Utilization of Musculoskeletal Sonography in Detecting Physiologic Changes of the Median Nerve in a Working Animal Model

THESIS

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Abstract

Currently the gold standard for the diagnosis of carpal tunnel syndrome (CTS) is through the use of nerve electrodiagnostic testing (EDX). The diagnosis is characterized by a drop in sensory and motor function within the median nerve. This increase in nerve dysfunction is associated with tissue injury, resulting from repetitive movements of the median nerve through the carpal tunnel. At this point of diagnosis inflammation or ischemic processes may have already begun compromising nerve function, both locally, and systemically. In order to prevent symptoms as severe and possibly irreversible such as these, the need for a method of earlier detection of median nerve trauma must be investigated.

Utilizing musculoskeletal (MSK) sonography, it is hypothesized that earlier, less afflictive physiologic changes to the median nerve can be visualized and quantified, with the goal of providing enough pathophysiologic information to aid in the diagnosis of CTS. By detecting the microtrauma within the nerve, it may be possible for a CTS diagnosis to be made before tissue injury, as well as before a decrease in nerve sensory or motor function occurs. Through the use of gray-scale ultrasonography, geometric measurements of the median nerve can be obtained and analyzed, providing a quantitative representation of nerve size. Similarly, the spectral Doppler function possesses the capability of quantifying intraneural microvasculature within the median nerve, by obtaining peak systolic velocities (PSVs) throughout.

These methods of using MSK sonography for early detection of symptoms related with CTS will be investigated through a longitudinal study involving an animal model. Two cohorts of macaca fascicularis monkeys will be used as participants in median mononeuropathy (MMN) research in which they will be exposed to a repetitive pinching task, putting them at risk for MMN. As the subjects move through the working and non-working stages of the study, MSK sonograms are performed longitudinally to allow for quantitative data comparison within each subject. Because the majority of evidence pertaining to the use of MSK sonography in aiding in the diagnosis of CTS exists only at the end stage of the disease process, a longitudinal examination of the disease progression would be the next logical step.

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Fields of Study

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Table of Contents

Abstract....ii

Acknowledgements....iv

Vita....v

List of Tables....x

List of Figures....xi

Chapter 1: Introduction....1

Background and Significance....1

Objective....3

Research Question....6

Research Approach....6

Definition of Terms....7

Limitations....9

Chapter 2: Utilization of Sonography Compared With Magnetic Resonance Imaging in Determining the Cross-Sectional Area of the Median Nerve in a Sample of Working *Macaca fascicularis*: A Preclinical Study....11

Abstract....11

Key Terms....12

Introduction....12

Methods and Materials....14

MRI Examination....15

Sonography Examination....16

Analysis....16
MRI Analysis....17
Sonogram Analysis....18
Statistical Analysis....19
Results....19
Discussion....22
Limitations....28
Conclusions....28
Future Directions....29
Acknowledgments....31
Declaration of Conflicting Interests....31
Funding....32
Chapter 3: Longitudinal Analysis of Median Nerve Perfusion Utilizing Spectral Doppler in a Working Animal Model....33
Abstract....33
Key Terms....34
Introduction....34
Systematic Review....36
Materials and Methods....40
Data Collection....40
Image Analysis....42
Data Analysis....43
Results....44
Inclusion of Waveforms....44
Descriptive Results of Waveforms....45

Discussion....45
Limitations....49
Conclusions....50
Chapter 4: Discussion....52
Chapter 5: Future Directions....60
References....65
Appendix A: Tables....71
Appendix B: Figures....73

List of Tables

Table 1. Mean direct trace cross-sectional areas for the Subjects' Left Hand Pre and Post-intervention by group.....71

Table 2. Correlations Between cross-sectional areas of Sonographic Residual Area (Epineurial Layer) Outer and Inner CSA by anatomical location of the Pisiform, Post-work Exposure.....72

List of Figures

Figure 1. WRMSD's Progression Chart.....4

Figure 2. WRMSD's Progression Chart.....53

Figure 3. WRMSD's Progression Chart.....73

Figure 4. Sonogram and Magnetic Resonance image of the median nerve.....74

Figure 5. Cross-sectional Diagram of a normal peripheral nerve.....75

Figure 6. Systematic Review Flow Chart.....76

Figure 7. Spectral Doppler tracing of the median nerve.....77

Figure 8. Trend Graph of maximum PSV of the working wrist.....78

Figure 9. Trend Graph of maximum PSV of the non-working wrist.....79

Figure 10. Trend Graph of mean PSV of the working wrist.....80

Figure 11. Trend Graph of mean PSV of the non-working wrist.....81

Figure 12. Waveform analysis Flow Chart.....82

Chapter 1

Introduction

Background and Significance

Carpal tunnel syndrome (CTS) is a form of median mononeuropathy (MMN) which has become the most common entrapment syndrome of the upper limbs, approximately 849,000 new cases reported each year in the United States.[1] This musculoskeletal (MSK) disorder has proven costly in the working population, resulting in more than $6.5 billion dollars in compensation claims, with an individual claim costing between $5000 and $8000.[2] Researchers have indicated the most common risk factors for CTS are exposure to vibrations, direct compression, and highly repetitive and forceful hand-wrist motions.[3] Because multiple studies have reported risk factors, they are fairly established, therefore next the step in CTS prevention is early diagnosis.

Employers, in an attempt to reduce the rising number of work-related musculoskeletal disorders (WRMSDs), have turned to electrodiagnostics (EDX) as a form of early diagnosis of CTS.[4] EDX has been studied extensively, as a set of diagnostic tests, to detect CTS however it is not well accepted by patients and has variable results which has hampered diagnosis.[5] Presently, EDX exams, such as nerve conduction studies, serve as the gold standard in the diagnosis of CTS, however, imaging studies

have shown a positive correlation between increasing cross sectional nerve area measurements obtained using musculoskeletal sonography and a decrease in nerve conduction velocities.[6] Further investigation into the use of high frequency musculoskeletal ultrasound as surveillance tool for individuals that are at an increased risk of developing CTS due to exposure to already established risk factors, could prove extremely beneficial.

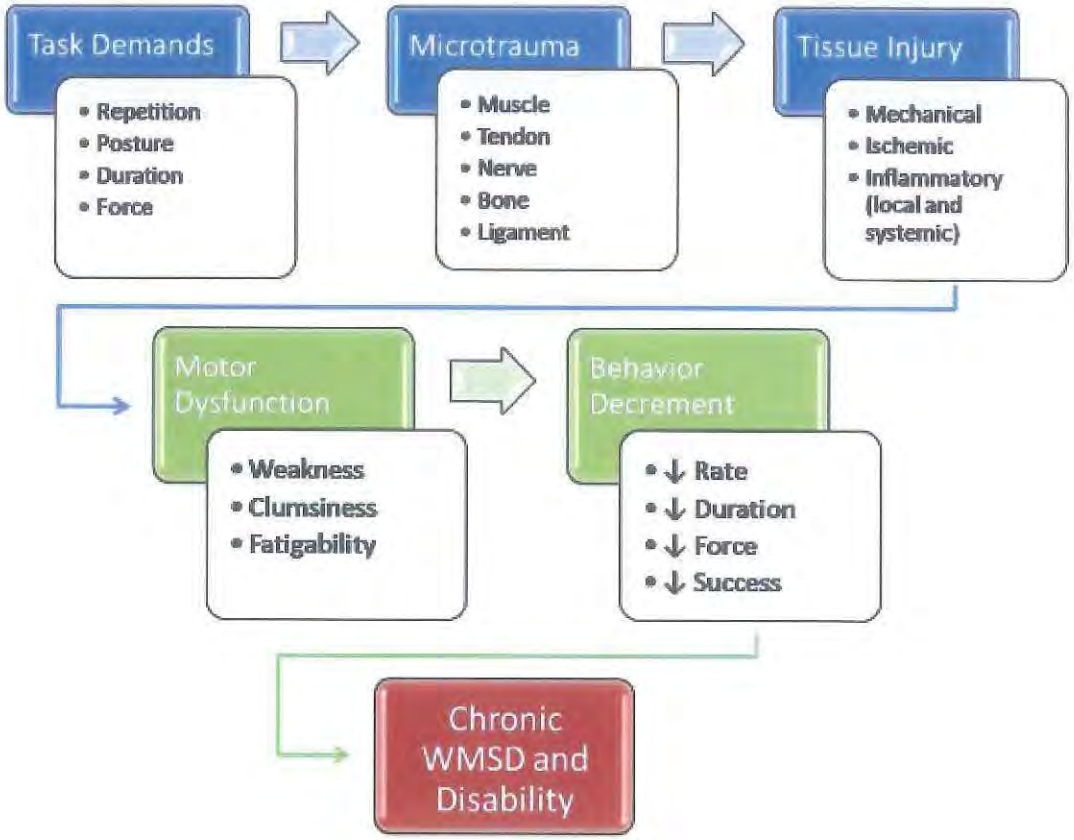
Within the sonography profession, WRMSDs are proving to become a major concern for employers and employees. In a study conducted in 2009 by Evans et al, it was found that of the representative sample interviewed, 90% of all diagnostic medical sonographers (DMS) and vascular technologists (VT's) experienced pain while scanning patients.[7] This demonstrated a rise from a similar study conducted in 1997 which reported only 81% of respondents experiencing pain while scanning.[8] Given these findings, it is to no surprise that in 2007 OSH

A ranks Health Care Related occupations as number six in number of reported cases of CTS throughout that year, with an incidence rate of 1.3/10,000 workers , making up approximately 5.4%.[9] Upon examination of this steady rise in CTS incidence in health related occupations, furthermore within the sonography profession, it is clear that further investigation into more effective diagnostic methods should be carried out.

Objective

To better understand the progression of CTS, from the early risk factors into the chronic stages of the disease, a flowchart (Figure 3) demonstrating the hypothesized steps taken while developing WRMSD, was utilized.[5] Currently the use of EDX testing is utilized in individuals with CTS symptoms in the Tissue Injury stage, as pertaining to the conceptual model. At this point in the disease progression, inflammatory as well as ischemic processes have already begun within and around the median nerve, causing discomfort and occupational restrictions for the individual inflicted. The objective of this study is to longitudinally examine the progression of CTS within the more acute Microtrauma stage of the disorder into the Chronic and Disability stage. This longitudinal examination will potentially provide evidence and determine if quantitative data exists which will provide and insight into the way CTS disorder advances.

Figure 1. Conceptual model depicting the hypothesized manner in which many factors contribute to WRMSDs.[5]



By returning to the Microtrauma stage of the disease, results consistent with acute CTS, such as hyperemia, intraneural edema, and median nerve inflammation, are expected to be occurring.[1] Through the use of high frequency MSK ultrasound, these pathophysiologic indicators are hypothesized to be evaluated quantitatively. Analyzing median nerve cross-sectional area (CSA) to evaluate nerve inflammation as well as obtaining intraneural spectral Doppler waveforms in order to evaluate the hyperemic

response of the median nerve associated with CTS will provide quantitative data representing the acute changes in a median nerve after being exposed to repetitive stressful maneuvers.

As median nerve compression throughout the carpal tunnel becomes chronic, trauma such as demyelination occurs due to repetitive excursion of the nerve through the restricted carpal tunnel space.[10] This continued confinement leads to median nerve ischemia at the site of compression.[11] Similarly to the detection of pathophysiologic changes in the acute stages, MSK ultrasound will also be utilized to observe changes to the median nerve in the chronic phase associated with nerve ischemia, such as decreased nerve size and diminished intraneural vascularity.

Previous literature on CTS has primarily consisted of data representing only the end stages of the disease. Therefore a systematic review will be needed in order to establish the level of evidence that already exists within the literature. After attaining the baseline evidence and existing knowledge reported in CTS disease progression, a study will be formed allowing for the longitudinal collection of quantitative data as a population progresses through the stages of the disease.

Research Question

The study currently being reported will explore and answer the following research question: *What ultrasonographic quantitative measures can be used to evaluate median nerve tissue injury and microtrauma, while examining an animal cohort longitudinally with suspected median mononeuropathy?* By investigating this question, further insights and conclusions can be made about the use of MSK ultrasonography in the diagnosis of CTS. The data and information gained will subsequently point future research on this topic in the correct direction so that advancements in the detection of median nerve microtrauma and CTS diagnoses can be made.

Research Approach

In order to answer the above research question, a research model should be established allowing the collection of quantitative data to be collected longitudinally. This study will be conducted in an animal model using a cohort of *macaca fascicularis* monkeys. Because this experiment is being conducted using an animal model, the level of evidence should be considered as pre-clinical, and is not able to be generalized to a human population. However the knowledge gained from this study regarding feasibility

and protocol appropriateness will assist in the next logical step of this study, which would be the translation of this experimental model into a human population.

