SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA
Azienda Ospedaliero - Universitaria di Ferrara

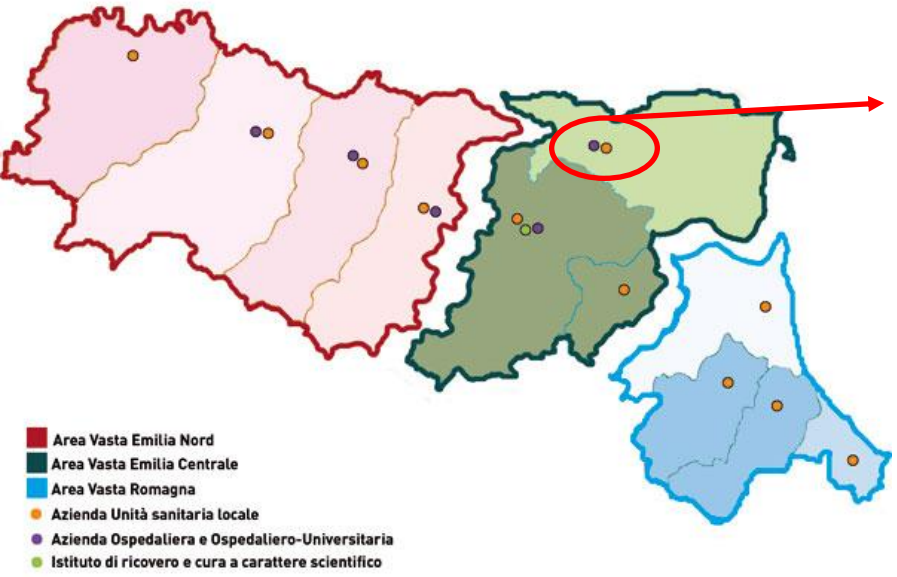
università di ferrara
DA SEICENTO ANNI GUARDIAMO AVANTI.

Regione Emilia-Romagna
Programma di ricerca Regione-Università 2010-2012
Area 1 - Strategic Programmes
Workshop 24.04.2015, Bologna

REHABILITATION AFTER CEREBRAL DAMAGE: FUNCTIONAL RECOVERY AND IDENTIFICATION OF BIOMARKERS RELATED TO THE CLINICAL OUTCOME

Nino Basaglia, MD
Strategic Programme Scientific Coordinator

Neuroscience and Rehabilitation Department
Rehabilitative Medicine Section, Ferrara University Hospital
(Prof. Nino Basaglia)



Settore di Medicina Riabilitativa
"Ospedale di Riabilitazione San Giorgio"
UO Medicina Riabilitativa
Direttore Nino Basaglia

Strutture Organizzative Semplici:
- Modulo Neuropsicologia Riabilitativa
- Modulo Laboratorio Analisi del Movimento
- Modulo Unità Spinale
- Funzione Interdipartimentale di Riabilitazione Cardiovascolare

UO Gravi Cerebrolesioni
Direttore Susanna Lavezzi

SOS Dipartimentale Attività Ambulatoriale

Servizio di Psicologia Clinica

PM&R Department, Ferrara

- 80 beds Neurorehabilitation

(stroke, SBI, MS, other neurological disabilities: 600 patients/year)

- Day- Hospital (1200 patients/year)

HUB center GRACER
(rehabilitation network for SBI Emilia Romagna)

HUB center stroke network (for Ferrara Province)

Ferrara

Inhabitants: 362.000

Density: 140/km^2

Number of new patients with “specific” neuropathology followed by San Giorgio:

- 70-80 patients/year with multiple sclerosis (EDSS 4-7)
- 140-150/year with stroke in subacute phase
- 75-80 traumatic brain injury

Roadmap

1. strategic program background
2. trials registrations
3. organization research staff and laboratories
4. enrollment subjects and data collecting

Strategic Program Background

Rationale

- Rehabilitation restores functions and reduces disabilities due to diseases sequelae

- The relationship between intensity of rehabilitation and clinical outcomes has generated a great interest for technological high-intensity interventions

- However, their effects compared to traditional interventions as well the involved biological mechanisms remain uncertain

Functional Recovery after CNS lesions

Biology

- vasculo-neurogenesis
- Inflammation
- Coagulation
- oxidative stress

Cortical reorganization

- fNIRS
- TMS
- EEG

Motor Control

Behaviour
Clinical tests

Quality of Life

Strategic Program aims

- Predict treatment efficacy in specific rehabilitation profiles
- Improve use of “targeted” therapies and individual management of patients with stroke, DOC and MS
- Transfer these findings into rehabilitative strategies

REHABILITATION AFTER CEREBRAL DAMAGE: FUNCTIONAL RECOVERY AND IDENTIFICATION OF BIOMARKERS RELATED TO THE CLINICAL OUTCOME

PI: Dr Nino Basaglia

WP#1	
Coordinator: Dr. Sofia Straudi (RU#1) Dipartimento di Neuroscienze e Riabilitazione – Sez. di Medicina Riabilitativa, Az. Ospedaliero-Universitaria di Ferrara **Involved Research Units: RU#1,2,3,4,5,6,8**	
Health problem/ disease	**STROKE SURVIVORS**
Primary Outcome Measure	**FUGL-MEYER ASSESSMENT (UE)**
Instrumental Biomarkers	**TMS, EMG, NIRS**
Circulating Biomarkers	Circulating Cell Populations, Markers of Inflammation/Angiogenesis, Neurotrophic factors, Coagulation factors, Metabolism biomarkers

WP#2	
Coordinator: Dr.ssa Susanna Lavezzi (RU#2) Dipartimento di Neuroscienze e Riabilitazione – Sez. di Medicina Riabilitativa, Az. Ospedaliero-Universitaria di Ferrara **Involved Research Units: RU#1,2,3,4,5,6,8**	
Health problem/ disease	**MINIMALLY CONSCIOUS STATE PATIENTS**
Primary Outcome Measure	**COMA RECOVERY SCALE**
Instrumental Biomarkers	**TMS, NIRS**
Circulating Biomarkers	Neurotrophic Factors and Markers of brain damage, Metabolism biomarkers

WP#3	
Coordinator: Dr. Fabio Manfredini (RU#1) Dipartimento di Neuroscienze e Riabilitazione – Sez. di Medicina Riabilitativa, Az. Ospedaliero-Universitaria di Ferrara **Involved Research Units: RU#1,4, 5, 6, 7,8**	
Health problem/ disease	**MULTIPLE SCLEROSIS PATIENTS**
Primary Outcome Measure	**TIMED 25 WALKING TEST**
Instrumental Biomarkers	**HAEMODYNAMIC MEASUREMENTS, NIRS**
Circulating Biomarkers (**RU#4** Prof. Secchiero) **(RU#5** Prof. Bernardi) **(RU#6** Prof. Pinton)	Circulating Cell Populations, Molecular markers of inflammation, Neurotrophic factors, Microparticles, Coagulation factors, Metabolism biomarkers

Biostatistical/Bioinformatics Studies (**RU#8**, Dr. Volpato**)**

Coordinating Unit (Dr. Bertelli)

Strategic Program

	Population	Interventions	Outcomes	
WP 1	Stroke (n=64)	•UE Robotics + Hand Functional Electrical Stimulation •Conventional training	Behavioural/TMS/ NIRS/EMG data	Biomarkers
WP 2	Minimally conscious state (n ≥10)	•DC electrical stimulation	Behavioural/EEG/ NIRS data	
WP 3	Multiple sclerosis (n=98)	•Robot-assisted gait training •Conventional Walking training	Behavioural/NIRS data	

Primary endpoints

- WP1→Upper extremity motor function:

  Fugl-Meyer UE score

- WP2→Awareness:

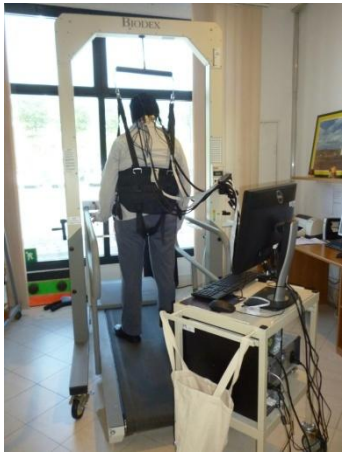
  Coma Recovery Scale-R

- WP3→Walking/mobility:

  T25FW

Secondary endpoints

- Brain plasticity



- QoL
- Proteins/Cells
- Variations of biomarkers

Strategic Program: Expected Results

- High-intensive rehabilitation interventions: effects on functional recovery on stroke and MS
- Non invasive brain stimulation: safety and feasibility in minimally conscious state, and modulation of behaviour and cortical excitability
- Biomarkers related to specific functional recovery

Strategic Program: Clinical Implications

- Better definition of rehabilitative strategies
- Better identification of patients eligible for specific treatments
- Increased clinical appropriateness
- Increase efficacy and effectiveness of rehabilitation care

Trials registrations

Protocol approvals and registration

The three clinical trials have been approved by **Ferrara University Hospital Ethics Committee** on September 29th 2012.

All trial have been registered in **Clinicaltrial.gov** (NCT02267798, NCT02288533, NCT02421731)

ClinicalTrials.gov PRS
Protocol Registration and Results System

Organization research staff and laboratories

1. Staff employment
2. Research team training
3. Permanent staff training
4. Development of communication tools
5. Purchase of neurophysiological and rehabilitative equipments
6. Brain Plasticity Lab
7. Biobank

Trial team

Staff employed

clinical RUs: 4 FT, 1 PhD

2 post-doc

bio RUs: 4 post-doc, 1 PhD student

Permanent staff

2 rehabilitation nurses

3 medical doctors

5-6 physiotherapists

1 university researcher

administrative staff

+ 1 PT student and 1 PM&R resident

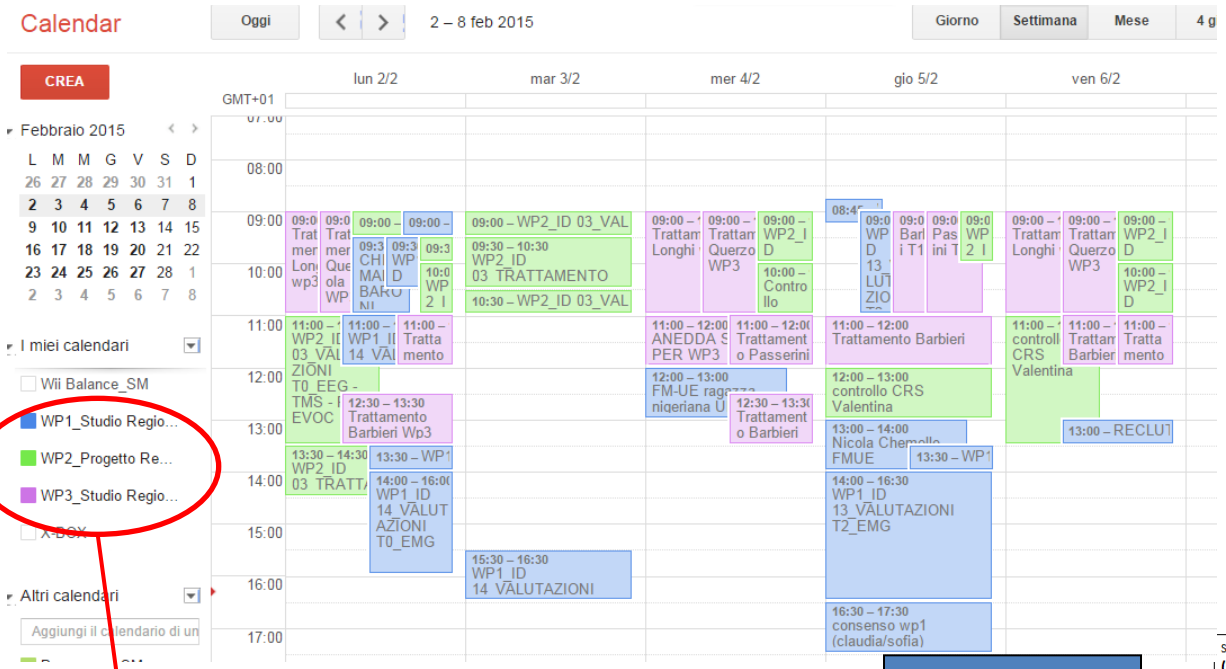
Research Units Leaders

RU1: **Neuroscience and Rehabilitation Department – Rehabilitative Medicine Section, Ferrara University Hospital (Prof. Nino Basaglia)** is responsible of the coordination of clinical research activities (recruitment, clinical assessment and treatments) and communication with other research units involved in neurophysiological assessments.

RU4: **Advanced Therapy Laboratory and Morphology and Embryology Department, Ferrara University (Prof. Paola Secchiero)** is responsible of the blood storage and coordination of pre-clinical RUs.

The activities of the aforementioned units allow a high degree of interactions among research groups favoured by frequent contacts between project leaders and staff meetings.

Communications tools



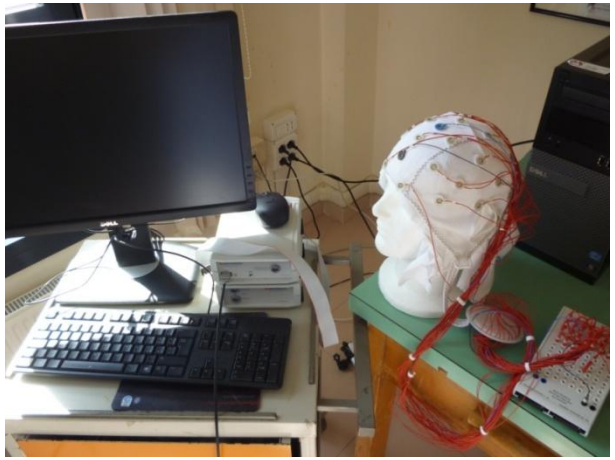
subject scheduling
(mailed to the trial team)

calendars (wp1,wp2,wp3)
with subjects scheduling
(shared with the trial team)

	Settimana 1 (T0)	Settimana 2	Settimana 3 (T1)	Settimana 4	Settimana 5	Settimana 6 (T2)	FU 6 mesi (T3)
Prelievi AMB.2 tel. 0532-238721	07/10/2014 ORE 8:45		28/10/2014 ORE 8:45			19/11/2014 ORE 8:45	18/05/2015 ORE 8:45
Valutazioni CLINICHE AMB. 6 tel. 0532-238720	07/10/2014 ORE 10:30		28/10/2014 ORE 9:00			19/11/2014 ORE 9:00	18/05/2015 ORE 9:00
Valutazione TMS AMB. 3 tel. 0532-238739	07/10/2014 ORE 9:00		28/10/2014 ORE 10:30			19/11/2014 ORE 10:30	18/05/2015 ORE 10:30
Valutazioni NIRS AMB. 3 tel. 0532-238739	07/10/2014 ORE 14:00		28/10/2014 ORE 14:00			19/11/2014 ORE 14:00	18/05/2015 ORE 13:30
Valutazione EMG AMB. 7 LAM tel. 0532-238716	07/10/2014 ORE 14:30					24/11/2014 ORE 14:30	18/05/2015 ORE 14:00

Riabilitazione	Reo-go	INIZIO: 08/10/2014	FINE: 18/11/2014
	Convenzionale	INIZIO: 08/10/2014	FINE: 18/11/2014

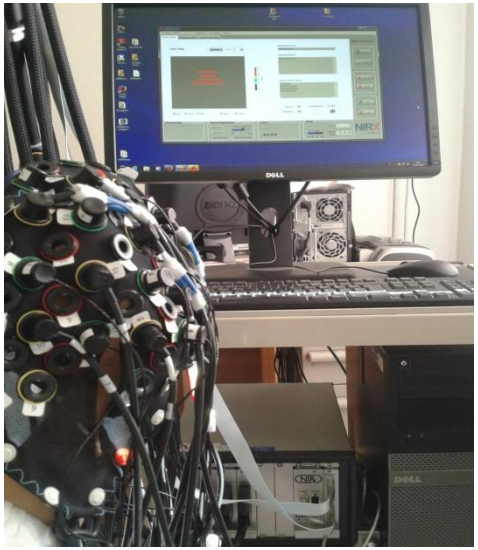
Brain Plasticity Lab



EEG Workspace

TMS Workspace

Clinical evaluation tools



NIRS Workspace

Laboratory Facilities and main Equipments

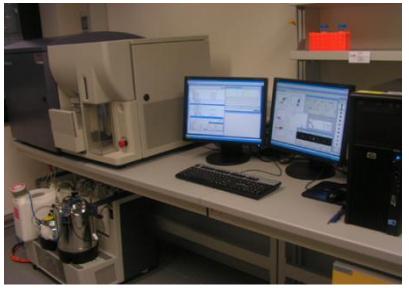
The laboratories of RU#4, #5 and #6 are part of the Interdepartmental Center of the "**Laboratory of Technology for Advanced Therapies**" of the University of Ferrara (http://ltta.tecnopoloferrara.it).

Location:
"Il CUBO"
Via Fossato di Mortara, 70
44124 Ferrara

The Center is divided into independent laboratory units equipped for:

a) cell culture and cell manipulation;
b) biologic bank and cryopreservation;
c) multiparametric flow Cytometry immunophenotyping and cell sorting;
d) molecular biology studies;
e) protein analyses and purification;
f) advanced microscopy analysis and proteomic studies.

BioBank Facility



FACS Facility

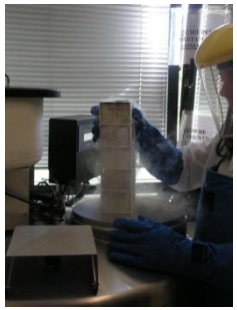
LTTA BioBank Facility

The entire infrastructure has been conceived and deployed in order to guarantee standards of high availability and security.

The technologies and expertise available at the BioBank facility allow:

- the purification of primary cell lines from blood and different tissue specimens and their morphological/functional characterization;

- the labeling of samples with specific barcode allowing the appropriate record and tracking of the samples;



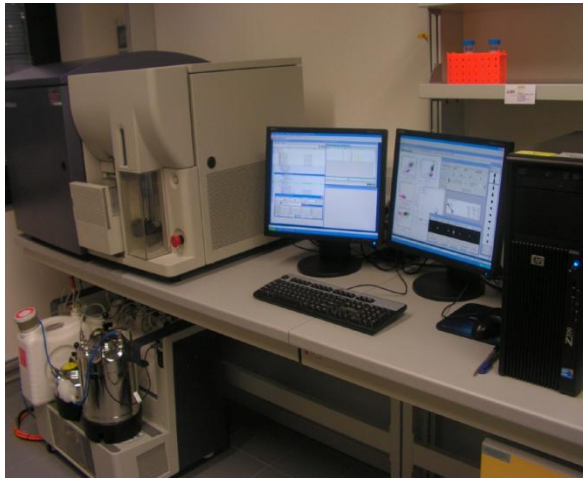
- the cryopreservation at -150°C in tanks for vapor-phase nitrogen storage for cells and at -80°C for plasma/serum samples.

LTTA FACS Facility

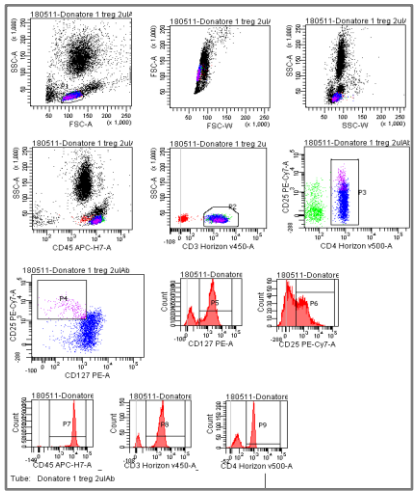
The LTTA FACS facility consists of a Becton-Dickinson Cell sorter FACS Aria, a Becton-Dickinson FACS Calibur and a Beckman Coulter Epics XL MCL.

The facility is managed by flow cytometry experts and was established to support biosciences research by providing capabilities which individual labs might not otherwise have access to.

