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14. ABSTRACT

This study is a ***double-blind randomized placebo-controlled clinical trial using repeated measures***. The ***objective*** is to improve recovery of functional skills for persons living in states of seriously impaired consciousness 3 to 12 months after severe TBI. This will be achieved by determining the neurobehavioral and neural effects of repetitive transcranial magnetic stimulation (rTMS), which is a non-invasive technique to stimulate the brain. The evidence of therapeutic efficacy from the literature in non-TBI related neurologic populations combined with our preliminary findings with severe TBI, indicate that rTMS merits investigation as a neurotherapeutic for severe TBI and that the proposed repetitive TMS protocol should be examined to determine effectiveness in inducing structural and functional neural plasticity and improving neurobehavioral recovery after severe TBI. ***Specific Aims***: Aim I will determine presence, direction and sustainability of rTMS-induced neurobehavioral effects measured with the Disability Rating Scale. Aim II will determine the presence, direction and sustainability of rTMS-induced changes in functional neural activation and whether or not these changes correlate with improving neurobehavioral function. Aim III will examine the effect of rTMS on white fiber tracts and whether or not the rTMS-related effects correlate with improving neurobehavioral function. Aim IV addresses the need to confirm rTMS safety for severe TBI.

15. SUBJECT TERMS

Disability Rating Scale (DRS), Neurobehavioral, Repetitive Transcranial Magnetic Stimulation (rTMS), Traumatic Brain Injury (TBI), Vegetative (VS), Minimally Conscious (MCS)

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1. INTRODUCTION

Advanced medical care saves and sustains the lives of persons incurring severe Traumatic Brain Injury (TBI), but for survivors there are few to no treatments that induce or accelerate functional and adaptive recovery. Two separate research awards were conferred to address the need for targeted treatments that effectively induce functional and structural changes in the brain that can, ultimately, enable neurobehavioral recovery. The first award supports a clinical trial (Project #1) examining the safety and therapeutic efficacy of repetitive Transcranial Magnetic Stimulation (rTMS) and is funded via the Congressional Directed Medical Research Program (CDMRP) (ID # W81XWH-14-1-0568). The CDMRP funded clinical trial, referred to here as Project #1, is ongoing and a separate final report will be provided at time of completion. This final report relates to the projects funded by the second research award from the Joint Warfighter Medical Research Program (JWMRP) (ID#: W81XWH-16-2-0023).

The JWMRP award address the challenges to measuring meaningful and accurate treatment effects for persons remaining in states of Disordered Consciousness (DoC) after TBI. As these scientific barriers hinder treatment development, the JWMRP award was conferred to advance capabilities to measure meaningful and accurate treatment effects at the neurobehavioral and molecular levels. To supplement the CDMRP funded clinical trial, the JWMRP award funded two additional research projects, referred to here as Projects # 2 and #3. Project #2 is titled "Advancing Clinically Reported TBI Outcomes using Modern Psychometrics" and Project #3 is titled "rTMS: miRNA as biomarkers for severe TBI and rTMS mediated gains in neurobehavioral activity." Both supplemental projects address the **overarching goal** of advancing and creating scientific capabilities to enable development of precision neuromodulatory treatments for persons with DoC after severe TBI.

2. KEYWORDS

Table 1 Keywords and Acronyms

Key Words	Acronyms
Coma Near Coma Scale	CNC
Coma Recovery Scale-Revised	CRS-R
Common Data Element	CDE
Disability Rating Scale	DRS
Disorders of Consciousness	DoC
Disorders of Consciousness Scale-25	DOCS
Emergence from Minimally Conscious State	eMCS
Glasgow Outcome Scale including Extended version	GOS and GOSE
Micro RNA	miRNA
Minimally Conscious State	MCS
National Institutes of Health	NIH
Operation Enduring Freedom	OEF
Operation Iraqi Freedom	OIF
repetitive Transcranial Magnetic Stimulation	rTMS
Traumatic Brain Injury	TBI
Vegetative State	VS

3. ACCOMPLISHMENTS

What were the major goals of the project?

The major goals/tasks of Supplemental Projects #2 and #3, according to specific objectives as outlined in the updated and approved Statement of Work (SOW) dated 05/27/2020, are reported here by planned and actual completion dates (or percentage completed). To enhance readability of this final report, the numbering of the SOW major goals and the numbering of these same goals in this final report are not the same. Thus, notes are provided in this report (in parentheses and *italicized font)* to enable cross referencing of this final report with the goals/tasks as listed in aforementioned SOW.

Project # 2: "Advancing Clinically Reported TBI Outcomes using Modern Psychometrics"

The **purpose** of project #2 is to determine how to make neurobehavioral outcomes more comparable, accurate and meaningful. For the five most widely used neurobehavioral DoC assessments, this project leverages the unique data collected in the rTMS clinical trial as well as existing data to address these **aims**: (a) Compare content and construct validity of widely used severe TBI outcome measures to determine the extent to which these assessments do (or do not) measure the same trait(s), for patients across states/levels of DoC (e.g., Vegetative State, Minimally Conscious State). (b) Increase the accuracy/precision of TBI outcome measures by quantifying and neutralizing the impact of rater severity/leniency on TBI outcome measures. (c) Based on the psychometric analyses for aims (a) and (b) above, develop meaningful indices of change (effect size, minimally detectable change, minimally clinically important difference, qualitative perceptions of change from caregivers and clinicians) for each TBI outcome measure.

Goal #1: Development and testing of meaningful change anchors

This goal *(Note: In SOW Goal #1 is referred to as Major Task 2a*) addresses the need for TBI outcomes/endpoints that can be used in clinical trials with severe TBI. The specific objectives, addressing this goal, involved examining existing endpoints as they are known to be limited by specificity and sensitivity.[1] To develop capabilities, this work also examined psychometrically strong but less commonly used endpoints. Specifically, four neurobehavioral assessments were examined.

Goal #1 Specific Objectives	Completion	
	Planned	Actual
1.1. Submit the Disorders of Consciousness Scale-25 (DOCS) as a federally qualified endpoint	8/2020	1/2022
1.2. Complete rating scale, item, and person analyses for each major assessment of neurobehavioral function (*Note: In SOW referred to as Major Task 2c*)	9/2020	6/2019
1.3. Conduct co-calibration of assessments	12/2018	1/2019
1.4. Examine specific psychometric properties including indices of responsiveness and rater severity/leniency (*Note: In SOW referred to as Major Task 2d)*	2/2019	5/2019

Goal #2: Describing the meaningful change during recovery of consciousness from the clinician's perspective

This goal also addresses the need for TBI outcomes/endpoints that can be used in clinical trials for patients with severe TBI. To explicate meaningful neurobehavioral change during DoC recovery, this work also examined existing TBI endpoints.

Goal # 2 Specific Objectives:	Completion	
	Planned	Actual
2.1. Describe clinicians' perceptions of evaluating and treating patients with DoC	8/2019	12/2019
2.2. Develop vignettes that represent varying amounts of patients recovering consciousness	8/2019	9/2019
2.3. Create a hierarchy of the vignettes Conduct co-calibration of major assessments	8/2020	60%

Goal #3: Dissemination of Information

This goal *(Note: In SOW referred to as Major Task 2f)* relates to dissemination of all findings reported below under accomplishments. This information also relates to the information reported Products Section (Section 6) below, which provides a comprehensive list of papers and presentations in different scientific and educational venues.

Goal # 3 Specific Objectives:	Completion	
	Planned	Actual
Manuscript: CNC Psychometrics	2018	12/2020
Manuscript: CNC meaningful change	2019	70%
Manuscript: DOCS Letter to Editor	2018	3/2020
Manuscript: CRS-R Psychometrics	2020	2/2022
Manuscript: CRS-R meaningful change	2019	50%
Manuscript: DOCS rater severity leniency calibration paper	2020	70%
Manuscript: Clinician's reasoning and decision making	2020	11/2021
Manuscript: CNC: DOCS co-calibration illustrating methods	2020	50%
Manuscript: Co-creating vignettes: Methods and Lessons learned	2020	50%
Manuscript: CNC-DOCS-CRS co calibration-neurobehavioral recovery hierarchy	2021	50%
Manuscript: Best Practices in Rasch Reporting Guidelines: Part I	2020	2022
Manuscript: Best Practices in Rasch Reporting Guidelines: Part II	2020	25%
Manuscript: Comparison of clinician and caregiver perspectives of meaningful change	2021	20%
Manuscript: Protocol for Scoping Reviews	Not planned	08/2021
Manuscript: Scoping Review 1 – Identifying & Mapping Clinical Endpoints	2021	02/2022
Manuscript: Scoping Review 2 – Delineating Domains of Neurobehavioral Function	2021	90%

Manuscript: Psychometric properties of DRS	2020	50%
Educational: How do clinicians use a Keyform – Archives	2021	50%
MHSRS Presentations	2016 - 2021	2017, 2020
ACRM Presentations	2016 - 2021	2017, 2018, 2019, 2020
Measurement Conferences	2016 - 2021	2017, 2018

Project # 3: "rTMS: miRNA as Biomarkers for Severe TBI and rTMS Mediated Gains in Neurobehavioral Activity"

The **purpose** of the 3rd project is to determine if it is feasible to develop whole blood micromolecular biomarker, micro-Ribonucleic acids (miRNA), of recovery and/or responsiveness to rTMS treatment. The **aims** are to, in whole blood, (a) Identify and validate miRNA associated with DoC after TBI, (b) Identify specific miRNA changing after rTMS, and (c) Correlate miRNA changes to clinical changes in neurobehavioral abilities.

As reported in previous technical quarterly reports Year 1, Quarter 1, Major Goals/Tasks #1 and #2 involved regulatory and project start-up activities (planned/actual completion dates: 10/2016 / 5/2016) and Major Goal/Task #3 involved validating sample collection/shipment processing and storage (planned/actual completion dates: 12/2016 / 10/2016). Here we report on the final major goals/tasks (#4 and #5).

Goal #4: Validation of miRNA in severe TBI Patients

This goal was addressed by examining whole blood from persons in states of DoC after TBI, in part, because miRNA profiles of this TBI sub-population will confirm or counter the idea that miRNA may be useful for diagnosing TBI across severities. Also, standard practice for DoC patients is to use neurobehavioral tests to distinguish between levels of DoC. At least 48% of the time, however, these tests yield inaccurate diagnoses.[2,3] As patients in the VS have poorer prognoses than patients in the MCS, accurate differentiation between levels or staets of DoC is critical for guiding long-term medical rehabilitation. We also theorized that miRNA may enable development of phenotypes of recovery and treatment responsivness.

Goal # 4 Specific Objectives:	Completion	
	Planned	Actual
4.1. Validation of miRNA in whole blood of TBI patients.	10/2018	10/2021
4.2. Collection, shipment, and processing of all patient blood samples.	4/2020	90%
4.3. miRNA analysis from whole blood from all patients.	4/2020	90%
4.4. Coordinate data monitoring and entry into database.	4/2020	90%
4.5. Perform analyses according to aims and hypotheses.	4/2020	100%

Goal # 5: Dissemination of information
This goal *(Note: In SOW referred to as Major Goal/Task 3e)* relates to disseminating miRNA findings in the scientific literature.

Goal # 5 Specific Objectives:	Completion	
	Planned	Actual
5.1. Manuscript: miRNA review paper	12/2018	06/2020
5.2. Manuscript: miRNA in severe TBI relative to healthy	12/2018	02/2022
5.3. Manuscript: rTMS induced changes in miRNA	12/2019	50%
5.4. Manuscript: rTMS induced miRNA changes relative to clinical changes-therapeutic targets	12/2019	50%

What was accomplished under these goals?

Accomplishments are described here in terms of significant results including major findings and developments. To enhance readability of this final report, the numbering of the SOW goals and the numbering of these same goals in this final report are not the same. Thus, notes are provided in this report (in parentheses and *italicized font)* to enable the reader to cross reference the accomplishments according to Major Goals/Tasks as they are listed in the SOW dated 05/27/2020.

Goal #1 Accomplishments: Development and testing of meaningful change anchors

Specific Objective 1.1. Accomplishments: Submit the Disorders of Consciousness Scale-25 as a federally qualified endpoint
We collaborated with consultants at ICON (Information Consultants) to prepare a Letter of Intent to the US Food and Drug Administration (FDA) to recognize the Disorders of Consciousness Scale-25 (DOCS) as a federally qualified TBI endpoint. ICON has successfully helped others gain approval of outcome assessments as federally qualified endpoints. This collaboration demonstrated that the Letter of Intent was not ready for submission because the field of severe TBI broadly lacks a conceptual model that describes both i) the totality of domains including neurobehavioral function as it relates to recovery of consciousness and ii) the specific concepts within neurobehavioral function. While the FDA has approved some TBI-specific biomarkers as endpoints, only the Glasgow Outcome Scale Extended (GOSE) is considered a federally "accepted" endpoint. In addition, only the Disability Rating Scale (DRS) is categorized as an NIH TBI-specific Common Data Element (CDE). The Coma Recovery Scale-Revised (CRS-R) and Galveston Orientation Amnesia Test (GOAT) are classified as supplemental CDEs for recovery of consciousness. To address this knowledge gap, identified with the ICON collaboration, the work conducted for this objective advanced the field of TBI endpoints. Specifically, our team conducted two scoping reviews that are described below, and each are registered with PROSPERO (https://www.crd.york.ac.uk/prospero/):

- PROSPERO 2017 CRD42017058383 (Scoping Review on Outcomes Mapping)
- PROSPERO 2017 CRD42017062599 (Scoping Review Delineating Domains and Concepts of Interest for Neurobehavioral Function)

Scoping Review on Outcomes Mapping: For this project, we have a protocol manuscript under review at BMJ Open (submitted August 2021; See Appendix 2), which describes the methods and protocol followed for the scoping review on outcomes mapping. The scoping review on outcomes mapping manuscript will be submitted for peer-review February 2022 which follows publication of the protocol paper in BMJ OPEN. This manuscript will report the results summarized next.

We conducted a 30-year scoping review consisting of approximately 17,000 articles (January 1986-December 2020). 489 of these articles required full text review; 292 articles formed the final data set after full text review. We assembled a multi-disciplinary team of 37 contributors including a research librarian to develop the search strategy. All contributors were trained in knowledge of inclusion/exclusion criteria, the data extraction form, and SIGN quality reporting. This work resulted in the most comprehensive database of severe TBI outcomes to date and will be a valuable resource for other researchers who wish to conduct reviews in this area. Dr. Weaver has already, for example, conducted a sub-study from these data as part of her dissertation research.

We utilized the World Health Organization International Classification of Disability and Functioning (WHO ICF) to categorize 74 primary outcome assessments from 292 articles into 6 categories. The majority of articles mapped to the Body Functions category (n=257, 88%) (**Table 2**). Of these, 86% of articles were categorized to the Consciousness domain.

Table 2 Primary outcome assessments by WHO ICF categories

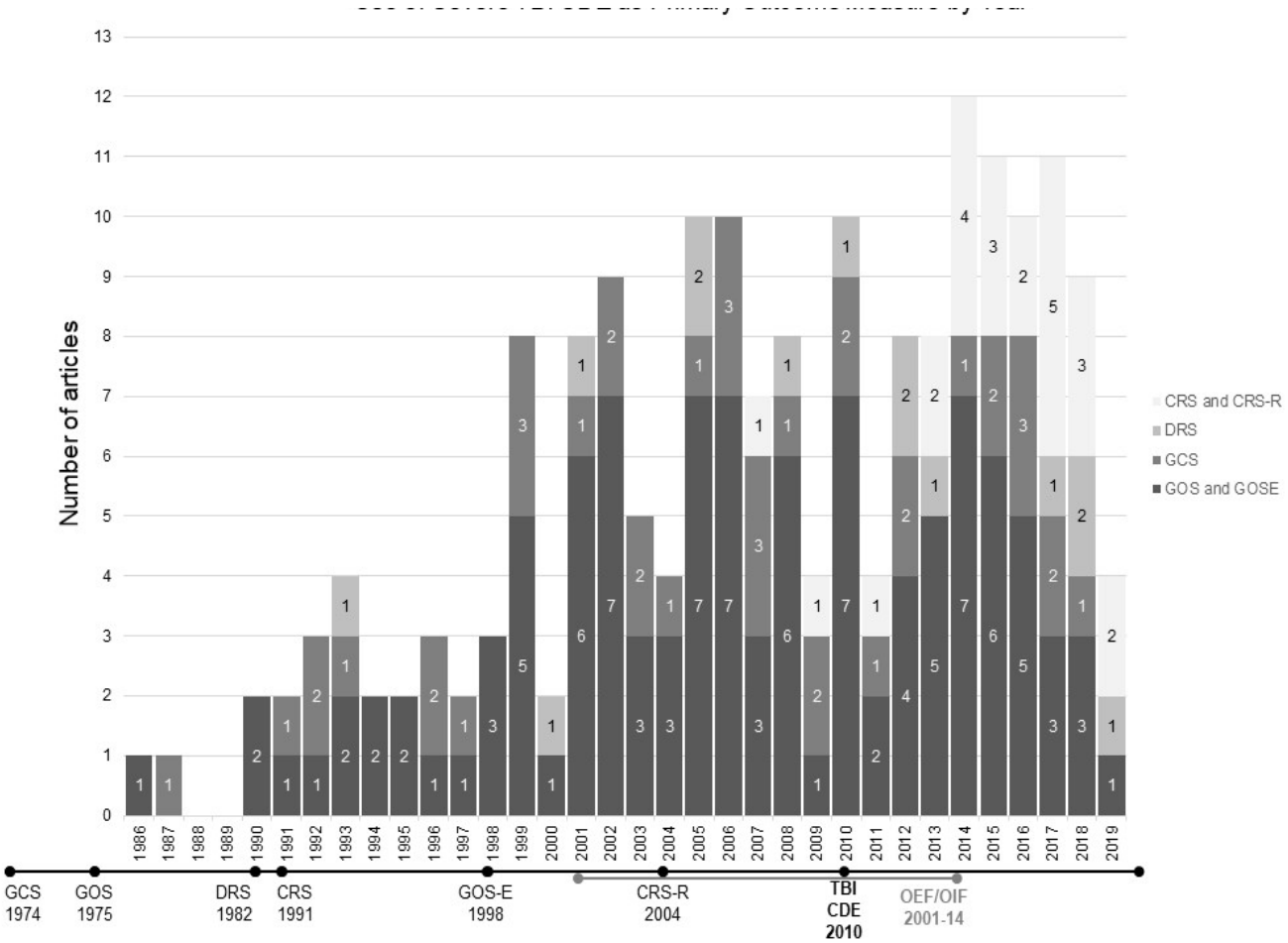
WHO ICF Category	Frequency	Percent of Total Articles
Body Function	257	88%
Body Structure	14	5%
Activities and Participation	14	5%
Environmental Factors	4	1%
Crosses Categories	2	>1%
Other Categories	1	>1%
Totals	**292**	**100%**

In the past 5 years, there has been increased use of primary endpoints in the Body Structures category, such as EEG, fMRI, and DTI. However, the most frequently used neurobehavioral assessments of body function were the Glasgow Outcome Scale (GOS including the extended version GOSE), Glasgow Coma Scale (GCS), Coma Recovery Scale (CRS, including the revised version, CRS-R), and Disability Rating Scale (DRS). These four assessments are also classified as NIH CDEs and accounted for 67% of articles (**Table 3**). The most commonly reported primary outcome assessment that is classified as a CDE was the GOS and GOSE.

Table 3 Primary outcome assessments classified as CDEs

Assessment	Frequency (Number of Articles)	Percent of Total Articles
GOS and GOSE	117	40%
GCS	41	14%
CRS and CRS-R	24	8%
DRS	14	5%
Totals	**196**	**67%**

We found that introduction of CDEs in 2010 had little or no impact on the frequency with which outcome assessments were selected as a primary endpoint (**Figure 1**). For Figure 1, Years of OEF (2003-2011) and OIF (2001-2014) are indicated as these likely resulted in greater number of severe TBI studies reported during these years. As indicated in Figure 1, the GOS and GOSE are the most frequently used primary endpoints with an increasing use of CRS-R staring in 2010. However, increasing use of more recently developed assessments is also evident, limiting evidence harmonization. This finding suggests that



traditional assessments classified as CDEs may not fully capture the concepts that researchers and/or clinicians seek to measure. [1]

Figure 1 Use of CDE as Primary Severe TBI Outcome Measure by Year

Scoping Review Delineating Domains and Concepts of Interest for Neurobehavioral Function: This scoping review seeks to describe the domains and concepts of interest for outcome measures related to neurobehavioral function in patients in states of DoC after severe TBI. All primary and secondary outcome measures of neurobehavioral function from the primary data set (n=38 measures) have been categorized as part of the primary data extraction. The main manuscript for this work will be submitted for peer-review after submission of the aforementioned Outcomes Mapping paper.

Specific Objective 1.2. Accomplishments: Complete rating scale, item, and person analyses for each major assessment of neurobehavioral function

Disorders of Consciousness Scale-25 (DOCS): Basic psychometrics were completed prior to the award[4] but to facilitate clinical translation an educational page summarizing the psychometric properties was published, in 2019.[5] This enabled us to focus on issues of rater severity/leniency described below.

Coma Near Coma Scale (CNC): The psychometrics paper was published in Archives of Physical Medicine and Rehabilitation.[6] The manuscript was originally submitted in 2017, revised and resubmitted to three different journals prior to being accepted in Archives of Physical Medicine and Rehabilitation with early online publication in 2020. (Appendix 3) During this publication process, we received a great deal of conflicting feedback from reviewers and editors at each journal. It became clear that the field of rehabilitation lacked unambiguous and consistent reporting guidelines for manuscripts using contemporary psychometric methods such as Rasch Measurement Theory. As a result, in tandem with ongoing work as part of this JWMRP, a task force was stood-up as part of the American Congress of Rehabilitation Medicine's (ACRM) Measurement Interdisciplinary Special Interest Group (M-ISIG).

The goal of the new ACRM 12-member task force was to develop reporting guidelines. This task force registered the Rasch Reporting Guideline for Rehabilitation Research (RULER) with the EQUATOR network in July 2020. https://www.equator-network.org/library/reporting-guidelines-under-development/reporting-guidelines-under-development-for-observational-studies/#RULER

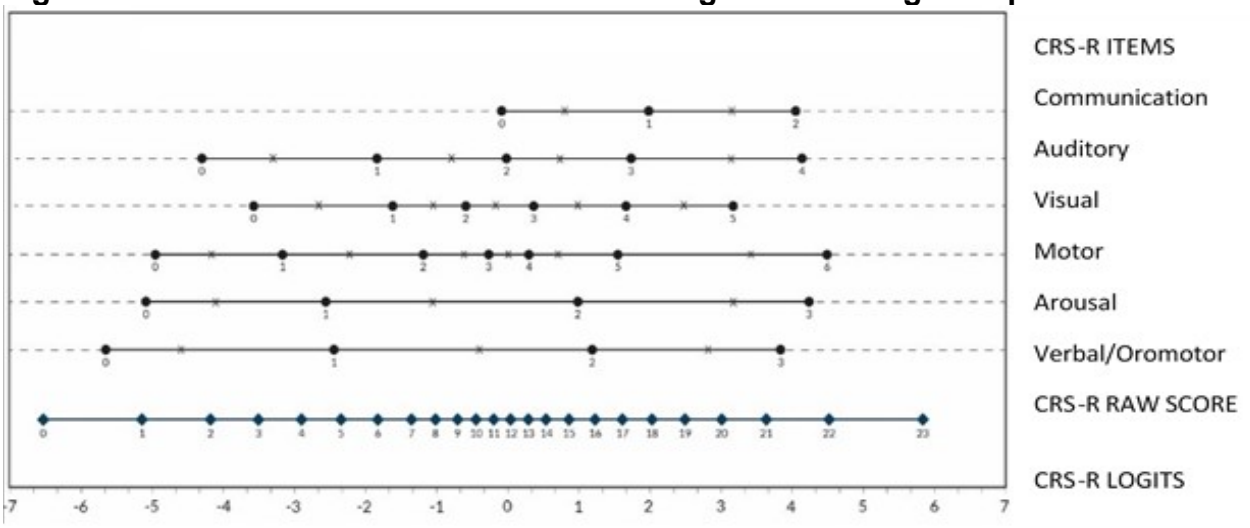
Following EQUATOR recommendations, the task force developed the RULER statement, Explanation and Elaboration document, and supplemental materials which were reviewed by more than 30 ACRM and International Objective Measurement (IOM) conference attendees and submitted for publication to Archives of Physical Medicine and Rehabilitation in July 2021. (Appendix 4) While this work proceeded independently of this JWMRP project, it was conducted in tandem with the JWMRP work as the JWMRP work spurred the necessity of these guidelines. This was particularly salient to the JWMRP work as our plans included submitting additional psychometric studies based on Rasch measurement as part of this project (e.g., the CRS-R paper currently under review). This is a critical research development, both broadly and specifically for recovery of consciousness research. If we are going to build better measures, we need better reporting guidelines.

Coma Recovery Scale-Revised (CRS-R): In addition to the rTMS clinical trial data, we located, requested, and received data from three additional sources as this larger data set enabled robust analytic decisions. These additional data sources are the Shirley Ryan Ability lab (n=50); Texas Institute for Rehabilitation Research (TIRR) (n=19); Amantadine trial[7] (n=184). Based on this data, a poster was accepted and presented at the ACRM Annual Conference in October of 2021 (see Appendix 5) and a manuscript has been submitted to Journal of Neurotrauma for review (see Appendix 6).

A key finding regarding classifying patients as having emerged from the Minimally Conscious State (eMCS) was that, in addition to rating scale step 6 on the Motor item and rating scale step 2 on the Functional Communication item, we found empirical evidence for

3 additional rating scale steps that align with Aspen consensus criteria for eMCS (**Figure 2**). These are step 3 (attention) on the CRS-R arousal item, step 4 (consistent movement to command) on the CRS-R auditory item, and step 3 (intelligible verbalization) on the CRS-R oromotor/verbal item. This is an important finding since up to 48% of patients with disorders of consciousness are misdiagnosed to a lower state of consciousness.[2,3] Also, as seen in **Figure 2**, we found that the most challenging CRS-R item is communication with the least challenging being verbal-oromotor. Because each CRS-R item combines a wide range of stimuli, Figure 2 also indicates that most items cover the full range of the trait. Some stimuli, such as response to pain (steps 2-3 and 4 of the motor item), cover very little range, probably because these reflect a different construct.

Figure 2 CSR-R Raw score-to-measure Nomogram and Wright map

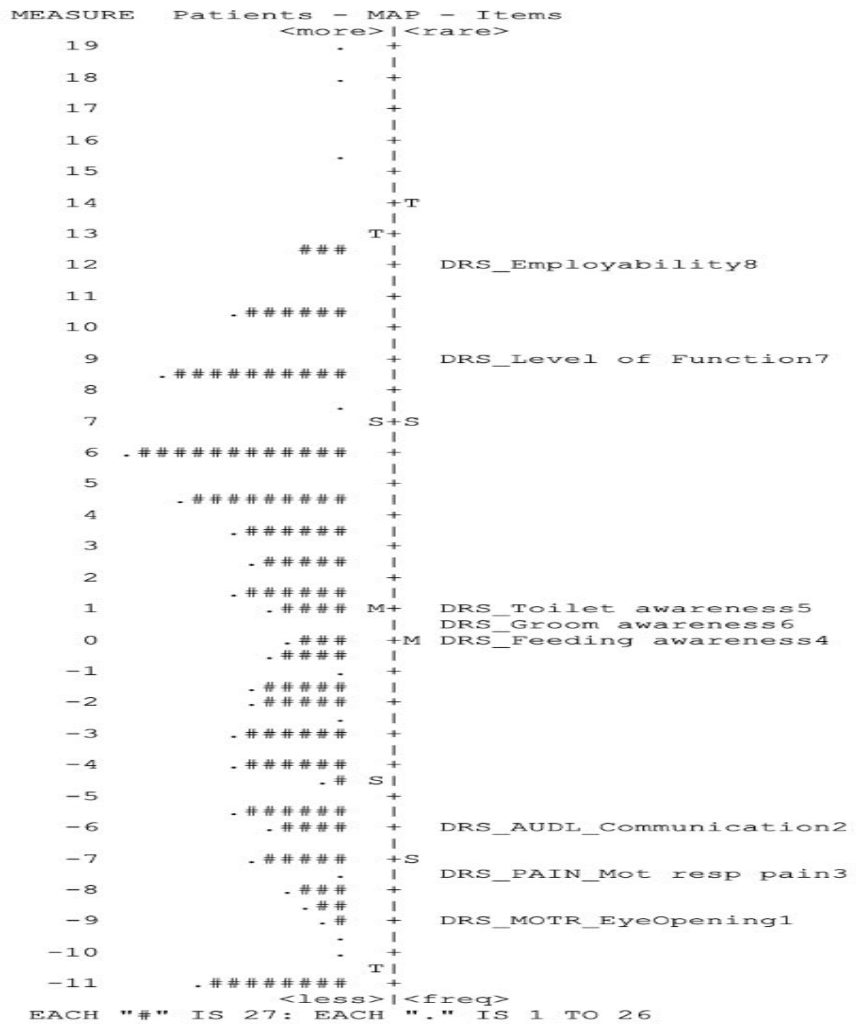


The six CRS-R items (y axis, Figure 2) each combines very different stimulus level data into larger domain-level items. For example, the motor function domain 'item' includes abnormal posturing, motor responses to pain, and functional object use (in response to auditory command). Our previous analyses of the CNC and DOCS assessment tools suggested that stimulus-level data, rather than CRS-R item level data would facilitate evaluation of the CRS-R. Specifically, we were concerned that the ordering of the stimuli within CRS-R items (i.e., the ordering of the rating scale steps from least to most challenging) reflect the actual level of challenge based on empirical evidence. Therefore, in addition to obtaining retrospective CRS-R data from TIRR, we prospectively collaborated with TIRR to pilot test a stimulus-level CRS-R data collection form. We collected 30 data points and proceeded with analysis of the CRS-R in its original format while this data collection was ongoing. This pilot data will be available to examine CRS-R stimulus level ordering in the future.

Disability Rating Scale (DRS): In addition to the rTMS clinical trial data, we acquired DRS data from the Amantadine trial[7] (n=184) and from the NIDILRR TBI Model systems (TBIMS) data set (n= 2,107) that included DRS item-level data. Results depicted in **Figure 3** indicate that there are clearly visible three distinct groups of patients (i.e., comatose, disordered consciousness, and eMCS) that are measured by 3 distinct sets of items (i.e.,

those reflecting disordered consciousness; those reflecting consciousness, employment, and overall function). However, our initial Rasch Measurement analysis demonstrated that DRS responses are especially Guttman-like; that is, once a patient has been scored on one or two items, their scores on the remaining items can be predicted with 100% accuracy. Also, additional items do not add information to measure person function more precisely. As a result, items representing least function appear “clumped” at the low end of the scale (Figure 3) and at the higher end of the scale items are stretched extremely far apart. This limits the ability of the DRS to detect change in function over time and makes the items very challenging to calibrate with other tools. Based on previously published findings from other labs, the intent of using the DRS in the rTMS clinical trial was to measure patients with severe TBI as they recovered consciousness and returned to the community. Our psychometric findings indicate, however, that the DRS measures neither group (i.e., disordered consciousness or community living) well. Disordered consciousness is a qualitatively different and not a quantitatively different state. Furthermore, four items fail the test for local independence with inter-item correlations greater than .90. The 3 awareness items; that are essentially the same item asked three times; and the Level of function item, which summarizes the other 7 items. This publication is in development.

Figure 3 DRS Person-item Hierarchy



Specific Objective 1.3. Accomplishments: Conduct co-calibration of major assessments of neurobehavioral function

Due to slower than expected recruitment for the rTMS clinical trial and the need for a larger than anticipated data set to conduct the co-calibration analyses, we assembled data sets from eight additional studies that each have a unique sample of patients in DoC due to TBI. Co-calibration requires some linkages in the data set, which was provided by the rTMS trial (ID # W81XWH-14-1-0568) as it includes all four assessments being co-calibrated (**Table 4**). That is, to link the data sets across studies, some of the patients in the co-calibration data set must be scored on more than one of the four assessments. We leveraged the 8 additional databases to complete the analyses, creating data use agreements with each organization sharing the data. We supplemented the clinical trial data with previously collected assessment data from Dr. Pape's FAST clinical trial [8]and observational post-acute care study.[9] We leveraged existing relationships with Kelsey Watters and Piper Hansen to receive DOCS and CRS-R data from Shirley Ryan Ability Lab and Katherine O'Brien at TIRR Memorial Hermann to receive additional CRS-R data. We also entered into a data use agreement with Dr. Giacino and Dr. Whyte to receive DRS and CRS-R data from the amantadine trial. Each data set required IRB amendments and data use agreements.

Table 4 Co-Calibration dataset: Combined data from nine studies

	Data Sets	**Assessments Being Co-calibrated**			
	n/r	**DRS**	**CRS-R**	**CNC**	**DOCS**
**rTMS Trial	18/126	X	X	X	X
FAST Clinical Trial	30/354			X	X
PACS (Observational)	175/851			X	X
R21 Clinical Trial	4/62			X	X
Open Label Trial	3/31			X	X
SRAL	52/876		X		X
Amantadine trial	184/1288	X	X		
TBIMS	2107/2312	X			
TIRR	19/25		X		

The rTMS clinical trial (ID # W81XWH-14-1-0568) included all four assessments (DOCS, CRS-R, CNC and DRS) thereby creating the necessary linkage across the co-calibration data set. **Abbreviations: n/r-sample of unique patients and total records**; rTMS trial** (ID # W81XWH-14-1-0568; 2016-2022); **FAST**-Familiar Auditory Sensory Training clinical trial; **PACS-** Post Acute Care Study data that collected at Hines VA as part of DOCS validation observational study. **R21**: clinical trial data collected at Hines VA; **Open Label:** Data collected at Hines VA; **SRAL:** clinical data from Shirley Ryan Ability Lab 2014-2018; **Amantadine trial**:-Clinical trial testing effect of Amantadine 2003-2010; **TBIMS**: NIDILRR TBI Model Systems Data 2012-2018; **TIRR**: Clinical data from Texas Institute of Rehabilitation Research 2019-2020.

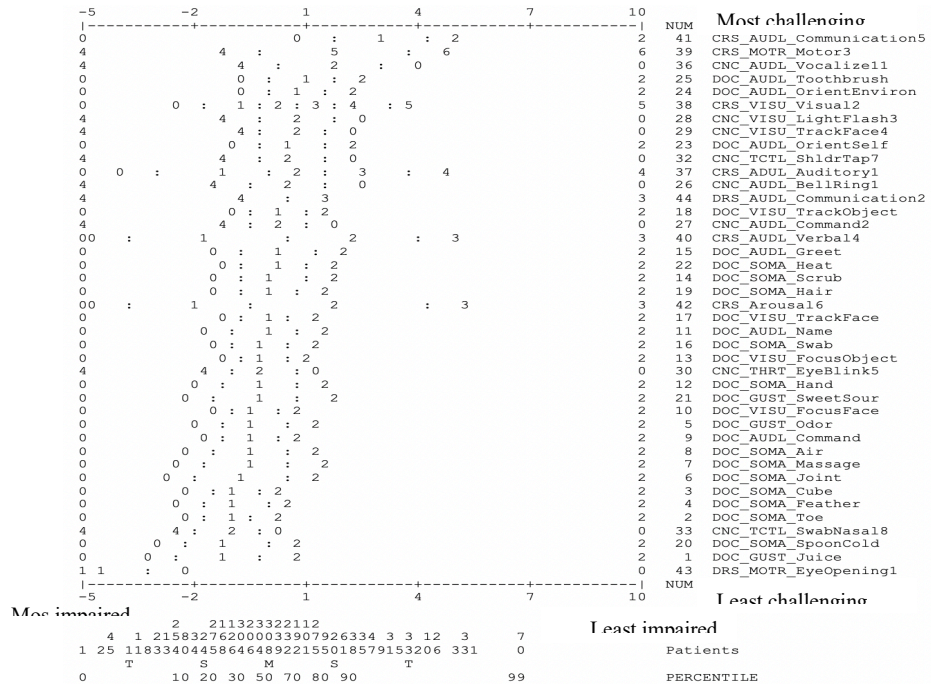
To arrive at the final co-calibration of 49 items across the four assessments of neurobehavioral function, we conducted a series of analyses, as follows:

1. Examined all 49 items and rating scale steps for DOCS, CNC, CRS, DRS
2. Removed DRS items 44-49 Awareness, communication, and global function items.
    a. Rationale for removing these items described above under DRS analyses.
3. Removed Pain items or rating scale steps from the CNC (CNC9-Pain to Finger; CNC10-Pain to ear), CRS pain rating scales steps of motor item (CRS3 Motor Function steps 3, 2, 1, 0) and the DRS pain rating scale categories (DRS1 Eye Opening step 3, 2; DRS3-Motor Response to Pain steps 5,4,3,2,1).
    a. Rationale for removing these items/step categories was evidence (fit statistics, principal component analysis of residuals) that pain is a distinct construct from neurobehavioral function.

The resulting item hierarchy (**Figure 4**) shows that DRS eye-opening is the easiest item as it is the first to be seen in the recovery of patients in states of DoC after TBI. Communication, orientation, and purposeful object use are the most challenging and align with eMCS. Next, many of the DOCS somatosensory items capture very low levels of function in patients classified as being in the VS and provide an important means of capturing change in this difficult to measure population. CNC items, which were originally designed to capture very low levels of patient function are, however, seen towards the top of the hierarchy. This is consistent with findings reported in our aforementioned CNC publication when the CNC items were examined separately.[6] As found with our analyses of the CRS-R items separately (Appendix 6),the wider range of these seen here with the CRS-R is related to the six CRS-R items being comprised of multiple stimuli levels within a single item domain.

In summary, the co-calibration of the CNC, CRS-R, DOCS, and DRS indicates that the DOCS somatosensory items are easier for patients to respond to, CRS-R and DOCS auditory and visual items are the most challenging. CRS-R arousal item appears higher in the hierarchy than my be expected because of rating scale step 3 which is attention; this aligns with other items of similar challenge (e.g., DOCS-25 face tracking, and responding to name called aloud). This is the first time that alignment of major assessment tools has been empirically described. It is clear that each of these four assessments capture different areas of the recovery spectrum for patients in states of DoC. Our findings clearly illustrate that no one assessment in isolation is able to effectively measure DoC after TBI.

Figure 4 Co-Calibrated Person-item Hierarchy (abbreviation DOC=Disorders of Consciousness Scale)



Specific Objective 1.4 Accomplishments: Examine specific psychometric properties including indices of responsiveness and rater severity/leniency

This objective was addressed by computing indices of change for each of the four neurobehavioral assessment tools used in aforementioned co-calibration. The indices of change examined included Minimally Detectable Change (MDC), Minimally Clinically Important differences (MCID), effect sizes, standardized response mean (SRM) and conditional MDCs (cMDC). This objective was also addressed by examining for the presence and impact of rater severity and leniency.

Other Achievements: Compute Indices of Change for Each of the Neurobehavioral Assessments

For each of the four assessments, we sought to compute indices of change *(Note: In SOW referred to as Major Task 2e). Results are presented below for each assessment tool.*

Disorders of Consciousness Scale-25 (DOCS): Indices of change including MDC, MCID, and effect size were published[10] prior to this award. However, during the course of the study we identified that the incorrect equation had been used to calculate the MDC. Thus, during the JWMRP we submitted a Letter to the Editor in the Journal of Head Trauma and Rehabilitation describing the revised MDC results.[11] (Appendix 7) As part of that revision, we identified a recent study by Kozlowski [12]and colleagues describing analyses for a conditional MDC (cMDC) which is more appropriate for Rasch-based measures. We conducted these analyses in consultation with Dr. Kozlowski for the DOCS. While cMDCs provide more correct estimates of change for individual patients, the literature is currently unclear how cMDCs can be used to evaluate change in groups such as different arms of a clinical trial. We continue to collaborate with Dr. Kozlowski to examine this issue.

Coma Near Coma Scale (CNC): Analyses to compute indices of change for the CNC were conducted and disseminated at the 2017 ACRM conference https://doi.org/10.1016/j.apmr.2017.09.031 (Appendix 8). Results indicate that the SRM for

improvers and non-improvers was 1.18 and 1.03, respectively. MDC_{95} indicates the amount of change needed to exceed measurement error; 8.3 for improvers and 6.9 for non-improvers. Distribution-based MCIDs based on Cohen effect size criteria for moderate differences was 5.2. 40% of the sample exceeded the MDC_{95} and 47% exceeded the moderate distribution based MCID. The SRM was relatively large for both improvers and non- improvers, suggesting the CNC is sensitive to detecting clinical change in this patient group. The moderate distribution- based MCID of 5.3 is the amount of change that may be considered clinically relevant for patients.

Coma Recovery Scale Revised (CRS-R): We conducted analyses to compute indices of change for the CRS-R. The SRM for improvers and non-improvers was 1.45 and -0.68, respectively. MDC_{95} indicates the amount of change needed to exceed measurement error; 1.32 for improvers and 0.93 for non-improvers. Distribution-based MCIDs based on Cohen effect size criteria for non-improvers were 0.3, 0.5, and 0.75 for .2SD, .33SD, and .50SD, respectively. Distribution-based MCIDs based on Cohen effect size criteria for improvers were 0.43, 0.70, and 1.06 for 0.2SD, 0.33SD, and 0.50SD, respectively. For 244 participants, 204 were considered improvers (change in CRS-R>0) and 40 were considered non-improvers. Of the non-improvers (n=40), 30% (n=12) declined on the CRS-R beyond measurement error. Of the improvers (n=204), 74% (n=151) improved on the CRS-R beyond measurement error.

Disability Rating Scale: Due to the poor psychometric properties of the DRS for patients with DoC, as we outlined in the section related to Specific Objective 1.2., we did not move forward with calculating indices of responsiveness.

Publications for Indices of Change: Brief reports regarding the CNC and CRS-R will be submitted in 2022. We are not writing a manuscript on the responsiveness of the DRS due to the poor psychometric properties (described above). A manuscript presenting the indices of change for each assessment in the same frame of reference based on the co-calibrated data is in development. We believe this work will substantively improve the state of the science relative to comparing across studies or using larger dataset (e.g., FITBIR and TBIMS). More specifically, by enabling evaluation of the amount of change in neurobehavioral function in comparable units, we believe this work has the potential to substantively improve the state of the science relative to cross-study comparisons and use of large data sets (e.g., FITBIR and TBIMS) in the future.

Other Achievements: Examine Issues of Rater Severity Leniency

This work*, (Note: In SOW referred to as Major Task 2d)* relates to Clinician-reported outcomes (ClinROs) of neurobehavioral function being critical aspects of evaluating recovery for patients in states of DoC. More specifically, clinician raters may introduce natural tendencies to be more lenient or more severe in their ratings of a patient's responses, such that change on the assessment reflects something other than improvement as a result of the treatment/intervention. Rater severity/leniency affects *all* rater-mediated assessments. It should not be confused with "interrater reliability." Rater severity/leniency is defined as: "The consistent tendency on the part of the rater to give a score that is higher or lower than appropriate, which is usually interpreted to mean higher or lower than the average of the other raters." [13] We examined the impact of rater severity/leniency on DOCS measures from the FAST and PACS data sets described above in Table 4. The data included 172 patients from 7 post-acute care facilities scored by 48 rehabilitation practitioners trained in

administration of the DOCS, measured weekly for 6 weeks. In about 20% of records, patients were scored simultaneously by two separate practitioners, creating the linkage necessary to conduct the Many Facets Rasch Measurement analyses.[14] Data were analyzed with and without adjustment for rater severity and the results examined for differences greater than the established MDC of 5 units.[10]

As indicated in **Figure 5**, we found that bias ranged from trivial to detrimental, and week to week variations suggested that specific raters at each assessment point are driving observed differences. Across all 6 weeks of observations, 15-20% of observations exceeded MDC limits of agreement (Week 1: 17.4%, n=37/213; Week 2: 16.8%, n=28/167; Week 3: 28.7%, n=33/115; Week 4: 7%, n=6/82; Week 5: 16.9%, n=11/65; Week 6: 18.4%, n=9/49). In summary, the plots in Figure 5 show association between adjusted and unadjusted data at three time-points; weeks 1, 3, and 6. Bias is most noticeable at Week 1, raters are more lenient. Approximately 20% of ratings were too severe or too lenient at each time point demonstrating the need for adjustment. Results were presented as a poster at the 2018 National Capital Area TBI conference at NIH (Appendix 9) and in an oral presentation at the 2018 International Objective Measurement Conference.

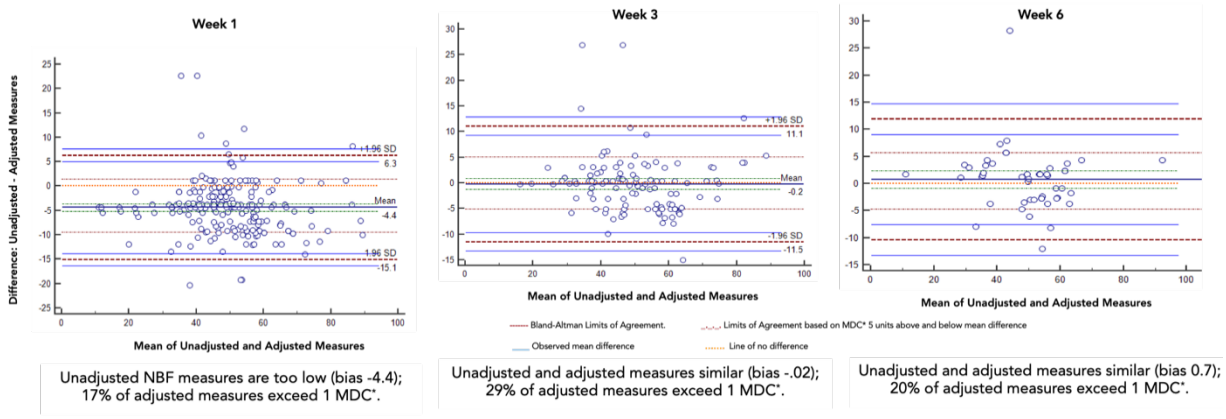


Figure 5 Bland-Altman plots: Agreement & bias in ratings with/out adjustment for rater severity/leniency

Rater severity/leniency makes a quantifiable difference in outcome measures for patients with DoC and has significant potential to misrepresent the effectiveness of a drug or a treatment's effect. To be useful as an endpoint for adequate and well-controlled clinical trials providing substantial evidence of drug effectiveness, rater-mediated measures of neurobehavioral function must account for rater severity.

Other Achievements: Creating patient video cases and collection of linking data

We originally planned *(Note: In SOW this is referred to as Major Task 2b)* to conduct rater severity/leniency analyses on each of the four neurobehavioral assessments being administered in the rTMS clinical trial (ID # W81XWH-14-1-0568). To do this, the JWMRP plan originally included creating patient video cases of each of the assessments used in the rTMS clinical trial. As the rTMS clinical trial originally had raters at multiple sites, the purpose of the videos was to link raters for the Many Facet Rasch Measurement Analysis conducted as describe above for the DOCS. We collected and reviewed the videos, but due to changes in the study sites (i.e., one site was eliminated due to low recruitment) this original plan was not feasible nor practical. Therefore, we did not create video cases or

collect clinical rater data on the videos. While we can pair raters in the available rTMS clinical trial data, the final sample size will likely be too small to conduct sufficiently robust rater severity/leniency analysis for each of the four neurobehavioral assessments. Also, the data sets we leveraged to conduct the psychometric analyses for the CRS-R and DRS (Table 4) did not contain information on raters. Thus, we have revised our goal and we are preparing a manuscript on the above summarized DOCS results.

Goal #2 Accomplishments: Describing the meaningful change during recovery of consciousness from the clinician's perspective

Specific Objective 2.1. Accomplishments: Describe clinicians' perceptions of evaluating and treating patients with DoC

We conducted 21 interviews with practitioners who work in a civilian or veteran DoC specialty inpatient rehabilitation program. A key result from these interviews was that practitioners work in a context of scientific uncertainty regarding accurate detection of states of DoC. At the same time, there is a lack of empirical data to guide clinical treatment, which creates ambiguity about treatment decision-making. We came to recognize that how practitioners perceive and make sense of diagnostic and prognostic uncertainty and therapeutic ambiguity remains an uncharted psychosocial domain and an unappreciated aspect of DoC rehabilitation treatment. We therefore described this psychosocial domain by analyzing practitioner interview data.

We found that ambiguity and uncertainty are omni-present in the clinical world of the rehabilitation practitioners we studied. Practitioners in our study stated that they "don't always know" what to do, that they "try things" in any way they can in order to help patients emerge to consciousness, all the while they second guessed themselves, they were unable to explain patient recoveries, experienced assessment discordance (i.e., different practitioners' clinical assessment scores were often not in agreement with each other's) and cognitive dissonance (such as 'double take'). They rarely used language that positioned themselves as knowers. Their stories provided us with an opportunity to become aware of taken-for-granted practices within the rehabilitation canon that may be otherwise invisible. Revealing these practices, for future clinician trainees is critical.

During the interviews, the clinician participants told stories of their reasoning in which they make sense of their actions. Our analyses show how practitioners make sense of (i.e., interpret) the clues patients give them to piece together a meaningful picture of the patient in DoC as a person, rather than as a mere body. In searching for consciousness, practitioners breach the canons of Evidence Based Medicine by tinkering with their treatment toolbox. As they tinker, they expose the limitations of the current state of scientific knowledge in the field of DoC. Tinkering, in this sense then, is a clinical reasoning practice that breaches the canon of rehabilitation science. As such, it has the potential to open the field of DoC practice to celebrating practitioners' ways of caring and treating. This may promote exploration and innovation of new treatment modalities and practices. A publication of the above findings is in press with PLOS ONE (Appendix 10).

Specific Objective 2.2 Accomplishments: Develop vignettes that represent varying amounts of patients recovering consciousness

We conducted narrative interviews with 21 clinicians to understand memorable, frustrating, and surprising change. We analyzed these interviews using thematic and narrative analysis. Throughout the analytic process, significant mentorship occurred as Dr. Ann Guernon, Dr. Elyse Walsh, and Dr. Jennifer Weaver were novice to qualitative data collection and analysis. Dr. Papadimitriou and Dr. Trudy Mallinson provided training to build the capacity of the research team.

We first developed vignettes starting with sentences in the interview data. We identified 20+ stories about behavioral decline, stability, and improvement. We then cognitively tested these stories with clinicians either 1:1 or in a focus group format. The purpose of the cognitive testing was to solicit impressions from participants as to whether the vignettes were easy to follow (comprehension), easy to compare, and reflected a continuum of change. We adjusted the vignettes three times and iteratively performed cognitive testing.

Next, we adjusted the vignettes using concepts from Labov[15] that describe the key elements for story-telling. We created our own checklist to ensure that each vignette had the same structural elements. During the cognitive testing and when using the Labov checklist, we heard from many participants about clinical reasoning and the data they felt they needed to make comparisons. From the Labov checklist and cognitive testing, we continued with 18 refined vignettes (Examples in **Table 5**) to address specific objective 2.3 as described below.

Table 5 Exemplar vignettes derived from qualitative interview data

I have been working with Yasmeen who is a 19-year-old college student and sustained a severe traumatic brain injury during a motor vehicle collision. One month ago, she didn't do anything when I pressed firmly on her legs but the monitor displayed a higher heart rate when I put ice on her big toe. I was thinking I really hadn't seen much response to therapy. Today, I went into her hospital room and put ice on her big toe, her heart rate and respiration rate increased. When I pressed my hand firmly on her legs, her toes moved. I hope this continues.
Ken is a 34-year-old man with a passion for watching the Boston Celtics. When he first came to rehabilitation two weeks ago it was hard to tell if he was responding to any of my stimuli or if it was just his baseline response. Anytime I would hold a basketball in front of his face or put the ball in his hands, I only noticed physiological responses with an increased heart rate. Today, I tossed a basketball in his lap and the ball rolled away towards his wife. His wife tossed the ball back into his lap and one arm jolted over towards the ball and then the ball rolled off his lap. His wife tossed the ball into his lap again and the same arm moved towards the ball again with his hand forming a curve as if to hold the ball. This was repeated multiple times, which was encouraging.
After his car accident, Jackson was not able to actively participate in Occupational Therapy. He did not follow commands or attempt to use functional objects like a toothbrush when I placed them in his hand. His family had told the therapist how important taking care of his beard was to him. I focused a lot of time in therapy with him sitting in his wheelchair at the sink to wash his face, apply shaving cream and then shave. After two weeks of therapy in acute care, he went to intensive rehabilitation for eight more weeks of therapy. By the time Jackson went home, he was able to brush his teeth independently, use an electric razor, move his wheelchair using his legs and arms, and communicate simple ideas to his family and friends. Everyone was happy with his progress.

Other Achievements

By developing the capacity of the team to address this objective, Dr. Ann Guernon and Dr. Jennifer Weaver now train undergraduate and graduate students in qualitative research. They have met weekly with 4 students from Oakland University and 4 students from George Washington University so that other students could learn the rigor of qualitative research. Building capacity has increased the dissemination output of the team but also provides a transformational experience as these trainees are interested in entering the

medical field.

Specific Objective 2.3 Accomplishments: Create a hierarchy of the vignettes
In order to generate a hierarchy of descriptors reflecting clinicians' descriptors of change (i.e., vignettes, see Table 5 above for examples), we utilized the paired comparison approach typically applied in the measurement of judgement and market research and more recently in education.[16] It has been reported that the most difficult aspect of the analysis is that it often feels monotonous to compare vignettes and it takes time to get a sufficient number of comparisons. [16]

We paired each of our 18 vignettes with one another, resulting in 306 comparisons. Knowing that collecting the data would be a challenge, we decided that the order in which we pair the vignettes was not important which reduced our comparisons to 153. 153 comparisons in one survey would be too much of a burden for future survey participants. Therefore, to reduce the number of comparisons in each survey, we drew upon the item-linking approach in Rasch Measurement Theory. 10 of the 153 pairs would occur at random throughout all 4 surveys, leaving 143 pairs to be distributed across 4 surveys. We created 4 surveys in RedCap, so that each survey only had 35 unique pairs plus the 10 pairs for item-linking. Data is currently being collected. We have collected a total of 63 paired comparison surveys and three of the four surveys have sufficient responses (Table 6). The survey website will remain open until we collect the remaining responses needed for survey four. We need a minimum of 10 fully completed surveys for each set, our ongoing data collection.

Table 6 Completed paired comparison surveys and respondents

Paired Comparison Surveys by Numbers	Partially Completed Surveys	Fully Completed Surveys	Total Number of Respondents
1	9	15	24
2	5	15	20
3	1	10	11
4	1	8	8

Other Achievements
The Vignettes will serve an important role in future studies. This is the first attempt that we know of to develop meaningful anchors from the perspective of clinicians. As this is possibly the first use of paired comparisons in rehabilitation research, we have a fully drafted outline for a manuscript describing the methodological process of transforming qualitative interview data into vignettes for a survey and ultimately creating a hierarchy of these vignettes.

Goal # 3 Accomplishments: Dissemination

For specific manuscripts, please see the above Goals section (Section 2) that provides a table of manuscripts by planned and actual completion dates for Goal #3. Also, please see the products Section (Section 6) below, which provides a comprehensive list of presentations in different scientific and educational venues, also addressing accomplishments for Goal # 3.

Goal #4 Accomplishments: Validation of miRNA in severe TBI Patients

Specific Objective 4.1. Accomplishments: Validation of miRNA in whole blood.
To validate presence of stable miRNA expression, we examined miRNA in whole blood for 20 healthy controls (10 male) distributed equally across 10 age categories covering the range of 18 to 75+ years of age. DoC patients were largely males (83%) of an average age of 38 years (SD: 7.8; median: 40) and their age/gender matched healthy controls were of a mean age of 41 years (SD: 0.7; median: 42).

Peripheral whole blood samples were collected in 2.5cc PAXgene blood tubes (2.5 mls, Qiagen, Germantown, MD) and 3-5cc r EDTA tube (5 mls) at two time points (separated by six weeks). Significance tests were conducted to determine presence/absence of differentially regulated miRNA between the two time-points. Findings indicate that healthy control miRNA profiles did not significantly change over six weeks validating that there was low to no variability, over the six weeks, in miRNA expression in the healthy control sample.

Specific Objective 4.2. Accomplishments: Collection, shipment, and processing of all patient blood samples
To examine the merits of using miRNA to diagnose states of DoC and for measuring responsiveness to rTMS treatment, we collected and processed whole blood miRNA profile of six patients and their individually age and gender matched healthy controls.

Prior to initiating any study procedures with patients, all patients were titrated off of pharmacological neurostimulants and sedatives. After titration, each patient underwent neurobehavioral, neuroimaging and EEG testing. A state of DoC was determined by clinical experts according to clinical observations and using established clinical consensus criteria. Patients randomized to receiving placebo rTMS were, after completion of the placebo study, offered the opportunity to be re-enrolled in the active rTMS arm; blinding and data independence were maintained during active rTMS study participation.

For patients and healthy controls, peripheral whole blood samples were collected in 2.5cc PAXgene blood tubes (2.5 mls, Qiagen, Germantown, MD) and 3-5cc r EDTA tube (5 mls). For patients, blood samples were collected at 12 unique timepoints during study participation (**Table 7**).

Regarding processing, after RNA isolation and sequencing of each specimen, reads were mapped to the most recent human genome release from Ensembl, GRCh38 using Bowtie2 (v. 2.2.1). An annotation file from miRBase release 21, describing miRNA coordinates, and the sequence alignment mappings were used as input for the Python package HTSeq (v. 0.6.1p1) to generate raw counts of miRNAs observed in the alignments. DESeq2 (v. 1.14.1) was used to determine differential expression between sample groups using these raw counts.

To date, all samples have been collected and shipped. For processing of samples using RNA isolation and sequencing all healthy control samples have been processed. For patients, samples from six timepoints for all participants except the final two participants in the rTMS clinical trial have been processed. These findings were used to conduct analyses for the JWMRP aims and hypotheses and these findings are summarized for the specific objective # 4.3 below. These same six timepoints, for the final two participants, are

scheduled for processing Spring of 2022. The results for the final two participants will be added to the dataset for use in examining the hypotheses for the rTMS clinical trial. These findings will be reported separately in the final report for the rTMS clinical trial.