Definition of Terms

Electrodiagnostic Testing (EDX)

- Theoretical: Medical tests used to detect and diagnose injuries or diseases of nerves throughout the body by examining sensory and motor functions
- Operational: Nerve conduction studies used as the clinical gold standard for diagnosis of carpal tunnel syndrome and detection of median nerve sensory impairment

Carpal Tunnel Syndrome (CTS)

- Upper extremity entrapment disorder characterized by the compression of the median nerve as it passes through the carpal tunnel

Cross-sectional area (CSA)

- Theoretical: The area within a set of boundaries, calculated in a cylindrical surface by $A = \pi r^2$
- Operational: Measurement made trans-axially around the median nerve for longitudinal geometric measurement comparison

Median Mononeuropathy (MMN)

- Disease or disorder affecting the median nerve characterized by nerve compression and ischemia

Median Nerve

- Upper extremity nerve which innervates the arm, forearm, and hand

Musculoskeletal (MSK) Sonography

- Theoretical: High frequency ultrasound utilized to image musculoskeletal components throughout the body
- Operational: High frequency ultrasound utilized to image the median nerve, providing quantitative information used to analyze the median nerve

Spectral Doppler

- Theoretical: Ultrasound imaging technique used to detect and quantify blood flow, by providing waveforms
- Operational: Ultrasound imaging techniques used to detect and quantify blood flow within the median nerve

T1 Weighted Magnetic Resonance Imaging

- Theoretical: A magnetic resonance imaging technique which demonstrates the amount of longitudinal relaxation time of the tissues being imaged

- Operational: Imaging gold standard of the median nerve used to compare with MSK ultrasound

Work Exposure

- Theoretical: The exposure to a task requiring strength intended to accomplish a specific physiologic change
- Operational: A repetitive pinching task intended to induce symptoms of MMN

Work Related Musculoskeletal Disorders (WRMSDs)

- Theoretical: A variety of disorders resulting from occupational hazards
- Operational: Carpal tunnel syndrome (CTS) or median mononeuropathy (MMN) resulting from the work exposure of a repetitive pinching task

Limitations

This study is limited first and foremost due to the of the level of evidence and should be considered pre-clinical and has threats to both internal and external validity. Additionally a direct measure of the force during the repetitive squeeze task was not reported and would have made this a more robust study. Furthermore, the addition of

more subjects into the experiment would have increased the data collected and statistical rigor.

Chapter 2

Utilization of Sonography Compared with Magnetic Resonance Imaging in Determining the Cross-Sectional Area of the Median Nerve in a sample of Working *Macaca fascicularis*: A Preclinical Study

Abstract

A preclinical study of 15 *Macaca fascicularis* monkeys was conducted to determine (1) the ability of detecting median mononeuropathy (MMN) within the median nerve after a work intervention and (2) the relationship between the layers of the median nerve during an acute inflammatory process by using sonography in conjunction with magnetic resonance imaging (MRI). Cross-sectional areas (CSAs) were imaged using MRI and sonography proximal to the carpal tunnel inlet (defined by the most distal portion of the radius) and further distal into the carpal tunnel (defined by the most proximal portion of the pisiform) at the pre-work exposure stage. Cross-sectional areas measured on the outer edge of the median nerve were obtained from both modalities, at both anatomical locations. An intermodality *t*-test demonstrated no statistical differences between the two sets of measurements (radius, $P \leq .01$; pisiform, $P \leq .46$). At the post-

work exposure stage, sonographic measurements were obtained on the outer and inner borders of the median nerve at both anatomical levels. A two-tailed *t*-test showed statistically significant differences within the carpal tunnel comparing pre- and post-work CSA measurements (radius, $P \leq .01$; pisiform, $P \leq .297$). The epineurial layer was then determined as the difference between outer-border and inner-border CSA's. Pearson correlations between the epineurial layer and overall median nerve CSA within the carpal tunnel demonstrated a strong positive correlation that was statistically significant ($r = 0.97$; $P \leq .01$) after post-work exposure. Possible factors contributing to this acute phase of MMN could be hyperemia within the layers of the nerve and the development of Renault bodies. This work would need to be translated to human studies for further confirmation of the anatomic and clinical significance of this effect.

Key Terms

median mononeuropathy, MRI, sonography, wrist, median nerve

Introduction

Carpal tunnel syndrome (CTS), also known as median mononeuropathy (MMN), is a musculoskeletal disorder characterized by the compression of the median nerve as it

passes through the carpal tunnel and under the transverse carpal ligament. This is the most common nerve entrapment syndrome of the upper limbs, with approximately 849,000 new cases reported each year in the United States.[1] Nerve conduction studies are presently the gold standard in the diagnosis of CTS; however, imaging studies have shown a positive correlation between increasing cross-sectional area measurements obtained using musculoskeletal sonography and a decrease in nerve conduction velocities.[6,12-15]

Sonography has also been demonstrated effective in evaluating other pathologies in the wrist such as masses or fluid collections, as well as the size and echotexture of the median nerve at distinct anatomical landmarks.[16] The National Guidelines Clearinghouse provides an evidence-based guideline for CTS diagnosed with sonography. This set of guidelines was the direct result of a meta-analysis published in 2011, which reported the diagnostic sensitivity of sonography to evaluate to evaluate the median nerve at 77.6% and a specificity of 86.8%.[17] This indicates a role for high-resolution sonography, which can capture median nerve enlargement and increased hypoechogenicity.[18] Recent studies have taken a different approach to improve the diagnostic accuracy of sonography of the median nerve. During the acute phase of CTS, the median nerve becomes enlarged and constricted by the transverse carpal ligament as it passes through the carpal tunnel inlet. Proximal to the inlet, a hyperemic response develops coupled with intraneural edema, causing an inflamed nerve. Swelling constricts the nerve as it passes through the inlet on its course to innervate the hand. By combining sonographic measures, including cross-sectional area, hypoechogenicity, and hypervascularity of the median nerve, the additive

effect of these measures raised the probability of a correct CTS diagnosis from 87% to 99%.[19,20]

Preliminary studies have suggested that magnetic resonance imaging (MRI) of the carpal tunnel is more sensitive than sonography for detecting compression of the median nerve by surrounding anatomy or pathology.[21] The American College of Radiology (ACR) Appropriateness Criteria rank MRI as the imaging gold standard for CTS, and is the recommended radiologic procedure with the clinical condition of chronic wrist pain with or without prior injury and normal or nondiagnostic radiographs.[22]

The objective of the current study is to investigate the use of cross-sectional area measurements of the median nerve to determine whether longitudinal sonography measures could be used to detect physiologic change. By investigating the size of the median nerve measured using sonography and compared with MRI, a preclinical investigation might discern whether this imaging modality can detect physiologic change in the median nerve.

Methods and Materials

A study approved by the Intuitional Animal Care and Use Committee (IACUC) board of review was completed using 15 *Macaca fascicularis* monkeys as participants in median mononeuropathy (MMN) research. During a 20-week working phase, the 15 *M*

fascicularis performed a repetitive pinching task affecting the left wrist, with the intention of inducing MMN.[23] A baseline MRI scan and two baseline sonograms were taken prior to work exposure. Ten additional sonograms were performed throughout the pre- and post-work exposure. The baseline MRI examinations were then retrospectively matched with sonograms that were completed closest to the same date.

MRI Examination

A modified MSK MRI protocol was completed on a BioSpec 94/30 MRI System equipped with a 9.4T horizontal bore magnet. The magnet operated at 400 MHz and runs ParaVision™ 4.0 software platform adapted from the TopSpin 1.5 (Bruker BioSpin; Billerica, MA). Each subject was positioned in a warm plastic cylinder with a 3.5 mm quadrature coil placed anterior to the wrist. A 20 cm spatial gradient was used for signal acquisition. The subjects were also monitored throughout the scan with a small animal monitoring system - Model 1025 (Small Animals Instruments, Inc. Stony Brook, NY). T1- and T2-weighted MRI scans were then performed in the axial plane, obtaining 32 images of the median nerve as the scans progressed distally down the arm.

Sonography Examination

A musculoskeletal sonography protocol was refined and replicated to evaluate the size and shape of the median nerve qualitatively and quantitatively.[24,25] Three sonographers used a GE Logiq *i* hand-carried unit (HCU) (GE Healthcare , Milwaukee, WI, USA) to scan the subjects, using a 12.0 MHz linear probe, with a bandwidth of 7-12 MHz. A 12.0 MHz frequency was selected; the time gain compensation controls were aligned vertically and centered, with Harmonics and CrossBeam® also being utilized. Each wrist was scanned in both the axial and longitudinal planes in order to obtain multiple views of the median nerve. In the axial plane, dynamic clips were stored as the scan progressed distally. Weekly quality control measurements were performed with the transducer and a tissue mimicking phantom throughout the study to monitor and document reliable equipment performance.

Analysis

Cross-sectional area (CSA) measurements were obtained from both imaging modalities in the axial plane, at the same levels of the distal radius (proximal to the carpal tunnel) and the proximal pisiform (within the carpal tunnel). In the previous studies, there has been some discrepancy in the manner with which sonographic CSA

measurements were acquired. Several studies have used the outer edge of the nerve epineurium to evaluate CSA of the nerve,[26,27] whereas other have advocated measuring inside the epineurium by tracing around the hypoechoic inner border.[16,28-31] For this study, both measurements were obtained on the sonograms by tracing around the outer edge of the epineurium as well as the inside of the epineurium (Figure 2A, 2B). To address measurement error that could be attributed to the researcher, a direct trace of the CSA was completed five times at each anatomical location. The highest and lowest values were eliminated and the middle three data points were averaged.

MRI Analysis

The appropriate T1-weighted MRI exam was selected and analyzed using the National Institute of Health, Center for Information Technology medical imaging analysis computer software package, Medical Image Processing, Analysis, and Visualization (MIPAV; NIH, Bethesda, Maryland). A credentialed MRI technologist selected images of the median nerve in the axial plane at the anatomical landmarks of the distal radius and the proximal pisiform. Proximal to the carpal tunnel inlet was identified in the axial plane as the slice at the most distal level of the radius, and within the carpal tunnel was identified by the most proximal portion of the pisiform. CSA measurements of the median nerve were then performed by using the direct trace function on the MIPAV software. The researcher carefully traced around the outer edge of the nerve

epineurium at these two locations. Once the traces were completed, the computer software automatically calculated the CSA. The image number within the imaging sequence for both MRI and sonography was recorded on a customized worksheet to ensure reproducibility for further data analysis.

Sonogram Analysis

The individual cases were analyzed by a credentialed sonographer on the same GE Logiq *i* HCU that was used to perform the original musculoskeletal sonogram. Using the analytical software found on the HCU, measurements at the corresponding locations to MRI measurements were taken. The dynamic clip of the axial median nerve was selected and matched by date to the corresponding MRI. Using the run/stop function, images were selected that captured an image at the distal radius and at the proximal pisiform. Using the direct trace function on the HCU, the outer edges of the median nerve were carefully traced, making sure to include all of the epineurium. On the same image, the nerve trace was repeated for the inner hyperechoic border. In a similar manner as with the MRI, the direct trace of the CSA was completed five times, and the high and low measures were eliminated from the average. Once both an outer CSA and an inner CSA were recorded, the inner CSA was subtracted from the outer CSA to obtain a residual area that represented the epineurial tissue.

Statistical Analysis

Descriptive statistics were generated using Excel 2010 (Microsoft Corp., Redmond, Washington) so that a mean CSA measurement could be objectively compared between both modalities. This analysis also allowed for the detection of measurement changes involving the nerve that could be attributed to work exposure. Mean CSA's were organized and grouped by method of analysis (inner border, outer border, and residual), anatomical location (proximal to the carpal tunnel inlet and within the carpal tunnel), and the time of point within the study the measurements were collected (pre-work vs. post-work exposure).

Two-tailed *t*-tests were used to compare the subjects' measurements made by MRI and sonography at the baseline time point at both anatomical locations. In addition, a Pearson correlation was used to test for a linear relationship between work exposure and ultrasound CSA measurements made at the same anatomical locations. A P value $\leq$ .05 was set a priori for statistical significance.

Results

Fifteen *M fascicularis* monkeys completed the 20 weeks of work exposure and were analyzed retrospectively to determine the potential effects on the left hand and the

resulting CSA measurement changes that may have taken place in the median nerve proximal to the carpal tunnel inlet and distal within the carpal tunnel. Mean CSA measurements of the median nerve taken both at baseline and post-work exposure are included in Table 1 for the subjects of the study. The mean days and hours worked for both groups were compared to imaging measurements taken at the distal radius, and proximal pisiform, with MRI and sonography.

At the baseline stage, a *t*-test comparison between outer direct trace CSA's at the pisiform for MRI and sonography yielded a P value $\leq .46$, indicating no significant differences in the measurements (and retaining the null hypothesis). The *t*-test was repeated in a similar fashion at the same time point at the distal radius, with a P value $\leq .15$, indicating that the hypothesis concerning statistical equality of the direct trace CSA's made including the median nerve epineurium using either MRI or sonography could be retained. The inner sonographic CSA measurements could not be compared with a corresponding MRI image because the inner border of the epineurium was not consistently resolved on the MRI, such that only an outer CSA could be compared at the baseline time point.

A *t*-test was also used to compare ultrasound CSA measurements, including the epineurium, obtained proximal to the carpal tunnel inlet and further distal into the carpal tunnel prior to and at the conclusion of the working phase. Proximal to the carpal tunnel inlet, a P value $\leq .297$ was found, indicating that the CSA's measured using sonography at this level were similar and no significant change was observed after the work intervention. However, further distal into the carpal tunnel, a P value $\leq .01$ was noted,

concluding that the direct-trace CSA's obtained from sonography were significantly different following the work interval.

The designed intervention was an opportunity for the subjects to perform a repetitive pinching task that could affect the left wrist. Given the statistical differences in CSA from the baseline examination and the conclusion of the working phase at the level of the pisiform, a Pearson correlation was done to examine if changes in CSA measurements were associated with the work exposure and if it would be possible to gauge the changes that might occur in the CSA of the epineurial layer occurring at the level of the pisiform (Figure 4A).