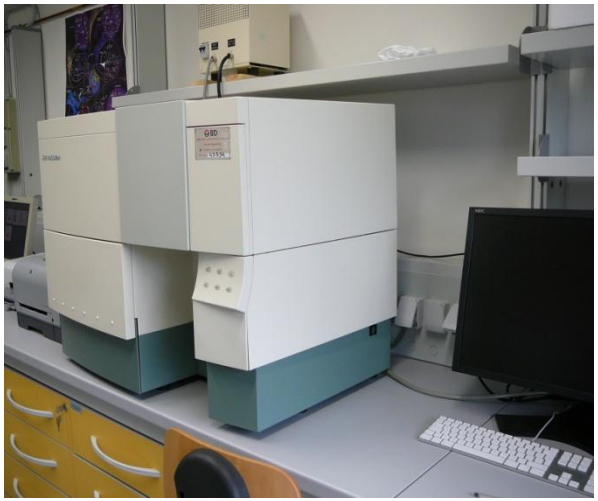
Beckman Coulter Epics XL MCL



Cell Sorter BD FACSAria II



Multiparametric-Treg Analysis

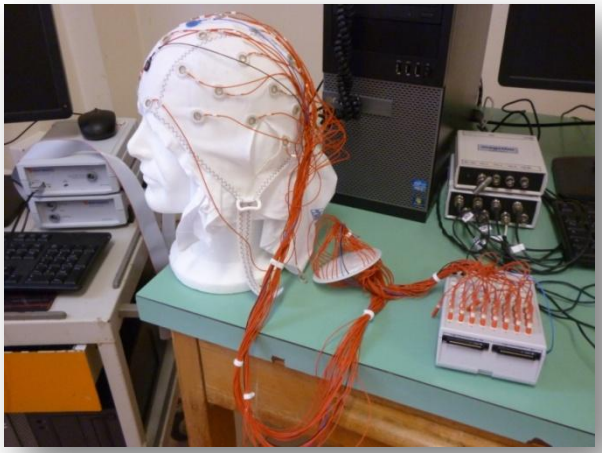


BD FACSCalibur

New Neurophysiological and Rehabilitative equipment

Hand FES (Bioness H200)

Single pulse TMS (MagStim 200)



EEG 32 channels (BrainAmp DC)

NIRS 16 +16 (NIRx Scout)

San Giorgio facility

Motion Analysis Lab

Robot-assisted gait training

Upper Limb Robotic Device

tDCS

Collaborations

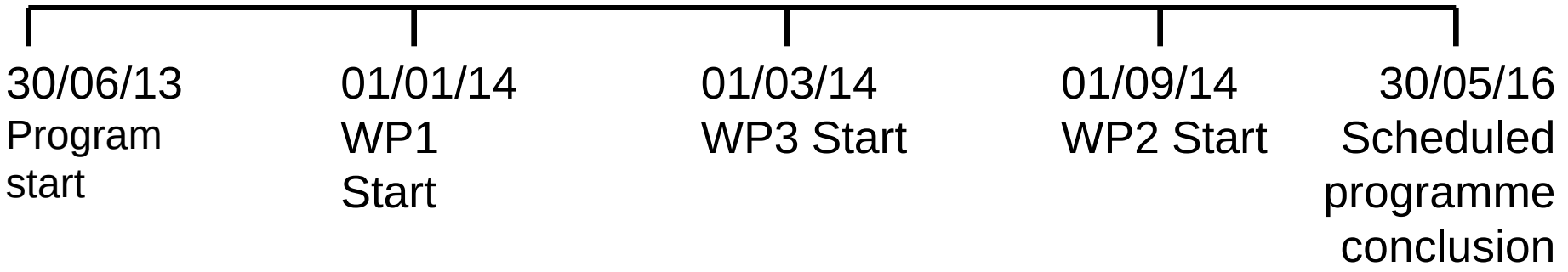
Giacomo Severini, PhD
Paolo Bonato, PhD

Motion Analysis Laboratory,
Spaulding Rehabilitation Hospital
Harvard Medical School, Boston, MA

Motion Analysis Lab

Enrollment subjects and Data collecting

Patients enrollment timeline



Research pathway

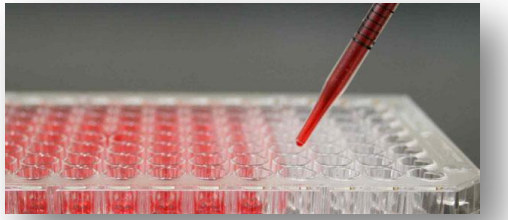
RU1

Blood sample

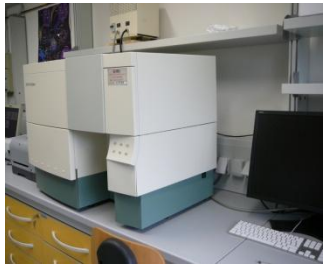
RU4

Biobank

16 aliquots



Assessments



flow-cytometry analyses

Treatment

Neurotrophic
Coagulation
Factors
Cytokines
Chemokines

Biological Samples Flow-chart Collection and processing

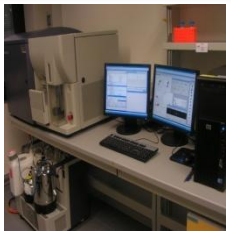
Clinical RUs

Peripheral blood patients' samples collection

Bio RUs

1 Vial
Collected in EDTA

Real-time blood sample processing for flow-cytometry analyses of circulating cell populations:
-ECP/CPC
-Treg
-MSC-like

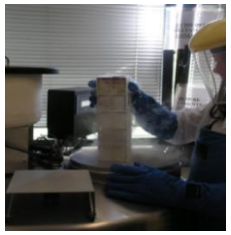


3 Vials
Collected in Sodium Citrate

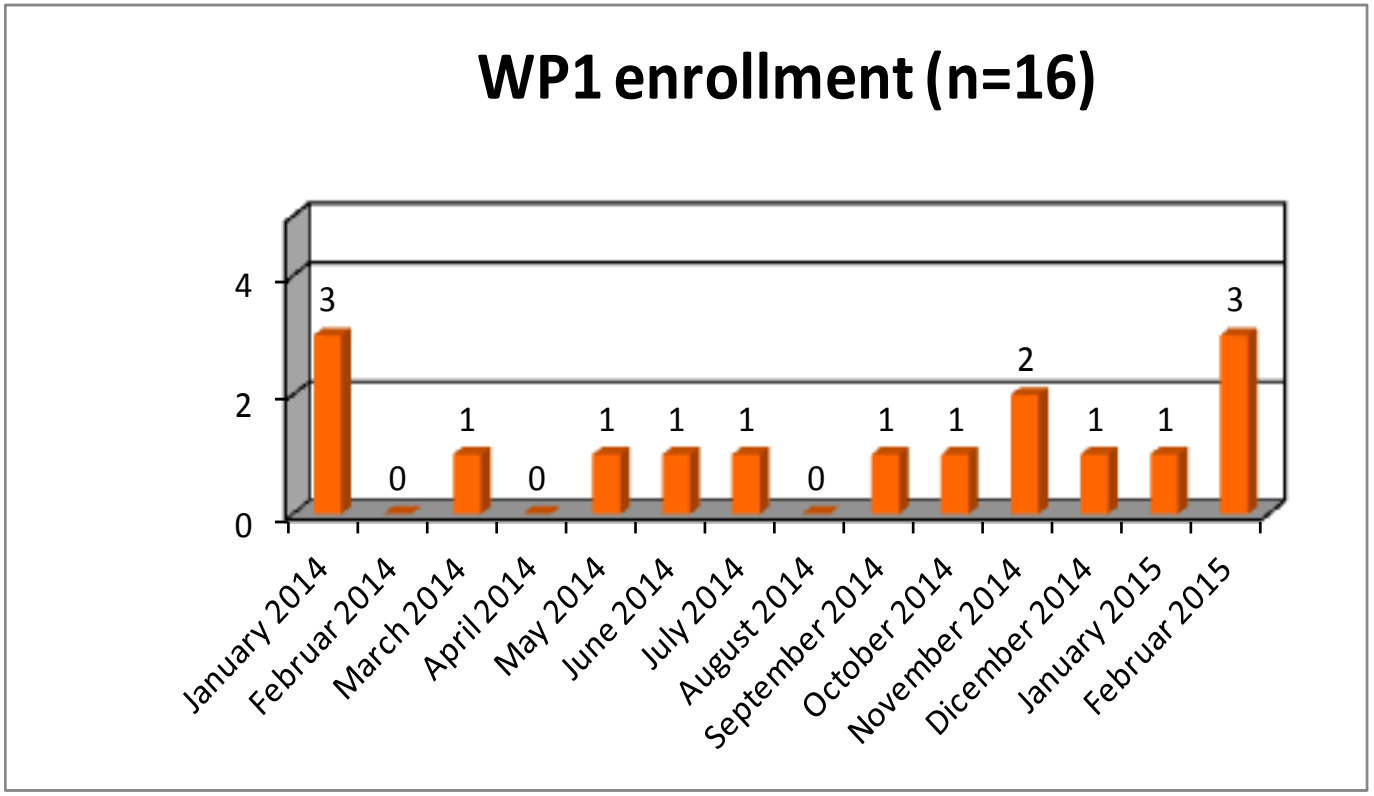
- Plasma isolation and biobanking in multiple aliquots stored at -80°C:
  - 1 aliquot for Metabolism markers analyses
  - 16 aliquots of 120 ul of plasma for circulating biomarkers analyses
  - Back-up aliquots of the remaining plasma for biological/functional assays
- Blood cells pellets collected and stored for DNA extraction and genetic analyses

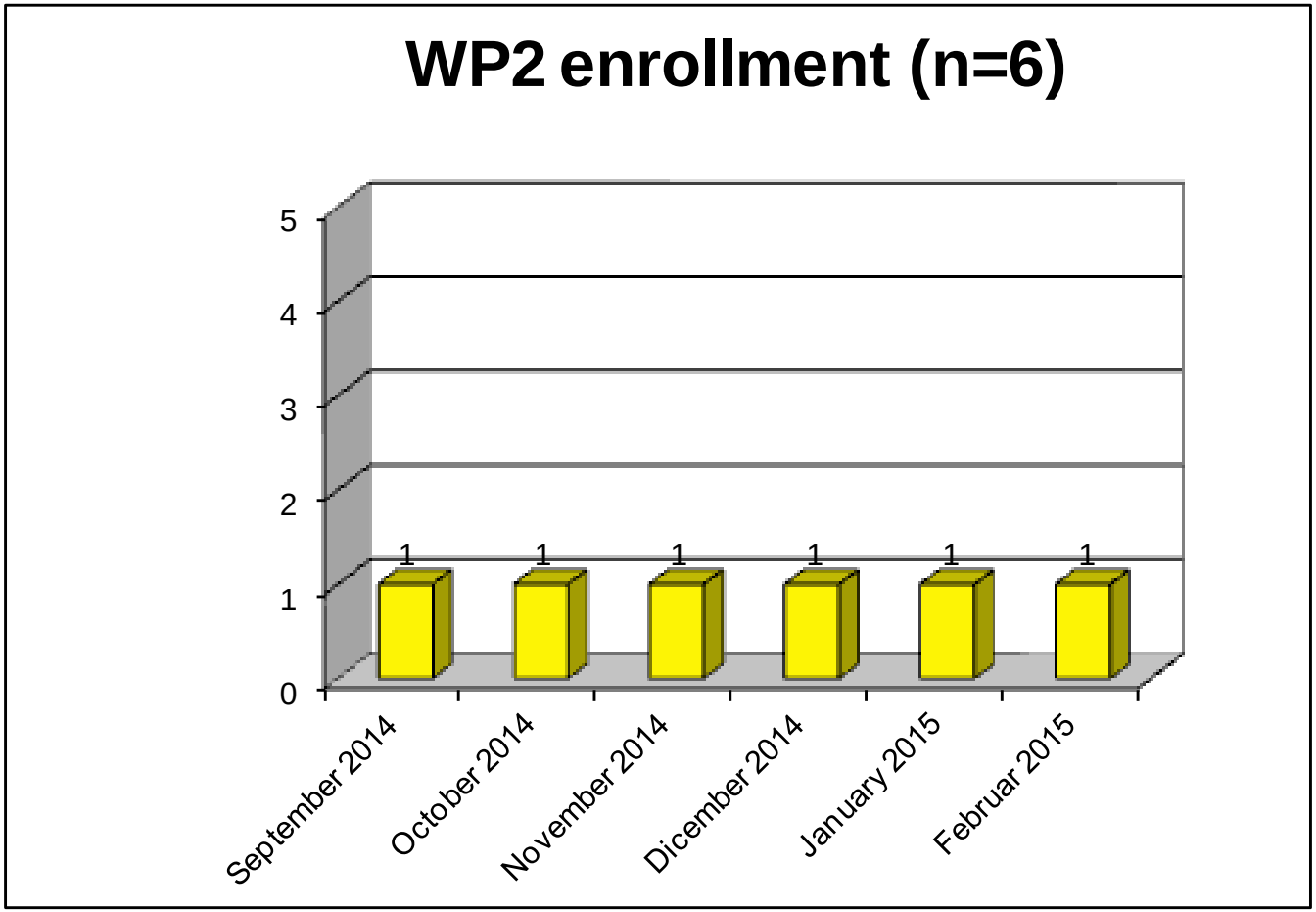
1 Vial
Collected in RNA-stabilizer

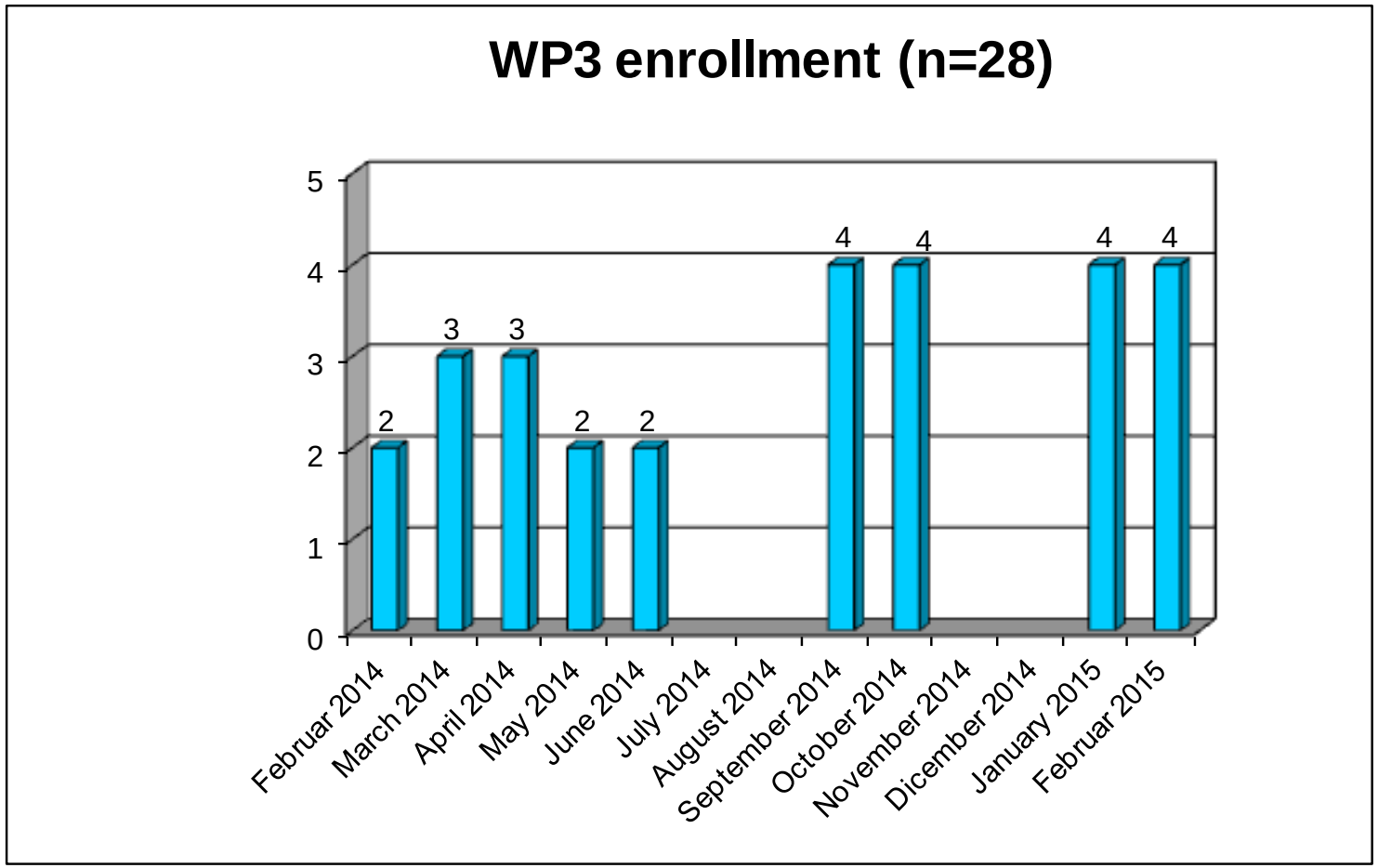
RNA extraction, quantification and storage for molecular analyses



Subjects' enrollment



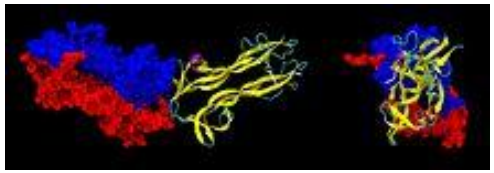

WP2 enrollment (n=6)
5
4
3
2
1
0
1
1
1
1
1
1
September 2014
October 2014
November 2014
Dicember 2014
January 2015
Februar 2015



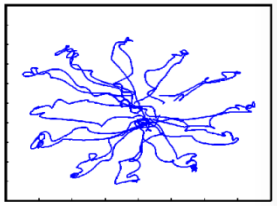
Waiting List: 25 patients

Measures

Circulating Biomarkers

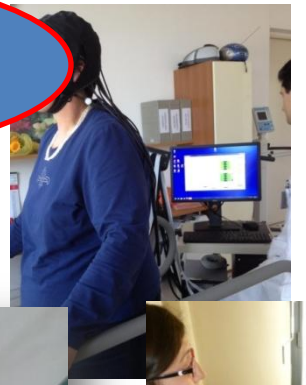


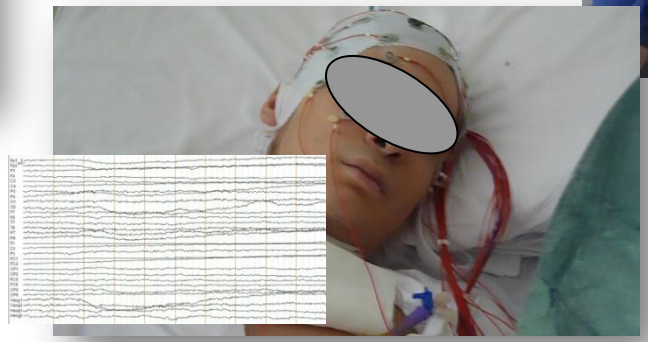
Upper Extremity Motor Synergies

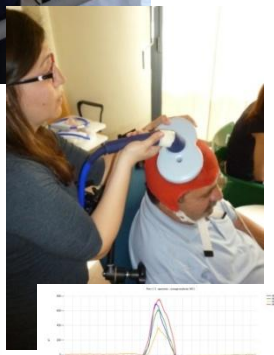


Functional Tests

Brain Plasticity (fNIRS, EEG, TMS)







First set of antigens selected for Circulating Biomarkers Screening by Luminex Technology

Pre-costumed panels

Human Angiogenesis / Growth Factor

Angiopoietin-2	HB-EGF
BMP-9	HGF
EGF	IL-8/CXCL8
Endoglin	Leptin
Endothelin-1	Placental Growth Factor (PLGF)
FGF-1/FGF-acidic	VEGF-A
FGF-2/FGF-basic	VEGF-C
Follistatin	VEGF-D
G-CSF	

Human Neurodegenerative Disease Panel 3

BDNF	PAI-1 (total)
Cathepsin D	PDGF-AA
sICAM-1	PDGF-AB/BB
MPO	RANTES
sNCAM	sVCAM-1

Human Cytokine / Chemokine Panel I

sCD40L	IL-9
EGF ♦	IL-10 ♦
Eotaxin/CCL11 ♦	IL-12 (p40) ♦
FGF-2/FGF-basic	IL-12 (p70) ♦
Flt3 ligand	IL-13 ♦
Fractalkine /CX3CL1	IL-15 ♦
G-CSF ♦	IL-17A ♦
GM-CSF ♦	IP-10/CXCL10 ♦
GRO	MCP-1/CCL2 ♦
IFNα2 ♦	MCP-3/CCL7
IFNγ ♦	MDC/CCL22
IL-1α ♦	MIP-1α/CCL3 ♦
IL-1β ♦	MIP-1β/CCL4 ♦
IL-1ra ♦	PDGF-AA Δ
IL-2 ♦	PDGF-AB/BB Δ
IL-3 ♦	RANTES/CCL5 ♦ Δ
IL-4 ♦	TGFα
IL-5 ♦	TNFα ♦
IL-6 ♦	TNFβ/LTA ♦
IL-7 ♦	VEGF-A ♦
IL-8/CXCL8 ♦	

Human CVD Panel 4

sE-Selectin	Pentraxin-3 (PTX3)
Follistatin (FST)	Tissue Factor (TF)
dPAPP-A	Thrombomodulin
sPECAM-1	Troponin T (TnT)

Human Cytokine / Chemokine Panel II

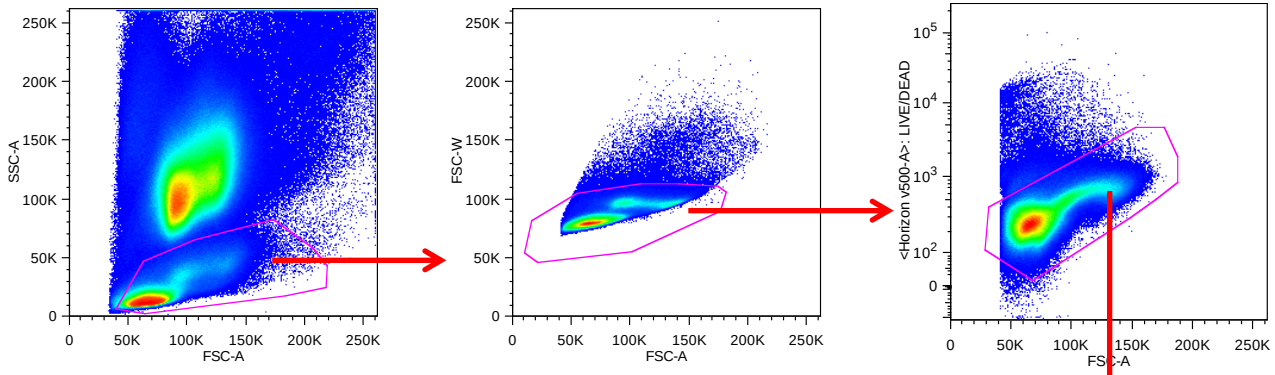
6Ckine/CCL21	I-309/CCL1
BCA-1/CXCL13	LIF
CTACK/CCL27	MCP-2/CCL8
ENA-78/CXCL5	MCP-4/CCL13
Eotaxin-2/CCL24/ MPIF-2	MIP-1δ/MIP-5/ CCL15
Eotaxin-3/CCL26	SCF
IL-16	SDF-1/CXCL12
IL-20	TARC/CCL17
IL-21	TPO
IL-23	TRAIL/TNFSF10
IL-28A	TSLP
IL-33/NF-HEV (mature)	

Selected single antigens

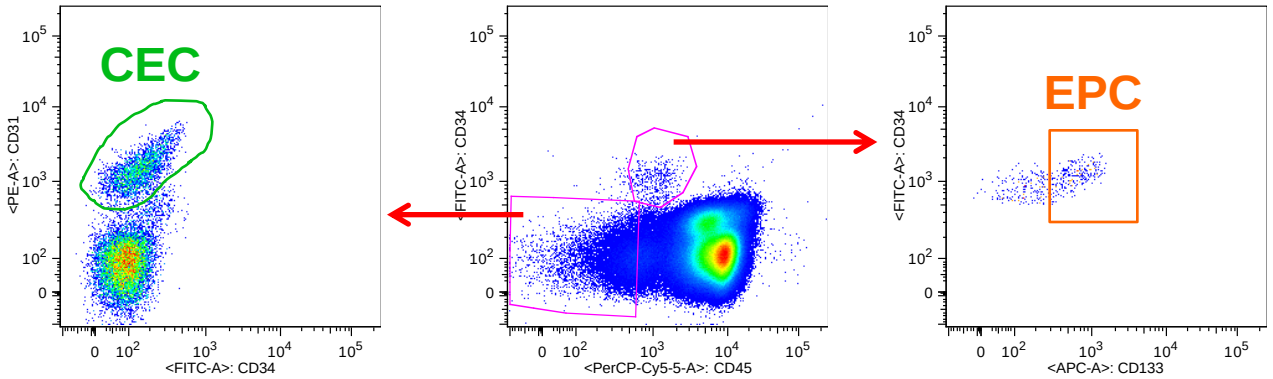
- IGFBP3
- IGF-1
- Thrombospondin-1 (TSP-1)
- Osteopontin (OPN)
- Adiponectin

