10 seconds after injection), late vascular phase (25-30 seconds after injection), and parenchymal phase (7-10 minutes after injection).[20] Enhancement pattern was divided subjectively based on the echogenicity comparison between lesion parenchyma and the surrounding normal parenchyma (hypoechoic, isoechoic, and heteroechoic). Statistical analyses determined a significance between benign and malignant lesions and the respective sensitivity and specificity.[20]

Of the 29 dogs included in the study, 13 dogs had benign nodules and 16 dogs had malignant tumors. The vessel appearance was not significantly different between malignant and benign lesions.[20] However, similar patterns were visible in 9 of the 16 malignant lesions and 10 of the 13 benign lesions.[20] Enhancement patterns between benign and malignant lesions were found to be significantly different during the early and late vascular phases.[20] Hypoechoic pattern was found to be significantly associated with malignancy, as a hypoechoic pattern was found in 6 of the 16 malignant lesions and in none of the 13 benign lesions.[20] Isoechoic and heteroechoic patterns were not found to be significantly different during the early vascular phase.[20] In the late vascular phase, hypoechoic pattern was significantly associated with malignancy and isoechoic pattern significantly associated with benignancy.[20] No significant difference was found between benign and malignant lesions in the parenchymal phase.[20]

The study concluded that the use of CEUS has significant value in differentiating between benign and malignant focal splenic nodules in dogs with high accuracy.[20] While the parenchymal phase of imaging was not significant in differing between benign and malignant lesions, detection of hypoechoic nodules in the late vascular phase of perflubutane microbubbles-enhanced ultrasound is suggestive of benign lesions.[20] Also, differentiation between benign and malignant lesions was determined to be highly accurate during the early vascular phase.[20]

Prostatic cancer is the 2nd leading cause of death among males in the United States.[21] This increased incidence sparked the advent of prostate-specific antigen (PSA) assessment and transrectal ultrasound imaging for prostate cancer detection.[21] Further diagnostic advancements resulted with researchers from the University Hospital Nijmegen (The Netherlands) creating a series of case studies to investigate contrast-enhance three-dimensional power Doppler angiography of the human prostate.[21] This study likewise explored the subject of ultrasonic contrast agents but used human patients instead of an animal model to complete the research. The overall objective of the study was to determine the practicability of contrast-enhanced 3D imaging of the prostate and to analyze whether symmetry and distribution of the vascular structures in the 3D images correlated with biopsy outcome.[21]

Eighteen male patients with a strong suspicion of prostate cancer were chosen for the study. 3D power Doppler angiography images were obtained in all patients before and after intravenous injection of the contrast agent Levovist (2 μm). All examinations were performed with a Voluson 530 D ultrasound machine with 3D capabilities. Enhancement of power Doppler signals was observed within 1 minute after beginning the contrast administration.[21] After images were obtained, all patients underwent sextant prostate biopsies and specimens were color-coded by site of origin and analyzed separately.[21]

Thirteen cases had positive biopsy results for prostate cancer (72%).[21] Vascular anatomy was judged abnormal in unenhanced images in 6 cases – 5 of which proved malignant.[21] Enhanced images were considered suspicious for malignancy in 12 cases; of these cases, biopsy results found 1 benign vasculature and 11 malignant vasculatures.[21] In 6 patients, B-mode images were considered not suspicious for cancer; however, in 4 of the 6 patients the final judgment on vasculature was changed from normal to abnormal after administration of the contrast agent.[21] In all these patients, biopsy specimens were found to be malignant.[21]

Researchers from the study concluded that the use of microbubble contrast agents, such as Levovist, combined with 3D imaging gives rise to clear enhancement of Doppler images in the human prostate.[21] In 11 of 13 patients with positive biopsies, contrast enhanced images showed abnormal prostate vasculature. The

enhancement of prostatic Doppler images increased the sensitivity from 38% in unenhanced images to 85% in enhanced images, a clear sign of the contrast agents' significance.[21]

Carpal tunnel syndrome (CTS) is an increasingly prevalent musculoskeletal disorder spanning the globe. CTS presently affects over 8-million Americans and is the number one reported medical problem – accounting for about 50% of all work-related injuries.[22] In relation to the clinical field, carpal tunnel syndrome has been discovered to be the most prominent work-related injury among Diagnostic Medical Sonographers.[5] One study found that 90% of sonographers are scanning in pain, with the majority suffering numerous symptoms of CTS.[5] Nearly all of the published research regarding carpal tunnel syndrome is targeted at prevention and rehabilitation; however, researchers are discovering the diagnostic capabilities of contrast-enhanced ultrasonography (CEUS) in identifying and diagnosing CTS. Additionally, researchers and medical experts praise its potential ability to provide a "gold standard" diagnostic tool for the detection of carpal tunnel syndrome.[22]

In regards of such a novel diagnostic tool, researchers at The Ohio State University developed a study designed to provide scientific evidence, gather preclinical safety information, and determine the efficacy of CEUS for detection of median nerve vascularity.[13] The main goal was to identify a contrast media dose that would consistently demonstrate perineural vascularity along the median nerve in the macaca fascicularis, and to develop a reproducible protocol for sonographically imaging the median nerve along the carpal tunnel inlet.[13]

Eleven young adult female monkeys (macaca fascicularis) were trained to complete a repetitive pinching task with their left thumb and finger, mimicking the pre-cursor occupational risks associated with developing carpal tunnel syndrome. During data collection, all subjects were anesthetized with ketamine and maintained with isofluorine gas provided from a mask. The equipment utilized was a GE Logiq 9 (GE Healthcare, Inc., Milwaukee, WI) complete with contrast settings and a GE Logiq i (GE Healthcare, Inc., Milwaukee, WI) which is considered a hand-carried unit. A 9.0 MHz linear broadband transducer was used with the GE Logiq 9 and a 12.0 MHz linear broadband transducer was used with the GE Logiq i hand-carried unit. Definity® (Lantheus Medical Imaging, Billerica, MA) was determinedly used as the contrast agent for the study because it possessed the smallest microspheres, 1.1-1.3 um, stability of < 10 minutes, and resonates at 4MHz.[13-16]