Table 7 Whole Blood Collection Timepoints for DoC-TBI Patients during rTMS Trial

Timepoints	Definition of Timepoint by Number of rTMS Treatment Sessions
B0	Baseline after acute bed admission and after CNS medication titration is complete
B1	Baseline 24 hours prior to first rTMS (Active or Placebo) treatment
T1	After rTMS session #2 and before rTMS Session # 3
T2	Before rTMS Session # 8 but after rTMS Session # 7
T3	After rTMS session #9 and before rTMS Session # 10
T4	Before rTMS Session # 15 but after rTMS session # 14
T5	After rTMS Session # 16 but before rTMS Session # 17
T6	After rTMS session # 21 but before rTMS Session # 22
T7	After rTMS Session # 23 but before rTMS Session # 24
T8	After rTMS Session #29 but before rTMS Session #30
F1	Within 24 hours of final rTMS Session (#30)
F2	At time of 3-week follow-up

Specific Objective 4.3. Accomplishments: miRNA analysis from whole blood from all patients

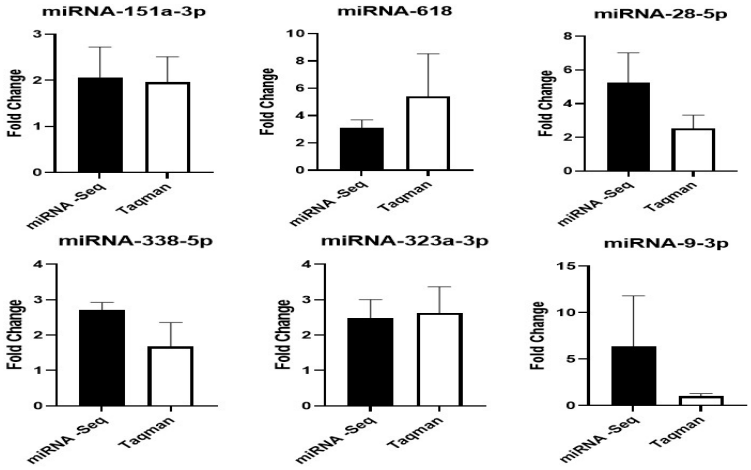
For the first six participants in the rTMS clinical trial, miRNA seq identified 2,598 miRNA. Of these 2,598 miRNA, Wald tests of significant difference, using raw counts of each miRNA, indicate that expression of 48 of these miRNAs was significantly ($p < 0.05$) different for the DoC participants relative to their age-gender matched healthy control. Specifically, there are 30 significantly up-regulated miRNAs and 18 down-regulated miRNAs in the DoC participants. (>2.0-fold change). When looking at tissue-specific miRNAs, we found that the only enriched tissue was brain, despite these being blood samples.

As miRNA-seq is a global approach, we validated the above miRNA-seq based findings using TaqMan. To validate, miRNAs were ranked by summing the absolute value of the difference of the expression of each DoC patient's miRNA from their matched healthy control. As this sum represents the magnitude of the difference of each DoC patient's miRNA compared to all persons in the healthy control group, the top ranked miRNAs were selected for Taqman validation. RT-qPCR was performed using TaqMan Advanced miRNA assays (ThermoFisher Scientific) according to manufacturer's instructions.

For validation, we examined the six highest ranked miRNA as these had the greatest distance between the patients and the healthy control group. Using TaqMan, we found the

direction of change to be the same for both miRNA-seq and RT-PCR (**Figure 6**). Although the magnitude of change did differ somewhat, no comparisons between the fold change found with miRNA-seq and RT-PCR were found to be statistically significant. More specifically, TaqMan validation of RNAseq findings indicate that expression was going in the same direction (up *vs* down regulation) with some differences in magnitude. However, we did notice that miR-9-3p had an unusually large standard error and found that the miRNA-seq fold change results were driven by an outlier, highlighting the need to continue using a validation procedure in the currently proposed study examining miRNA as an outcome for TBI in rodents and humans.

Figure 6 Validation Findings-TaqMan vs miRNA Seq



To better understand the molecular function of the differently regulated miRNAs in the DoC TBI patients, we conducted pathway analysis on the significantly regulated miRNAs. We found 9 pathways that included 10 or more miRNAs with a FDR<0.05. The top groups are all pathways that are likely to be involved in injury and repair, including inflammation, apoptosis, the immune response, and regulation of stem cells. The top pathway, by fold change, was brain development, followed by lipid metabolism and hematopoiesis. Pathway analysis also found that 12 miRNAs are involved in TBI in humans, including miRs-191, -223, -499a, -499b, -423, -23a, -142, -30e, -93, -155, -150 and -3945. Two of the miRNAs, miR-223 and miR-142, were also significantly regulated in our study. For the 48 miRNAs, the enriched pathways identified are implicated in secondary brain damage.

Given validation findings (summarized below under Specific Objective # 4.5. below, there is no indication to repeat the above miRNA analyses for validation purposes. Thus, the remaining participants in the rTMS trial. Thus, we will not repeat the above validation analyses for the entire sample as validating the use of miRNA in severe TBI has been adequately addressed.

Other Achievements

A potential/plausible limitation of the above findings is that they are, similar to all emerging work with miRNA, based on mi-RNA Seq, which was developed for RNA. This is one reason why we chose to validate the above findings with TaqMan. Considering ongoing efforts to create a microRNAome,[17] however, Dr. Pape is currently collaborating with the PI of this work, Dr. Matthew McCall. Dr. McCall is using data from healthy persons and

persons with many diseases to develop statistical methods that directly address the challenges unique to counting miRNA/measuring expression levels of microRNAs. He is specifically addressing challenges regarding the lack of independence between microRNA counts, significant variation in the overall transcription level across samples, and imbalance of over- and under-expressed features when comparing any two samples. Dr. Pape recently agreed to collaborate with Dr. McCall to enable inclusion of her severe TBI data in his recent effort funded in 2020 by the NIH. Based on Dr. McCall's preliminary statistical methods specific to miRNA, we are in the process of using our miRNA count of 2,598 miRNA identified with miRNA-seq to compare with Dr. McCall's analytical for the next generation of novel microRNA detection algorithm. Given Dr. McCall's past findings suggesting that it is plausible that there are many undetected miRNA in humans, it is possible that our findings above underestimate the number of miRNA's differentially expressed in severe TBI.

Specific Objective 4.4. Accomplishments: Coordinate data monitoring and entry into database

To enable specific objective 4.5., described below, all data values and variables were entered into a study database. All raw miRNA counts were transformed to standard counts for use in all analyses in objective 4.5. Data were extracted and inspected for data quality using descriptive statistics and scatterplots. After data is entered for the final 2 participants, the data quality inspections will be repeated for use in examining the rTMS clinical trial hypotheses.

Specific Objective 4.5. Accomplishments: Perform analyses according to specific aims and hypotheses

To examine the merits of using miRNAs as biomarkers of DoC after severe TBI and used as a measure of effects of rTMS treatments, analyses were conducted and completed (as noted above). All processing of blood was conducted as described above for SEpcific Objective # 4.2. The results are summarized below.

<u>Project # 3 Aim (a)-miRNA as Biomarkers of DoC after Severe TBI:</u> Baseline Expression Analyses of miRNA in Whole Blood for severe TBI, relative to each DoC-TBI patient's age/gender matched healthy control, were conducted to determine the merits of using miRNAs as biomarkers for DoC after severe TBI. These analyses were conducted with the first set of DoC-TBI patients (n = 6) who consented to participate in the additional fluid biomarker study of the rTMS clinical trial (ID # W81XWH-14-1-0568) and who remained in states of DoC for an average of 1.5 years after TBI. For the fluid biomarker component, all patients were matched by age and gender to a healthy control with no neurologic history (n = 6). Baseline whole blood miRNA profiles, as previously described (Specific Objective # 4.2.), were measured using miRNA-seq and Real Time-Polymerase Chain Reaction (RT-PCR).

The miRNA profiles were compared between patients and healthy controls using cluster analyses. The cluster analysis showed that healthy controls were most similar to each other and with two of the patients. The remaining four patients clustered separately, with one patient being an independent group.

For the patients, correlations were examined between the baseline miRNA levels and levels of neurobehavioral function using measures derived from a set of seven

neurobehavioral tests. To determine which of the 2,598 miRNA are correlated with neurobehavioral function, we first conducted mixed linear effects regression models, correcting for multiple comparisons, for each of the 2,598 miRNA with the neurobehavioral measures as outcomes. Thus, the findings account for inter-dependency and multiple comparisons. For all models where there was a significant relationship, models were re-run to determine if the relationships remained significant when accounting for covariates (Relative Volumes of Gray Matter Density, White Matter Density and Cerebral Spinal Fluid). After adjusting for covariates, 26 miRNA are significantly ($p < 0/05$; $r > .97$) correlated with at least one measure of neurobehavioral abilities and outcomes.

The findings, collectively, indicate that patients remaining in states of DoC an average of 1.5 years after TBI showed a different and reproducible pattern of miRNA expression relative to healthy controls, suggesting several candidate biomarkers for further study.

Project # 3 Aim (b)-Change in miRNA expression during rTMS Treatments: Examinations were conducted to determine if change in miRNA expression can be reliably detected and precisely measured in response to the experimental rTMS intervention. To exmaine the merits of using miRNA change as an index of rTMS treatment effect, we compared change in miRNA expression after at least two patients had been randomized to placebo rTMS (**Table 8**). For this sample of 10 DoC-TBI patients (8 active rTMS group and 2 Placebo rTMS), change was compared according to six of the timepoints specified in Table 7 (B0, baseline; T1, after 2 treatments; T3, after 9 treatments; T5, after 16 treatments/midpoint; T7, after 23 treatments; F1, after 30 treatments/endpoint).

Table 8 Demographics of active and Placebo Participants at Study Baseline

Active rTMS (n = 8)	**Placebo rTMS (n = 2)**
Mean age: 34	Mean age: 22
Gender: 88% Male	Gender: 100% Male
Vegetative State: 2	Vegetative State: 1
Minimally Conscious State: 6	Minimally Conscious State: 1
Mean days post TBI: 1.4 yrs.	Mean days post TBI: 1.2 yrs.

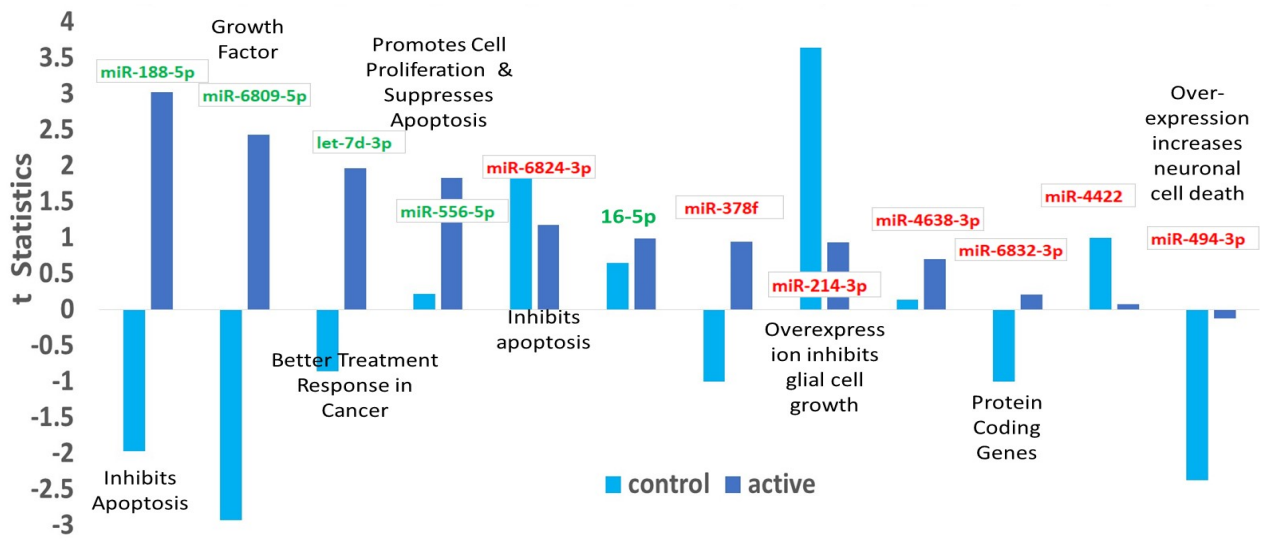
At each of the six timepoints, whole blood miRNA expression was computed using miRNA-seq and Real Time-Polymerase Chain Reaction (RT-PCR) (as described above or Specific Objective # 4.2.). Relative to each patient's age/gender matched healthy control, each patient's levels of expression were computed using raw counts. After standardizing the raw miRNA counts, change in expression for each DoC TBI patient was computed as the change in expression from B0 to Peak change across the five other timepoints. This index allowed us to measure maximum change as both a maximum increase and a maximum of decrease in expression. The apriori rules used to compute peak change for each miRNA were as follows:

- Increase = F1 is greater than B0
- Decrease = F1 is less than B0
- Whenever it is increasing from B0, take the maximum from T1 to F1
- Whenever it is decreasing from B0 take the minimum from T1 to F1

For each of the 2,598 miRNA we used peak change values to conduct mixed linear effects regression models as this approach accounts for inter-dependency. We also corrected for multiple comparisons. Each miRNA was modeled separately as joint models would not run (i.e., due to high variability in changes in expression across the set of miRNA). For all models where there was a significant change from B0 to peak expression ($p < 0.05$), models were re-run to determine if the change remained significant when accounting for covariates (Relative Volumes of Gray Matter Density, White Matter Density and Cerebral Spinal Fluid).

Accounting for covariates, we found 12 miRNAs that not only were significant in the DoC patients compared to their healthy controls at baseline, but that also significantly changed with rTMS treatments. Given small sample size in the placebo group, we addressed this aim by comparing the change between the two groups according to the standardized t-statistics (**Figure 7**) but did not conduct tests of statistical significance. As illustrated in Figure 7, the miRNA either significantly increased (**green font**) or decreased (**red font**) in expression over time for each group. While there is a dearth of knowledge regarding the neuronal roles of miRNA, known associations from current literature (to date) are depicted in the text boxes for each miRNA in Figure 7. Based on this knowledge, these findings suggest that during provision of active rTMS, the large proportion of the miRNA significantly changing in expression (e.g., miR_188-5) are implicated in secondary brain damage (e.g., apoptosis/cell death[18]). This is consistent with our chronic DoC-TBI sample, who were on average 1.2 to 1.4 years post TBI (Table 8), as secondary brain damage is known to occur gradually over time after TBI.[19]

Figure 7 miRNA Significantly changing (BO-Peak Expression) for active vs placebo rTMS treatment groups



Project # 3 Aim (c) -Correlation with neurobehavioral Change: Since miRNA can either inhibit or enhance gene expression, that correlate to certain pathways and neurobehavioral outcomes, we examined relationships between changes in miRNA expression and

neurobehaviorall function by computing Pearson correlations. These correlations, and the contrasting fonts in Figure 7 aid interpretation because there are two ways in which miRNA can be changed in the serum:

- Increased levels of miRNA are being produced in the brain and therefore there is increased levels secreted into the periphery. In this model, there would be a direct correlation between the increase in miRNA level in the serum and the behavioral change that the miRNA produces.
- The brain is clearing out the miRNA and therefore there will be an increased level in the periphery. In this model, there would be an inverse correlation between the increase in miRNA level in the serum and the behavioral change that the miRNA produces.

The Pearson correlation findings (Table 9) are preliminary and will be re-computed based on the co-calibrated neurobehavioral measures derived from previously described analyses (Figure 4). These preliminary correlational results do indicate, however, that rTMS induced changes in expression are significantly correlated with foundational and cortically based neurobehavioral skills. Considering the direct correlation between miRNA-188-5p and neurobehavioral gains, this suggests for example, that increasing levels of miRNA 188-5p in the serum is directly related to improving visual, somatosensory and auditory-language skills. As higher levels of expression of miRNA 188-5p is known to inhibit cell death (Figure 7), this suggests further that the rTMS may be attenuating, stopping or possibly reversing cell death to support recovery of these neurobehavioral skills.

Table 9 Preliminary correlations (r) between significantly changing miRNA and Neurobehavioral change

Expression	miRNA	Neurobehavioral Domain	r (p values)
Increasing	miR_188-5p	Visual	0.82 (0.004)
Increasing	miR_188-5p	Somatosensory	0.64 (0.43)
Increasing	miR_188-5p	Auditory-Language	0.44 (0.043)
Decreasing	miR_6832_3p	Gustation/Olfaction	-0.86 (0.003)
Decreasing	miR_16_5p	Gustation/Olfaction	-0.19 (0.005)
Decreasing	miR_4638_3p	Gustation/Olfaction	-0.78 (0.013)

Goal # 5 Accomplishments: Dissemination of information

Specific Objective 5.1. Accomplishments: miRNA Review Paper

To inform our analyses for Specific Objective 4.5, as described above, we conducted review of the evidence of miRNA alterations after TBI and evaluate the state of science relative to potential neurorehabilitation applications of TBI-specific miRNA. This review was published in 2021.[20]

Specific Objectives 5.2. through 5.4. Accomplishments: Manuscript Status
The analyses completed for Specific Objective 4.5, as described above, resulted in findings that will be disseminated in two manuscripts. For Specific Objective 5.2., the manuscript reporting findings of the merits of using miRNA as a biomarker of severe TBI is under review with the Journal of Head Trauma Rehabilitation (Appendix 11). We will combine objectives 5.3. and 5.4., as the above summarized findings support the development of a single the manuscript describing the results of rTMS induced changes in miRNA expression relative to neurobehavioral gains.

What opportunities for training and professional development has the project provided?

Pre-Doctoral and Post-Doctoral Mentorship/Training Experiences

Institution	***Trainee***	***Profession/Title***	***Mentor/Trainer***	***Training/Professional Develop. Activities***
University of Illinois-Chicago, Dept. of Neurosciences	Noor Chaudhry	BS Candidate Neurosciences, Degree conferred: 12/2021	Theresa Pape	1:1 mentoring in good research practices, clinical testing and treatment procedures and imaging analyses
University of IL at Chicago (UIC) School of Public Health	Yue (Annie) Wang,	PhD Geneticist and PhD Biostatistician	Theresa Pape	Doctoral Dissertation: Bayesian Multimodal Local False Discovery Rate in Neuroimaging
University of IL at Chicago (UIC) School of Public Health	Fei Jie	PhD Biostatistician	Theresa Pape	Doctoral Dissertation: Statistical Methodologies for Neuroconnectivity Analysis using fMRI data
University of IL at Chicago (UIC) School of Public Health	Fei Jie	PhD Biostatistician	Theresa Pape	Doctoral Dissertation: Statistical Methodologies for Neuroconnectivity Analysis using fMRI data
University of IL at Chicago (UIC) School of Public Health	Weihan Zhao	PhD Biostatistician	Theresa Pape	Doctoral Dissertation: Methods for Group Comparisons of Brain Connectivity with Multimodal Neuroimaging
Edward Hines Jr. VA Hospital	Julie Schwertfeger	Doctor of Physical Therapy (DPT) and PhD in Interprofessional Healthcare Studies,	Theresa Pape	1:1 Mentoring in Neural plasticity and Neuromodulation in TBI

		Polytrauma Post-Doctoral Fellow		
Edward Hines Jr. VA Hospital	Andre Lindsey	PhD Speech Language Pathologist, Polytrauma Pos-Doctoral Fellow	Theresa Pape	1:1 Mentoring in Neural plasticity and Neuromodulation in TBI and detecting meaningful treatment effects with EEG/EPs
University of IL at Chicago (UIC) college of Medicine	Paul Thomas	MD/PhD Candidate	Theresa Pape	Doctoral Dissertation: Predicting Treatment responsiveness using Diffusion Tensor Imaging
Edward Hines Jr. VA Hospital	Sandra Kletzel	PhD Neuroscience, Polytrauma Post-Doctoral Fellow, Health Research Scientist	Theresa Pape	1:1 mentoring in Clinical Rehabilitation Research, Neural plasticity and Neuromodulation
Edward Hines JR. VA Hospital	Alexandra Aaronson	MD Neuropsychiatrist	Theresa Pape	1:1 mentoring in Clinical Rehabilitation Research, Neural plasticity and Neuromodulation
George Washington University	Jennifer Weaver	Occupational Therapist	Trudy Mallinson	Completed a PhD in Translational Health Sciences while working full time as a Project Manager; this included taking courses in advanced statistical methods, qualitative inquiry for health professionals, and mixed methods research. Independent studies in Rasch Measurement Theory and the Many Facet Rasch Model were instructed by Dr. Trudy Mallinson.

Capacity Building

Direct Training				
Institution	***Trainee***	***Profession/Title***	***Mentor/Trainer***	***Training/Professional Develop. Activities***
George Washington University	Jennifer Weaver	Occupational Therapist	Christina Papadimitriou	Trained 1:1 to learn expertise in narrative and phenomenological qualitative inquiry
Edward Hines, Jr. VA Hospital	Elyse Walsh	Occupational Therapist		
Edward Hines, Jr. VA Hospital	Ann Guernon	Speech-Language Pathologist		
George Washington University and Oakland University	Elizbeth Elgin	Undergraduate and graduate students	Jennifer Weaver, Ann Guernon, Christina Papadimitriou	Trained 1:1 to learn expertise in qualitative data collection, analysis, and writing
	Andrew Jones			
	Sophia Slack			
	Calista Mueller			
	Christian Versin			
	Rua Almaat			
	Izra Abuzahra			
	Raneem Sabaaq			
National Rehabilitation Hospital	Samantha Johnson	Occupational Therapist	Trudy Mallinson	Collaborated with National Rehabilitation Hospital to train an occupational therapy fellow

Interdisciplinary Collaboration			
Caregiver Collaboration			
Institution or Organization	***Collaborator***	***Role***	***Activity***
No affiliate	Tisha Kot	Caregivers of loved ones (civilian) with disorders of consciousness	Founded collaborations with caregiver partners
	Paige Ford	Caregiver of loved one (veteran) with disorders of consciousness	
Professional Collaboration			
Institution or Organization	***Collaborator***		***Activity***

Edward Hines, Jr VA Hospital	Theresa Pape, DPH, CCC-SLP Marilyn Pacheco, MD Monica Steiner, MD	Collaborated with a multidisciplinary team to conduct a scoping review. Collaborators were educated in SIGN Quality rating criteria.
George Washington University	Trudy Mallinson, PhD Thomas Harrod, MS Parie Bhandari, BA Chantal Nguyen, MD Erica Jacobs, BS	
Colorado State University	Jennifer Weaver, PhD, OTR/L CBIS	
University of Michigan-Flint	Elizabeth Yost, OTD, OTR/L	
Lewis University	Ann Guernon, PhD, CCC-SLP	
Shirley Ryan Ability Lab	Catherine Kestner, PT. DPT Kelsey Watters, OTR/L, BCPR, CBIS Kelly Krese, PT, DPT, NCS Haylee Widen, PT, DPT Stephani Cleaver, PT, DPT Jennifer Nebel, MS, CCC-SLP Mary Kate Philbin, MS, CCC-SLP Erika Cooley Mary McLoughlin Alison Cogan	
MedStar Washington Hospital Center	Julianne Angel Ladan Hakima Elizabeth Burns Sarah Hollingsworth Bailey Widener Jessica Rudin	
MedStar National Rehabilitation Hospital	Coty B. Wardwell, PT, DPT Samantha Johnson, OTD, OTR/L	
Nevada State College	Andre Lindsey, PhD, CCC-SLP	
Northwestern University	Joshua Rosenow, MD	
Radboud UMC	Henk Eilander, PhD Berno Overbeek	
Shepherd Center	Angela N. Hartman, OTD, OTR/L	
Inova Fairfax Hospital	Elizabeth Burns, PT, DPT, CBIS	
Adventist HealthCare Rehabilitation	*Cody B Wardell, PT, DPT, NSC*	
Adler University	Vanessa Silva, MS Konner Nelson	
Creations of Communities of Practice		

Institution or Organization	*Collaborators*	*Facilitator*	*Activity*
George Washington University	Leslie Davidson Keith Cole Trudy Mallinson	Trudy Mallinson	Created multi-disciplinary study group to advance measurement of NBF, cognition, and examine recovery trajectories
Colorado State University	Jen Weaver		
VA Greater LA Health Care System	Alison Cogan		
George Washington University	Trudy Mallinson	Trudy Mallinson	Created a multi-disciplinary study group to advance our understanding of Rasch Measurement Theory and its applications
University of Minnesota	Ann van de Winckel		
Michigan State University	Allen Kozlowski		
University of South Carolina	Craig Velozo		
Colorado State University	Jennifer Weaver		
University of Texas-Medical Branch	Chin Ying (Cynthia) Li		
University of Washington	Namrata Grampurohit		
Edward Hines, Jr VA Hospital	Theresa Pape		

How were the results disseminated to communities of interest?

We disseminated to communities of interest via peer-reviewed publications, international, national, and local conference presentations. We specifically disseminated to communities that were discipline specific and interdisciplinary to tailor our messaging and reach a broader audience. We also created easily digestible products such as updating the Disorders of Consciousness psychometric properties on the Shirley Ryan Ability Lab Rehabilitation Measures Database platform and published in the Archives of Physical Medicine and Rehabilitation.

What do you plan to do during the next reporting period to accomplish the goals?

Not applicable, final report.

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?

Measurement and Psychometrics of Neurobehavioral Function

Our team provided contemporary psychometric analysis of existing neurobehavioral function assessments including indices of change, which are critical for interpreting treatment responsiveness, results of clinical trials, and recovery for patients with DoC. The

co-calibrated data set is the first of its kind and will transform the state of the science for leveraging existing databases such as, TBIMS and FITBIR. By co-calibrating items and rating scale categories across assessments, researchers are able to compare patient performance and change in patient performance across studies that may have used different primary outcome assessments. For example, outcomes on the Amantadine trial [21] using the CRS-R can be compared to the outcomes from the ProTECT trial using the DRS.[22]

Additionally, our team created a scoping review data set that contains intervention studies for patients with DoC from 1986 through 2020. Data abstracted from the studies include primary outcome measures, secondary outcome measures, study quality using SIGN (Scottish Intercollegiate Guidelines Network), interventions, and significant findings, and funding source. Outcome measures are coded by ICF category and domain as well as common data element status. We can imagine numerous reviews that leverage these data to better describe the conceptual landscape of DoC recovery.

miRNA Profiles and Neurobehavioral Recovery

The study findings indicate that patients remaining in states of DoC an average of 1.5 years after TBI have a different and reproducible pattern of miRNA expression relative to healthy controls. These findings demonstrate the merits and validity of using miRNA as a biomarker of DoC-TBI. To our knowledge, this is the first study that explored altered expression of miRNA at chronic time points in a controlled manner and, importantly, using a comprehensive battery of neurobehavioral tests.

The DoC-TBI phenotypes, defined by miRNA relative to profiles of persisting impairments in neurobehavioral abilities, provide the empirical foundation for future research to determine the miRNA profiles differentiating states of DoC and predicting functional outcomes. Considering that 2/3 of the survivors who receive specialty DoC rehabilitation recover consciousness[23] and continue to make gains for years thereafter [24] and that US reimbursement criteria determining candidacy for specialty rehabilitation is based on diagnosis of state of DoC as well as recovery prognosis, there is a critical need for precise evidentiary basis for diagnoses and prognoses. Precise diagnoses and prognoses have been elusive [2,3] as these determinations are based largely on serial neurobehavioral assessments. The DoC phenotypes reported here, demonstrate that it is possible to address the longstanding and critical need for an objective evidentiary basis for differentially diagnosing DoC states. Moreover, the use of peripheral blood and commonly used neurobehavioral tests makes future implementation of these DoC phenotypes feasible for use in daily practice of DoC-TBI neurorehabilitation. As we are also in the process of examining the contribution of the baseline miRNA profiles to predicting responsiveness to the rTMS intervention, this study will also determine if the miRNA profiles will provide the evidentiary basis needed to make precise prognoses regarding treatment responsiveness and functional outcomes. If future research confirms and further delineates the DoC phenotypes by states of DoC and advances evidentiary based prognostication, then the miRNA-neurobehavioral DoC phenotypes can be used to address the critical need for precise and objective evidence-based differential diagnoses and prognostication.

In addition to providing the foundation needed for researchers to develop an objective and empirical basis for differential diagnosis and prognostication, the DoC specific baseline miRNA profiles advance understanding of an individual patient's therapeutic targets. Specifically, the miRNA profiles are, largely, implicated in secondary brain damage and have known roles in supporting cognitive abilities. Thus, a patient's miRNA profile can inform clinicians about the pathways to be targeted to enable skill restoration and/or prevent persisting neurobehavioral impairments.

This study also demonstrated that change in miRNA expression can be reliably detected and precisely measured in response to an intervention (rTMS) and how these changes relate to neurobehavioral gains. This means that clinicians could use, in the future, miRNA change as an index of treatment effects. Specifically, by demonstrating that rTMS induced change in miRNA is related to neurobehavioral gains, there is now a basis for using miRNA to make evidence-based decisions regarding types of treatments, dosing (e.g., # of sessions) and the need for alternate treatments. The findings demonstrate that evidence-based DoC-TBI rehabilitation can become a reality.

The miRNA changing with rTMS are implicated in secondary brain damage and these changes are associated with neurobehavioral gains. Thus, these findings collectively suggest that rTMS may be attenuating, stopping or possibly reversing cell death to support recovery of these neurobehavioral skills. These findings provide evidence, for the first time, that even in the most damaged brains we can enable recovery and enhance the quality of lives for persons living in states of DoC after TBI. After replication in a larger scale research study, the findings indicate that clinicians will be able to use peripheral blood to identify their patient's critical pathways for neuromodulation, determine treatment effects on neural repair and, ultimately, advance capabilities to provide patient-centric DoC-TBI neurorehabilitation.

What was the impact on other disciplines?

Measurement and Psychometrics

The results process and products of this project will have impact on the field of rehabilitation beyond disorders of consciousness. Examples include the development of the Rasch Reporting Guidelines for Rehabilitation Research, which will foster greater consistency and transparency in reporting rehabilitation research studies using contemporary psychometrics.

Collaborative Care Practices in DoC

The information we learned about clinical practice, clinical reasoning and how clinicians manage ambiguity in the DoC population lead this team to seek additional resources to study caregiver perceptions of interacting with rehabilitation practitioners when caring for persons in DoC. Combining qualitative data of practitioners' perceptions collected from this study with data collected during a separate study of care partners' perceptions results suggested ways to facilitate effective communication between these two stakeholders when treatment planning. This work will support future educational/training opportunities for practitioners and family care partners to clinically reason collaboratively.

What was the impact on technology transfer?

Project #2

Co-calibration of Neurobehavioral Assessments

At the onset of this project the ability to compare results/interpret results from the five most commonly used DoC Neurobehavioral assessments. While each assessment/outcome measure had its independent interpretation of results, aligning the assessment tools (CNC, CRS-R, DOCS and DRS) allows clinician's to clearly understand where their individual DoC patient's falls within the spectrum of neurobehavioral recovery. The co-calibration also enables better comparison of evidence across studies using one or a few of these four commonly used DoC assessments. Moreover, the study dataset enables addition of other assessments, in the future, for co-calibration with these four assessments.

The knowledge produced by co-calibrating items and rating scale categories across assessments, advances the technological readiness of the utilization of these tools for this unique population and enables researchers to compare patient performance and change in patient performance across studies that may have used different primary outcome assessments. Based on the USAMRMC 'knowledge readiness levels' (KRL) (April, 2019) this advances the technology KRL from 3 level to level 6 (Knowledge Product '2') as it generates knowledge to perform a function or inform a tool's effect and confirmed such questions as whether a tool can work and, if so, how and for whom. For example, outcomes for three severe TBI trials, each using different outcomes, can now be compared (e.g., FAST trial using the CNC and DOCS,[8] the Amantadine trial[7] using the CRS-R and PROTECT[22] trial using the DRS).

Project #3

Gold standard analytic steps for miRNA biomarker assessment

Through the completion of a scoping review including 57 animal and human studies evaluating miRNA, we formulated a theoretical model of miRNA as a biomarker of DoC-TBI. For this project, we tested this model by following the Gold standard analytic steps for miRNA biomarker assessment models. The study findings, collectively, advance the technological readiness of miRNA as a DoC-TBI biomarker from "knowledge readiness level" (KRL) 2 to a KRL of 5. Findings from our first analysis advances the KRL from 2 to 4 as the findings address the question: Can a model of miRNA expression, based on peripheral blood, work as indicated by accurate detection of miRNA and measurement of differential expression of miRNA in DoC TBI? Our paper under review (Appendix 11) disseminates the initial knowledge regarding the application of peripheral blood to detect and measure miRNA in DoC-TBI patients. We are in the process of developing the manuscript reporting the findings (Summarized earlier in this report) that replicating the first findings from the first analysis thereby advancing the KRL to level 5. Specifically, as the findings from this second analysis are based on longitudinal data and inclusion of two placebo patient, these findings demonstrate how to use differential miRNA expression to detect a treatment effect that is, in this case, rTMS. In our separate final report for the rTMS clinical trial, where we include all active and placebo participants, we will further replicate these findings by examining testing our clinical hypotheses that miRNA expression levels are significantly altered in active rTMS, relative to placebo rTMS, and that these differences in expression are correlated with more neurobehavioral recovery.

What was the impact on society beyond science and technology?

Project #2

Although this project was not designed as a translational project, it does build the foundation for such undertaking. For example, the RULER guideline might end up changing how people report and review manuscript using Rasch. The work has future potential to change how clinicians think about their work with patients in DoC.

Project # 3

The study findings of differential miRNA expression with DoC-TBI and the relationship of the levels of expression with neurobehavioral function as well as how changes in miRNA expression key to recovery change with rTMS interventions, demonstrate that we can provide neuromodulation targeting messenger RNA that code for proteins and have key roles in forming the basis for neural plasticity, neural repair and neurobehavioral recovery. In addition to advancing the understanding that patients can recover from DoC-TBI, these findings provide the foundation to test and, ultimately, provide treatments targeting the pathways identified in this study as they are related to persistence of specific and basic neurobehavioral impairments. Furthermore, this provides the foundation to develop treatments that, when paired, will enable recovery of functional skills considered meaningful to the patient and/or their families. Since this work provides the foundation for realizing the dream of personalized precision neuromodulation, it is likely to change clinical attitudes toward DoC after TBI, rehabilitation medicine, policies about reimbursement criteria for DoC specialty rehabilitation.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

In year 4 of the project, we recognized the barriers to moving forward with Major Goal 2 to create patient video cases and collection of linking data and proposed to not move forward with this goal. We had collected and reviewed the videos. The original purpose of the videos was to link raters for the Many Facet Rasch Analysis because we would have raters at multiple sites. However due to changes in the study sites, we were able to pair all raters and planned to move forward with an item linking process if needed for our future analyses. Therefore, we did not create the patient video cases.

Actual or anticipated problems or delays and actions or plans to resolve them

We were not able to officially begin enrolling patients until June and August of 2016 due to several requested IRB/HRPO modifications which delayed initiating enrollment for the project. Once all approvals were obtained, problems and delays were encountered due to difficulties with enrollment and the impact of the COVID-19 pandemic.

Recruitment

Recruitment for the parent grant (# W82XWH-14-1-0568) had a direct impact on progress for project #3 as subjects recruited to the parent study are also recruited to this

supplement project. Strict eligibility criteria limited many potential enrollments. Most common reason for exclusion were that that patients were admitted to standard acute rehabilitation first and then referred to the study. During acute rehabilitation, many patients emerged from the minimally conscious state (i.e., are classified as conscious). Thus, at time of acute rehabilitation discharge, they were no longer eligible for study enrollment. Patients who did remain in a state of disordered consciousness were often referred to us after the 1-year cut-off, which was later even extended to a 2-year cut-off however with the same limitation persisting. Due to the risk of post-traumatic seizures, many patients were on an anti-epileptic medication and could not be titrated off of the medications safely. We worked to address these recruitment barriers by extending the initial exclusion criteria of greater than 1 year post injury, to greater than 2 years post injury. Additionally, we later reduced the required period to be off anti-epileptic medications from 3 months to 1 month prior to enrollment.

Over the course of the project, we also considered multiple approaches to bolster recruitment. Initial recruitment strategies focused on VA system referrals and referrals from clinical partners. Veterans diagnosed with severe TBI and admitted to a VA hospital or medical center with the primary reason for admission being severe TBI, were identified using the national inpatient files available at VA Informatics and Computing Infrastructure (VINCI). We accessed these data files on VINCI using the Data Access Request Tracker (DART) system and then searched the database according to the ICD-9-CM and ICD-10-CM codes that allowed us to search by three eligibility criteria. This national search yielded a list of 33,398 unique Veterans, unfortunately after further filtering and screening none of these were ultimately eligible.

We sought direct referrals from VA PRC through collaboration with the VA Central Office PM&R and Polytrauma Medical Director. During monthly leadership meetings with the PRC Chiefs (medical directors of the emerging consciousness programs at each PRC), the importance of referrals to the study was emphasized. However, this did not result in any PRC referrals. Thus, we continued to receive lists from the VA CO emerging consciousness program database as the method for identifying study candidates from PRC admissions, unfortunately with no resulting enrollments.

We further sought referrals nationally and locally through distribution of study flyers with national Defense and Veterans Brain Injury Centers (DVBIC) sites and salient websites. Study team members sent email reminders to specialty providers and provided in-services at Level I trauma centers and extended care facilities throughout the Chicago-land area.

In 2019 we sought to further bolster recruitment through the services of PatientWing, an online interface for potential families searching for clinical trials. Families and caregivers could search for clinical trials based on conditions and geography. The clinical trial had a “landing page” which provided trial specific information, such as inclusion criteria, as well as a contact form. The page also linked to “pre-screener” questions to collect further information about participant eligibility. When a potential participant’s family entered contact information and answered the pre-screener questions, an email notification was sent to the study coordinator for further follow up. We significantly increased inquiries regarding study enrollment with the addition of PatientWing, however caregivers and family members who initially contacted us would become less responsive to follow-up communication, change their position on participation, or further screening would

determine that patients did not meet inclusion criteria often due to anoxic brain injury or being conscious at time of screening.

Direct referrals of civilians from physicians at emerging consciousness programs at the Shirley Ryan Ability Lab and the Texas Institute for Rehabilitation Research, along with inquiries from PatientWing, were the most successful strategies with most of our enrolled patients coming from these referral sources.

Impact of Covid-19 Pandemic

The Illinois governor's Stay at Home order was effective March 21, 2020 through May 29, 2020. Although the Stay-at-Home order was lifted May 29, 2020, IRB approval to resume research activities was not immediately granted by either of our study sites, Edward Hines Jr. VA (Hines) or Northwestern University (NU). For Hines VA site, the requirements for re-starting were issued 6/2/2020, and we submitted our Hines VA specific plan on 6/22/2020. To date, we have not received approval to reactivate research activities and enroll Veterans for this study at Hines due to COVID surges making an inpatient bed unavailable for a research admission.

Civilian enrollment at Northwestern was also put on hold due to the Stay-at-Home order. This study population required an inpatient admission within a Clinical Research Unit at Northwestern Memorial Hospital, and additional research visits for imaging at Northwestern University. Therefore, approval to restart research activities was needed from both institutions to resume enrollment. Northwestern University instituted a phased re-opening plan.

In response to the financial impact on the hospital, Northwestern Memorial Hospital notified us on 7/2/2020 that there would be a restructuring of the inpatient Clinical Research Unit (CRU), including an increase in the fee structure. Despite having a contract in place that listed a daily per diem that was budgeted to meet recruitment goals of the grant, the new daily rate was a three-fold increase. Further, as we continued to work with leadership of the hospital to find a resolution to the budgeting problem, the fall COVID surge pulled all available nursing resources to immediate acute care nursing needs. We were subsequently notified that there would be no internal support for nursing staff for research admissions, and that the only option would be for the study to hire agency nurses at an estimated rate of $40,000/participant admission.

As we continued to receive more information about compounding costs at Northwestern Memorial Hospital, the Principal Investigator (PI), began pursuing options for other study sites to allow affordable enrollments to achieve the study goals. Shirley Ryan Ability Lab was considered as an admission site, but costs were similar to the new CRU rates and new non-essential research activities were considered to be too risky to the existing vulnerable inpatient population. Further investigation presented an option to consider a subacute rehabilitation facility located in the greater Chicagoland area. Initial meetings to pursue the viability of HealthBridge Complex Care and Rehabilitation (HB) as a research site occurred in October 2020. Multiple meetings were required to thoroughly vet the site with leadership of the facility and the study PI, to ensure that staffing and safety monitoring needs of the study could be met. Additionally, a private EEG company needed to be identified to provide bedside EEGs as part of the FDA IDE requirements of the parent

study. Contractual negotiations between the study team and HB, and HB and EEG companies took place over the ensuing months.

As negotiations were finalized, the research team began the regulatory work necessary to move the study location to a new site. The protocol modifications were submitted to the NU IRB on 1/21/2021 with approval to add HB as a site provided on 4/12/2021. As modifications were finalized with the IRB, a revision to the FDA IDE was prepared and ultimately submitted on 4/7/2021 with approval received on 5/10/2021. The final regulatory approval needed through HRPO was submitted on 4/15/2021, with approval granted on 6/1/21.

In addition to the COVID-19 Pandemic forcing a change in study site, it also impacted our ability to recruit and retain participants. Without a feasible plan for a study site, any participants that were in the screening process prior to the pandemic could not be enrolled while a new site was vetted, and regulatory approval was obtained. At the start of the pandemic, 4 candidates were being screened for enrollment. One candidate withdrew from consideration in February 2021 due to family concerns about additional COVID exposure risk for the participant with the necessary travel required for study participation. Another participant withdrew in June 2021 as they had identified home nursing support that they had been pursuing over a year and could not lose as a result of traveling to Chicago area to participate in the study. A third participant was scheduled to begin research procedures in July 2021, however as final screenings were completed to schedule transport it was determined that the participant had regained consciousness and therefore no longer met inclusion/exclusion criteria.

Changes that had a significant impact on expenditures

New recruitment was limited during the COVID-19 Pandemic in the interest of protecting remaining funds to support study procedures (e.g., MRI, EEG). All of the above referenced participants who were being screened and prepped for enrollment during the pandemic were obtained through Patient Wing, a web-based recruitment platform. While an effective recruitment tool, the cost (approximately $5,000 quarter) was considered to be too high risk to continue while we did not have an approved site to complete study procedures and considering the candidates we already had in the queue. Therefore, PatientWing services were discontinued in June 2021.

Funding to support staffing levels necessary to meet recruitment and study goals was also impacted by the delays due to the COVID-19 pandemic. More affordable staffing solutions via fellowship opportunities and recruiting new volunteers to support the study were pursued to accomplish the goals of the project.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Not applicable.

Significant changes in use or care of human subjects

In year 4 of the project we identified the need to modify our biomarker analysis based on mounting evidence that the apolipoprotein E gene (APOE) is linked to poor long term outcome following traumatic brain injury (TBI).[25,26] The apolipoprotein (APOE) ε4 allele, found on chromosome 19, is responsible for production of apolipoprotein, a protein that is produced in response to a central nervous system injury.[28] Presence of the APOE ε4 allele has been strongly linked to the development of Alzheimer's disease.[27] The APOE gene has three alleles, ε2, ε3, and ε4, with the ε3 as the most common allele.[28] There are three homozygous phenotypes ε2/ε2, ε3/ε3, and ε4/ε4 as well as three heterozygous phenotypes APOE ε2/ε3, ε3/ε4, and ε2/ε4 that arise from the expression of any two of the three alleles. The most common phenotype is ε3/ε3, which is estimated to be prevalent in 60-63% of the population.[29] Because ε3/ε3 is the most common phenotype and ε3 is the most common allele, ε3 is considered the parent form of the protein, and ε2 and ε4 are variants. Although the specific mechanism of involvement of the gene is unclear, it is believed that it has a role in binding to amyloid beta peptide which results in eventual development of neuritic plaques.[30] Others have postulated that the APOE ε4 allele is associated with the formation of neurofibrillary tangles[31], has a neurotoxic role in hippocampal cell death[32], and possibly reduces neuroplasticity.[33] Studies demonstrate that individuals with the APOE-ε4 are at greater risk for worse functional and cognitive outcomes following TBI.[25,26,34] Furthermore, carriers of the ε4 allele have been shown to have compromised default mode networks (DMN) as evidenced on fMRI.[35] Despite evidence of the important role of the APOE gene on neural brain networks and TBI outcomes, no studies had examined the influence of the APOE gene on response to rTMS in traumatic brain injury. Additionally, studies have shown that BDNF is up regulated with rTMS and thought to be positively correlated with TBI recovery acceleration by improving neuronal health.[36] Therefore, we amended our protocol to analyze DNA samples and BDNF levels of our participants and examine relationships of APOE status with rTMS treatment response. These modifications were approved by the NU IRB on 11/25/2019 and Hines VA IRB on 2/24/2020.

In year 5 of the project, we recognized the need for further contextual information to fully understand biomarker findings, therefore the protocol was modified to add a baseline endocrine panel and a complete blood count (CBC) with differential at each timepoint. We determined it was important to obtain a baseline endocrine panel in subsequent patients because it allows us to rule out hypogonadotropic hypogonadism as a contributor to the miRNA and behavioral changes seen after treatment with TMS. Certain hormones have been shown to alter miRNA levels, for example, activation of estrogen receptors have been shown to have effects on miR-218 pathway, which is one of the miRNA significantly changed in this study.[37] Estradiol levels have also been shown to influence several other miRNA that were significantly elevated in this study including miR-618[38], miR-329-3p[39], and miR-338-3p[40]. Additionally, persistent hypogonadotropic hypogonadism in TBI patients has been shown to be associated with worse outcomes. In one study persistent hypogonadotropic hypogonadism is defined as low testosterone/estradiol levels at 12-16 weeks post-injury, and this was associated with worse global outcome scores, more disability, and reduced functional cognition at 6- and 12-months post TBI[41]. In another study, persistent hypogonadotropic hypogonadism was found to influence later estradiol

synthesis, as well as being associated with worse overall outcome.[42] Given the influences estradiol has been shown to have on miRNA levels, this is an additional factor that needed to be taken into consideration when controlling for covariates. These changes were not As change in expression of specific miRNA during the provision of rTMS may serve as an objective measure of treatment efficacy and inform us about the neural pathways being modulated with rTMS, it was important that we added CBC panels to this study protocol based on the emerging understanding of how the expression of miRNA are influenced by common illnesses (e.g., the common cold[43], the flu[44], diabetes mellitus[45], weight[46], dietary habits[47], and illnesses more common for persons with chronic conditions (eg., bacterial infections, pneumonia, respiratory[48,49,50]. Thus, for each timepoint we added a CBC with differential. This was submitted to the Hines IRB as we never received approval to lift the COVID-19 administrative hold. These changes were approved by the NU IRB on 4/2/2021.

Significant changes in use or care of vertebrate animals

Not applicable. Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications

Project #2

1. **Weaver JA,** Liu J, Guernon A, Pape T, & Mallinson T. (2021). Psychometric Properties of the Coma Near-Coma Scale for Adults in Disordered States of Consciousness: A Rasch Analysis. *Archives of Physical Medicine and Rehabilitation,* 102(4): 591-597. DOI: 10.1016/j.apmr.2020.10.119
2. Bender Pape T, Livengood S, Blabas B, Kletzel S, Guernon A, Bhaumik DK, Bhaumik R, Mallinson T, **Weaver JA**, Wang X, Herrold AA, Rosenow JM, Parrish T. (2020). Neural Connectivity Changes Facilitated by Familiar Auditory Sensory Training in Disordered Consciousness: A TBI Pilot Study. *Frontiers in Neurology*, 11(1027): 1-19. DOI: 10.3389/fneur.2020.01027
3. Bender Pape TL, Herrold AA, Livengood SL, Guernon A, **Weaver JA**, Higgins JP, Rosenow JM, Walsh E, Bhaumik R, Pacheco M, Patil VK, Kletzel S, Conneely M, Bhaumik DK, Mallinson T, Parrish T. (2020). A Pilot Trial Examining the Merits of Combining Amantadine and Repetitive Transcranial Magnetic Stimulation as an Intervention for Persons with Disordered Consciousness after TBI. *Journal of Head Trauma Rehabilitation*, 35(6), 371-387. DOI: 10.1097/HTR.0000000000000634
4. Mallinson T, **Weaver JA**, Guernon A, & Pape T. (2020). Letter to the Editor: Updating the Disorders of Consciousness Scale. *Journal of Head Trauma & Rehabilitation*, 35(5): 363. DOI: 10.1097/HTR.0000000000000577.
5. Weaver JA, Cogan A, Bhandari P, Awan B, Jacobs E, Pape A, Nguyen C, Guernon A, Harrod T, RECON Team, **Pape TB**, Mallinson T (under review). Mapping Outcomes for Recovery of Consciousness in Studies from 1986 to 2020: A Scoping Review. BMJ Open

6. **Bender Pape T** Herrold A Livengood S Guernon A Weaver J Higgins J Rosenow J Walsh E Bhaumik R Pacheco M Patil V Kletzel S Conneely M Bhaumik D Mallinson T Parrish T (2020) A Pilot Trial Examining the Merits of Combining Amantadine and Repetitive Transcranial Magnetic Stimulation as an Intervention for Persons with Disordered Consciousness after TBI. Journal of Head Trauma Rehabilitation, 35(6): 371–387 (PMID: 33165151).

Project #3

1. Zilliox M** Foecking E** Kuffel G Conneely M Saban K Herrold A Kletzel S Radke J Walsh E Guernon A Ripley D Patil V Pacheco M Rosenow, J Bhaumik D **Bender Pape T** (under review). miRNA profiles of persons with chronic neurobehavioral impairments and remaining in states of Disordered Consciousness after Severe Traumatic Brain Injury, Journal of Head Trauma Rehabilitation (**authors contributed equally).
2. Herrold A** Kletzel S** Foecking E Saban K Szymanska M Bhaumik D Lange D Radke J Salinas I **Bender Pape T** (2021). miRNA as potential biomarkers for TBI: pathway from diagnosis to neurorehabilitation, Journal of Head Trauma Rehabilitation, 36 (3): E155 - E1691 (PMID: 33201038) (** authors contributed equally).

Books or other non-periodical one-time publications.

Nothing to report

Other publications, conference papers and presentations.

Project #2

Invited Reviews and Commentaries

1. **Bender Pape T** and Zasler N (2020) Provider Competencies for Disorders of Consciousness: Minimum Competency Recommendations Proposed by the ACRM-NIDLRR Workgroup. Brain Injury Professional, 17(2): 28.
2. **Bender Pape T** Herrold A Aaronson A Guernon A Rosenow J (2020) Preface, JHTR Topical Issue on Neuromodulation Interventions in TBI. Journal of Head Trauma Rehabilitation, 35(6): 365–370 (PMID: 33165150).

Practice Guidelines, Standards, and Consensus Statements

1. Giacino J Whyte J Nakase-Richardson R Rosenbaum A Yablon S Weintraub A Katz D **Bender Pape T** Seel R Greenwald B Zasler N Zafonte R Blum S Day K Hammond F Arciniegas D (2020) Minimal Competency Recommendations for Programs that Provide Rehabilitation Services for Persons with Disorders of Consciousness: A Position Statement of the American Congress of Rehabilitation Medicine and the National Institute on Disability and Rehabilitation Research Traumatic Brain Injury Model System. Archives of Physical Medicine and Rehabilitation, 101(6): 1072-1089 (PMID: 32087109).

Referred Abstracts

1. **Weaver J**, Mallinson T, Cogan AM, Guernon A, O'Brien K, Hansen P. (2021). Examining the Association Between States of Consciousness and the Coma Recovery Scale–Revised: A Rasch Analysis. *American Journal of Occupational Therapy*; 75 (Supplement_2): 7512500005. https://doi.org/10.5014/ajot.2021.75S2-RP5
2. **Weaver J**, Cogan A, Davidson L, Schlumpf K, Mallinson T. (2020). When is change meaningful?: A comparison of the MCID, MDC, and cMDC. *Archives of Physical Medicine & Rehabilitation*, 101 (11), E57. DOI: https://doi.org/10.1016/j.apmr.2020.09.169
3. **Weaver J**, Maisano K, Cogan A, Mallinson T, Leeds S, Guernon A, Watters K, Nebel J, & Pape T. (2020). Rehabilitation Interventions to Facilitate Recovery of Consciousness Following a

Traumatic Brain Injury: A Systematic Review. *American Journal of Occupational Therapy*, 74(4, Supplement 1). PMID: 7411515451. DOI: 10.5014.ajot.2020.74S1-PO8733.