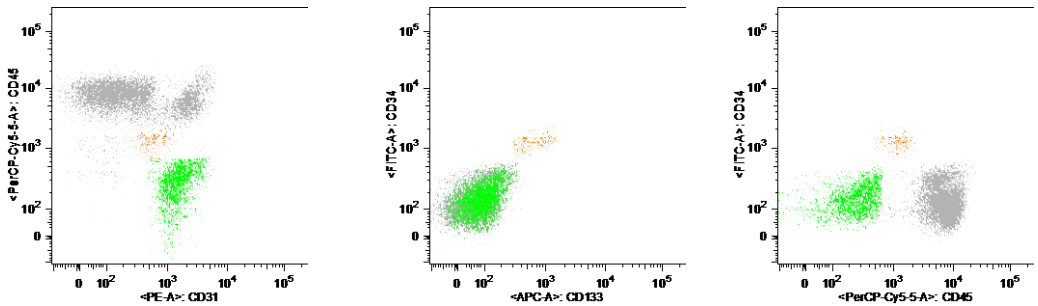
Circulating Cells Population Analyses

1. Circulating Endothelial Cells (CEC) and Endothelial Progenitors Cells (EPC) Gating Strategy (Duda G et al. Nat Protoc. 2007)



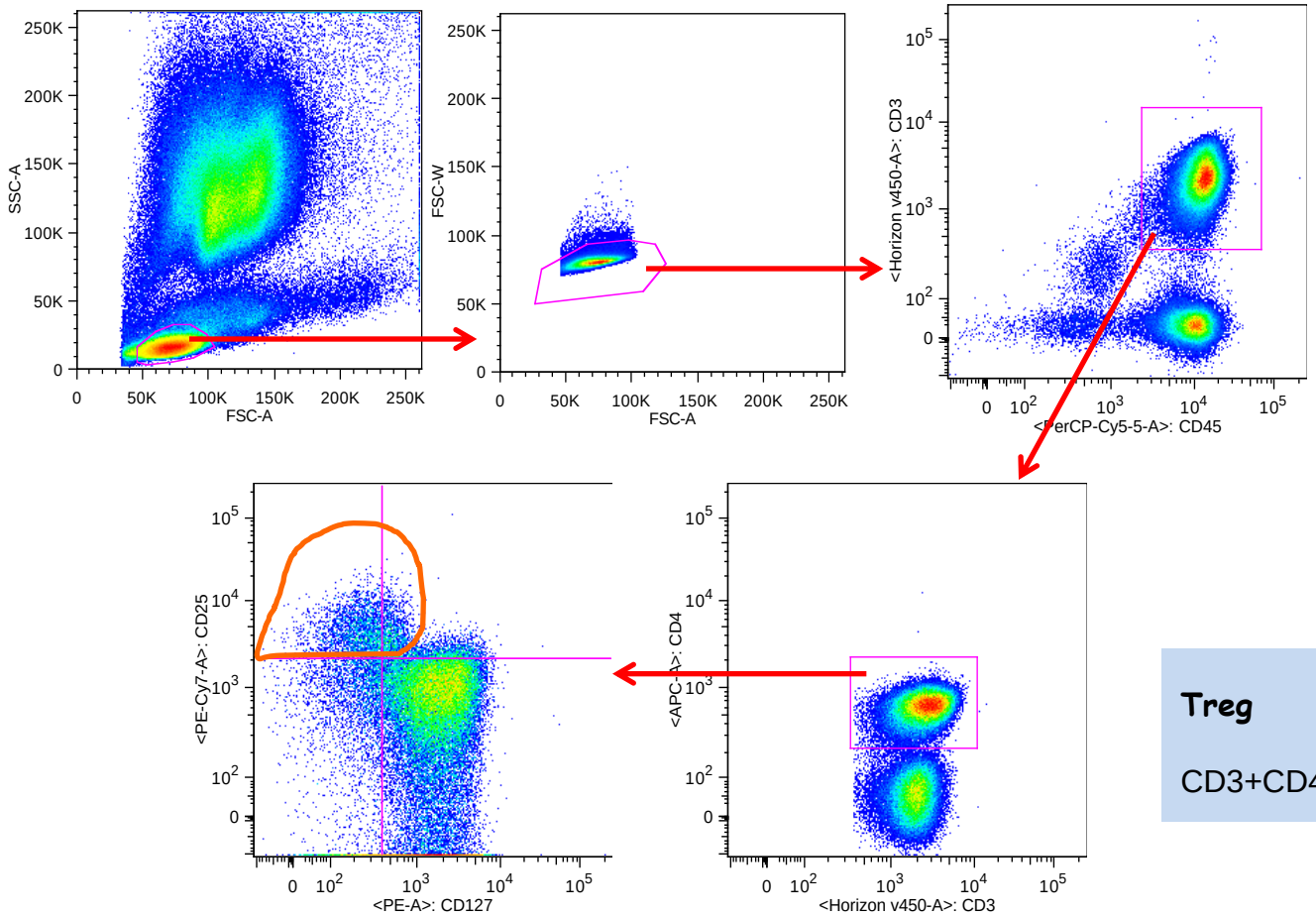
EPC CD45dimCD34brightCD133+
CEC CD45-CD34dimCD31+CD133-





Circulating Cells Population Analyses

2. T regulatory cells (Treg) Gating Strategy (Liu W. et al., J Exp Med. Jul 10, 2006)

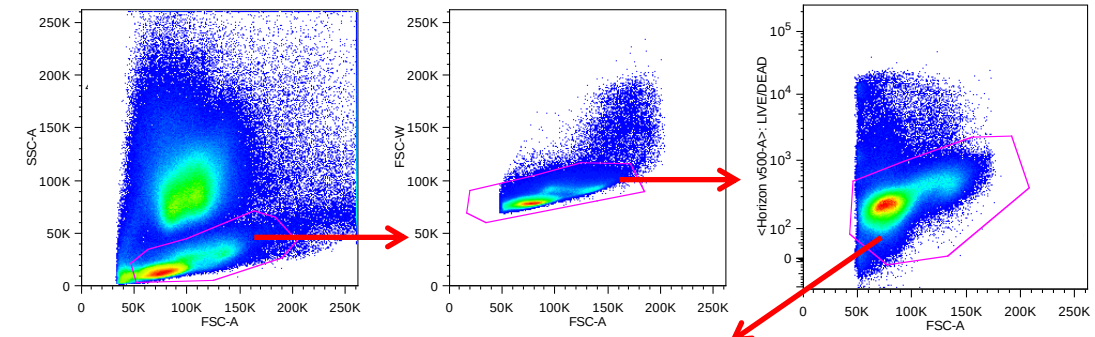


Treg

CD3+CD45+CD4+CD25+CD127-

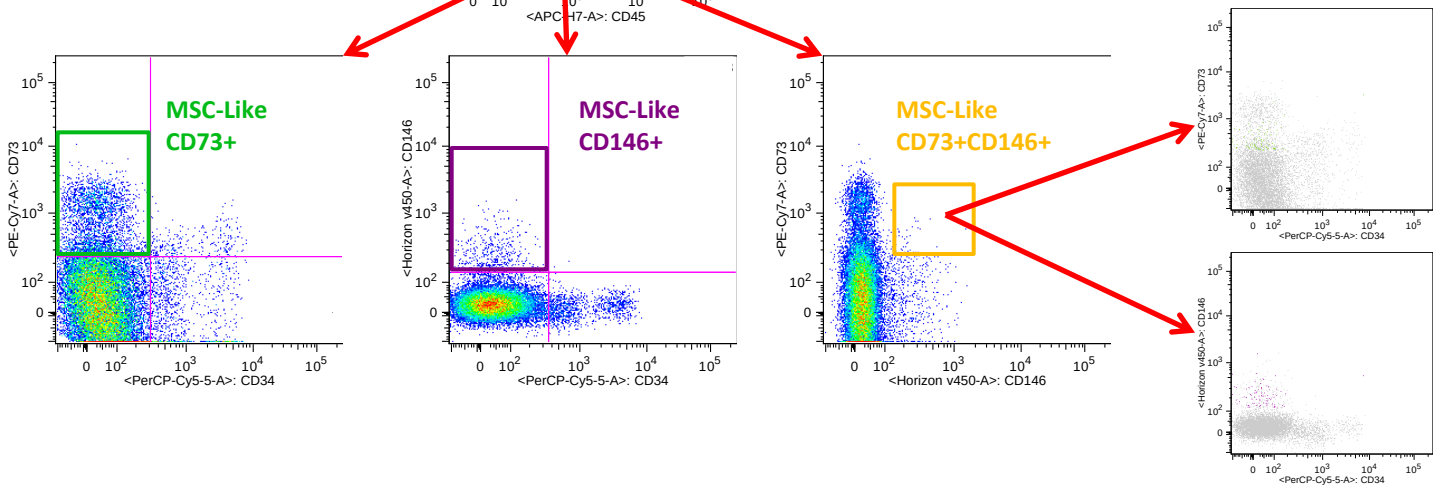
Circulating Cells Population Analyses

3. Mesenchymal Stem Cell-Like (MSC-Like) Gating Strategy



MSC-Like: CD45-CD14-CD34-CD73+

MSC-Like: CD45-CD14-CD34-CD146+



Interventions

Robot-assisted therapy
Functional Elettrical Stimulation (FES)
tDCS
High-intensity “conventional” therapy

Results achieved (1)

The evaluation of the rehabilitation efficacy after a primary analysis on n=16 (wp1), n=6 (wp2) n=27 (wp3) revealed a positive effect of the treatments received

However, the cohorts of patients is too small to develop hypothesis about the efficacy and the difference between the treatments

At the moment, the following goals have been reached in all the studies:

i. methodology of patients enrollment
ii. definition and execution of the three protocols
iii. clinical measures and questionnaires collection

Results achieved (2)

Regarding clinical/instrumental and circulating biomarkers the following goals have been reached:

1. the establishment of a Biobank of biological samples from patients

2. the identification of a panel of circulating antigens/cell populations to be analyzed in biological samples collected

3. few preliminary data on correlation between clinical, circulating markers and recovery are available (i.e. UE- motor synergies, MEPs, progenitors cells)

Strengths

1. high synergies between clinical and pre-clinical research groups
2. high adherence to treatments and low-rate drop-outs
3. no major adverse effects (falls, pain, other..)
4. subjects recruited from extra-ER regions (active mobility)

Limits

- equipment and software acquisition (+ 6 months)

- subjects recruitment (i.e. subacute stroke survivors: 16 subjects enrolled out of 167 subjects screened or 6 chronic traumatic minimally conscious state disorders out of 60 disorders of consciousness)

1- year extension will be necessary to complete the strategic program

Future goals

- Increase sample size
- Increase knowledge about biomarkers and networks that may be involved in recovery process


















Neurorehabilitation team

LTTA (Unife)
Fabio Casciano, PhD
Veronica Tisato, PhD
Silvia Meneghetti, PhD
Chiara Agnoletto, PhD
Massimo Bonora, PhD
Paola Secchiero, PhD
Paolo Pinton, PhD
Francesco Bernardi, PhD
Giovanna Marchetti, PhD

Medicina Riabilitativa (Ferrara)
Nino Basaglia, MD
Sofia Straudi, MD
Susanna Lavezzi, MD
Valentina Buonsangue, MD
Claudia Pavarelli, FT
Carlotta Martinuzzi, FT
Andrea Baroni, FT
Amira Sabbagh Charabati, FT
Marco da Roit, FT
Daniela Ripa, FT
Livio Balugani, FT
Laura Di Marco, FT
Donatella Marchetti, FT

Fabio Manfredini, MD
Nicola Lamberti, PhD

Paolo Zamboni, MD

Andrea Montis, MD

Sez. Fisiologia (Unife)
Sonia Mele, PhD
Laila Craighero, PhD
Saro Canto, MS

Motion Analysis Lab (HMS, Boston, MA)
Giacomo Severini, PhD
Paolo Bonato, PhD

Neuromodulation lab (HMS, Boston, MA)
Felipe Fregni, MD, PhD

Physical Medicine and Rehabilitation
Neuroscience and Rehabilitation Department
Ferrara University Hospital
Ferrara, Italy

SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA
Azienda Ospedaliero - Universitaria di Ferrara

università di ferrara
DA SEICENTO ANNI GUARDIAMO AVANTI.

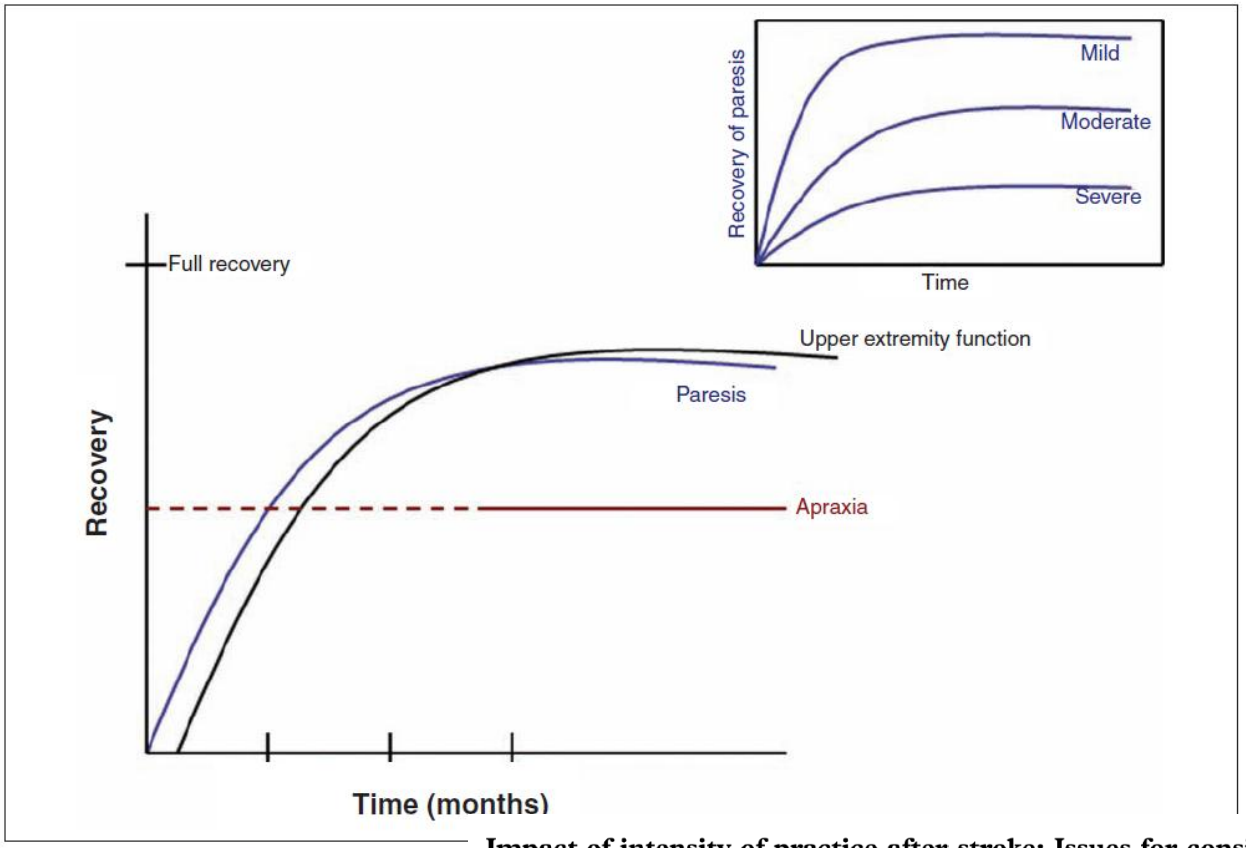
WP1: Scientific coordinator Sofia Straudi, MD

The effects of repetitive arm training combined with functional electrical stimulation on upper extremity motor recovery in subacute stroke survivors

Single center,single blinded, 2 arm-trial,
conducted at PM&R Dept, Ferrara

Stroke → **Upper Limb paresis** → ADLs Limitation → ↓ QoL

Recovery: fx (time / severity)



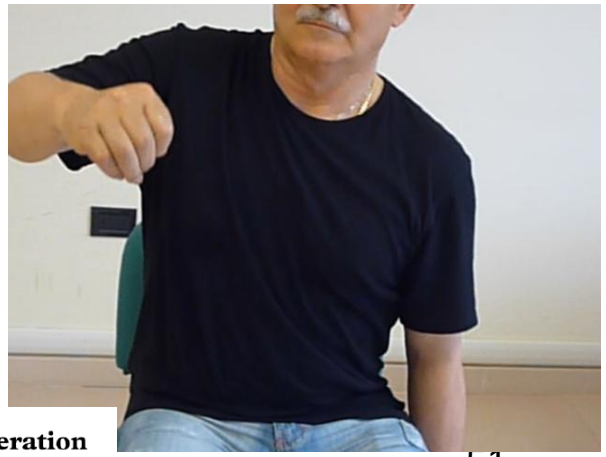


Figure 4. Schematic of time course of recovery af
The dashed line for apraxia represents the current

Primary aims

- to test the feasibility of an upper-arm intensive rehabilitation with the additional use of functional electrical stimulation during an early phase of rehabilitation in stroke subjects

- to test the hypothesis that this intervention could have higher benefit, compared with conventional therapy alone, in arm and hand function in sub-acute stroke subjects

- The evaluation of arm motor recovery will be performed by: i) clinical tests (i.e. Fugl-Meyer Assessment Score); ii) measures of motor cortical excitability (TMS) and cerebral perfusion (NIRS); (iii) muscle activation patterns during upper extremity movement (EMG)

Secondary aims

- to assess the potential role of circulating biomarkers in revealing the effects of these interventions and their possible correlations with clinical and instrumental outcomes

- to transfer these findings into rehabilitative strategies

Trial design (NCT02267798)

Ischemic stroke < 2 m
Arm paresis (FM-UE >10 e <55)

RAT+SEF

100 min/session

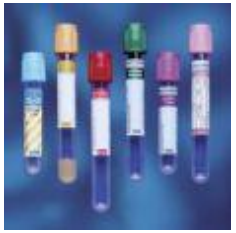
5 times/week over 6 weeks

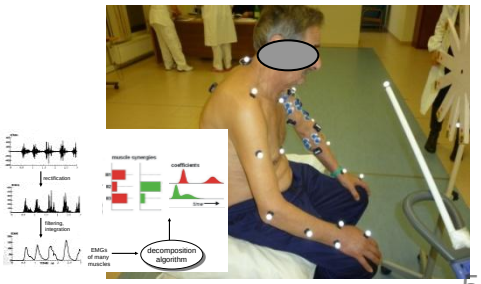
ICT

WP1
STROKE SURVIVORS
(n=64)

TRAINING

Time (weeks) 0 3 6 *Follow-Up (week 30)*





Robot-assisted Upper Limb rehabilitation

intensive
repetitive
task-specific
progressive
providing feedback

ReoGo (Motorika) device:

End-effector connected with a monitor
Multiplanar reaching movements

Hand - FES

Motor recovery

Muscle strenghtening

Cortical priming?

Sensory stimulation

H200 Wireless HandRehabilitation

REVIEW Open Access

The influence of functional electrical stimulation on hand motor recovery in stroke patients: a review

Fanny Quandt[1] and Friedhelm C Hummel[1,2*]

Efficacy of electrical stimulation as an adjunct to repetitive task practice therapy on skilled hand performance in hemiparetic stroke patients: a randomized controlled trial

Nevein MM Gharib, Ahmed M Aboumousa, Abeer A Elowishy, Soheir S Rezk-Allah and Fatma S Yousef

Clin Rehabil published online 14 August 2014
DOI: 10.1177/0269215514544131

Clinical Outcome Measures

Fugl-Meyer Upper Extremity (FMUE)

Wolf Motor Function Test (WMFT)

Modified Ashworth Scale (MAS)

Box and Block Test (BBT)

Motor Activity Log (MAL)

Barthel Index (BI)

Stroke Impact Scale 2.0 (Italian version)

UE – motor synergies

Muscles recorded

R1: infraspinatus
R2: latissimus dorsi
R3: superior trapezius
R4: rhomboid major / medial trapezius
R5: pectoralis major, clavicular head
R6: deltoid, anterior part
R7: deltoid, medial part
R8: deltoid, posterior part

L1: triceps, lateral head
L2: biceps, short head
L3: biceps, long head
L4: brachialis
L5: brachioradialis
L6: pronator teres

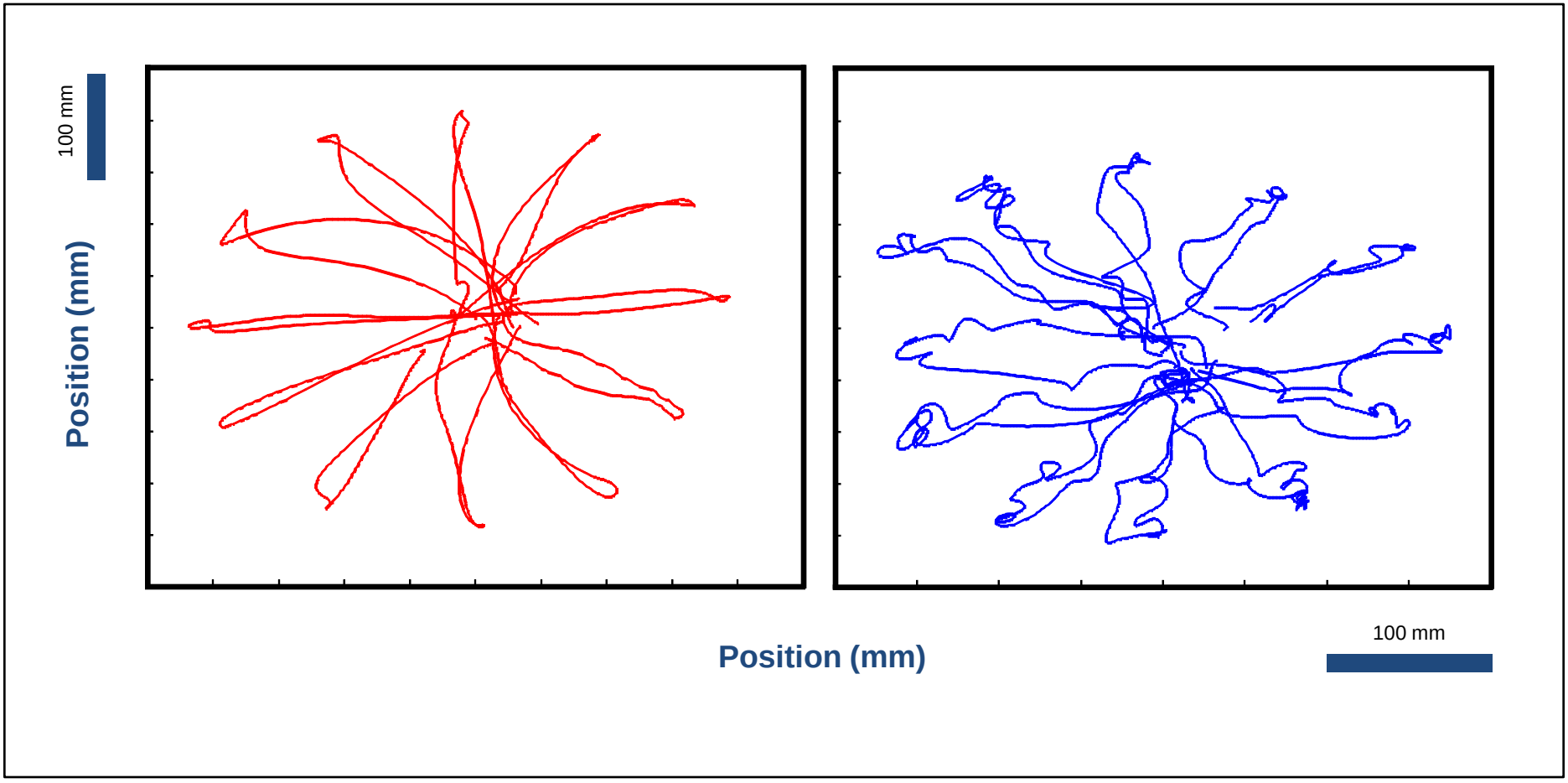
Muscle synergy patterns as physiological markers of motor cortical damage