After work exposure, a residual area, or area of the epineurium, at the post-work stage was calculated by subtracting the post-work inner CSA measurements from the post-work outer CSA measurements. This residual area was then correlated with both the post-work outer CSA and the post-work inner CSA measurements. The results of a Pearson correlation demonstrated a statistically significant low-level strength of association (r =.22, $P \leq .01$) between the residual area and inner CSA measurements. However, a statistically significant high-level association (r= .95, $P \leq .01$) was noted for a correlation between the residual area and outer CSA measurements (Table 2).

Further analysis was done to determine if the work exposure intervention presented a correlation between mean work exposure and the residual area (epineurial layer) of the median nerve at the level of the pisiform in the post-work stage. Again, a

Pearson correlation was used and determined that a slightly positive correlation ($r = .30$, $P \leq .01$) was present.

Discussion

A mononeuropathy is a disorder that involves a single peripheral nerve is caused by an injury that can result in short- or long-term debilitation. Unlike the central nervous system, peripheral nerves are stronger and much more resilient.[32] This resilience to injury in a peripheral nerve has been attributed to a series of connective tissue sheaths that help to insulate and enclose the delicate sensor and motor fibers.[32] This preclinical study was designed to investigate the use of cross-sectional area measurements across imaging modalities in order to provide diagnostic information on physiologic change and the stage of injury to the median nerve in a controlled cohort of *M fascicularis*. Using MRI and sonography to image the median nerve required a diagnostic ability to distinguish between the normal appearance of the nerve and the various changes resulting from repetitive injury.

This cohort of subjects began with pre-work measurements that were compared statistically using a *t*-test. Reviewing the outcomes during the pre-work phase of the study, the results demonstrated no statistical differences between MRI and sonography in the measurements proximal to the carpal tunnel or further distal into the carpal tunnel. This lack of statistical difference demonstrated the ability of MRI and sonography to

provide very similar measurements of the median nerve when the outer border of the epineurium was included in the CSA. Because MRI is considered the gold standard for obtaining median nerve dimensions, this data provides validity for the measurements made using sonography.

The choice to suspend further analysis of CSA's measured on the outer rim of the epineurium was the controversy and the need to standardize the measurement being explored in this experiment. The analysis of the CSA's measured inside of the epineurium with sonography is highly advocated; however, this could not be compared with MRI since this modality cannot accurately resolve the inner border of the epineurium (Figure 4B). Therefore, by subtracting the sonographic outer border CSA measurements, which included the epineurium, from the inner sonographic CSA measured along the inner border of the epineurium, a residual area representing epineurial layer thickness can be obtained. Outer sonographic measurements were used throughout this experiment due to the statistical similarity when compared with the gold standard MRI measurements.

The next step was to try to understand the relationship of the inner border sonographic CSA (outer border epineurium) measurements. The connective tissue making up the epineurium, which serves to pretest the nerve fibers, is known as epineurial connective tissue. Because sonography is able to accurately determine the CSA of the median nerve excluding the epineurium, it was possible to derive the interfascicular epineurial connective tissue area measurement, which surrounds the fascicles inside the nerve, by subtracting this from the sonographic measurements, which

included the epineurium.[16] This residual area or epineurium measurement would then allow for the analysis of the interaction between the outer and inner CSA measurements, and the epineurial connective tissue area, all obtained through sonography. While examining the area of the nerve at the inlet to the carpal tunnel (most distal point of the radius), a *t*-test between these residual area measurements before and after work exposure demonstrated no statistical differences. Therefore, further measurement analysis was deemed unnecessary.

Moving to the area of the nerve further distal within the carpal tunnel, identified by the most proximal portion of the pisiform, significant differences were seen comparing the pre- and post-work intervention. Using the calculated epineurial area, defined as the difference between the outer and inner median nerve CSA measurements, correlations could be made to allow for a better understanding of the relationship between the layers of the median nerve involved in the inflammatory process. A comparison of the outer CSA measurements, which included the epineurium, to the epineurial layer itself, demonstrated a strong positive correlation, suggesting that any increase in outer CSA could result in an increase of the epineurium area regardless of physiologic change. A second correlation between CSA measurements made along the inner border, which excludes the epineurial layer, and the same epineurial layer as used in the previous correlation demonstrated a weak positive correlation, suggesting that as the epineurial layer area increased, the inner CSA measurements remained somewhat constant. These findings suggest that as inflammation of the median nerve occurs, it is the enlargement of the epineurial layer causing the overall increase in size, as opposed to the inner area of

the nerve, which includes the fascicles and the perineurium (Figure 5). Continued study into alternative physiologic factors that could have influenced these results is important to explore, with the cautionary note that a strong statistical correlation does not necessarily represent causation.[33]

Previous studies have reported the acute constriction of the median nerve at the site of entrapment resulting in swelling.[34] In the current study, measurements were repeated after work exposure to detect acute measurement changes within the nerve that may be attributed to inflammation. Inflammatory changes in the nerve have been described as eliciting a hypoechoic appearance with sonography and enlargement of the nerve due to endoneural edema.[27,35] To determine the optimal diagnostic technique, it was necessary to repeat the statistical comparisons for post-work exposure at both anatomical locations, proximal to the carpal tunnel inlet and further distal into the carpal tunnel. Repeating the *t*-test demonstrated a significant *P* value at the location of the proximal pisiform, or distal inside the carpal tunnel, which indicated a significant difference in the CSA measurements obtained. Because differing time points in the study were being explored, it became important to isolate the single intervention, which was the only factor that had been altered between the pre- and post-work measurements.

Because work exposure was the single intervention between pre- and post-work measurements, the analysis of work exposure should be important to postulating what could be contributing to differences in the imaging measurements. Work exposure was the opportunity to complete a repetitive squeeze task, but it is not a direct measure of the

actual force exerted by the subjects. This would be a more direct measure of the impact of the repetitive movements on the median nerve.

The work exposure proved to be influential in the difference between measurements taken before and after work at the anatomical level inside the carpal tunnel. Although the mean hours worked are a relatively "soft" measure of intervention, this begins to describe the possible physiologic changes that may have been taking place within the median nerve. Increased CSA measurements traced outside the epineurium with concomitant residual CSA enlargement of the fascicules within the median nerve. Work exposure would be hypothesized to have more impact over a sustained time period, so the fact that this level of association was even demonstrated speaks to the influence of the repetitive pinch-pinch task.

These findings suggest a potential relationship between the physiologic response of the inflammation of the entire median nerve and that of just the epineurium, which is worth further investigation. The lack of entire nerve inflammation suggests that, as epineurial swelling compresses the interfascicular connective tissue area, this swelling in turn compresses the fascicles and affects electrical signaling. This ultimate compressive impact on the electrical signals is the underlying cause of the painful and symptomatic hands that are associated with CTS.[36] This would also explain the lack of sensitivity reported in a previous study in which CSA measurements were obtained excluding the epineurium.[19] Although the entire epineurial area of the nerve is accurately visualized through sonography, it is not the portion of the nerve where swelling is attributed. This data suggests that not only is the epineurium of the median nerve expanding and causing

difficulty in moving through the carpal tunnel, but also the expansion internally within the nerve causes a decrease in electrical signals being transmitted distally to the hand and fingers.

An additional factor that can be contributed to intrafascicular compression is Renaut bodies. Renaut bodies are fibroblasts that are believed to be derived from endoneurial connective tissue and are often noted to be deposited around the median nerve as a response to repeated mechanical stress.[37,38] In a cadaver study, Renaut bodies were found on all five females, and the deposition consistently surrounded the fascicles of the median nerve segments removed for microscopic investigation.[39] The presence of Renaut bodies is another potential cause for an increase in the epineurium and constriction of the perineurium from the connective tissue. Renaut bodies' deposition has been linked with the development of MMN.[34]

When examining the layers of the median nerve that has incurred a microinjury, the endoneurium would react acutely under hyperemic conditions and then progress to tissue repair once the trauma is relieved or, if the trauma is not relieved, become ischemic.[35] An injury longitudinally can result in regeneration of nerve fibers depending on the distance that regrowth must take place.[40] Continued study is needed to address the reaction of the nerve tissue surrounding the individual fascicles, as physiologic changes may be varied.

Limitations

This study is limited foremost by the level of evidence, which is correlative but not necessarily causative, and should be considered preclinical. In addition, a direct measure of the force during the repetitive squeeze task was not reported and would have made this a more robust analysis. Last, the positioning of the subjects for MRI in the warm plastic cylinder was difficult and could be the source of problems with consistent imaging of the wrist.

Conclusions

While comparing MRI CSA measurements and sonography CSA measurements, it was determined that without manipulation of the nerve, these two measurements suggest no statistical difference and can be accurately compared. Because MRI is defaulted to as the optimal diagnostic tool for imaging the wrist, it was possible to continue with the experiment using sonography as an optimal imaging technique.

Because of the statistical difference inside the carpal tunnel following work exposure, the next step taken was to correlate both inner and outer CSA sonographic measurements with the epineurial layer area. Given the strong positive correlation between outer CSA's and the epineurial layer, combined with the weak positive

correlation between inner CSA's and the epineurial layer, it can be suggested that an increased size in the epineurium may be the cause of many CTS symptoms. This relationship is critical in demonstrating the pathophysiologic change of the nerve. Possibilities suggested for this physiologic result were intraneural edema, which could be adding to the inflammation of the median nerve, therefore increasing the compression, and Renaut bodies.[16,34,27,38,40-42] Inflammation of the nerve was best displayed using a T2 MRI, which would be the next subsequent step in this research. Another important step would be to compare the blood flow in the perineural plexus using Doppler of the CSA areas to document any increased vascularity that could be linked to an increase in CSA.[41]

Future Directions

Although this study was able to report statistically significant findings that somewhat contradict previous studies, further research is needed at the level of the intraneural vessels and surrounding tissue. The results of this pilot study should be considered preclinical evidence and as such cannot be generalized. Our team is committed to further investigation and replication of this study to advance further median nerve imaging and CTS diagnosis.

To move this line of research forward and determine whether musculoskeletal sonography can indeed be accurately compared with MRI measurements of proximal and distal segments of the wrist area, this study needs to be replicated with more participants

and also translated to a human study. This current study involved only 15 animal subjects, yielding 30 scans at 60 locations to correlate, and more clinical data is needed if conclusions are to be drawn. Translating this work to humans is the next logical step in the progression of the research.

Another suggested direction that this research could undertake would be to compare these two imaging modalities using a direct overlay method. By manipulating images and matching anatomical landmarks, there would be less discrepancy in the measurements obtained because this would further facilitate a direct comparison. This would also remove the derived measurement of the connective tissue area and provide a more direct measurement of the area and shape during all time points throughout the study.

An intermediate step in the current study would be to obtain histological samples of the median nerve through dissection. This gold standard measurement would provide an even higher level of comparison between the two imaging modalities. It would also allow for a search for the deposition of Renaut bodies. Fueling further research based on this preliminary data may affect the current ACR Appropriateness Criteria and influence an updated version that would reflect a more effective use of musculoskeletal sonography in evaluating the painful wrist for patients. This would advance the diagnosis capabilities of musculoskeletal sonography.

Musculoskeletal sonography also offers additional techniques for the detection of acute nerve trauma such as spectral Doppler. In an acute traumatic stage, the nerve will

become hyperemic, therefore causing increased vascularity.[43] This physiologic response can be detected and visually sampled at any portion of the nerve.[44] The use of real-time scanning and the ability to see the live movement of the nerve and the way it interacts with surrounding tissue generates additional ways to monitor the physiologic process of nerve repair.

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Chapter 3

Longitudinal Analysis of Median Nerve Perfusion Utilizing Spectral Doppler in a Working Animal Model

Abstract

Objective: The objective of this study was to determine the ability of detecting median mononeuropathy (MMN) longitudinally in a working animal model utilizing spectral Doppler. *Methods:* Fourteen *Macaca fascicularis* monkeys were exposed to a repetitive pinching task in order to increase their risk for developing MMN. Spectral Doppler tracings were recorded within the median nerve longitudinally in this working animal model, during the pre-work, working, and post-work stage. Peak systolic velocities (PSVs) were obtained in the non-working and working arm of each of the subjects. Trend graphs were utilized to graph maximum and mean PSVs for each subject, as well as by the two cohorts of subjects which were involved, for a qualitative analysis of the data. *Results:* Retrospectively, 1445 waveforms were collected, and 633 found valid for analysis upon meeting a priori set of inclusion/exclusion criteria. Data points representing maximum PSVs, as well as mean PSVs were trended for both the non-

working and working wrist. After analysis of the graphs, no discernible trend could be established. *Conclusion:* It was found that Spectral Doppler alone does not possess the appropriate sensitivity to gather data that may be representative of median nerve hyperemia. In order for this research move forward, a replication study would be in order, addressing concerns of better control of the subject work being conducted, angle correction during spectral Doppler tracings, and an increased number of subjects. Amplification of velocity waveforms, through the use of contrast-enhanced ultrasound could also help to discriminate changes in the waveform velocity.