4. Papadimitriou, C., **Weaver, J.,** Guernon, A., Mallinson, T., Walsh, E., & Louise Bender Pape, T. (2018). Clinicians' Sense-Making When Working with Patients in Disordered States of Consciousness Following Brain Injury—Accepted Abstracts from the American Congress of Rehabilitation Medicine, *Archives of Physical Medicine and Rehabilitation*, 99 (12): e195. DOI: 10.1016/j.apmr.2018.09.019.
5. Guernon, A., Walsh, E., **Weaver, J.,** Louise-Bender Pape, T., & Mallinson, T. (2018). Aligning Two Neurobehavioral Assessment Tools for Patients with Disorders of Consciousness – Accepted Abstracts from the Federal Interagency Conference for Traumatic Brain Injury, *Archives of Physical Medicine and Rehabilitation*, 99(11): e154. DOI: 10.1016/j.apmr.2018.08.082
6. Guernon, A., Papadimitriou, C., Walsh, E., **Weaver, J.,** Louise-Bender Pape, T., & Mallinson, T. (2018). Clinical Reasoning when Treating Patients with Disorders of Consciousness Following Severe Traumatic Brain Injury – Accepted Abstracts from the Federal Interagency Conference for Traumatic Brain Injury, *Archives of Physical Medicine and Rehabilitation*, 99(11): e131. DOI: 10.1016/j.apmr.2018.08.011
7. **Weaver, J.,** Mallinson, T., Papadimitriou, C., Guernon, A., Walsh, E., & Pape, T. (2018). Communication Among Multidisciplinary Team Members Treating Patients with Disorders of Consciousness Following Traumatic Brain Injury – Accepted Research Abstracts from the American Occupational Therapy Association Conference, *American Journal of Occupational Therapy*, 72 (4: Supplement 1) PO 4041. DOI: 10.5014/ajot.2018.72S1-PO4041
8. **Weaver J**, Mallinson T, Pape T, Guernon A, Walsh E. (2017). Clinically Meaningful Change Using the Coma Near Coma Scale. *Archives of Physical Medicine and Rehabilitation, 98*(12): e159. DOI: 10.1016/j.apmr.2017.09.031
9. Lyons L, Mallinson T, Pape T, Guernon A, Cotton L, **Weaver J**, & Walsh E. (2017). SMARTraining: A novel approach for teaching clinicians, family members, and caregivers to conduct neurobehavioral assessments. 12th World Congress on Brain Injury, International Brain Injury Association, Paper: #645, *Brain Injury, 31.*
10. Papadimitriou C, Mallinson T, Pape T, Guernon A, **Weaver J**, & Walsh E. (2017). Identifying Exemplars of Meaningful Functional Change seen in Patients with Disorders of Consciousness Following Severe Traumatic Brain Injury. 12th World Congress on Brain Injury, International Brain Injury Association, Paper: #577, *Brain Injury, 31.*

Other Publications

1. Mallinson T, **Weaver J**, Pape T, Guernon A, Walsh E, Rosenow J, Ripley D, Pacheco M, Steiner M, & Harrod T. Delineating the domains and concepts of neurobehavioral function in adults in states of disordered consciousness following severe traumatic brain injury: a systematic review. *PROSPERO2017*: CRD42017062599. Available from http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42017062599
2. **Weaver J,** Mallinson T, Harrod T, Pape T, Guernon A, Walsh E, Rosenow J, Ripley D, Pacheco M, & Steiner M. Outcomes mapping study for adults with severe traumatic brain injury in states of disorders of consciousness: a systematic review. *PROSPERO 2017*:CRD42017058383 Available from http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42017058383
3. **Weaver J**, Elgin E, Jones A, Bender Pape T, Guernon A, & Mallinson T. (2019, January). Disorders of Consciousness Instrument Summary. Retrieved from: https://www.sralab.org/rehabilitation-measures/disorders-consciousness-scale

PRESENTATIONS
Regional Presentations
Peer Reviewed

2021 Bhandari P, Awan BZ, **Weaver JA**, Cogan A, Mallinson T. Mapping Outcomes for Recovery of Consciousness in severe Traumatic Brain Injury. GWU Research Days, April 2021. (Poster) Retrieved from: https://cpb-us-e1.wpmucdn.com/blogs.gwu.edu/dist/7/135/files/2021/04/2021-Abstract-Submissions.pdf (Poster, nominated for OVPR Cross-Disciplinary Research Award, p. 165)

2020 **Weaver J**, Maisano K, Cogan A, Harrod T, Pape T, Mallinson T. Rehabilitation Interventions for Adults with Disorders of Consciousness Following a Brain Injury: A Scoping Review. GW Research Days, Washington, DC. April 2020. Retrieved from: https://cpb-us-e1.wpmucdn.com/blogs.gwu.edu/dist/7/135/files/2020/05/2020-Abstract-Submissions-Booklet-FINAL-3.pdf (Accepted Poster, page 359) *Conference cancelled due to COVID-19

2018 **Weaver J**, Mallinson T, Guernon A, Walsh E, Pape T, Papadimitriou, C. Communication Among Multidisciplinary Team Members Treating Patients with Disorders of Consciousness Following Traumatic Brain Injury, GW Research Days, April 11, 2018. Retrieved from: https://hsrc.himmelfarb.gwu.edu/gw_research_days/2018/SMHS/195/ (Poster 281)

2018 Mallinson T, **Weaver J**, Guernon A, Herrold A, Kletzel S, & Pape T. Using Method Comparison Approaches to Evaluate the Impact of Rater Severity/Leniency on Patient Measures of Disordered Consciousness, National Capital Area TBI Research Symposium, March 6-7, 2018. (Poster)

2017 **Weaver J**, Mallinson T, Pape T, Guernon A, Walsh E. Coma Near Coma Scale and Traumatic Brain Injury, Center for Healthcare Innovation and Policy Research Works In Progress at The George Washington University, November, 13, 2017. (Oral)

2017 **Weaver J**, Mallinson T, Pape T, & Guernon A. Neurobehavioral Function in Adults Recovering Consciousness after Severe Traumatic Brain Injury: A Scoping Review, The George Washington University Research Day, April 1, 2017. (Poster)

2017 **Weaver J**, Mallinson T, Pape T, & Guernon A. Neurobehavioral Function in Adults Recovering Consciousness after Severe Traumatic Brain Injury: A Scoping Review, National Capital Area TBI Research Symposium, March 8-9, 2017. (Poster)

National Presentations

2021 **Weaver J**, Mallinson T, Cogan A, Guernon A, Hansen P, O'Brien K. Examining the Association Between States of Consciousness and the Coma Recovery Scale-Revised: A Rasch Analysis. AOTA, April 9, 2021. (Research Platform

2020 **Weaver J**, Mallinson T, Cogan A, Guernon A, Hansen P, O'Brien K. Examining the Association Between States of Consciousness and the Coma Recovery Scale-Revised: A Rasch Analysis. ACRM 97th Annual Conference Progress in Rehabilitation Research, Atlanta, GA. October 22nd, 2020. (Poster)

2020 **Weaver J**, Cogan A, Davidson L, Schlumpf K, Mallinson T. When is change meaningful?: A Comparison of the MCID, MDC, and cMDC. ACRM 97th Annual Conference Progress in Rehabilitation Research, Atlanta, GA. October 22nd, 2020. (Poster)

2020 Bender Pape T, Mallinson T, Papadimitriou C, Guernon A, Walsh E, O'Brien K, **Weaver J**. Co-calibrating Five Assessments of Neurobehavioral Sensory-Motor Function in Persons in Disordered States of Consciousness. Military Health System Research Symposium (MHSRS), Kissimmee, Florida. 2020. (Accepted Poster: Abstract # MHSRS-20-01966 for Improving Neurosensory Function after TBI) *Conference cancelled due to COVID-19

2020 **Weaver J**, Maisano K, Cogan A, Harrod T, Bender Pape T, Mallinson T. Rehabilitation Interventions to Facilitate Recovery of Consciousness Following a Traumatic Brain Injury: A Scoping Review. Summit of Scholars Annual Conference, Denver, Colorado. 2020. (Accepted Poster) *Conference cancelled due to COVID-19

2020 **Weaver J**, Maisano K, Cogan A, Leeds S, Guernon A, Watters K, Nebel J, Bender Pape T, Mallinson T. Rehabilitation Interventions to Facilitate Recovery of Consciousness Following

a Traumatic Brain Injury: A Systematic Review. American Occupational Therapy Annual Conference, Boston, MA. 2020. (Accepted Poster) *Conference cancelled due to COVID-19

2019 Van de Winckel A, Mallinson T, **Weaver J**, Terhorst L, Heinemann A W, Kozlowski A. Overview of Good Practices in Reporting Rasch-Based Analysis. 96th Annual ACRM Conference Progress in Rehabilitation Research Translation to Clinical Practice, Chicago, IL. November 7th, 2019. (Oral)

2018 Pape T, Mallinson T, Papadimitriou C, Guernon A, **Weaver J**, Elyse Walsh, Maurer-Karattup P, Jessie V. Addressing Clinician-Reported Outcomes for disordered consciousness: A concurrent mixed methods approach, American Congress of Rehabilitation Medicine, September 30, 2018. (Oral Symposia)

2018 **Weaver J** & Guernon A. Applying Measurement Principles in the Selection of Assessment Tools for Disorders of Consciousness, American Congress of Rehabilitation Medicine, September 30, 2018. (Oral Symposia)

2018 Papadimitriou C, **Weaver J**, Guernon A, Mallinson T, Walsh E, Pape T. Clinicians' Sense-Making When Working with Patients in Disordered States of Consciousness Following Brain Injury, American Congress of Rehabilitation Medicine, September 28-October 3, 2018. (Poster)

2018 Guernon A, **Weaver J,** Mallinson T, Walsh E, Pape T. Aligning Two Neurobehavioral Assessment Tools for Patients with Disorders of Consciousness, 4th Federal Interagency Conference on Traumatic Brain Injury, June 11-13th, 2018, Washington, D.C. (Poster)

2018 Pape T, Mallinson T, Guernon A, Walsh E, **Weaver J,** Papadimitriou C, Maurer P, Jessie V. Co-calibrating TBI endpoints for disordered consciousness and opportunities to improve translation. 4th Federal Interagency Conference on Traumatic Brain Injury, June 11-13th, 2018, Washington, D.C. (Oral Symposia)

2018 **Weaver J,** Guernon A, Walsh E, Pape T, Mallinson T, Papadimitriou C. Clinical Reasoning when Treating Patients with Disorders of Consciousness following Severe Traumatic Brain Injury, 4th Federal Interagency Conference on Traumatic Brain Injury, June 11-13th, 2018, Washington, D.C. (Oral Paper)

2018 **Weaver J**, Mallinson T, Pape T, Guernon A. Demystifying Neurobehavioral Function Outcome Assessments for Patients with Disorders of Consciousness Following Severe Traumatic Brain Injury. American Occupational Therapy Association Annual Conference, April 21, 2018, Salt Lake City, Utah. (Oral) SC: 343

2018 **Weaver J**, Mallinson T, Guernon A, Walsh E, Pape T, Papadimitriou C. Communication Among Inter-Professional Teams Treating Patients with Disorders of Consciousness Following Traumatic Brain Injury, American Occupational Therapy Association Annual Conference, April 20th, 2018. (Poster- Oral Presentation for Young Scientist Award) RDP: 4041

2018 Davidson L, Radomski M, Eakman A, Mallinson T, & **Weaver J**. Translating Post-911 Era Military and Veteran Occupational Therapy Research to Civilian Practice: An Annual Update, American Occupational Therapy Association Annual Conference, April 19th, 2018. SC: 148.

2017 **Weaver J**, Mallinson T, Pape T, Guernon A, Walsh E. Clinically Meaningful Change Using the Coma Near Coma Scale. American Congress of Rehabilitation Medicine: Progress in Rehabilitation Research, October 26, 2017, Atlanta, GA. (Poster)

2017 Mallinson T Howard K Saretsky T Pape T Guernon A Weaver J Walsh E (2017) TBI Treatment & Emerging Care - Steering a Clinician-Reported TBI Outcome Assessment Through the FDA Drug Development Tool Qualification Program: A collaboration between industry, academic and clinical partners, Military Health Systems Research Symposia, Poster, Abstract # MHSRS-17-1801.

2017 *Lyons L Mallinson T **Pape T** Guernon A Cotton L Weaver J Walsh E (2017) SMARTraining: A novel approach for teaching clinicians, family members, and caregivers to

conduct neurobehavioral assessments, 12th World Congress on Brain Injury, International Brain Injury Association.

International Presentations

2018 Mallinson T, Pape T, Guernon A, **Weaver JA**, & Herrold A. Quantifying the Impact of Rater Severity/Leniency on ClinROs, 23rd Annual International Meeting for the International Society for Pharmacoeconomics and Outcomes Research, May 19-23rd, 2018. (Poster)

2017 Guernon A, Mallinson T, **Weaver J** Pape T, & Walsh E. Co-calibration of Two Assessment Tools to Measure Neurobehavioral Function for Persons in Disordered States of Consciousness. International Outcome Measurement Conference, September 15th, 2017, Chicago, IL. (Oral)

2017 Mallinson T, Pape T, Guernon A, **Weaver J**, Rentas C, Milhorn D, Herrold A, & Kletzel S. Accounting for Rater Severity/Leniency in Endpoint Measures of Disorders of Consciousness in Adults with Severe Traumatic Brain Injury. International Outcome Measurement Conference, September 15th, 2017, Chicago, IL. (Oral)

2017 Lyons L, Mallinson T, Pape T, Guernon A, Cotton L, **Weaver J**, & Walsh E. SMARTraining: A novel approach for teaching clinicians, family members, and caregivers to conduct neurobehavioral assessments. 12th World Congress on Brain Injury, International Brain Injury Association. (Poster)

2017 Papadimitriou C, Mallinson T, Pape T, Guernon A, **Weaver J**, & Walsh E. Identifying Exemplars of Meaningful Functional Change seen in Patients with Disorders of Consciousness Following Severe Traumatic Brain Injury. 12th World Congress on Brain Injury, International Brain Injury Association. (Poster)

Project #3

Poster Presentations: Unpublished abstracts

1. ***Bender Pape T** Foecking E Zilliox M Saban K Herrold A Kletzel S Walsh E Guernon A Pape A Bhaumik D Conneely M (2020, August) A Primary study examining utility of miRNA for diagnosing TBI, developing phenotypes of TBI recovery and developing phenotypes of treatment responsiveness, Military Health System Research Symposium (MHSRS) (Poster Abstract # MHSRS-20-00883 for TBI Biomarkers: Diagnostic & Prognostic Indicators - Peripheral Blood miRNA Biomarkers for TBI).

Oral Presentations: Unpublished Abstracts

1. **Bender Pape T** (2022) Neuromodulation for Neurologic Recovery, Transcranial Magnetic Stimulation for TBI. North American Neuromodulation Society's Annucal COnferecne, Symposia, Orlando FL.

Oral Presentations: Published Abstracts

1. Foecking M Zilliox M Saban K Herrold A Kletzel S Walsh E Guernon A Pape A Bhaumik D **Bender Pape, T** (2021) Preliminary evaluation of the clinical utility of miRNA as neurobiological markers of TBI diagnosis and rTMS treatment responsiveness, 4th International Brain Stimulation Conference, Symposia, Charleston, SC, Brain Stimulation: 14 (2021) e1721 (FS4F.07).

- **Website(s) or other Internet site(s)**

None to report for Projects #2 and #3.

- **Technologies or techniques**

Co-calibration methods developed for Project #2 will be disseminated in a planned manuscript. The co-calibration map, to enable monitoring of DoC recovery will also be shared. Also, the miRNA sequencing approach and validation are being shared in a manuscript under review (Appendix 11).

- **Inventions, patent applications, and/or licenses**

Nothing to report for Projects #2 and #3.

- **Other Products**

Project #2

The co-calibrated assessments provide a comprehensive map/hierarchy of neurobehavioral recovery. This provides clinicians with the evidentiary basis necessary to understand a patient's neurobehavioral recovery trajectory from the VS to eMCS as well as effects of rehabilitation interventions on that recovery trajectory. The study dataset enables addition of other assessments, in the future, for co-calibration with these four assessments.

Project #3

This JWMRP study allowed us to collect specimens from 12 timepoints during study participant (2-baseline, 10-during treatment, 1-follow-up). To date, all of the above analyses have examined six of these 12 timepoints. The additional timepoints were collected to enable future analyses that, with additional research funding, can advance this work to KRL6. That is, future analyses based on the existing specimens across the 12 timepoints and the behavioral data from the rTMS clinical trial can address more nuanced questions such delineating the number of rTMS treatment sessions needed to change miRNA expression to the level that supports maximum gains in neurobehavioral function.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Projects #2 and #3

Name: Theresa Pape. Dr. PH
Project Role: Principal Investigator
Role: Project #2 and #3, Dr Pape has overseen protocol development, staffing at each study site and overall project flow.

Name: Dulal Bhaumik, PhD
Project Role: Biostatistician
Role: Project #2 and #3, Statistical analysis support for articles and presentations

Name: Andrea Billups
Project Role: Research Assistant
Role: Project #2, Research support for coordinating and documenting of collected data

Name: Brett Blabas, MS

Project Role: Research Assistant
Role: Project #2 and #3, Ms. Blabas provided research support for coordinating and documenting of collected data

Name: Kenneth Blank, MS
Project Role: Research Assistant
Role: Project #2, Mr. Blank provided research support and data coordination

Name: Eileen Foecking, PhD
Project Role: Neurobiologist
Role: Project # 3, Dr. Foecking helped develop framework and process for miRNA analysis

Name: Ann Guernon, MS
Project Role: Clinical Research Manager
Role: For Project #2 and #3, Dr. Guernon provided the oversight of regulatory submissions and clinical data collection. She worked closely with Drs. Walsh, Mallinson and Pape to ensure the quality of data collected in the project.

Name: Catherine Kestner, DPT
Project Role: Research Therapist
Role: Project #2 and #3, Dr. Kestner, provided research support for coordinating and documenting of collected data. She assisted in the documentation of reporting requirements for projects.

Name: Lisel Kwartnik
Project Role: Project Manager
Role: Project #2 and #3, Ms. Kwartnik was responsible for the development and monitoring of study budgets and ensuring all financial allocations and expenditures were in accordance with the grant and VA requirements for the currently funded research clinical trial and the supplemental projects. She provided daily operational assistance to project staff.

Name: Trudy Mallinson, PhD
Project Role: PI of Supplemental Project # 2
Role: Project #2, Dr. Mallinson provided oversight to Dr. Weaver and Dr. Papadimitriou, building capacity for the team to examine the psychometric properties of each outcome measure and complete systematic reviews to strengthen the content validity of Neurobehavioral Functional outcome measures. She has trained Dr. Weaver in Rasch Analysis and in the many-facet Rasch model. She worked closely with Drs. Pape, Guernon, Walsh, Weaver and Papadimitriou on the revision of the caregiver guide and protocols.

Name: Christina Papadimitriou, PhD
Project Role: Research Associate
Role: For Project #2, Christina Papadimitriou, PhD led a study exploring healthcare practitioners' perceptions of caring for persons in DoC. This study was needed in order to incorporate practitioners' experiences in the refinement of clinical assessments as well as understand their use of assessments with this patient population.

Name: Jay Radke, PhD
Project Role: PI of Project #3, but moved to Portland and Dr. Foecking assumed this role after his move

Role: For Project #3, served as PI with developing miRNA protocols.

Name: Elyse Walsh, DPT
Project Role: Research Therapist
Role: Project #2 and #3, Dr. Walsh managed the specific IRB submission for the Clinical Language Protocol for all study locations. She managed screening of potential participants and scheduled procedures.

Name: Jen Weaver, PhD, OTR/L
Project Role: Research Associate
Role: Project #2, Dr. Weaver created two systematic review protocols that are published on PROSPERO, an international database for prospectively registered systematic reviews. She assisted in the letter of intent to the FDA regarding the DOCS-25 endpoint. She was an active participant in the coding of the qualitative interview and completed a psychometric analysis of the CNC scale.

Name: Michael Zilliox, PhD
Project Role: Bioinformatics
Role: For Project #3, Dr. Zilliox lead analyses of miRNA and biomarkers

What other organizations were involved as partners?

PROJECT #2:

Organization Name: George Washington University
Location of Organization: Washington, DC, USA
Partner's Contribution to the Project: Collaboration

PROJECT #3

Organization Name: Northwestern University
Location of Organization: Chicago, IL, USA
Partner's Contribution to the Project: Collaboration

Organization Name: Santa Clara Valley Medical Center **(STUDY SITE CLOSED-5/10/2019)**
Location of Organization: San Jose, CA, USA
Partner's Contribution to the Project: Collaboration

Organization Name: Loyola Genomics Facility
Location of Organization: Maywood, IL, USA
Partner's Contribution to the Project: Quality control testing of blood cell RNA

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: None to report

QUAD CHARTS: Submitted separately

9. APPENDICES:

Appendix 1	Cited References in the Final Report
Appendix 2	BMJ Open protocol manuscript, “Mapping Outcomes for Recover of Consciousness in Studies from 1986 to 2020: A scoping review” (Under Review)
Appendix 3	“Psychometric Properties of the Coma Near-Coma Scale for Adults in Disordered States of Consciousness: A Rasch Analysis” *Archives of Physical Medicine and Rehabilitation* (In Press)
Appendix 4	Manuscript: “Psychometric Properties of the Coma Near-Coma Scale for Adults in Disordered States of Consciousness: A Rasch Analysis” *Archives of Physical Medicine and Rehabilitation* (Under Review)
Appendix 5	Poster Presentation, “The Coma Recovery Scale-Revised (CRS-R): A Rasch Regression using States of Consciousness” ACRM Annual Conference, October 2021
Appendix 6	Manuscript: “Determining the Hierarchy of Coma Recovery Scale-Revised Rating Scale Categories and Alignment with Aspen Consensus Criteria for Patients with a Brain Injury: A Rasch Analysis” Journal of Neurotrauma (Under Review)
Appendix 7	Letter to the Editor in the Journal of Head Trauma and Rehabilitation describing the revised MDC results
Appendix 8	Poster, “Defining Clinically Meaningful Change with the Coma Near-Coma Scale” Disseminated at the 2017 ACRM conference
Appendix 9	Poster, “Using Method Comparison Approaches to Evaluate the Impact of Rater Severity/Leniency on Patient Measures of Disordered Consciousness”, presented as a poster at the 2018 National Capital Area TBI conference at NIH
Appendix 10	Manuscript, “Fluctuation is the norm: Rehabilitation practitioner perspectives on ambiguity and 1 uncertainty in their work with persons in disordered states of consciousness after traumatic 2 brain injury” PLOS ONE (Under Review)
Appendix 11	Manuscript, “miRNA profiles of persons with chronic neurobehavioral impairments and remaining in states of disordered consciousness after severe traumatic brain injury” *Journal of Head Trauma Rehabilitation* (Under Review)

APPENDICES

AWARD NUMBER: W81XWH-16-2-0023

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Appendix 2

BMJ Open protocol manuscript, “Mapping Outcomes for Recover of Consciousness in Studies from 1986 to 2020: A scoping review” (Under Review)

BMJ Open

Mapping Outcomes for Recovery of Consciousness in Studies from 1986 to 2020: A scoping review protocol

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Complete List of Authors:	Weaver, Jennifer; Colorado State University College of Health and Human Sciences, Department of Occupational Therapy; The George Washington University School of Medicine and Health Sciences Cogan, Alison; Veterans Affairs Greater Los Angeles Healthcare System Bhandari, Parie; The George Washington University School of Medicine and Health Sciences Awan, Bint-e; The George Washington University School of Medicine and Health Sciences Jacobs, Erica; The George Washington University School of Medicine and Health Sciences Pape, Ariana; The George Washington University School of Medicine and Health Sciences Nguyen, Chantal; The George Washington University School of Medicine and Health Sciences Guernon, Ann; Lewis University - College of Nursing and Health Professions; Hines Veterans Affairs Hospital Harrod, Tom; The George Washington University School of Medicine and Health Sciences Team, Recovery of Consciousness; The George Washington University School of Medicine and Health Sciences Pape, Theresa; Hines Veterans Affairs Hospital Mallinson, Trudy; The George Washington University School of Medicine and Health Sciences
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Mapping Outcomes for Recovery of Consciousness in Studies from 1986 to 2020: A scoping review protocol

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Abstract

Introduction: Historically, heterogeneous outcome assessments have been used to measure recovery of consciousness in patients with disorders of consciousness (DoC) following traumatic brain injury (TBI), making it difficult to compare across studies. To date, however, there is no comprehensive review of clinical outcome assessments that are used in intervention studies of adults with DoC. The objective of this scoping review is to develop a comprehensive inventory of clinical outcome assessments for recovery of consciousness that have been used in clinical studies of adults with DoC following TBI.

Methods and Analysis: The methodological framework for this review is: 1) identify the research questions, 2) identify relevant studies, 3) select studies, 4) chart the data, 5) collate, summarize and report results and 6) consult stakeholders to drive knowledge translation. We will identify relevant studies by searching the following electronic bibliographic databases: PubMed, Scopus, EMBASE, PsycINFO, and The Cochrane Library (including Cochrane Database of Systematic Reviews, Cochrane Central Register of Controlled Trials, and Cochrane Methodology Register). Criteria for article inclusion are published in the English-language, peer-reviewed studies of interventions aimed at facilitating recovery of consciousness among adults (≥ 18 years) with DoC following a severe TBI, published from January 1986 to December 2020. Articles meeting inclusion criteria at this stage will undergo a full text review. We will chart the data by applying the World Health Organization International Classification of Functioning, Disability and Health Framework to identify the content areas of clinical outcome assessments. To support knowledge translation efforts, we will involve clinicians and researchers experienced in TBI care throughout the project from conceptualization of the study through dissemination of results.

Dissemination: Results will be presented at national conferences and published in peer reviewed journals.

Keywords: Traumatic Brain Injury; Disorders of Consciousness; Common Data Elements, Clinical Outcome Assessments

Strengths and limitations of this study

- The proposed scoping review will result in a comprehensive catalogue of outcome assessments utilized in traumatic brain injury research aimed at facilitating recovery of consciousness among adults with DoC. These outcome assessments will be grouped according to the WHO ICF domains and sub-domains in order to identify key trends and gaps in concepts of interest.
- To the authors' knowledge, this will be the first study to identify whether the introduction of NINDS CDEs influenced outcome assessment reporting among studies that received federal funding in the United States.
- Our search is limited to articles published since 1986, therefore we may miss outcome assessments for DoC that were used prior to this date.
- It is possible that our search strategy will miss relevant studies; we will mitigate this risk by searching multiple databases and manually searching review articles and meta-analyses.
- Studies reporting US federal funding published after the introduction of NINDS CDEs may have been conducted prior to 2010 and therefore the authors may not have been strongly encouraged to use NINDS CDEs.

INTRODUCTION

Rationale

To date, there has been limited success in clinical trials for treatment of patients with severe traumatic brain injury (TBI) that result in disorders of consciousness (DoC).[1-3] Representing a continuum of impaired consciousness, DoC is based on a person's ability to demonstrate arousal and/or awareness. The DoC continuum includes comatose, vegetative state/unresponsive wakefulness syndrome, minimally conscious state, and emergence from the minimally conscious state.[4] Recovery of consciousness for people with DoC following a severe TBI is uncertain and difficult to predict.[5-7] Accurate measurement of recovery of consciousness for people in DoC is essential for diagnosis and prognosis as well as determining the efficacy and effectiveness of interventions.[5,8-10] To date, there has been no review of the range of clinical outcome assessments used in measuring recovery of consciousness.

Historically, measuring recovery of consciousness in clinical trials has involved a range of clinical outcome assessments measuring different concepts of interest (e.g., response to pain, awareness), making it difficult to compare results across studies.[11-14] The National Institute of Neurological Disorders and Stroke (NINDS), part of the US National Institutes of Health (NIH), established a set of Common Data Elements (CDEs) for TBI in 2010 with the goal of promoting comparability of study findings. Traumatic brain injury researchers applying for United States (US) federal funding sources including NIH, Department of Defense, Department of Veteran's Affairs are strongly encouraged to use NINDS CDEs for outcome measurement to improve comparability across trials. Further, a data repository for TBI research was created as a result of collaboration between NIH and the Federal Interagency Traumatic Brain Injury Research

Informatics System; [15] federally funded researchers may be required to submit their data to this repository in the future. This requirement provides additional incentive to use NINDS CDEs.[15-17]

CDEs are categorized as core, basic, or supplemental. The 'core' designation indicates data elements pertinent for all TBI studies. Basic CDEs are specific to studies of populations within TBI, such as 'concussion/mild TBI', 'acute hospitalized', 'moderate/severe TBI: rehabilitation', and 'epidemiology'. Basic CDEs for 'moderate/severe TBI: rehabilitation' include, but are not limited to, pupil reactivity, death date and time, hospital discharge destination, and alteration of consciousness duration.[18] Supplemental CDEs are optional and may be appropriate depending on the research question and scope.[16] Only two supplemental CDEs are related to recovery of consciousness in adults: the Galveston Orientation Amnesia Test and JFK Coma Recovery Scale-Revised (CRS-R) (Table 1).[18]

Table 1. Examples of Common Data Elements

Type of CDE	Definition	Example of CDE
General Core	Recommended for all NIH-funded studies: General	C00031: Race Expanded Category
Disease-specific Core	Recommended for all NIH-funded studies: Disease specific (TBI)	C01001: Glasgow Coma Scale (GCS) - motor response scale
Basic*	Recommended for all TBI NIH-funded studies: Specific to sub-diseases (e.g., Epidemiology and Moderate/Severe: Rehabilitation)	C07155: Disability Rating Scale Total Score
Supplemental	Recommended for NIH-funded studies: Specific to study design or type of research	C07145: JFK Coma Recovery Scale-Revised – Total Score

*Basic CDEs are comparable to Supplemental-Highly Recommended CDEs for other diagnostic categories.

Two studies have described the implementation of CDEs in TBI research.[13,19] Yue et al (2013) described the implementation of CDEs for a multicenter prospective study and note recommendations for future data collection procedures as well as the success in transferring the data to FITBIR. Stead et al (2013) used CDEs to describe TBI patients in emergency

departments and were able to compare results to several other published studies. Although the goal of the NINDS CDE project is to improve consistency and comparability across clinical studies of patients with DoC following severe TBI by encouraging more consistent use of clinical outcome assessments, there is currently no evidence to indicate whether this outcome has been achieved.

Objective

The primary objective of this scoping review is to develop a comprehensive inventory of clinical outcome assessments in clinical trials aimed at recovery of consciousness for patients with DoC after TBI. Secondary objectives are to examine the trends in primary outcomes over time and whether reporting of NINDS CDEs increased after their introduction in 2010 in studies that received US federal funding.

METHODS AND ANALYSIS

A scoping review is an appropriate method to achieve the stated objectives because we want to identify characteristics of clinical outcome assessments used to evaluate the recovery of consciousness following a severe TBI.[20] The scoping review will be conducted based on the Arksey and O'Malley[21] methodological framework that has been refined by Levac et al[22]. The methodological framework for this review will include: 1) identify the research questions, 2) identify relevant studies, 3) select studies, 4) chart the data, 5) collate, summarize and report results, and 6) stakeholder engagement to drive knowledge translation.[21,22]

1. Identify the Research Questions

Primary question

- What clinical outcome assessments have been used in published studies about recovery of consciousness for adults with severe TBI in states of disordered consciousness?

Secondary questions

- How have the outcomes assessments used to measure DoC in adults with severe TBI changed over time?
- Did frequency of reporting clinical outcome assessments classified as NINDS CDEs change after their introduction in 2010 among federally funded studies in the US?

2. Identify Relevant Studies

The search strategy was developed in collaboration with a research librarian. Our search terms are broad to identify all eligible studies. These search terms encompass three primary categories: severe TBI, recovery of consciousness, and outcomes.

Search terms

An in-depth outline of the full search strategy is reported in Table 2.

Table 2. Examples of the search strategy that will generate the articles to review for the research question.

Database	Search Terms	Customization
Cochrane	((“traumatic brain injury”) OR (coma) OR (“persistent vegetative state”) OR (“minimally conscious state”) OR (“consciousness disorder*”) OR (“disorder* of consciousness”)) AND ((recovery) OR (“activities of daily living”) OR (awareness) OR (wakefulness)) AND ((“critical care outcome*”) OR (“treatment outcome*”) OR ("outcome assessment”) OR (evaluation) OR (assessment))	1987-2020, all publication types
Embase	((exp traumatic brain injury/ OR traumatic brain injur*.ti,ab.) OR (exp coma/ OR coma*.ti,ab.) OR (exp persistent vegetative state/ OR persistent vegetative state*.ti,ab.) OR (exp minimally conscious state/ OR minimally conscious state*.ti,ab.) OR (exp consciousness disorder/ OR consciousness disorder*.ti,ab. OR disorder* of consciousness.ti,ab.)) AND ((exp convalescence/ OR convalescence.ti,ab. OR recover*.ti,ab.) OR (exp daily life activity/ OR daily life activit*.ti,ab. OR activit* of daily living.ti,ab.) OR (exp awareness/ OR awareness.ti,ab.) OR (exp wakefulness/ OR wakefulness.ti,ab.)) AND ((exp critical care outcome/ OR critical care outcome*.ti,ab.) OR (exp treatment outcome/ OR treatment outcome*.ti,ab.) OR (evaluation*.ti,ab.) OR (exp outcome assessment/ OR assessment*.ti,ab.))	English, 1986-2020
PsycInfo	(SU (“traumatic brain injur*”) OR TI (“traumatic brain injur*”) OR AB (“traumatic brain injur*”) OR SU (coma*) OR TI (coma*) OR AB (coma*) OR SU (“persistent vegetative state*”) OR TI (“persistent	1/1987-12/31/2020, English only

	vegetative state*") OR AB ("persistent vegetative state*") OR SU ("minimally conscious state*") OR TI ("minimally conscious state*") OR AB ("minimally conscious state*") OR SU ("consciousness disorder*") OR TI ("consciousness disorder*") OR AB ("consciousness disorder*") OR SU ("disorder* of consciousness") OR TI ("disorder* of consciousness") OR AB ("disorder* of consciousness")) AND (SU (recover*) OR TI (recover*) OR AB (recover*) OR SU ("activit* of daily living") OR TI ("activit* of daily living") OR AB ("activit* of daily living") OR SU (awareness) OR TI (awareness) OR AB (awareness) OR SU (wakefulness) OR TI (wakefulness) OR AB (wakefulness)) AND (SU ("critical care outcome*") OR TI ("critical care outcome*") OR AB ("critical care outcome*") OR SU ("treatment outcome*") OR TI ("treatment outcome*") OR AB ("treatment outcome*") OR SU ("outcome assessment*") OR TI ("outcome assessment*") OR AB ("outcome assessment*") OR SU (evaluation*) OR TI (evaluation*) OR AB (evaluation*) OR SU (assessment*) OR TI (assessment*) OR AB (assessment*))	
PubMed	(Severe Traumatic Brain Injury [tiab] OR Brain Injuries, Traumatic [mesh] OR traumatic brain injury [tiab] OR coma, post-head injury [mesh] OR persistent vegetative state [mesh] OR minimally conscious state [tiab] OR consciousness disorders [mesh] OR disorders of consciousness [tiab]) AND (recovery [tiab] OR recovery of function [mesh] OR activities of daily living [mesh] OR awareness [mesh] OR awareness [tiab] OR wakefulness [mesh] OR wakefulness [tiab]) AND (Critical care outcomes [mesh] OR treatment outcome [mesh] OR "outcome assessment (health care)" [mesh] OR disability evaluation [mesh] OR evaluation [tiab] OR patient outcome assessment [mesh] OR assessment [tiab])	Humans, English, 1/1/1986-12/31/2020
Scopus	(TITLE-ABS-KEY ("traumatic brain injur*") OR TITLE-ABS-KEY (coma*) OR TITLE-ABS-KEY ("persistent vegetative state*") OR TITLE-ABS-KEY ("minimally conscious state*") OR TITLE-ABS-KEY ("consciousness disorder*") OR TITLE-ABS-KEY ("disorder* of consciousness")) AND (TITLE-ABS-KEY (recover*) OR TITLE-ABS-KEY ("activit* of daily living") OR TITLE-ABS-KEY (awareness) OR TITLE-ABS-KEY (wakefulness)) AND (TITLE-ABS-KEY ("critical care outcome*") OR TITLE-ABS-KEY ("treatment outcome*") OR TITLE-ABS-KEY ("outcome assessment*") OR TITLE-ABS-KEY (evaluation*) OR TITLE-ABS-KEY (assessment*))	English

147 *Search dates will include January 1, 1986 to December 31, 2020

148 ***Information sources***

We will search the following electronic bibliographic databases: PubMed, Scopus, EMBASE, PsycINFO, and The Cochrane Library (including Cochrane Database of Systematic Reviews, Cochrane Central Register of Controlled Trials, and Cochrane Methodology Register).

Synthesis of eligibility criteria

This review will include all published, peer-reviewed studies using an intervention/treatment to facilitate recovery of consciousness for adults (≥18 years) with DoC following severe TBI (Table 3).

Table 3. Inclusion and exclusion criteria for the scoping review.

Category	Inclusion Criteria	Exclusion Criteria
Language	English	
Publication Date Range	January 1986 to December 2020	Before 1986
Participant Age	Participant age: ≥ 18 years of age At least one participant in the study was ≥ 18 years of age	All participants were under 18 years of age
Participant Diagnosis	Participant diagnosis: Disordered Consciousness (DoC) following severe TBI DoC was established utilizing a known assessment for evaluating states of consciousness such as the Coma Recovery Scale-Revised (CRS-R) or Glasgow Coma Scale ≤ 8 At least one participant in the study was diagnosed with DoC from a TBI	Participants had brain pathologies such as Alzheimer's Disease or non-traumatic brain injury, and/or were conscious, alert, and oriented Participants had a Diagnostics and Statistical Manual of Mental Disorders (5th edition) diagnosis of psychiatric disorders
Intervention	Intervention aimed at facilitating recovery of consciousness	Purpose of intervention was not described as facilitating recovery of consciousness
Study Design	All designs of primary, peer-reviewed studies including randomized control trials, observational studies, cohort studies, case control studies, case series, and case reports	Qualitative studies; meta analyses, systematic reviews, and scoping reviews

Language: English

Publication date: January 1986 to December 2020

Study Design: This review will consider all designs of peer-reviewed studies including randomized control trials, observational studies, cohort studies, case control studies, case series, and case reports. Meta-analyses and review articles will be excluded.

Setting: This review will include intervention studies delivered in any setting to adults with DoC following a severe TBI. There is no restriction on country of origin.

Participtants: For a study to be included in this review, at least one participant in the study must have DoC following a severe TBI. A severe TBI resulting in DoC is defined as: a) Glasgow Coma Scale (GCS) score of 3-8[12] or b) an assessment known for evaluating states of consciousness, such as the CRS-R.[5,8] Studies will be excluded if all participants were under 18 years of age, had a Diagnostic and Statistical Manual of Mental Disorders (5th edition) diagnosis of a psychiatric disorder, had brain pathologies such as Alzheimer's Disease or non-traumatic brain injury, or were conscious, alert, and oriented. All non-human studies will be excluded.

Interventions: Examples of interventions to be included are medication, nutrition, rehabilitation therapy, non-invasive brain stimulation, and surgery. Studies will be excluded if the purpose of the intervention/treatment provided was not described as facilitating recovery of consciousness.

3. Select Studies

Following the search, each identified article will be uploaded to Endnote, a reference management system. Duplicate articles will be removed. Titles and abstracts will be screened by two independent reviewers to assess whether articles meet inclusion criteria (Table 4). If studies are meta-analyses or reviews that are relevant to the research question, we will search the reference list. Articles that are included by the screening process will undergo a full text review. Two independent reviewers will read the full text articles to make a final determination of inclusion. Articles that do not meet inclusion criteria at this stage will be excluded from the final sample, with rationale documented. Discrepancies about inclusion of articles will be resolved through further discussion and/or input by a third reviewer.

Table 4. Title and abstract review form

Questions	
1. Is the article written in English?	☐ Yes ☐ No
2. Is the article published after 1985?	☐ Yes ☐ No
3. Is the article about human subjects?	☐ Yes ☐ No
a. Are the human subject's adults (≥ 18 years)	☐ Yes ☐ No ☐ Unsure, requires full text review
b. Do the adults have a traumatic brain injury?	☐ Yes ☐ No ☐ Unsure, requires full text review
c. Are the adults unconscious?	☐ Yes ☐ No ☐ Unsure, requires full text review
4. Is the article about an intervention?	☐ Yes ☐ No ☐ Unsure, requires full text review
a. Is the purpose of the intervention to facilitate recovery of consciousness?	☐ Yes ☐ No ☐ Unsure, requires full text review
b. Is it a meta-analysis, scoping review, or systematic review?	☐ Yes → Exclude & search the reference list. ☐ No

4. Chart the Data

Data will be extracted from included articles by independent reviewers using a uniform data extraction tool developed for the study. A sample data extraction table is shown in Table 5. Reviewers will use the Scottish Intercollegiate Guideline Network (SIGN) rating form to evaluate study quality.[23] For each included article, data extraction will include details about the

year of publication, funding source, study aims, study design, number of participants (including number lost to follow up), recruitment, study completion rate, demographics (age, injury severity, days post-injury) of participants, clinical setting, specific intervention (including control conditions, if applicable), primary and secondary outcomes, timing, and location of outcomes.

Table 5. Data extraction form for full text review.

Study Information	
Study Title	
Year	
Funding Source	
Inclusion/Exclusion Criteria	
Is the paper relevant to our research question, "What are the content areas of outcomes related to recovery of consciousness that have been used in clinical trials and/or intervention studies for adults with severe traumatic brain injury in states of disordered consciousness?" (i.e. there are outcome measures for people in disorders of consciousness following an intervention)	
Inclusion Criteria: • Adults (≥18 years) with primary diagnosis of severe traumatic brain injury; • Identified brain injury is noted to be severe by Glasgow Coma Scale of 8 or less; • At least one of the study participants are in states of disorders of consciousness following a traumatic brain injury; • Addressed outcome related to recovery of consciousness; • Written in English	

Exclusion Criteria: • People with documented history of psychiatric illness (DSM criteria), and/or organic brain syndrome such as Alzheimer's Disease. • All study participants are fully conscious; • All study participants are <18 years of age; • Study participants include non-traumatic brain injury *only*	
Study Details	
Study design	
Sample/number of participants: Include sample size and diagnoses (i.e. DoC following TBI, stroke, anoxia)	
Sample/demographics: age, injury severity, days post injury (if reported)	
Sample: The study's inclusion criteria	
Sample: The study's exclusion criteria	
Data Collection Procedures	
Intervention characteristics (intervention(s), control condition(s), duration and protocol information)	
Primary outcome measure	
Context of use for primary outcome measure	
Endpoint measure	
Secondary outcome measures	
Were outcome measures transformed? (Yes/No)	
Timing of outcome measures	
Results	
Observed sample	
Number of excluded participants	

Number of participants lost to follow up	
Primary Outcome (mean, proportion, other effect size index)	
Statistical analyses (description of groups, comparison of groups)	
Key Findings	
***Complete SIGN Quality Rating Based on Study Design*	

5. Collate, Summarize and Report Information

Data analysis

We will transfer information from the data extraction forms into STATA to complete descriptive analyses.

Conceptual Framework and Key Concepts

***World Health Organization International Classification of Functioning, Disability and Health*:** Clinical outcome assessments will be categorized based on the World Health Organization (WHO) International Classification of Functioning, Disability and Health (ICF) framework using relevant concept of interest. This framework has two major components: *Functioning and Disability* which includes the domains of Body Function, Body Structure, and Activities and Participation that impact an individual's daily life; and *Contextual Factors* which includes the domains of Personal Factors and Environmental Factors. Environmental Factors consider the "physical, social and attitudinal environment in which people live and conduct their lives."[24] Personal Factors include age, gender, and education; we will not apply this domain in classifying outcome assessments since these generally represent covariates rather than outcomes/endpoints.

Clinical outcome assessments will first be categorized into one of the four relevant WHO ICF domains (body structures, body functions, activities and participation, environmental factors) based on the concept of interest they are intended to measure. These categorizations will be mutually exclusive in that each outcome assessment will only be assigned to one domain. ICF domains can be further classified into subdomains.[24] We will also assign each outcome assessment to a relevant sub-domain. Should an outcome assessment not fit into a WHO ICF domain, we will create an 'Other' domain. Once all outcome assessments are categorized to a

domain, we will thematically analyze the outcome assessments in the 'Other' domain to determine if a new domain is needed. For example, previous literature argues for the inclusion of quality of life as a domain.[25]

Common Data Elements: We will also categorize outcome assessments as to whether they are a NINDS CDE for moderate/severe TBI. We will test the significance of the introduction for CDEs on outcome reporting before and after 2010 using a chi-square test.

Presentation of results

Results will be presented via detailed quantitative and narrative summaries. First, we will present the PRISMA-Scr flow diagram demonstrating the inclusion of studies.[26,27] We will also create an outcome map table that categorizes outcome assessments by WHO ICF domain and sub-domain. We will create two figures to display (1) the frequency of WHO ICF sub-domains in order to show the gaps in the concepts of interest that outcome assessments address by domain, and (2) the number and percent of studies that received US federal funding by year to show the proportion that used a CDE as a primary outcome. In addition, we will present a 2x2 table of CDE status and whether the publication was pre/post the introduction of CDEs.

Stakeholder Engagement

Clinicians and researchers with extensive experience treating and studying recovery of consciousness following a TBI have been involved in the development of this scoping review protocol. We have formed the Recovery of Consciousness (RECON) study team to continuously engage these stakeholders throughout the scoping review process, inclusive of study selection through dissemination of results.

Patient and Public Involvement

No patient involvement.

ETHICS AND DISSEMINATION

No ethical approval is required for this study as it is not determined to be human subjects research. Results will be presented at a national rehabilitation conference and submitted to a peer-reviewed journal for publication.

Reporting of protocol and study records

We registered this scoping review with PROSPERO (CRD42017058383). This study protocol and future reports will follow PRISMA-ScR guidelines for the publication of scoping reviews.[26]

Contributorship statement: All authors meet ICJME authorship criteria.

Competing interests: Authors have no disclosures.

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PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item	Page or Line number
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	Lines 1-2
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	N/A
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	Line 272
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	Title page
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	Line 274
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	N/A
Support:			
Sources	5a	Indicate sources of financial or other support for the review	Lines 276-277
Sponsor	5b	Provide name for the review funder and/or sponsor	Lines 276-277
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	N/A
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	Lines 2-115
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	Lines 116-121
METHODS			
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	Lines 152-194
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	Lines 148-151
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits,	Lines 143-147

		such that it could be repeated	
Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	Lines 196-197
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	Lines 197-204
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	Lines 208-221
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	Lines 208-221
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	Lines 208-221
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	Lines 208-221
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2, Kendall's τ)	
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	Lines 251-258 (Scoping review quantitative and narrative summaries planned)
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	N/A
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	Lines 196-197

*** It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.**

From: Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart L, PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ. 2015 Jan 2;349(jan02 1):g7647.

Appendix 3

"Psychometric Properties of the Coma Near-Coma Scale
for Adults in Disordered States of Consciousness:
A Rasch Analysis" *Archives of Physical Medicine and Rehabilitation* (In Press)

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AMERICAN CONGRESS OF REHABILITATION MEDICINE

Archives of Physical Medicine and Rehabilitation

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Archives of Physical Medicine and Rehabilitation 2021;102:591-7

ORIGINAL RESEARCH

Psychometric Properties of the Coma Near-Coma Scale for Adults in Disordered States of Consciousness: A Rasch Analysis

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Abstract

Objectives: To examine the construct validity and measurement precision of the Coma Near-Coma scale (CNC) in measuring neurobehavioral function (NBF) in patients with disorders of consciousness receiving postacute care rehabilitation.

Design: Rasch analysis of retrospective data.

Participants: Participants (N = 48) with disordered consciousness who were admitted to postacute care rehabilitation.

Interventions: Not applicable.

Main Outcome Measure: CNC.

Results: Assessment with CNC repeated weekly until the participant was conscious or discharged from the postacute care facility (451 participant records). Rating scale steps were ordered for all items. Eight of the 10 CNC items evaluated in this study fit the measurement model ($\chi^2 = 5332.58$; $df = 11$; $P = .17$); pain items formed a distinct construct. The ordering of the 8 items from most to least challenging makes clinical sense and compares favorably with other published hierarchies of NBF. Tactile items are more easily responded to. Visual and auditory items requiring higher cognitive processing were more challenging. In the full sample, the CNC achieved good measurement precision, with a person separation reliability of 0.87.

Conclusions: The items of the CNC reflect good construct validity and acceptable interrater reliability. The measurement precision achieved indicates that the CNC may be used to make decisions about groups of individuals but that these items may not be sufficiently precise for individual patient treatment decision-making.

Archives of Physical Medicine and Rehabilitation 2021;102:591-7

Disorders of consciousness (DoC) are classified according to clinical consensus criteria as being in a comatose state, vegetative state (VS) or unresponsive wakefulness syndrome (UWS),[1-4] minimally conscious state (MCS), or emerging from MCS.[5,6] Neurobehavioral assessments are the current clinical standard for evaluating individuals with a brain injury (BI) resulting in DoC. Assessments are based on clinician observation of behavioral responses elicited with sensory stimuli. Clinicians ascribe a score to the elicited behavioral responses. The attribution is based on the notion that elicited responses serve as clinical indicators of neurobehavioral function (NBF).[7]

The Coma Near Coma scale (CNC) was designed to capture small clinical changes in individuals with lower levels of NBF.[8] Scores on the CNC are assigned according to the consistency of specified behavioral responses elicited with 11 different test stimuli. Based on scoring criteria, elicited behavioral responses are interpreted as implying varying degrees of NBF.[9] A systematic review by the American Congress of Rehabilitation Medicine concluded that investigations of the psychometric properties of the CNC are scarce.[10] To date, the CNC has little published evidence of reliability and validity, although reasonable interrater reliability has been demonstrated in one small-scale study.[9,10]

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The purpose of this study is to contribute to the body of psychometric evidence by examining the construct validity of the CNC and the extent to which this tool can detect differences in a patient's NBF within the context of the Rasch measurement model. Rasch analysis assumes that items in an assessment capture an underlying, unidimensional construct and that each of the items reflects a different amount of that construct. Items of the CNC are believed to reflect varying levels of NBF when scored in conjunction with the CNC rating scale, which distinguishes quality and consistency in patient responses. For example, reflexive behaviors such as visual threat or flexion and withdrawal to pain may be observed in VS and UWS patients.[11] By comparison, behavioral signs of awareness such as visual pursuit and localization to stimuli are seen in MCS.[6] Thus, the items of the CNC can be expected to form a unidimensional hierarchy and distinguish among patients with varying levels of DoC.

Three research questions guided this study. First, does the construct represented by the ordering of the CNC items reflect increasing NBF in individuals with DoC (construct validity)? Second, can raters reliably score responses of individuals with a severe BI in states of DoC? Third, is the CNC sufficiently precise for making clinical decisions about individuals with DoC?

Methods

Setting and participants

This retrospective analysis included 48 adults with DoC after severe BI. Data were obtained from 2 separate studies: (1) Post Acute Care Study, an observational study conducted between 2007 and 2010 of patients receiving postacute care (n=34), and (2) Familiar Auditory Sensory Training, a clinical trial examining the effect of a familiar voice intervention on recovery of NBF (n=14). Demographics and health history were collected via medical record review and interviews with a family member or surrogate. Participants were evaluated at baseline and then weekly until recovery of full consciousness or discharge from the enrolling facility, whichever occurred first. All participants were measured at least once and as many as 35 times (n=451 total records) by 24 practitioners across both studies. Further details and results of these studies have been reported elsewhere.[12,13] Ethical approval for this secondary data analysis was obtained from the institutional review boards at the George Washington University and the Edward J. Hines Jr. VA Hospital. Eligibility criteria for participants were (1) age of 18 years or older, (2) had incurred a severe BI, and (3) had remained in a state of DoC for at least 28 days consecutively. Exclusion criteria were individuals with BI owing to cancer; nonmalignant tumors; or inflammatory, infectious, or toxic metabolic encephalopathies. Post Acute Care Study participants were within 180 days of injury at study enrollment; Familiar Auditory Sensory Training participants were within 1 year of injury at study enrollment.

List of abbreviations:

BI	brain injury
CNC	Coma Near-Coma
CI	confidence interval
DoC	disorders of consciousness
DOCS-25	Disorders of Consciousness Scale
KA	Krippendorff's alpha
LID	local item dependency
MnSq	mean square
MCS	minimally conscious state
NBF	neurobehavioral function
PCAR	Principal Component Analysis of Residuals
PSR	person separation reliability
SI	Separation Index
UWS	unresponsive wakefulness syndrome
VS	vegetative state

Main outcome measure: CNC

The CNC includes 11 test stimuli (items) scored using 3 response options: 0 (consistent responsive state), 2 (partially responsive state), and 4 (no response). Each rating scale step is defined uniquely for each of the test stimuli (items). For example, a score of 2 indicates tentative or inconsistent response to verbal command and partial tracking 1 or 2 times for the moving face item. The olfactory item was not administered because of difficulty controlling the consistency of ammonia and restrictions in shipping to study sites.

Analytic procedures

Rasch analysis

Data were analyzed using Winsteps software (version 4.1.0).[a] Rasch analysis is a probabilistic model that estimates the amount of an underlying trait reflected by the test items (stimuli) independently of the ability levels of the persons in the sample.[14] Person measures and item calibrations are expressed as logits (log odds units). The current study consisted of 451 records contributed by 48 individuals. Owing to concerns with local item dependency (LID) resulting from repeated measures, the first or last record for each individual were randomly assigned to calibration (n=48) and validation samples (n=48) (see supplemental appendix S1 and supplemental fig S1, available online only at http://www.archives-pmr.org/). Accordingly, although item calibrations and person measures will be stable within 1.0 logits and 99% confidence intervals (CI), results are best considered as preliminary.[15]

Rating scale structure

Each CNC item is scored on a 3-point rating scale unique to that item. To facilitate interpretation, the existing rating scale (ie, 0, 2, 4) was rescored to 2, 1, 0, so that higher scores represent better NBF. Data were analyzed using a partial credit model allowing threshold calibrations for the rating scale steps to be determined separately for each item (see supplemental appendix S1).

Construct validity: item hierarchy, item fit, and principal components analysis

The ordering of items from least to most challenging reflects these expectations and is the operational definition of NBF as defined by the CNC items. Items were considered to fit the model if mean square infit statistics (MnSq) were between 0.6 and 1.4.[16,17] LID was examined for standardized residual item correlations that exceeded .70.[18] Principal Component Analysis of Residuals (PCAR) was to identify dimensionality within the item residuals (see supplemental appendix S1).

Applicability: targeting, alignment, and precision

The separation index (SI), person separation reliability (PSR), and Wright's sample-independent method for strata in non-normally distributed data[19] were used to evaluate measurement precision. PSRs of.80 and .90 or greater were considered acceptable for group and individual level decision-making, respectively. Person fit is used to detect person response strings that are improbable, ambiguous, or too

probable. We considered person infit MnSq greater than 1.4 and standardized Z greater than 2.0 to indicate person measures that are unproductive for establishing item calibrations (see supplemental appendix S1).[20]

Validating results

Results for each psychometric property above were compared with those of the validation sample. Meaningful deviations in person measures or item calibrations were evaluated by crossplotting values and 95% CIs described by Luppescu.[21]

Interrater reliability

We examined interrater reliability using Krippendorff's alpha (KA). KA was calculated on a subset of data consisting of 6 raters paired on 24 separate occasions for 8 participants. KA coefficients were produced for each item rather than over all items. In general, values greater than .80 are acceptable (.90 is preferred), values from .66 to .80 are tentative, and values lower than .66 are inadequate.[22]

Concurrent validity

We compared person measures obtained from the CNC with those from the Disorders of Consciousness Scale (DOCS-25) to determine the extent to which the CNC compares to another assessment known to measure NBF.

Results

Sample

The study sample (n=48) was largely male (n=39; 81%). The average age of participants at the time of the injury was 35.6±14.9 years. Most had sustained traumatic brain injury (n=41; 85%) and were within 90 days of injury (n=35; 73%) (table 1).

Table 1 Sample characteristics

Characteristic	Total (N=48)
Age, mean years at injury ± SD	35.6±14.9
Sex, n (%)	
Men	39 (81)
Women	9 (19)
Veteran status, n (%)	
Veteran	10 (21)
Civilian	38 (79)
Time from onset to enrollment, n (%)	
<90 d	35 (73)
91-180 d	12 (25)
>180 d	1 (2)
Etiology of BI, n (%)	
Traumatic	41 (85)
Nontraumatic	7 (15)
State of consciousness at baseline, n (%)	
MCS	15 (31)
VS	24 (50)
Missing	9 (19)
Tracheostomy at baseline, n (%)	
Present	41 (86)
Absent	4 (8)
Missing	3 (6)

Sequence of analysis and analytical decisions

Table 2 presents the sequence of analytical steps undertaken. Initial analysis of the calibration sample indicated that the 10 items had moderate precision for detecting differences among individuals (PSR=.70) and no misfitting items. No items were found to have problematic LID. The items were modestly more challenging than the individuals' ability to respond (mean person measure −.38+1.09 logits) and 2 individuals had NBF levels that could not be distinguished with the easiest item (floor effect). The PCAR indicated that a contrast was present (eigenvalue, 2.34; percent variance explained by the first contrast, 15.6%). Inspection of the loadings indicated a contrast between pain items and visual items. To further inspect this contrast, we correlated person measures calibrated with and without the 2 pain items. The resulting high correlation, (r=.91) suggested that pain items have little effect on person measures (supplemental fig S2, available online only at http://www.archives-pmr.org/). Given that other authors have also suggested that pain is not a reliable indicator of consciousness, the 2 pain items were removed from subsequent analyses.[23] Analysis of the 8 remaining items revealed similar results as the first. Step and item anchors from the calibration sample were applied to the validation sample, except for vocalization, which was left unanchored. The results obtained compared favorably with the calibration sample (details are described later), and the step and item calibrations were then applied to the full sample. All the following results refer to the calibration sample and the 8 remaining items unless otherwise specified.

Rating scale structure

Thresholds for all items were ordered monotonically. Four items had fewer than 10 responses for select rating scale steps: Vocalization, Moving Face, and Light Flashes at step 0 and Verbal Command at step 2. Eight individuals were not administered the vocalization item. All rating scale steps demonstrated good fit except vocalization step 4 (MnSq, 1.7).

Construct validity

Item hierarchy

The item hierarchy describes the ordering of items from those that are easier to those that are more challenging for the participants to respond. The easiest item for participants to respond to was Nasal Swab (mean item calibration, −1.14); easier items also included Hand to Face and Bell Ringing. Verbal Command was in the middle of the scale; Shoulder Tap and both of the visual items were more challenging items. The most challenging was Vocalization (mean item value 0.74) (table 3).

Item fit

All items except Vocalization (MnSq, 1.47) fit the measurement model. We compared these results with those based on a more conservative approach advocated by Smith et al.[24] We generated 10 simulated data sets that approximate the calibration sample but which are designed to fit the model.[25] The average upper bound for fit values obtained from these 10 simulations was 1.31. Although slightly lower than the a priori limit of 1.4, no other items would have been judged as misfitting. Overall, fit of items to the Rasch model was good (χ^2=5332.58; df=11; P=.17).

Table 2 Sequence of Rasch analyses and summary psychometrics

Analysis	Items	Rating Scale Steps	Person Mean ± SD Logits	RMSE	Adjusted SD	SI	PSR	Number of Misfitting Items	PCA Eigenvalue First Contrast (%)	Ceiling Effect n (%)	Floor Effect n (%)
Calibration sample (n=45), PCM	10	29	−0.38±1.09	0.60	0.91	1.53	0.70	0	2.34 (15.6)	NA	2 (4.4)
Calibration sample (n=45), PCM, removed 2 pain items	8	23	−0.43±1.23	0.71	1.00	1.41	0.67	1 (Vocalize)*	1.89 (13.6)	NA	2 (4.4)
Validation sample (n=45), PCM, removed 2 pain items, anchored	8	24	−0.31±1.27	0.70	1.05	1.49	0.69	1 (Vocalize)*	1.99 (13.8)	2 (4.4)	5 (11)
Full sample (n=451), PCM, removed 2 pain items, anchored	8	24	−.30±1.18	0.68	0.96	1.40	0.66	0	1.81 (13.0)	11 (2.4)	13 (2.9)

Abbreviations: NA, not applicable; PCA, principal components analysis; PCM, partial credit model; RMSE, root mean square error.
* Vocalization item unanchored.

Validation of item anchors

Person measures were examined for the validation sample with and without item anchors. After crossplotting these values, no person measures exceeded the 95% CI and correlated at 0.99 (supplemental fig S3, available online only at http://www.archives-pmr.org/). Item calibrations were examined for the calibration and validation samples (unanchored). After crossplotting these values, no items exceeded the 95% CI or had a displacement of greater than .50 logits, indicating that these item measures can be adequately replicated across samples[26] (supplemental fig S4, available online only at http://www.archives-pmr.org/).

PCAR

For the calibration sample, 57.6% of the total raw variance was explained by the items. The eigenvalue for the first contrast was 1.89, indicating that any secondary dimension is not greater than chance.[27] The percent raw variance explained by the items was 22.9% and by the first contrast 13.6%. The ratio of these 2 values was 1.68, suggesting that any secondary dimension is weak. PCAR analyses on the 10 simulation data sets obtained results of a mean eigenvalue at 1.90 and mean percent variance explained by the first contrast at 12.5%; this was very similar to the calibration sample results (see supplemental table S1, available online only at http://www.archives-pmr.org/). Disattenuated correlation was 1.0 for all clusters in the calibration sample, although this was lower for cluster 1 to 3 in the validation sample ($r=0.63$), probably reflecting the small sample size.[28]

Measure applicability

Targeting and alignment

Mean person measures were acceptably well aligned with item calibrations (within 0.5 logits of item mean) for the calibration, validation, and full samples (see table 2, fig 1). In the calibration and full sample, we identified minimal ceiling and floor effects.

Precision and reliability

The PSR of .67 for the calibration sample compared favorably with that of the validation sample (.69) and full sample (.66). The sample was non-normally distributed (skewness, .36; $P=.0023$; Kurtosis, 4.15; $P=.0004$). Wright's sample-independent method identified the maximum statistically different levels of performance (strata) of 2.3; Wright's Sample-independent person reliability based on maximum strata is .84. For the full sample, these values were 2.6 and 0.87, respectively. A score to measure conversion table is provided in supplemental table S2 (available online only at http://www.archives-pmr.org/).

Person misfit

Six of the 45 (13.3%) individuals exhibited misfitting response patterns in the calibration sample, as well as 7 of 45 (15.5%) and 71 of 451 (15.7%) in the validation and full samples, respectively.

Interrater reliability

Interrater reliability varied by item (table 4). We found strong interrater reliability for 2 items (Moving Face and Hand to Face) and poor interrater reliability for 6 items (Vocalization, Light Flashes, Shoulder Gap, Verbal Command, Bell Ringing, and Nasal Swab).

Concurrent validity

Pearson correlation of person measures for participants scored on both CNC and the DOCS-25 (unique individuals, n=34; records, n=183) indicated a moderate association ($r=0.65$).