Vincent C. K. Cheung[a], Andrea Turolla[b], Michela Agostini[b], Stefano Silvoni[b], Caoimhe Bennis[c], Patrick Kasi[c], Sabrina Paganoni[c], Paolo Bonato[c], and Emilio Bizzi[a,1]

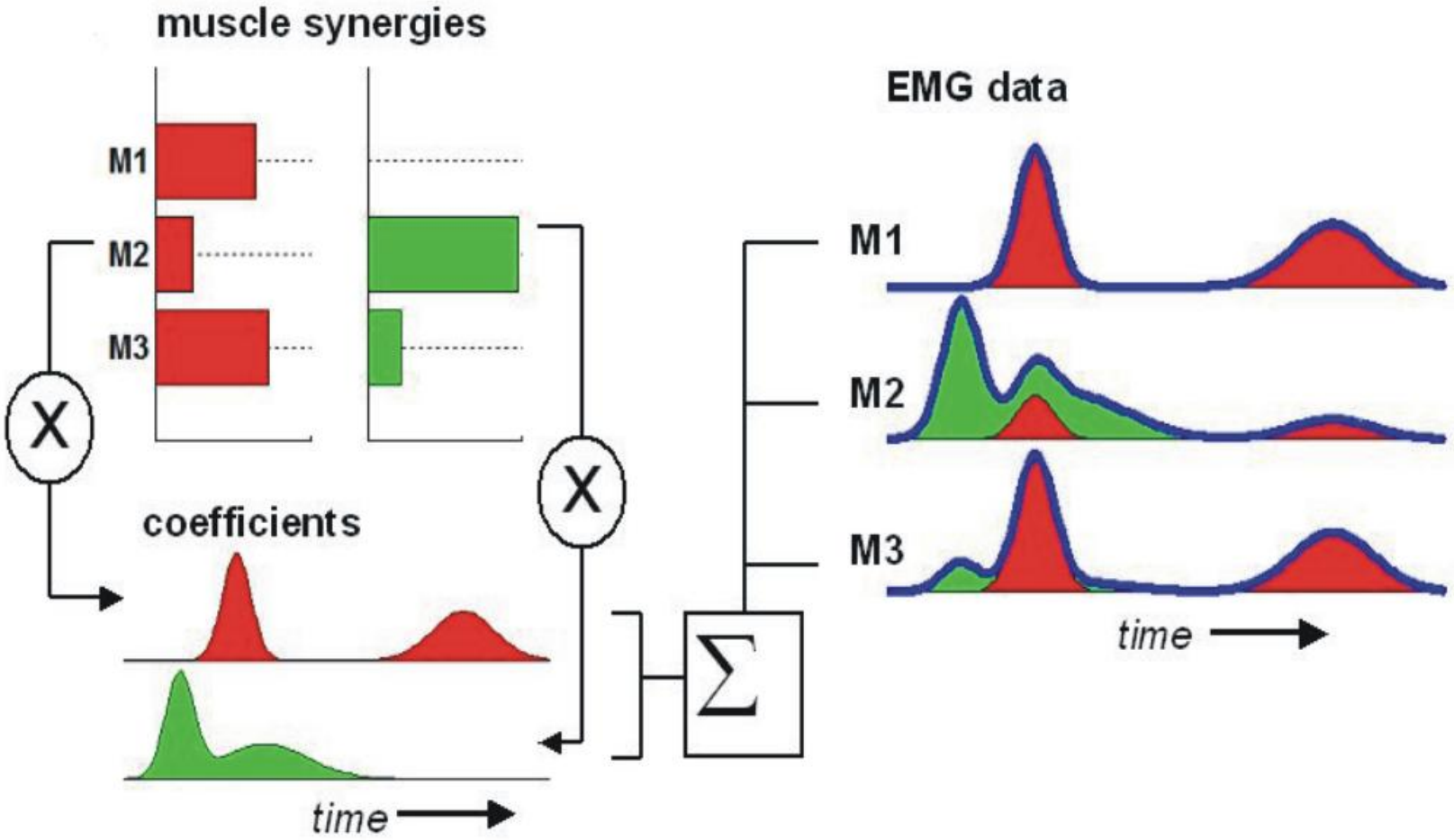
[a]McGovern Institute for Brain Research and Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139; [b]Istituto di Ricovero e Cura a Carattere Scientifico Fondazione Ospedale San Camillo, 30126 Lido di Venezia, Italy; and [c]Department of Physical Medicine and Rehabilitation, Harvard Medical School, Boston, MA 02114

Contributed by Emilio Bizzi, July 16, 2012 (sent for review June 15, 2012)

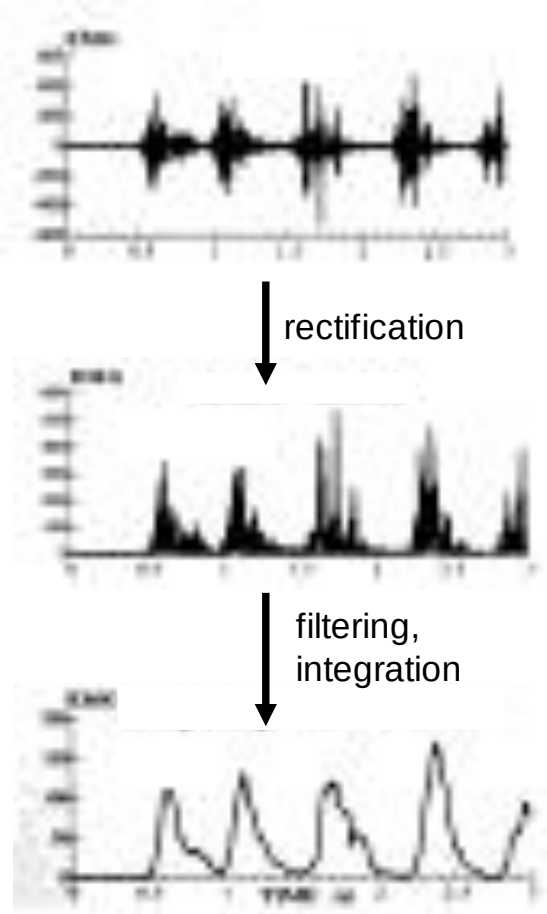
Reaching Trajectories

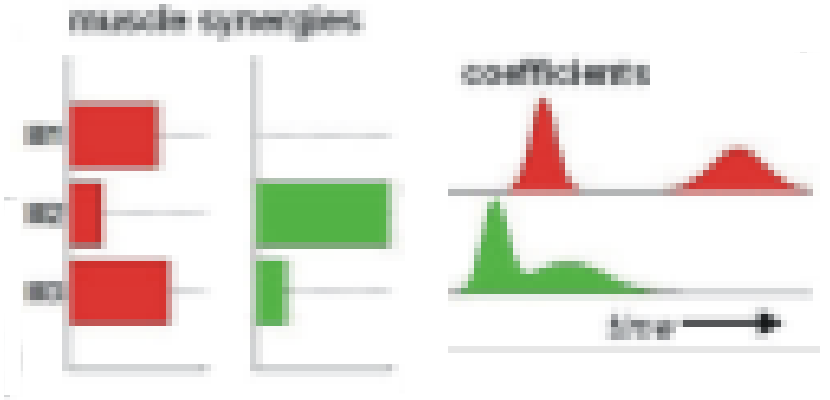


A Group of Muscles Controlled as a Single Unit



Identifying Muscle Synergies





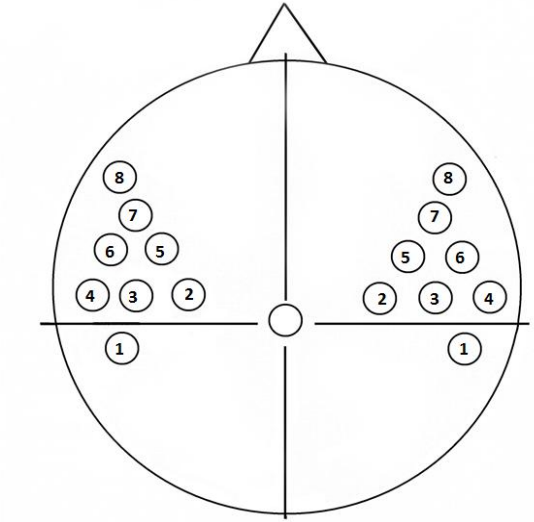
EMGs of many muscles

decomposition algorithm

Motor cortex excitability (TMS)

I. Resting motor threshold (MT)

II. mapping (8 points/each hemisphere) : 4 MEPs (110% MT)



III. MEPs recruitment curve (110%, 130%, 150% di MT)

fNIRS

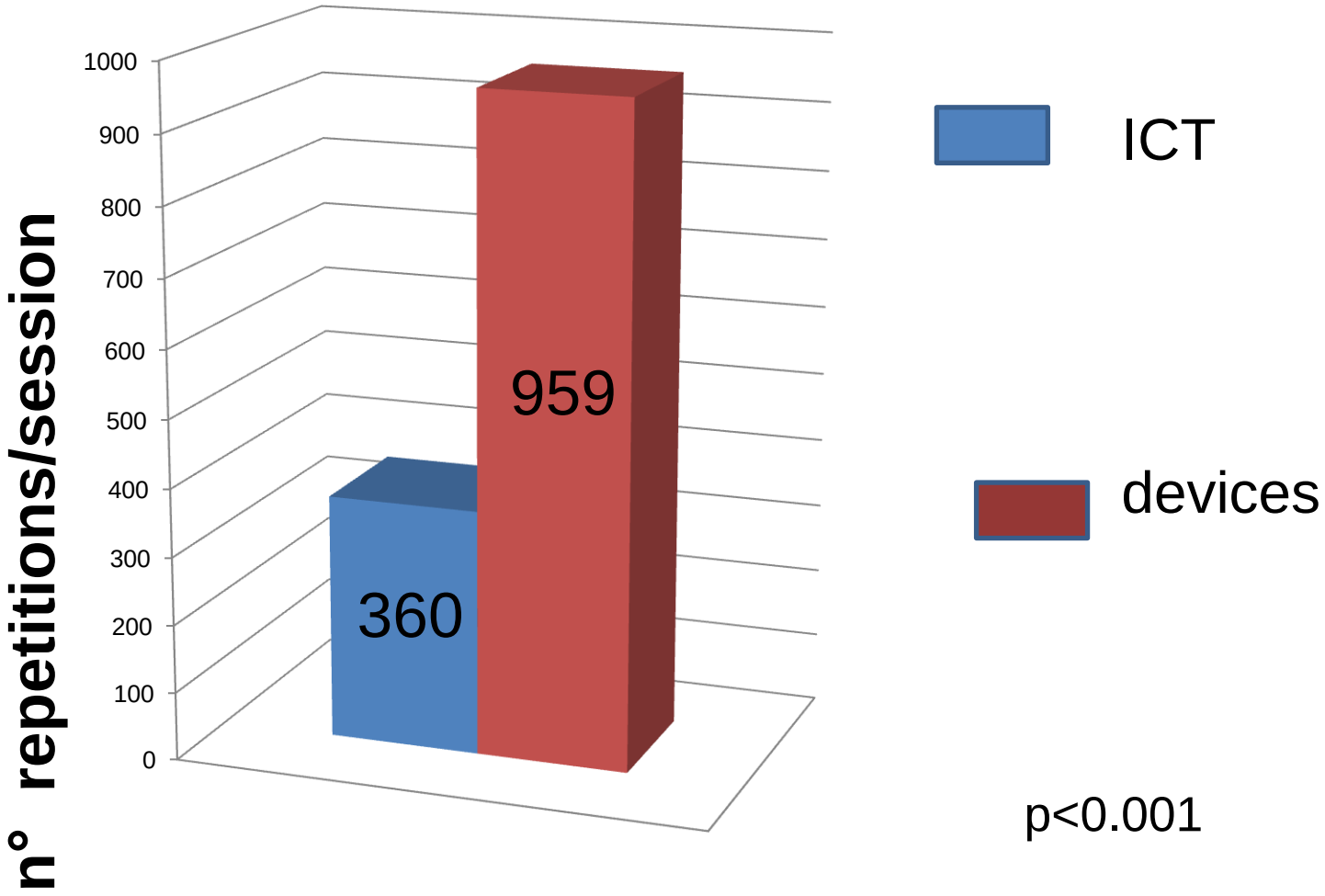
NIRScout system 48 channels
16 sources – 16 detectors
Record of Oxygenated and Deoxygenated hemoglobin from M1 in both hemispheres.

Experimental condition:

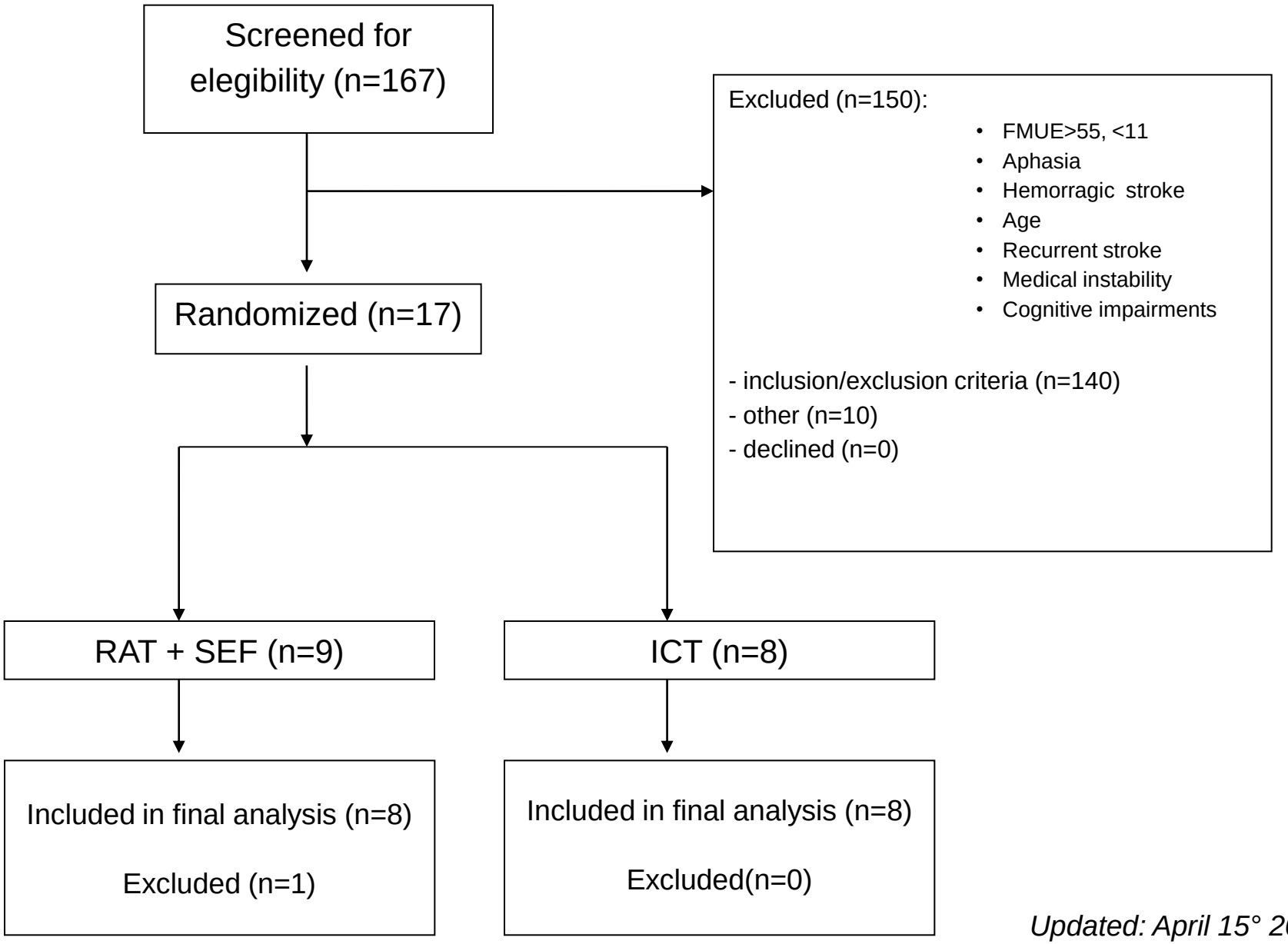
x6 times	Task: reaching and grasping 15”	Rest 45”	Healthy forearm
x6 times	Task: reaching and grasping 15”	Rest 45”	Paretic forearm

Preliminary Results

Intensity (n=60 sessions)

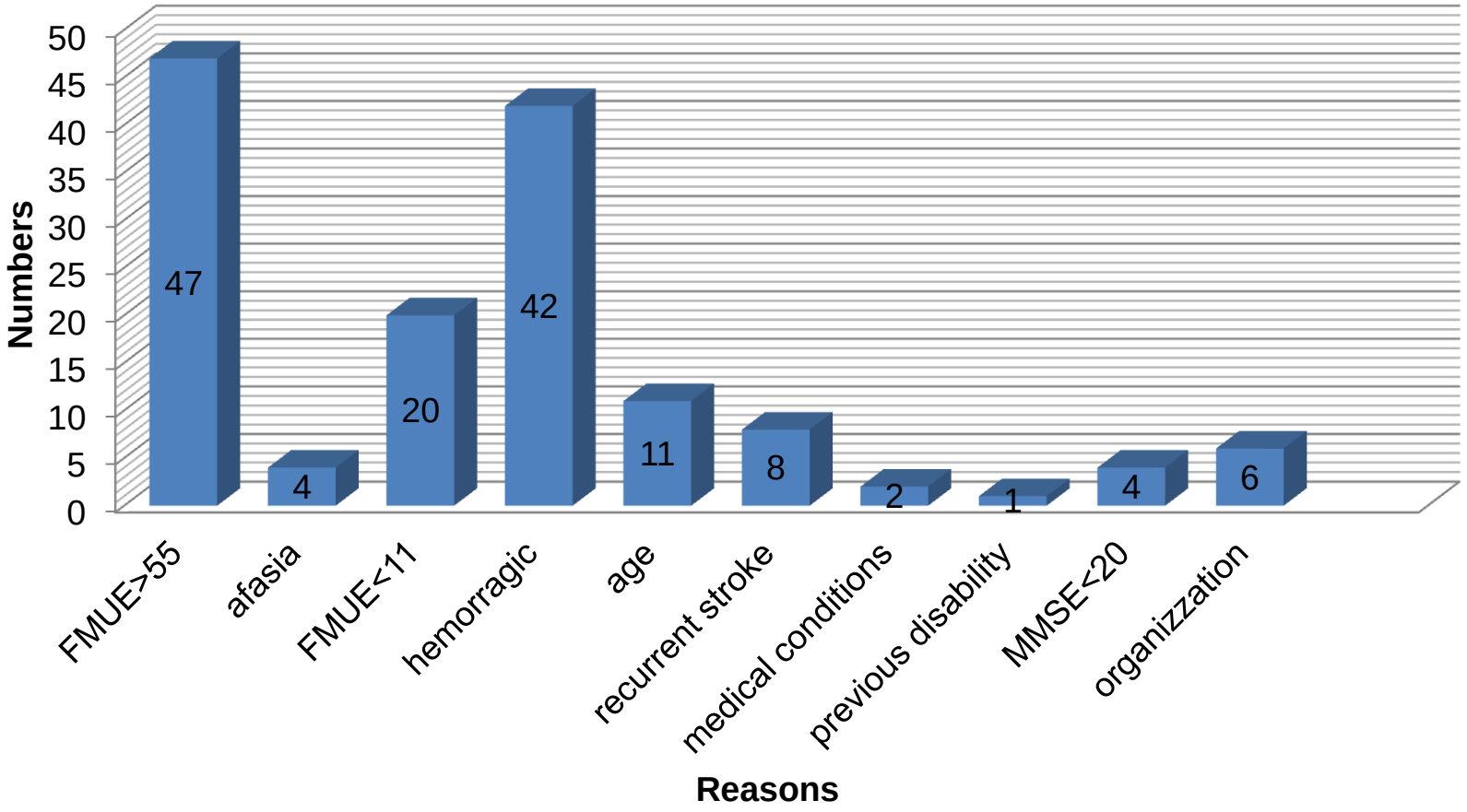


CONSORT flow diagram



Updated: April 15° 2015

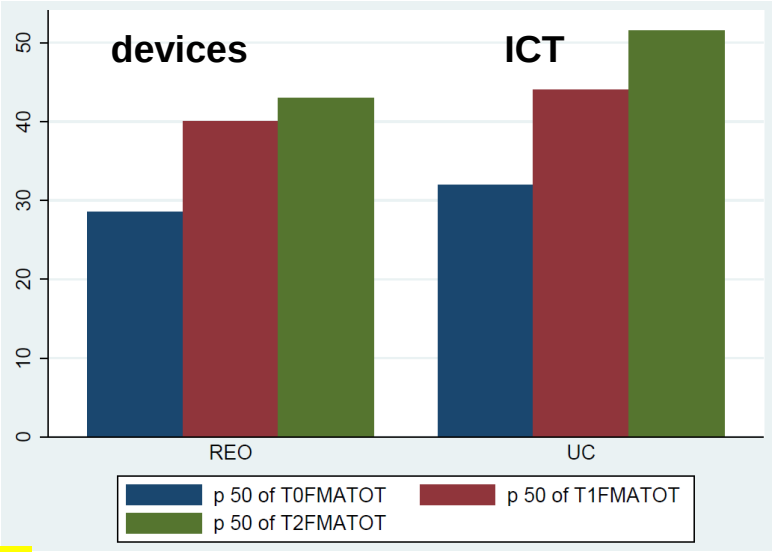
Exclusion reasons



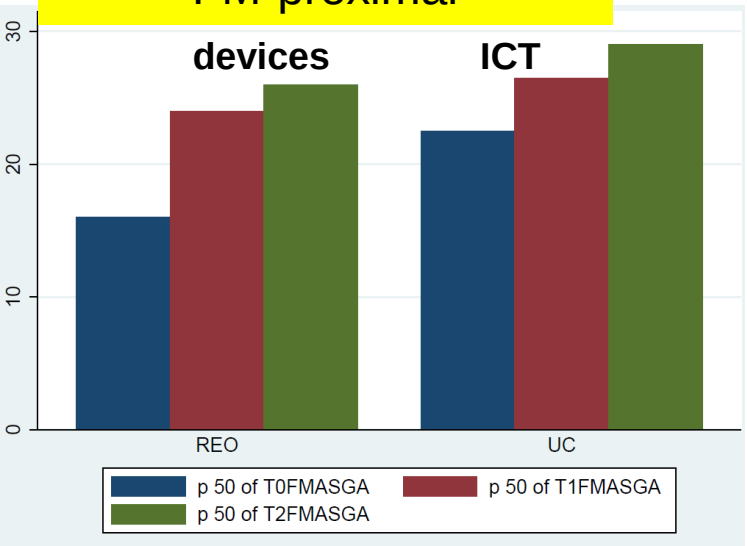
Sample characteristics (n=16)