Key Terms

carpal tunnel syndrome, median mononeuropathy, median nerve, spectral Doppler, sonography

Introduction

Carpal tunnel syndrome (CTS) is a form of median mononeuropathy (MMN) of the wrist, which has become the most common nerve entrapment syndrome of the upper limbs. CTS affects just under five million people in the U.S. working population, with approximately 849,000 new cases reported each year in the United States.[1,45,46] This

musculoskeletal disorder is characterized by the compression of the median nerve as it passes through the carpal tunnel in the distal upper forearm. The carpal tunnel, which is defined by the area surrounded by the anatomical boundaries of the transverse carpal ligament superiorly and the first row of carpal bones inferiorly, provides space for the median to travel distally for innervation of parts of the hand and digits.[44] However, the most common risk factors for CTS are exposure to vibrations, direct compression, and highly repetitive and forceful hand-wrist motions, will ultimately cause this space in the carpal tunnel to diminish.[3] Furthermore, individuals suffering from other pathologies such as rheumatoid arthritis, hyperthyroidism, acromegaly, and diabetes mellitus are also at an increased risk for developing this entrapment disorder.[47]

During the acute phase of CTS the median nerve becomes hyperemic, and accompanied by intraneural edema, becomes enlarged as it begins to be compressed by the transverse carpal ligament.[1] However, these acute physiologic changes are often subtle and go unnoticed by the afflicted individual, as they most often occur at night.[16,44] As the compression becomes chronic and CTS symptoms become evident, microtrauma consisting of demyelination of the nerve sheath occurs from the repetitive excursion of the median nerve through the constricted carpal tunnel space.[44,49] This continual compression ultimately leads to median nerve ischemia at the site of compression.[11] Concentrating on the arterial innervation aspect of the pathophysiologic response within the median nerve, it is plausible to attempt to observe changes in the arterial blood flow, in and around the nerve, throughout the progression of this disorder. Previous studies have been carried out to determine the vascularity of the median nerve and its

contribution to the disorder. However, few have utilized spectral Doppler in obtaining a quantitative value of the perfusion of the median nerve. Because of the lack of existing research in this specific focus of the evaluation of the median nerve in CTS, a systematic review of the published literature was conducted to determine the current level of research that has been published as well as to ensure the appropriateness of the methodology being conducted in this experiment being reported.

Systematic Review

A systematic review was conducted in order to determine the current level of evidence that has been published with regard to the sonographic spectral Doppler detection of blood perfusion within the median nerve. A diligent process was established to conduct a systematic review of this topic and gather all published information regarding this subject area. (Figure 6) The review process began with the selection of key terms which described the research focus: 1) Doppler, ultrasound, sonography, carpal tunnel syndrome, and CTS. 2) Doppler, ultrasound, sonography, carpal tunnel syndrome, CTS, and median nerve. 3) Musculoskeletal ultrasound, sonography, carpal tunnel syndrome, and CTS. Next, Pubmed, Cinahl, Google Scholar, and Scopus, search engines were utilized in conjunction with the previously stated phrases, within the time parameter of January 1, 2000 – Present (June 12, 2012). A total of 1123 abstracts were returned

(Pubmed=88, Cinahl=297, Google Scholar=729, Scopus=9) as a result of this first level of investigation.

Once the abstracts were compiled from the four search engines and duplicates removed, n=728 abstracts were retained. These abstracts were then evaluated using a set of inclusion criteria to determine their relevance to the topic being explored. The a priori criterion for this review was that the articles must be focused on CTS pathology, the experiment must be examining the median nerve, and the research design must include the use of spectral Doppler. Also, if an abstract was not provided for a given study, the full article was retrieved in order to ensure completeness. Once the abstract was compared against this criterion, 714 abstracts were excluded. This yielded n=14 abstracts whose full articles' were retrieved for further evaluation.

The full published articles retrieved were then read and evaluated independently by two researchers. Each made the determination to include or exclude the articles as part of the systematic review. The blinded and independent decisions of each researcher were then tabulated by a third blinded researcher. Of the 14 articles, ten were excluded due to unbiased consensus between the two researchers, while four were included in the same manner. No disagreement occurred over an articles' exclusion/inclusion status during the researchers' blinded evaluation, therefore a jury was not necessary. The final number of included articles in the systematic review was n=4. The evaluation and information gained from four research articles was the starting point and became the underpinning for a potential research project.

It was first necessary to determine the utility of musculoskeletal sonography to detect the microvasculature present within the median nerve, as well as quantifying the blood flow within it. This was verified in a study which successfully demonstrated the capability of MSK sonography in detecting and quantifying arterial blood flow within the median nerve in a population of diagnostic medical sonographers. Five neonatal sonographers were followed longitudinally, and for 10 weeks received MSK sonograms of the wrist to evaluate median nerve perfusion. These exams were completed before and after each sonographer conducted a neonatal head sonogram. Peak systolic and end diastolic measurements throughout the median nerve were recorded, and although differences in these quantitative variables were not statistically significant, the study was successful at demonstrating the feasibility of utilizing spectral Doppler as a method of interrogating median nerve perfusion.[49]

In a retrospective analysis conducted by Evans et al, spectral Doppler tracings of patients who were symptomatic and asymptomatic for CTS were analyzed. One hundred and sixty-six wrists were analyzed 47 symptomatic and 44 asymptomatic patients reporting to have nerve conduction studies done. This yielded a total of 435 spectral tracings that was then reduced to 245 waveforms after a set of exclusion criteria was applied. Upon examination of the peak systolic velocities (PSV) obtained, no appreciable difference was found between the symptomatic and asymptomatic recruitment groups.[44] This study also evaluated the possible correlation between spectral Doppler tracings and provocative testing which was conducted to obtain an indirect measurement of CTS

symptoms. Polynomial trending was used to compare these, however due to limited sample sizes, no statistically significant data could be recovered.[44]

Taking this study a step further, these same 245 spectral Doppler waveforms were then compared with electrodiagnostic testing results obtained on those same patients. Peak systolic velocities were correlated with nerve conduction velocity (NCV) results, and determined there may be an inverse relationship between them, in patients with symptoms consistent with CTS.[49]

Finally a more detailed retrospective analysis was carried out in a population of subjects which divided the hands in the study into the categories of highly-likely CTS patients and indeterminate CTS patients based on nerve conduction studies. This nerve conduction data was then analyzed along with CSA area nerve measurements of the median nerve and intraneural spectral Doppler tracings. In the wrists evaluated which contained elevated intraneural PSV, there was a sensitivity of 83% while diagnosing patients with CTS from the highly-likely CTS group. Furthermore, when the use of spectral Doppler was combined with an increase in median nerve CSA, the sensitivity increased again to 90%, while diagnosing patients with CTS from the highly-likely CTS group.[11]

The few studies that collected spectral Doppler waveforms, as a part of the research design, were limited to evaluating the median nerve based on symptomatic and asymptomatic individuals with CTS, at the time of their appointment. Based on the different pathophysiologic changes that occurred throughout progression of CTS, it is

only reasonable that the median nerve be evaluated longitudinally, at different stages of the disorder. Since a lack of evidence exists, a controlled longitudinal study which prospectively collected spectral Doppler tracings, indicative of intraneural vascular flow, would be the next level of evidence. Therefore, the objective of this study is to longitudinally quantify the amount of perfusion of the median nerve using spectral Doppler, in a working animal model as the cohort prospectively increases their risk of MMN through a repetitive pinching task. This data could in turn answer the research question as to the utility of spectral Doppler tracings gathered during a musculoskeletal sonogram. This research has the potential to detect changes in the microvasculature within the median nerve, in a working animal model at risk for developing MMN.

Materials and Methods

Data Collection

A study approved by the institution's The Institutional Animal Care and Use Committee (IACUC) board of review, was completed using 14 macaca fascicularis monkeys as participants in MMN research. During a 20 week working phase, the 14 macaca fascicularis performed a repetitive pinching task involving the left hand, at risk for developing MMN.[23] Two baseline sonograms were completed prior to work exposure

in order to obtain baseline data. Ten subsequent sonograms were completed on each subject during the working phase of the study to capture any longitudinal physiologic changes. Lastly, two additional sonograms were completed post-work exposure.

A musculoskeletal sonography protocol was refined and replicated to evaluate perfusion of the median nerve quantitatively using spectral Doppler.[24,25] Three sonographers used a GE Logiq *i* hand-carried unit (HCU) (GE Healthcare , Milwaukee, WI, USA) to scan the subjects, using a 12.0 MHz linear probe, with a bandwidth of 7-12 MHz. A 12.0 MHz frequency was selected; the time gain compensation controls were aligned vertically and centered, with Harmonics and CrossBeam® also being utilized. Each wrist was scanned in the longitudinal plane in order to obtain an optimal view of the median nerve. Power and spectral Doppler was applied to the gray-scale image and optimized using a standardized set of technical settings (Figure 7). Technical factors standardized for the gray-scale image were frequency (12MHz), power output (100%) and B-mode gain (66). Power and spectral Doppler was applied to the gray-scale image and again, optimized using a standard set of technical settings. Multiple tracings were obtained for each subject during each scan at a variety of locations within the median nerve throughout the carpal tunnel. Boundaries of the carpal tunnel were defined by the anatomical landmark of the most distal portion of the radius. Proximal to this location was defined as the carpal tunnel inlet, whereas distal to landmark was defined as the carpal tunnel outlet. Spectral Doppler tracings were obtained using the flow as displayed by the power Doppler. Once again the technical settings of the spectral Doppler was optimized and kept consistent throughout the experiment to promote reproducibility. Due

to the very small size of the intraneural vasculature and the inability to determine the course in which the blood vessels were traveling, no angle correction was applied when obtaining the spectral Doppler tracings.[44] Weekly quality control measurements were performed with the transducer and a tissue mimicking phantom throughout the study to monitor and document reliable equipment performance.

Image Analysis

All spectral Doppler waveforms were analyzed retrospectively by two credentialed sonographers. The research sonographers were blinded to the subject, as well as the time point in the study in which images were obtained. This was done to guard against researcher bias which might have influenced the results. All analysis was completed on the same GE Logiq *i* hand-carried unit (HCU) (GE Healthcare, Milwaukee, WI, USA) that was used to collect the data.

A strict inclusion criteria was developed and applied to both the power Doppler portion and spectral tracings in order for these data points to be added to the study.[44] The criteria was as follows:

1. Must have at least three cardiac cycles in the spectral tracing
2. Must have the spectral Doppler gate positioned within the median nerve
3. Must have a power Doppler pixel in the gate

4. Must not be utilizing angle correction

5. Must have an optimized gray-scale image

6. Must have more signal than noise in the spectral tracing

The peak systolic velocity (PSV) and end diastolic velocity (EDV), as determined from the automatic trace function present on the HCU software, was then recorded for each waveform, in order to meet the inclusion criteria. Other data documented included: location of tracing (carpal tunnel inlet or outlet), phasicity of the waveform (mono-, bi, or triphasic), and presence or not of spectral broadening.

Data Analysis

Frequencies and means of the peak systolic velocities at both anatomical locations were gathered and calculated for waveforms meeting the inclusion criteria. The mean PSV as well as the maximum PSV, was retained for each subject at the proximal portion of the carpal tunnel. The maximum PSV of the median nerve gathered in left (working) and right (non-working) wrists were trended longitudinally in order to give a qualitative representation of the data throughout the working stages of the study. (Figures 8 and 9) This also allowed for the comparison of the maximum PSV velocities gathered in the left (working) wrist, and the right (non-working) wrist. Similarly, the mean PSV's recorded in the proximal portion of the median nerve were trended longitudinally in an attempt to

observe patterns in the data throughout the different stages of the study. (Figures 10 and 11) Furthermore, this data was then broken down into graphs, trending the maximum PSV's for the two cohorts of the study, and then into each individual subject, for further and more detailed analysis of the data.

Results

Inclusion of Waveforms

Retrospectively, 1445 waveforms were collected, representing the 14 macaca fascularis subjects. Of those waveforms collected, 812 failed based on the aforementioned exclusion criteria, yielding a total of 633 waveforms that were considered valid for further analysis. Starting with the 633 valid waveforms, 235 duplicate dated waveforms were removed, along with three others because of missing key data points, the final total number of waveforms being reported ended at 395. A flow chart (Figure 12) is provided to better demonstrate the inclusion process. It is important to note that each waveform had the ability to be excluded from the study based on more than one error located within the image. Therefore, since the exclusion criterion was not mutually exclusive, the sum of individual exclusions is greater than the total number of waveforms excluded.

Descriptive Results of Waveforms

The maximum peak systolic velocities were collected and a table of descriptive data is provided such as mean PSV for both the working and non-working arm of the subjects. Subjective analysis of the collective data was more readily appreciated by constructing trend graphs, which depicted the non-working PSV. (Figure 9) Similarly, a trend graph was created for the working wrist, (Figure 8) which depicted the PSV collected over the length of the study.

The mean PSV's were then gathered for each subject at the anatomical location of the proximal carpal tunnel. These data points were trended for the non-working and working wrists of each individual subject in an identical manner to that of the maximum PSV. (Figures 10 and 11)

Discussion

This study is the first attempt to analyze spectral Doppler waveforms in order to gather information on MMN longitudinally. By gathering quantitative data depicting the blood flow within the microvasculature of the median nerve longitudinally, with the intention of determining the utility of this application of sonography. Since the cohorts

of subjects participated in different phases throughout the study (baseline, working, and recovery), this represented a unique opportunity to examine the development of this disorder. The decision was made to investigate only the proximal portion of the median nerve proximal to the carpal tunnel. As reported previously, the median nerve has been described with swelling in the segment of the nerve just proximal to the site of compression.[50] Because of the associated hyperemic response associated with the inflammation process, this proximal segment of the nerve was deemed most likely to demonstrate the greatest change in intraneural blood flow activity. Given the research question proposed, this site was regarded as most appropriate for investigation with spectral Doppler.