Perineural vessels were imaged with a suspension solution of 0.04 mL Definity®/0.96 mL saline introduced over five minutes for a total dose of .8 mL of contrast solution.[13] No side effects or negative reactions were documented post-injection of the UCA into the Macaca fascicularis. In order to gather objective data from the images collected, the use of an image analysis software program was employed to quantitize the amount of perineural vascularity. In conclusion of the study, researchers both determined the most appropriate ultrasound machine settings to image the median nerve of the macaca fascicularis and also developed an appropriate dose of the ultrasonic contrast agent (Definity®) to properly visualize the perinueral vasculature for a defined time scale[13]. Furthermore, the study found the automated PixelFlux software to be statistically significant in the purpose of obtaining objective data in the evaluation of the vascularity of the median nerve.[13] Researchers concluded the article by challenging future CEUS studies to not only determine the reproducibility of their successful imaging protocol for the median nerve, but also to implement a higher level of evidence into such a study.[13]

Comparative analysis

All articles similarly concluded that UCAs provide extremely positive effects in sonographic images.[13,16-21] In the myocardial opacification study, CEUS allowed for prolonged enhancement of the myocardium along with differentiation in the LAD occlusion of the coronary artery.[18] The early placental study concluded that CEUS allows for the determination of intervillous flow in early primate pregnancy, leading to the possibility of a similar analysis in human pregnancy.[19] UCAs provided differentiation between benign and malignant splenic lesions in one canine study.[20] And even more impressive were the results of the human prostate study- CEUS provided a 47% increase in the sensitivity of determining benign versus malignant prostate vasculature.[21] Most innovatively, researchers at The Ohio State University developed a protocol for the CEUS imaging of the median nerve and found significant, objective measurements with the use of a novel, automated CEUS software.[13] None of the articles provided evidence of negative or null effects of CEUS, significantly showing that contrast-enhanced ultrasound has an extremely high rate of positive effects in the demonstrated fields.[13,16-19] This growing level of evidence should provide increased scientific confidence in CEUS and its potential impact on screening for earlier signs of disease.

Also demonstrated within the articles is the importance of microbubble size.[13,16-19] As scientific evidence is increasingly published, the diameter of the ultrasonic contrast agents produced by pharmaceutical manufacturers correlationally decrease in size. To further explain, within the animal studies a microbubble size of 2.5 to 3.8 µm was administered (SonoVue, Perflubutane bubbles, and Albunex, respectively); however, within the human study a contrast agent with microbubble size of 2µm was administered (Levovist). Most noteworthy, the microbubble size of Definity® within the macaca fascicularis study is of miniscule size; measuring at 1.1-1.3 µm. Essentially, research studies such as the ones outlined above show that the smaller the microbubble diameter, the more potential the contrast agent has to traverse various body systems and allow for enhancement of images.[16] Table 2.1 summarizes the increasingly advanced characteristics of clinically evaluated contrast agents, including diameter size.[16] The advancement of UCA diameter size, along with others seen in the table, provides even more possibilities for enhancement in images and diverse applications for screening early stages of disease.

Agent	Physical Components	Size (m)	Stability	Transpulmonary	Application
Albunex	Air, human albumin shell	3.8	<1 min	Yes	Endocardial border delineation
Echovist	Air, galactose matrix	2	<1 min	No	Right heart cavities, cardiac shunts
Levovist	Air, galactose matrix with palmitic acid	2	<5 min	Yes	Heart, liver, kidney imaging
EchoGen	Dodecafluoropentine	2-5	>5 min	Yes	Cardiac
Optison	Octafluoropropane	2-4.5	>5 min	Yes	Opacification of heart chambers, left ventricular endocardial border
SonoVue	Sulfurhexafluoride polyethylene glycol, phospholipids, palmitic acid	2.5	>5 min	Yes	Opacificaiton of heart chambers; left ventricular endocardial border; cerebral, carotid, and peripheral arteries breast and liver vascularity
Definity	Liposome encapsulated perfluoropropane	1.1-3.3	<10 min	Yes	Opacification of heart chambers, left ventricular endocardial border, prostate

Table 2.1. Contrast Agent Characteristics. *Ultrasound Physics and Instumentation 4th ed.*

Conclusion

Overall, the preceding articles offer compelling evidence of UCAs providing enhancement of sonographic images.[13,16-19] All studies explored various areas of the human anatomy and provided similar, observable enhancement of sonography images by means of contrast. Enhancement of images not only assisted in viewing complex capillary and musculoskeletal systems within the body, but also helped to identify differences between benign and malignant lesions.[13,20] The fact that using ultrasonic contrast agents has been found to have similar, positive opacification effects regarding such a broad range of analyses provides undeniable evidence for the diagnostic usefulness of CEUS. The EFSUMB Guideline also provides an extensive list of anatomical fields, other than those in the above articles, that contrast agents can aid in.[17] Advancements in CEUS, and the continuing research regarding UCAs, will only continue to press forward the positive effects found of contrast-enhanced ultrasound. Although animal studies are considered a relatively high level of evidence, human trials are necessary to promote the benefits of CEUS and ultimately provide the significance necessary for the FDA's approval of diagnostic UCAs in the United States.

Since the literature is heavily dominated by animal studies that prove the effectiveness of ultrasonic contrast agents, a human pilot study that determines the feasibility of utilizing CEUS is necessary to increase the levels of evidence in support of UCAs. Given that the researchers at The Ohio State University have concluded an immensely successful study, determining not only a protocol for the imaging of the median nerve in macaca fascicularis but also the significance of a novel automated software program previously unbeknownst to the U.S. medical imaging community, the most appropriate course of action would be to determine the translational quality of the study's results in a human-model. The benefits of the continuation of such a study are trifold – first, to provide sufficient background and an increased level of evidence for the FDA's approval of CEUS, outside of echocardiography studies; second, to establish increased verification of the use of contrast-enhanced sonography as a diagnostic tool and eventual "gold standard" for identifying entrapment neuropathy in symptomatic CTS patients; and third, to reinforce the success of the retrospective study's regulatory equipment protocol for imaging the median nerve.