Characteristics	Sample	RAT + SEF	ICT
subjects (n)	16	8	8
age (mean ± SD)	(66.5 ± 10.46)	(65.38 ± 12.16)	(67.14 ± 9.77)
male (n)	9	5	4
female (n)	7	3	4
lacunar stroke (n)	10	5	1
big vassels stroke (n)	6	3	3
Right hemiplegia (n)	5	3	2
Letf hemiplegia (n)	11	5	6
Stroke onset (days)	40.88	35.63	45.43
Fibrinolysis yes (n)	3	1	2
Fibrinolysis no (n)	12	6	6

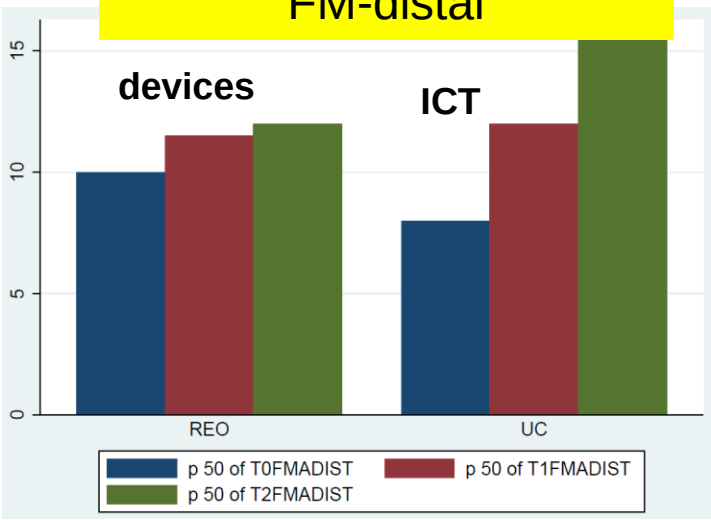
Primary outcome: FM-UE



FM-proximal

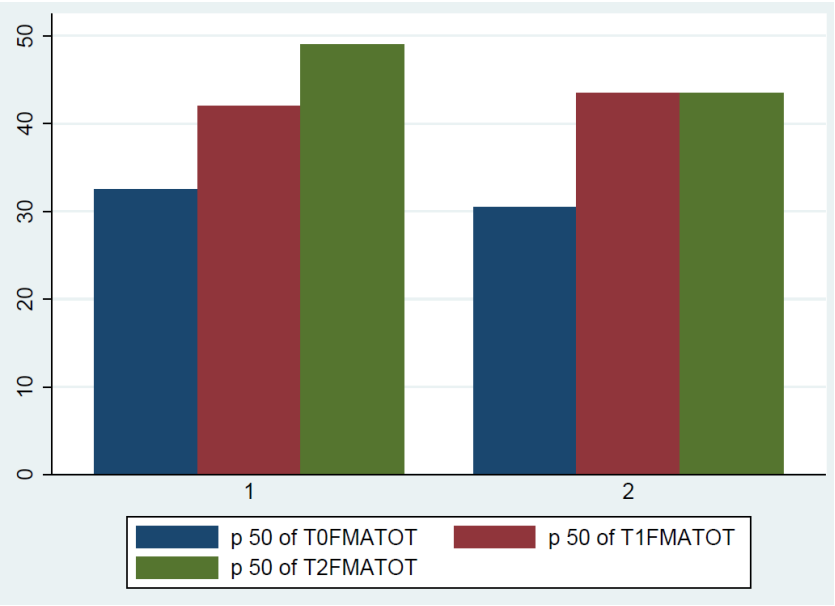


FM-distal

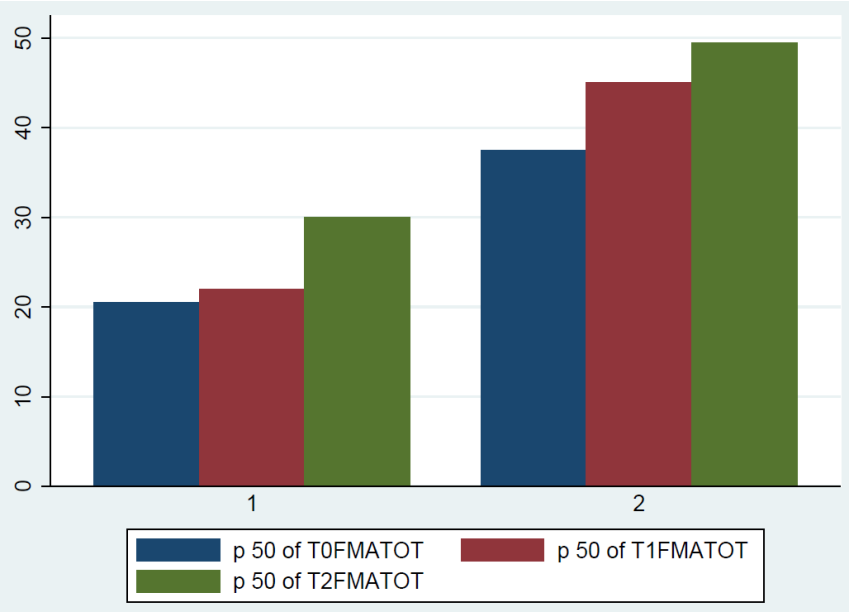


FM-UE: time and impairment

Early-Late

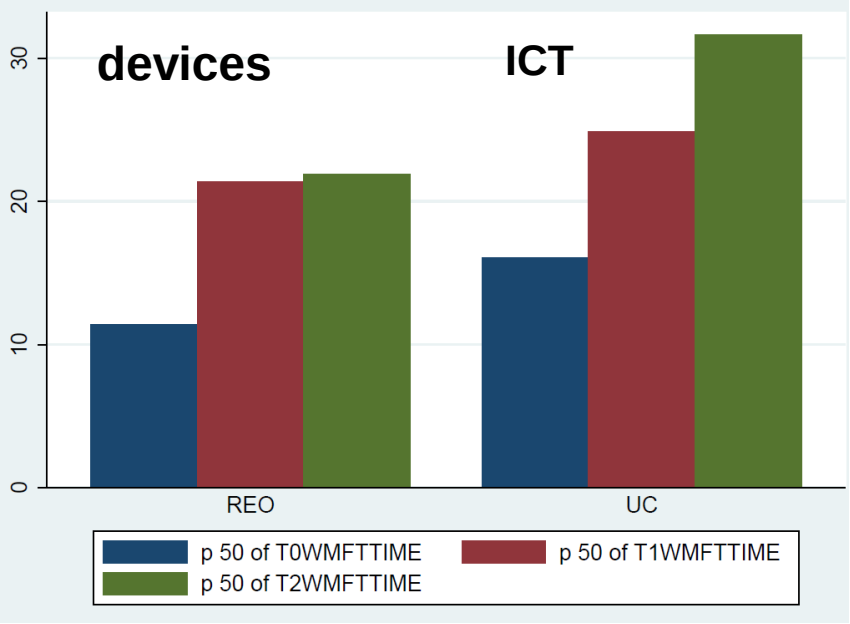


Low-High functioning

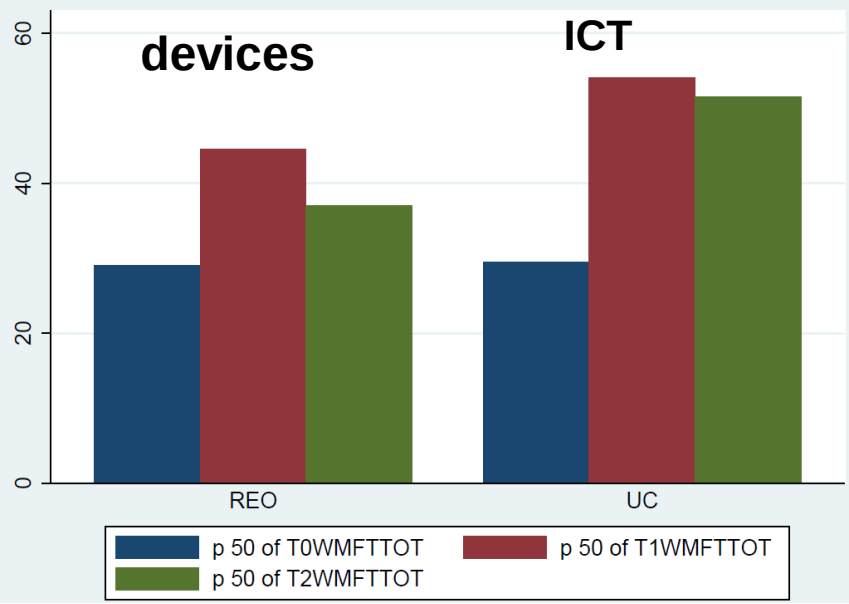


Wolf Motor Function Test

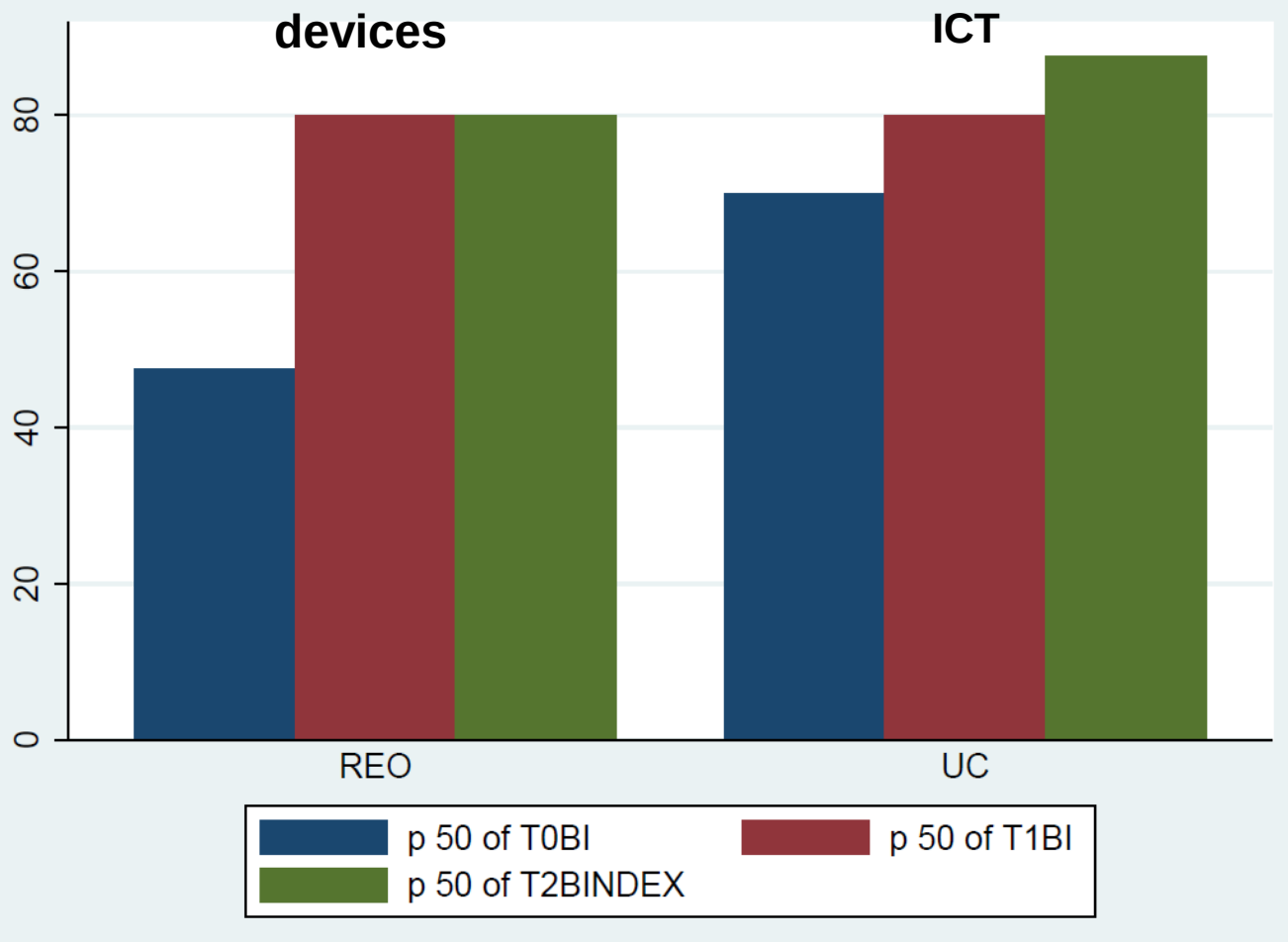
rate: n tasks/60s



Quality of movements

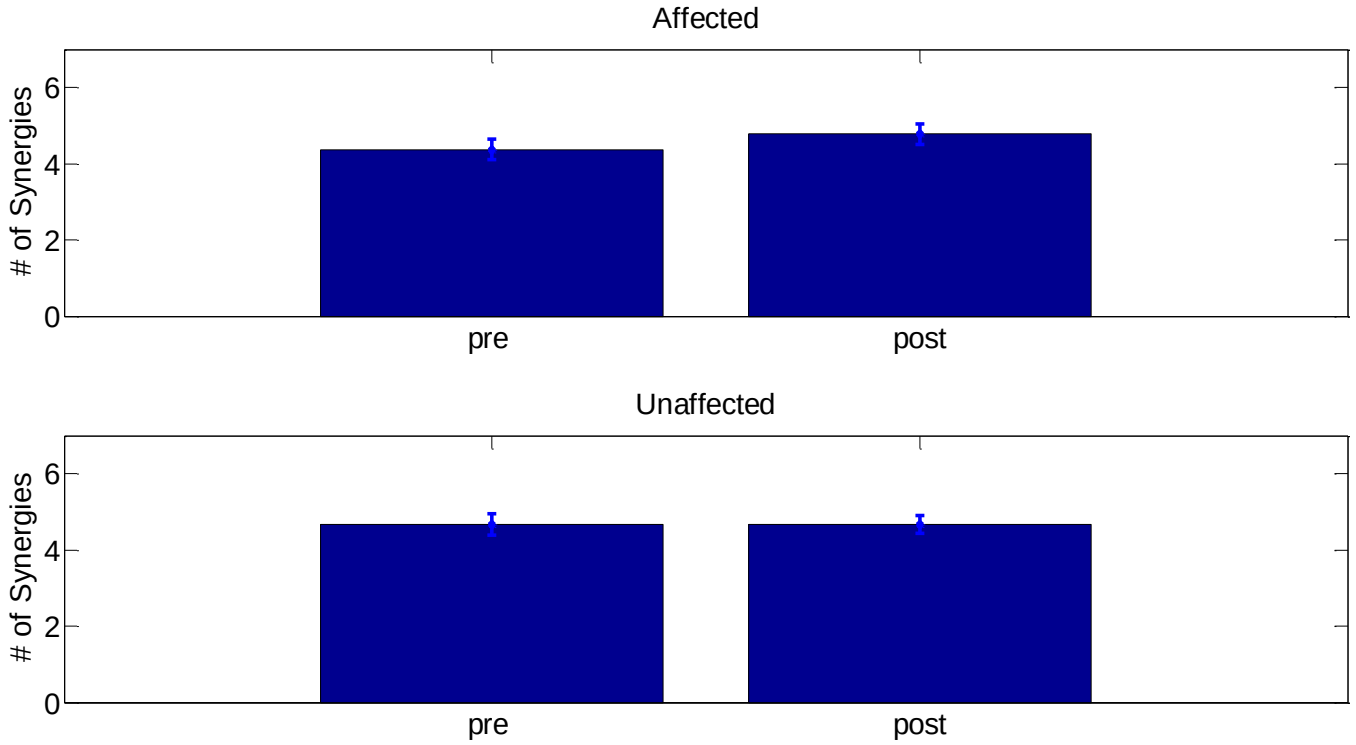


Barthel Index (ADL)



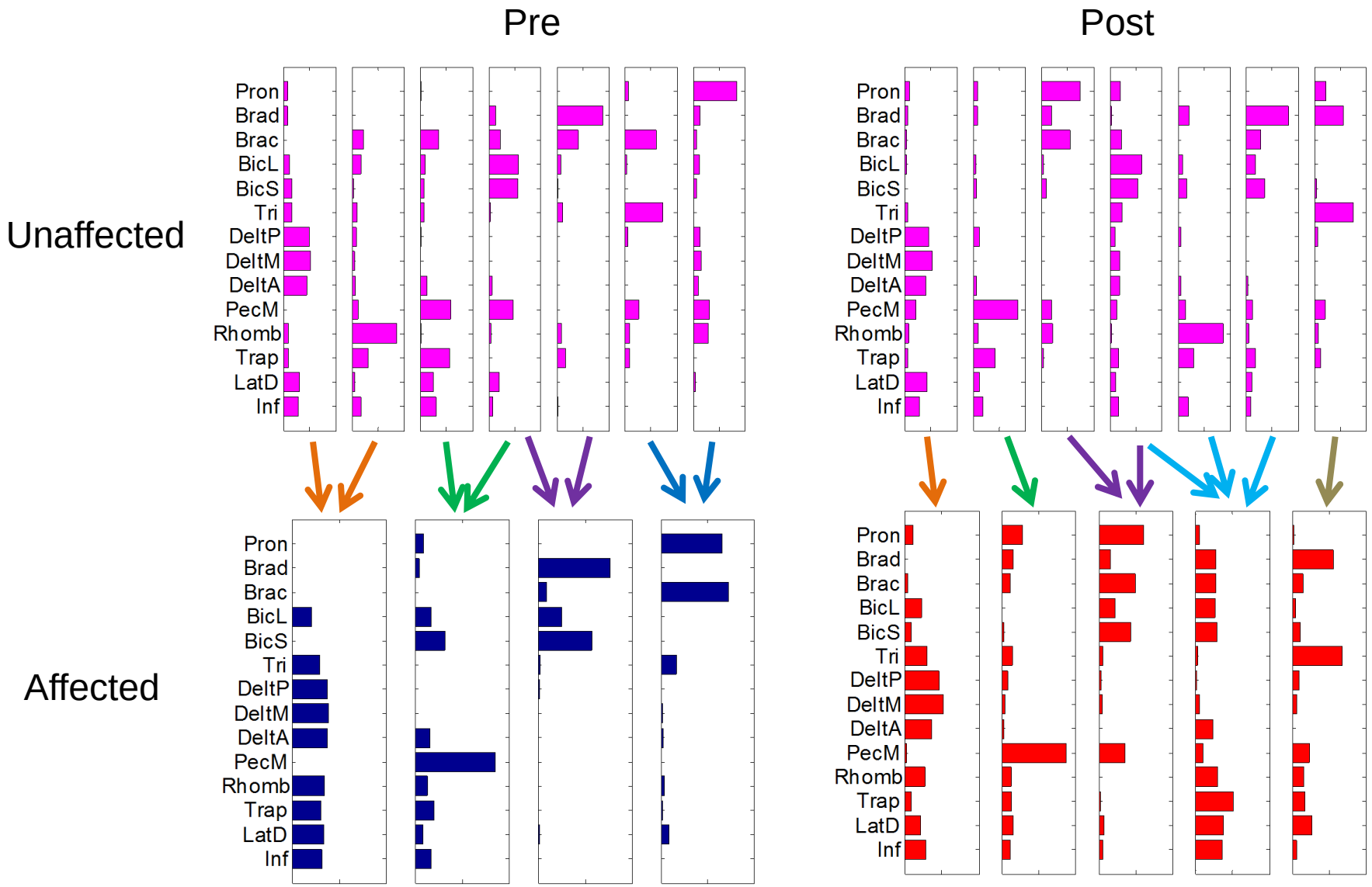
Preliminary results on UE Motor Synergies

of Synergies

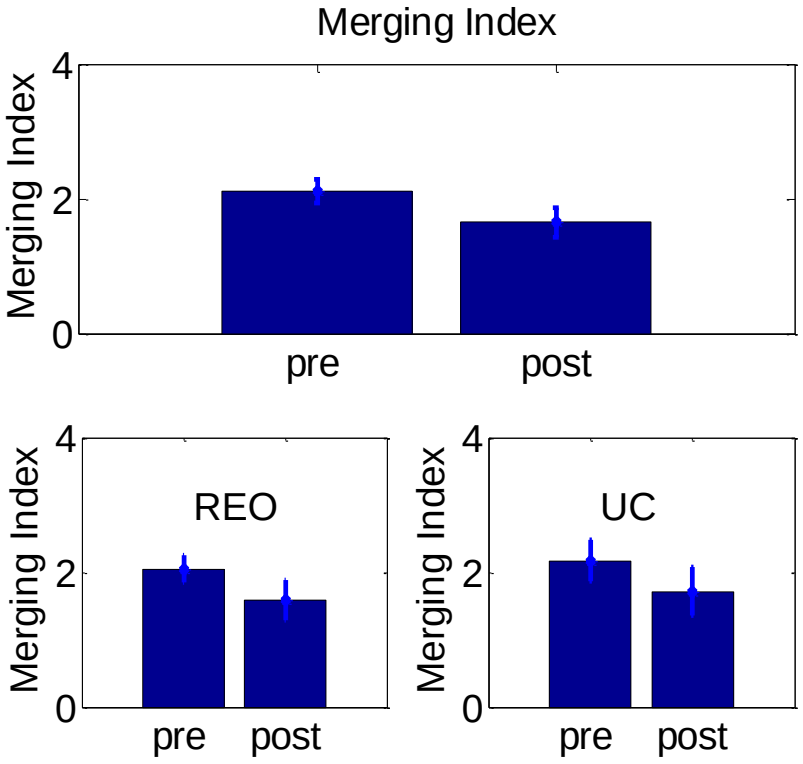


- smaller number of synergies in the affected side with respect to the sound one
- the number of synergies on the unaffected side does not change across time
- increase in number of synergies for the affected side