Theoretically the baseline scans, completed before the subjects began the repetitive working phase of the study, should have displayed consistency throughout subsequent bi-weekly scans. Once the working phase began, the goal was to increase the risk of acute physiologic changes to the median nerve. As repetitive wrist movements occur, the inflammatory response of the median nerve begins. Subsequently, the enlarged nerve has experienced microtrauma, due to the repetitive movement, through a restricted space within the carpal tunnel.[44] This microtrauma and swelling solicits the hypervascularity of the median nerve which was the basis of the expected rise in peak systolic velocities during the working phase. This was not in fact documented with the data collected during this study. Similarly, corresponding qualitative changes were expected in the trended data which reflected the subjects' completion of work exposure and recovery. Throughout the working stage, this risk of injury to the median nerve

increased as the subject completed a repetitive squeezing task. Once the recovery phase began, the subjects' risk was reduced and the constant irritation of the nerve was ameliorated. However due to the microvasculature damage that may have occurred, resulting chronic changes could have developed. Unlike the acute phase, this is characterized by ischemia and scarring of the median nerve, which can impair sensory and motor abilities.[7,51,52] Given our understanding of this pathophysiology, a drop in peak systolic velocities to approximately equal or below the original baseline values was expected. However, like the transition from the baseline to working stage of the study, no detectable differences in peak systolic values could be demonstrated qualitatively.

These results are consistent with study conducted by Evans et al. in which power and spectral Doppler was utilized to evaluate median nerve behavior in a group of working DMS and VT sonographers. Data was collected the first week of the study in a pre-scanning MSK exam, then five weeks later in a post-scanning MSK exam.[7] This study presented a limited data set, therefore no statistical tests were able to be conducted. However, change scores of the average peak systolic values recorded within the median nerve were able to be carried out, which resulted in very little difference indicated between the pre- and post-scanning exams. This result of MSK sonography lacking the sensitivity to detect any reasonable microvasculature change is consistent with the data generated in the current study, as no discernible pattern could be distinguished. Reconfirming the Evans et al. study, Ghasemi-Esfe et al. reported similar results of indeterminate data while analyzing intraneural vascularity. In this experiment patients with a certain diagnosis of CTS were evaluated using a multivariate approach in

analyzing multiple potential risk factors discovered in the musculoskeletal wrist sonogram. These factors were analyzed along with the nerve conduction data of the patient inflicted with CTS, and again spectral Doppler was not sensitive enough to be used as a single discriminator of CTS.[20]

Despite the unsuccessful results of the current study, utilizing nerve hypervascularity as a diagnostic tool to aid in the diagnosis of CTS, still holds some promise but needs further investigation. In a study conducted by Mallouhi et al., a musculoskeletal sonogram was completed on 206 wrists among patients reporting symptoms of CTS. Factors such as nerve appearance, edema, and the presence of nerve hypervascularity were examined simultaneously. The findings of this study demonstrated, that when compared with nerve conduction studies, nerve hypervascularization had the highest accuracy over all other sonographic measures.[53] However where this study differed from the current research, is the method by which hypervascularity was determined. Mallouhi et al. only captured color Doppler signals as a means of determining the presence of a hyperemic nerve. The color Doppler signals were characterized by the presence of any intraneural structures not related to the persistent median artery. Furthermore, this presence or lack thereof, of increased nerve vascularity was reported statistically in a 2-point nominal fashion: present or absent.[53] This method of determining hypervascularity was nearly duplicated in a more recent study in which any intraneural vascularity demonstrating pulsatile blood flow was considered as hypervascularity of the median nerve.[19] Further, representative velocities

are more accurately captured through the use of angle correction within spectral Doppler gate. Therefore a replication study would advocate the use of angle correction.

Throughout the current study, it became apparent that with the proper technical settings, intraneural vascularity, unrelated to the persistent median artery, was present within the median nerve during every sonogram performed. Adopting this methodology, in the current study, for determining median nerve hypervascularization, would not have possessed adequate sensitivity. This underscores the need to further investigate utilizing spectral Doppler for quantifying intraneural vascularity. An innovative approach to assessing nerve vascularity which may alleviate the problem of diminished sensitivity, would be to amplify the intraneural waveforms as recorded by spectral Doppler, through the use of contrast enhanced ultrasound (CEUS). By injecting a microbubble-based contrast agent, detection of low-volume blood flow can be improved by increasing the signal to noise ratio.[54,55]

Limitations

This preclinical study is limited foremost due to the level of evidence and has threats to both internal and external validity. Furthermore, the addition of more subjects into the experiment would have increased the data collected and statistical rigor. A replication of this study would require that a direct measure of the force during the repetitive squeeze task was not reported and would have made this a more robust study,

as the work effort would have had the ability to be quantified. This would have also ensured that the repetitive squeezing task was truly being completed by the subjects, ruling out any threats to construct validity. Statistically, while comparing the left (working wrist) to the right (non-working wrist), the non-working wrist would ideally be used as the controlled variable, allowing a comparison to be made intra-subject. However, caution is given to the use of a subject serving as its own control due to the changes within the median nerve being attributed to pregnancy, diabetes, or leprosy. This confounding variable causes the unilateral manipulation to not be the exclusive variable causing median nerve changes. Subsequently this comparison is not appropriate; therefore no true controlled variable was established.

Conclusion

This study provides low level evidence that the use of spectral Doppler to accurately detect longitudinal changes in vascularity within the median nerve may be diagnostically inadequate. As the subjects in the study moved through the working and non-working stages of study, spectral Doppler alone proved to lack the level of sensitivity needed to detect physiologic changes within the nerve. This outcome, although inconclusive when it comes to detecting changes in median nerve vascularity, does suggest the need for this method to be analyzed in conjunction with other sonographic features and provocative testing. Cross-sectional area (CSA), nerve echogenicity, and

Color/Power Doppler signal counts have all demonstrated validity and the ability to provide useful data while being interrogated in previous studies in the diagnosis of CTS. The investigation of sonographic features being used as a diagnostic adjunct should not be abandoned, a longitudinal study combining all these factors and analytically comparing the changes observed would appear to be the next logical step.

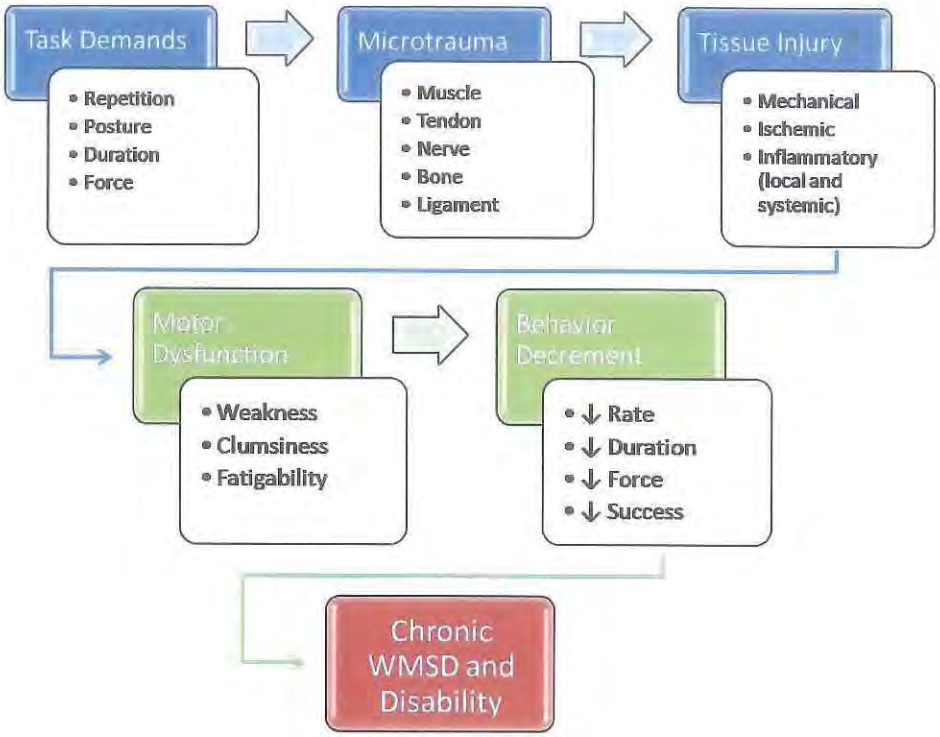
While examining the hypervascularity of the median nerve, the next step as suggested in previous experiments would be the utilization of a contrast agent to amplify the spectral Doppler signal returning from the microvasculature in the nerve.[54] These amplified waveforms would then hopefully possess the sensitivity to demonstrate a detectable difference as the progression of MMN occurs. Taking this technique a step further, these contrast enhanced Doppler waveforms could then be compared to T2 weighted MRI musculoskeletal scans. As set forth by the American College of Radiology Appropriateness Criteria, MRI is the imaging gold standard pertaining to acute wrist pain. Therefore a direct comparison between intraneural vascular intensity, collected through the use of musculoskeletal ultrasound, and median nerve signal to noise ratio (SNR) as collected through the use of MRI, would be appropriate.[22]

Chapter 4

Discussion

The objective of this study was to determine the feasibility and evaluate which ultrasonographic measures can be utilized to quantitatively portray pathophysiologic changes to the median nerve. This was done in a preclinical animal model using a cohort of *macaca fascicularis* monkeys which were exposed to repetitive pinch maneuvers. The data collected from these subjects through the use of musculoskeletal sonography could then potentially provide some insight in the disease progression of CTS. The premise of this experiment was derived from the conceptual model of the hypothesized development of work related musculoskeletal disorders, by Barr and Barbe.[5]

Figure 2. Conceptual model depicting the hypothesized manner in which many factors contribute to WRMSDs.[5]



In an attempt to provide more information regarding acute median nerve pathophysiology, this study attempted to identify risk factors associated with CTS in the Microtrauma stage, immediately following the exposure to Task Demands. This was done in hopes of reaching an earlier diagnosis of CTS, before the disease progressed into further stages such as Motor Dysfunction, Behavior Decrement, and Chronic or Debilitating damage was done.

In the first study conducted, an attempt was made to monitor the CSA of the median nerve while the animal cohorts underwent work exposure to a repetitive pinching task. This measurement was done in two ways, one including the outer covering or epineurium surrounding the nerve, and one excluding the epineurium. These CSA measurements were also made in two locations, first proximal to the carpal tunnel as defined by the most distal portion of the radius and second, within the carpal tunnel as defined by the most proximal portion of the pisiform.

First and foremost, this study demonstrated the feasibility of musculoskeletal ultrasound to conduct a reproducible protocol which evaluates the median nerve while collecting data obtaining data longitudinally. The portability and non-ionizing nature of MSK sonography are among the ideal characteristics which support longitudinal point of care imaging examinations. One main drawback to this experiment is that it was conducted in an animal model, which is considered preclinical, low level evidence; however this was the only conceivable option to test this imaging modality in a cohort of subjects over a long period of time and attempt to control confounding variables. Given these findings which support the feasibility of the use of MSK sonography longitudinally, further research can be explored into the use of this imaging modality as a possible screening tool, in which the care provider could travel to the work site and evaluate individuals at a high risk of developing CTS, a very common WRMSD.

Another successful outcome of this study was the ability of CSA measurements made using MSK sonography to demonstrate consistency with CSA measurements obtained using the gold standard of MRI. Because of our execution of the study's

experimental design, the only opportunity to compare MRI CSA measurements with MSK sonography CSA measurements was at the baseline time point in the study. Therefore it was necessary to determine that CSA measurements were comparable between the two imaging modalities in order to give validity to the MSK sonography CSA measurements which were used throughout the remainder of the study. This statistical similarity which was discovered may lead into further investigation into similarities of these two imaging modalities, which may have the potential to raise the ranking of MSK sonography in the ACR Appropriateness Criteria for the imaging modality of choice in patients with wrist pain.

Further investigation into the geometrical changes of the median nerve conducted in this study provided limited evidence. While comparing CSA measurements obtained including the epineurium and obtained excluding the epineurium, no significant differences were able to be demonstrated between the pre- and post-work exposure scans at both locations evaluated. Future studies conducting similar research may consider evaluating more than two locations along the course of the median nerve. With CSA median nerve measurements obtained from multiple sites, a change score could then be used in order to evaluate for focal points of compression or inflammation.

It is important to note that MSK ultrasound is a dynamic imaging modality. By only obtaining measurements at two predetermined anatomical locations, as was done in this study; the full utilization of this imaging modality is being limited, as the point of disease has the potential to be located anywhere throughout the nerve. One suggested improvement on the imaging protocol conducted in this study would be the use of the

run/stop function available on the ultrasound unit. Subjects' median nerves could be scanned axially moving distal down the arm, and once the scan reaches the most distal desired portion of the median nerve, a cine clip can be taken, capturing the entire path of the median nerve. Later, during analysis, the researcher then has the ability to play back the clip, which provides an entire representation of median nerve pathophysiologic activity. Additionally, movement can be recorded so that continued research into nerve excursion which is a potential important diagnostic variable that could not be measured with MRI. This suggestion of this more innovative method of scanning and analysis demonstrates another success of this experiment, which is that it provides insight which would lead to a more refined protocol being established for future studies. In a study conducted by Tagliafico et al, nerve density recorded with sonography demonstrated promise as a imaging parameter, then this might be an additional variable to measure besides geometry and could account for swelling (inner and outer) of the acutely damaged nerve.[56] This would be worth including as a variable in a replication of this geometric study.

Although this study did not possess the proper sensitivity to demonstrate changes in the CSA of the median nerve between pre- and post- work exposure, a statistically significant difference was demonstrated in the residual area measurement of the median nerve. The residual area measurement, which represents the epineurial layer of the median nerve, was obtained by subtracting the inner CSA measurements (which exclude the epineurium) from the outer CSA measurements (which exclude the epineurium). Statistical tests support that between the time of pre- and post- work exposure, these

epineurium measurements demonstrated a significant increase in area. This possible representation of inflammation may be considered a good measurement associated with an increased risk in developing CTS, and further investigation of this measurement could be considered.