Table 3 Item calibrations and fit statistics, arranged in hierarchical order from most to least challenging*

Items	Measure (Calibration)	Standard Error	Infit MnSq	Infit zstd	Outfit MnSq	Infit zstd	Displacement
Vocalization†	1.50	0.11	1.37	4.7	1.34	3.7	0.00
Visual (Moving Face)	0.58	0.08	0.86	−2.3	0.75	−3.1	−0.08
Visual (Light Flashes)	0.31	0.08	0.69	−5.7	0.64	−5.4	0.27
Tactile (Shoulder Tap)	0.26	0.08	0.93	−1.2	0.86	−1.7	−0.16
Responsivity (Verbal Command)	0.14	0.07	0.98	−0.3	1.16	1.1	−0.20
Auditory (Bell Ringing)	−0.25	0.08	1.01	0.2	0.98	−0.3	0.38
Threat (Hand to Face)	−0.55	0.07	1.04	0.6	1.06	0.7	0.14
Tactile (Nasal Swab)	−1.14	0.08	1.23	3.3	1.45	3.7	−0.27

Abbreviation: zstd, standardized Z value.
* n = 451 records. Item calibrations were anchored on values from the calibration sample.
† This item was unanchored.

Discussion

The results of this small-scale study indicate that the ordering of CNC items from least to most challenging makes clinical sense and corresponds with the literature indicating that reflexive and stereotypical responses to loud sounds and threatening types of stimulation are often the first visible changes in patient's NBF observed after severe BI.[29] The easiest items, Nasal Swab and Hand to Face, represent more generalized responses to perceived threat. The next most challenging items, Bell Ringing, Verbal Command, and Shoulder Tap, require environmental awareness and fundamental coordination of ocular motor movement. Next, items including light flashes and visual tracking of a moving face require more precise and sustained coordination of ocular movement. The most challenging CNC item was vocalization. The highest rating for this item represents the spontaneous production of words, which differs from other items because the response is not elicited by a sensory stimulus; the quality and consistency of response is not prescribed.

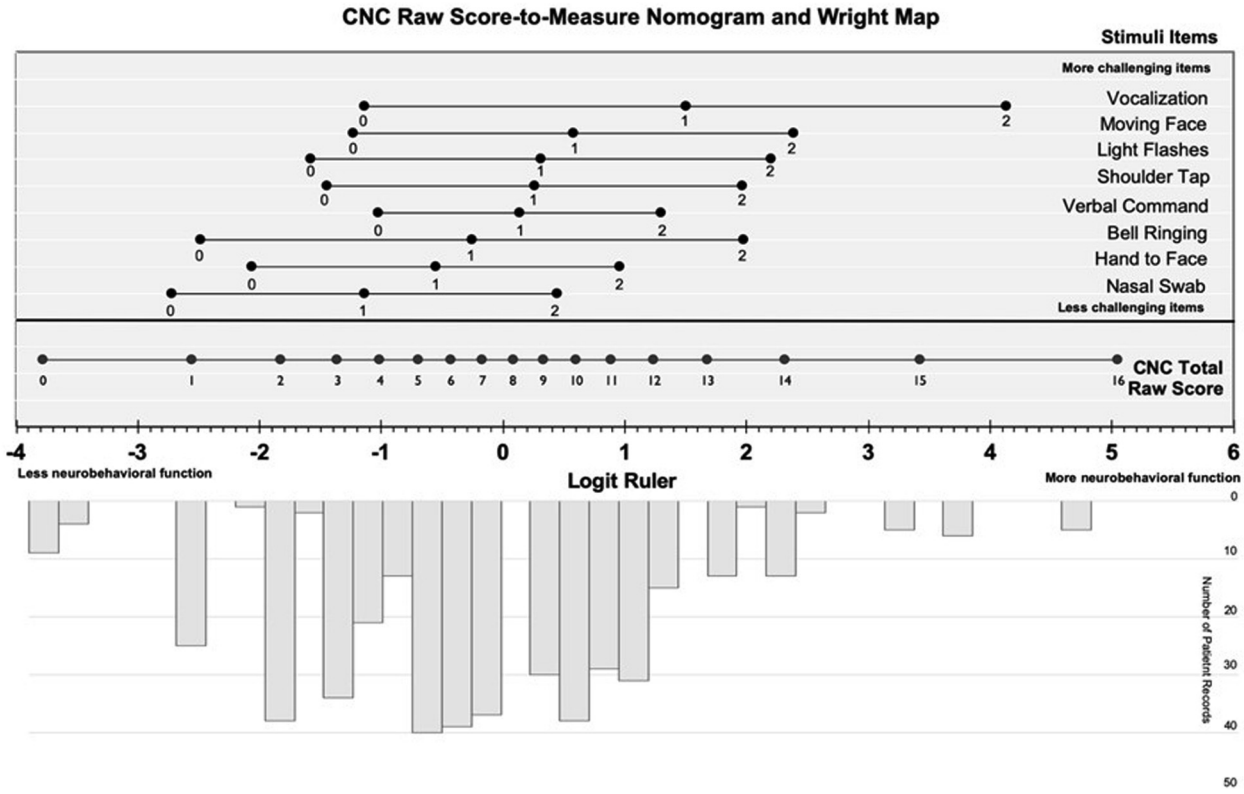

Fig 1 CNC raw score-to-measure nomogram and Wright map. CNC stimuli items arranged vertically in order from least challenging (bottom) to most challenging (top). Rating scale steps for each item are arranged to align horizontally with logit ruler. CNC total raw score arranged to align with logit ruler. Raw scores are non-linear, so a 1-point change at the extremes represents a greater logit change than in the midrange of the scale. Person measures are in logits. The histogram is aligned with the logit ruler so that those with less neurobehavioral function on the left and those with more neurobehavioral function on the right.

Table 4 Interrater reliability for each CNC item in hierarchical order from most to least challenging

CNC Item	Krippendorff's Alpha
Vocalization	0.34
Visual (Moving Face)	0.95
Visual (Light Flashes)	0.56
Tactile (Shoulder Tap)	0.28
Responsivity (Verbal Command)	0.51
Auditory (Bell Ringing)	0.15
Threat (Hand to Face)	0.84
Tactile (Nasal Swab)	0.32

Furthermore, CNC item order aligns with the literature describing different states of DoC. Coma is characterized clinically by the absence of eye opening and no response to the most vigorous stimulation.[30,31] VS and UWS are defined clinically as arousal without awareness of self or environment[2,3] but may respond to threats,[29] which aligns with the easier CNC items of Nasal Swab and Hand to Face. Patients in an MCS demonstrate minimal but definite behavioral evidence of environmental awareness[29] with increasing degrees of volitional response that, although inconsistent, can be differentiated from reflexive responses.[32] CNC items including Bell Ringing, Verbal Command, Shoulder Tap, Light Flashes, and Moving Face, which require the individual to fixate 1 or more times, may be indicative of an MCS. For example, Wannez et al[32] found fixation, visual pursuit, and reproducible response to command were most commonly seen in patients in MCS.[32] For the CNC item Vocalization, the highest score represents spontaneous production of words, but not functional communication, suggesting that it also aligns with the MCS. Because there are no items that reflect functional communication or object use, the CNC is not able to capture patients emerging into consciousness according to the published consensus criteria.[6,31,33]

We found that pain and noxious stimuli items did not cohere conceptually with other items; removing these items produced a more unidimensional scale. The CNC pain items are designed to reflect pain perception and not conscious neurobehavioral function.[34] The 2 pain items share an underlying trait that is conceptually distinct from the other 8 CNC items. In addition, patient measures with and without the pain items were highly correlated, suggesting these pain items do not add value to measuring NBF. This is consistent with the findings of Schnakers et al,[23] indicating that pain stimuli may not be useful for determining levels of consciousness and note that the clinical purpose of monitoring pain response is to identify patients in need of pain management.

Our results demonstrate that the amount of NBF represented by a single rating scale step (ie, 0-2 or 2-4; revised scoring, 2-1 or 1-0) varies within and across items. A gain of 1 point on light flashes represents 50% more gain in NBF than a 1-point gain on verbal command (see fig 1). The nomogram highlights that a 1-point gain in CNC total raw score implies more gain in NBF at the lower and upper ends of the scale compared with the middle of the scale. Using Rasch-transformed measures can help researchers and practitioners more clearly interpret a patient's change in NBF.

The moderately strong relationship between the CNC and DOCS-25 person measures reflects the difference in person distributions. CNC measures clustered at the lower end of the scale but were more dispersed on the DOCS-25.

Interrater reliability for CNC was quite variable by item. Visual tracking items showed excellent reliability, whereas bell ringing and shoulder tap showed particularly poor interrater reliability. This variability may be related to administration procedures. Bell ringing and shoulder tap items require the assessor be out of the person's visual field, whereas for visual tracking and threat items, the assessor is positioned in front of the person.

Future studies could enhance the measurement precision of the tool as well as provide indices of responsiveness such as minimal detectable change and minimally clinically important differences. Research is needed to provide evidence that this tool can capture meaningful change in neurobehavioral function.

Study limitations

This study examined a relatively small sample of adults with DoC and results may not be generalizeable.[27,28] Although we provided a practical and novel solution to dependence in repeated clinical data, we recognize that item estimates are based on a relatively small number of participants. Also, differential item functioning generally requires samples larger than 100[35] to identify whether clinical groups have different response patterns. These analyses should be undertaken in the future with a larger sample.

Conclusions

This is the first study to examine the construct validity of the CNC using Rasch analysis. Overall, we found important psychometric assets: the rating scale was operating as intended. Most items adequately fit the assumptions of the Rasch model, items were sufficiently precise to identify 2.6 levels of NBF and PSR of 0.87, and person response strings showed acceptable fit. These preliminary findings suggest that pain and noxious stimuli items did not cohere conceptually with the visual, tactile, and auditory items, which hierarchically align with current literature of DoC.

Supplier

a. Winsteps, version 4.1.0; Winsteps.

Keywords

Brain injuries; Consciousness disorders; Rehabilitation

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Appendix 4

Manuscript: “Psychometric Properties of the Coma Near-Coma Scale for Adults in Disordered States of Consciousness: A Rasch Analysis” *Archives of Physical Medicine and Rehabilitation* (Under Review)

Psychometric Properties of the Coma Near-Coma Scale for Adults in Disordered States of Consciousness: A Rasch Analysis

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Running head: CNC Rasch Analysis

Psychometric Properties of the Coma Near-Coma Scale for Adults in Disordered States of Consciousness: A Rasch Analysis

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Psychometric Properties of the Coma Near-Coma Scale for Adults in Disordered States of Consciousness: A Rasch Analysis

Abstract

Objective: To examine the construct validity and measurement precision of the Coma-Near Coma Scale (CNC) in measuring neurobehavioral function (NBF) in patients with disorders of consciousness receiving post-acute care rehabilitation.

Design: Rasch analysis of retrospective data.

Participants: 48 participants with disordered consciousness admitted to post-acute care rehabilitation.

Interventions: Not applicable.

Main Outcome Measure: Coma Near Coma Scale.

Results: Assessment with CNC repeated weekly until participant was conscious or discharged from post-acute care facility (451 participant records). Rating scale steps were ordered for all items. Eight of the 10 CNC items evaluated in this study fit the measurement model (χ^2=5332.58; df=11; P=0.17); pain items formed a distinct construct. The ordering of the 8 items from most to least challenging makes clinical sense and compares favorably with other published hierarchies of NBF. Tactile items are more easily responded to. Visual and auditory items requiring higher cognitive processing were more challenging. In the full sample, the CNC achieved good measurement precision; person separation reliability of 0.87.

Conclusions: The items of the CNC reflect good construct validity and acceptable interrater reliability. Measurement precision achieved indicates the CNC may be used to make decisions about groups of individuals but that these items may not be sufficiently precise for individual patient treatment decision-making.

Keywords: Brain Injuries; Outcome Assessment (Health Care); Consciousness Disorders

Abbreviations

Brain Injury (BI)

Coma Near Coma (CNC)

Confidence intervals (CI)

Disorders of consciousness (DoC)

Disorders of Consciousness Scale (DOCS-25)

Krippendorff's Alpha (KA)

Local item dependency (LID)

Mean square (MnSq)

Minimally conscious state (MCS)

Neurobehavioral function (NBF)

Person separation reliability (PSR)

Principal Component Analysis of Residuals (PCAR)

Separation Index (SI)

Unresponsive wakefulness syndrome (UWS)

Vegetative state (VS)

Disorders of consciousness (DoC) are classified according to clinical consensus criteria as being in a comatose state, vegetative state/unresponsive wakefulness syndrome (VS/UWS) [1-4], minimally conscious state (MCS), or emerging from MCS [5,6]. Neurobehavioral assessments are the current clinical standard for evaluating individuals with a brain injury (BI) resulting in DoC. Assessments are based on clinician observation of behavioral responses elicited with sensory stimuli. Clinicians ascribe a score to the elicited behavioral responses. The attribution is based on the notion that elicited responses serve as clinical indicators of neurobehavioral function (NBF) [7].

The Coma Near Coma scale (CNC) was designed to capture small clinical changes in people with lower levels of NBF [8]. Scores on the CNC are assigned according to the consistency of specified behavioral responses elicited with 11 different test stimuli. Based on scoring criteria, elicited behavioral responses are interpreted as implying varying degrees of NBF [9]. A systematic review by the American Congress of Rehabilitation Medicine concluded that investigations of the psychometric properties of the CNC are scarce [10]. To date, the CNC has little published evidence of reliability and validity although reasonable inter-rater reliability has been demonstrated in one small-scale study [9,10].

The purpose of this paper is to contribute to the body of psychometric evidence by examining the construct validity of the CNC and the extent to which this tool can detect differences in patient's NBF within the context of the Rasch measurement model. Rasch analysis assumes that items in an assessment capture an underlying, unidimensional construct and that each of the items reflects a different amount of that construct. Items of the CNC are thought to reflect varying levels of NBF when scored in conjunction with the CNC rating scale, which distinguishes quality and consistency in patient responses. For example, reflexive behaviors such as visual threat or

flexion/withdrawal to pain may be observed in VS/UWS patients [11]. By comparison, behavioral signs of awareness such as visual pursuit and localization to stimuli are seen in MCS [6]. Thus, the items of the CNC can be expected to form a unidimensional hierarchy and distinguish among patients with varying levels of DoC.

Three research questions guided this study. First, does the construct represented by the ordering of the CNC items reflect increasing NBF in people with DoC (construct validity)? Second, can raters reliably score responses of people with a severe BI in states of DoC? Third, is CNC sufficiently precise for making clinical decisions about individuals with DoC?

Methods

Setting and Participants

This retrospective analysis includes 48 adults with DoC following severe BI. Data were obtained from two separate studies: a) PACS: An observational study (2007-2010) of patients receiving post-acute care (n=34); b) FAST: A clinical trial examining the impact of a familiar voice intervention on recovery of NBF (n=14). Demographics and health history were collected via medical record review and/or family/surrogate interview. Participants were evaluated at baseline and then weekly until recovery of full consciousness or discharge from the enrolling facility, whichever occurred first. All participants were measured at least once, and as many as 35 times (n=451 total records) by 24 practitioners across both studies. Further details and results of these studies have been reported elsewhere.[12,13] Ethical approval for this secondary data analysis was obtained from the institutional review boards at [redacted for review and redacted for review]. Eligibility criteria for participants were: 1) 18 years of age or older, 2) had incurred a severe BI, and 3) had remained in a state of DoC for at least 28 days

consecutively. Exclusion criteria were: persons with BI due to cancer, non-malignant tumors, inflammatory, infectious, or toxic metabolic encephalopathies. PACS participants were within 180 days of injury at study enrollment; FAST participants were within 1 year of injury at study enrollment.

Main Outcome Measure: CNC

The CNC includes 11 test stimuli (items) scored using 3 response options: 0 (consistent responsive state), 2 (partially responsive state), and 4 (no response). Each rating scale step is defined uniquely for each of the test stimuli (item). For example, a score of 2 means tentative or inconsistent response to verbal command and partial tracking 1 or 2 times for the moving face item. The olfactory item was not administered because of difficulty controlling the consistency of ammonia and restrictions in shipping to study sites.

Analytic Procedures

Rasch Analysis.

Data were analysed using Winsteps® software (version 4.1.0) [14]. Rasch analysis is a probabilistic model that estimates the amount of an underlying trait reflected by the test items (stimuli) independently of the ability levels of the persons in the sample.[15] Person measures and item calibrations are expressed as logits (log odds units). The current study consists of 451 records contributed by 48 persons. Due to concerns with local item dependency (LID) due to repeated measures, the first or last record for each person were randomly assigned to calibration (n=48) and validation samples (n=48) (see Supplemental File & Supplemental Figure 1). Accordingly, while item calibrations and person measures will be stable within 1.0 logits and 99% confidence intervals (CI),

results are best considered as preliminary.[16]

Rating Scale Structure.

Each CNC item is scored on a the 3-point rating scale unique to that item. To facilitate interpretation, the existing rating scale, i.e., 0, 2, 4, was rescored to 2, 1, 0, so that higher scores represent better NBF. Data were analysed using a partial credit model allowing threshold calibrations for the rating scale steps to be determined separately for each item (see Supplemental File).

Construct Validity: Item hierarchy, Item Fit, & Principal Components Analysis

The ordering of items, from least to most challenging, reflects these expectations and is the operational definition of NBF as defined by the CNC items. Items were considered to fit the model if mean square infit statistics (MnSq) were between 0.6 and <1.4. [17,18] Local item dependence (LID) was examined for standardized residual item correlations that exceeded .70.[19] Principal Component Analysis of Residuals (PCAR) was to identify dimensionality within the item residuals (see Supplemental File).

Applicability: Targeting, Alignment, and Precision

The separation index (SI), person separation reliability (PSR) and Wright's sample-independent method for strata in non-normally distributed data[20] were used to evaluate measurement precision. PSR of.80 and $\geq$.90 were considered acceptable for group and individual level decision-making, respectively.

Person fit is used to detect person response strings that are improbable, ambiguous or too probable. We considered person infit MnSq >1.4 and standardized Z >2.0 to indicate person measures that are unproductive for establishing item calibrations (see

Supplemental File).[21]

Validating Results

Results for each psychometric property above were compared to those of the validation sample. Meaningful deviations in person measures or item calibrations were evaluated by crossplotting values and 95% confidence intervals (CI) described by Luppescu (1995).[22]

Interrater Reliability

We examined interrater reliability using Krippendorff's Alpha (KA). KA was calculated on a subset of data consisting of 6 raters paired on 24 separate occasions for 8 participants. KA coefficients are produced for each item rather than over all items. In general, values above .80 are acceptable (.90 preferred), values from .66 to .80 tentative and below .66 inadequate [23].

Concurrent Validity

We compared person measures obtained from the CNC to those from the Disorders of Consciousness Scale (DOCS-25) to determine the extent to which the CNC compares to another assessment known to measure NBF.

Results

Sample

The study sample (n=48) was largely male n= 39 (81%). The average age of participants at the time of the injury was 35.6±14.9 years. Most had sustained traumatic brain injury n=41 (85%) and were within 90 days of injury n=35 (73%) (See Table 1).

[Insert Table 1 near here].

Sequence of analysis and analytic decisions

Table 2 presents the sequence of analytic steps undertaken. Initial analysis of the calibration sample indicated that the 10 items had moderate precision for detecting differences among individuals (PSR=.70) and no misfitting items **[Insert Table 2 near here]**. No items were found to have problematic LID. The items were modestly more challenging than these individuals' ability to respond (mean person measure (-.38 + 1.09 logits) and 2 individuals had levels of NBF that could not be distinguished with the easiest item (floor effect). The PCAR indicated a contrast was present (Eigenvalue 2.34; percent variance explained by the first contrast 15.6%). Inspection of the loadings indicated a contrast between pain items and visual items. To further inspect this contrast, we correlated person measures calibrated with and without the two pain items. The resulting high correlation, (r=.91) suggests pain items have little impact on person measures (see supplemental materials figure 2). Given that other authors have also suggested that pain is not a reliable indicator of consciousness the two pain items were removed from subsequent analyses.[24] Analysis of the 8 remaining items revealed similar results as Round 1. Step and item anchors from the calibration sample were applied to the validation sample except for vocalization that was left unanchored; results obtained compared favorably with the calibration sample (details are described below) and the step and item calibrations were then applied to the full sample. All results below refer to the calibration sample and the 8 remaining items unless otherwise specified.

Rating Scale Structure

Thresholds for all items were ordered monotonically. Four items had fewer than 10 responses for select rating scale steps: Vocalization, Moving Face, and Light Flashes at step 0 and Verbal Command at step 2. Eight individuals were not administered the

vocalization item. All rating scale steps demonstrated good fit except vocalization step 4 (MnSq 1.7).

Construct Validity

Item hierarchy. The item hierarchy describes the ordering of items from those that are easier to those that are more challenging for the participants to respond to **[Insert Table 3 near here]**. The easiest item for participants to respond to was Nasal Swab (mean item calibration -1.14); easier items also included Hand to Face and Bell Ringing. Verbal Command was in the middle of the scale; Shoulder Tap and both of the visual items were more challenging items. The most challenging was Vocalization (mean item value 0.74) (see Table 3).

Item Fit. All items except Vocalization (MnSq 1.47) fit the measurement model. We compared these results to those based on a more conservative approach advocated by Smith.[25] We generated 10 simulated data sets that approximate the calibration sample but which are designed to fit the model.[26] The average upper bound for fit values obtained from these 10 simulations was 1.31. Although slightly lower than the a priori limit of 1.4, no other items would have been judged as misfitting. Overall, fit of items to the Rasch model was good (χ^2= 5332.58; df= 11; P= 0.17).

Validation of Item Anchors. Person measures were examined for the validation sample with and without item anchors. After crossplotting these values, no person measures exceeded the 95% CI and correlated at 0.99. See supplemental materials figure 3. Item calibrations were examined for the calibration and validation samples (unanchored). After crossplotting these values, no items exceeded the 95% CI or had a displacement of

greater than .50 logits indicating these item measures can be adequately replicated across samples.[27] See supplemental materials figure 4.

Principal Component Analysis of Residuals (PCAR). For the calibration sample, 57.6% of the total raw variance was explained by the items. The eigenvalue for the first contrast was 1.89 indicating any secondary dimension is not greater than chance.[28] The percent raw variance explained by the items was 22.9% and by the first contrast 13.6%. The ratio of these two values is 1.68 suggesting any secondary dimension is weak. PCAR analyses on the 10 simulation data sets obtained results of a mean Eigenvalue at 1.90 and mean percent variance explained by the first contrast at 12.5%; very similar to the calibration sample results (See supplemental materials table 1). Disattenuated correlation was 1.0 for all clusters in the calibration sample; although lower for cluster 1-3 in the validation sample (r=0.63), probably reflecting the small sample size.[29]

Measure Applicability

Targeting and Alignment: Mean person measures were acceptably well aligned with item calibrations (within 0.5 logits of item mean) for the calibration, validation, and full samples (see Table 2, Figure 1). In the calibration and full sample, we identified minimal ceiling and floor effects.

Precision and Reliability. The PSR of .67 for the calibration sample compared favorably with that of the validation sample (.69) and full sample (.66). The sample was non-normally distributed (Skewness .36 p=.0023; Kurtosis 4.15 p=.0004). Wright's sample-independent method identified the maximum statistically different levels of performance (strata) of 2.3; Wright's Sample-independent Person Reliability based on maximum strata is .84. For the full sample these values are 2.6 and 0.87, respectively. A

score to measure conversion table is provided in the supplemental materials (Supplemental Table 2).

Person Misfit. Six of the 45 (13.3%) persons exhibited misfitting response patterns in the calibration sample; 7/45 (15.5%) and 71/451 (15.7%), in the validation and full samples, respectively.

Interrater Reliability. Interrater reliability varied by item (see Table 4) **[Insert Table 4 near here]**. We found strong IRR for 2 items (Moving Face and Hand to Face) and poor IRR for 6 items (Vocalization, Light Flashes, Shoulder Gap, Verbal Command, Bell Ringing, and Nasal Swab).

Concurrent Validity. Pearson correlation of person measures for participants scored on both CNC and the DOCS-25 (unique individuals n=34; records n=183) indicated a moderate association r=0.65.

Discussion

The results of this small-scale study indicate that ordering of CNC items from least to most challenging makes clinical sense and corresponds with literature indicating that reflexive and stereotypical responses to loud sounds and threatening types of stimulation are often the first visible changes in patient's NBF observed following severe BI.[30] The easiest items, nasal swab and hand to face, represent more generalized responses to perceived threat. The next most challenging items, bell ringing, verbal command, and shoulder tap, require environmental awareness and fundamental coordination of ocular motor movement. Next, items including light flashes and visual tracking of a moving face, require more precise and sustained coordination of ocular movement. The most challenging CNC item was vocalization. The highest rating for

this item represents spontaneous production of words, which differs from other items because the response is not elicited by a sensory stimulus; the quality and consistency of response is not prescribed.

Further, CNC item order aligns with literature describing different states of DoC. Coma is characterized clinically by the absence of eye opening and no response to the most vigorous stimulation.[31,32] VS/UWS is defined clinically as arousal without awareness of self or environment[2,3] but may respond to threats,[30] which aligns with the easier CNC items; nasal swab and hand to face. Patients in a MCS demonstrate minimal but definite behavioral evidence of environmental awareness[30] with increasing degrees of volitional response that while inconsistent can be differentiated from reflexive responses.[33] CNC items including Bell Ringing, Verbal Command, Shoulder Tap, Light Flashes, and Moving Face, which require the person to fixate 1 or more times, may be indicative of a MCS. For example, Wannez et al (2017) found fixation, visual pursuit, and reproducible response to command were most commonly seen in patients in MCS.[33] For the CNC item Vocalization, the highest score represents spontaneous production of words, but not functional communication, suggesting that it also aligns with the minimally conscious state. Since there are no items that reflect functional communication or object use, the CNC is not able to capture patients emerging into consciousness according to the published consensus criteria.[6,32,34]

We found that pain/noxious stimuli items did not cohere conceptually with other items; removing these items produced a more unidimensional scale. The CNC pain items are designed to reflect pain perception and not conscious neurobehavioral function.[35] The two pain items share an underlying trait that is conceptually distinct from the other eight CNC items. In addition, patient measures with and without the pain items were highly correlated suggesting these pain items do not add value to measuring

neurobehavioral function. This is consistent with Schnakers et al (2009) findings that pain stimuli may not be useful for determining levels of consciousness and note that the clinical purpose of monitoring pain response is to identify patients in need of pain management.[24]

Our results demonstrate that the amount of NBF represented by a single rating scale step i.e., 0-2 or 2-4 (revised scoring 2-1 or 1-0) varies within and across items. A gain of one point on light flashes represents 50% more gain in NBF than a one-point gain on verbal command (see Figure 1). The nomogram highlights that a one-point gain in CNC total raw score implies more gain in NBF at the lower and upper ends of the scale compared to the middle of the scale. Using Rasch-transformed measures can help researchers and practitioners more clearly interpret their patient's change in NBF.

The moderately strong relationship between the CNC and DOCS-25 person measures, reflects the difference in person distributions; CNC measures clustered at the lower end of the scale but were more dispersed on the DOCS-25.

Interrater reliability for CNC was quite variable by item; visual tracking items showed excellent reliability while bell ringing and shoulder tap showed particularly poor interrater reliability. This variability may be related to administration procedures; bell ringing and shoulder tap items require the assessor be out of the person's visual field; for visual tracking and threat items, the assessor is positioned in front of the person.

Future studies could enhance the measurement precision of the tool as well as provide indices of responsiveness such as minimal detectable change and minimally clinically important differences. Research is needed to provide evidence that this tool can capture meaningful change in neurobehavioral function.

Study Limitations

This study examined a relatively small sample of adults with DoC and results may not generalize.[28,29] While we provided a practical and novel solution to dependence in repeated clinical data, we recognize that item estimates are based on a relatively small number of participants. Also, differential item functioning generally requires samples larger than 100[36] to identify if clinical groups have different response patterns. These analyses should be undertaken in the future with a larger sample.

Conclusions

This is the first study to examine the construct validity of the CNC using Rasch analysis. Overall, we found important psychometric assets: the rating scale was operating as intended; most items adequately fit the assumptions of the Rasch model, items were sufficiently precise to identify 2.6 levels of NBF, PSR of 0.87, and person response strings showed acceptable fit. These preliminary findings suggest that pain/noxious stimuli items did not cohere conceptually with the visual, tactile, and auditory items, which hierarchically align with current literature of DoC.

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Tables

Table 1. Sample characteristics.

Table 2. Sequence of Rasch analyses and summary psychometrics.

Table 3. Item calibrations and fit statistics arranged in hierarchical order from most to least challenging.

Table 4. Inter-rater reliability for each CNC item in hierarchical order from most to least challenging.

Figures

Figure 1. CNC raw score-to-measure nomogram and Wright map.

Figure Captions

Figure 1. CNC stimuli items arranged vertically in order from least challenging at the bottom to most challenging at the top. Rating scale steps for each item are arranged to align horizontally with logit ruler. CNC total raw score arranged to align with logit ruler; raw scores are non-linear so a one-point change at the extremes represents a greater logit change than in the mid-range of the scale. Person measures are in logits; histogram is aligned with logit ruler so that those with less neurobehavioral function on the left and those with more neurobehavioral function on the right.

Supplemental Material

Supplemental Material File. Expanded Rasch Analytic Procedures.

Supplemental Figure 1. Generation of the Calibration and Validation Samples.

440 Supplemental Figure 2. Comparison of Person Measures Calibrated with and without
441 Pain Items.
442 Supplemental Figure 3. Comparison of Person Measures from the Validation Sample
443 Unanchored and Anchored.
444 Supplemental Figure 4. Comparison of Item Measures from the Calibration and
445 Validation Samples.
446 Supplemental Table 1. Results of principal components analysis of residuals across 10
447 simulated samples.
448 Supplemental Table 2. Raw Score to Rasch Measure Conversion Table.

Table 1. Sample Characteristics.

Characteristic	Total n=48
Age, Mean years at Injury (SD)	35.6 (14.9)
Gender, n (%)	
Male	39 (81)
Female	9 (19)
Veteran Status, n (%)	
Veteran	10 (21)
Civilian	38 (79)
Time from Onset to Enrollment, n (%)	
Less than 90 Days	35 (73)
91-180 Days	12 (25)
More than 180 Days	1 (2)
Etiology of Brain Injury, n (%)	
Traumatic	41 (85)
Non-Traumatic	7 (15)
State of Consciousness at Baseline, n (%)	
Minimally Conscious State	15 (31)
Vegetative State	24 (50)
Missing	9 (19)
Tracheostomy at Baseline, n (%)	
Present	41 (86)
Absent	4 (8)
Missing	3 (6)

Table 2. Sequence of Rasch Analyses and Summary Psychometrics.

Analysis	Items	Rating Scale Steps	Person Mean (SD) logits	RMSE	Adj. SD	SI	PSR	Number of Misfitting Items	PCA Eigenvalue 1st contrast (%)	Ceiling Effect n (%)	Floor Effect n (%)
Calibration sample (n=45), PCM	10	29	-0.38 (1.09)	0.60	0.91	1.53	0.70	0	2.34 (15.6%)	N/A	2 (4.4%)
Calibration sample (n=45), PCM, removed two pain items	8	23	-0.43 (1.23)	0.71	1.00	1.41	0.67	1 (Vocalize)	1.89 (13.6%)	N/A	2 (4.4%)
Validation sample (n=45), PCM, removed two pain items, anchored	8	24	-0.31 (1.27)	0.70	1.05	1.49	0.69	1 (Vocalize)	1.99 (13.8%)	2 (4.4%)	5 (11%)
Full Sample (n=451), PCM, removed two pain items, anchored	8	24	-.30 (1.18)	0.68	0.96	1.40	0.66	0	1.81 (13.0%)	11 (2.4%)	13 (2.9%)

Abbrev. RMSE=Root Mean Square Error; Adj. SD=Adjusted Standard Deviation; SI=Separation Index, PSR=Person Separation Reliability, PCA=Principal Components Analysis, PCM=Partial Credit Model, N/A=Not Applicable

*Vocalization item unanchored.

Table 3. Item calibrations and fit statistics, arranged in hierarchical order from most to least challenging.*

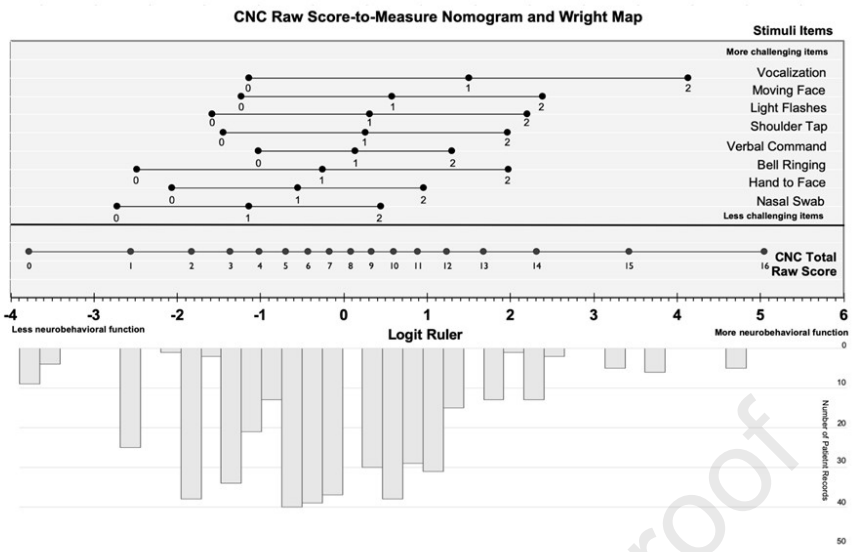
Items	Measure (calibration)	Std. Error	Infit MnSq	Infit zstd	Outfit MnSq	Infit zstd	Disp.
Vocalization**	1.50	0.11	1.37	4.7	1.34	3.7	0.00
Visual (Moving Face)	0.58	0.08	0.86	-2.3	0.75	-3.1	-0.08
Visual (Light Flashes)	0.31	0.08	0.69	-5.7	0.64	-5.4	0.27
Tactile (Shoulder Tap)	0.26	0.08	0.93	-1.2	0.86	-1.7	-0.16
Responsivity (Verbal Command)	0.14	0.07	0.98	-0.3	1.16	1.1	-0.20
Auditory (Bell Ringing)	-0.25	0.08	1.01	0.2	0.98	-0.3	0.38
Threat (Hand to Face)	-0.55	0.07	1.04	0.6	1.06	0.7	0.14
Tactile (Nasal Swab)	-1.14	0.08	1.23	3.3	1.45	3.7	-0.27

*n=451 records; item calibrations anchored on values from calibration sample.
**This item is unanchored.
Abbreviations: Std. Error=Standard Error; MnSq=Mean Square; zstd=Standardized Z value; Disp.=Displacement.

Table 4. Inter-rater reliability for each CNC Item in hierarchical order from most to least challenging.

CNC Item	Krippendorff's Alpha
Vocalization	0.34
Visual (Moving Face)	0.95
Visual (Light Flashes)	0.56
Tactile (Shoulder Tap)	0.28
Responsivity (Verbal Command)	0.51
Auditory (Bell Ringing)	0.15
Threat (Hand to Face)	0.84
Tactile (Nasal Swab)	0.32

Abbreviations: CNC=Coma-Near Coma Scale



Appendix 5

Poster Presentation, “The Coma Recovery Scale-Revised (CRS-R): A Rasch Regression using States of Consciousness” ACRM Annual Conference, October 2021

The Coma Recovery Scale-Revised (CRS-R): A Rasch Regression using States of Consciousness

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BACKGROUND

- Misdiagnosis of state of consciousness is around 40% (Giacino et al, 2018)
- CRS-R item scores inform classification of disordered state of consciousness in patients with severe brain injury (Schnakers et al, 2009)
- Items scores are ordinal and can lead to misinference (Merbitz, 1989) ... and misdiagnosis
- Misdiagnosis matters because classification may limit
  - Patient's access to care,
  - Reimbursement for services, and
  - Evaluation of treatment effectiveness

OBJECTIVE

To empirically examine the association between the CRS-R and current Aspen guideline criteria for classification of disordered states of consciousness (Giacino et al, 2002).

DESIGN

Retrospective cohort study using a Rasch analysis.

METHODS

Setting

- Four cohorts: two clinical trials and two rehabilitation centers

Participants

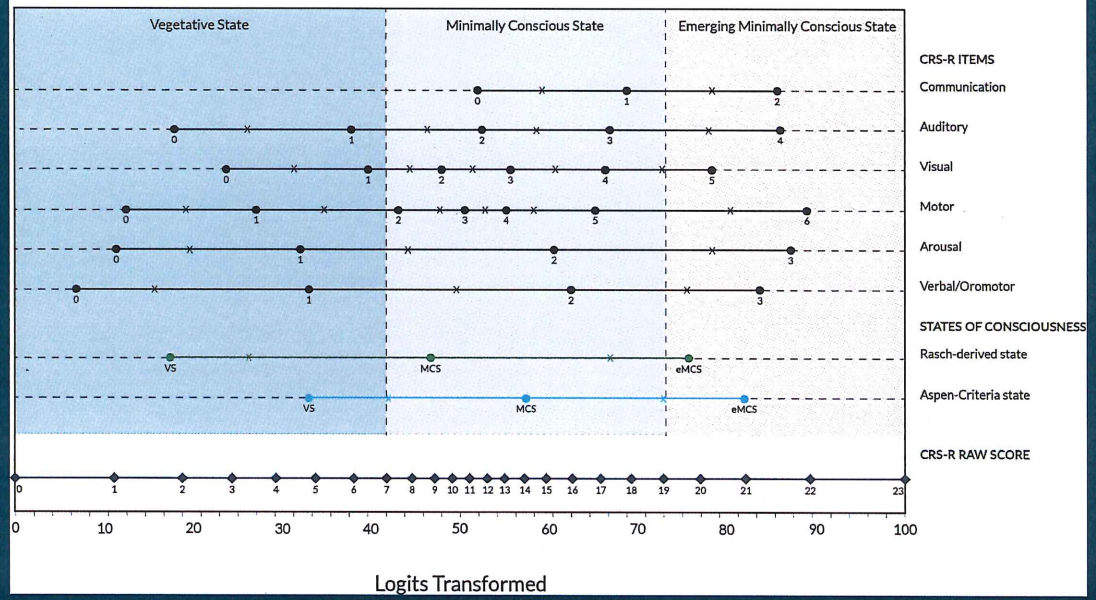
- 264 adults with disorders of consciousness receiving treatment and assessed using the CRS-R.
- 244 Participants had repeated assessments (n=1142 records)

Rasch Analytic Approach

- Generated calibration & validation sub-samples (n=252)
- Calibration sample generated step and item anchors
- Applied calibration anchors to validation subsample
- Applied calibration anchors to full sample (n=1142)

Additional CRS-R items should be considered to indicate eMCS and MCS. This may benefit patients as being in a MCS has implications for access to care.

CRS-R: Raw Score-to-Measure Nomogram and Wright Map



State of Consciousness	Current CRS-R Items Aligning to Aspen Criteria for State of Consciousness	Additional Items Aligning to State of Consciousness based on similar Item Difficulty
MCS	"3 or 4" on Auditory " 2, 3, 4, or 5" on Visual "3-Intelligible Verbalization" on Verbal/Oromotor "3, 4, or 5" on Motor "1-Non-functional: Intentional" on Communication	"2-Localization to Sound" on Auditory "2-Eye Opening without Stimulation" on Arousal "2-Vocalization/Oral Movement" on Verbal/Oromotor
eMCS	"2-Functional: Accurate" on Communication OR "6-Functional Object Use" on Motor	"4-Consistent Movement to Command" on Auditory, "3-Attention" on Arousal, and "3-Intelligible Verbalization" on Verbal/Oromotor

Generating an Empirically Derived Classification for States of Consciousness

- Aspen Criteria (blue line) used to determine states of consciousness
  - MCS: raw score 7, logits 42
  - eMCS: raw score 19, logits 74
- Identified 2 additional item scores (See table 2) within MCS state; 3 additional within eMCS →created new Rasch-Derived State Variable
  - MCS: raw score 3, logits 26
  - eMCS: raw score 17, logits 68
  - Recategorized patients based on new Rasch-derived state of consciousness
- Examined level of agreement between Aspen-criteria and Rasch-derived state of consciousness

RESULTS

Reproducibility

- Calibration and validation sub-samples demonstrated excellent R^2 (>0.95) for Item Measures and Person Measures

Structural Validity (Full Sample)

- All rating scale steps have 10 or more observations and all thresholds proceed monotonically (no disordering)
- Unidimensionality
  - All items fit the model: PCAR Eigenvalue (%variance from the 1st contrast)=1.61 (8.8)
- Measurement Accuracy
  - Wright's PSR (0.95) sufficiently precise for individual-level decisions
  - Person Fit: 15% misfit (n=223)

Level of Agreement: Fisher's Exact Test

Vegetative State

- VS Aspen Criteria: 506 patients
- Rasch-derived Criteria: 327 reclassified as MCS (65%, p<0.001)

Minimally Conscious State

- MCS Aspen Criteria: 711 patients
- Rasch-derived Criteria: 95 reclassified as eMCS (13%, p<0.001)

Emerging Minimally Conscious State

- 100% Agreement between Aspen and Rasch-derived criteria

CONCLUSION

- Transformation of raw scores to logits implies additional CRS-R items scores for MCS and eMCS
- Patients considered VS by clinical criteria may be more accurately classified as MCS
- Implications for access to care

REFERENCES

Available upon request.

Appendix 6

Manuscript: “Determining the Hierarchy of Coma Recovery Scale-Revised Rating Scale Categories and Alignment with Aspen Consensus Criteria for Patients with a Brain Injury: A Rasch Analysis” Journal of Neurotrauma (Under Review)

Determining the Hierarchy of Coma Recovery Scale-Revised Rating Scale Categories and Alignment with Aspen Consensus Criteria for Patients with Brain Injury: A Rasch Analysis

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Abstract

This study aimed to empirically evaluate the hierarchical structure of the Coma Recovery Scale-Revised (CRS-R) rating scale categories and their alignment with the Aspen consensus criteria for determining disorders of consciousness (DoC) following a severe brain injury. DoC includes a range of states from comatose, Vegetative State/Unresponsive Wakefulness Syndrome (VS/UWS), to the Minimally Conscious State (MCS). The CRS-R includes rating scale categories that indicate whether a patient has emerged from MCS (eMCS). CRS-R data from 262 patients with DoC following a severe brain injury were analyzed applying the partial credit Rasch Measurement Model. Rasch Analysis produced logit calibrations for each rating scale category. 28 of the 29 CRS-R rating scale categories were operationalized to the Aspen consensus criteria. We expected the hierarchical order of the calibrations to reflect Aspen consensus criteria. We also examined the association between the CRS-R Rasch person measures (indicative of performance ability) and states of consciousness as determined by the Aspen consensus criteria. Overall, the order of the 29 rating scale category calibrations reflected current literature regarding the continuum of neurobehavioral function: category 6 'Functional Object Use' of the Motor item was hardest for patients to achieve; category 0 'None' of the Oromotor/Verbal item was easiest to achieve. Of the 29 rating scale categories, six were not ordered as expected. Four rating scale categories reflecting the VS/UWS had higher calibrations (reflecting greater neurobehavioral function) than the easiest MCS item (category 2 'Fixation' of the Visual item). Two rating scale categories, one reflecting MCS and one not operationalized to the Aspen consensus criteria, had higher calibrations than the easiest eMCS item (category 2 'Functional: Accurate' of the Communication item). CRS-R person measures (indicating amount of neurobehavioral function) and states of consciousness, based on Aspen consensus criteria,

showed a strong correlation (r_s=0.86, p<0.01). Our study provides empirical evidence for revising the diagnostic criteria for MCS to also include category 2 ‘Localization to Sound’ of the Auditory item and for eMCS to include category 4 ‘Consistent Movement to Command’ of the Auditory item. The strong association between the CRS-R person measures and states of consciousness further supports the use of the CRS-R for diagnostic purposes.

Keywords: disorders of consciousness, measurement, outcome assessment, brain injury

Abbreviations

Coma Recovery Scale-Revised (CRS-R)

Disorders of Consciousness (DoC)

Emerged from Minimally Conscious State (eMCS)

Minimally Conscious State (MCS)

Principal Components Analysis of Residuals (PCAR)

Person Separation Reliability (PSR)

Unresponsive Wakefulness Syndrome (UWS)

Vegetative State (VS)

Accurate diagnosis of state of consciousness among adults with disorders of consciousness (DoC) following brain injury is critical because it is associated with prognosis. In the United States, prognosis for recovery from DoC influences access to specialty DoC rehabilitation services.[1] DoC includes a range of states from coma (no arousal, sleep wake cycles, or awareness), vegetative state/unresponsive wakefulness syndrome (VS/UWS, presence of wakefulness without awareness), to the minimally conscious state (MCS, inconsistent volitional behavior).[2] Patients who have emerged from MCS (eMCS) demonstrate consistent functional behavior.[2] To address the need for accurate diagnosis, the Aspen Neurobehavioral Conference Workgroup defined diagnostic criteria for VS/UWS and MCS and proposed criteria for eMCS based on an evidence review and expert consensus.[3] Nonetheless, differentiating DoC based on behavioral observations remains challenging because certain behaviors may occur infrequently and random movements can be interpreted as volitional behavior.[4]

The Coma Recovery Scale-Revised (CRS-R) is the reference standard for assessment of neurobehavioral function and is used to diagnose DoC.[5] The CRS-R consists of six items (i.e., subscales): Auditory, Visual, Motor, Oromotor/Verbal, Communication, and Arousal. Previous psychometric analysis of the CRS-R demonstrated unidimensionality and monotonicity, indicating all items reflect the concept of neurobehavioral function and logit values for the rating scale categories (i.e., scores on each subscale) occur in order.[6] Analysis using the Rasch Partial Credit Model established the item hierarchy based on average item measure, from most to least neurobehavioral function, as Communication, Oromotor/Verbal, Auditory, Visual, Motor, and Arousal for individuals with a traumatic brain injury.[7] For the purposes of applying and interpreting the Rasch model, the CRS-R subscales are treated as ‘items’ and each score

achieved within a subscale is a 'rating scale category'. The prior analysis did not describe the hierarchy of the 29 rating scale categories.[7]

Prior work operationalized 28 of the 29 CRS-R rating scale categories to the Aspen consensus criteria for VS/UWS, MCS, and eMCS (Table 1).[8] More specifically, the CRS-R criteria for diagnosis of VS/UWS is delineated with 15 categories, MCS is delineated with 11 categories, and eMCS is delineated with 2 categories (Table 1).[8] Diagnosis of VS/UWS requires the patient to achieve a rating scale category operationalized to VS/UWS on every item; whereas, diagnosis of MCS or eMCS requires the patient to achieve an operationalized rating scale category for only one item. Although the CRS-R item hierarchy is established, empiric evidence is required to support whether the rating scale categories are accurately operationalized to VS/UWS, MCS, and eMCS.

One study demonstrates that eMCS could be diagnosed with an additional CRS-R rating scale category[9], which differs from how the Aspen consensus criteria are operationalized to the CRS-R. Specifically, two rating scale categories indicative of eMCS (category 6 'Functional Object Use' of the Motor item and category 2 'Functional: Accurate' of the Communication item), were compared with the occurrence of category 4 'Consistent Movement to Command'of the Auditory item (indicative of MCS). Findings indicated these three rating scale categories occured at the same time for 50% of the participants.[9]

The current study builds on this work in several important ways: the primary purpose of this study is to empirically evaluate the hierarchical ordering of the CRS-R rating scale categories and their alignment with each of the Aspen consensus criteria for VS/UWS, MCS, and eMCS. We hypothesize that the two rating scale categories indicative of eMCS will be in the same approximate upper logit region of the neurobehavioral function continuum while the 11

rating scale categories indicative of MCS and 15 rating scale categories indicative of VS/UWS will be approximately located in markedly different and lower regions of the continuum (Table 1). A secondary purpose of this study is to examine the association between CRS-R Rasch person measures (indicative of person ability level and akin to a total raw score) and Aspen consensus criteria to substantiate that the VS/UWS, MCS, and eMCS are distinctly different.

Methods

Data Sources

The CRS-R data set for this study was assembled from four cohorts: two clinical trials and two rehabilitation hospitals. One clinical trial administered amantadine or placebo and all participants received inpatient rehabilitation services.[10] The other clinical trial administered active or placebo repetitive transcranial magnetic stimulation.[11] The two rehabilitation hospitals are located in metropolitan areas in the Midwest and Southern regions of the United States. Following IRB approval from [institution], data were aggregated into a single data set.

Participants (n=262) were included if they were ≥ 14 years old and had DoC from a brain injury. Participants had at least one CRS-R assessment and up to 37 re-assessments, resulting in a dataset of 1,142 CRS-R records. The CRS-R was administered and scored by a rehabilitation practitioner or trained researcher. Data were also collected on age, time from onset to study enrollment or rehabilitation admission, and etiology of the brain injury.

Measure

Coma Recovery Scale-Revised. Each CRS-R item (i.e., subscale) includes a hierarchical ordering of rating scale categories (i.e., scores); a higher score indicates more neurobehavioral function. The assessor begins with the highest rating scale category; if a response is observed and

meets the scoring criteria, the assessor moves to the next item.[12] If no response is observed or the response does not meet the scoring criteria, the assessor continues down to the next rating scale category for that item. The number of rating scale categories varies by item; for example, Communication has three rating scale categories (2, 1, 0) while Motor has seven rating scale categories (6, 5, 4, 3, 2, 1, 0). Total CRS-R raw scores range from 0-23. The CRS-R Administration and Scoring Guidelines can be found on the Rehab Measures database.[13]

States of Consciousness. The CRS-R items and rating scale categories used to diagnose VS/UWS, MCS and eMCS, based on the Aspen consensus criteria (Table 1), were applied to categorize all CRS-R records (n=1142) using STATA SE 14.[8] All analytic procedures and results refers to **states of consciousness**, based on the Aspen consensus criteria, unless otherwise specified.

Analytic Procedures

The partial credit Rasch Measurement Model was applied using Winsteps version 4.0.1.[14] Since a score of a 1, for example, is qualitatively different for each item, the partial credit model allows for this item by item variation, enabling each item to have its own rating scale structure .[15] Following the Rasch Reporting Guideline for Rehabilitation Research (RULER),[16] we examined the reproducibility and structural validity of the CRS-R in order to identify the hierarchy of the CRS-R rating scale categories. Reproducibility refers to whether the CRS-R assessment results are comparable across individuals. Structural validity refers to whether the items, rating scale categories, and persons cohere on the measure and reflect the requirements of the Rasch model. Second, we evaluated the extent to which Aspen consensus criteria align with the rating scale category hierarchy. Finally, we examined the association between the CRS-R Rasch person measures and states of consciousness.

Reproducbility

We addressed the potential for local dependency among persons in the full sample (since the dataset included repeated measures of the same individuals) by generating two random subsamples—calibration and validation sub-samples—in which each individual is represented once by either their first or last record.[17] The calibration and validation sub-samples were represented by 242 participants with either their first or last record. Twenty participants with a single record were randomly assigned for a total of 252 participants in the calibration and validation sub-sample, respectively (Figure 1).

The calibration sub-sample was used to produce step and item anchors; these were validated with the validation sub-sample. Luppescu's method of cross-plotting person measures and item calibrations with 95% confidence intervals was used to evaluate whether there were significant deviations between the calibration and validation sub-samples.[18] Step and item anchors were validated by evaluating item displacement >.50 logits.[19] Once validated, the step and item calibrations from the calibration sub-sample were applied as anchored values to the full sample.[15]

Structural Validity

Structural validity was examined in terms of rating scale category structure, unidimensionality, hierarchical order, and measurement accuracy.

Rating Scale Category Structure. Rating scale categories for each item were examined to ensure that each had sufficient observations and that the Andrich thresholds proceeded monotonically.[20] Rating scale categories are defined by the average category difficulty measure.[21,22] Categories with low frequencies (fewer than 10 observations) do not provide enough observations for stable category measures.[15]

Unidimensionality. Unidimensionality refers to the items measuring one underlying trait, neurobehavioral function, in the case of the CRS-R.[15] Unidimensionality was evaluated by level of item fit, principal component analysis of residuals, and by amount of local item dependence. Items with an infit mean square >1.4 or <0.6 were considered misfitting (i.e., may not represent the same underlying trait of neurobehavioral function).[23] Principal component analysis of residuals (PCAR) and disattenuated correlations were also used to evaluate the extent to which items and categories share a similar underlying trait. Disattenuated correlations above 0.82 indicate items are likely measuring the same underlying trait.[24] We also examined the residuals of each item to determine if items are duplicative.[25] Local item dependence was analyzed by evaluating the inter-item correlations. Inter-item correlations >0.70 indicate local dependence which violates the assumptions of the Rasch measurement model.

To confirm item fit and PCAR, we used a more stringent technique in which we generated ten simulated data sets based on the calibration data and fit model assumptions to identify more precise upper and lower bounds for infit mean square, Eigenvalue, and percent variance of the first contrast.[26] In Winsteps, the Simulated Data File (SIFILE) output was specified based on 1) the request for 10 data files, 2) using the data for the simulation, 3) no resampling of persons, 4) allowing for missing data to maintain the same data pattern, and 5) allowing for extreme scores. The ten data sets were imported into STATA to calculate the more precise upper (97.5%) and lower (2.5%) bounds for infit mean square, ZSTD, Eigenvalue, and percent variance of the first contrast.

Hierarchical Order. We generated logit calibrations for the average item difficulty and each rating scale category. We examined the hierarchical order of the CRS-R rating scale categories as they relate to the Aspen consensus criteria.

Measurement Accuracy. The separation index and person separation reliability generated from Winsteps software program were used to examine the measurement precision and ability of the assessment to distinguish among patients with different states of consciousness. Wright's sample-independent PSR is reported for our analysis as a Shapiro Wilk test determined our data were non-normal (test statistic=0.93, $p<0.01$). The person strata index indicates how many statiscally distinct states of consciousness the assessment can distinguish.[27,28]

We evaluated the alignment between the distribution of persons and items by comparing the mean person measures and mean item calibrations. Ceiling and floor effects were reported to describe how well the items aligned with the range of person neurobehavioral function measures. Persons with unexpected patterns of responses were identified via infit mean squares. These unexpected patterns can often be clinically useful in identifying people with particular conditions.[29]

Score to Measure Conversion. The Rasch model transforms ordinal scores into equal-interval logit measures. To enhance clinical interpretation of findings, we generated a CRS-R raw score conversion to Rasch person measures.

Alignment of Aspen Consensus Criteria, Rasch-based Person Measures, and CRS-R Rating Scale Categories

The distribution of the CRS-R Rasch person measures was described by mean and SD for VS/UWS, MCS, and eMCS. To confirm that the VS/UWS, MCS, and eMCS were statistically significantly different, we used a one-way ANOVA. The Bartlett test indicated unequal variances, so we also used a Kruskal Wallis test to describe the presence of differences in the mean CRS-R Rasch person measures across states of consciousness. The association between Rasch person measures and states of consciuosness was examined via Spearman's correlation

coefficient. The strength of the correlation coefficients <0.25 were interpreted as having little or no association, 0.25 to 0.50 a low to fair association, >0.50 to 0.75 a moderate to good association, and >0.75 a strong association.[30]

Results

Participants by Samples

Of the 262 participants, ninety-seven percent (n=254) were receiving therapy at an intensive rehabilitation setting; seventy-three percent (n=192) were enrolled in a clinical trial. Participants were mostly male (70%), in a MCS (74%), after sustaining a traumatic brain injury (92%) (Table 2). The average age of participants was 36.5±15.2 years (range: 14-82 years).

Analytic Process and Reproducibility

Table 3 presents the sequence of analytic steps. During the first iteration (calibration sample) (Table 3, row 1), the six CRS-R items had good precision (Wright's PSR=.95) and no misfitting items; inter-item correlations indicated no local item dependence.[25,31] Items were slightly more challenging than the person ability (mean person measure (-0.35 ± 1.98 logits). Twenty-two (8.7%) of individuals reached the assessment's ceiling and there was a negligible floor effect. PCAR indicated items generally reflect the same underlying trait (Eigenvalues 1.63; percent variance of the first contrast 8.1%) and this was further confirmed via inspection of the loadings (disattenuated correlations >0.82 for all item contrasts). The second iteration, the validation sub-sample (Table 3, row 2), had comparable results to the calibration sub-sample. Therefore, for the third iteration (Table 3, row 3), we applied the step and item anchors from the calibration sub-sample to the validation sub-sample. Comparison of the person measures from the validation sample unanchored (Table 3, row 2) and anchored (Table 3, row 3) were consistent (Person $R^2 = 0.99$; Supplemental Figure S1). Comparison of the item calibrations were

also consistent across validation sub-samples (Items $R^2 = 0.98$; Supplemental Figure S2); no displacement was greater than 0.50 logits.[19] For the final iteration (Table 3, row 4), the step and item calibrations from the calibration sub-sample were applied to the full sample. All results below refer to the ***full sample*** unless otherwise specified.

Structural Validity

Rating Scale Category Structure. All rating scale categories had 10 or more responses for the calibration and full sample indicating confidence in the stability of the category measures. The validation sub-sample had less than 10 responses for rating scale category 0 on Motor (n=6) and Arousal (n=8). Rating scale categories were monotonic for all items in the calibration, validation, and full samples indicating category logits all proceeded in the same direction.

Unidimensionality. Items from the calibration, validation, and full sample each fit the measurement model with the infit mean square ranging from 0.80 to 1.36 across samples (Table 4). The ten simulated datasets identified a more stringent infit mean square criteria range of 0.78 to 1.22 and ZSTD of -2.21 to 2.00.[26,32] The calibration sample met this stringent infit mean square criteria with all items falling between 0.84 and 1.12. Two items misfit for the validation and full samples using the more restrictive criteria: Motor (1.31 and 1.21, respectively) and Oromotor/Verbal (1.36 and 1.27, respectively).

The PCAR Eigenvalue for the first contrast of the full sample (Table 3, row 4) was 1.61 with 8.8% unexplained variance from the first contrast, which was comparable with average values derived from the 10 simulated data sets (Eigenvalue of 1.44 and 5.4% unexplained variance in the first contrast, Supplemental Table S1).[26,32] Disattenuated correlations were >0.85 suggesting the same underlying trait, posited to be neurobehavioral function, was being captured by the items.