Example of synergies



Merging index



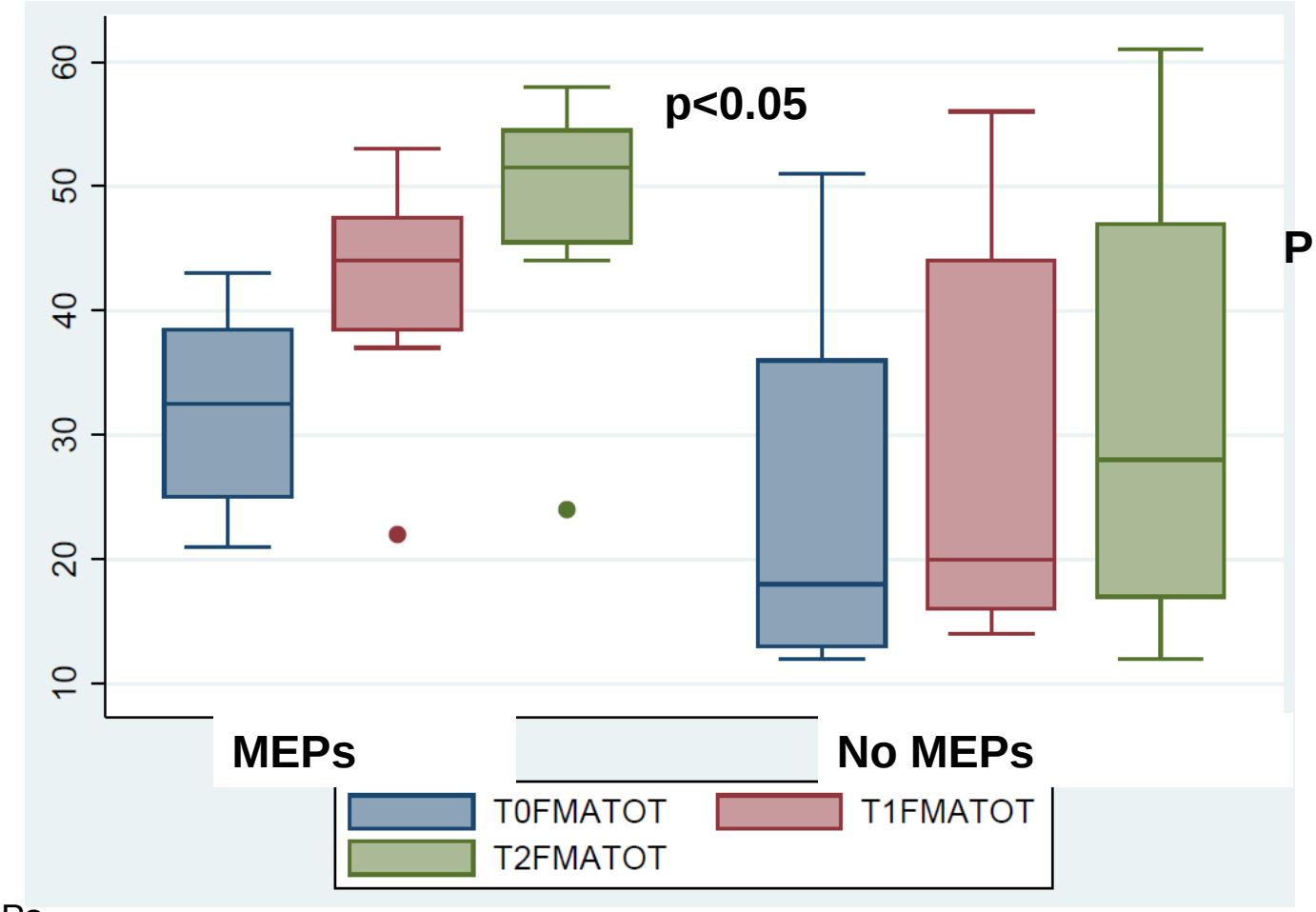
MI = number of merged synergies

at least 2 synergies for each patient can be identified as merged versions of two or more unaffected synergies

Merging Index decreased after rehabilitation

Preliminary results on Motor Cortex Reorganizations

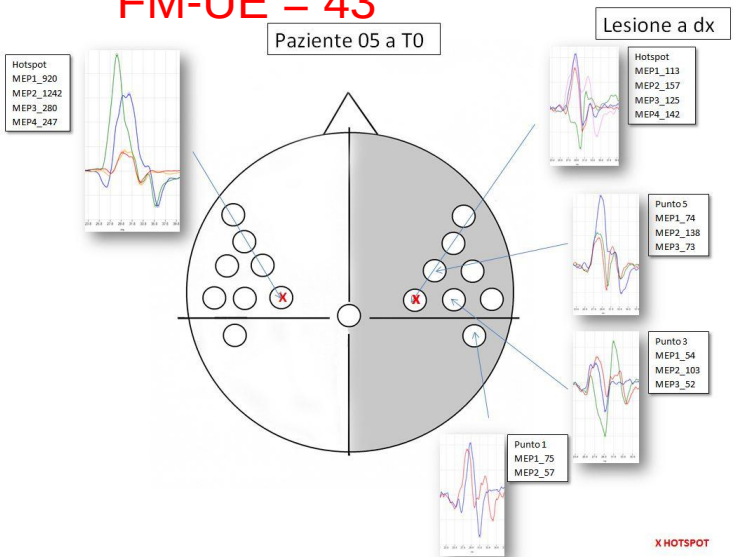
FM-UE and MEPs



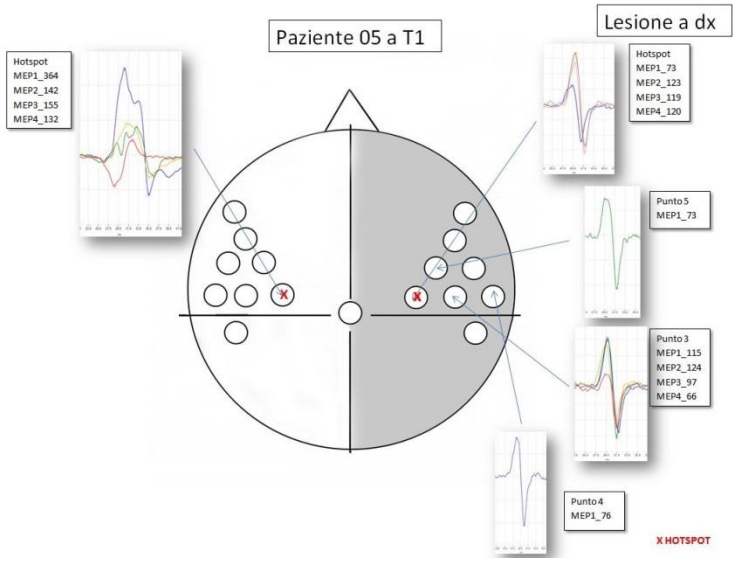
n=8 MEPs
n=5 no MEPs
n=3 MEPs nv

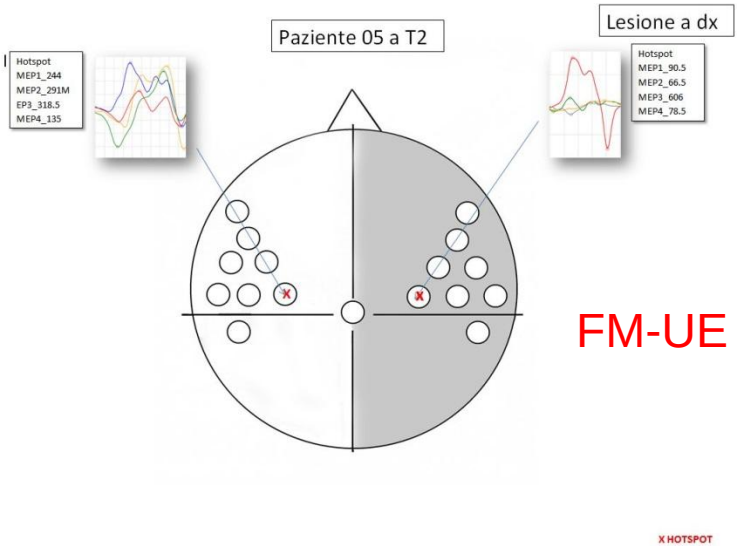
Subject #05 (good recovery)

FM-UE = 43



FM-UE = 49

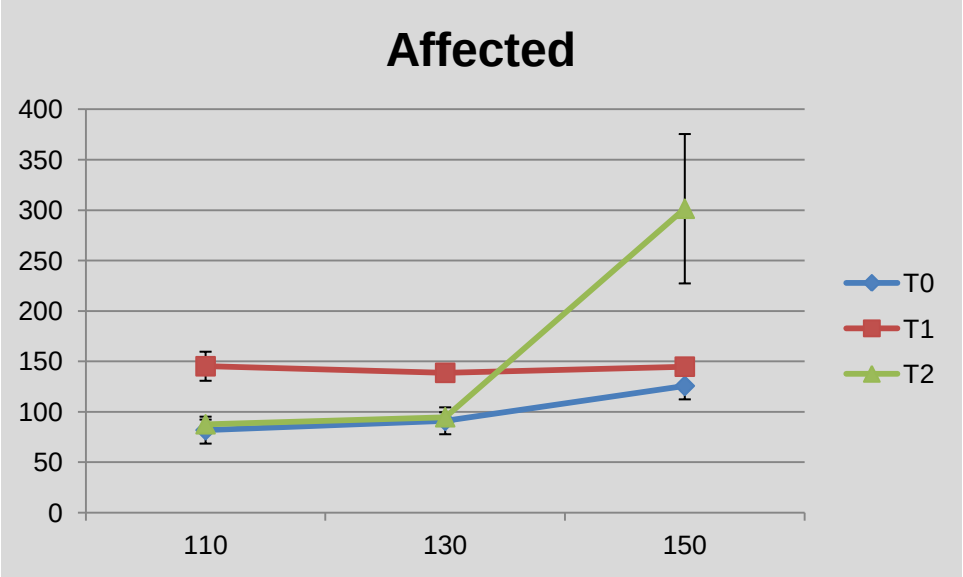




FM-UE = 53

MEPs recruitment curve

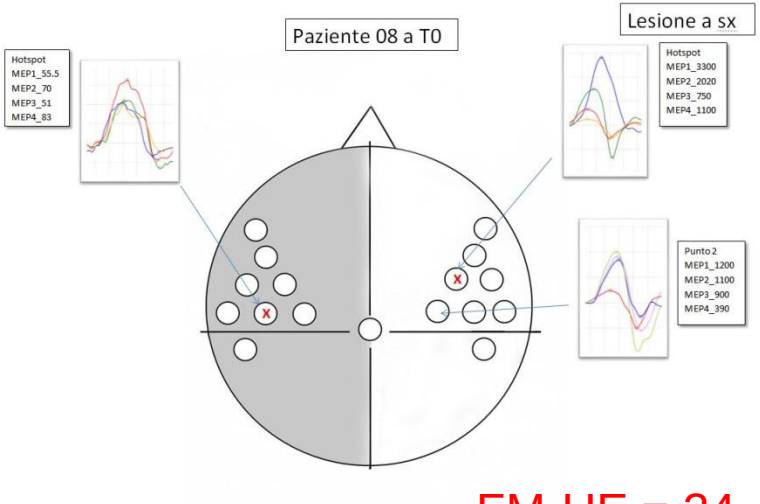
	MT
T0	48
T1	39
T2	45



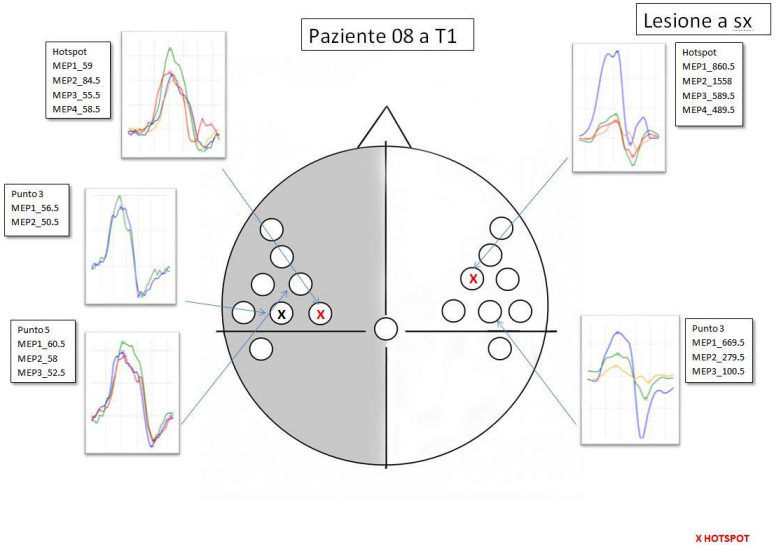
T0, T1 no curve
T2: increased MEPs amplitude (150%)

Subject #08 (poor recovery)

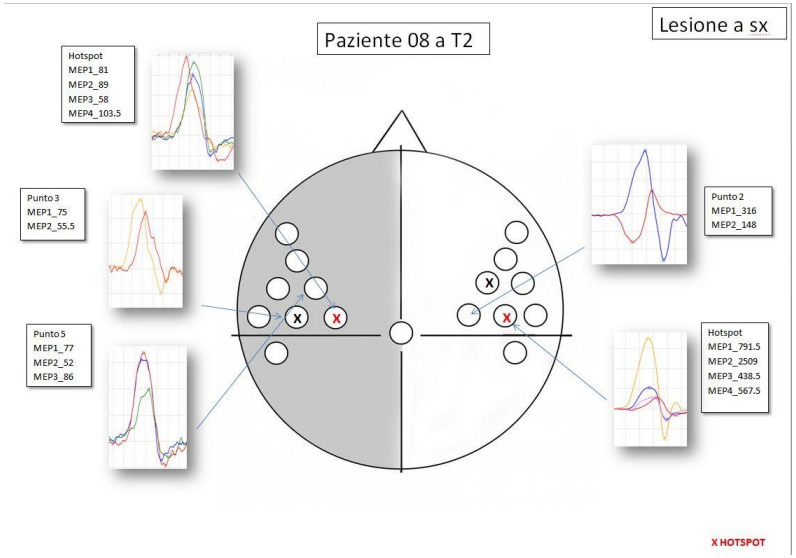
FM-UE = 21



FM-UE = 22

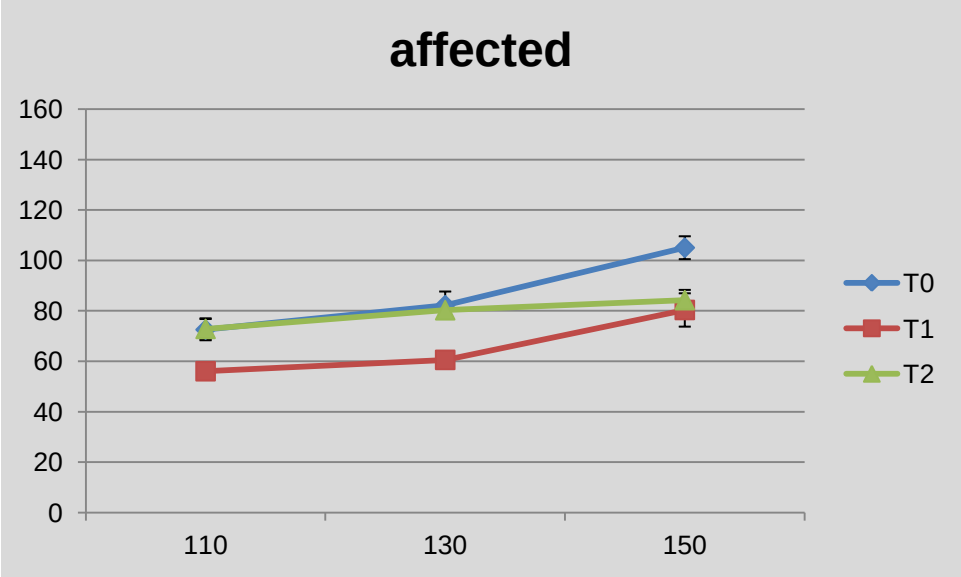


FM-UE = 24



MEPs recruitment curve

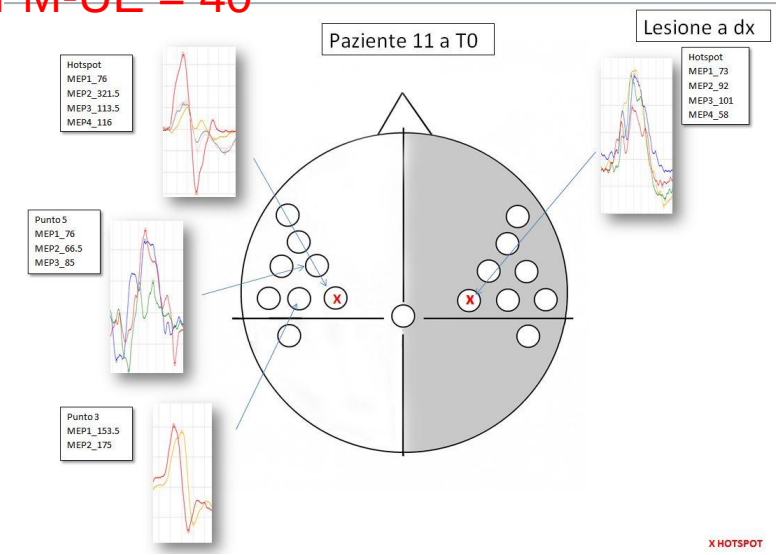
	MT
T0	43
T1	41
T2	45

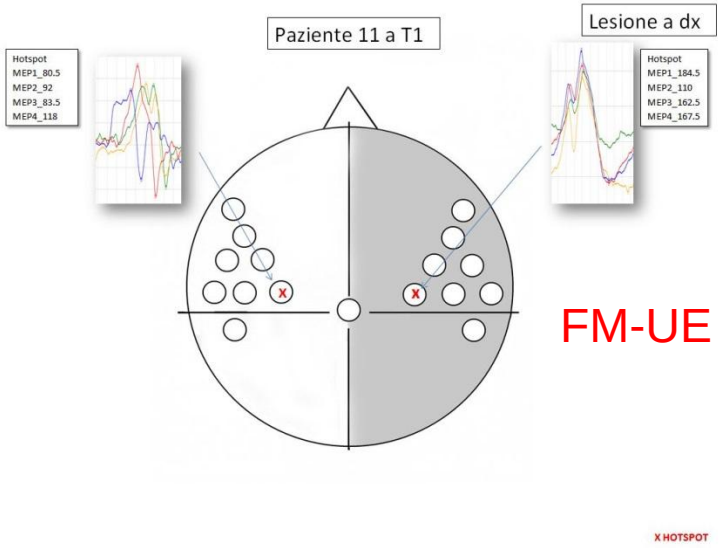


Low MEPs amplitude

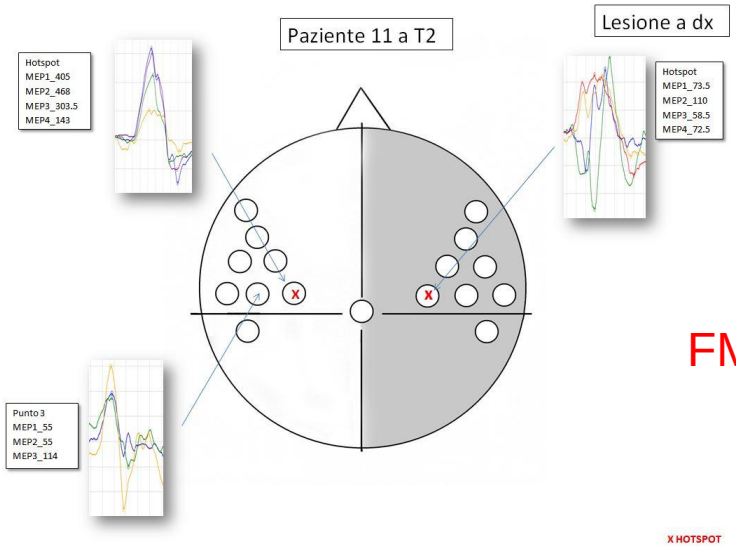
Subject #11 (high-functioning)