The second study carried out in an attempt to obtain more information into the pathophysiologic response of the median nerve associated with a repetitive pinching task was through the use of spectral Doppler. Spectral Doppler tracings obtained throughout the median nerve provide a quantitative representation of median nerve perfusion throughout the nerve's microvasculature. Similar to the first study conducted, this study attempts to revert back to the Microtrauma stage of WRMSD disease process as conceptualized in the Barr and Barbe diagram. Because this stage of acute CTS is characterized by hyperemia of the median nerve, associated with inflammation, it was plausible to expect to observe an increase in median nerve perfusion throughout the beginning and into work exposure.[1]

As was demonstrated in the first study, the feasibility of obtaining spectral Doppler tracings within the median nerve longitudinally was confirmed. However, although the feasibility of obtaining these waveforms was possible, the discrimination of the exact location of the waveforms be gathered was questionable. Ideally, with enough blood flow coursing through the median nerve microvasculature, more accurate determination of location as well as the ability to use angle correction while obtaining spectral tracings may have been possible. For these reasons, this study provided inadequate evidence to quantitatively or qualitatively demonstrate any significant change

or trend in median nerve perfusion throughout the work exposure and non-work exposure stages of this experiment; and therefore also unable to refine an appropriate protocol for evaluating median nerve perfusion.

Because this inability to detect changes in the perfusion of the microvasculature of the median nerve arose from the lack of sensitivity demonstrated through the use of MSK sonography, future studies may consider a methodology which accounts for this hindrance. One way that has been demonstrated to increase the visualization of microvascularity is through the use of contrast enhanced ultrasound (CEUS), by increasing the signal to noise ratio.[55] In a study conducted by Klauser et al., CEUS was used in an attempt to increase detection of the microvasculature blood flow within the proximal interphalangeal (PIP) and metacarpophalangeal (MCP) joints in patients with rheumatoid arthritis (RA). It was concluded that the use of CEUS significantly improved detection of the microvasculature blood flow within the joints investigated, and significant differences in blood flow associated with the level of RA disease was demonstrated.[54] Although this research is focused primarily on evaluating low-volume blood flow associated with inflammation and synovitis in RA patients, the premise is translatable to the detection of the microvasculature within the inflamed median nerve in patients suffering from CTS. Since the pathophysiologic response involved with the two aforementioned diseases both involve vessel microtrauma and revascularization, it can hypothesized that utilizing CEUS in the evaluation of the perfusion of the median nerve will increase the sensitivity of spectral Doppler and allow for more accurate information to be gathered.

In summary, the success of these two studies was involved mainly with demonstrating the feasibility of MSK sonography to evaluate the median nerve longitudinally, through the Microtrauma stage into the Chronic and Disability stage. With the exception of the significant increase in epineurium area, which may merit further investigation, the geometric analysis of the median nerve did not possess the proper sensitivity to demonstrate any significant changes in CSA throughout the pre- and post-work exposure stages of the experiment. Similarly, the use of spectral Doppler tracings to evaluate the microvasculature within the median nerve associated with median nerve perfusion, also currently lacked appropriate sensitivity. Spectral Doppler sensitivity is contextually limited by the current detection of low blood flow of the ultrasound equipment utilized.

Although these studies were not able to identify statistically significant differences between pathophysiologic responses of the median nerve, in an animal model, exposed to a repetitive pinch task, this research should not be abandoned. As portrayed in WRMSD's progression diagram, the symptoms associated with the Microtrauma and Tissue Injury stages of WRMSD's should be detectable through the use of MSK sonography. These two studies demonstrate the first step in the quest to document preclinical evidence and provide a basis on how to continue this investigation. Because this study was unsuccessful at establishing a proper protocol and technique and utility at the preclinical level, the next natural step would be to translate these experiments into a human model to achieve the next level of evidence based practice.

Chapter 5

Future Directions

The objective of this study was to longitudinally examine the progression of MMN within a working animal model, via ultrasound imaging techniques. By controlling the work exposure of the cohorts, it was hypothesized that quantitative data would be able to be collected before and throughout the acute Microtrauma stage and into the Chronic and Disability stage of the disorder. This data, which represents pathophysiologic changes of the median nerve, would then potentially provide evidence and determine if quantitative data is able to provide any insight into the way CTS/MMN progresses.

This experiment demonstrated the feasibility of MSK ultrasound to possess the ability of quantifying pathophysiologic changes of the median nerve. Some insight was also provided into the way in which the different layers of the median nerve behave in a subject with suspected CTS/MMN. However, the main conclusion to be drawn from this study is the inability of MSK ultrasound to detect significant changes of median nerve size and perfusion longitudinally, due to lack of sensitivity. Because of this, a replication study should take place in order to increase this sensitivity, first and foremost.

Since this study was conducted in an animal model and all evidence derived from it should be considered as preclinical, the next logical step would be its replication utilizing a human model. As stated previously, the sensitivity of MSK ultrasound needs to be increased in order to detect pathophysiologic changes within the median nerve longitudinally. By doing this is the only way of achieving the ultimate goal, which is to use MSK ultrasound to detect biomarkers of MMN and aid in the diagnosis of CTS.

One way in which this increased level of sensitivity can be obtained is by combining different measures attainable through MSK ultrasound and analyzing their effectiveness individually, as well as in combination. This study examined the effectiveness of CSA obtained through gray-scale ultrasound as well as PSV's obtained through spectral Doppler to monitor changes in the median nerve. However other ultrasonographic measures could also be used in conjunction with the aforementioned techniques, such as median nerve excursion. By obtaining a longitudinal view of the median nerve in the distal wrist, the sliding of the median nerve through the carpal tunnel can be evaluated as the subject retroflexes the wrist. This subjective measure of the median nerve's gliding ability can also be considered in the detection of MMN risk factors.

Another method of median nerve perfusion analysis, which could be combined with PSV data, is the Klauser pixel count approach. In this method, color or power Doppler and the amount of pixels illuminated are subjectively counted and scored. This could be done on each subject with a standard dimension ROI, to provide more information regarding median nerve perfusion. Taking this a step further, computer

software such as PixelFlux Scientific® can be used to retrospectively quantify pixel intensities within the median nerve semi-automatically. By manually drawing and ROI around the median nerve displayed longitudinally, this software will calculate a variety of data points, of those including average and maximum pixel intensities within the ROI.

As described in previous chapters, an innovative approach to increasing the sensitivity of MSK ultrasound is through the use of CEUS. Previous studies have demonstrated success in increasing the visualization and sensitivity of spectral Doppler while detecting microvasculature blood flow in patients with rheumatoid arthritis.[54] Although this research is focused primarily on evaluating low-volume blood flow associated with inflammation and synovitis in RA patients, the premise is translatable to the detection of the microvasculature within the inflamed median nerve in patients suffering from CTS. Since the pathophysiologic response involved with the two aforementioned diseases both involve vessel microtrauma and revascularization, it can hypothesized that utilizing CEUS in the evaluation of the perfusion of the median nerve will increase the sensitivity of spectral Doppler and allow for more accurate and sensitive information to be gathered. It could be suggested that an intermediate step, before the transition of a study evaluating median nerve perfusion in humans, would be a second preclinical study, in which the safety and feasibility of CEUS is tested in an animal model.

Other non-sonographic measures can also be used in conjunction with MSK ultrasound to increase the overall sensitivity of MMN/CTS risk factor detection. Provocative tests, such as Phalen, Tinels, and Durkin's can be utilized to provide a

subjective measurement of pain within the wrist. Provocative testing is not possible with an animal model, however these tests can be easily executed in a human study, which would add another dimension during data analysis. Likewise, once the appropriate sensitivity has been reached through the use of imaging techniques coupled with provocative testing, a human study that monitors physiologic changes within the median nerve, longitudinally, could be completed. Returning to the conceptual flow chart created by Barr and Barbe, the hypothesized progression of WRMSD's is a theoretical frame work for conducting such a study. As demonstrated in this study, it is feasible to detect changes within the median nerve during which the subject is suspected to be in the Microtrauma stage of the disease progression. Given this preclinical evidence, the next logical step would be to attempt to detect such changes associated with the Microtrauma stage in human subjects.

In order to analyze and translate the desired outcomes, subject recruitment is a crucial part of the experimental design. One method of recruitment would be to collect data from patients at the time of their electrodiagnostic testing. By doing this, EDX data can be combined with data obtained through MSK ultrasound, which could provide more representative data of pathophysiologic changes that are occurring within the median nerve. However, it is possible that patients receiving EDX testing may be experiencing symptoms associated with a more advanced stage of the disease, such as Motor Dysfunction or Tissue Injury. To avoid this potential pitfall, subjects who are experiencing acute symptoms, associated with the Microtrauma stage, should be recruited from a primary care physician's office. Since this would be an initial step for patients

seeking CTS treatment, it would provide possible volunteers with the most acute symptoms. Once these subjects that are suspected as being at the Microtrauma stage, are recruited, it would be possible to longitudinally follow these patients. Data collected on these patients would then allow for physiologic biomarkers to be analyzed throughout the subsequent stages of the Barr and Barbe diagram, all the way to the Chronic and Disability stage.

Taking the idea of analyzing median nerve activity throughout as many stages of the Barr and Barbe diagram as possible, a research design could be constructed to allow for the analysis of the median nerve during the Tasks Demands stage. Since this study provides evidence that it is feasible to collect quantitative data through MSK ultrasound in a portable fashion, it would be possible to travel to the workplace and recruit subjects which participate in occupations with a high prevalence of MMN/CTS and other WRMSD's. By recruiting individuals who may be new to the high-risk work environment or who do not yet report symptoms associated with MMN/CTS, it may be possible to place the individual at the beginning of the Task Demands stage. This would provide the next level of scientific evidence, a cohort study of individuals beginning an occupation associated with a high prevalence of CTS/MMN, where the researcher has the opportunity to obtain prospective pathophysiologic data.

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Appendix A

Tables

Table 1: *Mean Direct Trace cross-sectional areas for the Subjects' Left Hand Pre and Post-intervention by Group*

	US Pisiform OCSA	US Pisiform ICSA	US Radius OCSA	US Radius ICSA	MRI Pisiform OCSA	MRI Radius OCSA
Preintervention						
Year 1 (n = 8)	2.76	0.97	2.21	0.70	2.09	1.91
Year 2 (n = 7)	2.54	1.10	1.85	0.66	2.87	3.46
Postintervention						
Year 1 (n = 8)	2.59	0.91	1.94	0.65		
Year 2 (n = 7)	2.79	0.83	1.68	0.49		

Proximal to the carpal tunnel inlet was indicated by the most distal portion of the radius, and within the carpal tunnel was indicated by the most proximal portion of the pisiform. ICSA, inner cross-sectional area; OCSA, outer cross-sectional area; MRI, magnetic resonance imaging, US; ultrasonography.

Table 2: *Correlations Between cross-sectional areas of Sonographic Residual Area (Epineurial Layer) Outer and Inner CSA by anatomical location of the Pisiform, Post-work Exposure*

	r Value	P Value
Inner CSA	.22	.01
Outer CSA	.95	.01

Within the carpal tunnel is identified by the most proximal portion of the pisiform. CSA; cross-sectional area.

Appendix B

Figures

Figure 3: *Conceptual model depicting the hypothesized manner in which many factors contribute to WRMSDs.*[5]