Hierarchical Order. Item order from least to most challenging was Verbal (average item calibration -0.72), Arousal, Motor, Visual, Auditory, and then Communication (average item calibration 1.99) (Table 4). The average rating scale category calibrations from least to most challenging were category 0 'None' of the Verbal item and category 6 'Functional Object Use' of the Motor item, respectively. Table 5 provides logit values (calibrations) for each rating scale category in order from least to most challenging; also indicated are the items (i.e.,subscales) and state of consciousness.

Measurement Accuracy. Wright's PSR for the full sample is 0.95 and equates to 4.5 statistically different strata. The unadjusted person separation reliability was 0.83 (separation index was 2.20) (Table 3).

The mean person measure was -0.44 (±1.75) logits less than the mean item calibration. There was no appreciable floor effect (0.1%) and a minimal ceiling effect (4.9%) (Table 3).[33-35] Person misfit was consistent across samples at 18%, 20%, and 19% for the calibration, validation, and full samples, respectively.

Score to Measure Conversion. Total CRS-R raw scores range from 0 to 23 and correspond to person measures of -6.52 to 5.85 logits. A full score-to-measure table is provided in Supplemental Table S2. Figure 2 displays a visual ruler for converting the CRS-R total raw scores into Rasch logit calibrations.

Alignment of Aspen Consensus Criteria with CRS-R Rating Scale Categories and CRS-R Rasch Person Measures

The 15 CRS-R rating scale categories for VS/UWS have Rasch category calibrations ranging from -5.66 to 1.2 logits (Table 5). The 11 CRS-R rating scale categories for MCS have Rasch category calibrations ranging from -0.59 to 4.13 logits. The two CRS-R rating scale

categories for eMCS had Rasch category calibrations ranging from 4.06 to 4.49 logits. Four rating scale categories reflecting VS/UWS had higher logit calibrations than the lowest MCS rating scale category (2 'Fixation' of the Visual item; Table 5). Two rating scale categories, 4 'Consistent Movement to Command' of the Auditory item reflecting MCS and 3 'Attention' of the Arousal item (not aligned to the Aspen consensus criteria), had higher logit calibrations than the lowest eMCS rating scale category (2 'Functional: Accurate' of the Communication item; Table 5). Rating scale category 3 'Intelligible Verbalization' of the Oromotor/Verbal item reflecting MCS was within 0.25 logits of the the lowest eMCS rating scale category (2 'Functional: Accurate' of the Communication item; Table 5) indicating comparable difficulty.

CRS-R person measures were summarized for each state of consciousness: VS/UWS mean -2.0±0.87SD, range -5.12 to 1.19 logits (raw score 0 to 16), MCS mean -0.01± 1.00SD, range -2.88 to 3.63 logits (raw score 4 to 21), and eMCS mean 2.65 ± 1.86SD, range -0.43 to 5.84 logits (raw score 10-23) (Table 6). The Bartlett test from the one-way ANOVA (F=453.5, df=2, p=0.00) confirmed the variances of the CRS-R person measures were statistically different for each state of consciousness (VS/UWS, MCS, and MCS; X^2=20.43, p<0.001) thus we conducted an equivalent non-parametric test; the non-parametric Kruskal-Wallis test indicated the mean ranks of CRS-R person measures were statistically different across each state of consciousness (H(2)=194.74, p<0.01). Correlation between states of consciousness and CRS-R person measures indicates a strong relationship (r_s=0.86, p<0.01).[33]

Discussion

The empirical evaluation of the CRS-R rating scale categories, as they are operationalized to the Aspen consensus criteria, indicated the order of the 29 rating scale category calibrations reflected current literature regarding the continuum of neurobehavioral

function: category 6 ‘Functional Object Use’ of the Motor item was hardest for patients to achieve; category 0 ‘None’ of the Oromotor/Verbal item was easiest to achieve. Six categories do not occur in the expected sequential hierarchical order. Two rating scale categories, one reflecting MCS and one not operationalized to the Aspen consensus criteria, had higher calibrations than the easiest eMCS item (category 2 ‘Functional: Accurate’ of the Communication item). A third rating scale category was within 0.25 logits of the easiest eMCS item; rating scale categories that are close together on the hierarchy are of comparable difficulty and reflect a similar level of neurobehavioral function. There are also four rating scale categories reflecting the VS/UWS that had higher calibrations (reflecting greater neurobehavioral function) than the easiest MCS item (category 2 ‘Fixation’ of the Visual item) (Figure 2, Table 5). CRS-R person measures (indicating amount of neurobehavioral function) and states of consciousness, based on Aspen consensus criteria, showed a strong correlation (r_s=0.86, $p<0.01$).

The three rating scale categories near the two eMCS categories all had average category calibrations greater than the mean person measure for eMCS (3.65 logits, Table 6). Further, the rating scale hierarchy exhibited category 4 ‘Consistent Movement to Command’ of the Auditory item to be slightly more challenging than category 2 ‘Functional:Accurate’ of the Communication item and less challenging than category 6 ‘Functional Object Use’ of the Motor item. Prior work established that category 4 ‘Consistent Movement to Command’ of the Auditory item is a behavior that occurs approximately at the same time as the two categories reflecting eMCS.[9,36] Our study substantiates this finding when examining average category difficulty and category 4 ‘Consistent Movement to Command’ of the Auditory item should be included in the diagnostic criteria for eMCS.

This is the first study to demonstrate that category 3 ‘Attention’ of the Arousal item and category 3 ‘Intelligible Verbalization’ of the Oromotor/Verbal item may also reflect comparable ability to other eMCS categories. For a patient to achieve category 3 ‘Attention’ of the Arousal item, the patient must respond to all but three of the verbal or gestural prompts demonstrating sustained attention and consistency throughout the administration of the CRS-R. For a patient to achieve category 3 ‘Intelligible Verbalization’ of the Oromotor/Verbal item, the patient must be able to vocalize, write, or use an alphabet board to communicate two words with a consonant-vowel-consonant triad. Patients who are able to achieve these rating scale categories are demonstrating an ability level that is similar to a patient demonstrating functional communication. These empirical findings suggest these additional categories are also indicative of eMCS and warrant further substantiation in future studies.

Four rating scale categories that reflect VS/UWS covered ranges of the continuum that overlapped with the range of some MCS rating scale categories. These four rating scale categories include: 0 ‘None’ of the Communication item, 2 ‘Eye Opening Without Stimulation’ of the Arousal item, 2 ‘Vocalization/Oral Movement’ of the Oromotor/Verbal item, and 2 ‘Localization to Sound’ of the Auditory item. However, because the range of these categories were wide, and most of the range aligned with other VS/UWS categories, it is likely that only category ‘2 Localization to Sound’ of the Auditory item is really indicative of MCS. Whereas, the other three rating scale categories may be indicative of VS/UWS and/or MCS dependent upon the patient’s behavior.

Category 2 ‘Localization to Sound’ of the Auditory item is likely between -0.6 to 0.83 logits (Figure 2), which is of similar difficulty to the range for category 2 ‘Fixation’ of the Visual item (-0.89 to -0.09 logits, Figure 2). ‘Localization to Sound’ requires the patient to orient

towards the auditory stimulus twice in at least one direction demonstrating awareness and a behavior in response to a specific stimuli, a key feature of MCS diagnostic criteria.[3] Thus, empirically and qualitatively category 2 'Localization to Sound' of the Auditory item should be included in the diagnostic criteria for MCS.

The other three rating scale categories reflective of VS/UWS have average calibrations within the range of both VS/UWS and MCS. Each covers a wide range of more than 3 logits: category 0 'None' of the Communication item ranges from -6.52 to 0.85 logits, category 2 'Eye Opening Without Stimulation' of the Arousal item from -1.05 to 3.32 logits, and category 2 'Vocalization/Oral Movement' of the Oromotor/Verbal item from -0.51 to 2.88 logits. These wide ranges reflect there is a range of person ability. Of note, the Communication item is only scored when there is evidence of command following on the Auditory item (e.g., rating scale categories 3 and 4, a rating scale category of 3 is achieved on the Oromotor/Verbal item, or if there is evidence of spontaneous communication).[13] So while the average category measure aligns with MCS categories, category 0 'None' of the Communication item does not qualitatively describe MCS behavior. Similarly, category 2 'Eye Opening Without Stimulation' of the Arousal item may reflect patients in a VS/UWS that continuously have their eyes open, patients in MCS who are able to have their eyes open and attend to some verbal or gestural prompts, and patients eMCS. Lastly, category 2 'Vocalization/Oral Movement' of the Oromotor/Verbal item includes patients demonstrating non-reflexive oral movements and those expressing one intelligible word that is contingent or spontaneous.[13] These rating scale categories require further investigation to better align them to an appropriate diagnostic category. The wide logit ranges for these three category measures suggests the need to split the category, which may help better distinguish patient behaviors reflective of VS/UWS and MCS.

Our work determined that patients categorized as VS/UWS, MCS, and eMCS are distinctly different groups when measured by Rasch analysis. We examined the CRS-R Rasch person measures relative to the states of consciousness and found a strong positive correlation, providing further empirical support for using the CRS-R for diagnostic purposes. This study provides empiric evidence that the CRS-R is useful for diagnosis and that additional CRS-R rating scale categories should be considered for diagnosing MCS and eMCS.

Limitations & Next Steps

The present study is a retrospective analysis of CRS-R data from individuals with DoC after brain injury who were receiving inpatient rehabilitation services or participating in a clinical trial. Our analysis did not include patients in a comatose state, which is the lowest level on the continuum of DoC. The previous study that operationalized the CRS-R rating scale categories to the Aspen consensus criteria[8] did not consider the comatose state, limiting the ability to identify patients who transition from comatose to VS/UWS. To our knowledge, this is the first study to align category 2 'Localization to Sound' of the Auditory item with diagnostic criteria for MCS; thus it should undergo further validation. Interrater reliability of each CRS-R rating scale category was not examined; it is possible rating scale categories with better interrater reliability may reflect increased confidence in scoring particular behaviors reflective of a particular state of consciousness. We did not evaluate the influence of rater severity/leniency, a rater who consistently scores more severely or more leniently, on person measures.[37]

Future research should examine rater severity/lenience and interrater reliability as these may impact which rating scale category is selected and therefore influence diagnosis. Future research is also needed to examine cut-points for the transition from VS/UWS to MCS and MCS to eMCS based on the Rasch measures. The hierarchy of the rating scale categories may be

impacted by the administration guidelines (e.g., administer the communication item only when certain criteria have been achieved). Therefore, a future study should replicate these findings when all rating scale categories are administered for each item.

Conclusion

Accurately diagnosing disorders of consciousness following a severe brain injury is important as it relates to clinical decision making. This Rasch analysis indicated a hierarchy of the CRS-R rating scale categories that support a unidimensional construct of neurobehavioral function. The CRS-R's high person separation reliability in this analysis indicates it is sufficiently precise for making reliable and consistent individual-level decisions. The strong association between the CRS-R person measures and states of consciousness further supports the use of the CRS-R for diagnostic purposes. Our study provides empirical evidence for revising the diagnostic criteria for MCS and eMCS. A patient achieving category 2 'Localization to Sound' of the Auditory item is empirically and qualitatively indicative of MCS and a patient achieving category 4 'Consistent Movement to Command' of the Auditory item is indicative of eMCS. The CRS-R should be used in lieu of unstructured clinical observations for evaluating disorders of consciousness when critical decisions about care are being made.

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Authors' Contribution Statements:

JW: Conceptualization (lead), Data curation (lead), Formal analysis (lead), Project administration (lead), Software (lead), Writing-original draft (lead), Writing-review and editing (lead). AC: Formal analysis (supporting), Writing -reviewing and editing (supporting). KO: Resources (supporting), Writing-reviewing and editing (supporting). PH: Resources (supporting), Writing-reviewing and editing (supporting). JG: Resources (lead), Writing-reviewing and editing (supporting). JW: Resources (lead), Writing-reviewing and editing (supporting). TBP Resources (supporting), Writing-reviewing and editing (supporting). PVDW: Conceptualization (supporting), Supervision (supporting), Writing-reviewing and editing (supporting). TM: Conceptualization (supporting); Formal analysis (supporting); Methodology (lead); Supervision (lead); Writing-reviewing and editing (supporting).

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Appendix 7

Letter to the Editor in the Journal of Head Trauma and Rehabilitation describing the revised MDC results

J Head Trauma Rehabil
Vol. 35, No. 5, p. 363

Special Feature

Letter to the Editor

Trudy Mallinson, PhD, OTR/L; Jennifer Ann Weaver, MA, OTR/L; Ann Guernon, MS, CCC-SLP/L; Theresa Bender Pape, DrPH, MA, CCC-SLP/L

IN OUR 2016 ARTICLE, examining the responsiveness of the Disorders of Consciousness Scale (DOCS-25), we reported the minimal detectable change (MDC) along with several other indices of responsiveness including anchor and distribution-based minimally clinically important differences (MCIDs).[1] Similar to others, we used a formula in which the SEM was included within the square root. Bland[2] points out the correct formula for the MDC is when the standard error of measurement (SEM) is external to the square root ($MDC_{95} = 1.96 \times SEM \times \sqrt{2}$).[2] We have recalculated the MDC for Rasch-transformed DOCS-25 person measures using this formula: 9.98, 11.22, and 11.47 for nonimprovers, improvers, and all participants, respectively. These MDC indices apply to Rasch-transformed person measures and not to total raw scores. The revised MDC is somewhat larger than our previously reported anchor-based MCID (8.6).[1]

As noted in our earlier article, MDCs can be clinically useful, particularly in early phases of recovery when patients may demonstrate fluctuating levels of neurobehavioral function on a day-to-day basis. Knowing when such change is beyond measurement error better enables clinicians to identify when variation is consequential enough to warrant attention. Clinicians may find MCIDs useful for informing treatment decisions such as when a change in intervention strategy may be warranted. In addition, anchor-based MCIDs may support clinicians to engage families in discussions about treatment goals.

We encourage readers to use discretion when applying this type of MDC since the calculation assumes that measurement error is consistent across all total raw scores, which it is not.[3] For Rasch-based measures, the standard error is larger at the ends of the range and smaller in the middle of the range; for raw score scales the standard error is larger at the middle of the range and smaller in the ends.[3] To provide rehabilitation clinicians with person-centered indices of change, future studies could examine alternative MDC approaches conditioned on patient admission and discharge measures.[4]

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Appendix 8

Poster, “Defining Clinically Meaningful Change with the Coma Near-Coma Scale” Disseminated at the 2017 ACRM conference

Defining Clinically Meaningful Change with the Coma Near-Coma Scale

Jennifer Weaver, MA, OTR/L, CBIS[1,2]; Trudy Mallinson, PhD, OTR/L, FAOTA[1,3] ; Theresa Pape, DrPH, MA, CCC-SLP/L[4]; Ann Guernon, MS, CCC-SLP/L, CCRC[4,5]; Elyse Walsh, PT, DPT, NCS[4]

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RESEARCH OBJECTIVES

To determine the minimal detectable change (MDC_{95}) and minimally clinically important difference (MCID) of the Coma-Near-Coma scale (CNC) for better assessment of neurobehavioral function (NBF) in patients with severe traumatic brain injury (TBI).

STUDY DESIGN

- Design: Retrospective cohort
- Setting: Post-acute rehabilitation hospitals
- Participants: 30 patients with disorders of consciousness following TBI
- Intervention: N/A
- Data Analyses: Stata 14.2 SE & MedCalc version 17.6

Characteristics	Baseline
Age, mean (SD), y	36.8 (13.7)
Sex	
Male	26
Female	4
Veteran status	
Civilian	24
Veteran	6
Time from onset	
<90 d	23
91-180 d	6
>180 d	1
Nature of Injury	
Traumatic	27
Anoxia	3
State of Consciousness at Baseline (n, %)	
Vegetative	17 (57)
Minimally Conscious	9 (30)
Missing	4 (13)

Table 1. Participant Demographics

MAIN OUTCOME MEASURE

COMA NEAR COMA SCALE

- 11 items
  - Olfactory item omitted
- Raw Score
  - Ranges: 0-40
  - Lower score=Greater NBF
- Transformed to Person Measures
  - Ranges: 0-100
  - Higher score=Greater NBF

Parameter	Item No.	Stimulus	# of Trials	Response Measure	Score Outcome	Scale Criteria
ALERTNESS	1	Self-ringing 3 sec, at 10 sec. intervals	3	Eye opening or toward sound	3 2 1 0	1 & 2 1 or 2 No Response
COMMAND RESPONSIVITY	2	Request patient to open or close eyes, mouth or move finger, hand or leg.	3	Response to command	4 3 2 1 0	Responds to command 2 or 3X Tentative or inconsistent 1x No Response
TACTILE	7	Shoulder tap—Top shoulder briskly 2X without speaking to patient; each side	3	Head or eye orientation of stimulator movement to tap	3 2 1 0	Orients toward tap 3 or 2x Partially orients 1x Did not orient to response
PAIN	10	Deltoid nerve pinch/pull x2; each side	3	Withdrawal or other response linked to stimulus	3 2 1 0	Responds 3 or 2x Gen. agitation/non-specific movement 1x No response

Table 2. Examples of CNC Items

RESULTS

All Participants Show Moderate Effect Compared to Large Effect Indicated when participants are grouped by Improvers and Non-Improvers

CNC Status	Baseline, mean (SD)	Follow-up, mean (SD)	SD_{pooled}	r	SEM	Hedge's G ES	Lower 95% CI	Upper 95% CI	SRM
Non-Improvers (n=8)	48.7 (12.3)	37.3 (8.9)	10.7	0.67	6.15	1.06	-1.72	-0.63	1.54
Improvers (n=22)	38.1 (16.8)	49.3 (14.1)	15.5	0.67	8.90	0.73	0.50	1.04	1.19
All participants (n=30)	40.9 (16.2)	46.1 (13.9)	15.1	0.67	8.67	0.35	-0.01	0.627	0.39

Table 3. Responsiveness Indices for the CNC

Calculations & Results

- Participant is an Improver if CNC person measure change was ≥ 0.0
- $SEM = SD_{pooled} \times \sqrt{(1-r)}$
- Hedge's G Effect Size accounts for small sample size
- All participants ES is moderate but ES becomes moderate to large when participants are split based on "Improvers" and "Non-Improvers"
- SRM is a ratio of observed change and the SD
- SRM is moderate for all participants but large when split into Improvers and Non-improvers

Improvers and All Participants With Similar Estimates of the MDC_{95}

CNC Status	MDC_{95}	MCID 0.20/0.33/0.50 SD
Non-improvers (n=8)	6.9	N/A
Improvers (n=22)	8.3	N/A
All participants (n=30)	8.2	3.0/5.0/7.6

Table 4. The Minimally Detectable Change and Clinically Important Difference Estimates for the CNC

Calculations

- $MDC_{95} = 1.96 \times \sqrt{(2 \times SEM)}$
- $MCID\ distribution\ method\ based\ on\ SD_{pooled}$

RESULTS

Patients who Met or Exceeded the Criteria for a Clinically Meaningful Change

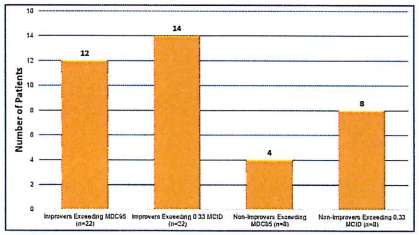

Figure 1. Frequency patients exceeded indices of responsiveness

Grouping Participants

- 55% of Improvers and 50% of Non-Improvers Exceeded the MDC_{95}
- 64% of Improvers and 100% of Non-Improvers Exceeded the 0.33MCID
- Average CNC Person Measure change does not reflect true change beyond measurement error

CNC Measure Change Between Two Timepoints for Non Improvers, Improvers, and All Patients

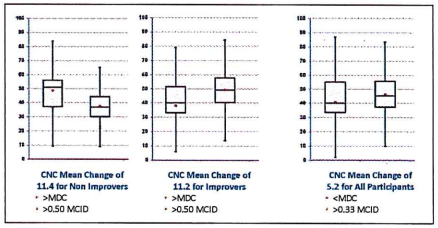

Figure 2. Box and Whisker Plot for Time 1 and Time 2 for each group

DISCUSSION

Indices of Responsiveness

- The size of the Standardized Response Mean (SRM) was relatively large for both improvers and non improvers. Although the SRM was larger for non-improvers, caution should be applied in interpreting this value as the n-size was very small (n=8).
- MDC_{95} gives clinicians an indication of amount of change in NBF needed to surpass measurement error (Copay et al, 2007). In this study, we found that about 8 Rasch-based units of change in CNC is needed to surpass measurement error.
- MCID which reflects distribution (variation) of CNC measures, was less than MDC_{95}. This may reflect limited variability in this small sample or limited ability of the CNC to detect differences among patients.

Implications for Practice

- MDC_{95} allows clinicians to feel more confident in determining a patient's progress beyond measurement error
- Researchers may find the MDC_{95} useful to evaluate clinical outcomes
- Further study on larger samples may be need to confirm the size of clinically meaningful changes using the CNC, particularly for individuals who do not improve.

Limitations

- Small sample size, no external anchor available for determining anchor-based MCIDs

FUTURE DIRECTIONS

- Compare distribution-based MCID with alternative analysis, such as an anchor-based approach
- Assess MCID change on CNC in relation to family and clinician reports of change to determine if it meets the criteria that the change corresponds with family perception of important meaningful change.

FUNDING SOURCE

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Appendix 9

Poster, “Using Method Comparison Approaches to Evaluate the Impact of Rater Severity/Leniency on Patient Measures of Disordered Consciousness”, presented as a poster at the 2018 National Capital Area TBI conference at NIH

Using Method Comparison Approaches to Evaluate the Impact of Rater Severity/Leniency on Patient Measures of Disordered Consciousness

Trudy Mallinson, PhD, OTR/L[1]; Theresa Pape, DrPH, MA, CCC-SLP/L[2]; Ann Guernon, MS, CCC-SLP/L, CCRC [2,3]; Jennifer Weaver, MA, OTR/L[1]
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Research Objective

- To describe a novel application of method-comparison analysis, for quantifying the impact of rater severity/leniency (RSL) on clinician-reported measures of neurobehavioral function in patients with severe disorders of consciousness.

Background

Little attention has been paid to evaluating the extent to which rater severity/leniency affects clinician-reported outcomes (ClinROs) or the extent to which adjusting for RSL results in more precise measurement of neurobehavioral function.

Common interrater reliability coefficients e.g., ICCs, are only useful as a population parameter since they cannot be used to adjust individual patient scores for a particular rater's judgments (Stemler, 2004). Many-faceted Rasch model (MFRM) removes the effect of RSL on patient measures (Linacre, 1994).

This study adapted the Bland-Altman method-comparison technique to evaluate the difference in patient measures with and without adjustment for RSL. Bland-Altman plots compare the difference between two measurement methods to the average of the two measurement methods. We report on the interpretation of agreement and bias in the context of RSL.

Agreement: The extent the two methods produce patient measures that are within the limits of agreement, in our case, within 5 units (a minimally detectable change).

Bias: The overall difference in values obtained and can be either positive (adjusting for RSL results in higher measures) or negative (adjusting for RSL results in lower measures).

Study Design & Methods

Prospective, observational cohort study of 172 patients at 7 post-acute rehabilitation facilities.

Participants: Patients with severe TBI, classified as vegetative or minimally conscious at enrollment, <180 days post-injury.

Raters: 48 rehabilitation clinicians trained to score the DOCS (occupational therapists n=13, physical therapists n=8, speech-language pathologists n=20, other disciplines n=7).

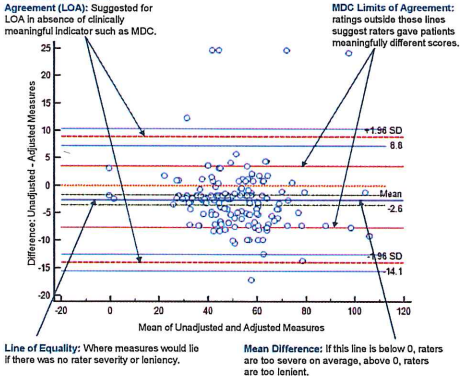
Outcome Measure: Behaviors elicited by 25 DOCS sensory stimuli, weekly for 6 weeks.

Data Analysis: Data were analyzed with and without adjustment for RSL. Previous work established the minimally detectable change (MDC) for the DOCS is 5 units.

MFRM: Facets® software, which adjusts for rater severity.

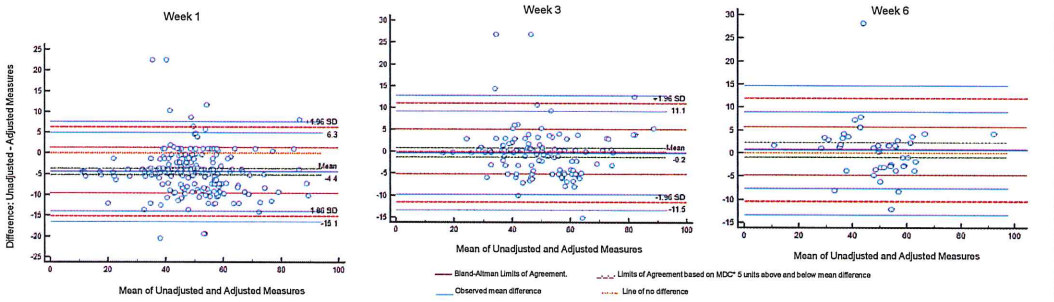
Bland-Altman Plots: MedCalc® software.

Anatomy of a Bland-Altman Plot



Principal Findings

Figure 1. Bland-Altman Plots Showing Agreement and Bias in Ratings With and Without Adjustment for Rater Severity/Leniency

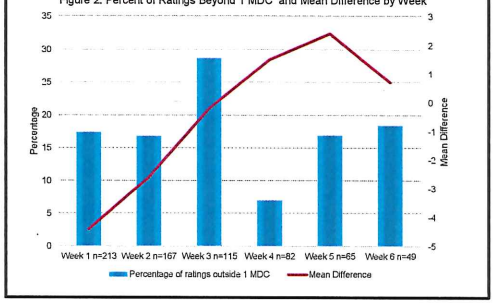


Unadjusted NBF measures are too low (bias -4.4); 17% of adjusted measures exceed 1 MDC*.

Unadjusted and adjusted measures similar (bias -.02); 29% of adjusted measures exceed 1 MDC*.

Unadjusted and adjusted measures similar (bias 0.7); 20% of adjusted measures exceed 1 MDC*.

Figure 2. Percent of Ratings Beyond 1 MDC and Mean Difference by Week



Percentage of measures exceeding 1 MDC (lack of agreement) and mean difference (bias) varies by week, based severity/leniency of different raters.

Conclusions

- Bias ranged from trivial to detrimental, and the changes in direction from week to week indicate that specific raters at each assessment period are driving observed differences.
- Further, more than 5% of patient measures exceeded the limits of agreement at each assessment period indicating that adjusting for RSL produces important impact on outcome measures. Urinary and fecal incontinence
- RSL makes a quantifiable difference in outcome measures for patients with disordered consciousness, affecting ALL rater-mediated ClinROs (Eckes, 2009) and has significant potential to misrepresent a drug's or a treatment's effect.
- To be useful as an endpoint for adequate and well-controlled clinical trials providing substantial evidence of drug effectiveness, rater-mediated measures of neurobehavioral function must account for rater severity.

Funding Source & References

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Appendix 10

Manuscript, “Fluctuation is the norm: Rehabilitation practitioner perspectives on ambiguity and 1 uncertainty in their work with persons in disordered states of consciousness after traumatic 2 brain injury” PLOS ONE (Under Review)

"Fluctuation is the norm": Rehabilitation practitioner perspectives on ambiguity and uncertainty in their work with persons in disordered states of consciousness after traumatic brain injury

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Abstract

The purpose of this study is to describe the clinical lifeworld of rehabilitation practitioners who work with patients in disordered states of consciousness (DoC) after severe traumatic brain injury (TBI). We interviewed 21 practitioners using narrative interviewing methods from two specialty health systems that admit patients in DoC to inpatient rehabilitation. The overarching theme arising from the interview data is "Experiencing ambiguity and uncertainty in clinical reasoning about consciousness" when treating persons in DoC. We describe practitioners' practices of looking for consistency, making sense of ambiguous and hard to explain patient responses, and using trial and error or "tinkering" to care for patients. Due to scientific uncertainty about diagnosis and prognosis in DoC and ambiguity about interpretation of patient responses, working in the field of DoC disrupts the canonical meaning-making processes that practitioners have been trained in. Studying the lifeworld of rehabilitation practitioners through their story-making and story-telling uncovers taken-for-granted assumptions and normative structures that may exist in rehabilitation medical and scientific culture, including practitioner training. We are interested in understanding these canonical breaches in order to make visible how practitioners make meaning while treating patients.

In this paper, we describe the clinical lifeworld in which rehabilitation practitioners work when treating persons remaining in states of disordered consciousness (DoC) after severe traumatic brain injury (TBI) [1-6]. About 59% of persons who receive specialty rehabilitation will recover from DoC within the first year of recovery [7]. For those remining in DoC recovery will continue for several years, but the odds of substantive recovery incrementally decreasing each year thereafter [7].

Recovery from DoC is described by a gradient of consciousness where less consciousness is associated with more disruption of functional and structural neural connectivity.[8-21] While the gradient is delineated clinically as the vegetative state (VS), minimally conscious state (MCS), and emergence from MCS (eMCS) recovery [22-25], is not necessarily a linear progression along this gradient [13, 26-28]. Persons remaining in states of DoC, often experience fluctuating levels of wakefulness and external awareness [29, 30] and, even with highly specialized care [31], this inherent variability in neurobehavioral performance obscures clinical observations of functioning during rehabilitation. This fluctuation challenges practitioners' day-to-day work because it is hard to unequivocally determine patients' level of consciousness. In this way, practitioners work in a context of scientific uncertainty regarding accurate detection of changes in levels and states of consciousness, which is the basis for monitoring recovery. At the same time, there is a lack of empirical data to guide clinical treatment [32], which creates ambiguity about treatment decision-making. How practitioners perceive and make sense of diagnostic and prognostic uncertainty and therapeutic ambiguity remains an uncharted psychosocial domain [33] and an unappreciated aspect of DoC rehabilitation treatment [34].

We report the ways practitioners provide rehabilitation services in spite of the day-to-day uncertainty and ambiguity. We also report the way practitioners talk about fluctuations in patient behavior through story-telling and story-making to make sense of patient recovery and treatment decisions. In addressing the uncharted domain of understanding how practitioners manage the uncertainty and ambiguity intrinsic in their day-to-day practice, this paper takes a non-traditional stance for rehabilitation science. Rather than positioning this study as an examination of how practitioners' clinical decision-making influences patient outcomes in order to inform quality improvement initiatives or advance person-centered care, we describe the more elementary practice of decision-making processes and underlying reasoning that enable monitoring of recovery and the inter-related treatment decisions. Doing so enables a more nuanced understanding of practitioners' everyday clinical reasoning and the strategies they use to cope with diagnostic uncertainty and therapeutic ambiguity. This is turn can lead to new insights and innovation regarding clinical practice and knowledge in sTBI and DoC.

Epistemological underpinnings

Using hermeneutic and narrative approaches, we posit that "meaning and the processes by which meanings are created and negotiated within a community" form culture [35-40]. Making sense (i.e., the act of interpretation) is a fundamental part of the human condition and provides the basis to understand patient recovery and make treatment decisions.

Inpatient rehabilitation culture is dominated by medical and evidence-based scientific models in which practitioners are treated as experts who know what to do and can diagnose and prognosticate with confidence. This culture is driven by the positing of theory-driven, empirically-proven, measurable outcomes-based clinical practices, where patient recovery is

the ideal outcome. As Mattingly et al note, "Culture gives us the possibility of reading other minds because a cultural world is one where meanings are public and communal, rather than individual and private"[41]. The culture of inpatient rehabilitation provides a foundation or canon by which practitioners can make sense of their work, and guides actions when canonical breaches or violations occur [35]. Practitioners in DoC work within a world of canonical breaches due to the combined scientific uncertainty [32] about diagnosis and ambiguity about treatment decisions in the face of fluctuating patient behaviors, which disrupt the canonical meaning-making processes in which practitioners are trained and in which health organizations operate. We are interested in understanding these canonical breaches and disruptions that exist within rehabilitation culture as they are exhibited in the stories practitioners in DoC told because they expose taken-for-granted assumptions and normative cultural structures that otherwise remain invisible. To do so, we used insights from the traditions of narrative medicine, grounded theory, and the first author's phenomenological training [1, 4, 5, 42].

Story-telling and therapeutic emplotment play important roles in rehabilitation practitioner sense-making [35, 41, 43]. In narrative interviews, we asked practitioners to tell us about times when interactions with patients were frustrating, surprising, or memorable (exciting, impactful, strange). These interview questions enabled practitioners to share their "stories from the field" [44], allowing us to see them as actors, even protagonists, in their story-telling, story-making, and meaning-making as they treated patients. These stories are how practitioners make sense of their day-to-day work. They are stories of unexpected patient responses, practitioners' explorations, improvisations, and successes. While we didn't design our study to focus on uncertainty and ambiguity in clinical practice, our epistemological approach allowed us to bring

to the surface the ways practitioners in DoC make sense of the challenging interpretive process of treating patients.

Methods

This study is nested within a larger clinical trial (NCT02366754) examining neurobehavioral, neural and molecular responses to repetitive transcranial magnetic stimulation provided to patients with DoC after severe TBI. This qualitative study aimed to advance understanding of how rehabilitation practitioners understand and communicate neurobehavioral change of these patients.

Collectively, the authors have many years of experience working with these patients and their families. The team includes occupational therapists, speech language pathologists, physical therapists, a phenomenological sociologist, and caregivers.

Data Collection: We conducted in-person interviews with 21 rehabilitation practitioners in two health systems from multiple rehabilitation disciplines (e.g., medical doctors, nurses, social workers, occupational therapists, physical therapists, and speech language pathologists) who each had at least six months experience working with patients with DoC after TBI. We used a purposive sampling strategy as our objective was to hear from practitioners who work in specialized DoC programs since they would have multiple patient encounters to reflect upon during interviewing. This is a common strategy in qualitative research designs where the goal is to gain in-depth understanding [36, 45] of process-oriented phenomena such as understanding behavioral change and meaning-making [37].

"Interviews are speech events that produce narratives that are jointly constructed by interviewers and respondents"[46]. Two rehabilitation practitioners (EW and AG) with expertise treating DoC patients conducted the interviews. They were trained by the first author, an expert in qualitative interviewing. All interviews were audio-recorded and transcribed verbatim. Institutional Review Board approvals were obtained from Northwestern (NU IRB: STU00203840) and Edward Hines, Jr. Veteran Affairs Hospital (Hines IRB: 16-037). Participants were provided with information letters about the study and verbally consented.

Data Analysis and Reflexivity: We are aware that these data are "our own constructions of other people's constructions of what they and their compatriots are up to" [47]. Using the principles of grounded theory and narrative analysis, we analyzed interviews into major topics and themes based on participants' direct quotes [1, 2, 6, 48]. Our analyses were inductive in that we did not apply *a priori* constructs or theories to coding. We began with open, line-by-line coding [1, 48]. Three members of our team (JW, AG, CP) coded separately and discussed codes during weekly meetings. Each member read transcripts a minimum of three times.

Data collection and analysis occurred simultaneously and iteratively. We created categories from codes and memos [1, 48]. For this study, categories served as organizing 'buckets' including multiple codes that described similar topics or experiences and which later became themes. Codes and categories were not mutually exclusive, rather one code might fit within two categories. Themes typically involved combining more than one category (Fig 1). We developed a codebook to organize and appraise our decisions, and engaged in constant comparison analysis of analytic themes [1, 48]. We used the qualitative software NVIVO 11 to organize our data and work on codes and categories.

[Figure 1 here]. Figure 1. Example of methodological approach to generating themes.

We practiced reflexivity,[49, 50] that is, critical awareness of our own positionality, biases, and emotions regarding the data, by writing personal and analytic memos [1]. We engaged in member-checking with the practitioners in our team by practicing "dialogical intersubjectivity"[1, 50], in which we exchanged positive and challenging emotions and thoughts related to the data, practiced active listening of each other's perspectives, and challenged each other to acknowledge our unique perceptions and predispositions (including personal, disciplinary, and professional). We documented these discussions in minutes to audit our decisions. When we disagreed, we re-read the transcripts until we reached simple group consensus [1]. In spite of all the careful work to not impose our experiences onto the data, it is possible that we have highlighted findings that are meaningful to us because of our experiences.

Findings

Participants were recruited and enrolled from two North American, mid-western clinical settings— a civilian (n=7) and a veteran inpatient rehabilitation facility (n=14) (Table 1). The majority of participants were female (n=20), rehabilitation therapists (n=13), with more than twenty years' experience in their profession (n=11), and more than 5 years' experience working specifically with patients with DoC (n=11). In an effort to preserve participants' anonymity, when quoting, we identify them with their professional designation (e.g., PT for Physical Therapist) followed by a number (e.g., PT 2). As is common in most qualitative reporting traditions, we do not enumerate how many participants agreed or mentioned a particular topic or category. Participant quotes are chosen because they best represent the themes we report

in this paper. Participant characteristics are representative of the rehabilitation workforce. The American Speech-Language-Hearing Association (ASHA) reported in 2019, that 96% of the 175,000 SLPs certified by ASHA are female [51]. The results of the 2017 National Nursing workforce survey, indicate 90.9% of the RN workforce in the United States identifies as female [52]. The American Occupational Therapy Association Workforce and Salary Survey of 2019 reports that 91% of the OT workforce identifies as female [53]. Similarly, statistics published by the American Physical Therapy Association indicate that 65% of physical therapists and 71% of physical therapy assistants identify as female [54].

Table 1. Participant Demographics

Demographic Information		Number of Participants
Setting	Veteran Facility	14
	Civilian Facility	7
Discipline	Occupational Therapist	4
	Physical Therapist	4
	Speech and Language Pathologist	3
	Nursing	2
	Physical Medicine & Rehabilitation Medical Doctor	2
	Psychologist	2
	Recreational Therapist	2
	Certified Nursing Assistant	1
	Social Worker	1
Gender	Female	20
	Male	1
Age	25-35	9
	36-45	4
	46-55	6
	>55	2
Years Practicing in Profession	<5	5
	5-10	3
	11-20	7
	More than 20	11
	0-5 years	9
	6-10	3

"Fluctuation is the norm"

Years Practicing with Patients in DoC	More than 10	9

Experiencing ambiguity and uncertainty in clinical reasoning about consciousness

Ambiguity (inexactness) and uncertainty (unpredictability) are related. Ambiguity may arise in situations where multiple persons have differing interpretations of the same experience. In DoC, ambiguity arises due to imprecision of clinical assessments to document states of consciousness, multiple expert interpretations of patient states of consciousness, and uncertainty arises with limited evidence regarding efficacy of chosen treatments [32]. A common aspect of ambiguity is experiencing lack of confidence because there is imprecise or unknown information which render situations difficult to be sure about. These epistemic limitations make a patient's states of consciousness largely unknown. In the context of DoC, uncertainty exists due to individual patient variation in responses to treatment and fluctuating patient response. As such, detecting and determining signs of consciousness in patients in DoC is challenging. Practitioners made sense of patients' ambiguous signs of consciousness via patient stories whose leitmotif was 'looking for a person' in the patient in DoC' during treatment [55]. In other words, practitioners in DoC observe signs that can point to patients' intention, motivation, or volition that could not be classified as mere bodily reflexes or responses [56]. 'Looking for a person' was one of the ways practitioners talked about searching for consciousness when treating their patients.

"Fluctuation is the norm"

We identify two major categories to describe ambiguity (inexactness) and uncertainty (unpredictability) when treating persons in DoC: "Fluctuation is the norm" and "Trying stuff" (Table 2). The first describes practitioners' experiences of clinical reasoning about diagnosing patients' levels or states consciousness by searching for consistency and making sense of ambiguous patient responses to describe their recovery. The second describes what practitioners do in spite of uncertainty in the face of paucity of empirical evidence and brings to the surface that practitioners go outside their canonical training in order to make treatment decisions. Both categories represent ways in which practitioners make meaning and clinically reasons about patients' consciousness in the midst of ambiguity and uncertainty regarding treatment decisions.

Table 2. Codebook example from the main theme of 'ambiguity and uncertainty among rehabilitation practitioners in DoC'

Main categories supporting theme	Supporting Subcategories (Description)	Participant Quotes	Profession, Participant #
"Fluctuation is the norm" Describes practitioners' experiences of clinical reasoning about diagnosing patients' levels or states consciousness by searching for consistency and making sense of ambiguous patient responses to describe their recovery		"We expect fluctuation in this patient population. Fluctuation is the norm. We don't expect consistent performance."	Physical Therapist, 7
		"I had a patient, he was in our emerging consciousness program, and he was making some gains but still wasn't consistently following commands … he was here for 12 weeks and for the majority of that time he was kinda at a similar level and … it was just very variable. One day he seemed to be occasionally responding or doing things more consistently, while other days he was doing nothing."	Physical Therapist, 8
	Searching to observe consistent responses to stimuli as	"You know, is she consistent? Is she *truly* consistent? Like 100% consistent? Or is she still	Physical Therapist, 7

	indications that the patient is improving	inconsistent enough where you'd say she was technically still minimally conscious? Or had she truly emerged into that conscious state?"	
		"I can remember when [patient] would follow a command for the first time. [I thought] 'Whoa, did they actually just do that? Did I actually just see that? Or was that sort of random?'"	Physical Therapist, 7
		"What I typically like to see when I'm following patients is, you know, that they are beginning to show some localized and purposeful activity. We might start to see first some sort of intentional motor cognitive behavior and then that that's consistent. You're seeing that consistently and then that's kind of building into even more than that. Either following a command, like a yes/no or whether that's nodding or thumbs up or thumbs down. So something consistent."	Physiatrist, 16
	C*ollaborating* with others in teams to identify consistency of responses thus documenting <u>level of</u> consciousness	"as a team we talk about [possible change] and I'll say 'I am seeing a localized response, they are localizing to this'… and speech may say 'I see that but I'm not seeing it consistently.' So that is why sometimes we will wait until it's consistent cross disciplines before we jump between levels [of consciousness.]"	Occupational Therapist, 6
	Observing nuances in patient responses, and *grappling with* u nexplained recoveries or stalls in patient progress.	"is more of a gradual thing. I don't feel like one day you walk in and they are emerged. …we're doing serial daily exams on the person and nursing is getting a 24/7 view. You have all this information that you are gathering all the time; so in my experience I would say it's more of taking all of that input in and it's not a black and white thing."	Physiatrist, 16

		"I feel like it's usually a pretty long and slow process and [patients] go from kind of a vacant stare with no recognition and no following or tracking movements. Usually the first thing we see is some sort of eye contact, some sort of effort to follow an object, or just pulling away if you touch them, or if you put your hand in their hand and they respond in some way with a hand movement. Usually, those are kind of the first signs that I start to notice."	Recreational Therapist, 11
		"Mr. Jones was our worst-case scenario patient. We, maybe, expected that he might regain some small level of function; and [yet] he's functioning on a level that no one can explain."	Recreational Therapist, 11
		"He never tracked in any way, he never focused on anything. At one point we were suspecting, 'could he be blind?' Because no matter what, we never saw anything visual with him."	Speech Language Pathologist, 1
		"[Patient] is really doing well from a physical perspective; much beyond my initial expectations were. So, it was actually a really good learning case for me because I thought I knew a lot at that point in my career and it was a good reminder to me of the things we don't always know."	Physical Therapist, 7
"Trying Stuff" Describes what practitioners do in spite of uncertainty in the face of paucity of empirical evidence and brings to the surface that practitioners go outside their canonical training in order to		"I was trained by my colleagues to just try stuff. Because there is a lack of research with disorders of consciousness as far as interventions that actually work. A lot of the times I feel like we are trying stuff, and we are just [waiting to] seeing what happens."	Occupational Therapist, OT4
		"So my intern went 'stop, collaborate,' and he stopped and the patient mouthed the word 'listen'. We didn't hear anything at	Recreational Therapist, 11

make treatment decisions		that time, but as we continued on with the song, [the patient] would finish the sentence and gradually we started to actually hear him verbalize the right word. So, we had tried everything, including songs that his wife said he liked. He didn't respond to those, but this was a song that he would have known as a young teenager, like 12 or 13 years old. And so somehow it stirred something different."	
		"The patient's head was down and he wasn't making any eye contact or an effort to raise his head. And when the dog came in, we had to cue him to look, and then he raised his head and his eyes widened and he started to smile. And then when the dog came closer to him, he leaned in towards the dog more and when we put his hand on the dog's head, we saw him moving his fingers as if he was trying to scratch. He wasn't able, at that point, to reach purposefully to do it, but when we put his hand in place, he moved his fingers. His sustained attention was longer when the dog was there; I could get him to really focus for ten to fifteen minutes."	Physical Therapist, 6
		"I'm a very non-traditional, sort of out of the box therapist, and sometimes what these young males respond to is not necessarily a clinically standard and appropriate type of approach. There's a TV show called "Jackass" where these guys do ridiculous things and oftentimes they're just gross and inappropriate and in every way unacceptable behavior. But, I get a better response from "Jackass" than I do almost anything and so I put it on for this young man.... The first thing that I noticed, he was watching the screen and not just sitting there, you know, just	Recreational Therapist, 3

		unaware. He was focusing on the screen and he smiled at an appropriate time. So he recognized that the moment was funny and he smiled at the right time; and so that was my first, I guess, sign that he was starting to emerge."	
		"There was singing, there was praying, there was shaking of rattles and drums and things like that. There was two people working with the patient and then two people that worked with his wife. They did breathing work with the wife to release emotional stuff and they did some massage. There was prayers in the Christian tradition and prayers in the Mayan tradition. Overall, it was a very emotional and amazing experience. The patient had been here for months and had no real response that we could see. So, immediately after that experience, he kind of went into this even deeper sleep, it was like he was knocked out for three days and on the third day when he woke up, he was present. His eyes had changed. He was tracking and showing responsiveness and he just went on this remarkable recovery process that nobody here can explain it. People talk about it and nobody has an explanation. People say it was, he was a miracle. "	Recreational Therapist, 11
		"we need to try and stimulate [patient's] level of alertness in any way we can."	Physical Therapist, 7

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210 ## Clinical reasoning about consciousness when "fluctuation is the norm"

211 Fluctuation, variability, and lack of consistency were expressions all rehabilitation practitioners
212 used to describe and interpret the recovery process of patients with DoC. One participant, PT7,

elegantly captures this experience: "We expect fluctuation in this patient population. Fluctuation is the norm. We don't expect consistent performance." "Fluctuation is the norm" resonated with practitioners in our research team, and is echoed in the DoC literature [29, 30, 57]. PT8 similarly described: "I had a patient in our emerging consciousness program, he was making some gains but still wasn't consistently following commands. He was here for 12 weeks and for the majority of that time he was kinda at a similar level and it was just very variable. One day he seemed to be occasionally responding or doing things more consistently, while other days he was doing nothing."

When patients' responses are inconsistent and fluctuating, it is challenging for practitioners to be certain about how to interpret them. For example, are patients improving or deteriorating? In which state of consciousness do their responses best fit? PT7 wonders about her patient: "You know, is she [patient] consistent? Is she *truly* consistent? Like *100%* consistent? Or is she still inconsistent enough where you'd say she was technically still minimally conscious? Or had she *truly* emerged into that conscious state?" (Italics denotes emphasis in the audio transcript). Wondering about patients' recovery, rather than being confident in their assessment of patient progress or decline, characterized practitioners' ways of talking about their work.

PT7 exemplifies her search for consistency by repeating: "is she consistent", "*truly* consistent", "*100%* consistent." Her words parallel the specialized language of commonly used clinical assessment tools (such as the CRS-R [58] or Coma Near Coma Scale [59, 60]) where patient responses are scored according to consistency, defined by consensus of practitioners, and serves as a clinical marker for recovery [61, 62]. Seeking consistency where "fluctuation is the norm" points to efforts to cope with the ambiguity inherent in practitioners' work.

Practitioners stated the experience of a 'double take' when patients responded to a command for the first time. "I can remember when [patient] would follow a command for the first time. [I thought] 'Whoa, did they actually just do that? Did I actually just see that? Or was that sort of random?'" (PT7) A double take is a behavioral response to the cognitive dissonance practitioners may experience when treating these patients whose responses fluctuate so much and are inconsistent [63]. It may also be an example of doubt—"Did I actually just see that?" perhaps indicates not trusting one's own senses.

We turn next to examples of how practitioners make meaning in this treatment environment.

Examples of meaning-making when "fluctuation is the norm"

Fleming and Mattingly assert that practitioners "simultaneously observe, assess, and interpret patient's actions" and this "thinking in action" is tacit and involves experiential knowledge (i.e. disciplinary and practical training, prior experience with patients, and astutely observing during treatment sessions) [37]. Practitioners make meaning as they act out their reasoning in treatment sessions with patients. The process of making meaning is active and creative, even though it is tacit. We show examples of practitioners' meaning-making and clinical reasoning when "fluctuation is the norm" around four themes: *searching to observe consistent* responses to stimuli as indications that the patient is improving, *collaborating* with others in teams to identify consistency of responses thus documenting level of consciousness, *observing nuances* in patient responses, and *grappling with* unexplained recoveries or stalls in patient progress.

P16 explains a clinical reasoning logic she uses in her searches for *consistency*: "What I typically like to see is, you know, that they are beginning to show some localized and purposeful activity.

We might start to see first some sort of intentional motor cognitive behavior and then that that's consistent. You're seeing that consistently and then that's kind of building into even more than that. Either following a command, like a yes/no or whether that's nodding or thumbs up or down. So, something consistent."

Clinical reasoning in inpatient rehabilitation is not just an individual practitioner's process, it is *collaborative* and team-based. Deciding whether patients were consistent was discussed often in team meetings. OT6: "as a team we talk about [possible change] and I'll say 'I am seeing a localized response, they are localizing to this'… and Speech may say 'I see that but I'm not seeing it consistently.' So that is why sometimes we will wait until it's consistent cross disciplines before we jump between levels [of consciousness.]" Multiple practitioners need to agree that indeed the patient is responding consistently in order to formally document a state of consciousness.

In order to determine whether patients are responding consistently, practitioners described the importance of *observing* fine *nuances* in patient responses as an important clinical reasoning practice. Recovery "is more of a gradual thing. I don't feel like one day you walk in and they are emerged. … we're doing serial daily exams on the person and nursing is getting a 24/7 view. You have all this information that you are gathering all the time; so, in my experience I would say it's more of taking all of that input in and it's not a black and white thing." (P16) The action of "taking it all in" is a form of clinical reasoning practitioners engage in to look for consistent indications of recovery of function and consciousness. Noticing is an important observational tool to achieve this: "Usually the first thing we see is some sort of eye contact, some sort of effort to follow an object, or just pulling away if you touch them, or if you put your hand in their

hand and they respond in some way with a hand movement. … those are kind of the first signs that I start to notice." (RT11)

Though practitioners notice fine nuances and "take all the information in," they sometimes *can't explain* patients' recovery using formal clinical assessment information or their own expertise and scientific training. RT11: "Mr. Jones was our worst-case scenario patient. We expected that he might regain some small level of function; and [yet] he's functioning on a level that no one can explain." SLP1 shares an example of a patient whose assessment information showed "no response," but that didn't stop the clinical team from wondering why that was the case: "He never tracked in any way, he never focused on anything. At one point we were suspecting, 'could he be blind?' Because no matter what, we never saw anything visual with him." When clinical assessment information does not satisfy explanations for why a patient isn't responding to stimuli, plausible wondering may be a way of grappling with ambiguity.

While analyzing these data, we wondered: What do practitioners do with unexplainable recoveries? How do practitioners work with clinical information they can't explain? PT7 sees a chance to learn: "[Patient] is really doing well from a physical perspective; much beyond my initial expectations were. So, it was actually a really good learning case for me because I thought I knew a lot at that point in my career and it was a good reminder to me of the things we don't always know."

Clinical reasoning takes place during the act of treating patients, it is not a purely cognitive, thinking process. It is "thinking in action" that practitioners engage in when they provide different stimuli to observe nuances of behavior, collaborate with team members to better

understand patient responses, and make sense of what they are observing in the moment to assess of patients' recovery status. During this "thinking in action," practitioners make meaning using prior knowledge and experience, assessing-in-the-moment information, and by comparing their observations of present behavior with patients' past performance. We turn next to explore further practitioners' "thinking in action" through practitioners' patient stories shared during interviews.

Trying to find consciousness by "trying stuff"

Through practitioners' patient stories we can learn how practitioners "think in action" and what they do during clinical sessions to evaluate patients' consciousness status, elicit responses, facilitate consistency in responses to various stimuli associated with a particular state of consciousness, or generate emerging responses for the next state of consciousness [62, 64, 65]. Through these stories we learn how they make sense of their interactions with patients' inherently fluctuating and inconsistent responses. "Thinking in action" involves trial and error. OT4: "I was trained by my colleagues to just try stuff. Because there is a lack of research with disorders of consciousness as far as interventions that actually work. A lot of time I feel like we are trying stuff, and we are just [waiting to] see what happens." Working in a clinical environment where practitioners "try stuff" and wait to "see what happens" is unlike other rehabilitation fields where recovery trajectories are more predictable and there is less clinical equipoise.

"Trying stuff" and "seeing what happens" frame the stories practitioners told us. Their stories communicate more than strategies or tools they use to evaluate and treat. We see the creation

and enactment of plots that help organize their observations and give meaning to unfamiliar or hard to explain situations. The uncertainty of responses to treatments and recovery trajectories for persons with DoC is a breach or challenge to the canonical scientific reasoning practitioners are trained in and comfortable with; it is no surprise that they share stories in which they narrate how they make sense of challenging interactions with patients. In narrating, they tell sense-making stories of complex or impactful situations, and position themselves as actors, even protagonists. In a medical culture where practitioners are supposed to know what to do and how to do it, treating patients in DoC may disrupt these suppositions.

In the examples that follow, we use practitioners' stories of patient interactions that focus on the theme of "trying stuff". In these stories, practitioners use music, video, prayer, and a dog to elicit responses and treat patients. They cast themselves sometimes in the role of explorer, improviser, or rebel, and they tell stories of miracles, informed experimentation, and lucky happenstances. In these stories, practitioners become tinkerers [66-69].

"Trying Stuff:" Examples of "thinking in action"

RT11 used music to elicit responses from a patient who was alert and used hand gestures but was not verbalizing. RT11 works alongside a young and energetic male intern, who sang a popular song by Vanilla Ice called 'Ice, Ice, Baby'. The refrain is 'stop, collaborate, listen':

"[the intern] sang 'stop, collaborate,' and stopped, and the patient mouthed the word 'listen'. We didn't hear anything at that time, but as we continued on with the song, [the patient] would finish the sentence and gradually we started to actually hear him verbalize the right word. We had tried *everything*, including songs that his wife said he liked. He didn't respond to those, but

this was a song that he would have known as a young teenager, like 12 or 13 years old. And so somehow it stirred something."

What can't be read in this passage is the excitement in the practitioner's voice about the increasing consistency of and improvement in the quality of elicited responses, starting first with mouthing and then verbalizing the song words. In this story, RT11 explains some of the reasoning strategies clinicians use including gathering information from individuals with close knowledge of patient preferences, such as the patient's wife, and also the in-the-moment lucky serendipity of a song sung by a team member. In declaring they had tried "everything," RT11 is acknowledging the interplay of clinical judgement and guesswork/ trial and error aspects in treatment planning. Sound clinical choices, such as the patient's past preferred music, are supplemented with in-the-moment lucky happenstance. RT11 exemplifies one way of "thinking in action": building implicit, individualized theories to explain what worked or didn't work with patients.

Another instance of practitioners "trying stuff" comes from a collaboration with family to bring a dog to a patient's room [70]. PT6 told us, "The patient's head was down and he wasn't making any eye contact or an effort to raise his head. When the dog came in, we had to cue him to look, and then he raised his head and his eyes widened and started to smile. When the dog came closer to him, he leaned in towards the dog more and when we put his hand on the dog's head, we saw him moving his fingers as if he was trying to scratch. His sustained attention was longer when the dog was there; I could get him to really focus for ten to fifteen minutes." In this example, the practitioner is reflecting on how the patient's attention when the dog is present is longer and more sustained than prior sessions without the dog. In this brief story, we

see the practitioner making sense of the patient's improvement (more consistent, sustained attention) as being related to the presence of a dog (with whom he felt connected). The practitioner explored bringing a dog in treatment as part of "trying things" and now builds her own knowledge base of possible interventions that might work with these patients.

Practitioners operate with few validated approaches in their treatment toolbox. As a result, they perceive their informed experimentation as radical and norm-breaking. Yet, in reality, normative rehabilitation practice in this area offers little guidance since recovery is unpredictable and the tool box of options for treatment with established efficacy are limited. In the next example, RT3 casts herself in the role of a rebel, or acting 'outside the box' to tell a story of non-conformity and success.

RT3 used a TV show in her treatment: "I'm a very non-traditional, sort of out of the box therapist, and sometimes what these young males respond to is not a clinically standard and appropriate type of approach. There's a TV show called "Jackass" where these guys do ridiculous things and often times they're just gross and inappropriate and, in every way, unacceptable behavior. But, I get a better response from "Jackass" than I do almost anything and so I put it on for this young man.... The first thing that I noticed, he was watching the screen and not just sitting there, you know, just unaware. He was focusing on the screen and he recognized that the moment was funny and he smiled at the right time. That was my first sign that he was starting to emerge." This practitioner's "out of the box" treatment points to the importance of transgressing disciplinary and normative training to "try things" in order to provide treatments that elicit responses indicative of alertness or arousability or that elicit contextually appropriate responses. This story also shows us that each and every clinical

observation a practitioner makes is an additional way for them to collect information that can bring clarity amidst uncertainty of treatment responses to help determine whether patients' responses can be seen as progress in the recovery trajectory.

We report a final example of "thinking in action" and trial and error process as it shows that rehabilitation practitioners are willing to go outside their comfort zones to enable, facilitate, and support patient recovery.