FM-UE = 40





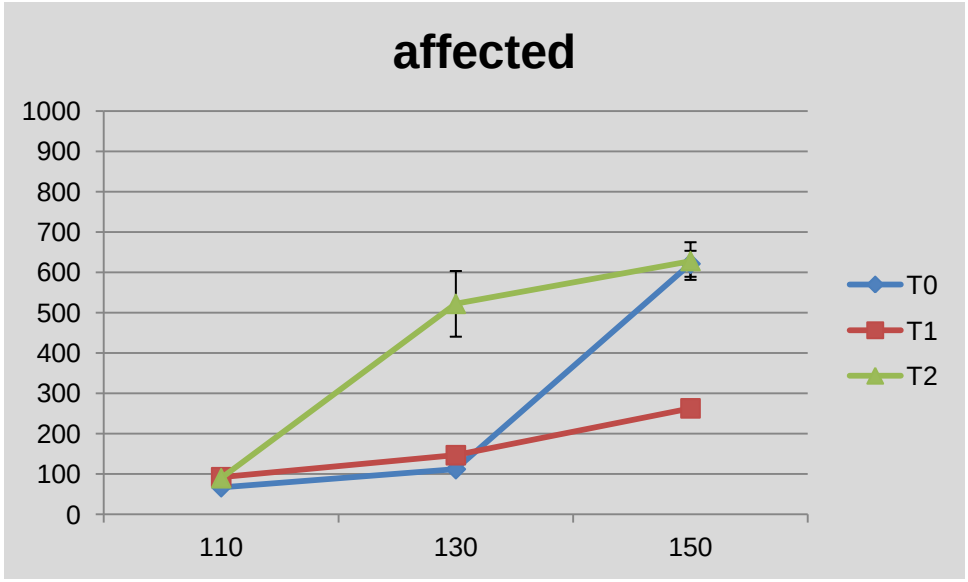
FM-UE = 46



FM-UE = 56

MEPs recruitment curve

	MT
T0	35
T1	32
T2	32

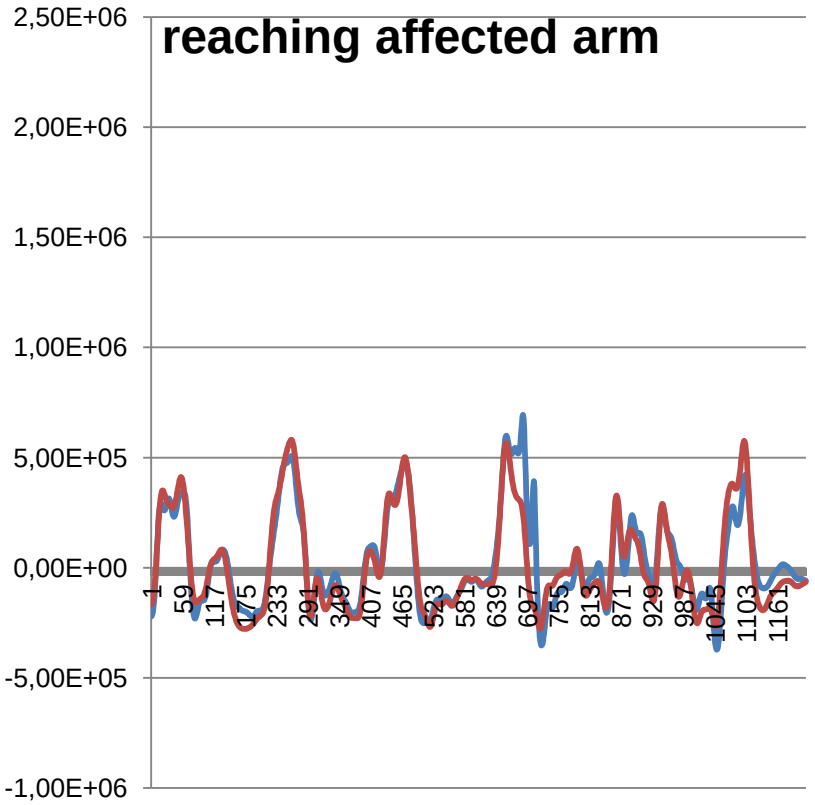


T0, T1 increased MEPs amplitude (150%)
T2 regular curve

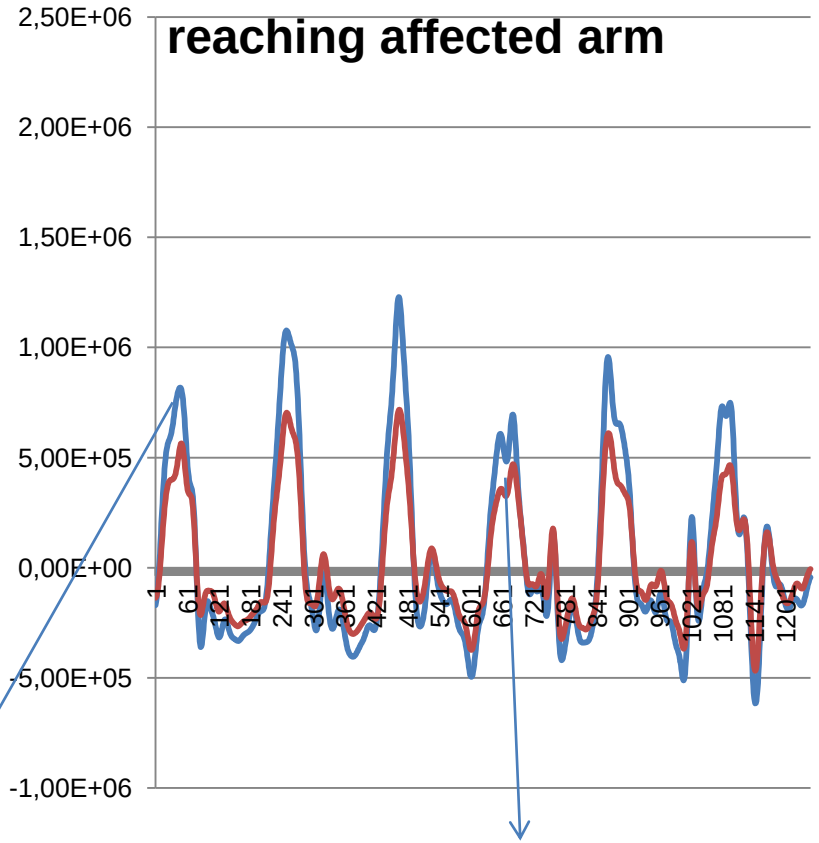
Preliminary results on Functional near-infrared spectroscopy

Oxy-HB raw data

pre-treatment



post-treatment



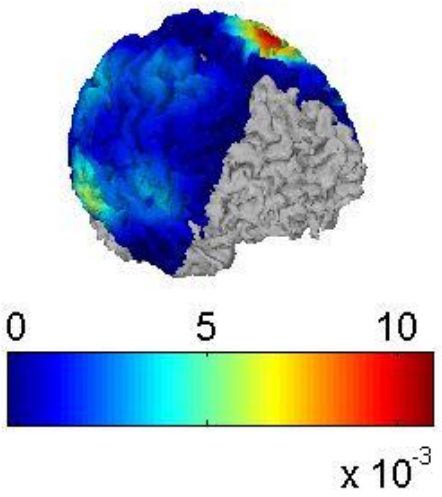
data collected 13/16 subjects

Unaffected M1 (blue)

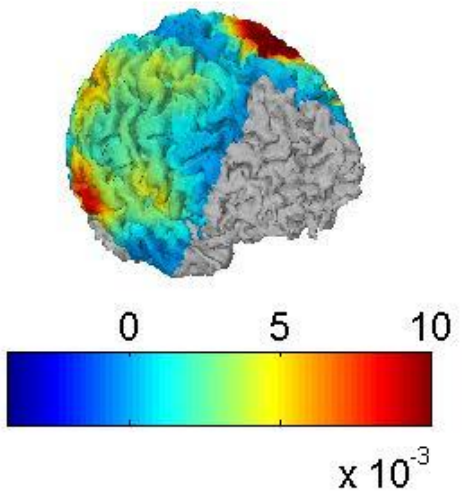
Affected M1 (red)

fNIRS brain maps

pre-treatment (FM-UE=36)



post-treatment (FM-UE = 47)



right hemisphere stroke: reaching task with the affected arm (15 seconds)

Preliminary results on circulating biomarkers

- **significant changes in circulating endothelial cells and endothelial cell progenitors are highlighted over time**

- **they might represent damage/repair biomakers after stroke**

Circulating endothelial progenitor cells

The Increase of Circulating Endothelial Progenitor Cells After Acute Ischemic Stroke Is Associated With Good Outcome

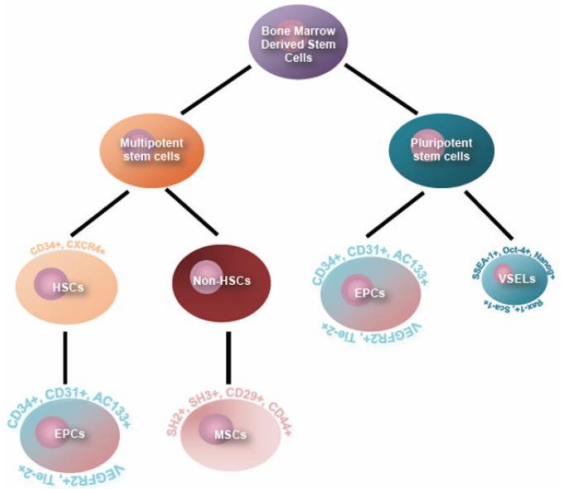
Tomás Sobrino, PhD; Olivia Hurtado, PhD; María Ángeles Moro, PhD; Manuel Rodríguez-Yáñez, MD, PhD; Mar Castellanos, MD, PhD; David Brea, BSc; Octavio Moldes, BSc; Miguel Blanco, MD, PhD; Juan F. Arenillas, MD, PhD; Rogelio Leira, MD, PhD; Antonio Dávalos, MD, PhD; Ignacio Lizasoain, MD, PhD; José Castillo, MD, PhD

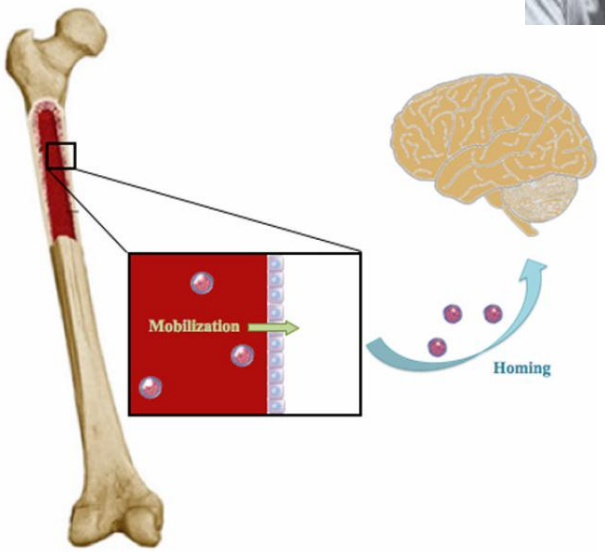
Background and Purpose—Increased circulating endothelial progenitor cells (EPC) have been associated with a low cardiovascular risk and may be involved in endothelial cell regeneration. The present study was designed to evaluate the prognostic value of EPC in acute ischemic stroke.

Methods—Forty-eight patients with a first-ever nonlacunar ischemic stroke were prospectively included in the study within 12 hours of symptoms onset. Stroke severity was evaluated by the National Institutes of Health Stroke Scale, and functional outcome was assessed at 3 months by the modified Rankin Scale (mRS). Infarct volume growth between admission and days 4 to 7 was measured on multiparametric MRI. EPC colonies were defined as early outgrowth colony-forming unit-endothelial cell (CFU-EC). The increment of CFU-EC was quantified during the first week and defined as the absolute difference between the number of CFU-EC at day 7 and admission. The influence of CFU-EC increase on good functional outcome (mRS ≤2) and infarct growth was analyzed by logistic regression and linear models.

Results—Patients with good outcome (n=25) showed a higher CFU-EC increment during the first week (median [quartiles], 23 [11, 36] versus −3 [−7, 1], $P<0.0001$) compared with patients with poor outcome. CFU-EC increment ≥4 during the first week was associated with good functional outcome at 3 months (odds ratio, 30.7; 95% CI, 2.4 to 375.7; $P=0.004$) after adjustment for baseline stroke severity, ischemic volume and thrombolytic treatment. For each unit increase in the CFU-EC the mean reduction in the growth of infarct volume was 0.39 (0.03 to 0.76) mL ($P=0.033$).

Conclusions—The increase of circulating EPC after acute ischemic stroke is associated with good functional outcome and reduced infarct growth. These findings suggest that EPC might participate in neurorepair after ischemic stroke. (***Stroke.*** **2007;38:2759-2764.**)





The Great Migration of Bone Marrow-Derived Stem Cells Toward the Ischemic Brain: Therapeutic Implications for Stroke and Other Neurological Disorders

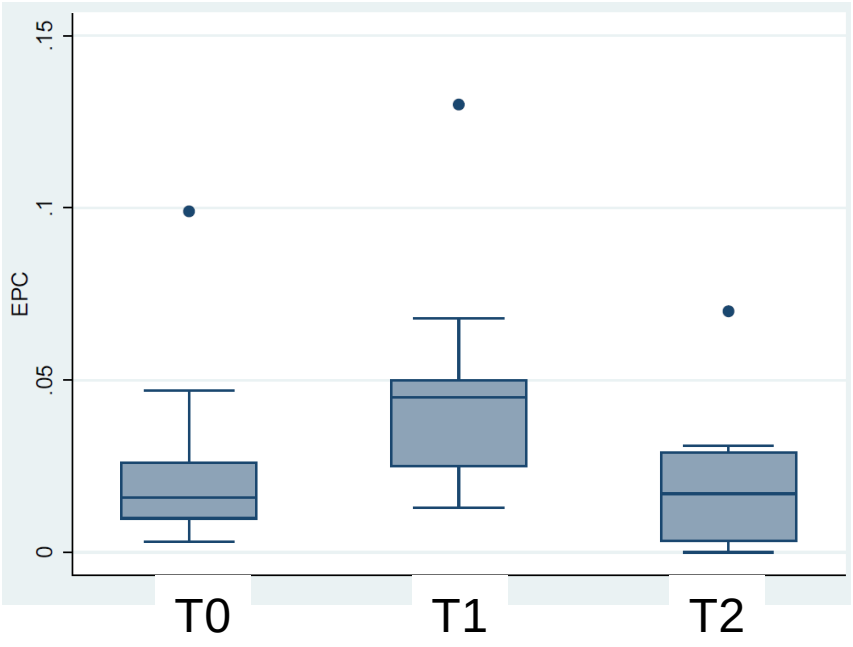
Cesar V. Borlongan, Ph.D., Loren E. Glover, M.S., Naoki Tajiri, Ph.D., Yuji Kaneko, Ph.D., and **Thomas B. Freeman, M.D.**
Department of Neurosurgery and Brain Repair, University of South Florida College of Medicine, 12901 Bruce B. Downs Blvd, Tampa, Florida 33612

Abstract

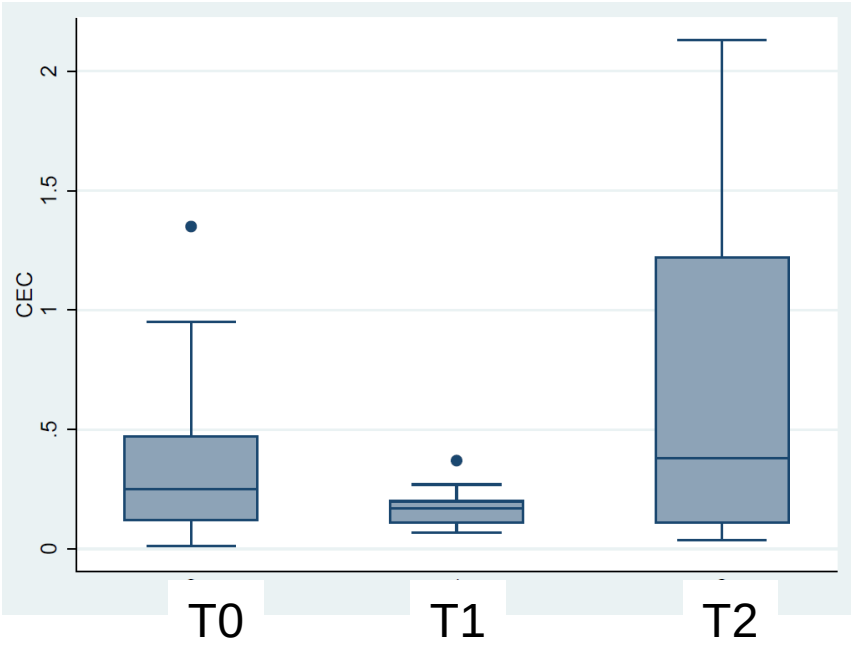
Accumulating laboratory studies have implicated the mobilization of bone marrow (BM)-derived stem cells in brain plasticity and stroke therapy. This mobilization of bone cells to the brain is an essential concept in regenerative medicine. Over the past ten years, mounting data have shown the ability of bone marrow–derived stem cells to mobilize from BM to the peripheral blood (PB) and eventually enter the injured brain. This homing action is exemplified in BM stem cell mobilization following ischemic brain injury. Various BM-derived cells, such as hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), endothelial progenitor cells (EPCs) and very small embryonic-like cells (VSELs) have been demonstrated to exert therapeutic benefits in stroke. Here, we discuss the current status of these BM-derived stem cells in stroke therapy, with emphasis on possible cellular and molecular mechanisms of action that mediate the cells' beneficial effects in the ischemic brain. When possible, we also discuss the relevance of this therapeutic regimen in other central nervous system (CNS) disorders.

Circulating endothelial cell populations

Endothelial progenitor cell (EPC)

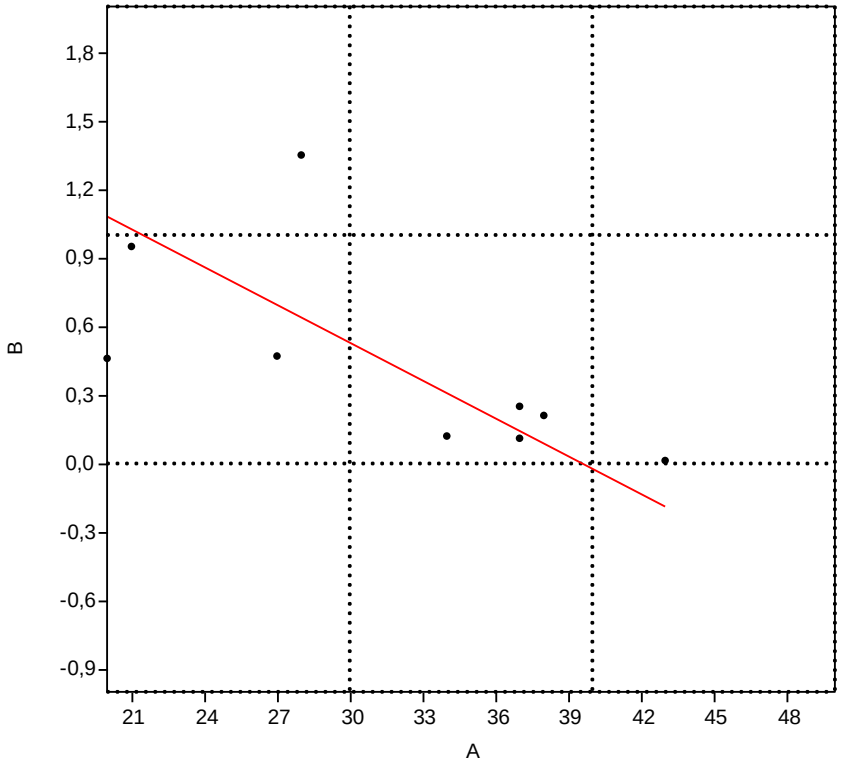


Circulating endothelial cell (CEC)



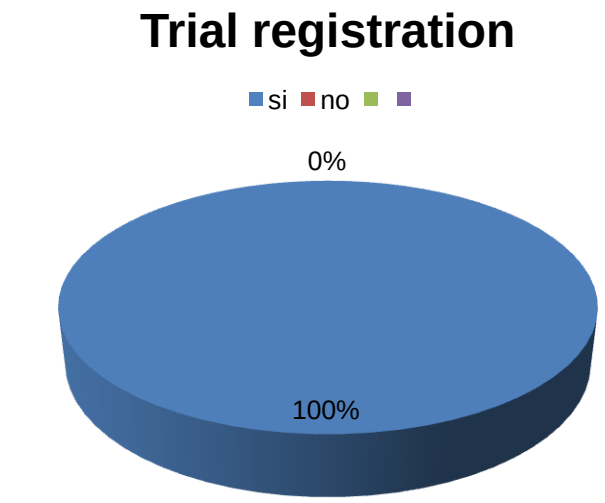
p<0.05

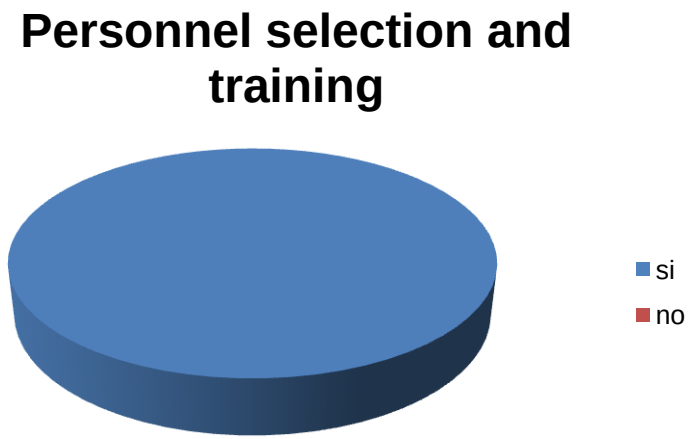
Baseline FM-UE-CEC

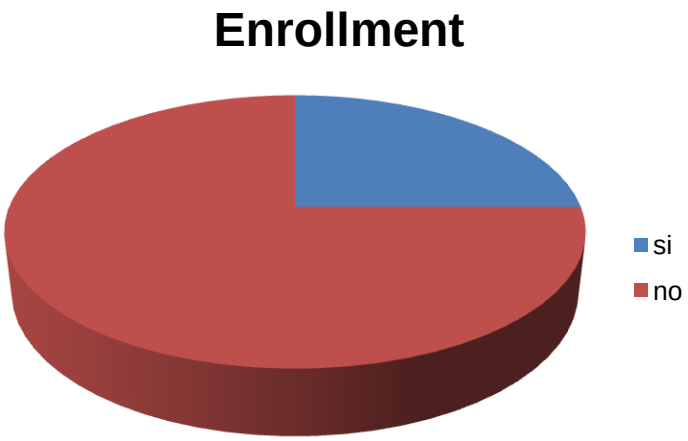


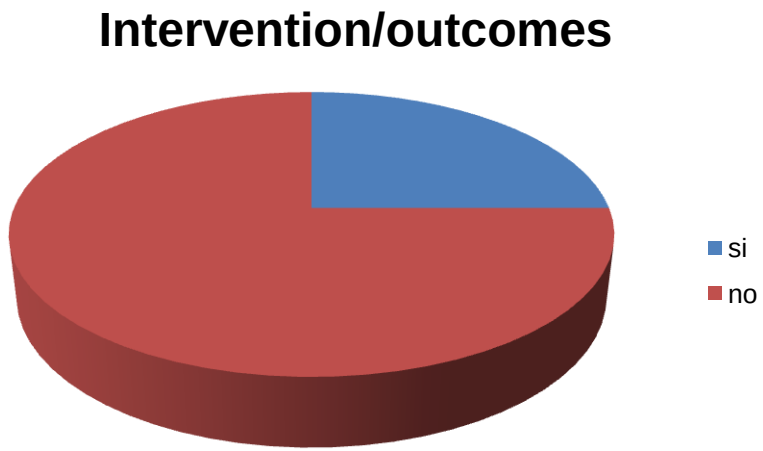
r=-0.66; p=0.05

Milestones


Trial registration
si
no
0%
100%


Personnel selection and training
si
no


Enrollment
si
no


Intervention/outcomes
si
no

Strenghts

clinical outcomes alone failed to prove the relevance of

early-high –intensity arm rehabilitation

combining clinical outcome with neurophisiological/neuroimaging, bological and motor control biomarkers might shed light on arm recovery after stroke

Limits

subacute stroke recruitment rate:

1 subject/month, 10% of the whole stroke population (*in line with literature*)

1-year extension

SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA
Azienda Ospedaliero - Universitaria di Ferrara

università di ferrara
DA SEICENTO ANNI GUARDIAMO AVANTI.