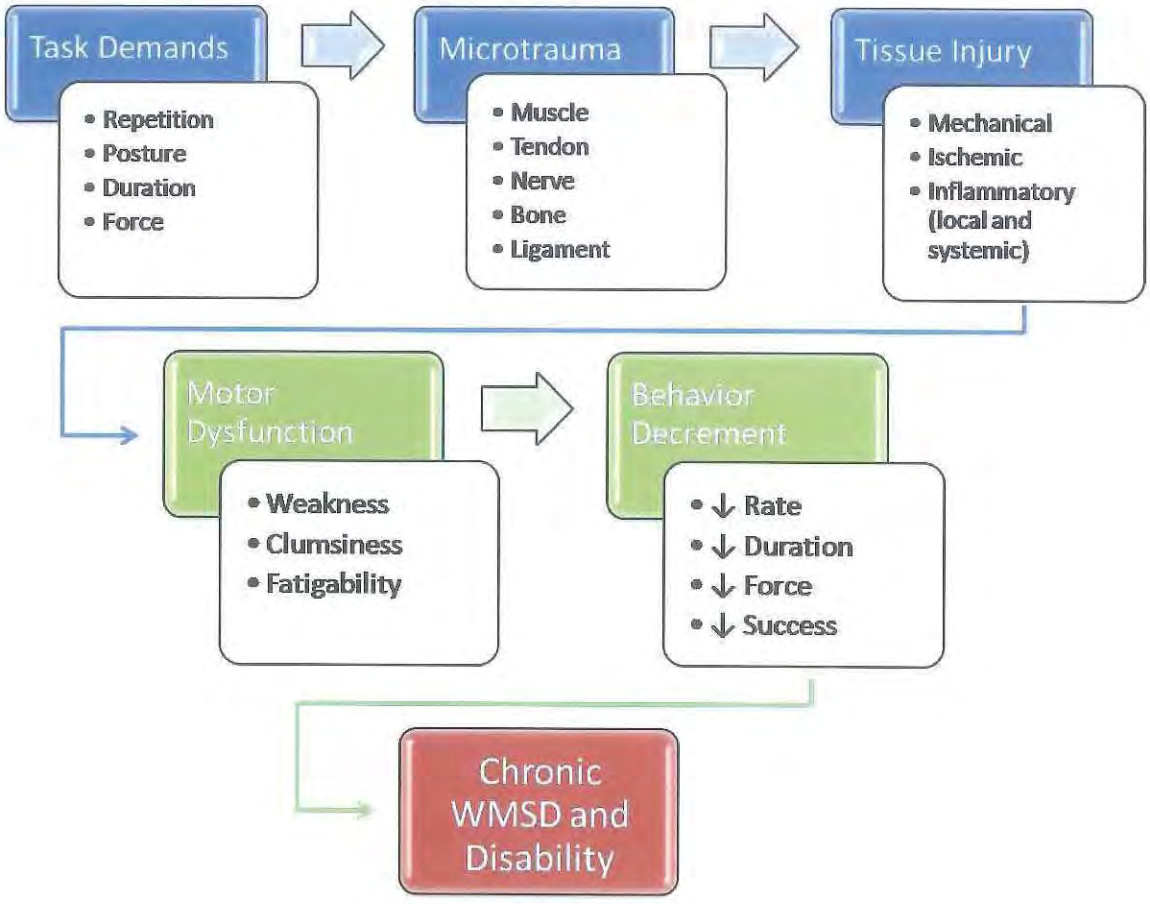

Figure 4: (A) *Sonogram of the median nerve imaged in the axial plane at the level of the radius, proximal to the carpal tunnel inlet. Note the echogenic outer edge (white arrow), known as the epineurium, which surrounds the nerve, and the hypoechoic inner edge (red arrow), which surrounds the perineurium and endoneurium.* (B) *Axial magnetic resonance image of the left working wrist of a subject that provides a high-resolution image of the outer border of the median nerve, at the level of the radius, proximal to the carpal tunnel inlet.*

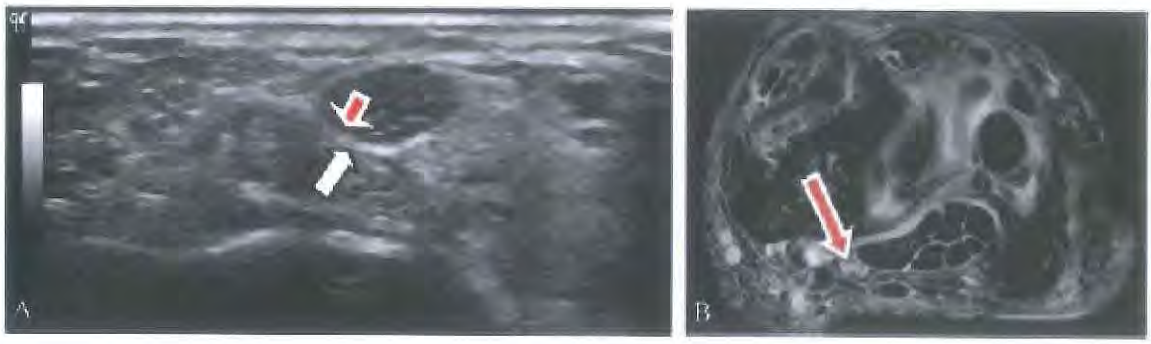

Figure 5: (A) *Cross-sectional representative diagram of a normal peripheral nerve and the associated vascularity, without compression of the nerve.* (B) *A hypothesized cross-sectional representative diagram of an acute mononeuropathy of a peripheral nerve with associated hyperemia. The solid blue arrow depicts the compression on the nerve and the small open blue arrow demonstrates the swelling directed outward on the epineurium.*

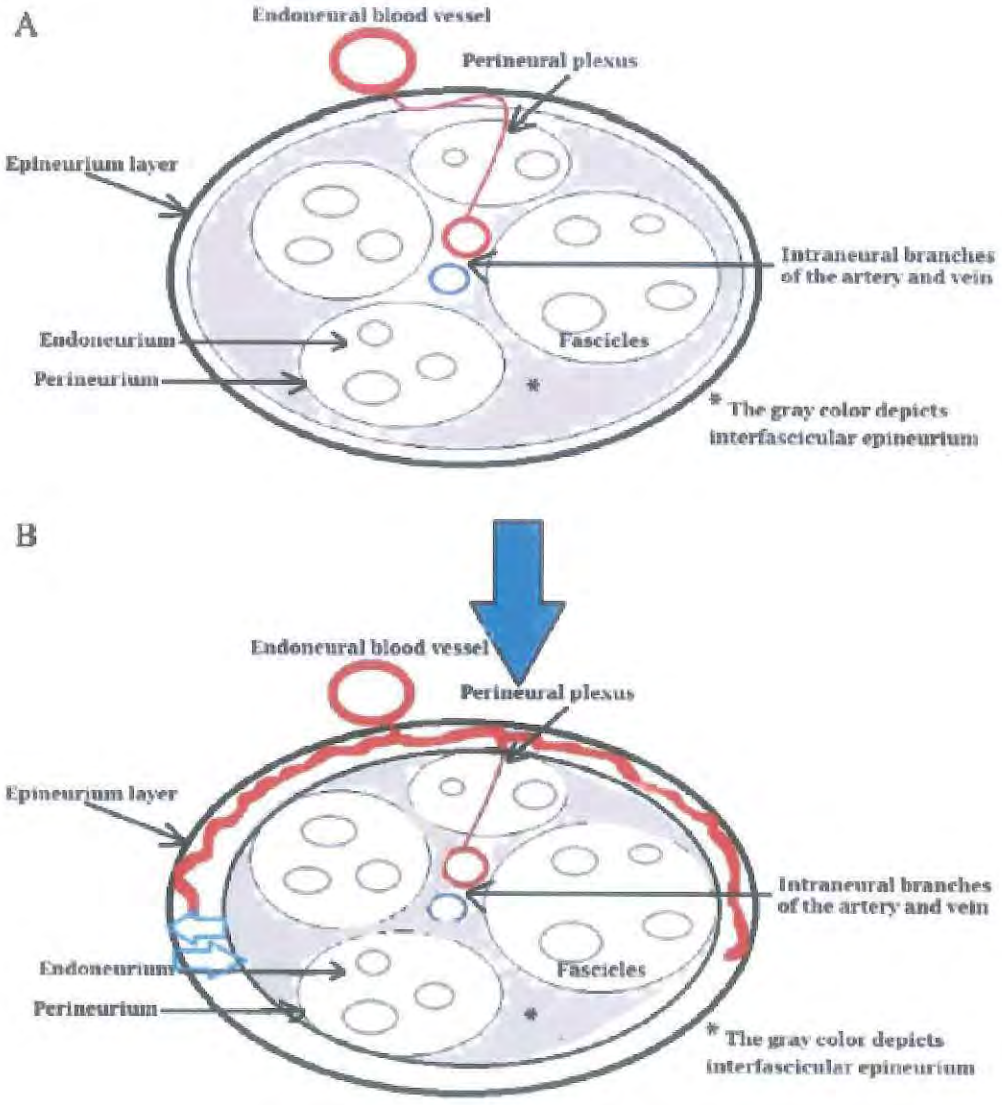

Figure 6: *Flow chart demonstrating the inclusion/exclusion process of the systematic review conducted to determine the current level of evidence regarding sonographic spectral Doppler detection of median nerve perfusion.*

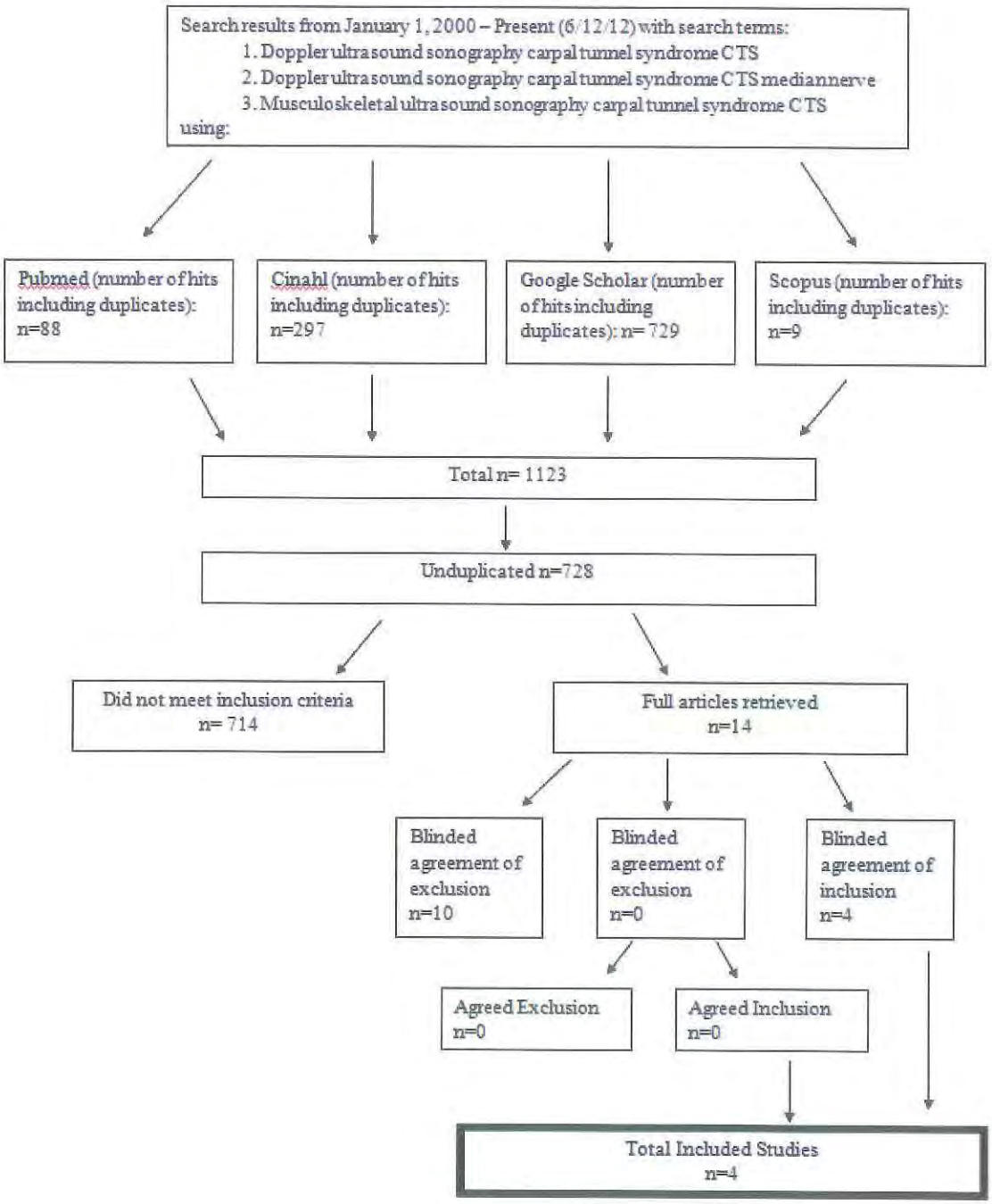

Figure 7: *Longitudinal view utilizing power and spectral Doppler to demonstrate triphasic arterial flow within the median nerve.*

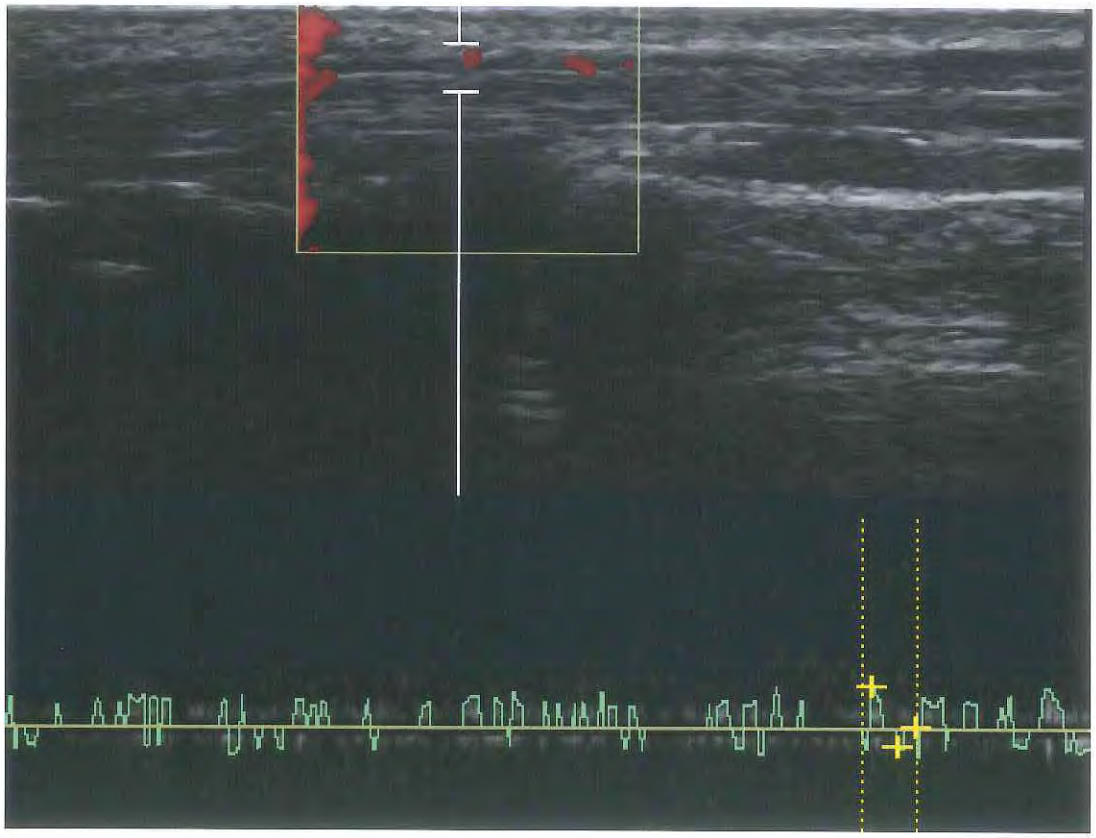

Figure 8: *Trend graph of data points representing maximum peak systolic velocities of individual subjects, gathered in the proximal portion of the left (working) wrist.*