RT11 describes being part of a prayer gathering with a patient's family. She expresses that this activity was out of her comfort zone and had difficulty making sense of the effects this prayer gathering had on the patient. "There was singing, praying, shaking of rattles and drums and things like that. There was two people working with the patient and then two people that worked with his wife. They did breathing work with the wife to release emotional stuff and they did some massage. There were prayers in the Christian and Mayan traditions. Overall, it was a very emotional and amazing experience. The patient had been here for months and had no real response that we could see. So, immediately after that experience, he kind of went into this even deeper sleep, it was like he was knocked out for three days and on the third day when he woke up, he was present. His eyes had changed. He was tracking and showing responsiveness and he just went on this remarkable recovery process that nobody here can explain it. People talk about it and nobody has an explanation. People say it was, he was, a miracle."

In this story of "miracle" recovery, RT11 is not in control of what takes place in the patient's room; she is a participant, not an expert. She can't explain why the patient recovered; her story is cast as a miracle and expresses her own sense making. In the lifeworld of ambiguity and

uncertainty that practitioners in DoC navigate, they are explorers, rebels, witnesses of miracles, and improvisers. In the stories presented, practitioners continue trying even though they may not know what might work and why.

"Trying stuff" is an intentional practice. Rehabilitation practitioners "wait to see" how patients respond (experimentation/trial and error), even when they "don't really know why a response is happening." PT7 stated, "we need to try and stimulate [patients'] level of alertness in any way we can." To say that practitioners try to stimulate patients "in any way they" can doesn't mean that 'anything goes.' Rather, practitioners use formal knowledge, prior experiences with previous patients, extrapolation from previous successes, and experimentation (trial and error.) They tell stories to create and enhance meaning making and clinical reasoning. In studies of medicine, this practice is called "doctoring" or "tinkering" [66, 69].

Discussion

Rehabilitation practitioners are trained in the scientific model of evidence-based medicine (EBM), which includes rational hypothetical-deductive reasoning and logical induction-based algorithms to produce reliable, accurate and valid diagnosis, prognosis, and treatment decisions. In recent years, they are also trained to provide services in person-centered and culturally competent ways. Medical training and EBM create a cultural framework within which practitioners are cast as experts who know what to do and when to do it. In this framework, empirical knowledge and theory are expected to inform clinical practice with confidence and replicability. Practitioners learn the norms of EBM, the technical tools to assess patients, and the medical language to communicate in. In the EBM literature, uncertainty is viewed as a potential threat to be minimized [71, 72]. EBM has become the canonical framework, and as

such it holds epistemic privilege. Some uncertainty is always involved in clinical reasoning. In the clinical lifeworld of rehabilitation practitioners in DoC, ambiguity and uncertainty are omni-present [73, 74]. Practitioners in our study stated that they "don't always know" what to do, that they "try things" in any way they can in order to help patients emerge to consciousness, all the while they second guessed themselves [75], they were unable to explain patient recoveries, experienced assessment discordance (i.e. different practitioners' clinical assessment scores were often not in agreement with each other's) and cognitive dissonance (such as 'double take'). They rarely used language that positioned themselves as knowers [75]. **They tell patient stories in discordance to the cultural frameworks they have been trained in.** They tell patient stories of fluctuation, multiple interpretation, dissonance and doubt, and of transgression from canonical training or treatment. Their stories give us an opportunity to become aware of taken-for-granted practices within the rehabilitation canon that may be otherwise invisible.

In this paper, we discuss the practice of tinkering [69] to make visible the ways practitioners enact clinical and narrative reasoning in this context. Our overall goal is to show the challenges of working in the field of DoC and exhibit practitioners' dedication and creativity to respond to these challenges. In doing so, we show the value that practitioner clinical practices bring to the field of DoC and thus expose the epistemic injustice of treating "thinking in action" as inferior to EBM [76]. We hope that future research and scholarship continue to explicate practitioners' experiences, practices and ways of working with patients in DoC as valuable ways of knowing and doing. Our data and research in clinical practice suggest that "thinking in action" and

tinkering is one of the tools/ ways practitioners use in clinical practice. Yet, there is very little in the peer-reviewed literature about the value this practice can offer rehabilitation medicine.

The Practice of Tinkering: Clinical reasoning in the midst of ambiguity and uncertainty

Humans reason logically, but also by analogy and through narrative: they use information from familiar areas to link to present situations or problems and tell stories that align with relevant cultural frameworks. This reasoning may be explicit and shareable, or it may be tacit [77]. In our study, practitioners rely on their own clinical expertise, past experiences, and on teammates to interpret patients' responses and make recovery and planning decisions. They make decisions based on judgments, not exactitude [78]; that is, they use a "treasure store of tacit tricks of the trade" such as "a working hypothesis, tradeoffs, risks, intuition" [78]. These "tricks of the trade" are clinical reasoning practices called "doctoring" or "tinkering" [66, 67, 69, 79].

Tinkering is *a way of caring* for patients that involves curiosity, experimentation, struggle, possibly "failing and trying again," being flexible and adapting to complex clinical settings [69]. Tinkering is not an approach where "anything goes." It involves casting oneself into particular narrative roles (rebel, experimenter, observer). The very expression "trying things" that all practitioners used to linguistically express their common practice, suggests that tinkering is part of their everyday lifeworld. Tinkering is how practitioners sometimes have to reason-- with creativity and dedication to do what is best for patients, in spite of the ambiguity and

uncertainty surrounding them. Tinkering as a practice and as a way of caring, however, is not generally taught in educational curricula and it is not celebrated as a creative response to caring for complex patients. Tinkering is not perceived as important in the peer reviewed literature since there is a paucity of studies that explicate it as a practice, even though our practitioners clearly use it daily.

Tinkering and the search for consciousness

When asked how they made sense of the fluctuation of patients' responses to treatment, practitioners reported that they constantly looked for signs of consciousness. They described this through their stories of looking for "a person being in there,"[63] which means observing signs of intention, motivation, or volition that could not be classified as mere bodily reflexes. Considering the high misdiagnosis rates in this population [80-82], efforts to find capacities that signal recovery of volitional abilities, i.e., of consciousness, are significant. 'Looking for a person in the patient in DoC' was the plot, the leitmotif, of many practitioners' patient stories told during interviews. In their stories, practitioners used expressions such as "paying attention to fine nuances" (RT11), "searching for consistency" (SLP1), "trying things" (OT6), and "trying anything to help patients emerge" (PT7). These are examples of tinkering with dedication.

From hermeneutic and narrative perspectives, we recognize interpretation as embedded in clinical reasoning [83-85] and see how it tacitly informs the ways practitioners are trained and work. In their day-to-day work, practitioners actively engage in interpretation. For instance, OTs find patterns in patients' expressions to produce narrative explanations of patients' problems [86]. PTs engage in "piecing clues together to form meaningful wholes" by "a continuing and

cyclical process of cue acquisition, hypothesis generation and evaluation of both" [87]. The inclination to find consciousness and, therefore, signs of personhood formed a part of narrative reasoning and "thinking in action" involving piecing together information to make sense of patients' data and circumstances. In other words, while treating patients, practitioners are enacting narrative plots in which they create meaning to make sense of what is happening in their treatments. During interviews, they told stories of their reasoning in which they make sense of their actions. In this paper we have shown how practitioners make sense of (i.e., interpret) the clues patients given them to piece together a meaningful picture of the patient in DoC as a person, rather than as a mere body. In searching for consciousness, practitioners breach the canon of EBM. In looking for consciousness, practitioners tinker with their treatment toolbox. As they tinker, they expose the limitations of the current state of scientific knowledge in the field of DoC. Tinkering, in this sense then, is a clinical reasoning practice that breaches the canon of rehabilitation science. As such, it has the potential to open the field of DoC practice to celebrating practitioners' ways of caring and treating. This may promote exploration and innovation of new treatment modalities and practices.

Yearning for consistency in the midst of uncertainty

Practitioners used linguistic expressions such as "is she truly consistent?" and "did I just see that?" signifying their disbelief, ambivalence, lack of certainty and confidence [75] because of patients' fluctuating or inconsistent signs [88]. Philosophers identify yearning for consistency as part of the human condition: in the face of fear and ambiguity, we want certainty [89, 90]. Practitioners may experience self-doubt; they may not know how to make sense of what they

are observing. Treating patients from a position of "not knowing" is challenging for practitioners because they "are trained to be experts, [their] job is to know things, to have answers, to educate... Doubt, uncertainty, openness, and reflexivity, however, are essential to avoid stasis, to move rehabilitation in creative directions that best meet the needs of the people and communities we serve" p.141) [68, 91].

In this all too human predicament, practitioners continued to treat, care, and "try things" with patients. They didn't waver, even when not knowing whether or how their interventions impacted their patients. They tried things, observed nuances, adjusted treatments, and didn't give up. Practitioners marshaled ethics, virtues, experiences, and insights. Clinical reasoning is not just about using the "tricks of the trade" [92]. It is part of the "detective" work of piecing clues together, and of tinkering [66, 67, 69, 79, 87]. Continuous critical review of new evidence and constructive doubting of one's decisions -- in other words, practicing with humility-- are important elements of being a practitioner in DoC rehabilitation.

Implications to the field of rehabilitation

In this study, practitioners show us the limitations of a canonical medical culture that focuses on EBM training and valorizes the credentialed professional as the expert. But practicing in the field of DoC is practicing in a borderland.[93] Practitioners are challenged by scientific uncertainty about diagnosis and prognosis and by the ambiguity inherent in treating patients whose responses fluctuate while there is limited evidence to guide treatment decisions. These epistemic limitations have day-to-day consequences for practitioners: they experience lack of confidence and doubt their expertise; they become tinkerers (innovators, improvisers, heroes,

rebels, humble observers) in order to respond and treat patients. One implication for the training of practitioners in DoC is to encourage the explicit use of tinkering as a form of clinical reasoning. Uncertainty poses epistemic challenges to EBM. But uncertainty is not necessarily a threat when we practice medicine. Uncertainty poses epistemic challenges to EBM. But uncertainty is not necessarily a threat to effectively practice rehabilitation medicine.

Uncertainty may make practitioners uncomfortable and vulnerable and while these are difficult experiences, they open possibilities for creative tinkering that can benefit patients. In the EBM model, uncertainty is a threat. In everyday rehabilitation practice, practitioners' narrative reasoning shows us how uncertainty opens up tinkering. In the field of DoC, where there is epistemic uncertainty, practitioners' ways of knowing and doing are valuable contributions to the treatment process, and "I don't know" is evidence of practicing with humility. Practicing with humility is a strength, not a liability. We hope future studies in the field of medical rehabilitation and DoC in particular will continue to make visible the creative ways that practitioners use to respond to epistemic uncertainty when they care for complex patients. Making visible how practitioners engage in tinkering practices is one way that we contribute to the field of rehabilitation and DoC. Whether tinkering is efficacious to supporting emergence to consciousness for patients in DoC remains unclear and an area of study that needs to be further explored.

Limitations

This study involves a small number of participants from two Midwestern rehabilitation facilities with specialized DoC programs and does not represent experiences across facilities and

553 settings. Patients with DoC are often overlooked and not admitted for inpatient rehabilitation,
554 which means our data reflect a vantage point of patients admitted to specialty rehabilitation.
555 Another limitation is recall bias; we were asking practitioners to describe past and current
556 experiences with patients, which may mean that we only heard about the most memorable,
557 frustrating, and surprising experiences. We may have missed opportunities to hear about
558 different types of patients with DoC after TBI. Our study focused on interview narratives and
559 the stories participants created for the purposes of interviews. We didn't have ethnographic
560 and video data of their clinical encounters. As such, we could not analyze using the tools of
561 conversational analysis and ethnography which would have allowed for more detailed analyses
562 and nuanced discussions of the ways in which practitioners organize their interpretation
563 processes. Future studies should pay more attention to the everyday practices of practitioners
564 by collecting video and ethnographic data of clinical encounters.

565 # Conclusion

566 Rehabilitation practitioners who care for patients in DoC work in an environment of ambiguity
567 and uncertainty. Ambiguity exists when there is either no evidence base or there is an
568 imprecise scientific basis to guide diagnoses, prognostication and treatment decisions.
569 Uncertainty occurs when there is high variability in patient responses to treatment and
570 recovery patterns are unpredictable. EBM rehabilitation training curricula do not provide the
571 tools to manage ambiguity, and the diagnostic and prognostic uncertainty of DoC challenges
572 practitioners. The practitioners in our study responded to ambiguity and uncertainty by using
573 their observation skills to monitor nuances in patients' responses that might indicate emerging
574 consciousness. They did so by *searching for consistent* behavioral responses to stimuli as

indications that the patient is improving, *observing* fine nuances, and *collaborating* with peers to grapple with unexplained recoveries or stalls in patient progress. While uncertainty raises discomfort, practitioners in our study used "thinking in action" tools such as tinkering to respond to uncertainty in order to care for their patients. They "tried things," used trial and error, worked "outside the box", tweaked things –they tinkered in order to provide optimal care. They sometimes admitted they didn't know why patients recovered the way they did. In admitting they didn't know, they showed their capacity for humility and vulnerability. Practitioners do not simply provide care to patients according to pre-established guidelines; they generate important knowledge by "thinking in action" and tinkering described in this paper. Understanding these practices can lead to new knowledge; practitioners' innovations can generate new insights that can move the science and practice of DoC forward. This study described the innovative ways rehabilitation practitioners deal with ambiguity and uncertainty in working with patients in DoC through tinkering, and as such, opens up the black box of rehabilitation practice.

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Appendix 11

Manuscript, “miRNA profiles of persons with chronic neurobehavioral impairments and remaining in states of disordered consciousness after severe traumatic brain injury” *Journal of Head Trauma Rehabilitation* (Under Review)

Journal of Head Trauma Rehabilitation

miRNA profiles of persons with persisting neurobehavioral impairments and states of disordered consciousness after severe traumatic brain injury

--Manuscript Draft--

Manuscript Number:	
Full Title:	miRNA profiles of persons with persisting neurobehavioral impairments and states of disordered consciousness after severe traumatic brain injury
Article Type:	Original Article (unsolicited)
Section/Category:	Unsolicited (Focus on Clinical Research)
Keywords:	Traumatic Brain Injury; micro RNA; Disorders of Consciousness; Biomarker; Vegetative State; Minimally Conscious State; Phenotype
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Abstract:	Objective Examine the merits of using microRNAs (miRNAs) as biomarkers of Disorders of Consciousness (DoC) due to traumatic brain injury (TBI). Settings Acute and sub-acute in-patient beds. Participants Patients in states of DoC an average of 1.5 years after TBI (n = 6) who were enrolled in a randomized clinical trial and healthy controls (n = 5). Design Comparison of whole blood microRNA profiles between patients and their age/gender matched healthy control. For patients, correlational analyses between miRNA profiles and measures of neurobehavioral function. Main Measures Baseline measures of whole blood microRNAs. MicroRNAs were measured using miRNA-seq, which interrogates both the cellular and fluid components of blood, and Real Time-Polymerase Chain Reaction. Baseline neurobehavioral measures derived from seven tests. Results For patients, relative to healthy controls, 48 miRNA were significantly ($p < 0.05$) /differentially expressed. Cluster analysis showed that healthy controls were most similar to each other and with two of the patients, 1- VS and 1- MCS. Three patients, all in MCS, clustered separately. The only female in the sample, also in MCS, formed an independent group. For the 48 miRNAs, the enriched pathways identified are implicated in secondary brain damage and 26 were significantly ($p < 0.05$) correlated with measures of neurobehavioral function. Conclusions Patients remaining in states of DoC an average of 1.5 years after TBI showed a different and reproducible pattern of miRNA expression relative to age/gender matched healthy controls. The phenotypes, defined by miRNA profiles relative to persisting

	neurobehavioral impairments, provide the basis for future research to determine the miRNA profiles differentiating states of DoC and the basis for future research using miRNA to detect treatment effects, predict treatment responsiveness, and developing targeted interventions. If future research confirms and advances reported findings, then miRNA profiles will provide the foundation for patient-centric DoC neurorehabilitation.

DEPARTMENT OF VETERANS AFFAIRS
Edward Hines Jr. VA Hospital
Neural Plasticity in Neurorehabilitation Lab

John Corrigan, PhD
Editor-In-Chief, *Journal of Head Trauma Rehabilitation*

Dear Dr. Corrigan:

Thank you for your consideration of the submitted paper, 'miRNA profiles of persons with persisting neurobehavioral impairments and states of disordered consciousness after severe traumatic brain injury.' We report, the first ever, miRNA phenotypes for persons remining in states of Disordered Consciousness (DOC) 1.5 years after Traumatic Brain Injury (TBI). The phenotypes are defined by changes in miRNA expression, relative age and gender matched healthy controls, and according to a comprehensive set of measures of neurobehavioral function. The phenotypes demonstrate the merits of using miRNA as DoC-TBI specific biomarkers to, ultimately, enable evidence-based diagnoses and prognoses.

Let me provide background on the reported findings. To address the need for targeted treatments that induce functional and structural changes in the brain of persons remaining in states of DoC after TBI, we have two research awards. The first award is for a recently completed Phase I double blind randomized clinical trial examining therapeutic effects of repetitive Transcranial Magnetic Stimulation (rTMS). The second award includes two measurement projects to advance capabilities for detecting meaningful treatment effects at the behavioral, neural network and molecular levels, specifically micro RNA (miRNA). For miRNA, the objective was to determine the accuracy with which whole blood derived miRNA profiles distinguish, from healthy controls, persons in states of DoC after TBI.

How are our findings relevant for the readers of *JHTR*? We report miRNA profiles, representing chronic miRNA levels. Findings indicate that the miRNA profiles of healthy controls remain stable over six weeks and further that DoC patients showed a different and reproducible miRNA expression pattern compared to healthy controls. These profiles, and how they relate to levels of neurobehavioral function, provide the basis for future research addressing critical knowledge gaps for this patient population including differential diagnoses, prognoses (i.e., factors influencing recovery and treatment responsiveness), treatment induced modulation of pathways important to recovery, and developing targeted neuromodulatory treatments. The use of peripheral blood provides the clinical capability to feasibly examine how treatments such as rTMS induce neuroplasticity and repair.

We think that this report will appeal to a broad clinical, scientific and lay audience, in part, because miRNA derived from peripheral blood can be feasibly obtained at the bedside and in daily practice of neurorehabiitaotn across settings. Considering this feasibility, the reported findings support further investigation of the utility of peripheral blood miRNA as a biomarker for states of DoC, measuring magnitude of treatment effect, informing optimal rTMS doses/sessions and possibly predicting responsiveness to rTMS treatments. If future research confirms and further advances these findings, then miRNA profiles can be used to provide evidence-based patient-centric DoC TBI neurorehabilitation. To enable future replication and

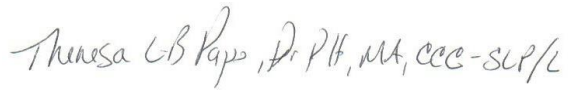
further advancement of miRNA – clinical neurobehavioral phenotypes, we explicate each participants neuropathology in Supplement A and report all of the correlation results in Supplement B. Collectively, the findings reported in the manuscript and these supplements provide the details necessary for those scientists seeking to replicate our approach and advance our findings in a larger and independent study sample.

The Principal Investigator and senior author, Theresa L. Bender Pape, takes responsibility for the integrity of the data and the accuracy of the data analysis and that all authors had full access to all the data in the study. This paper has 18 authors, and each contributed their unique expertise, without which this work in multi-modal neurobehavioral recovery relative to injury mechanisms with this challenging patient population would not be possible.

As we just completed subject enrollment, the primary paper reporting the RCT findings will be submitted for peer review late in 2022. The paper submitted here represents our first report of original RCT data regarding miRNA. Prior reports, regarding miRNA, include a miRNA review paper (published in the May-Jun JHTR 2021 issue . Thus, the submitted manuscript does not contain any data, patient information, or other material or results that have already been published or are in press, submitted, or nearly submitted. All authors are responsible for reported research. All authors participated in the concept and design, analysis, or interpretation of data; drafting or revising of the manuscript; and approve of the manuscript as submitted.

If you have any questions, please do not hesitate to contact me. Thank you for considering our work. We look forward to hearing from you.

Sincerely,



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miRNA profiles of persons with persisting neurobehavioral impairments and states of disordered consciousness after severe traumatic brain injury

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Keywords: Traumatic Brain Injury miRNA, Disorders of Consciousness, Biomarker, Vegetative State, Minimally Conscious State

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Abstract

Objective

Examine the merits of using microRNAs (miRNAs) as biomarkers of Disorders of Consciousness (DoC) due to traumatic brain injury (TBI).

Settings

Acute and sub-acute in-patient beds.

Participants

Patients in states of DoC an average of 1.5 years after TBI (n = 6) who were enrolled in a randomized clinical trial and healthy controls (n = 5).

Design

Comparison of whole blood microRNA profiles between patients and their age/gender matched healthy control. For patients, correlational analyses between miRNA profiles and measures of neurobehavioral function.

Main Measures

Baseline measures of whole blood microRNAs. MicroRNAs were measured using miRNA-seq, which interrogates both the cellular and fluid components of blood, and Real Time-Polymerase Chain Reaction. Baseline neurobehavioral measures derived from seven tests.

Results

For patients, relative to healthy controls, 48 miRNA were significantly ($p < 0.05$) /differentially expressed. Cluster analysis showed that healthy controls were most similar to each other and with two of the patients, 1- VS and 1- MCS. Three patients, all in MCS, clustered separately. The only female in the sample, also in MCS, formed an independent group. For the 48 miRNAs, the enriched pathways

identified are implicated in secondary brain damage and 26 were significantly ($p < 0.05$) correlated with measures of neurobehavioral function.

Conclusions

Patients remaining in states of DoC an average of 1.5 years after TBI showed a different and reproducible pattern of miRNA expression relative to age/gender matched healthy controls. The phenotypes, defined by miRNA profiles relative to persisting neurobehavioral impairments, provide the basis for future research to determine the miRNA profiles differentiating states of DoC and the basis for future research using miRNA to detect treatment effects, predict treatment responsiveness, and developing targeted interventions. If future research confirms and advances reported findings, then miRNA profiles will provide the foundation for patient-centric DoC neurorehabilitation.

Introduction

Persons experiencing severe traumatic brain injury (TBI) and remaining in states of disordered consciousness (DoC), after acute medical care, are evaulated with neurobehavioral tests to distinguish between states of DoC and to inform rehabilitation management. [1] When considering the heterogeneity of primary and secondary brain damage triggered by TBI [2-5] it becomes evident that differential DoC diagnoses, based solely on neurobehavioral testing, will result in a proportion of inaccurate diagnoses thereby providing an inadequate empirical foundation for evidence-based neurorehabilitation. The long-standing problem of diagnostic inaccuracy [6-9] highlights the need for phenotypes of who will/will not progress from a vegatative state (VS) to the minimally conscious state (MCS) relative to emergence from MCS (eMCS).[10-13]

Although 68% of DoC patients who receive specialty rehabilitation recover consciousness, US rehabilitation reimbursement criteria limits access to these services. [13, 14] As the criteria are based largely on diagnosis and prognosis, the need for injury phenotypes is critical to allowing access to care for those who will benefit from it. The purpose here is to report findings demonstrating the promise of microRNA (miRNA) for enhancing differential DoC diagnoses and prognoses to, ultimately, enable evidence-based neurorehabilitation.

MicroRNA are single-stranded, 20-24 nucleotide, stable RNA molecules conserved across species[15-17] and, as previously reviewed, [18] the secondary damage triggered by TBI make miRNA potentially useful as acute, sub-acute

and/or chronic biomarker(s). Specifically, severe TBI triggers pathological cellular processes (e.g., excitotoxicity), thought to be active through chronic recovery phases, causing excitotoxicity, apoptosis, inflammatory events, seizures, demyelination, white matter pathology, and diminished neurogenesis; all of which are correlated with persisting neurobehavioral impairments.[2-5] For clinical neurorehabilitation research and practice, miRNA are particularly promising because humans have over 2500 miRNAs regulating 30-60% of messenger RNAs.[19-23] Additionally, 70% of miRNAs are thought to be specific to the brain, spinal cord and nerves.[24] Furthermore, miRNAs cross the blood-brain barrier, have altered expression after TBI and brain miRNA have been detected in serum <1 hour after TBI.[24-27] When considering that protein biomarkers, such as GFAP and UCH-L1 in blood and cerebrospinal fluid do not consistently pass the blood brain barrier[28-30] miRNAs have the potential to address the need for reliable biomarkers for differential DoC diagnosis and prognosis.

The few published reports [19, 21, 25, 26,] of miRNA expression, in severe TBI, studied acute recovery (within 0 to 30 days) and provide evidence of 19 up-regulated and 33 down-regulated miRNAs with the most significant being miR-16, miR-92a and miR-765.[25] One of these studies demonstrated peak expression 24-72 hours post-injury, with miR-142-3p and miR-423-3p showing the highest expression at time '0' that decreased by day 30. This same study also demonstrated that miR-425-5p and miR-502 differentiated between acute mild and severe TBI (i.e., within 12 hours). [31] ,[27] , [32]

Regarding chronic recovery phases, a study in persons > 2 years post severe TBI (n = 9) found that miR-9 and miR-451 in CSF microparticles were significantly higher.[33] However, specimen collection by time after TBI varied considerably and only the Glasgow Coma Scale (GCS) was reported for clinical neurobehavioral phenotyping.

Based on limited knowledge of miRNA for severe TBI, we conducted a study examining global miRNA expression. Since the few severe TBI miRNA studies vary by time post-TBI and biofluids used, [25-27, 31-37] there is no established method for detecting miRNA and determining changes in expression. Leukocytes, for example, are known to increase after trauma. [38] Thus, the biofluid, cell type and fluid subcomponent interrogated will influence miRNA detection and computation of expression. Taqman or microarrays are also known to be limited in the miRNAs they can detect. Considering these factors, we examined global miRNA expression using a miRNA-seq assay to interrogate both the cellular and fluid components of whole blood. Based on this global approach, we report miRNA profiles and their relationships with neurobehavioral function for six persons remining in states of DoC chronically, 1.5 years after TBI.

Methods

The study was one component of a clinical trial, approved by each site's human subject's institutional review board (IRB) and the US Army Medical Research and Development Command, Human Research Protection Office. The study was overseen by an independent data safety monitoring board. MiRNA experiments were performed at Edward Hines Jr. VA Hospital and the Genomic Centers of

Loyola University Chicago's and Northwestern University Feinberg School of Medicine.

Patients remaining in states of DoC post TBI were eligible; their legally authorized representatives provided consent. Each patient was matched, by gender and age categories (18-20, 21-22, 23-24, 25-34, 35-44, 45-54, 55-64, 65-69, 70-74, 75+), to a healthy control with no history of neurologic conditions.

Baseline Procedures

This study is based on baseline tests and specimens collected at least 24 hours after patients were titrated off of pharmacological neurostimulants and sedatives. Each patient underwent a 3T MRI and lesions were coded by the study neuroradiologist by type, cortical and sub-cortical locations.[39, 40] States of DoC were determined clinically by experts and according to current consensus criteria defining VS and the MCS [41-46] using the Coma Recovery Scale-Revised (CRS-R) [47] and the Disorders of Consciousness Scale-25 (DOCS). [48-52] While CRS-R guidance specifies responses consistent with VS, MCS and eMCS, evidence indicates that sole reliance on cut-points contributes to diagnostic inaccuracies.[7, 53] Thus, a combination of the CRS-R, DOCS and clinical observations during these tests provided the basis to classify states of DoC according to clinical consensus criteria. Additional tests included the Coma-Near-Coma (CNC) scale, Disability Rating Scale (DRS), Spaulding Limb Movement, Glasgow Outcome Scale-Extended (GOSE) and the GCS. [54-60]

For patients and healthy controls, peripheral whole blood samples were collected in 2.5 cc PAXgene Blood tubes (2.5 mls, Qiagen, Germantown, MD) and then frozen at -80°C, until RNA was isolated. Whole blood was processed, and small RNAs (<200 bp) were sequenced. First, RNA was isolated on the Qiagen Qiacube using the automated protocol of the PAXgene Blood RNA Kit (Qiagen). Total RNA in each sample was quantified using the Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA) and quality was measured using the RNA6000 Nano chip on the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). Samples with an RNA integrity number greater than 7 were used for sequencing.

cDNA libraries were generated using the TruSeq Small RNA library prep kit (Illumina, San Diego, CA). Specifically, adapters were ligated using the 5' phosphate and 3' hydroxyl group common to most mature miRNAs as a result of the cellular pathways used to create them. After adapter ligation, samples were reverse transcribed and amplified. Finally, the libraries were size selected using a 6% polyacrylamide gel and concentrated using an ethanol precipitation. Purified libraries were normalized and pooled to create a double stranded cDNA library ready for sequencing. The samples were sequenced on the Illumina MiSeq and NextSeq to render 50 base pair single end reads.

Adapter sequences were removed and low quality reads were trimmed from raw sequencing reads using Cutadapt (v.1.11).[61] The resulting reads were mapped to the most recent human genome release from Ensembl, GRCh38 using Bowtie2 (v.2.2.1)[62] An annotation file from miRBase release 21, describing miRNA

coordinates, and the sequence alignment mappings were used as input for the Python package HTSeq (v.0.6.1p1) to generate a table of raw counts of miRNAs observed in the alignments.[63] DESeq2 (v.1.14.1) was used to determine differential expression between sample groups using the raw miRNA counts. [64] Wald tests were conducted to determine significance of differential expression.

TaqMan was used to validate the miRNA assay findings. To select the miRNA for validation, we ranked the set of significantly expressed miRNA according to the absolute value of the difference of expression between each patient and their matched healthy control. As the sum of the absolute values represents the magnitude of the difference of each patient's miRNA compared to all healthy control participants, we selected the top six miRNA.

We examined the relationship between significantly expressed miRNAs and neural pathways as well as neurobehavioral function. For pathways, we plotted the miRNAs in a heatmap using R (v3.5.1) and gplots (v3.0.1). Significantly regulated pathways were determined using TAM 2.0, with cutoffs of 10 miRNAs in a pathway. False Discovery Rate (FDR; q=0.05) was used, except for the tissue and family pathways, which used cutoffs of 2 miRNAs.[65] For neurobehavioral function, we examined correlations between miRNA standardized counts and clinical phenotype measures. To control for covariates, we derived each correlation and tested it for significance, using a regression model for each clinical phenotype measure (e.g. DOCS Total) fitted for each miRNA and including one of each of three covariates (Relative volumes of Cerebral Spinal

Fluid, CSF; Gray Matter Density, GMD; White Matter Density; WMD)[66]. To compute correlations (r), Test statistic (t) and degrees of freedom (df) were used [(r2= t2/(df+t2), [67]]. For large values of t2, corresponding r2 and hence r will be very large when based on small df. The sign (r, positive/negative) is determined from that of t. As we have a small df (df = 4), this approach will yield correlations close to 1.00. Thus, correlations with p values ≤ 0.05 are considered significant.

Results

The study sample included the first six participants of the trial. At baseline (Table Ia/Ib), these patients had remained in a state of DoC for an average of 1.5 years after TBI. Five patients presented in the MCS (83%), five were biologically male (83%) with half being Caucasian (3/6) and Hispanic (3/6). Average age of patients was 40 years (SD: 8.0) and 41 years (SD: 9.7) for healthy controls. For patients, specimens and neurobehavioral tests were collected an average of 534 (SD: 132; median 520) days after TBI. Proportion of diffuse axonal injury (DAI) lesions was the greatest across all patients followed by chronic microhemorrhages and contusions (Supplement A).

Correlations between patients and healthy controls (Figure 1) indicate that all controls clustered into one group along with 2 DoC patients (Far right arrow, Patients E-VS and F-MCS). Three patients clustered separately (Middle arrow, Patients C, A and D, all in MCS,). The sole female patient clustered alone (Far left arrow, Patient B in MCS,). Relative to controls, the six patients shared

significantly up-regulated (yellow) and down-regulated (blue) miRNAs, demonstrating that consistent differences in expression can be detected.

Wald tests identified 30 significantly up- and 18 down-regulated miRNAs (>2.0 fold change, Table IIa/IIb). Fold changes, or the ratio of differences between the patient and their matched control, indicate a 30.5-fold up-regulation for miR-218-5p, 29.7-fold for miR-9-3p and 12.1-fold for miR-582-3p. While we looked at tissue-specific miRNAs, the only enriched tissue was brain, despite these being blood samples. The miRNA with fold change >5.0 are largely involved in regulating secondary biochemical processes that impede neural plasticity and repair.

Validation findings indicate that, for all six miRNAs examined using TaqMan, direction of change is the same for both miRNA-seq and RT-PCR (Figure 2). Although magnitude of change differs somewhat, comparisons between the miRNA-seq fold change and RT-PCR were not statistically significant. The large standard error for miR-9-3p indicates, however, that miRNA-seq findings for miR-9-3p were driven by an outlier.

Pathway analysis, conducted to examine the relationship between neural pathways and the 48 significantly regulated miRNAs, identified 9 pathways that included 10 or more miRNAs per pathway (FDR<0.05) (Table III). These included general pathways such as inflammation, apoptosis and immune

response. Brain specific pathways, such as brain development, were also identified.

Accounting for GMD, WMD and CSF, 26 of the 48 miRNAs are significantly ($p \leq 0.05$) correlated with neurobehavioral measures (Table IIc and Supplement B). Of these 26 miRNAs, the majority (58%; 15/26) are significantly up-regulated and about one-third (38%, 10/26) have, to date, known neuronal roles (Table IIa,/IIb/IIc). While miR-381-3p is correlated with four different neurobehavioral measures, the majority of the 26 miRNAs (54%, 14/26) are each correlated with one measure followed by three (23%, 6/26) or two (20%, 5/26) neurobehavioral measures (see Supplement B for all correlation results).

Discussion

This study examined miRNA signatures of six DoC-TBI patients in chronic recovery stages, relative to their healthy controls. As patients were titrated off medications acting on the CNS prior to study procedures, reported differences in levels of expression of the 48 miRNAs represent the native CNS of these patients. The identified pathways of these miRNAs are, largely, implicated in secondary brain damage as they are involved in gradual degradation of cellular processes known to result in pathological conditions (e.g., excitotoxicity, inflammation, apoptosis, altered neurogenesis) thereby establishing an undesirable basis for neural plasticity and repair that can result in persisting neurobehavioral impairments. Aligned with pathway findings, 26 of the 48 miRNAs were correlated with clinical phenotype measures indicating a relationship between

the up- and down-regulation of these miRNAs and persisting neurobehavioral impairments. To our knowledge, this is the first study that explored altered expression of miRNA at chronic time points in a controlled manner and using a comprehensive battery of neurobehavioral tests.

Study findings for the six patients in chronic stages of severe TBI recovery, relative to each patient's age- and gender-matched healthy control, identified 48 miRNAs significantly and differentially expressed. Seven of these 48 are consistent with previously published acute and sub-acute severe TBI studies (miR-335, miR-144, miR-151a, miR-618, miR-142-3p, miR-769-5p, and miR-10b-5p).[24, 27, 31] While one of the seven miRNAs (miR-10b-5p) has a known role in lumbar motor neuron patterning,[68, 69] four of the seven are (miRs-335, -144, -151a and -618) known to regulate cell cycle, cell proliferation, inflammation, synaptic plasticity, functions of neurotransmitters, neurogenesis, all of which are implicated in secondary brain damage[2]. Moreover, our findings indicate that three of these miRNAs (miRs-335, -151a-3p and -151a-5p) are correlated with persisting neurobehavioral impairments. Specifically, miR-335 up-regulation is correlated with auditory-language skills while miRs-151a-3p and -151a-5p down-regulation are correlated with motor skills.

Expanding on acute/sub-acute findings of miRNA implicated in secondary brain damage, our study of the chronic population found nine novel miRNAs, that are also implicated in secondary brain damage. Specifically, nine miRNAs with large (>5.0 fold) differences in expression with healthy controls (Tables IIa/ IIb;

Upregulated miRNAs: miR-218-5p,miR-9-3p, miR-582-3p, miR-1246, miR-199b-5p, miR-409-5p; Down-regulated miRNAs: miR-199b-3p, miR-6515-3p, miR-7155-5p) are also each involved in regulation of cell cycle, proliferation, migration, differentiation of cells, neuroplasticity, and inflammation.

This study also found 26 correlations between miRNAs and clinical phenotype measures of neurobehavioral functioning. While the significant correlations are all important (Supplement B), there is a dearth of evidence on the neuronal roles of several of the miRNAs. Thus, here we discuss the correlations involving the miRNAs with known neuronal roles (denoted in Table IIc by black shading).

Of the 15 correlations with up-regulated miRNAs, the findings of higher levels of miR-1246 being correlated with better neurobehavioral function across multiple modalities (DOCS Total) is consistent with evidence that miR-1246 regulates Dyrk1A gene. This gene is associated with alterations in cognitive and motor deficits as well as altered neurogenesis in the brains of persons with down syndrome.[108] The PRKAR1A gene is also regulated by miR-1246 known to encode the type 1 alpha subunit of protein kinase A and has a known role in cAMP signaling that promotes cell metabolism, growth, proliferation and apoptosis.

As higher DRS and CNC scores indicate poorer function, whereas higher CRS-R Auditory scores indicate better function, the negative (DRS and CRS-R Auditory) and positive (CNC) correlations with miR-4482-3p highlight the usefulness of miRNA as well as the challenges with interpreting miRNA and neurobehavioral

relationships. More specifically, these findings indicate that as expression of miR-4482-3p increases there is less disability, impairment, and handicap (DRS), but poorer arousal/attention (CNC) and poorer CRS-R auditory skills. Notably, the down-regulated miR-939-5p is also negatively correlated with the DRS and CRS-R Auditory measures indicating that as miR-939-5p decreases in expression there is also less disability, but better auditory-languages skills. As a single miRNA can regulate multiple targeted messenger RNAs, [70] it is plausible that these findings indicate a previously undiscovered role for miR-4482-3p and miR-939-5p.

MiR-381-3p is known to be involved in neurogenesis and while several miRNAs are correlated with three neurobehavioral measures miR-381-3p is correlated with the most (4 measures). The positive correlations indicate that as miR-381-3p increases in expression there is better overall (GOSE) and motor recovery (CRS-R Oromotor; Spaulding upper limb function), but negative correlations with CRS-R Arousal indicate that as 381-3p increases there is poorer arousability. Of the genes known to be regulated by miR-381-3p, TWIST1 is notable given its demonstrated role with developmental delays, related pathways including cytokine signaling in the immune system and that one known action is to inhibit myogenesis or formation of skeletal muscular tissue.

There are four miRNAs correlated with the DOCS Gustation-Olfaction measures of swallowing and olfactory skills and three of these miRNAs are known to regulate genes associated with conditions involving impairments in swallowing

and/or olfaction. Correlated with improved swallowing and olfaction is miR-10b-5p, which is involved in lumbar motor neuron patterning and known to target regulation of most genes associated with motor development and dysfunction [i.e., Hox10, Neurofibromytosis type 1 (NF 1); T-cell lymphoma invasion and metastasis 1 (Tiam1);Phosphatase and tensin homolog (PTEN)]. Disruption of the Hoxd10 gene pathway for example, results in severe hindlimb defects.[68] while NF1 gene mutation plays a primary role in developing motor deficits.[71] In contrast, inhibition of the Tiam1 gene, results in death of motor neurons.[72] miRNA-10b-5p also regulates expression of the PTEN gene and PTEN inhibition in mice results in motor dysfunction and impaired response to dopamine.[73] PTEN mutations have also been associated in human demyelinating motor neuropathy syndrome.[74] Considering the cognitive components supporting swallowing and olfaction, the relationship between these skills improving and the increasing expression of miR-145-3p is also noteworthy because it is associated with regulating the protein phosphatase 3, catalytic subunit, alpha isoform (PPP3CA) gene. PPP3CA is involved with severe neurodevelopmental disease with seizures as well as the insulin receptor substrate 1 (IRS1) gene associated with Alzheimer's disease[75].

Regarding motor skills, improving CRS-R motor scores is correlated with miR-151a-3p, which regulates expression of myeloid cell leukemia (Mcl1). This is important as, in Parkinson's disease, reduction of Mcl1 levels result in dopaminergic neuron loss and motor impairments.[76]

Findings where miRNA have known neuronal roles also include relationships with improving auditory-language abilities. Specifically, improving auditory-languages skills is correlated with incremental down regulation of miR-7641 and miR-6087. MiR-7641, involved in working memory and neural plasticity, is known to regulate COX20 that impairs cytochrome c oxidase assembly and is associated with ataxia and muscle hypotonia. It is also known that miR-6087 regulates ZNF490 (BCL-6), which represses genes that function in lymphocyte differentiation, inflammation, and cell cycle control. The negative correlation between miR-10b-5p and DOCS auditory-language measure indicates that as 10b-5p increases these DOCS scores are worse. Considering the role of miR-10b-5p noted above (lumbar motor function) and that the DOCS test measures best responses elicited with auditory stimuli presented within the context of the patient's motor skills and abilities (e.g., sound localization, one step commands, answering yes-no questions), it is possible that this is novel discovery for miR-10b-5p. Advancing understanding of this relationship, however, requires further investigation.

In summary, this study identified three miRNAs implicated in secondary brain damage that were previously identified in studies of the acute or sub-acute stages of recovery. For chronic DoC-TBI, however, this study identified, at least, 9 other miRNAs also implicated in mechanisms of secondary brain damage. This study also identified significant correlations between 26 miRNA and one or more neurobehavioral measures. Of these, nine of the miRNAs have known neuronal roles and the correlated neurobehavioral measure(s) are largely consistent with

these roles. Despite these novel findings, it is important to note that measuring miRNAs is a rapidly evolving field and will continue to improve in sensitivity and specificity. For example, although our TaqMan validation studies mostly showed agreement between miRNA-seq and RT-PCR results, one miRNA was found to be driven by one outlier measurement, highlighting the need for improved processing methods for miRNA-seq data.

In conclusion, these findings indicate that DoC-TBI patients, an average of 1.5 years after TBI, showed a different and reproducible miRNA expression pattern relative to healthy controls. This conclusion becomes evident when considering that the findings are based on individual matching of patients with a healthy control according to the patient's age and gender and further by the consistency between our findings and previously published reports of the involvement of these miRNAs in secondary brain damage known to be related to persisting neurobehavioral impairments. Confirmatory evidence also includes the correlations between neurobehavioral measures, derived from a comprehensive testing battery, and the nine miRNAs having known neuronal roles that are important to these measures as they are indicative of multi-modal abilities, modality specific skills and overall functional recovery measures. Collectively, the findings support the merits of further research investigating miRNA signatures of severe TBI across acute and chronic stages of recovery. Future research is needed to advance the precision of diagnosis of states of DoC, phenotypes of recovery from DoC and treatment responders and research developing targeted treatments.

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Table Ia. Severe TBI Participants: Time of Injury and Study Baseline Characteristics

Participant	Time of Injury: Demographics and Etiology				Study Baseline: Time Post Injury and Clinical Characteristics			
Identifier	Age	Gender	Race/Ethnicity	TBI Etiology	Days after TBI	Age	DoC State	MCS Clinical Criteria Met
A	39	M	Caucasian	MCA- (No Helmet) *vs* Semi	494	41	MCS	Commands Reproduced
B	43	F	Hispanic	Bicycle- (No Helmet) *vs* Auto	545	45	MCS	Commands Reproduced & Yes/No
C	41	M	Caucasian	MVA- Unrestrained driver	636	43	MCS	Commands Reproduced
D	24	M	Caucasian	MVA- Unrestrained driver	398	25	MCS	Social Greetings Reproduced
E	46	M	Hispanic	MCA- (No Helmet) *vs* Semi	399	47	VS	None
F	36	M	Hispanic	Blunt Trauma, Assault	730	38	MCS	Commands Reproduced
Mean	**38**	**NA**	**NA**	**NA**	**533.7**	**40**	**NA**	**NA**
SD	**8**	**NA**	**NA**	**NA**	**132.1**	**8**	**NA**	**NA**
Median	**40**	**NA**	**NA**	**NA**	**519.5**	**42**	**NA**	**NA**

MCA= Motorcycle accident; MVA= Motor vehicle accident; DoC=Disordered consciousness; MCS=Minimally conscious state; VS= Vegetative state.

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Table Ia. Continued

Participant Identifier	Baseline: Neuropathology (% of all Pathology)		
	DAI	Contusions	Encephalomalacia
A	0.50	-	0.50
B	0.58	0.04	0.12
C	0.50	0.04	0.13
D	0.63	0.03	0.09
E	0.58	0.04	0.13
F	0.63	0.03	0.09
Mean	**0.54**	**0.03**	**0.19**
SD	**0.06**	**0.02**	**0.15**
Median	**0.54**	**0.04**	**0.13**

Table Ib. Healthy Control Participants: Demographics

Identifier	Age	Gender	Race/Ethnicity
HC-01	42	M	Caucasian
HC-02	54	F	Caucasian
HC-01	42	M	Caucasian
HC-03	29	M	Caucasian
HC-04	51	M	Asian/Pacific Islander
HC-03	29	M	Caucasian
HC-05	43	M	Caucasian
Mean	**41**	**NA**	**NA**
Standard Deviation	**9**	**NA**	**NA**
Median	**42**	**NA**	**NA**

Table IIa: Up-regulated miRNAs Across TBI patients compared to healthy controls (Gray shaded cells identify those miRNAs significantly correlated with at least one neurobehavioral measure)

miRNA	Average miRNA-seq Fold Change	mRNA Targets	Involved in
miR-218-5p	30.5	EGFR, IKBKB, ROBO1, RICTORBIRC5, LASP1, LAMB3, VOPP1, MBNL2	Cognition/Mood[77]; Spatial Memory and Synaptic Plasticity[78]; Neuronal Distribution[79]
miR-9-3p	29.7	RCOR1, ITGB1, NOTCH2, E2F1, CBX7, HES1, TAZ, GNAI1, FOXO1, CDH1, PPARA,	Neuronal Proliferation/ Differentiation[80]; Neuronal Migration[81]; Axon Growth[82]
miR-582-3p	12.1	AXIN2, DKK3, SFRP1, RREB1, KCNC1, LRRK2, RAB27A, DIXC1, ALDH9A1, ARL10	Neurogenesis[83-85]; Neural Stem Cell Differentiation[86-89]; Myelination[90, 91]; Axon Formation[92, 93]; Neural Cell Proliferation[94, 95]; Repolarization[96]
miR-1246	8.1	DYRK1A, NFIB, PRKAR1A, AXIN2,	Synaptic Plasticity and Neurodegeneration[97]; Neuronal Differentiation[98]

		GSK3B, CBX3, SRRT, CTC1, CKS2, TAOK1	
miR-199b-5p	6.3	HES1, SET, PODXL, DDR1, ERBB2, SETD2, JAG1, ITGA3, CCNL1, NLK	Inhibition of Neurite Outgrowth[99]; Neurogenesis[100]
miR-409-5p	5.1	STAG2, RSU1, GPBP1L1, ZNF512B, GNAI1, QSER1	Neurogenesis[101, 102]; Memory Formation[103]
miR-4482-3p	4.6	VAV3, USP42, SOD2, PLEKHF2, KIF13A, ZCCHC9, TNRC6A	Myelination[104]; Nerve Regeneration[105]; Cerebellar Development[106]; Neurogenesis[107-109]
miR-145-3p	4.3	SMAD1, MTDH, MMP16, PLCE1, CDC5L, DNTTIP2, TMEM106B, JMJD1C, BUB1, ABRACL	Axon Regeneration[110, 111]; Neurogenesis[112-114]; Reactive Astrogliosis[115]; Myelination[116, 117]; Synaptic Depression[118]; Neuroinflammation[119]
miR-149-5p	3.8	FOXM1, ZBTB2, GIT1, FGFR1, GPC1, IL6, BBC3, PTGER2, MYD88, PPM1F, FASLG	Motor neuron degeneration[120]; Neurite Outgrowth and Synaptogenesis[121]

miR-7855-5p	3.7	PCNA, SLC25A36, SRM, PP1R16B, BPTF, IPPK, GABRR2, STX6, NEDD4L,	Associated with platelets and inflammation[122]
miR-335-5p	3.7	BIRC5, LRG1, MAPK1, BCL2L2, RB1, SOX4, RASA1, TFF2, RUNX2, TNC	Inhibition of Neurite Outgrowth[123]; Neurogenesis[124, 125]
miR-3690	3.3	C2orf72, SLIT4, CCDC71, STX1B, TMEM239, RIMS4	GABAergic Transmission[126, 127]; Neuronal Arborization[128]
miR-618	3.1	STRN, MBD2, ABCG8,DVL3, ZNF529,IFNAR1,PDPK1, RDHG11, MTRNR2L3	Osteoclastogenesis[129]; Tumorigenesis[130]; Suppression of Cell Proliferation[131]
miR-337-3p	2.8	RAP1A, STAT3, CSNK2A1, MZF1, MTA3, IBA57, TFAM, COIL, MBD2, SNX16,	Synaptic function and Learning/Memory[132]; Neuronal migration and polarization[133, 134]; Axon formation[134]; Reactive astrogliosis[135]
miR-381-3p	2.8	ID1, WEE1, TBC1D9, NKKB1A, CD1C, TWIST1, GJA1, ANO1,	Neurogenesis[136]

		HDAC4, P2RX5, SMARCB1	
miR-5001-3p	2.8	MGAT5, C20orf144, PCDHA6, ORAI2, PITPNM3, TOGRAM2, MDGA1, C19orf47,	Upregulated in Alzheimer Disease[137, 138]
miR-338-5p	2.8	LRP1, BMI1, EFEMP1, NRP1, LPAR1, SPRY1, CD274, RSBN1, BTG3, PSMD7, FEM1C, MSI2, GTF2A1	Microglial polarization[139]; Synaptic plasticity[140]
miR-28-5p	2.7	CDKN1A, IGF1, IL34, MPL, MAD2L1, RAP1B, MAPK1, E3F6, TEX261, OTUB1	Neural stem cell differentiation[141]
miR-504-5p	2.7	DRD1, VEGFA, TFF1, BAX FAS, TCEAL1, GADD45A, BBC3, TP5313	Learning and memory[142]; Neurogenesis[143, 144]
miR-2115-5p	2.7	IFNAR2, MYLIP, PSAT1, NRF1, YARS	Neural Homeostasis[145, 146]; Neurite Outgrowth[147, 148]; Synaptic Transmission[149]

miR-329-3p	2.7	TIAM1, KDM1A, MCAM, MAPK1, BCAR1, GRB2, BRD4, UBN2, REST	Learning and memory[150, 151]; Synaptic plasticity[151, 152]; Neurogenesis[153]
miR-10b-5p	2.7	HOXD10, KLF4, PPARA, NCOR2, NF1, BCK2L11, TFAP2C, CDKN1A, CDKN2A, PTEN, TIAM1	Lumbar motor neuron patterning[68, 69]
miR-212-5p	2.6	MAF, TJP1, CTC1, PAIC5, RBM28, GNB1L, LRRC32,	Neuronal development[154]
miR-581	2.6	DICER1, EDEM1, KPNB1, TACC2, ZNF117,	Learning/Memory[155]; Neurogenesis[156]
miR-142-3p	2.4	RAC1, ARNTL, IL6, DOCK6, PRKCA, THBS4, TGFBR1, HMGB1, KAT2B, TIPARP	Learning/Memory[157]; Fear[158]; Synaptic plasticity[159]; Neurogenesis and Axon Formation[160]; Circadian Rhythm[161]
miR-4659b-3p	2.4	NAGK, CDKN1A, ZNF747	Neural stem cell differentiation[141]
miR-323a-3p	2.2	SMAD2, SMAD3, STAT3, CDKN1B, WDR45B, SDE2, HMGXB4	Long term memory[162]
miR-145-5p	2.2	BNIP3, KLF5, SOX2, KLF4, MUC1, MYO6,	Neuronal and Oligodendrocyte Cell

		CDKN1A, STAT1, YES1, PPP3CA, IRS1	Death[163]; Neurogenesis[164, 165]; Axon Growth[166, 167]; Neurogenesis Inhibition[168, 169]
miR-769-5p	2.2	GSK3B, LZIC, LRPPRC, PABPC1L2A, TRAPPC2B	Demyelination & Myelination[170-173]; Neuronal Hypo-proliferation[174]; Neuronal Survival[175]
miR-151a-3p	2.0	SEPT8, POTED, INTU, KCNJ6, MCL1, SLC39A9	Synaptic Plasticity[176]; Gabanergic Transmission[177]; Noradrenergic Transmission[178]; Neuronal Survival[179]

Table IIb: Down-regulated miRNAs (Gray shaded cells identify those miRNA significantly correlated with at least one neurobehavioral measure)

miRNA	Average miRNA seq Fold Change	mRNA Targets	Involved in

miR-199b-3p	12.9	Dyrk1a, PAQR5, PAK4, ITGA3, Jag1, MAPK1, MET	Inhibition of neurite outgrowth[99]; Neurogenesis[100]
miR-6515-3p	5.5	ZNF99, ZBTB18, GRAMD1B, CELF1	Neuronal Development/Differentiation[180-182]
miR-7155-5p	5.2	SPATA6, BMP8B, FXYD5, DIS3L, CACUL1, TOR2A, NCOA3	Synaptic plasticity[183]
miR-144-3p	4.2	NOTCH1, MTOR, PTEN, NFE2L2, Celf2, Abca1, ZFX, CFTR, meis1b, TTN, EZH2,	
miR-6815-5p	4.1	ZBTB40, ZNF451, HIST1H2BB, BCL2L13, PARP2, HSPA1B	Neurogenesis[184]; Neuroinflammation[185]; Neuronal Death/Survival[186]
miR-3180-5p	3.8	PODN, CYP2C19, GPM6B	Myelination[187, 188]; Axonal Growth[189]
miR-6087	3.5	HASPIN, ZNF490, FADS1, NCKAP1, HOXD3, CSTF2, GNB1L	Synaptogenesis[190]
miR-144-5p	3.5	ROCK1, ROCK2, MET, SMAD4, RUNX1, TGIF1, CCNE1, XXNE2, LBR,	Cell Proliferation[191, 192]; Memory[193]

		MDM4, FAM217B, ZNF529	
miR-3613-5p	3.4	LHFPL6, LCOR, ANP32B, F11R, H3F3C, VSP14B, MYO10, MTF2, LMNB2	Calcium Signaling Pathway, Temporal Lobe Epilepsy[194]
miR-6800-3p	2.8	KLHDC3, PPP1R15B, IGSF3, ZBTB39, ASAH2, CLIC4,	Neuronal Morphogenesis[195]; White Matter Damage[196]
miR-7641	2.7	PIM3, BANK1, TRIP4, TAOK1, REL, ARL5C, COX20,	Working Memory[197]; Neurogenesis[198]; Neuronal Plasticity[199]; Neuronal Survival[200]
miR-939-5p	2.6	IL6, SLC34A2, SEPT8, UNC13A, MSN, QSOX2, CLSTN1	Neurogenesis[201]
miR-4508	2.5	VAV3, BRSK2, BARHL1, CERS1, LYPLA2, CAPNS1, RGS6	GABA signaling[202]; Neuronal differentiation[203]
miR-15a-5p	2.4	VEGFA, CRKL, WNT3A, BCL2, CCNE1, BMI1, MYB, HMGA1, HMGA2, RECK, CCND1, CCND2,	Pathogenesis of Alzheimer Disease[204]

miR-126-3p	2.1	SPRED1, HOXA9, TOM1, RGS3, PLK2, VEGFA, IRS1, PIK3R2, CRK, TWF1, TWF2	Attenuates Blood-Brain Barrier Disruption[205-207]; Hypopituitarism in Patients Post TBI[208]
miR-101-3p	2.1	VEGF, SOX9, RAC1, CCDC88A, ACKR3, TLR2, STMN1, RAB1A, FOS, EZH2	Regulator of RAC1-Centred Network[209]
miR-342-5p	2.1	GFAP, NAA10, ENG, Akt1, PLCG2, DDX39B, CACUL1, MUC17, CYPW2, SPATA6	Neurogenesis[210]
let-7b-5p	2.0	KRAS, CDC34, ICF2BP1, HMGA1, HMGA2, CDC25A, CDK6, CCND1, CCND2, IGF2BP2	Associated with Fatigue Post TBI[211]

Table IIc: Significant Correlations by Neurobehavioral Constructs (Gray & Black Shaded cells identify the miRNA significantly ($p < 0.05$) differing in expression relative to matched healthy controls, all correlations are > 0.90. Black cells also denote the 9-miRNA with, to date, known neuronal roles)

	Neurobehavioral Constructs*									
	Multi-Modal	**Disability**	**Recovery**	**Injury Severity**	**Arousal/Attention**	**Aud-Lang**	**Motor**	**Gustation**	**Visual**	**Somato**
15 Up-regulated miRNAs										
miR-1246	**DOCS**									
miR-199b-5p		**DRS**			**CNC**	**CRS-R**				
miR-4482-3p		**DRS**			**CNC**	**CRS-R**				
miR-145-3p								**DOCS**		
miR-149-5p	**CRS-R**									
miR-7855-5p									**CRS-R**	
miR-335-5p						**DOCS**				
miR-381-3p			**GOSE**		**CRS-R**		**CRS-R Spaulding**			
miR-504-5p			**GOSE**		**CRS-R**				**DOCS**	
miR-2115-5p			**GOSE**		**CRS-R**				**DOCS**	
miR-10b-5p						**DOCS**		**DOCS**		
miR-4659b-3p		**DRS**				**CRS-R**				
miR-323a-3p									**DOCS**	
miR-769-5p									**CRS-R**	**DOCS**
miR-151a-3p							**CRS-R**			
11 down-regulated miRNAs										
miR-199b-3p				**GCS**						
miR-6515-3p			**GOSE**	**GCS**						
miR-7155-5p	**CRS-R**									
miR-6815-5p					**CRS-R**				**DOCS**	
miR-3180-5p								**DOCS**		
miR-6087						**DOCS**		**DOCS**		
miR-7641						**DOCS**				
miR-939-5p		**DRS**			**CNC**	**CRS-R**				
miR-15a-5p							**CRS-R**			
miR-126-3p			**GOSE**		**CRS_R**		**CRS-R Spaulding**			
miR-342-5p				**GCS**						

*Ten neurobehavioral constructs are derived from the seven tests, all administered at baseline except for one, as follows: **Multi-modal:** Total scores from Disorders of Consciousness Scale-25 (DOCS) and Coma Recovery Scale-Revised (CRS-R); **Disability Extent**: Disability Rating Scale (DRS); **Overall Recovery:** Glasgow Outcome Scale Extended (GOSE); **Injury Severity**: Glasgow Coma Scale (GCS) immediately after 1st treatment; **Arousal/Attention:** Coma Near Coma Scale (CNC) & CRS-R Arousal sub-scale; **Auditory-Language (Aud-Lang):** DOCS & CRS-R sub-scales; **(7) Motor:** Spaulding Upper Limb scale, CRS-R Oromotor/Verbal and Motor sub-scales**; (8 -10)** Gustation, Visual and Somatosensory **(Somato)**: DOCS Visual, Somatosensory and Gustation-Olfaction modality measures.