Research Question

To address the possibility of CEUS providing diagnostic criteria in association with carpal tunnel syndrome, the developed small cohort study ultimately addresses the two following research questions:

As previously identified within the macaca fascicularis study, is there a quanitifiable vascular difference in the median nerve in CTS symptomatic humans versus CTS asymptomatic humans? Furthermore, are the identified protocol and equipment settings optimal to capture consistent and replicable CEUS images along the course of the human median nerve while simultaneously providing little to no risk for patient well-being?

By investigating these research questions, valuable information may be attained that will increase the knowledge and support of the identification and evaluation of CTS by use of CEUS.

Chapter 3 – Methods and Analysis

The following materials and methods were determined by the published materials and methods defined in a retrospective study conducted by researchers at The Ohio State University. A detailed description of the following materials and methods have been published in a referenced article and comparatively used for this research study.[13]

Materials and Methods

Patients

This study was designed to obtain a higher level of clinical information, verify the effectiveness of equipment settings determined in a retrospective CEUS study, and determine the efficacy of CEUS for detection of median nerve vascularity in a human-model. Nine human patients from The Ohio State University Wexner Medical Center (OSUWMC) were voluntarily recruited to participate in the musculoskeletal CEUS study alongside their originally scheduled CEUS echocardiography studies. The subjects were briefed about the purpose and associated risk of the study and properly directed to sign the appropriate consent forms required by The Ohio State University's (OSU's) Internal Review Board (IRB). The subjects varied in age, gender, and symptomatic or asymptomatic syndromes of the wrist, hand, and fingers. Individuals were excluded from the study if the participant had major trauma in the distal upper extremity, the participant had previous carpal tunnel surgery in the right hand, the participant was previously diagnosed with polyneuropathy, the participant had a dialysis shunt in the right upper extremity, or if the participant was pregnant or within 3 months post-partum. Physical examinations and pain-severity surveys were completed before the sonographic imaging proceeded. Evaluations were performed at Ohio State's Ross Heart Hospital – Noninvasive Peripheral Vascular Laboratory in Columbus, OH. Vital signs and adverse reactions were monitored by a registered nurse employed by OSUMC. The research study was granted approval by OSU's Internal Review Board.

Equipment

The equipment utilized was a GE Logiq i, which is considered a hand-carried unit. A 12.0 MHz linear broadband transducer, downshifted to a transmit frequency of 9.0 MHZ, was consistently used to examine the median nerve of the patient. The output power was reduced to 4% in order to preserve the contrast activity. Throughout the series of experiments, quality control was maintained on the units and transducers with weekly checks based on imaging of the tissue mimicking phantom.

Previously determined equipment settings, as outlined in an earlier published study, were maintained to record consistent imaging data of the human median nerve. At the end of each CEUS session, the output power was increased to 100% for one minute to clear any residual contrast and a saline flush was also applied.

Contrast dosing

Definity®[23] was used as the contrast agent for this study because it possesses the smallest microspheres, 1.1-1.3 um, stability of < 10 minutes, and resonates at 4MHz.[16] These unique features of Definity® made it ideal for this experimental application. The dosing protocol was developed in consultation with the manufacturer and with cardiac sonographers who had experience using the product for human studies. A registered nurse employed by OSUMC managed the preparation of the doses designed to increase visualization of selective anatomical structures. The contrast was activated according to the instructions provided by the manufacturer and was also vigorously agitated in the syringes prior to being injected.[23] A work-sheet was kept that contained quantitative and qualitative data relating to the injection of contrast for each session and subject. Each subject had a 20-gauge catheter placed in either the right antecubital area or back of the right hand. The number of injections and contrast dosing amounts varied by patient, and were at the discretion of the lead echocardiography sonographer.

Nine subjects were imaged with a series of random injections as determined by the lead echocardiography sonographer. Research sonographers (AMH, KDE, & KRV) were blinded to the dosing series and the injections were concealed so that all immediate CEUS data was collected and evaluated without knowledge of the injection type or the order they were administered. This subset of experiments was added to confirm the rigor and reliability of the results that were reported.

CEUS image analysis

The equipment settings were maintained as outlined in a previously determined imaging protocol of a CEUS study of the median nerve. Additionally, a multi-incremental sampling method for imaging was similarly preserved. The imaging samples were captured at baseline and then every 30 seconds until seven minutes elapsed from the initial injection.

In the original study, choices were limited as to the method for image analysis given the restrictions imposed by the United States' FDA on the use of CEUS. Given this situation, a manual system was chosen for the pre-clinical study and maintained in the human study for assessing enhanced vascularity around the median nerve. To accomplish this, manual counting of PD pixels on the multi-incremental images, captured throughout the series of imaging trials, was conducted. The Klauser method[2] for counting PD pixels within the region of interest (ROI) was also maintained in the prospective study.

Statistical Analysis

Descriptive data was collected during the equipment optimizing trials as well as logs of equipment set up. These were used to record settings and resulting subjective image quality. Descriptive data was also charted on injection quantity, contrast activity beginning and ending times, and subject physiologic response. The final images of the respective median nerves were analyzed with a manual method and the resultant data was recorded for future analysis. Due to the manual method chosen to assess the data, measures of agreement and reliability were completed to verify the intra-rater reliability of the author of this study (AMH) in comparison to the original study's researchers (KDE & KRV). The information gleaned from the Pixel Flux Scientific software provided the foundation for the subjective and qualitative analysis of the recorded images. The image data was evaluated within subjects and over the intermittent time of imaging; specific research numbers were then recorded for further data analysis and are detailed in the following chapter.