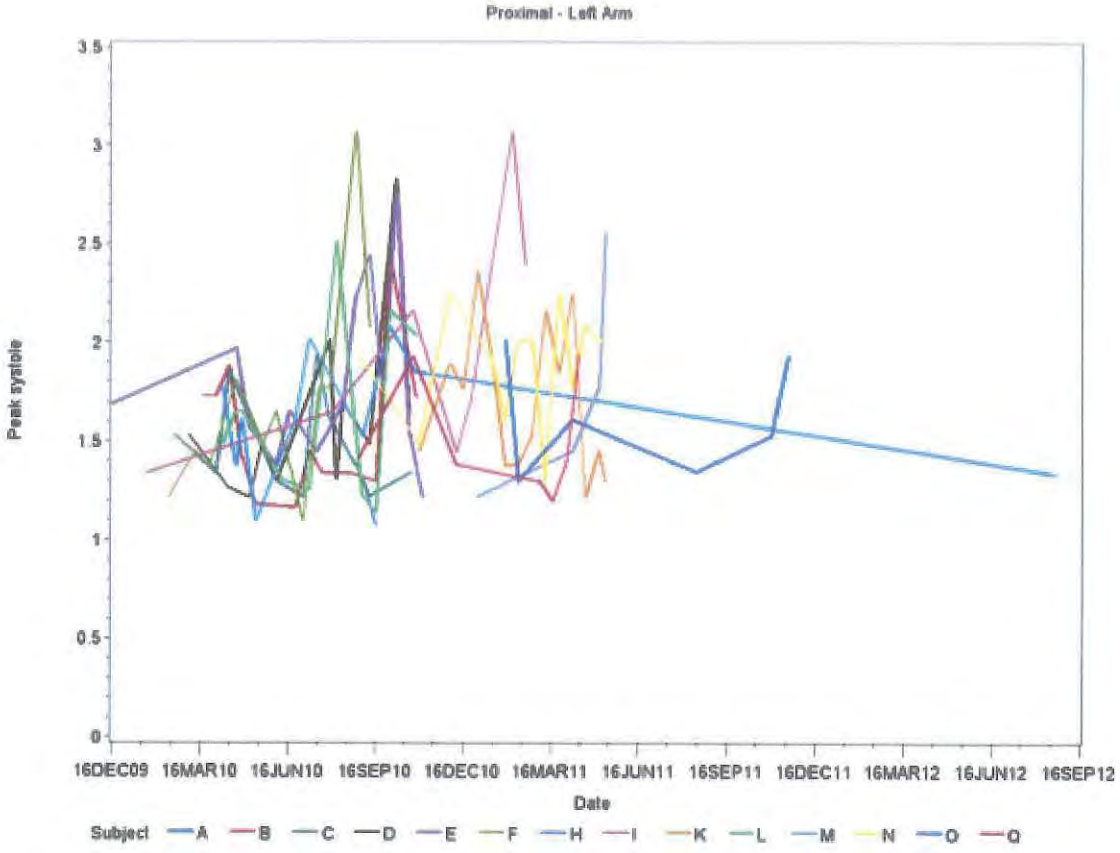

Figure 9: *Trend graph of data points representing maximum peak systolic velocities of individual subjects, gathered in the proximal portion of the right (non-working) wrist.*

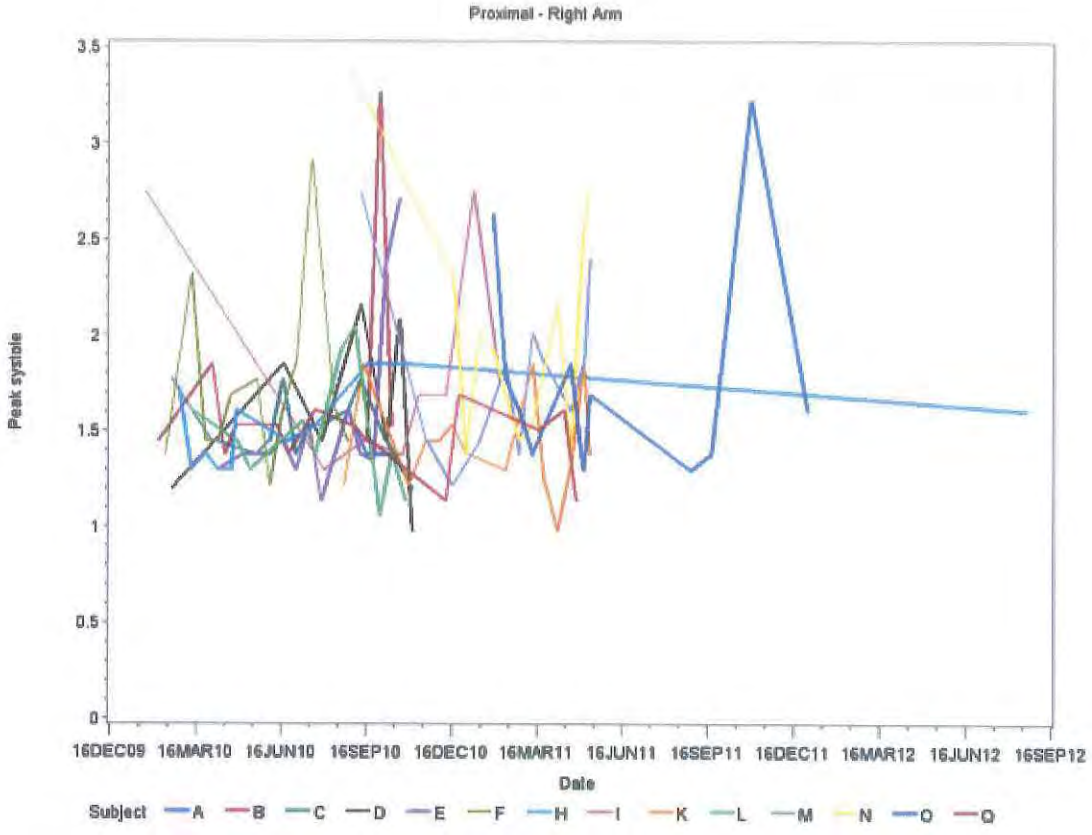

Figure 10: *Trend graph of data points representing the mean peak systolic velocities in the proximal portion of the left (working) wrist.*

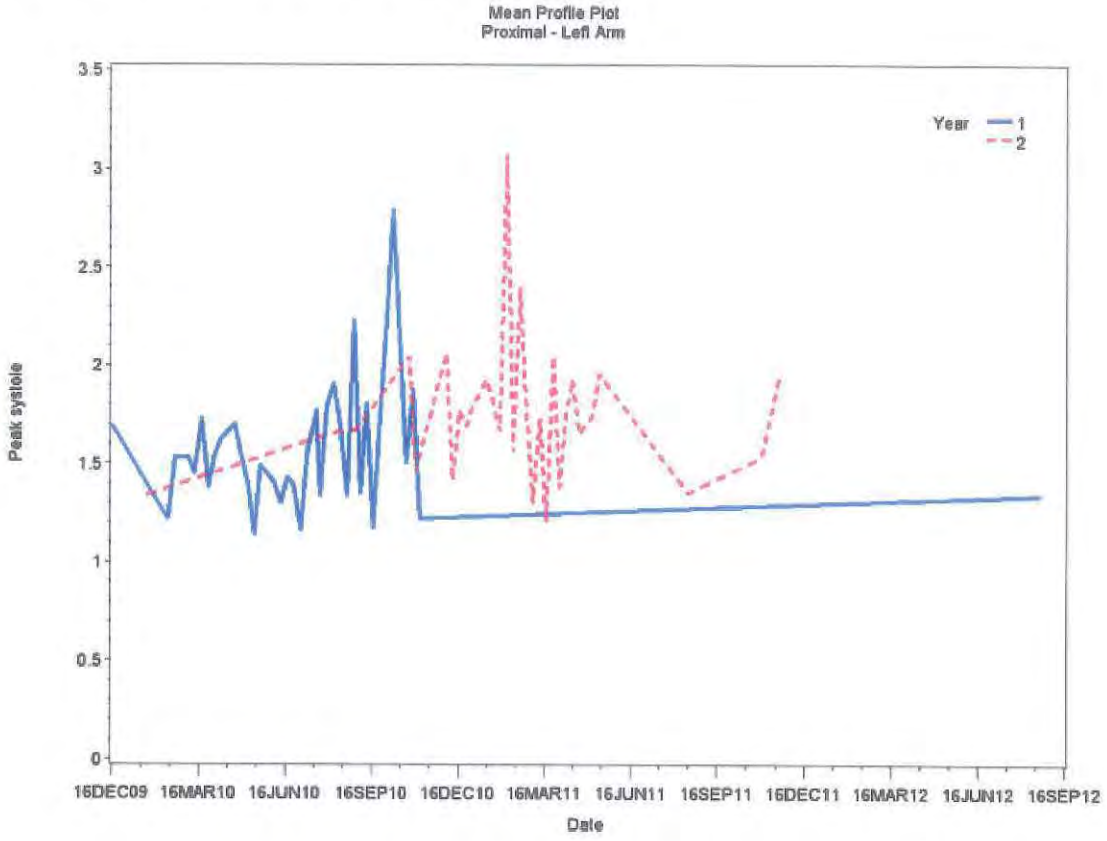

Figure 11: *Trend graph of data points representing the mean peak systolic velocities in the proximal portion of the right (non-working) wrist.*

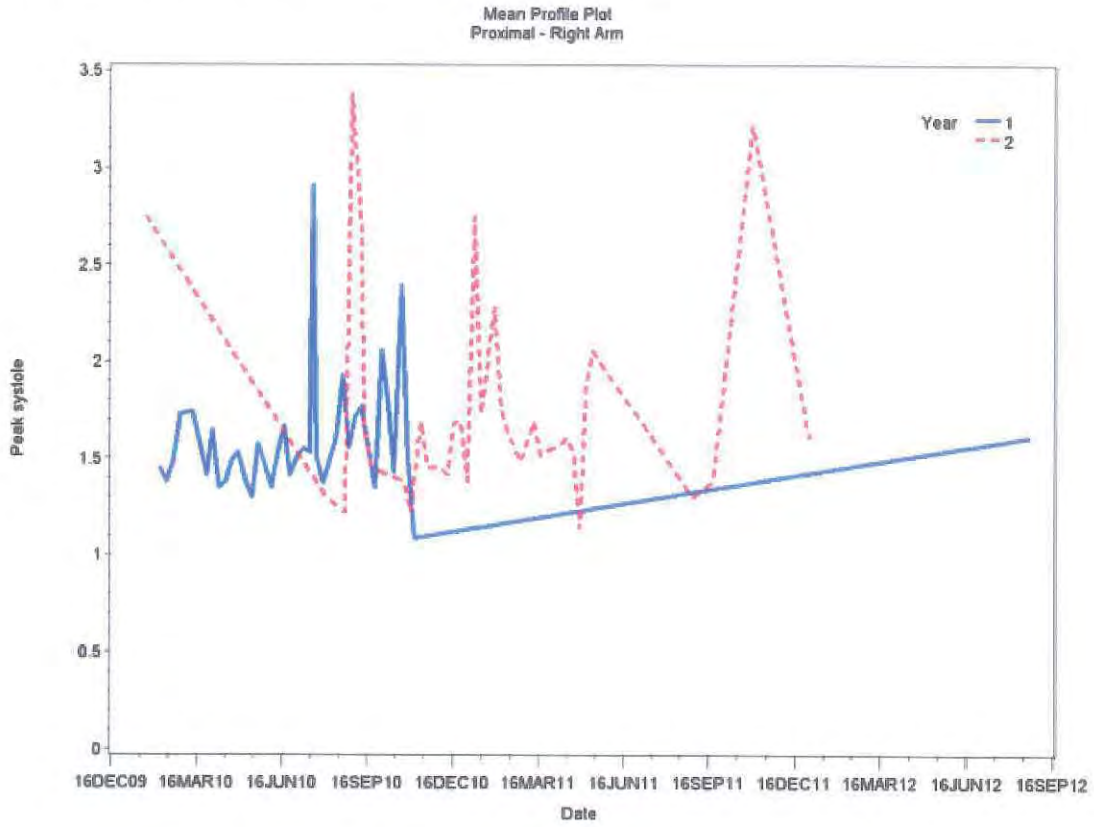

Figure 12: *Inclusion/Exclusion flowchart for assessment of Doppler waveform morphology and quantitative data points. *Criteria are not mutually exclusive, with some waveforms failing multiple criteria; numbers following each criterion indicate the count of waveforms that did not meet that individual inclusion criterion.* [44]