Table III. Pathway Analyses of the significantly up- and down--regulated miRNAs (miRNA count = Number of the 48 miRNAs included in the pathway; FDR- False Discovery Rate)

	miRNA Count	**Fold Change**	**FDR**
Inflammation	22	2.7	5.65E-04
Apoptosis	21	2.7	7.63E-04
Immune Response	18	2.7	3.14E-03
Regulation of Stem Cell	14	2.4	0.025
Epithelial-to-Mesenchymal Transition	14	2.3	0.0365
Brain Development	13	5.0	1.87E-04
Hematopoiesis	13	3.1	8.08E-03
Aging	13	2.8	0.0169
Lipid Metabolism	11	3.4	0.0124

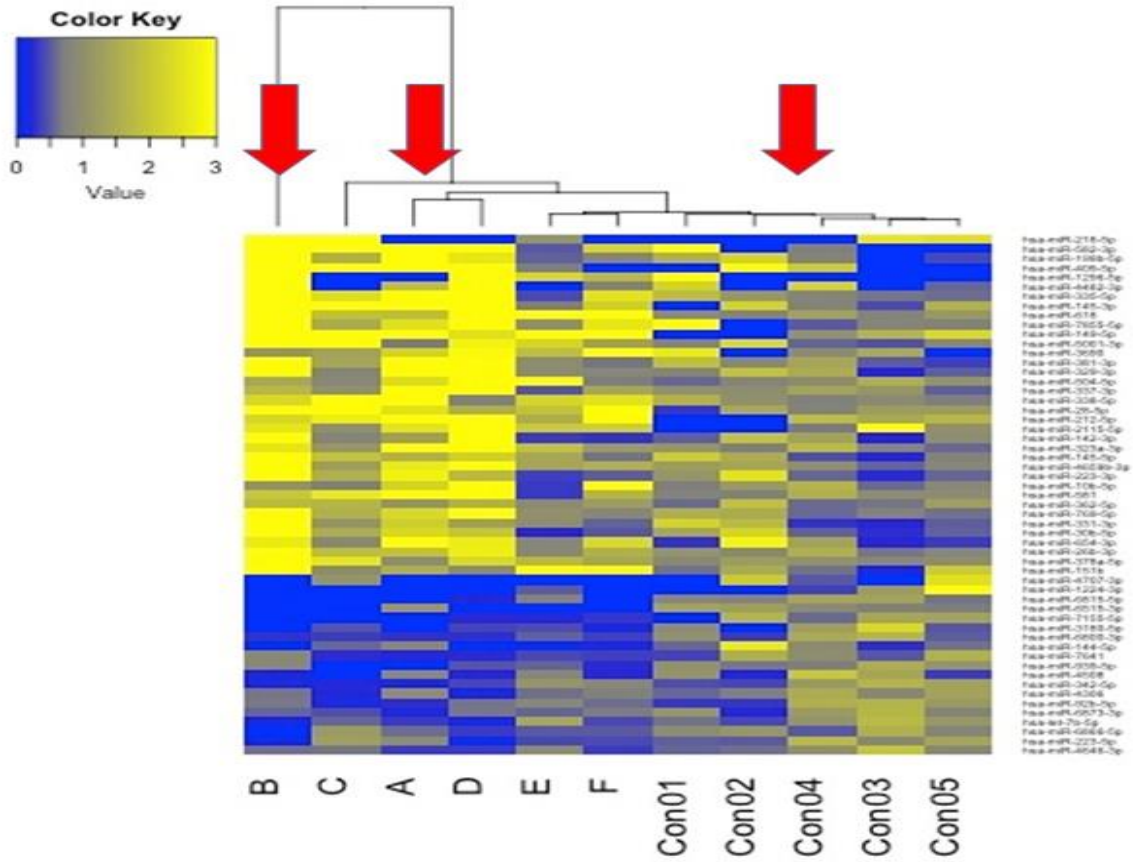

Figure 1: Cluster analysis of significantly regulated miRNAs across samples. Patients and healthy controls were clustered according to the distance between significantly-regulated miRNA profiles, with up-regulated miRNAs shown in yellow, and down-regulated miRNAs shown in blue. Red arrows denote the groups discussed in the text. Patient's A, B, C, D and F were in MCS and patient E was classified as being in the VS.

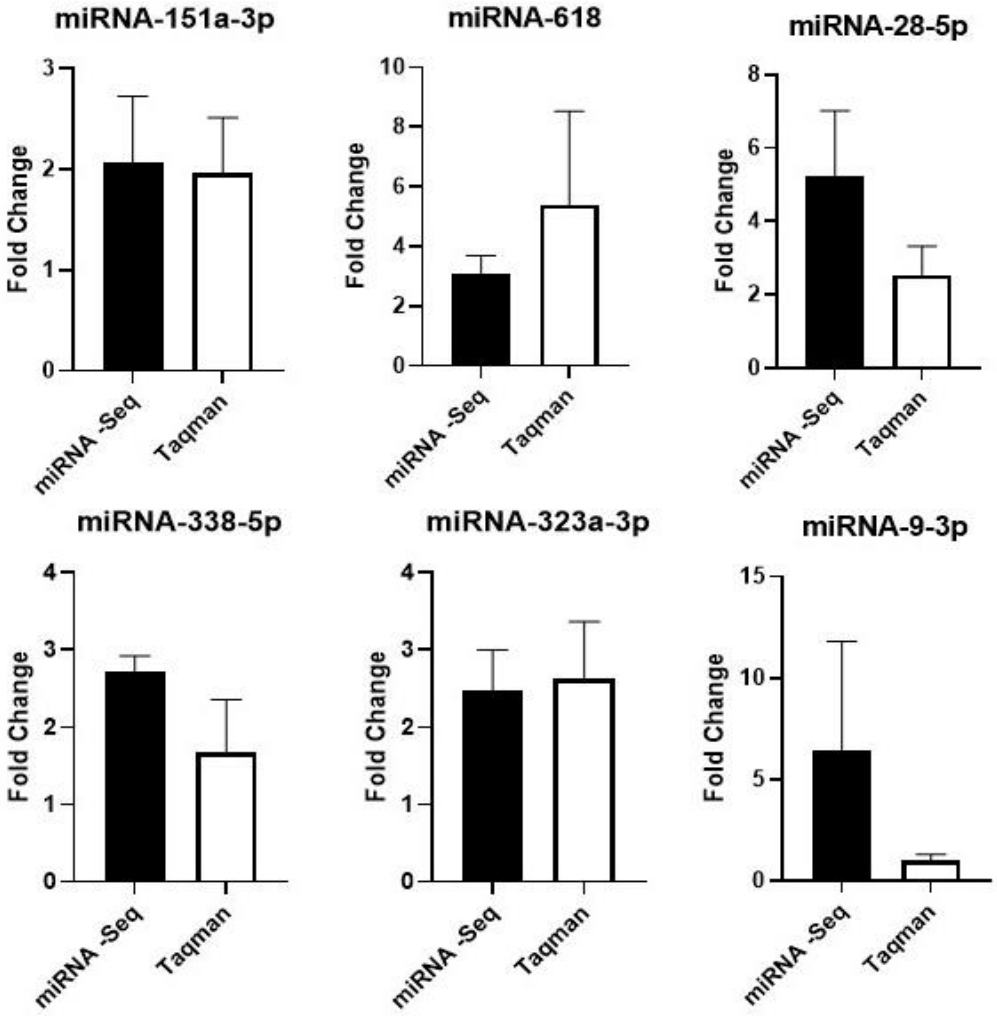

Figure 2: RT-PCR validation of miRNA-seq results. Select significantly regulated miRNAs were validated using RT-PCR. Fold change values for miRNA-seq (black bars) and RT-PCT (white bars) are shown for 6 miRNAs. None of the 6 miRNAs were found to be statistically different between the miRNA-seq and RT-PCR fold change results.

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Figure

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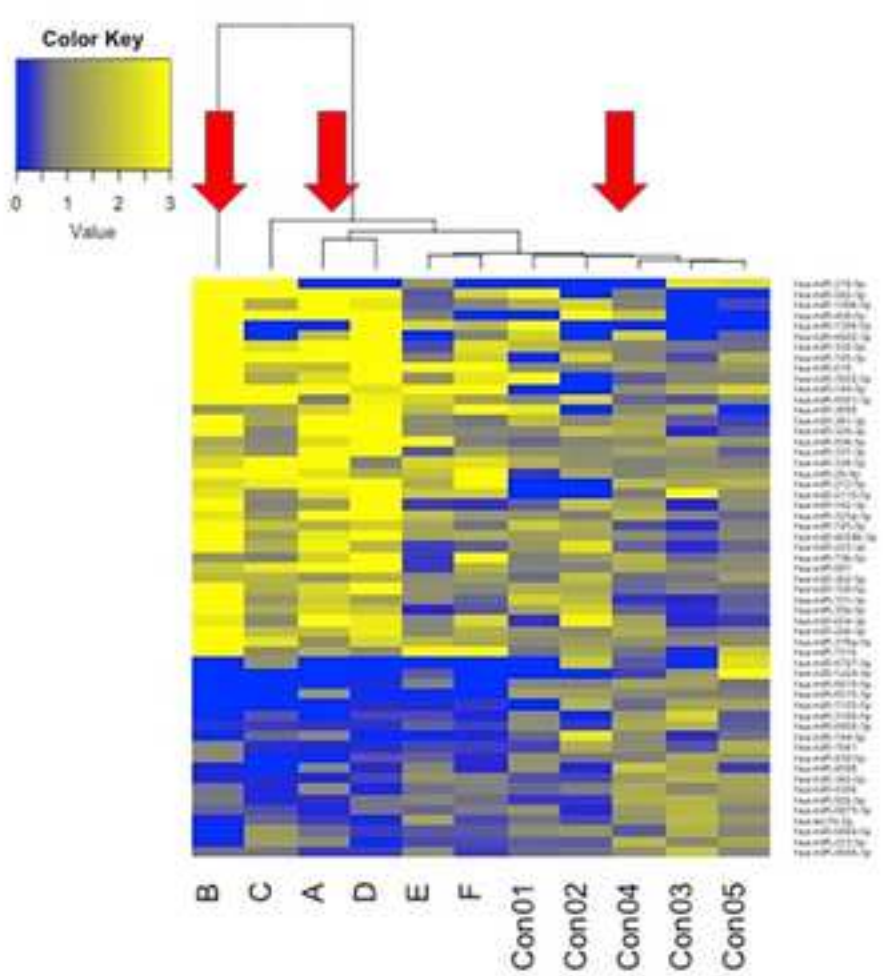

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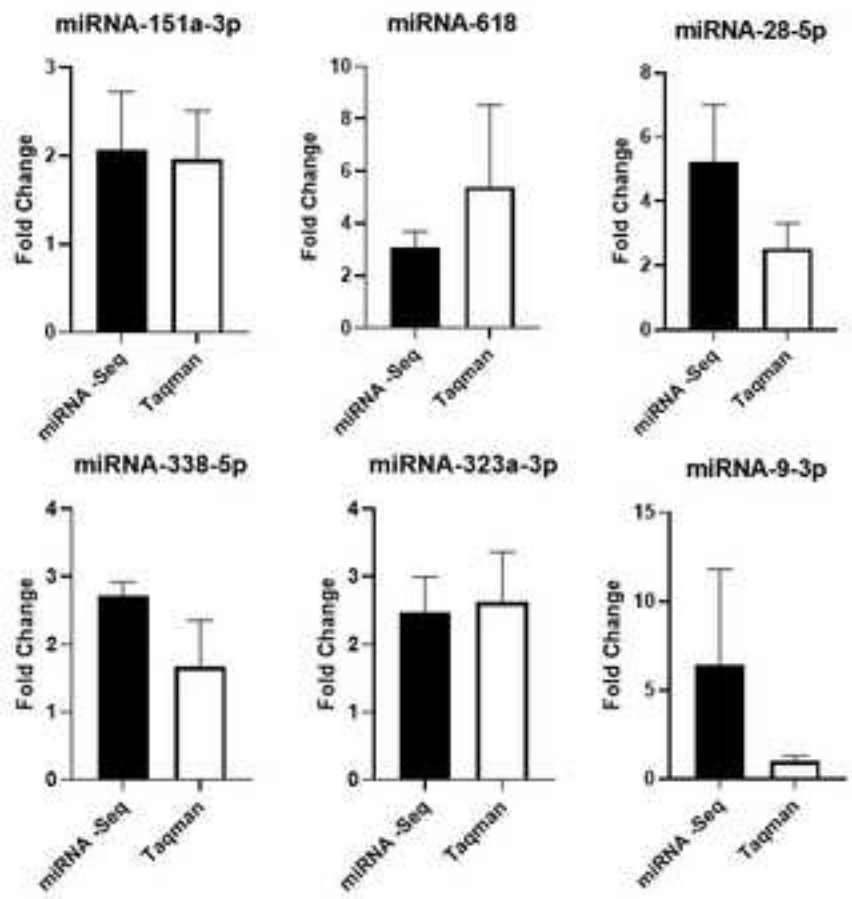
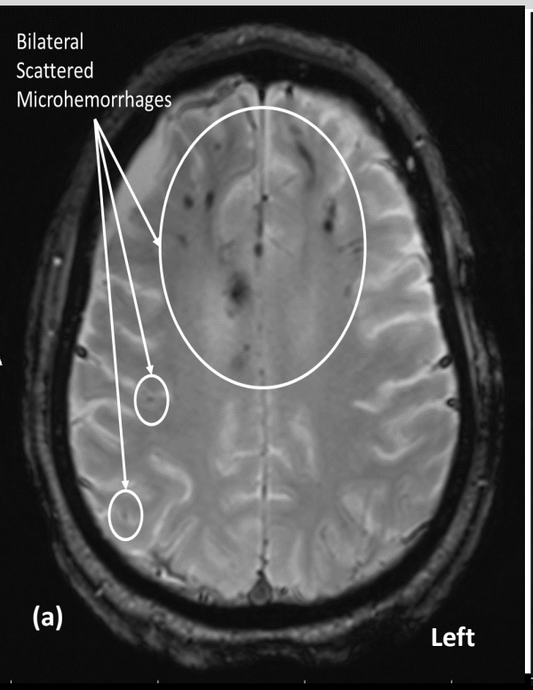
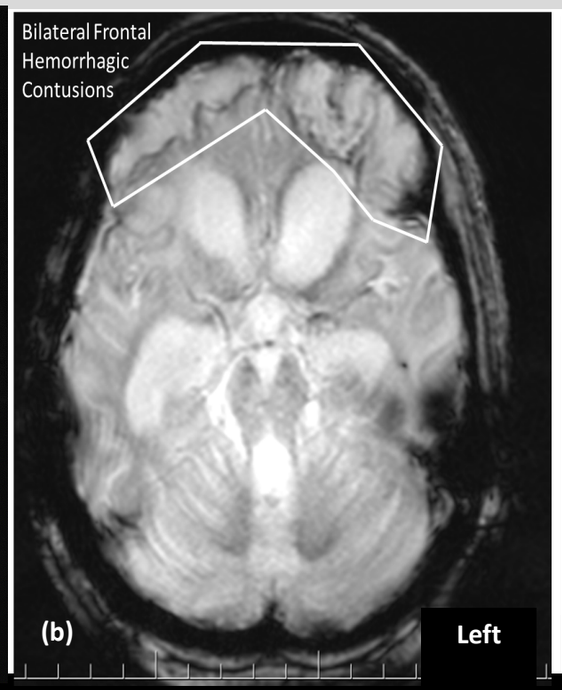
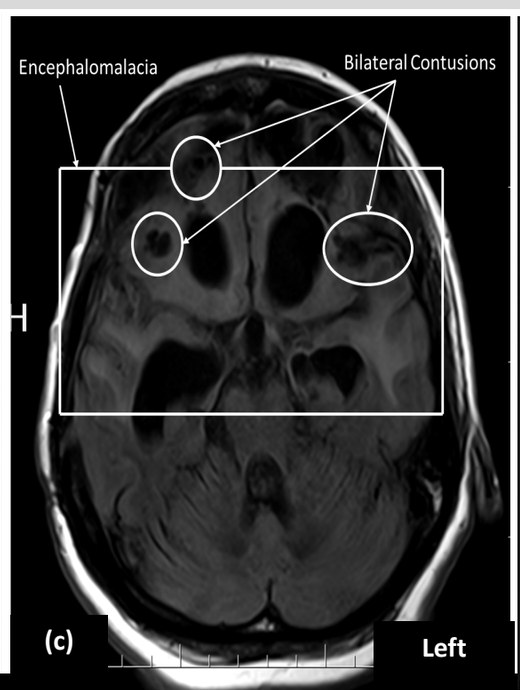


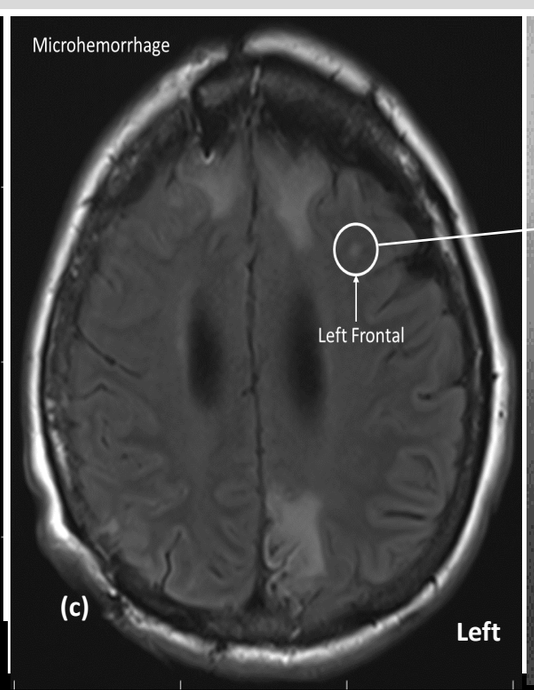
Supplement A

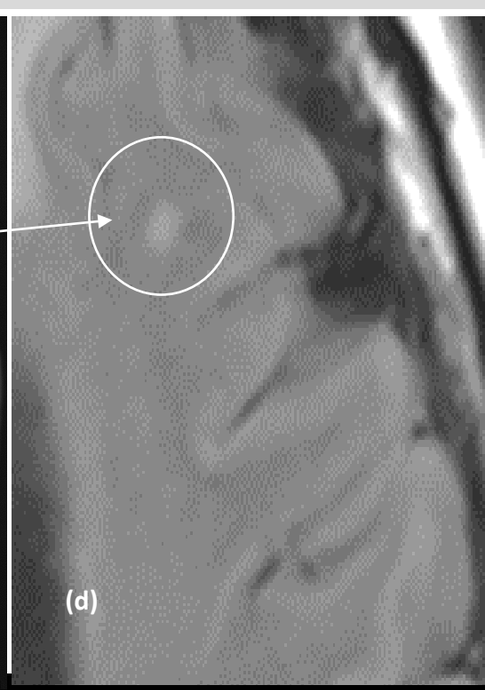
Predominant Brain Lesions Across Participants and for Each Participant; All images are in radiologic convention without flipping as denoted in lower right corner of each image











The three types of brain lesions illustrated above were common across participants. Using radiologic convention of not flipping the images. Images illustrated here are:

(a): Microhemorrhages identified using Axial T2* weighted gradient echo image. The scattered punctate signal voids in the subcortical white matter of the frontal and parietal lobes bilaterally are compatible with chronic microhemorrhages.

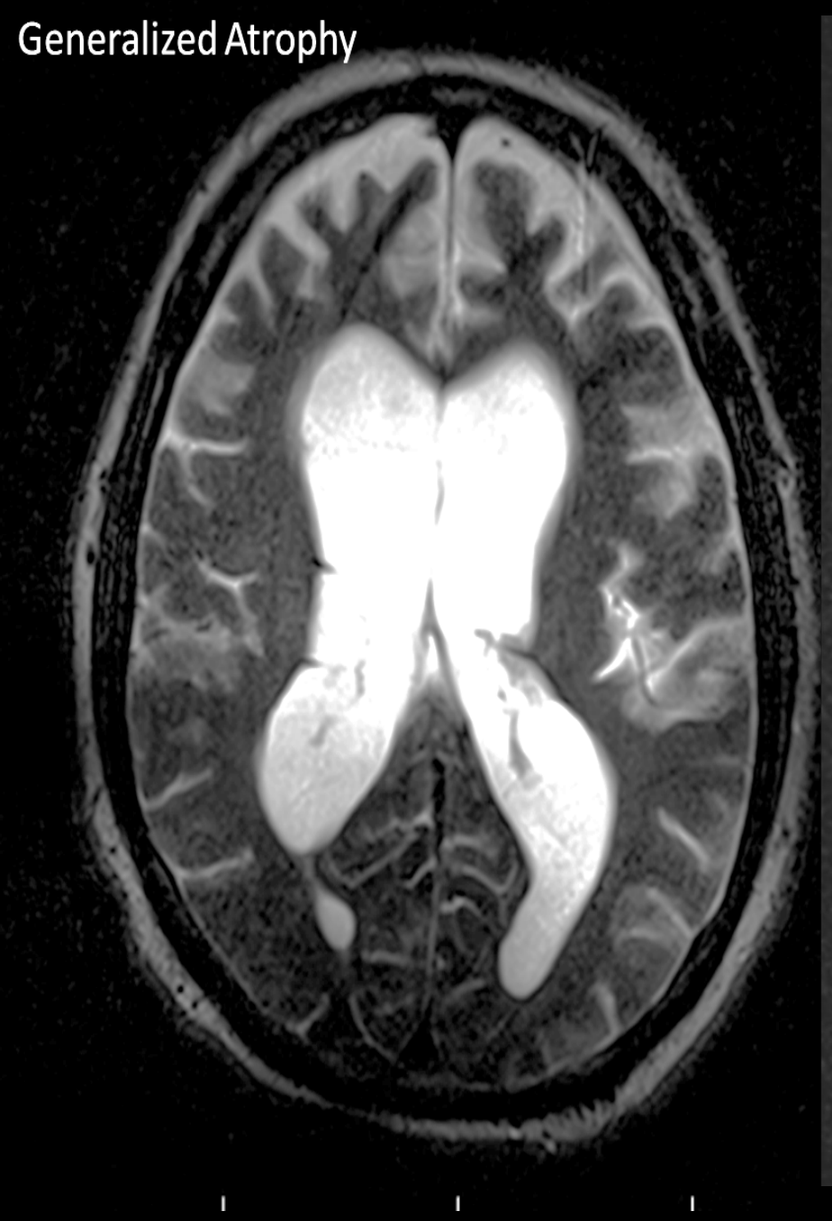
(b) – (c): Axial T2 * weighted gradient echo (GRE) (b) and fluid attenuated inversion recovery (FLAIR) (c) images demonstrate bilateral chronic hemorrhagic frontal contusions.

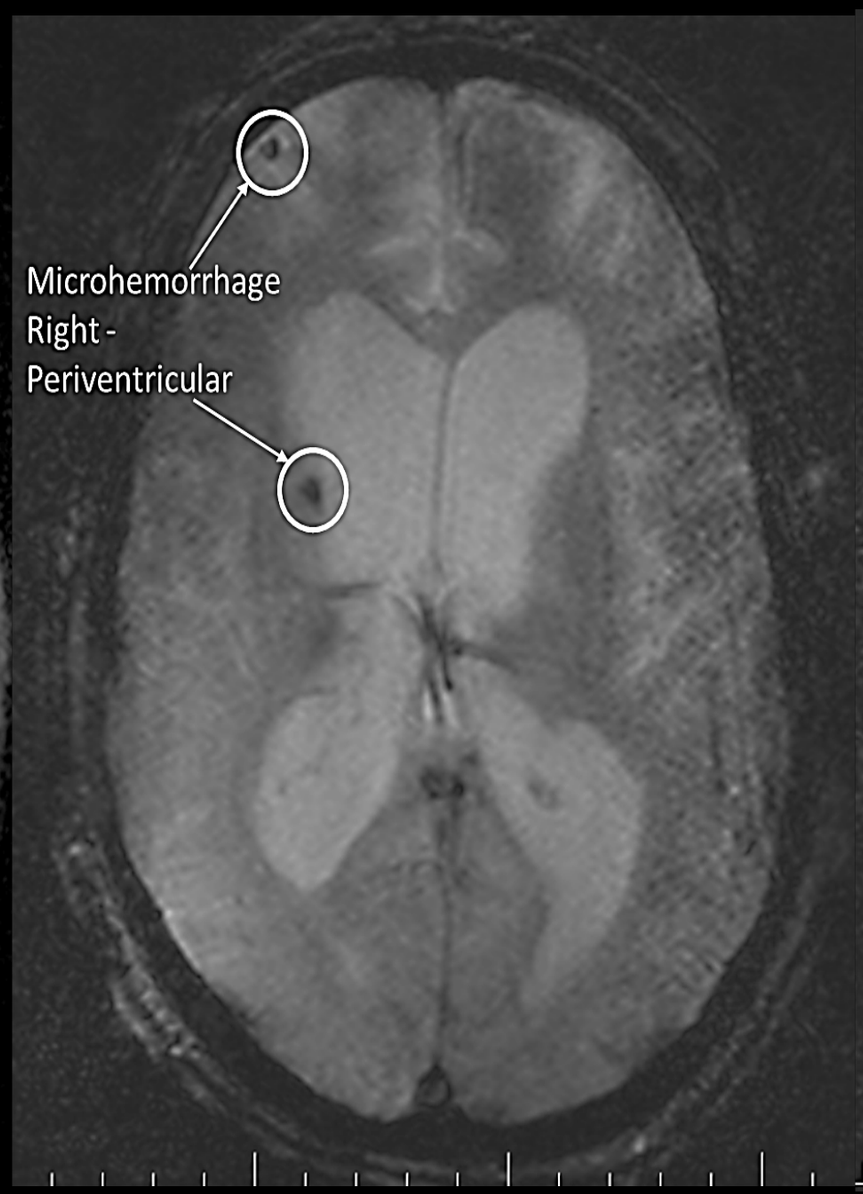
(d) – (e): Axial FLAIR image demonstrates a left frontal T2 hyperintense focus compatible with diffuse axonal injury (DAI) (d) with enlarged detail (e) from an axial FLAIR image demonstrating an approximately 3 mm T2 hyperintense lesion in the left frontal subcortical white matter, typical for DAI.

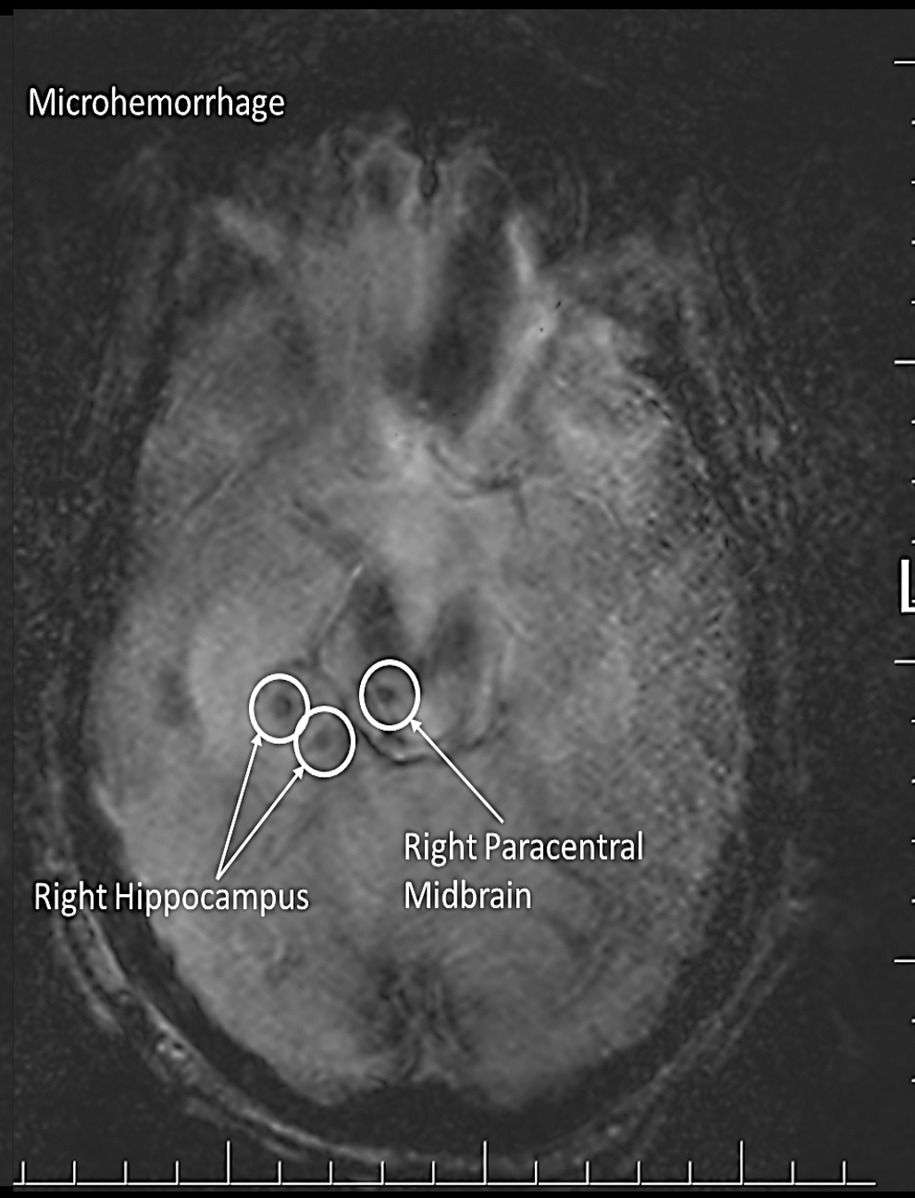
<u>Methods:</u>

- Baseline 3T MRI sequences included: T1, T2, Gradient Echo (GRE), Fluid Attenuated Inversion Recovery (FLAIR), Diffusion Weighted Imaging (DWI) and Susceptibility Weighted Imaging (SWI).
- Lesion Coding: Lesions, by type as well as cortical and sub-cortical locations, were coded by study

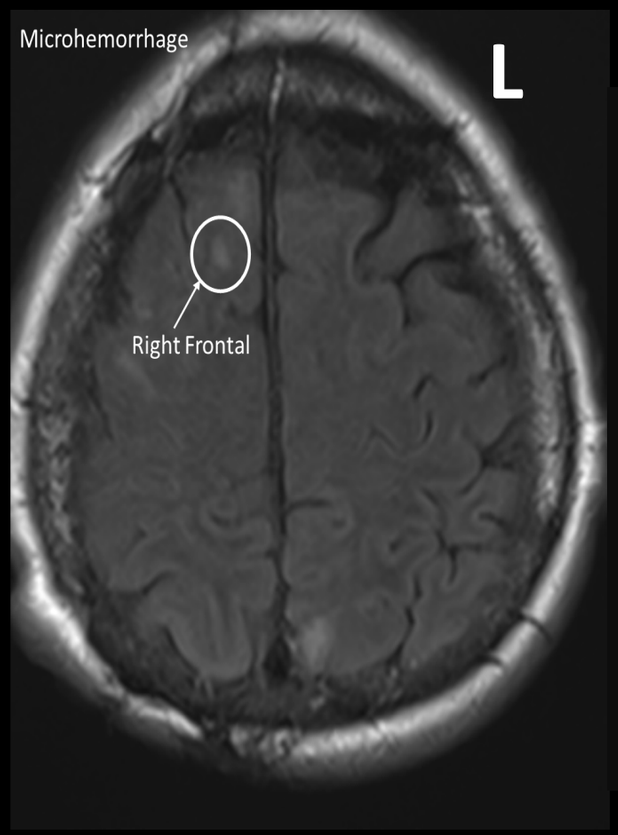
<u>Participant A (PA):</u> As illustrated below Participant A presented largely with generalized atrophy and microhemorrhages

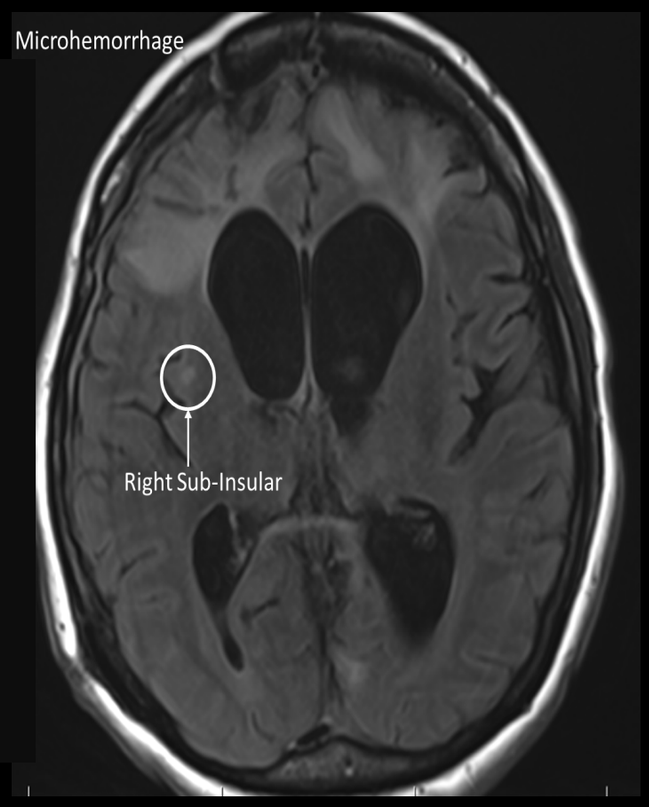


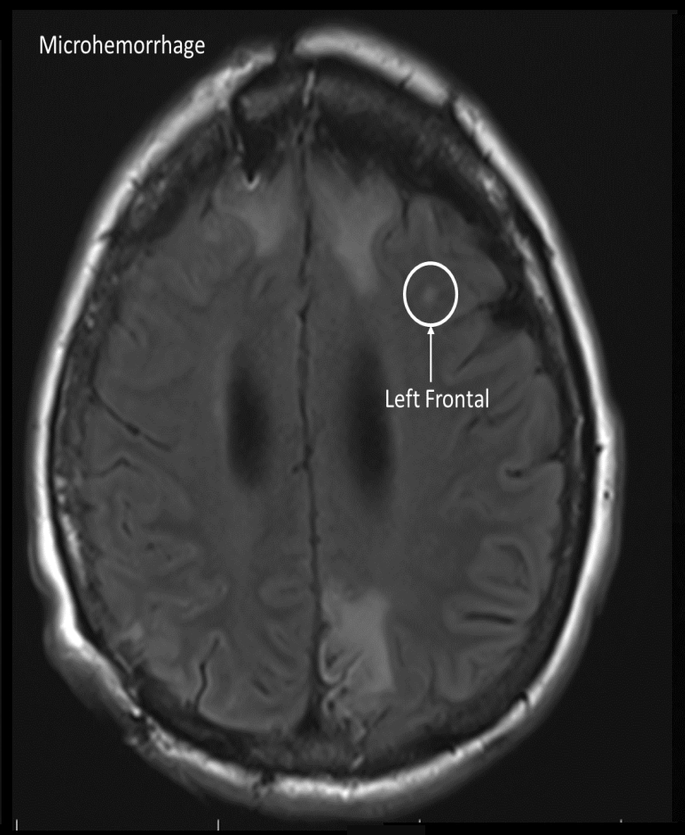


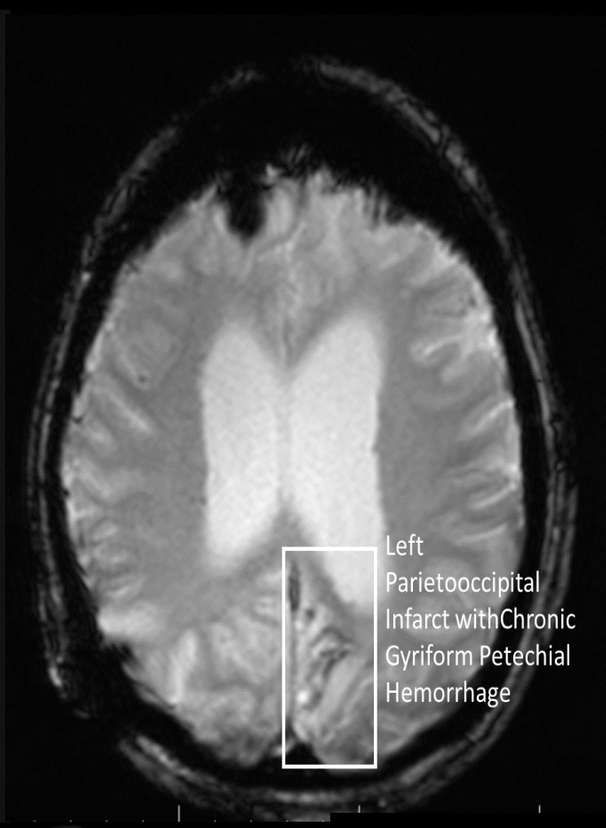


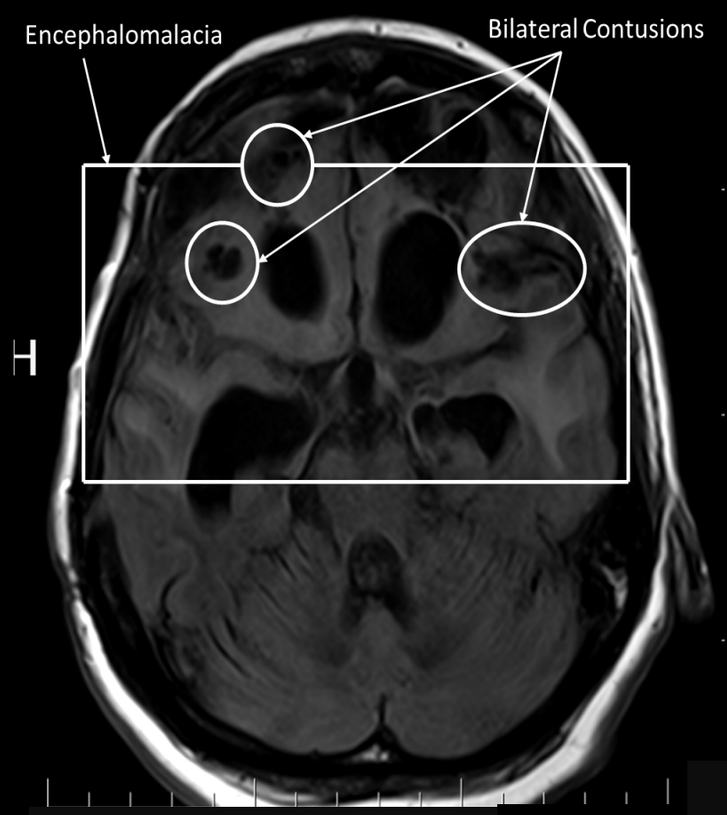
Participant B (PB): PB presented largely with microhemorrhages, contusions, encephalomalacia/chronic microhemorrhages & small infarcts

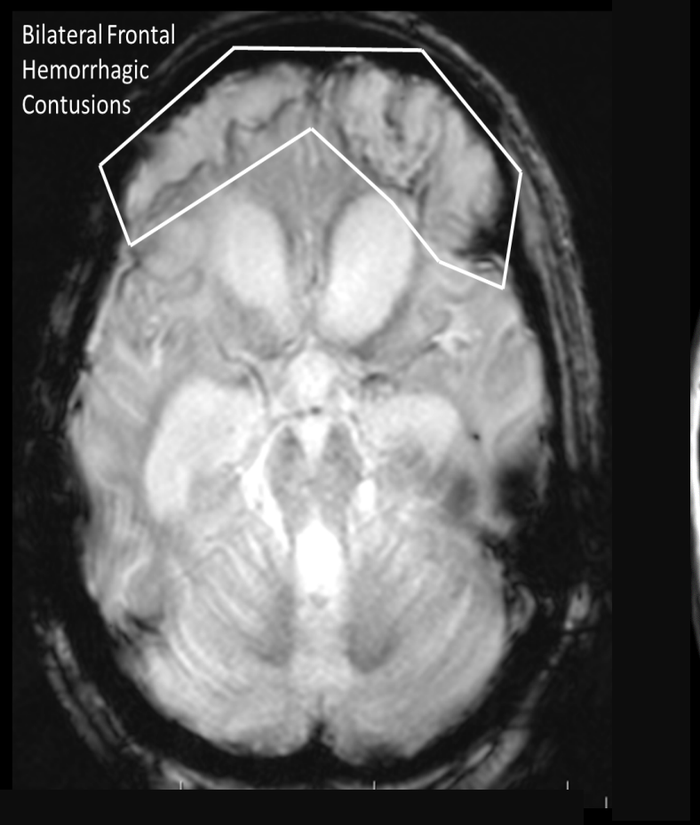


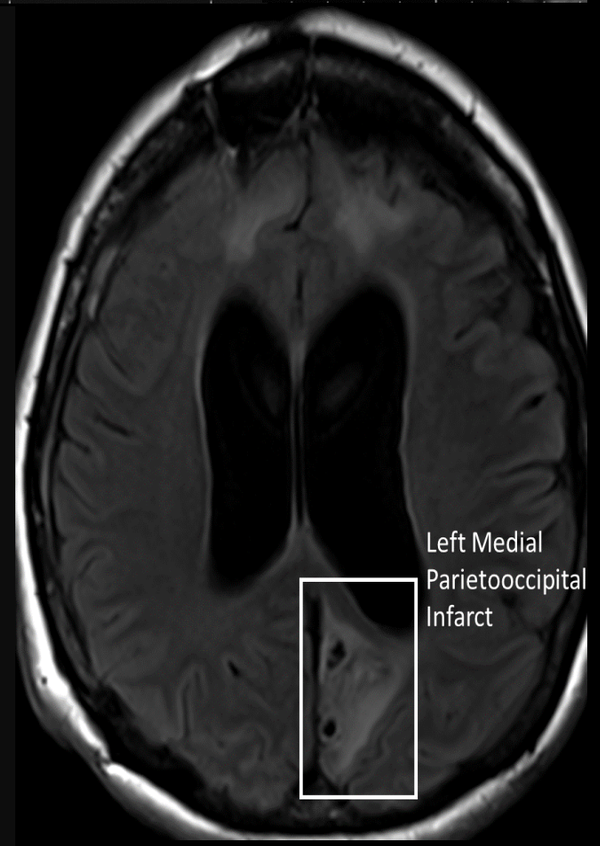




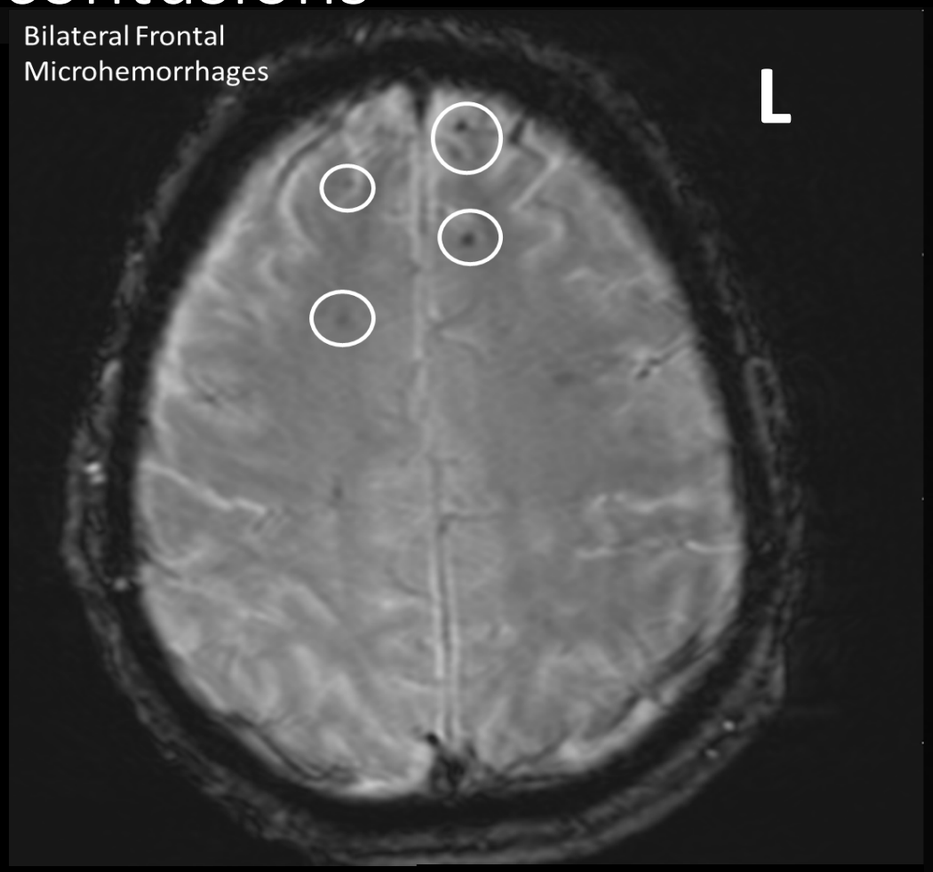


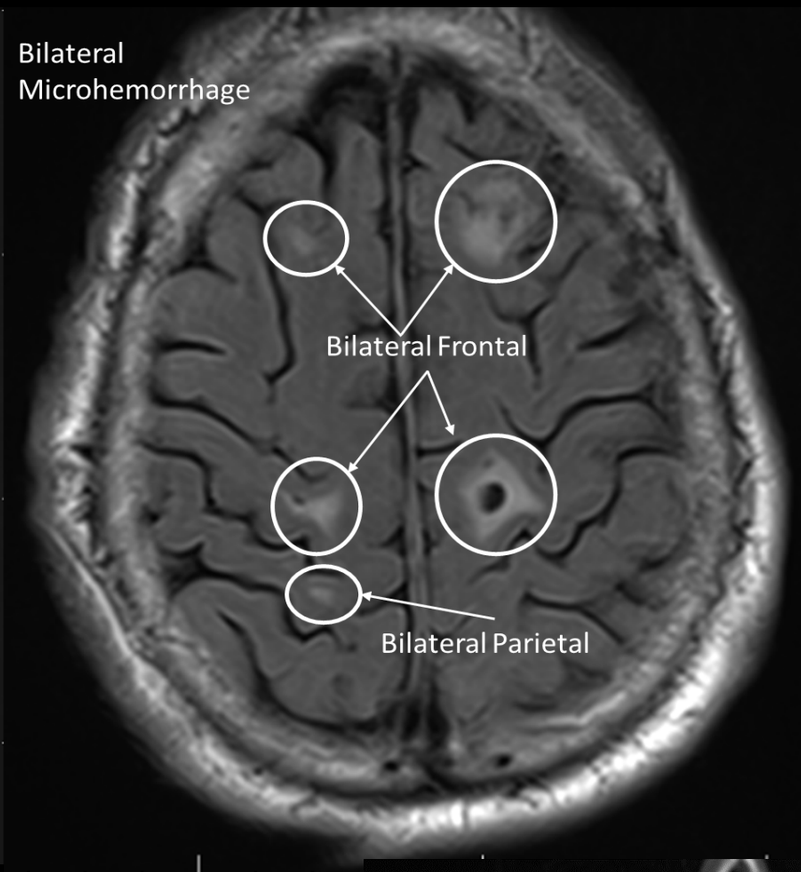


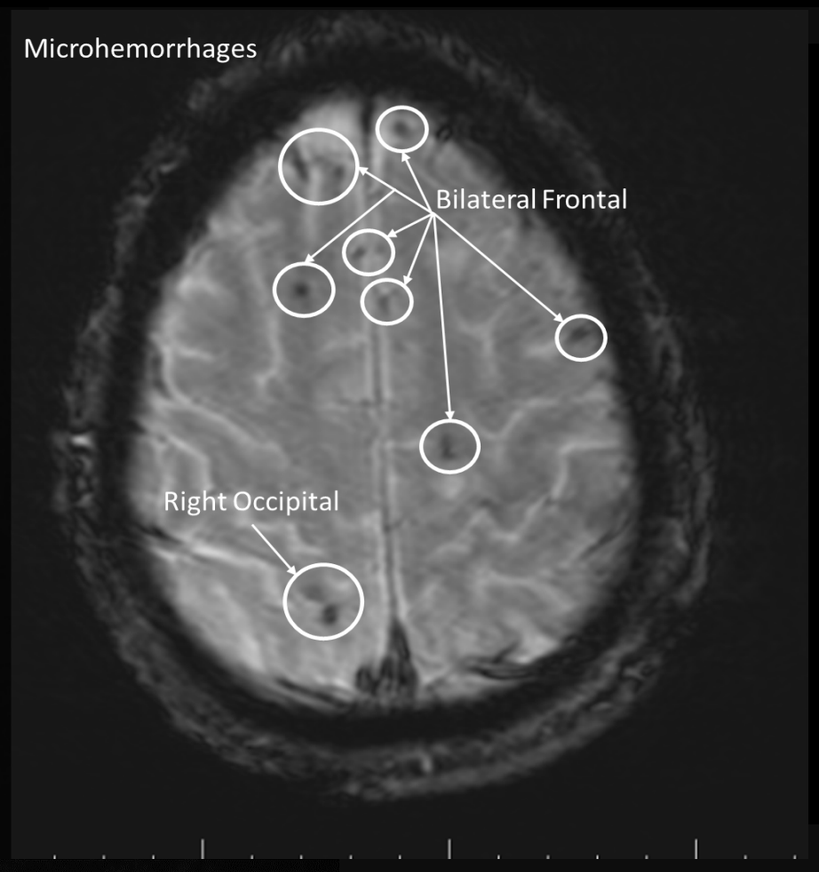


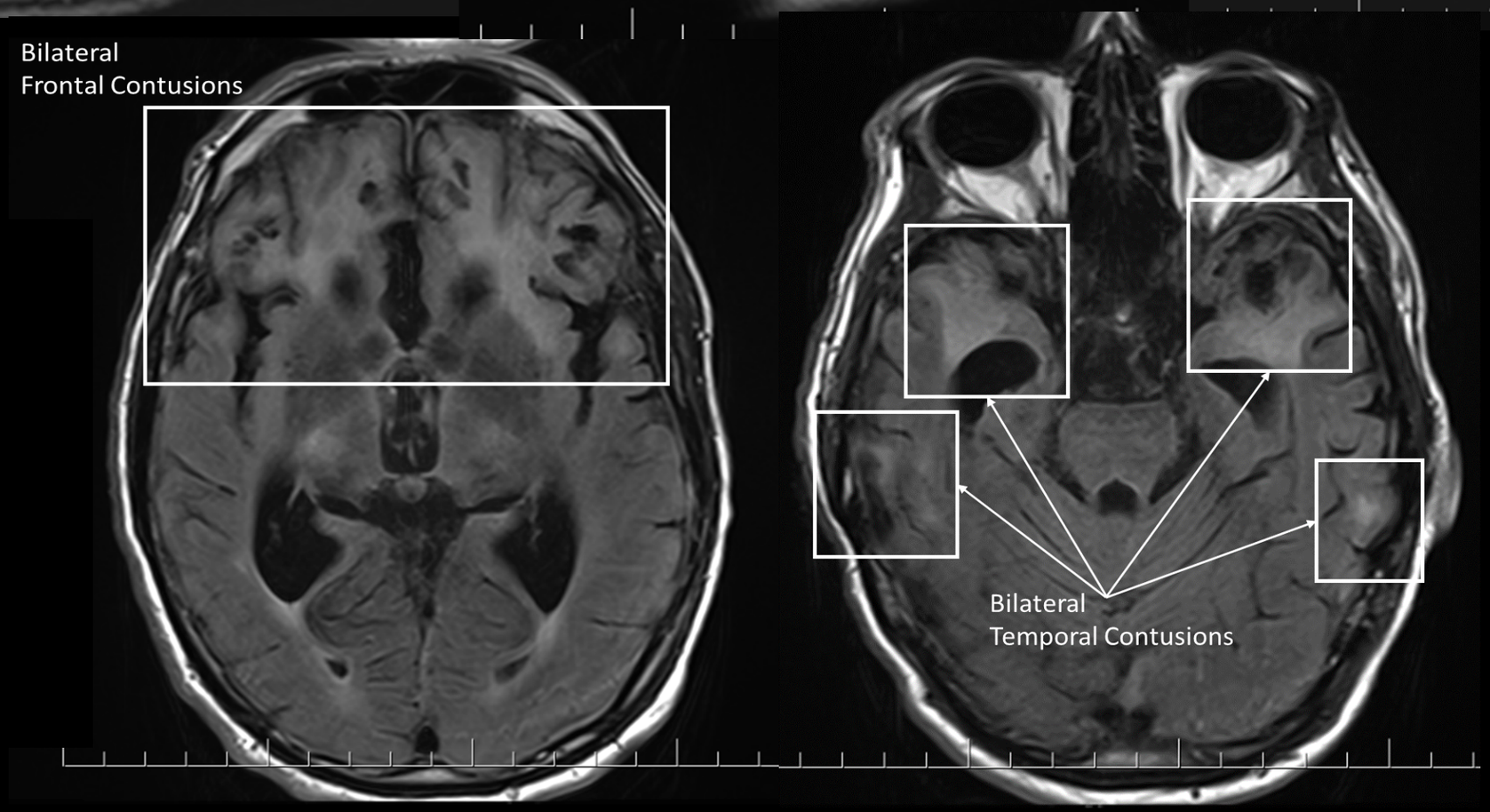


Participant C (PC): PC presented largely with microhemorrhages and contusions

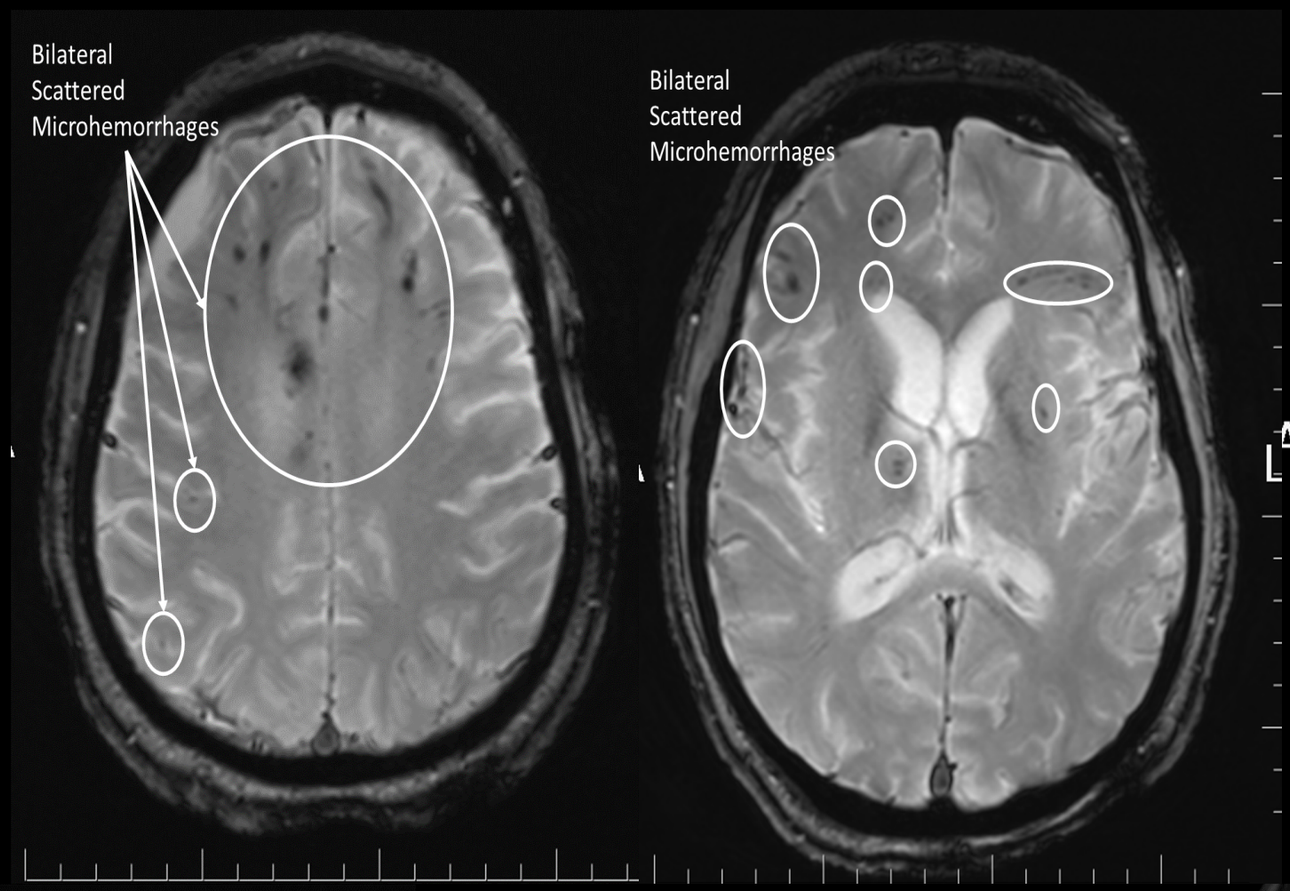


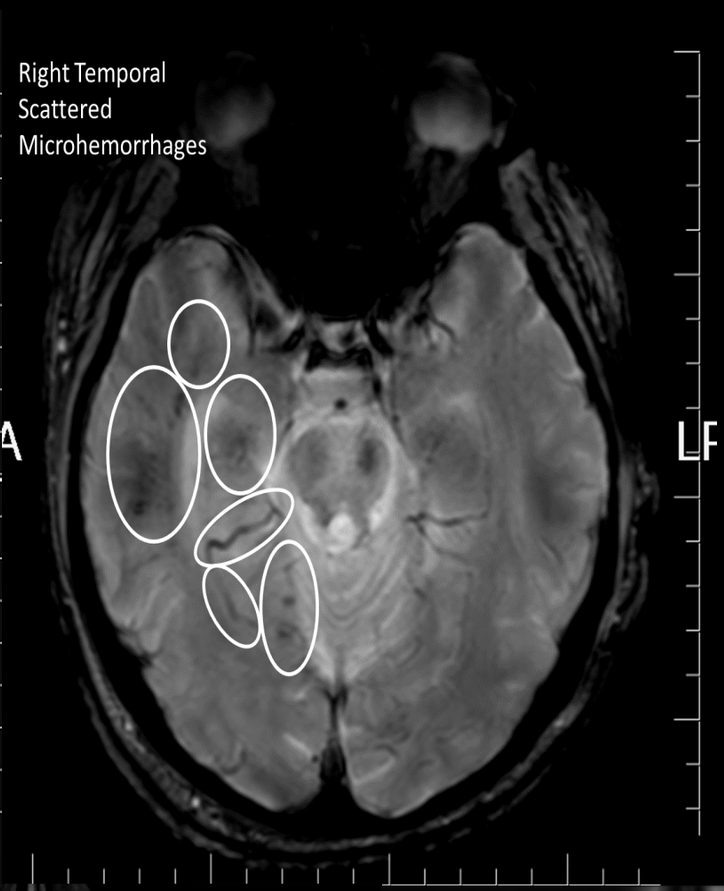


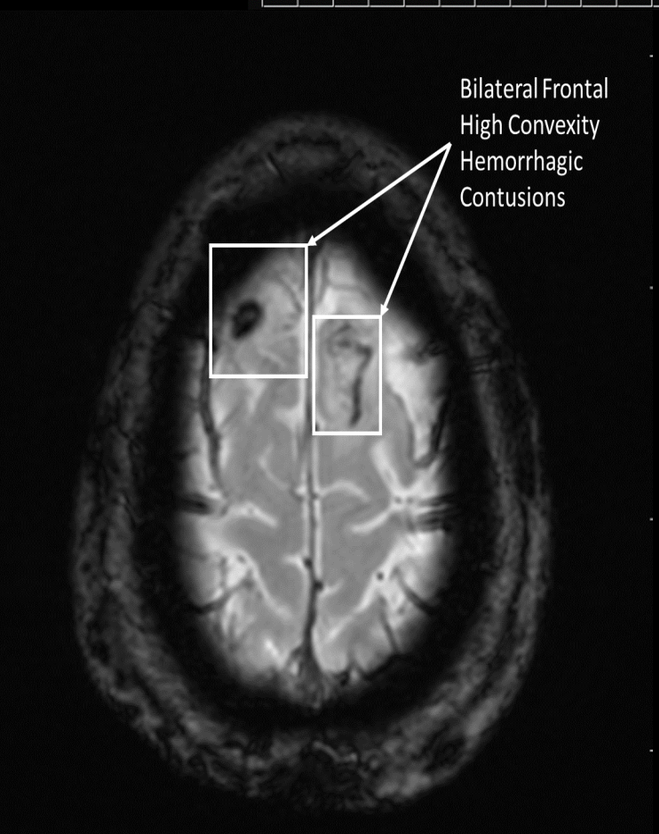


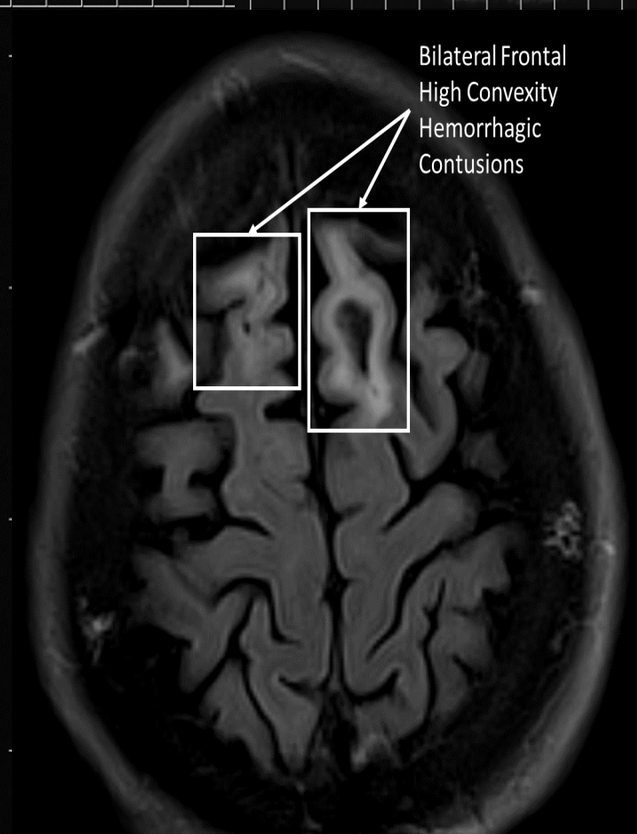


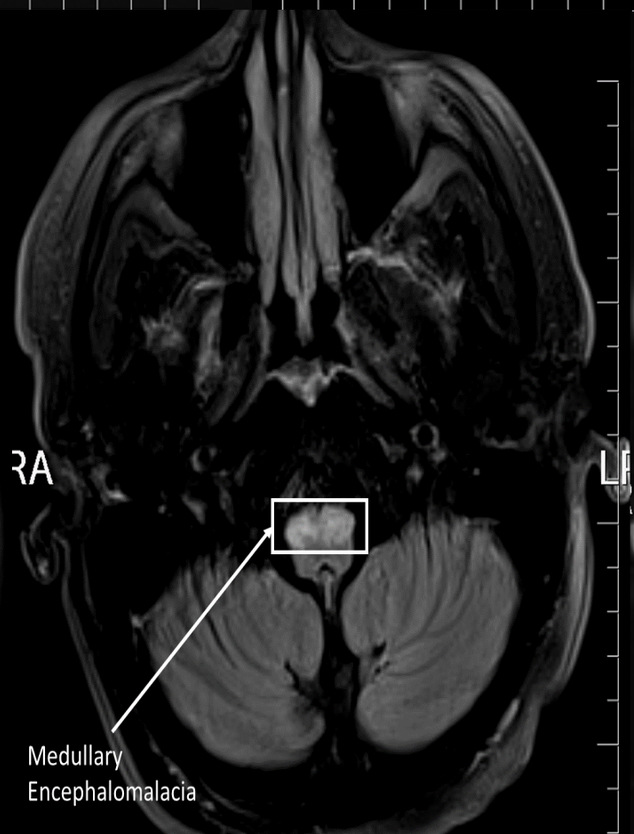
<u>Participant D (PD)</u>: PD presented largely with microhemorrhages, contusions and some encephalomalacia