Chapter 4 – Results

Demographics

The subject cohort consisted of 9 individuals (9 male, 100.00%). Symptoms were reported in 4 subjects (44.4% prevalence). Subject age ranged from 47-77 years (mean age, 62 ± 15 years) at the time of examination. All nine subjects were imaged with CEUS to determine the vascularity associated with the median nerve. The imaging parameters remained constant throughout the examination for all subjects while contrast dosing varied. Although some reactions have been associated with the use of Definity® as the contrast agent used for cardiac imaging, our subjects exhibited **no** reactions and tolerated multiple injections of the UCA without incident.

CEUS imaging technique

The subject trials were completed to consistently image CEUS of the median nerve at the carpal tunnel inlet and validate the imaging technique previously determined in the referenced Macaque study. The contrast dosing amounts were determined at the discretion of the lead sonographer, as previously noted, and varied in both dosing amount and number of dosing injections. The contrast dosing amounts varied from 3 ml (Patient 1) to 5 ml (Patient 5), with a mean dose of 3.33 ml over all patients. The contrast dosing solution for all subjects contained 1.3 ml of Definity® to 8.7 ml of saline. In addition, the equipment parameters were maintained at 4% output power and a mechanical index (MI) of 0.13. The transmit frequency of the linear transducer was held at a constant of 9 MHz throughout the trials.

Dosing trials

Two syringes were drawn up; one contained a suspension solution of 1.3 ml of Definity®- perflutren lipid microspheres to 8.7 mL saline and a second contained a saline solution, designated as a post-examination flush. All injections were introduced via a venous catheter at the antecubital space or behind the patient's right hand. Examinations began with an initial contrast injection at 0 seconds and were followed by booster injections of various amounts and times at the discretion of the main echo sonographer. All dosing trials were followed by a saline flush of the venous catheter by the attending OSUWMC nurse.

The descriptive results for recording the median start time in detecting CEUS perivascular flow of the median nerve, was 30 seconds after the primary injection of contrast was given. All nine of the subjects were imaged over 7 minutes to detect the vascularity within the median nerve. The subjective start time for detecting Definity® within the median nerve was 30 seconds for the 9 trials. The subjective time of enhanced detection of vascularity was between 30 seconds to 2 minutes post-contrast injection. The subjective extent of vascular filling was minute but still evident at the end of the 7 minute imaging trial.

Measurement Reliability

The author of this study (AMH) completed an individual, blinded analysis using Pixel Flux® to evaluate inter-rater reliability with the investigators of the original CEUS preclinical study study (KDE & KRV). The first 5 animal-subjects that received Definity® were analyzed by all investigators at each 30 second incremental frame between 00:00 min to 07:00 mins. Investigators individually drew a manual ROI around the median nerve within which contrast pixels were counted and evaluated for intensity. Each examiner evaluated 15 frames for a pixel count and recorded the average and maximum intensity within ROI as calculated by the software. Inter-rater reliability was determined between the investigators for manual signal counts and Iavg within the ROI using Chronbach's alpha and ICC. Chronbach's alpha for signal counts and Iavg were 0.90 and 0.80 and ICCs were 0.90 ($p< 0.01$) and 0.80 ($p<0.01$), respectively.

CEUS Kinetics

With high reliability established, descriptive and comparative data analyses were completed based on the evaluation of images across 15 time points in the 9 human subjects (n=135 images). The mean for pixel count across all subjects and all time points was 7.97 (SD, 11.50) pixels. In further statistical comparison, asymptomatic patients' pixel counts averaged 4.01 (SD, 3.27) pixels and varied between 1 pixels per image to 17 pixels per image; whereas symptomatic patients' pixel counts averaged 12.85 (SD, 15.52) pixels and varied between 1 pixels per image to 78 pixels per image.

To correlate the research results with similar CEUS studies being performed in European and East Asian countries, the Klauser method of counting perfusion intensities was also used. This categorical method is based on

a scale of correlating perfusion intensity to an ordered ranking, on a scale from 0 (least intense) to 3 (most intense). In analysis of the data, the Klauser method[24] determined the mean perfusion intensity for asymptomatic patients to be 1.18 (SD, 0.58) pixels and symptomatic patients to be 1.83 (SD, 0.86) pixels. Further explanation of the Klauser method[24] and how it is correlated to this study is demonstrated in Tables 4.1 and 4.2.

Grade Key	Pixel Count
0	0
1	1-5
2	6-10
3	>11

Table 4.1. The Klauser method[24] for perfusion intensity.

	Pixel Count Mean	Klauser Method Mean
Subject 1 *	27.2	2.40
Subject 2	3.87	1.20
Subject 3	2.34	0.86
Subject 4 *	12.4	2.07
Subject 5	5.87	1.53
Subject 6	5.93	1.40
Subject 7 *	6.53	1.67
Subject 8	1.93	0.87
Subject 9 *	5.26	1.40
Asymptomatic Overall	4.01	1.17
Symptomatic Overall	12.85	1.89

Table 4.2. Mean pixel count and mean Klauser[24] scores per patient.

Chapter 5 – Discussion

Within the past decade, research has provided compelling evidence that sonography may be used as a first-line diagnostic tool for the identification and evaluation of CTS.[13,17-21] The purpose of this study was to explore the ability of CEUS to determine minute yet quantifiable vascular differences in the periphery of the asymptomatic vs. symptomatic median nerve. Additionally, this research sought to confirm the reproducibility of previously published sonographic parameters for imaging and evaluating the median nerve.

Clinical translation

The foundation of this research revolved around the successful transition from a pre-clinical study to a clinical cohort study. Previous literature[5] provided significant evidence in a study of eleven macaca fasciculari that detection of hypervascularity in the median nerve of monkeys with CTS-like symptoms could be detected by CEUS. The successful translation of previously identified imaging protocol and sonographic equipment settings achieved by this subsequent study provides further support for the ability of CEUS to detect perineural vascularity.

It is important to note that an immense strength of this study is founded on the basis of patients' tolerance to the contrast agent Definity®. No UCA reactions were documented in the preclinical model nor demonstrated within the human cohort. Contrast safety is of utmost importance to not only the demonstration of quality patient care within the clinical field, but also to the establishment of CEUS as a diagnostic tool recognized and approved by the FDA. As outlined specifically within the Food and Drug Administration Safety and Innovation Act (FDASIA), novel medical tests and devices must provide definitive safety and protection for patients before clinical implementation for medical use.[25]

This study ultimately sets the stage for further investigation of perineural vascularity – the accomplishment of translating a preclinical model to clinical model without risk to patient well-being adds an undeniable level of evidence to the prospect of CEUS becoming a first-line diagnostic tool for the detection of CTS.