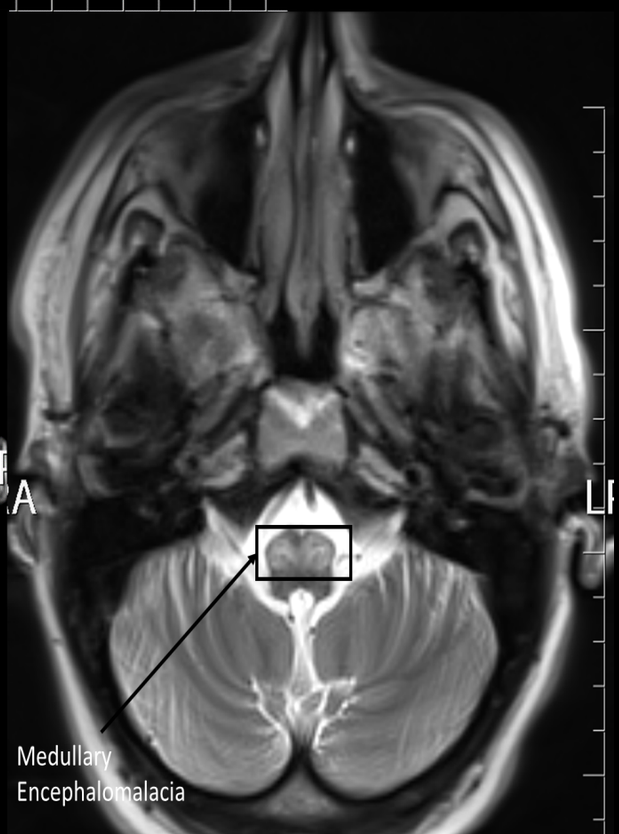




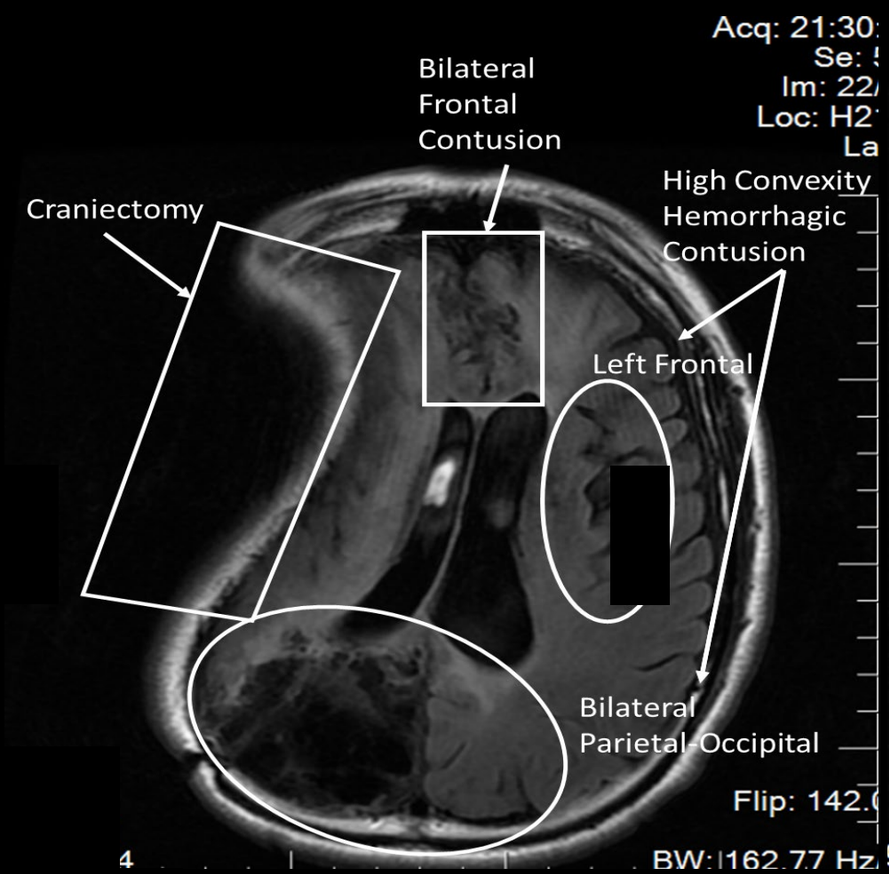


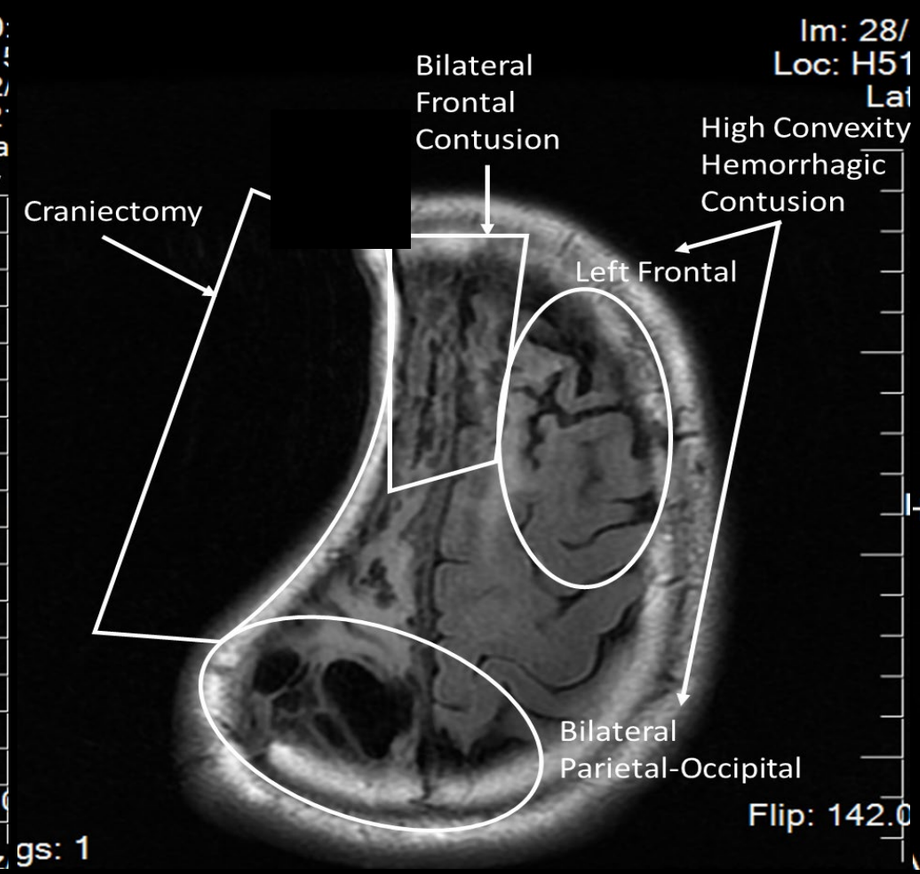


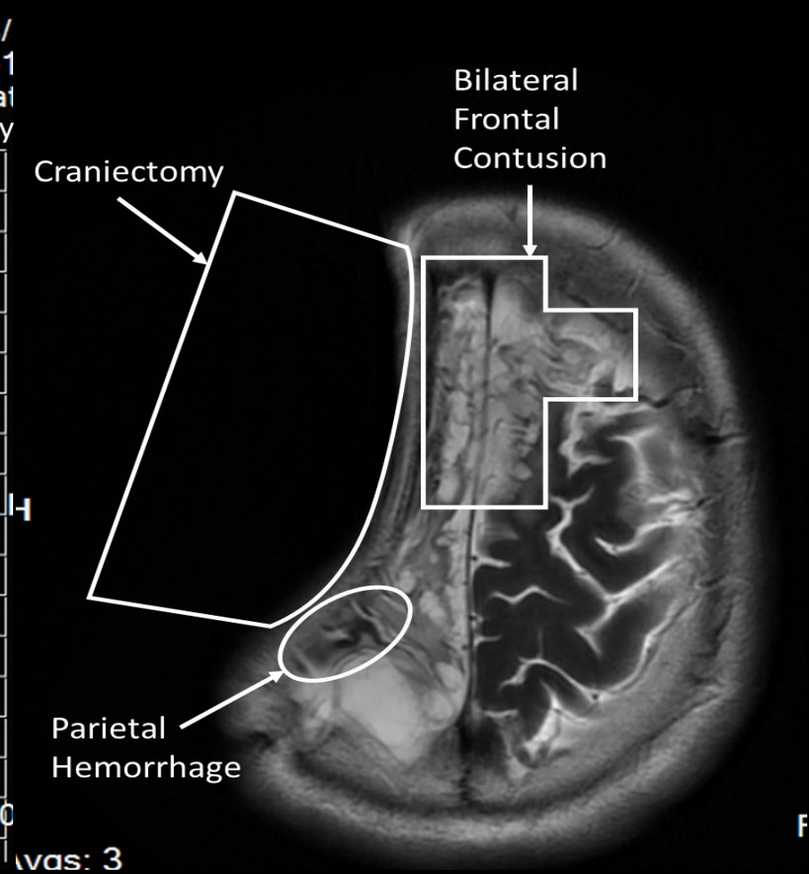


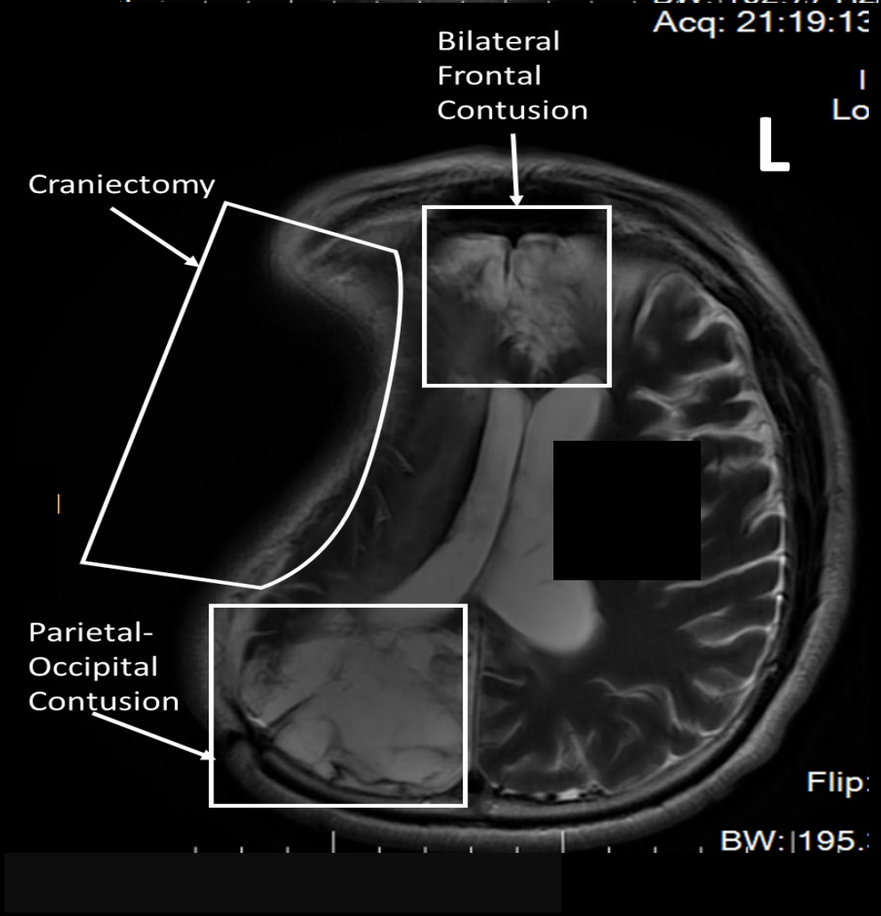


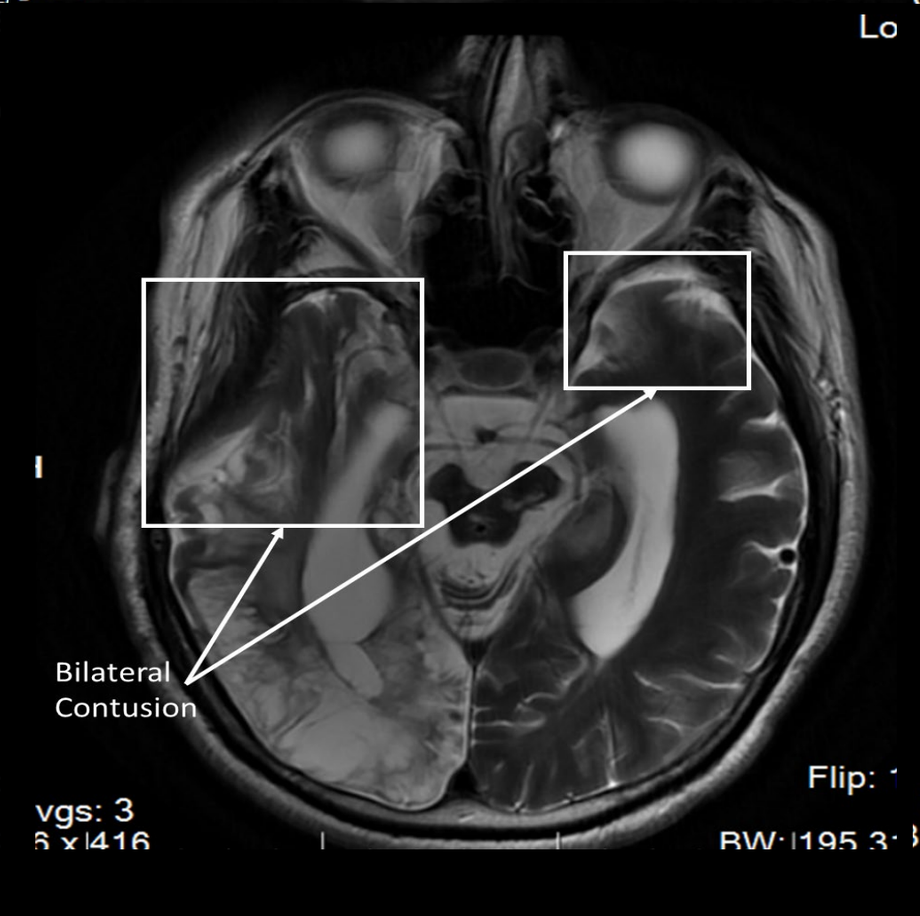
Participant E (PE): PE presented largely with contusions

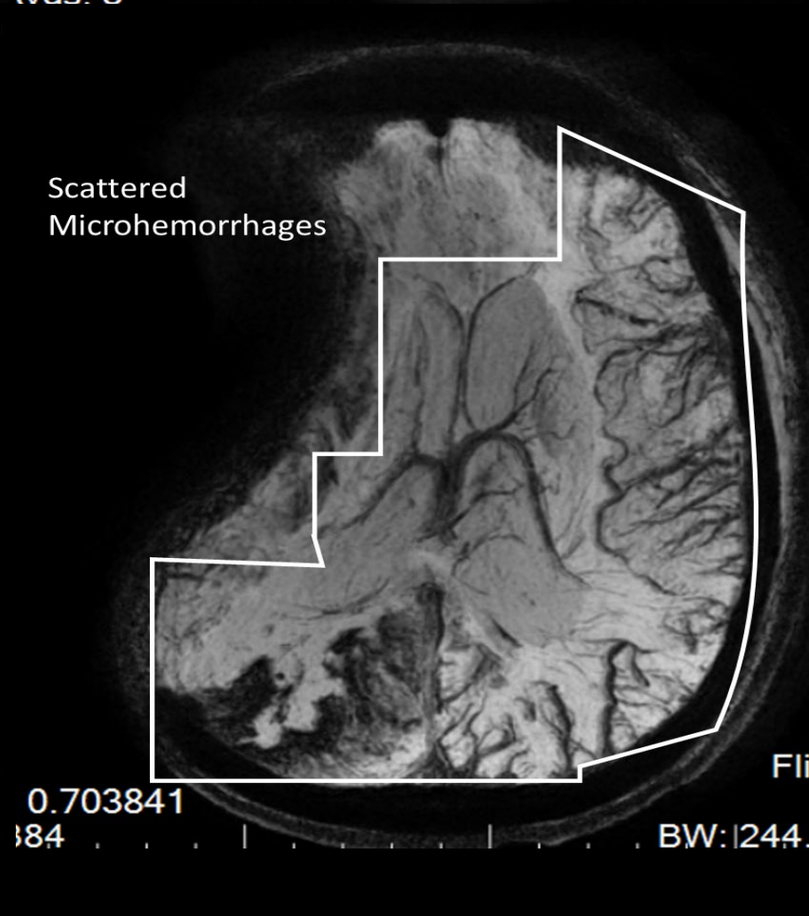




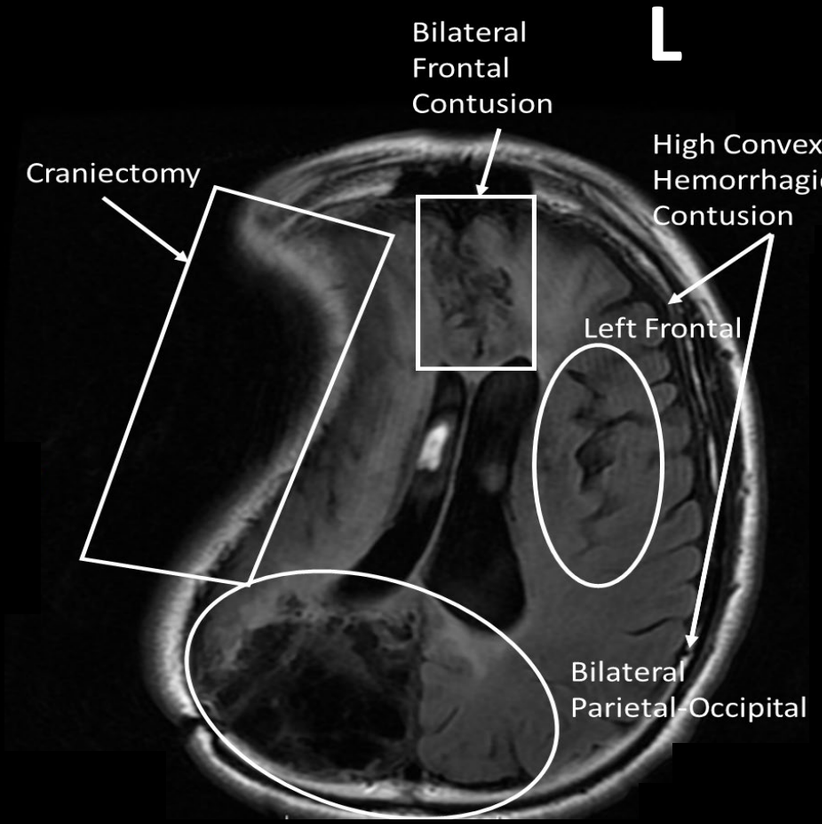


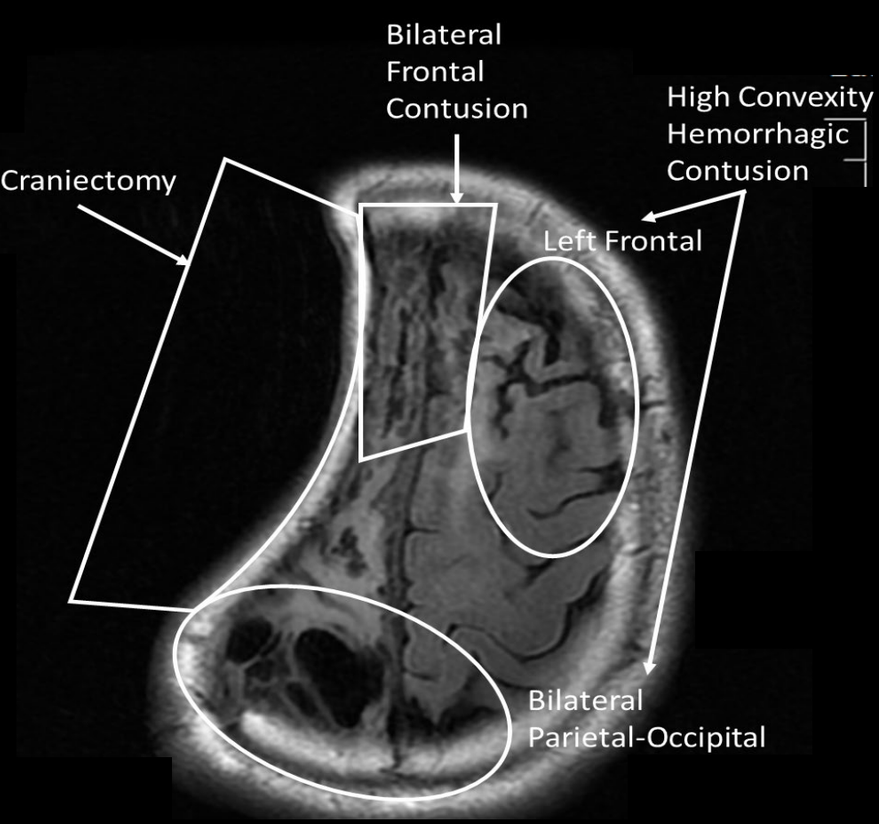


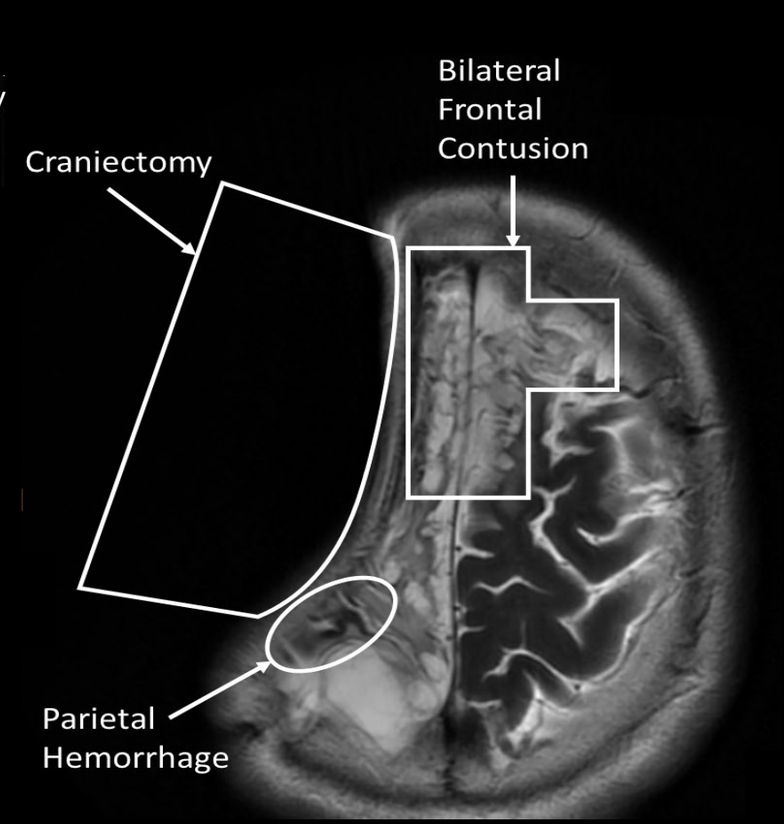


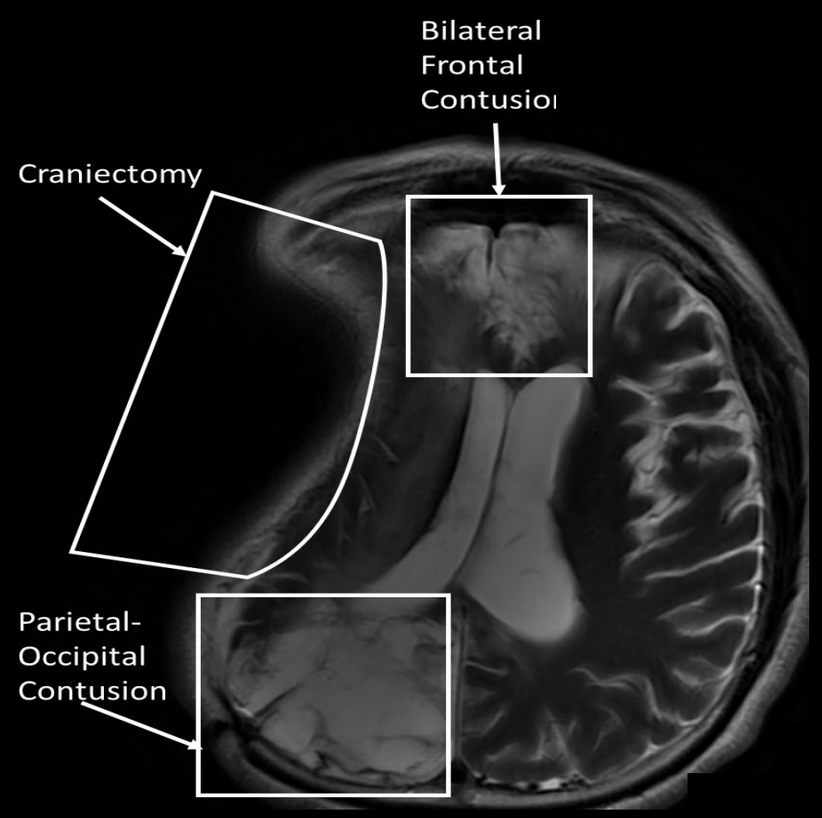


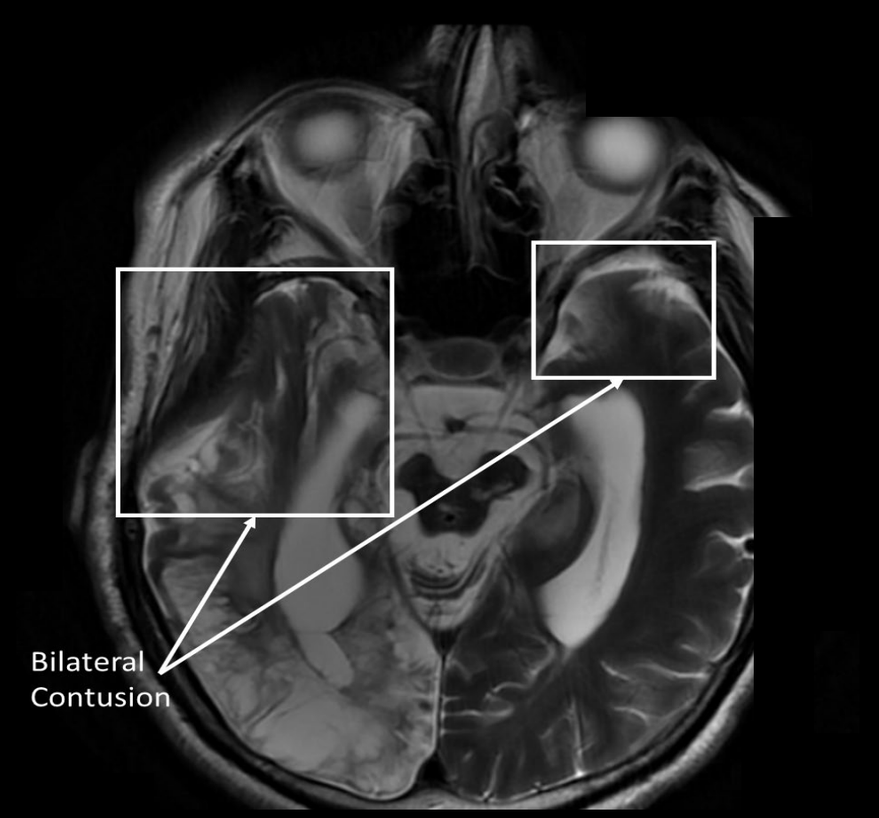
Participant F (PF): PF presented with craniotomy, contusions, microhemorrhages and chronic microhemorrhages/encephalomalacia

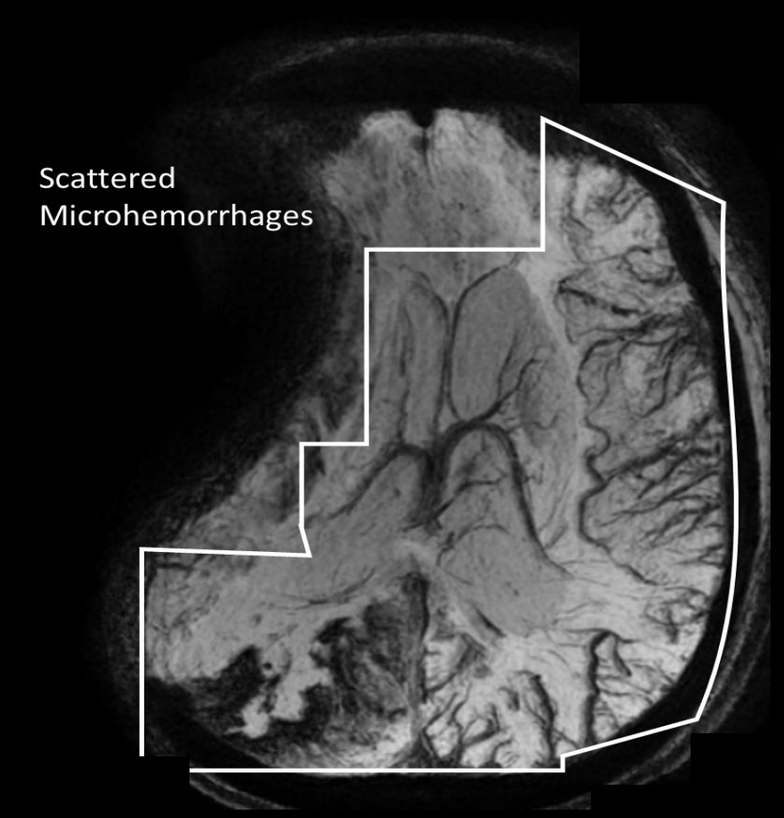












Supplement B

All correlations and p values between Clinical Phenotype Measures and Significantly Up- (a) and Down- (b) regulated microRNAs.

This supplement provides all correlations, and corresponding p values, between each baseline clinical phenotype measure and each of the significantly up- and down-regulated miRNAs. Correlations and p values are derived from a regression model fitted for each miRNA. Three models per miRNA were conducted and included one of each of three covariates (Cerebral Spinal Fluid relative volume, Gray Matter relative Volume and White Matter relative volume). Correlations with p values ≤ 0.05 while accounting for each of the three covariates, are considered significant. Each significant correlation, with p values, is highlighted in each table using red font. **Acronyms in reported tables are as follows:**

GCS T1 = Glasgow Coma Scale, obtained after one treatment session; **DOCS-25** = Disorders of Consciousness Scale – 25 items; **DOCS Aud-Lang, Visual Somto, Gust/Olf** = DOCS-25 Auditory-Language sub-scale, Visual Items, Somatosensory Items and Gustation/Olfaction Items; **CRS-R** = Coma Recovery Scale – Revised Total Converted Measure and the Auditory, Visual, Motor, Oromotor/Verbal and Arousal sub-scales; **DRS** = Disability Rating Scale; **CNC** = Coma Near Coma Scale; **GOSE =** Glasgow Outcome Scale Extended.

Table 1a. Correlations between Clinical Phenotype measures of Neurobehavioral Abilities and Outcomes and Significantly Up-reglated miRNAS

Clinical Phenotype Measures	Significantly Up-regulated miRNAs														
	miR-218-5p	miR-9-3p	miR-582-3p	miR-1246	miR-199b-5p	miR-409-5p	miR-4482-3p	miR-145-3p	miR-149-5p	miR-7855-5p	miR-335-5p	miR-3690	miR-618	miR-337-3p	miR-381-3p
DOCS Total	0.614	0.973	0.096	0.990	0.014	0.467	0.022	0.555	0.801	0.103	0.425	0.225	0.114	0.602	0.285
CRS-R Total	0.948	0.656	0.108	0.822	0.195	0.211	0.186	0.900	-0.996	0.329	0.753	0.485	0.090	0.311	0.066
DRS Total	0.293	0.070	0.946	0.025	-1.000	0.443	-0.999	0.337	0.172	0.909	0.454	0.716	0.924	0.328	0.662
CNC Total	0.254	0.103	0.979	0.008	0.993	0.492	0.996	0.296	0.137	0.857	0.408	0.657	0.964	0.371	0.721
GOSE Total	0.001	0.381	0.780	0.264	0.636	0.898	0.650	0.035	0.109	0.454	0.124	0.303	0.811	0.753	0.997
GCS Total	0.802	0.823	0.012	0.961	0.095	0.322	0.086	0.739	0.961	0.217	0.589	0.354	0.005	0.436	0.164
DOCS Aud-Lang	0.943	0.393	0.297	0.530	0.401	0.033	0.390	0.979	0.773	0.574	0.990	0.771	0.276	0.120	0.106
CRS-R Auditory	0.270	0.090	0.967	0.000	-0.998	0.472	-0.999	0.312	0.151	0.879	0.426	0.681	0.949	0.353	0.697
DOCS Visual	0.026	0.416	0.734	0.295	0.592	0.934	0.606	0.009	0.136	0.417	0.097	0.272	0.765	0.799	0.985
CRS-R Visual	0.407	0.020	0.810	0.116	0.937	0.324	0.927	0.457	0.272	0.996	0.592	0.870	0.779	0.223	0.516
DOCS Somato	0.520	0.098	0.672	0.198	0.817	0.232	0.803	0.577	0.368	0.977	0.727	0.972	0.642	0.139	0.403
CRS-R Arousal	0.001	0.381	0.780	0.264	0.636	0.898	0.650	0.035	0.109	0.454	0.124	0.303	0.811	0.753	-0.997
DOCS Gust/Olf	0.956	0.409	0.282	0.549	0.385	0.045	0.375	0.987	0.794	0.554	0.983	0.750	0.262	0.133	0.094
CRS-R Oro-Motor/Verbal	0.017	0.358	0.811	0.244	0.666	0.870	0.681	0.053	0.091	0.479	0.142	0.324	0.841	0.721	1.000
CRS-R Motor	0.310	0.846	0.332	0.680	0.234	0.820	0.244	0.267	0.452	0.110	0.170	0.005	0.354	0.949	0.580
Spaulding Total	0.045	0.322	0.859	0.212	0.716	0.823	0.730	0.081	0.062	0.522	0.172	0.360	0.887	0.672	0.994

Table 1a Continued. Correlations between Clinical Phenotype measures of Neuorbehavioral Abilities and Outcomes and Significantly Up-reglated miRNAS

Clinical Phenotype Measures	Significanlty Up-regulated miRNAs														
	miR-5001-3p	miR-338-5p	miR-28-5p	miR-504-5p	miR-2115-5p	miR-329-3p	miR-10b-5p	miR-212-5p	miR-581	miR-142-3p	miR-4659b-3p	miR-323a-3p	miR-145-5p	miR-769-5p	miR-151a-3p
DOCS Total	0.674	0.607	0.622	0.356	0.355	0.513	0.516	0.916	0.149	0.273	0.011	0.383	0.372	0.115	0.668
CRS-R Total	0.982	0.315	0.326	0.125	0.124	0.246	0.860	0.573	0.056	0.549	0.221	0.146	0.683	0.344	0.361
DRS Total	0.252	0.324	0.313	0.566	0.568	0.400	0.369	0.120	0.874	0.643	-0.996	0.533	0.513	0.891	0.281
CNC Total	0.214	0.367	0.355	0.621	0.623	0.447	0.327	0.155	0.923	0.587	0.977	0.587	0.463	0.837	0.321
GOSE Total	0.036	0.747	0.731	0.988	0.988	0.849	0.060	0.449	0.867	0.253	0.595	0.972	0.165	0.436	0.684
GCS Total	0.862	0.440	0.453	0.227	0.226	0.361	0.695	0.736	0.039	0.409	0.120	0.250	0.526	0.231	0.493
DOCS Aud-Lang	0.894	0.124	0.133	0.048	0.049	0.064	-0.994	0.329	0.237	0.843	0.435	0.028	0.958	0.594	0.161
CRS-R Auditory	0.229	0.350	0.338	0.598	0.600	0.428	0.344	0.141	0.904	0.609	0.987	0.565	0.483	0.859	0.305
DOCS Visual	0.062	0.793	0.777	0.999	-0.999	0.891	0.034	0.487	0.824	0.224	0.553	-0.991	0.138	0.400	0.729
CRS-R Visual	0.361	0.220	0.210	0.431	0.433	0.287	0.494	0.030	0.719	0.800	0.965	0.403	0.659	-0.990	0.180
DOCS Somato	0.468	0.136	0.126	0.328	0.330	0.198	0.619	0.048	0.586	0.925	0.858	0.303	0.796	0.986	0.099
CRS-R Arousal	0.036	0.747	0.731	0.988	0.988	0.849	0.060	0.449	0.867	0.253	0.595	0.972	0.165	0.436	0.684
DOCS Gust/Olf	0.912	0.136	0.145	0.036	0.037	0.076	0.998	0.344	0.223	0.823	0.418	0.016	0.944	0.574	0.174
CRS-R Oro-Motor/Verbal	0.018	0.716	0.700	0.975	0.976	0.819	0.078	0.424	0.895	0.274	0.625	0.955	0.184	0.462	0.653
CRS-R Motor	0.354	0.953	0.963	0.678	0.675	0.871	0.238	0.922	0.398	0.047	0.207	0.713	0.128	0.098	0.986
Spaulding Total	0.010	0.666	0.651	0.945	0.947	0.770	0.107	0.386	0.935	0.308	0.673	0.919	0.215	0.504	0.605

Table 1b. P VALUES for Correlations between Clinical Phenotype measures of Neurobehavioral Abilties and Significantly Up-reglated miRNAS

Clinical Phenotype Measures	Significantly Up-regulated miRNAs														
	miR-218-5p	miR-9-3p	miR-582-3p	miR-1246	miR-199b-5p	miR-409-5p	miR-4482-3p	miR-145-3p	miR-149-5p	miR-7855-5p	miR-335-5p	miR-3690	miR-618	miR-337-3p	miR-381-3p
DOCS Total	0.364	0.075	0.878	**0.046**	0.983	0.483	0.972	0.409	0.227	0.870	0.520	0.725	0.856	0.373	0.658
CRS-R Total	0.106	0.332	0.864	0.212	0.760	0.740	0.770	0.151	**0.030**	0.612	0.262	0.467	0.886	0.631	0.916
DRS Total	0.650	0.912	0.108	0.968	**0.004**	0.504	**0.014**	0.605	0.786	0.143	0.494	0.289	0.130	0.613	0.328
CNC Total	0.692	0.869	0.066	0.990	**0.039**	0.461	**0.028**	0.647	0.829	0.186	0.536	0.331	0.088	0.571	0.286
GOSE Total	0.999	0.561	0.243	0.681	0.347	0.153	0.337	0.955	0.863	0.494	0.844	0.640	0.221	0.262	**0.023**
GCS Total	0.227	0.211	0.985	0.091	0.880	0.620	0.891	0.272	0.090	0.733	0.383	0.588	0.993	0.510	0.795
DOCS Aud-Lang	0.112	0.550	0.646	0.430	0.542	0.958	0.552	0.066	0.248	0.395	**0.045**	0.249	0.668	0.849	0.866
CRS-R Auditory	0.675	0.887	0.083	0.993	**0.022**	0.478	**0.011**	0.630	0.812	0.169	0.519	0.314	0.105	0.588	0.303
DOCS Visual	0.966	0.528	0.276	0.648	0.380	0.120	0.370	0.988	0.830	0.527	0.877	0.673	0.254	0.229	0.056
CRS-R Visual	0.536	0.975	0.221	0.854	0.117	0.617	0.127	0.491	0.673	**0.030**	0.380	0.176	0.244	0.727	0.441
DOCS Somato	0.437	0.876	0.321	0.755	0.216	0.716	0.226	0.392	0.574	0.069	0.281	0.076	0.343	0.826	0.540
CRS-R Arousal	0.999	0.561	0.243	0.681	0.347	0.153	0.337	0.955	0.863	0.494	0.844	0.640	0.221	0.262	**0.023**
DOCS Gust/Olf	0.096	0.535	0.661	0.415	0.557	0.943	0.567	**0.051**	0.233	0.410	0.060	0.264	0.684	0.833	0.881
CRS-R Oro-Motor/Verbal	0.978	0.584	0.220	0.704	0.325	0.175	0.314	0.933	0.885	0.472	0.822	0.617	0.198	0.285	**0.000**
CRS-R Motor	0.633	0.194	0.610	0.314	0.714	0.214	0.704	0.678	0.496	0.861	0.789	0.993	0.587	0.104	0.390
Spaulding Total	0.942	0.620	0.184	0.740	0.289	0.211	0.278	0.897	0.921	0.436	0.786	0.581	0.162	0.321	**0.036**

Table 1b Continued. P VALUES for Correlations between Clinical Phenotype measures of Neurobehaioral Abilities and Outcomes and Significantly Up-regulated miRNAS

Clinical Phenotype Measures	Significantly Up-regulated miRNAs														
	miR-5001-3p	miR-338-5p	miR-28-5p	miR-504-5p	miR-2115-5p	miR-329-3p	miR-10b-5p	miR-212-5p	miR-581	miR-142-3p	miR-4659b-3p	miR-323a-3p	miR-145-5p	miR-769-5p	miR-151a-3p
DOCS Total	0.319	0.369	0.358	0.585	0.587	0.444	0.441	0.137	0.813	0.672	0.986	0.560	0.570	0.855	0.323
CRS-R Total	0.062	0.627	0.615	0.843	0.845	0.701	0.183	0.395	0.929	0.414	0.729	0.817	0.312	0.597	0.581
DRS Total	0.694	0.617	0.629	0.401	0.400	0.543	0.573	0.849	0.173	0.342	**0.027**	0.427	0.444	0.159	0.663
CNC Total	0.737	0.575	0.586	0.359	0.357	0.500	0.615	0.807	0.131	0.384	0.070	0.384	0.486	0.201	0.621
GOSE Total	0.955	0.266	0.278	**0.050**	**0.049**	0.192	0.924	0.498	0.178	0.693	0.378	0.076	0.794	0.510	0.312
GCS Total	0.182	0.506	0.495	0.722	0.724	0.581	0.304	0.274	0.950	0.535	0.849	0.697	0.433	0.718	0.460
DOCS Aud-Lang	0.156	0.845	0.833	0.939	0.938	0.919	**0.034**	0.613	0.711	0.196	0.511	0.965	0.095	0.379	0.799
CRS-R Auditory	0.720	0.592	0.603	0.376	0.374	0.517	0.598	0.824	0.148	0.367	**0.053**	0.401	0.469	0.184	0.638
DOCS Visual	0.922	0.233	0.245	**0.017**	**0.016**	0.159	0.957	0.465	0.211	0.726	0.411	**0.043**	0.827	0.543	0.279
CRS-R Visual	0.581	0.731	0.742	0.514	0.513	0.656	0.459	0.962	0.286	0.228	0.086	0.540	0.330	**0.046**	0.776
DOCS Somato	0.482	0.830	0.841	0.614	0.612	0.755	0.360	0.939	0.385	0.129	0.185	0.639	0.231	**0.053**	0.875
CRS-R Arousal	0.955	0.266	0.278	**0.050**	**0.049**	0.192	0.924	0.498	0.178	0.693	0.378	0.076	0.794	0.510	0.312
DOCS Gust/Olf	0.141	0.829	0.818	0.954	0.953	0.904	**0.019**	0.598	0.726	0.212	0.526	0.980	0.110	0.394	0.784
CRS-R Oro-Motor/Verbal	0.977	0.289	0.300	0.073	0.071	0.214	0.901	0.521	0.155	0.670	0.356	0.098	0.772	0.487	0.335
CRS-R Motor	0.588	0.100	0.089	0.317	0.318	0.175	0.710	0.131	0.545	0.941	0.745	0.291	0.839	0.877	**0.055**
Spaulding Total	0.987	0.325	0.336	0.109	0.107	0.250	0.865	0.557	0.119	0.634	0.320	0.134	0.736	0.451	0.371

Table 2a.Correlations between Clinical Phenotype Measrues and Significanlty Down-regulated miRNAs

Clinical Phenotype	Significanlty Down-regulated miRNAs																	
Measures	miR-199b-3p	miR-6515-3p	miR-7155-5p	miR-144-3p	miR-6815-5p	miR-3180-5p	miR-6087	miR-144-5p	miR-3613-5p	miR-6800-3p	miR-7641	miR-939-5p	miR-4508	miR-15a-5p	miR-126-3p	miR-101-3p	miR-342-5p	let-7b-5p
DOCS Total	0.898	0.898	0.694	0.606	0.355	0.555	0.492	0.099	0.981	0.485	0.425	0.015	0.874	0.675	0.271	0.588	0.86	0.432
CRS-R Total	0.949	0.949	0.989	0.942	0.124	0.899	0.834	0.105	0.673	0.225	0.753	0.193	0.529	0.365	0.054	0.928	0.974	0.185
DRS Total	0.111	0.111	0.239	0.299	0.567	0.337	0.39	0.943	0.06	0.426	0.454	-1	0.149	0.276	0.683	0.312	0.136	0.478
CNC Total	0.077	0.077	0.202	0.259	0.622	0.296	0.346	0.977	0.094	0.473	0.408	0.993	0.185	0.317	0.742	0.272	0.101	0.529
GOSE Total	0.17	0.17	0.047	0.004	-0.988	0.035	0.076	0.784	0.369	0.879	0.124	0.638	0.49	0.677	0.993	0.015	0.144	0.932
GCS Total	-0.999	-0.999	0.88	0.794	0.226	0.739	0.667	0.01	0.839	0.338	0.589	0.093	0.687	0.498	0.151	0.775	-0.989	0.293
DOCS Aud-Lang	0.67	0.67	0.876	0.948	0.048	0.979	0.999	0.294	0.405	0.045	0.99	0.4	0.295	0.165	0.119	0.96	0.712	0.009
CRS-R Auditory	0.09	0.09	0.217	0.275	0.599	0.313	0.364	0.965	0.08	0.454	0.426	-0.998	0.17	0.3	0.718	0.288	0.115	0.508
DOCS Visual	0.198	0.198	0.073	0.022	-0.999	0.009	0.05	0.738	0.403	0.918	0.097	0.595	0.53	0.723	0.976	0.011	0.172	0.962
CRS-R Visual	0.205	0.205	0.346	0.414	0.432	0.458	0.518	0.806	0.029	0.309	0.592	0.936	0.057	0.176	0.535	0.429	0.232	0.355
DOCS Somato	0.295	0.295	0.451	0.528	0.329	0.577	0.646	0.668	0.108	0.219	0.727	0.815	0.021	0.095	0.42	0.545	0.324	0.26
CRS-R Arousal	0.17	0.17	0.047	0.004	0.988	0.035	0.076	0.784	0.369	0.879	0.124	0.638	0.49	0.677	-0.993	0.015	0.144	0.932
DOCS Gust/Olf	0.691	0.691	0.895	0.962	0.037	-0.987	1	0.28	0.422	0.057	0.983	0.384	0.309	0.178	0.106	0.972	0.733	0.021
CRS-R Oro-Motor/Verbal	0.151	0.151	0.029	0.022	0.975	0.053	0.094	0.815	0.346	0.85	0.142	0.669	0.464	0.646	0.999	0.033	0.126	0.907
CRS-R Motor	0.539	0.539	0.368	0.304	0.676	0.267	0.22	0.335	0.83	0.841	0.17	0.236	0.957	-0.988	0.56	0.291	0.502	0.778
Spaulding Total	0.121	0.121	0.001	0.05	0.946	0.082	0.123	0.863	0.311	0.802	0.172	0.718	0.423	0.599	-0.998	0.061	0.097	0.864

Table 2b. P VALUES for Correlations between Clinical Phenotype Measrues of Neurobehavioral Abilities and Outcomes and Significantly Down-regulated miRNAs

Clinical Phenotype	Significantly Down-regulated miRNAs																	
Measures	miR-199b-3p	miR-6515-3p	miR-7155-5p	miR-144-3p	miR-6815-5p	miR-3180-5p	miR-6087	miR-144-5p	miR-3613-5p	miR-6800-3p	miR-7641	miR-939-5p	miR-4508	miR-15a-5p	miR-126-3p	miR-101-3p	miR-342-5p	let-7b-5p
DOCS Total	0.153	0.153	0.305	0.37	0.586	0.409	0.461	0.875	0.063	0.467	0.52	0.981	0.173	0.319	0.674	0.384	0.184	0.513
CRS-R Total	0.104	0.104	0.047	0.112	0.844	0.152	0.203	0.867	0.32	0.725	0.262	0.761	0.43	0.576	0.932	0.126	0.074	0.771
DRS Total	0.86	0.86	0.709	0.644	0.4	0.604	0.553	0.111	0.924	0.519	0.494	0.005	0.814	0.668	0.312	0.63	0.83	0.473
CNC Total	0.903	0.903	0.751	0.686	0.358	0.647	0.595	0.068	0.881	0.477	0.536	0.037	0.771	0.625	0.27	0.672	0.872	0.431
GOSE Total	0.789	0.789	0.94	0.995	0.049	0.955	0.903	0.24	0.573	0.169	0.844	0.346	0.463	0.317	0.038	0.981	0.819	0.122
GCS Total	0.016	0.016	0.168	0.233	0.723	0.272	0.324	0.988	0.2	0.604	0.383	0.882	0.31	0.456	0.811	0.247	0.047	0.65
DOCS Aud-Lang	0.322	0.322	0.171	0.105	0.938	0.066	0.014	0.649	0.538	0.942	0.045	0.544	0.648	0.794	0.851	0.092	0.292	0.989
CRS-R Auditory	0.886	0.886	0.734	0.669	0.375	0.629	0.578	0.086	0.899	0.494	0.519	0.02	0.788	0.642	0.287	0.655	0.855	0.448
DOCS Visual	0.756	0.756	0.907	0.972	0.016	0.988	0.936	0.273	0.54	0.136	0.877	0.379	0.43	0.284	0.071	0.986	0.786	0.089
CRS-R Visual	0.747	0.747	0.596	0.53	0.514	0.491	0.439	0.224	0.963	0.633	0.38	0.119	0.927	0.781	0.426	0.517	0.716	0.587
DOCS Somato	0.648	0.648	0.496	0.431	0.613	0.392	0.34	0.323	0.864	0.732	0.281	0.218	0.974	0.88	0.525	0.418	0.617	0.686
CRS-R Arousal	0.789	0.789	0.94	0.995	0.049	0.955	0.903	0.24	0.573	0.169	0.844	0.346	0.463	0.317	0.038	0.981	0.819	0.122
DOCS Gust/Olf	0.307	0.307	0.156	0.09	0.954	0.051	0.001	0.664	0.523	0.927	0.06	0.559	0.633	0.779	0.866	0.077	0.276	0.973
CRS-R Oro-Motor/Verbal	0.811	0.811	0.963	0.972	0.072	0.933	0.881	0.217	0.595	0.191	0.822	0.323	0.485	0.339	0.016	0.958	0.842	0.145
CRS-R Motor	0.422	0.422	0.573	0.639	0.317	0.678	0.73	0.607	0.206	0.198	0.789	0.712	0.096	0.05	0.405	0.652	0.453	0.244
Spaulding Total	0.847	0.847	0.999	0.936	0.108	0.897	0.845	0.181	0.631	0.227	0.786	0.287	0.521	0.375	0.02	0.922	0.878	0.181