Reliability

The strength of this study is based upon the blinded, individual measurement analysis completed to establish an inter-rater reliability with the use of the novel software Pixel Flux. The importance of reliable objective measurements within a team of medical researchers is essential for the dependability of translating perfusion measurement from research to clinical practice. As advanced medical software such as Pixel Flux is increasingly implemented into ultrasound exams, and the possible groundwork for neural vascularity detection as proposed by this research, there is a need for a direct measure of reliability and reproducibility in a group of sonographers conducting a CEUS within a clinical lab.

Research variables

One central variable was evaluated to assess the hypervascularity of the median nerve in clinical patients: the ability of contrast-enhanced ultrasound to image tissue perfusion of the median nerve. This variable was analyzed using a designated protocol referenced in earlier published literature, and a manual method for counting PD pixels by use of the novel tissue perfusion software Pixel Flux. Two counting methods were used to examine the ability of CEUS to detect vascular changes in the median nerve- Individual pixel counts and their respective means, as well as the Klauser method.[24]

Following qualitative analysis of the pixel counts in all asymptomatic and symptomatic patients' images, two conclusions were revealed. First, there in fact appears to be a successful differentiation in the vascularity of the median nerves by subjective pixel counts alone. As outlined with further detail in Table 2, the overall mean for asymptomatic patients was 4.01 pixels per image; whereas, the overall mean for symptomatic patients was 12.85 pixels per image. This increase in pixels per image from asymptomatic to symptomatic patients provides pre-imaging evidence of CEUS having the ability to determine minute vascular changes in CTS affected patients. The perfusion data provides further verification that as clinical symptoms and the pathophysiology of CTS manifests, an increase in vascularity occurs. This increase in vascularity can then be detected by CEUS, and ultimately is translated to a higher overall mean in the pixel counts for the symptomatic patient when using a semi-automatic software to analyze the images. In further qualitative analysis, it is important to note that while asymptomatic patients' pixel counts varied from only 1 to 17 pixels per image, symptomatic patients' pixel counts varied from a

vast 1 to 72 pixels per image. This increase in the variation of pixel count amounts also adds substantial evidence supporting the ability of CEUS to detect minuscule vascularity changes in the median nerve.

However, the second conclusion was that the Klauser method[24] of scoring pixel intensities appears to ineffectively represent the minute vascular changes that occur in asymptomatic vs. symptomatic median nerves. While the subjective pixel counts are notable, their respective rankings among the Klauser scale are less than impressive (see Figure 2). This result concurs with previous literature and similarly challenges the use of categorical scoring of CEUS for accurately categorizing the minute changes in vascularity within symptomatic median nerves[13]. The lack of correlation between subjectively counting pixels and ranking intensities into categories indicates the need for continued work to determine which measurement method is most representative of perineural vascularity.[13]

Sonography and its subcategory of CEUS have presented as an exciting and novel diagnostic test for the diagnosis of carpal tunnel syndrome. Advantages of sonography as a first-line diagnostic test for CTS include wide availability, cost-efficiency, portability, and high tolerance by patients. Recent literature has concluded that the benefits and reliability of sonography in the detection and diagnosis of CTS are growing exponentially. As stated earlier, it is entirely plausible that with increasing levels of evidence and data from research trials – such as the one described here – sonography may replace EDX as a first-line test to confirm clinically diagnosed CTS, and potentially provide for a "gold standard" in carpel tunnel syndrome diagnosis.

Chapter 6 – Conclusion

While the initial research results seem promising, due to the current restrictions on the use of CEUS as imposed by the US Food and Drug administration, this small cohort study inherently encountered several limitations. The most notable of these limitations is the small sample size. Due to the official patient count concluding at nine human subjects, the results obtained within this research study cannot be generalized to larger populations. Additionally, because subjects were recruited to the study out of convenience and on a volunteer basis, the results lack random assignment and subsequent generalizability to the public. Prospective research studies with increased recruitment and randomization are necessary to determine the clinical significance and prospect of CEUS becoming a preferred diagnostic test for evaluating carpal tunnel syndrome.

Inability to maintain controlled variables was another intrinsic limitation. Due to federal restrictions, researchers were unable to define a control dosing of the contrast agent Definity®. Amounts of the contrast injection varied per personal preference of the main sonographer performing the echocardiogram. This lack of consistency fails to identify a contrast media dose that would consistently demonstrate perineural vascularity along the human median nerve. Continued research is necessary to provide a resolute and reliable contrast dose to consistently image the minute vascularity of the median nerve.

Missing parameter values was another significant limitation to this cohort study, potentially causing an under-estimation of patient symptom severity and asymptomatic pixel counts. One symptomatic patient did not complete the full symptom severity evaluation, and parameters used to score his pain levels were missed. While this missing information did not hinder this particular study, future studies will need to evaluate the correlation between symptom severity and pixel perfusion. Additionally, pixel counts were missing in one symptomatic patient's sample increment at 0:30 minutes. Patient error was to blame as he moved his wrist during the image sampling and caused that specific image timeframe to be missed.

Finally, the research productively supports the utilization of CEUS for evaluating the vascularity of the median nerve in asymptomatic versus symptomatic CTS patients. Despite the inherent limitations, the research provides increasing levels of evidence for the prospective use of CEUS for the detection and evaluation of CTS and its associated hypervascularity of the median nerve. The use of perfusion software to subjectively detect increased

vascularity provides a promising foundation for the quantified measurement of median nerve hypervascularity. However, the discovered lack of sensitivity in the Klauser method of categorical scoring signifies the need for continued work to determine a measurement method that accurately represents minute changes in perineural vascularity. One of the most important discoveries of this study relies on the fact that no UCA reactions were documented in the preclinical model nor demonstrated within the human cohort. As stated previously, contrast safety is of utmost importance to the establishment of CEUS as a diagnostic tool recognized and approved by the FDA. Given that this novel diagnostic tool has no risk to patient well-being, it can be established as an extremely safe exam for the evaluation of human median nerves.

Finally, the results of this study encourage continued research into the most consistent and valid techniques for evaluating entrapment neuropathy, and offers increasing levels of evidence for the use of CEUS as a primary diagnostic tool for CTS. The potential benefits of CEUS are limitless, and more research is encouraged for continued validation of CEUS as an invaluable diagnostic tool to the medical community

Appendices

List of Abbreviations

CEUS	Contrast-enhanced ultrasound
CSA	Cross-sectional area
CT	Computed tomography
CTS	Carpal tunnel syndrome
DMS	Diagnostic medical sonography
EDX	Electrodiagnostic testing
EFSUMB	European Federation of Societies for Ultrasound in Medicine and Biology
EMG	Electromyogram
FDA	Food and Drug Administration
FSS	Functional status scale
IRB	Institutional review board
MI	Mechanical index
MRI	Magnetic resonance imaging
NCS	Nerve conduction study
OSU	The Ohio State University
OSUWMC	The Ohio State University Wexner Medical Center
ROI	Region of interest
SD	Standard deviation
SSS	Symptom severity scale
UCA	Ultrasonic contrast agent

Patient Screening and Chart Review

The Ohio State University
IRB Protocol #: 2011H0191
Symptomatic Participant Screening and Chart Review

Participant #: _____
Date of Exam: _____
MR #: _____

SCREENING

Circle the appropriate answer. To qualify all answers in bold lettering must be selected. Any non bold answer will disqualify the individual from participating.

Is the participant between the ages of 18 and 65?	**YES** or NO
Has the participant had numbness, tingling, or pain in the first 3 digits during the day or at night for at least 3 weeks?	**YES** or NO
Has the participant had major trauma in the distal upper extremity resulting in broken bones (e.g. radius, ulna, carpals)?	YES or **NO**
Has the participant had previous carpal tunnel surgery or any other surgery in the distal upper extremity, wrist, or hand?	YES or **NO**
Has the participant been previously diagnosed with polyneuropathy, or thyroid disease?	YES or **NO**
Does the participant have a dialysis shunt or any other permanent ports placed in the upper extremity?	YES or **NO**
Is the participant pregnant or within 3 months post-partum?	YES or **NO**

CHART REVIEW

Demographic Data

Date of Birth: _____

Gender: MALE / FEMALE

Hand Dominance: RIGHT / LEFT

Previous RA Diagnosis: YES or NO

Biomarkers

1. _____ ↓ / N / ↑
Date Obtained: _____

2. _____ ↓ / N / ↑
Date Obtained: _____

3. _____ ↓ / N / ↑
Date Obtained: _____

Clinical Data

BP: _____ **HR**: _____

	Right	**Left**
Phalen's:	+ / -	+ / -
Tinel's:	+ / -	+ / -
Durkin's:	+ / -	+ / -
Pain in 1st Digit	+ / -	+ / -
Pain in 2st Digit	+ / -	+ / -

Symptom Severity Assessment

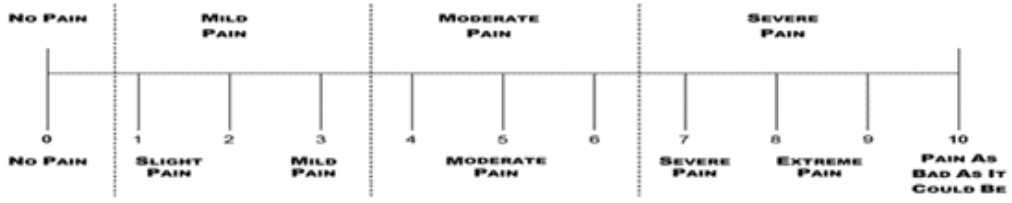
The Ohio State University
IRB Protocol #: 2010H0118
Initial Participant Questionnaire

Participant #: _____
Date of Exam: _____
MR #: _____

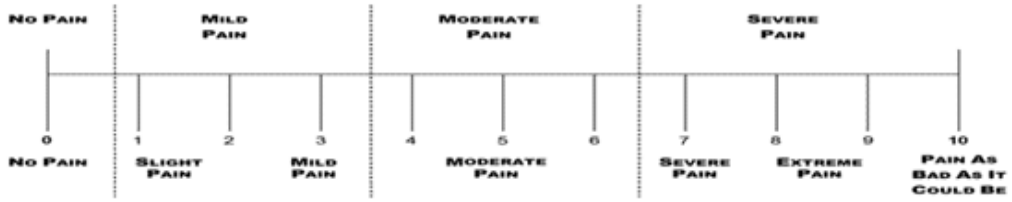
JOINT PAIN SCREENING

1. *Please circle the number that best describes the severity of pain you have experienced at the given locations* **within the past week**.

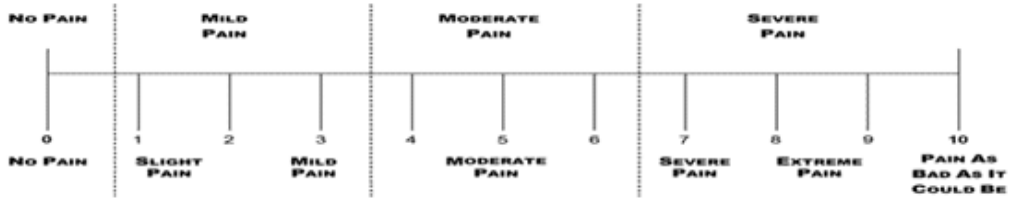
Wrist:



Thumb:

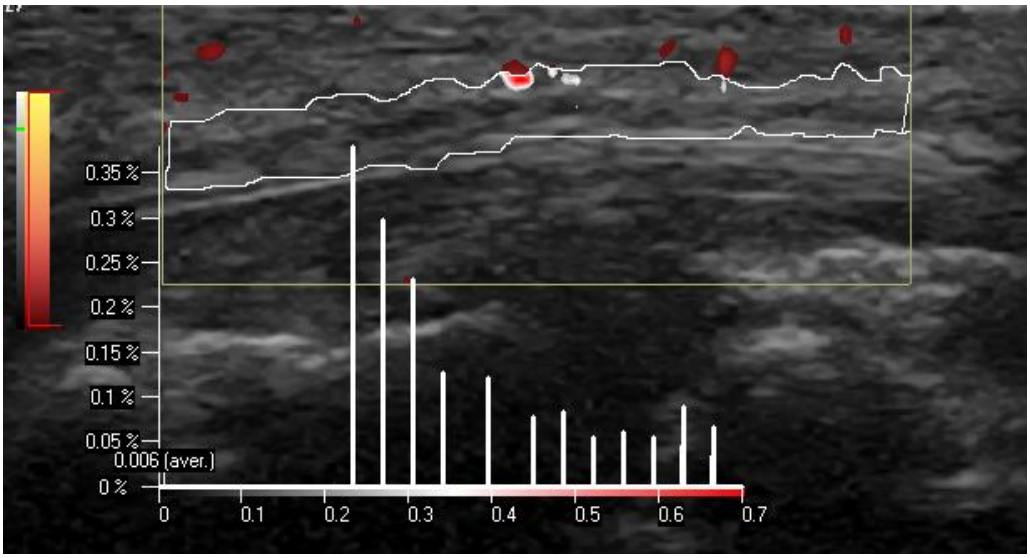


Index Finger:

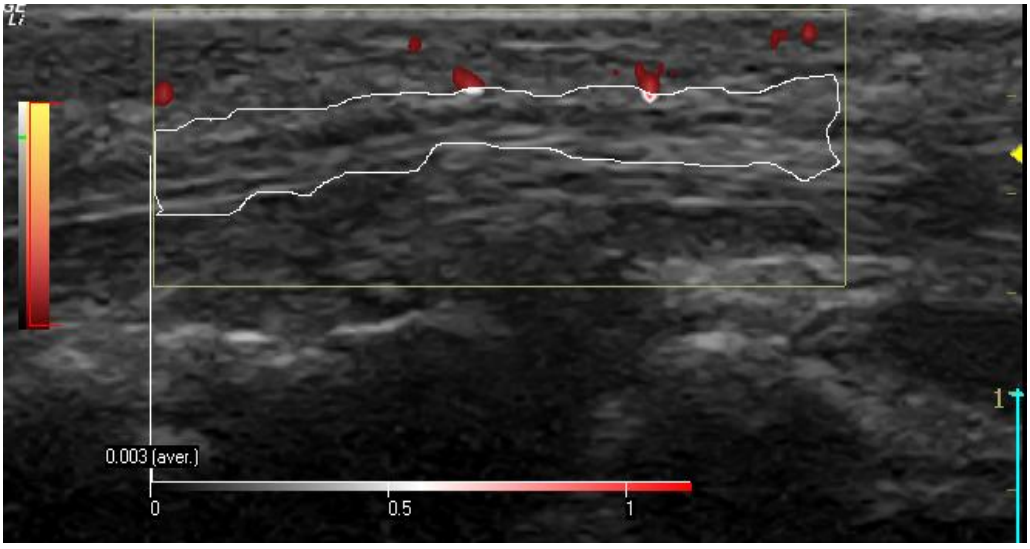


Sonographic Images: Pixel Flux Evaluation of the Human Median Nerve

Asymptomatic Patient



0:00 minutes, pre-injection

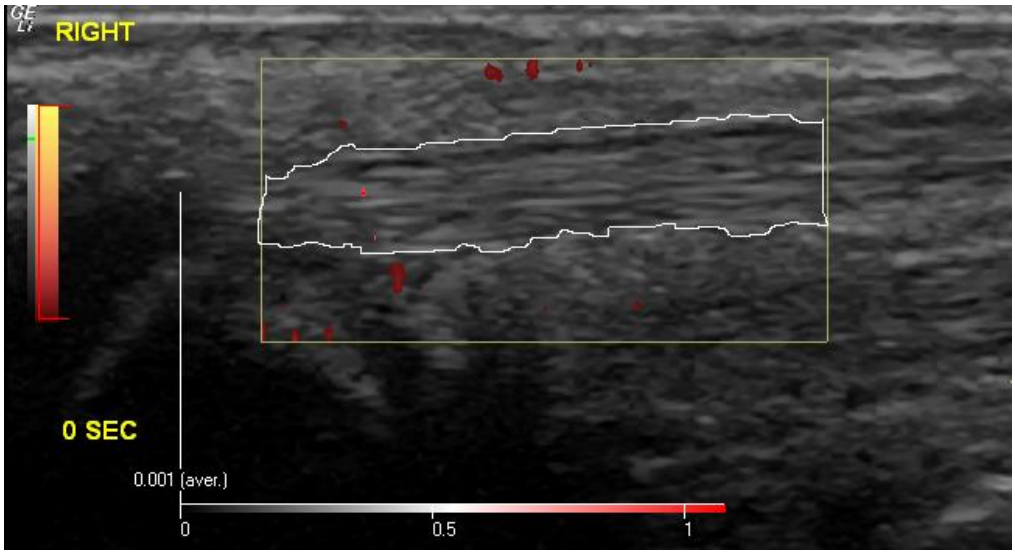


2:00 minutes, post-injection

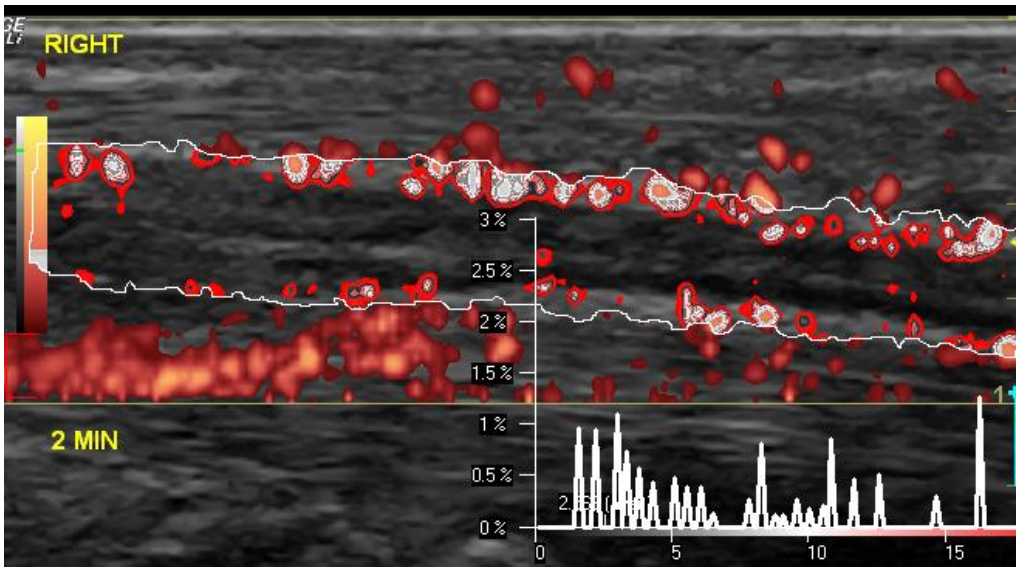
No increased vascularity noted in the asymptomatic median nerve

Sonographic Images: Pixel Flux Evaluation of the Human Median Nerve

Symptomatic Patient



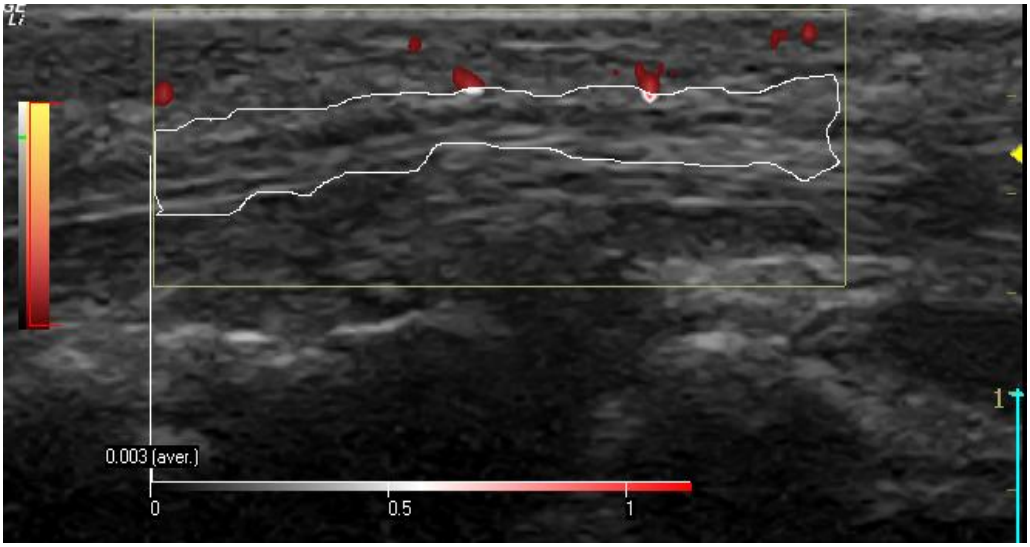
0:00 minutes, pre-injection



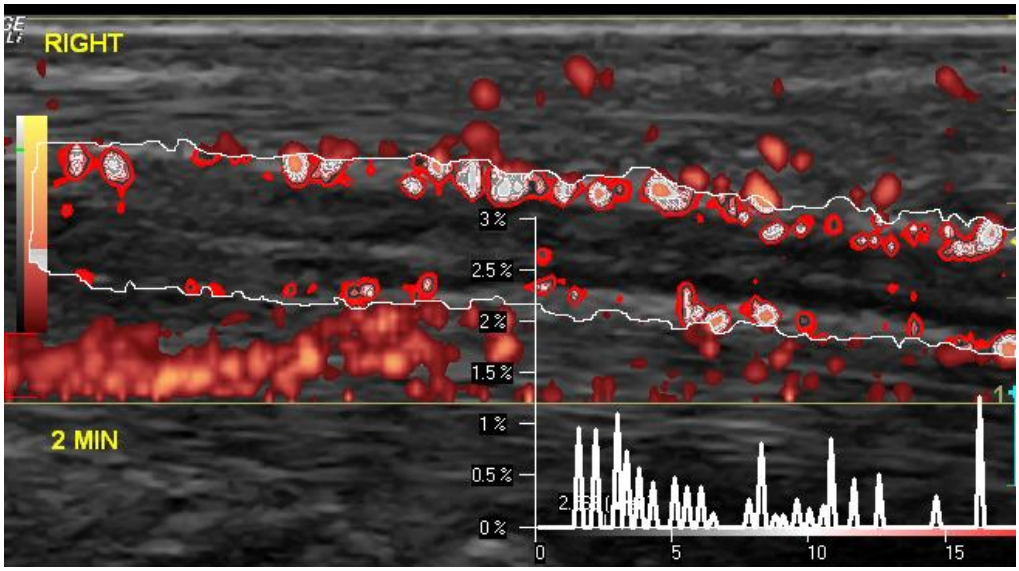
2:00 minutes, post-injection

The contrast agent, Definity®, acts as a catalyst for sonograhpic sensitivity and enhances the hypervascularity of the symptomatic median nerve.

Sonographic Images: Comparison of asymptomatic vs. symptomatic patients



Asymptomatic patient. 2:00 minutes, post-injection.



Symptomatic patient. 2:00 minutes, post-injection

Hypervascularity is readily visualized in the symptomatic patient following injection of the contrast agent Definity®. The asymptomatic patient shows no increased vascularity post-injection, confirming the physiologic pathophysiology of CTS.

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