WP2: Scientific coordinator Susanna Lavezzi, MD

The role of transcranial direct current stimulation in minimally conscious state

Feasibility study,
conducted at PM&R Dept, Ferrara

AMERICAN ACADEMY OF NEUROLOGY

Special Article

CME

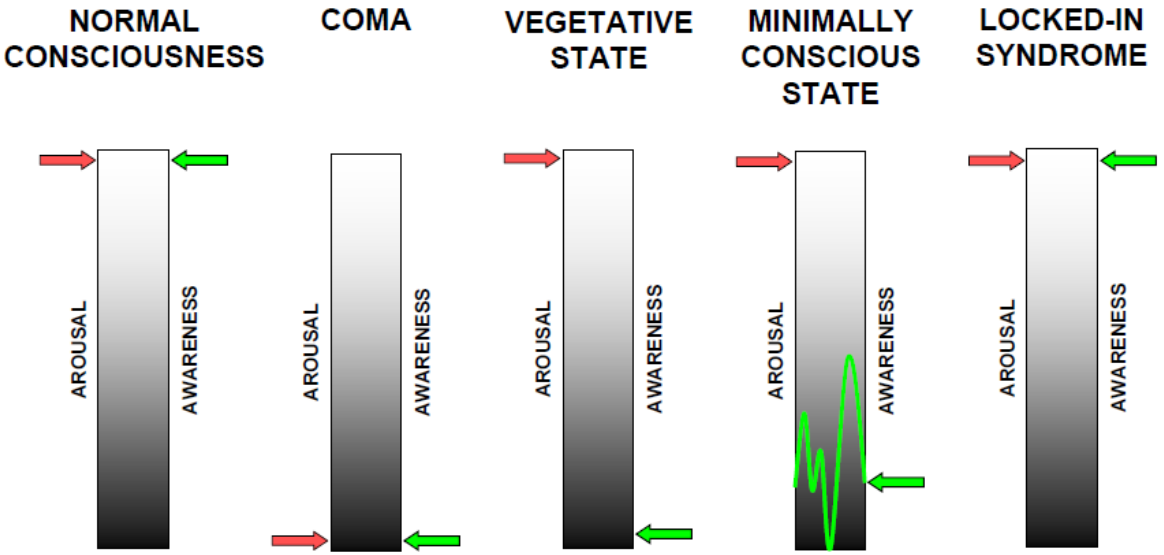
The minimally conscious state

Definition and diagnostic criteria

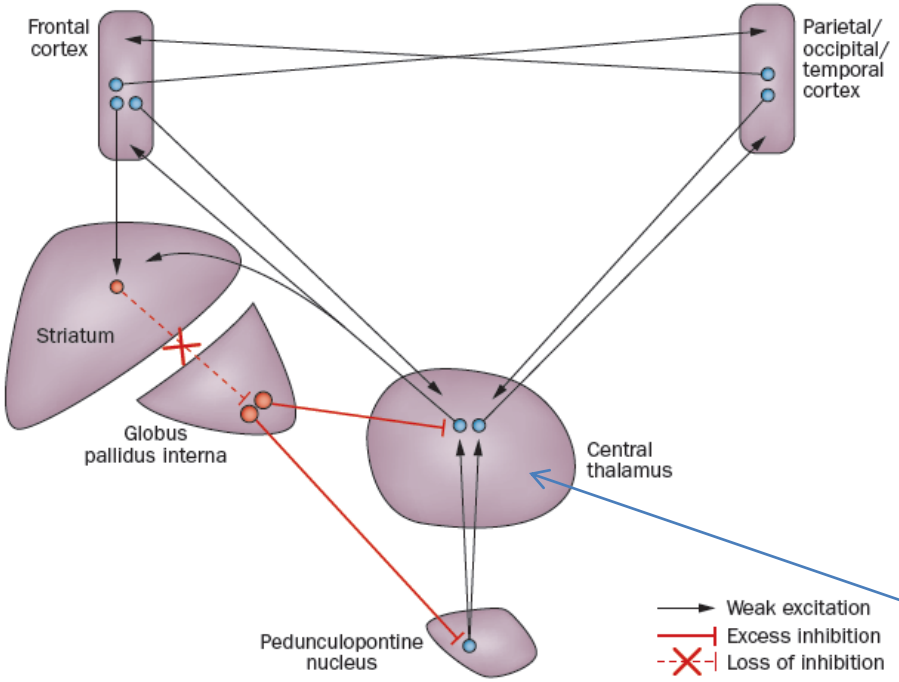
J.T. Giacino, PhD; S. Ashwal, MD; N. Childs, MD; R. Cranford, MD; B. Jennett, MD; D.I. Katz, MD; J.P. Kelly, MD; J.H. Rosenberg, MD; J. Whyte, MD, PhD; R.D. Zafonte, DO; and N.D. Zasler, MD

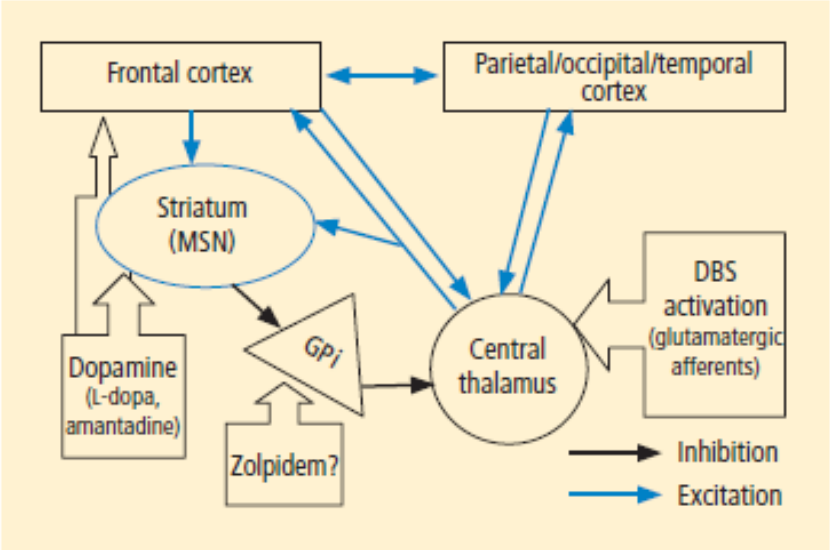
2002.

The minimally conscious state is a condition of severely altered consciousness in which minimal but definite behavioral evidence of self or environmental awareness is demonstrated.



RATIONALE





M1 bilaterally

- *Joseph T. et al* 2014.
- Schiff ND et al. 2010.

AIMS

- To test the feasibility of tDCS in chronic MCS

- To test the hypothesis that it might be beneficial in modulating behaviour

- To verify any correlations between clinical and brain mapping measures (EEG, fNIRS, ERP)

Inclusion/exclusion criteria

Inclusion criteria:

- males and females aged-> 18 years and <60 years
- diagnosis of disorders of consciousness classified as minimally conscious state (MCS)
- traumatic etiology (> 12 months after the acute event)

Exclusion criteria:

- tDCS contraindications such as the presence of metallic implants that can be stimulated, misplaced or over-heated by electric current
- the presence of skull defects or skull plates
- severe cardio-pulmonary, renal, hepatic diseases

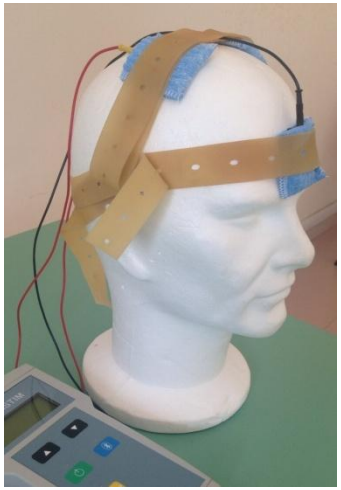
Procedures

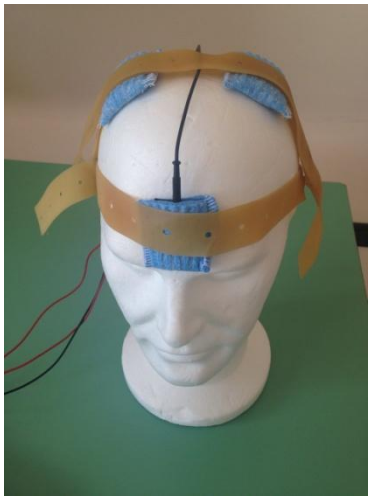
Each subject has received 10 sessions of tDCS (5 sessions / week) for two weeks.

tDCS protocol

- ✓ 2 electrodes (anode) in the primary motor cortex (M1) bilaterally
- ✓ electrode cathode (reference electrode) on the nasion
- ✓ the electrode sponge surface area of 16 cm2 (4x4), (soaked in saline solution)
- ✓ constant current stimulator (Brainstim, EMS, Italy)
- ✓ 2 mA intensity
- ✓ duration of stimulation 40 minutes.

Questionnaire reporting adverse events related to tDCS





According to International System of electrode placement for EEG

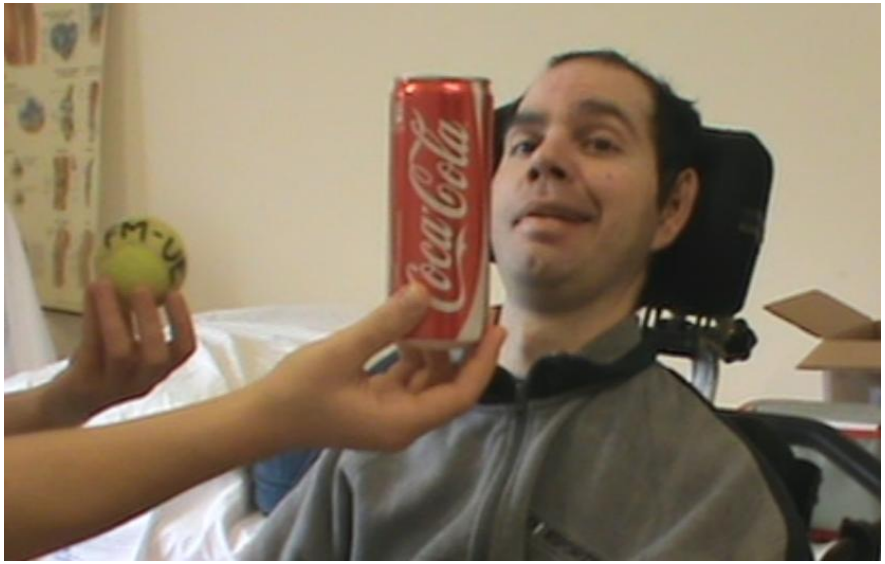
Outcome Measures

1- Clinical evaluations

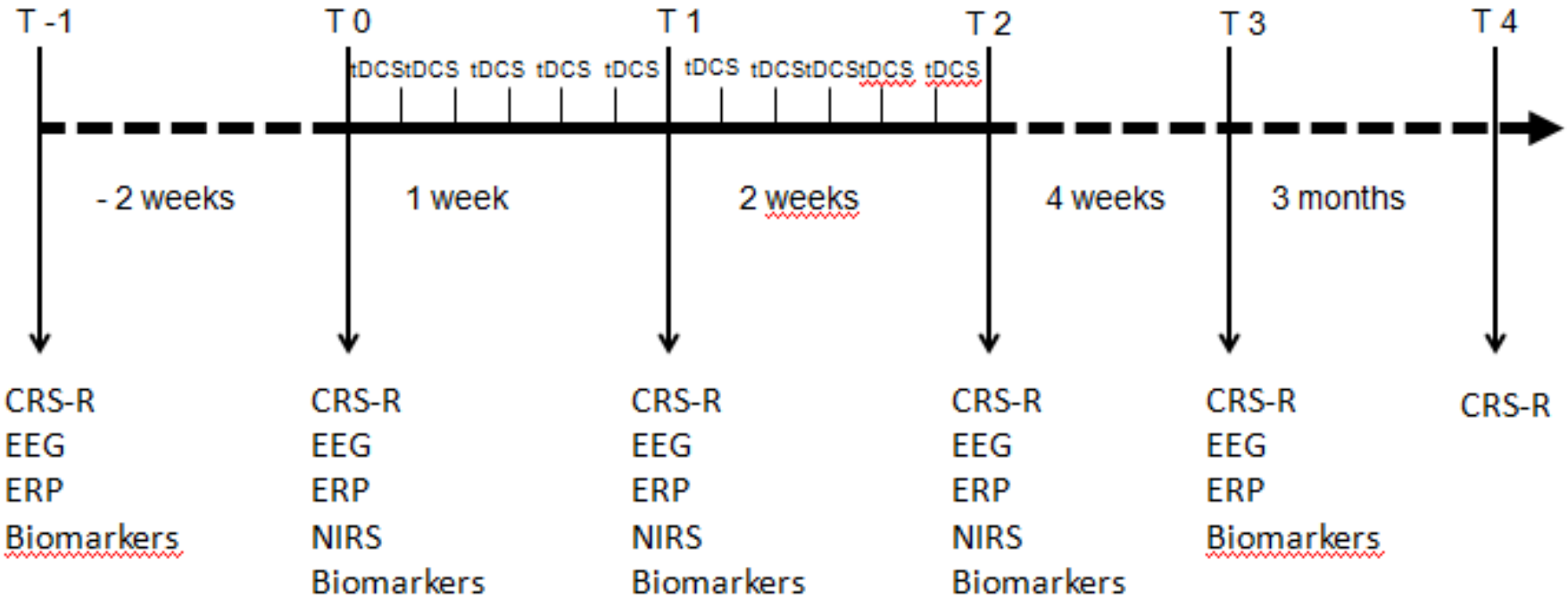
- JFK Coma Recovery revised scales (CRS-R)
- Disability Rating Scale (DRS)
- Coma Nociception Scale
- Caregivers diary

2- Strumental evaluations

- EEG
- Event-Related Potentials (**ERPs)**
- NIRS
- Circulating Biomarkers



Study timeline



Clinical evaluations

BRAIN STEM REFLEX GRID ©2004

Record Form

Patient:	Date:						
Pupillary Light	Reactive						
	Equal						
	Constricted						
	Dilated						
	Pinpoint						
	Accommodation						
Corneal Reflex	Absent						
	Present Unilateral						
	Present Bilateral						
Spontaneous Eye Movements	None						
	Skew Deviation						
	Conjugate Gaze Deviation						
	Roving						
	Dysconjugate						
Oculocephalic Reflex	None						
	Abnormal						
	Full						
	Normal						
Postural Responses (Indicate Limb)	Abnormal Extension						
	Abnormal Flexion						

JFK COMA RECOVERY SCALE - REVISED ©2004

Record Form

This form should only be used in association with the "CRS-R ADMINISTRATION AND SCORING GUIDELINES" which provide instructions for standardized administration of the scale.

Patient:	Diagnosis:	Etiology:
Date of Onset:	Date of Admission:	

Date															
Week	ADM	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
AUDITORY FUNCTION SCALE																
4 - Consistent Movement to Command *																
3 - Reproducible Movement to Command *																
2 - Localization to Sound																
1 - Auditory Startle																
0 - None																
VISUAL FUNCTION SCALE																
5 - Object Recognition *																
4 - Object Localization: Reaching *																
3 - Visual Pursuit *																
2 - Fixation *																
1 - Visual Startle																
0 - None																
MOTOR FUNCTION SCALE																
6 - Functional Object Use †																
5 - Automatic Motor Response *																
4 - Object Manipulation *																
3 - Localization to Noxious Stimulation *																
2 - Flexion Withdrawal																
1 - Abnormal Posturing																
0 - None/Flaccid																
OROMOTOR/VERBAL FUNCTION SCALE																
3 - Intelligible Verbalization *																
2 - Vocalization/Oral Movement																
1 - Oral Reflexive Movement																
0 - None																
COMMUNICATION SCALE																
2 - Functional: Accurate †																
1 - Non-Functional: Intentional *																
0 - None																
AROUSAL SCALE																
3 - Attention																
2 - Eye Opening w/o Stimulation																
1 - Eye Opening with Stimulation																
0 - Unarousable																
TOTAL SCORE																

Denotes emergence from MCS†

Denotes MCS*

Giacino, 2004.

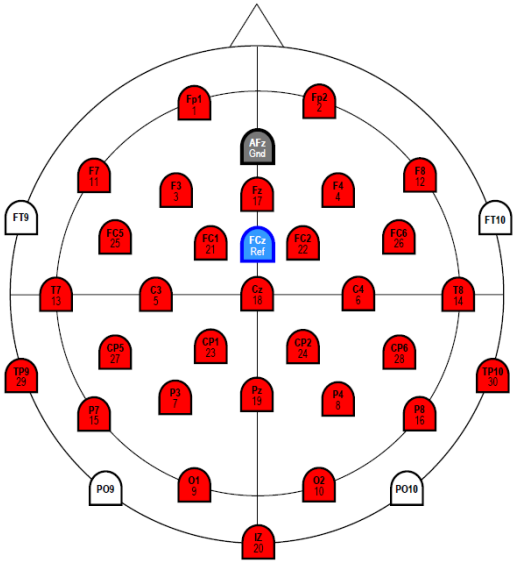
Instrumental evaluations

EEG: TMS-compatible EEG equipment (BrainAmp, Brain Products GmbH, Munich, Germany) was used to record EEG signals (BrainVision Recorder). The EEG activity was continuously recorded from a Fast'n Easy placed according to the 10–20 International System. Additional electrodes were used as reference and ground and for the electro-oculogram. The ground electrode was placed in AFz.

Examination: 15 EEG registration minutes done after stimulation and clinical evaluation (CRS-R).

Analysis: - frequence of alpha, theta and delta bands

- coherence (measure of connectivity between electrode sites).



fNIRS

NIRScout system 48 channels
16 sources – 16 detectors
Record of Oxygenated and Deoxygenated hemoglobin from M1 in both hemispheres.

Experimental condition:

NIRS registration 20’	tDCS 40’	NIRS registration 20’

Comparison of the traces collected for Oxygenated hemoglobin of the “Pre-tDCS period” and the “Post-tDCS period”.

Area Under Curve of Oxygenated hemoglobin for all channels in both hemispheres.

SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA
Azienda Ospedaliero - Universitaria di Ferrara

università di ferrara

Arcispedale S. Anna
Dipartimento Neuroscienze/Riabilitazione
Settore di Medicina Riabilitativa "San Giorgio"
Referente : Nino Bacaglia

Per il familiare

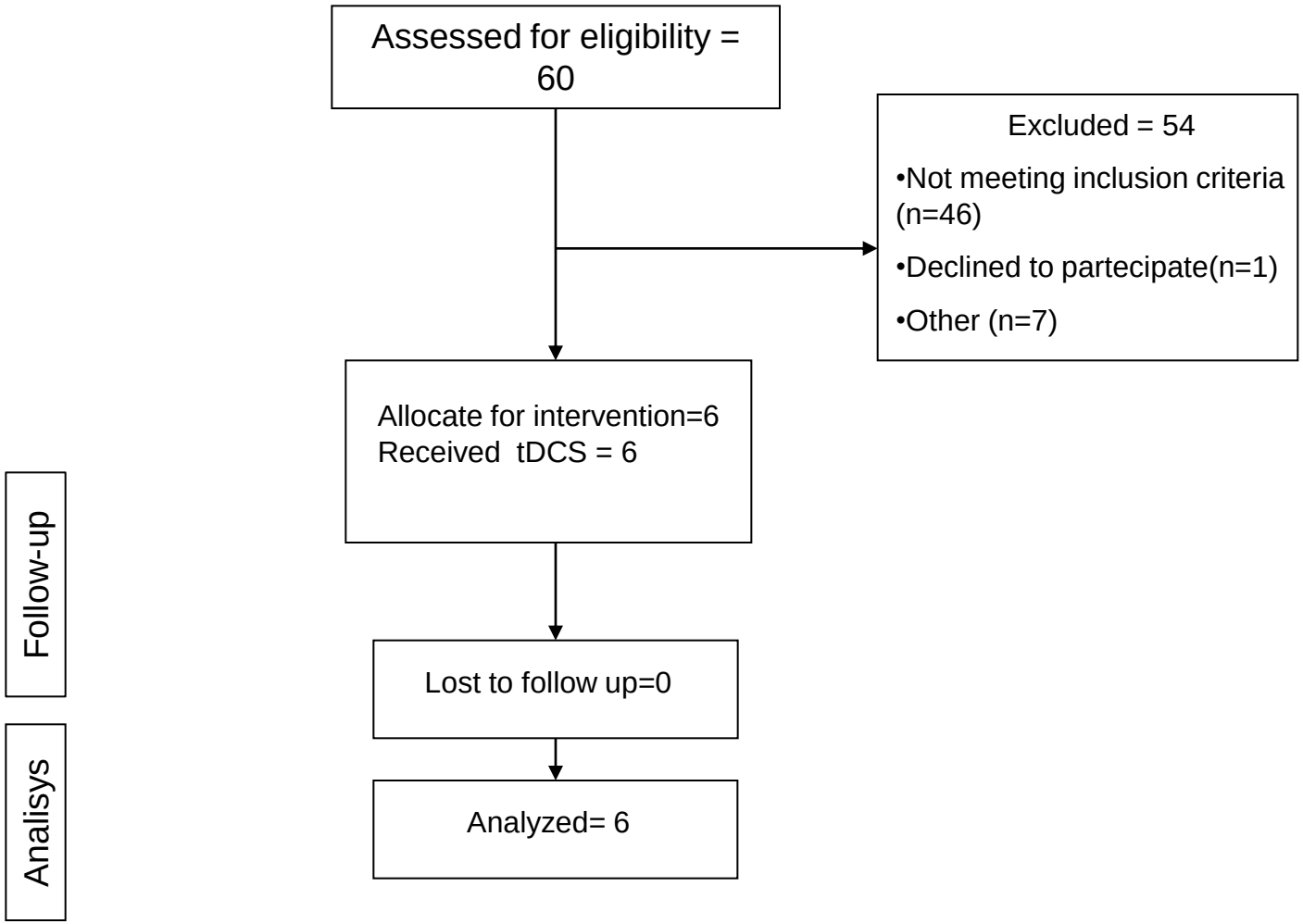
OSSERVAZIONE PAZIENTE

Cognome e Nome .....

Le chiediamo di riportare qui di seguito eventuali modificazioni nel comportamento del suo familiare che lei ha notato durante tutto il periodo di studio, dalle valutazioni due settimane prima di iniziare la somministrazione della tDCS, fino alla conclusione dello studio con le ultime valutazioni a due settimane dalla fine del trattamento.
Vorremmo che lei ci riferisse, qui di seguito, tutto quello che nota possa essersi modificato (alcuni esempi potrebbero riguardare: atteggiamento e postura della testa, movimento degli occhi come seguire con lo sguardo, espressione del viso, pronuncia di parole o suoni, movimenti delle braccia, mani, gambe, cambiamenti postura seduta...).

Se non dovesse notare nulla di diverso, non scriva nulla.

Giorno 1 data
Giorno 2 data
Giorno 3 data
Giorno 4 data


Assessed for eligibility = 60
Excluded = 54
•Not meeting inclusion criteria (n=46)
•Declined to partecipate(n=1)
•Other (n=7)
Allocate for intervention=6
Received tDCS = 6
Follow-up
Lost to follow up=0
Analisys
Analyzed= 6

Exclusions causes

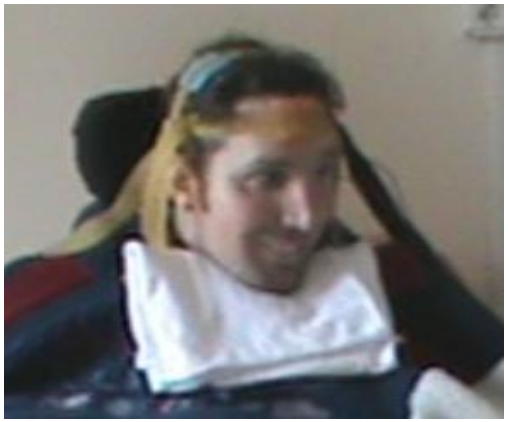
- Unresponsive Wakefulness Syndrome (UWS)
- Difficult diagnosis
- Skull plates and skull defects
- Non traumatic acquired brain injury
- Organization and logistic issues (extra-ER subjects)

Participant characteristics

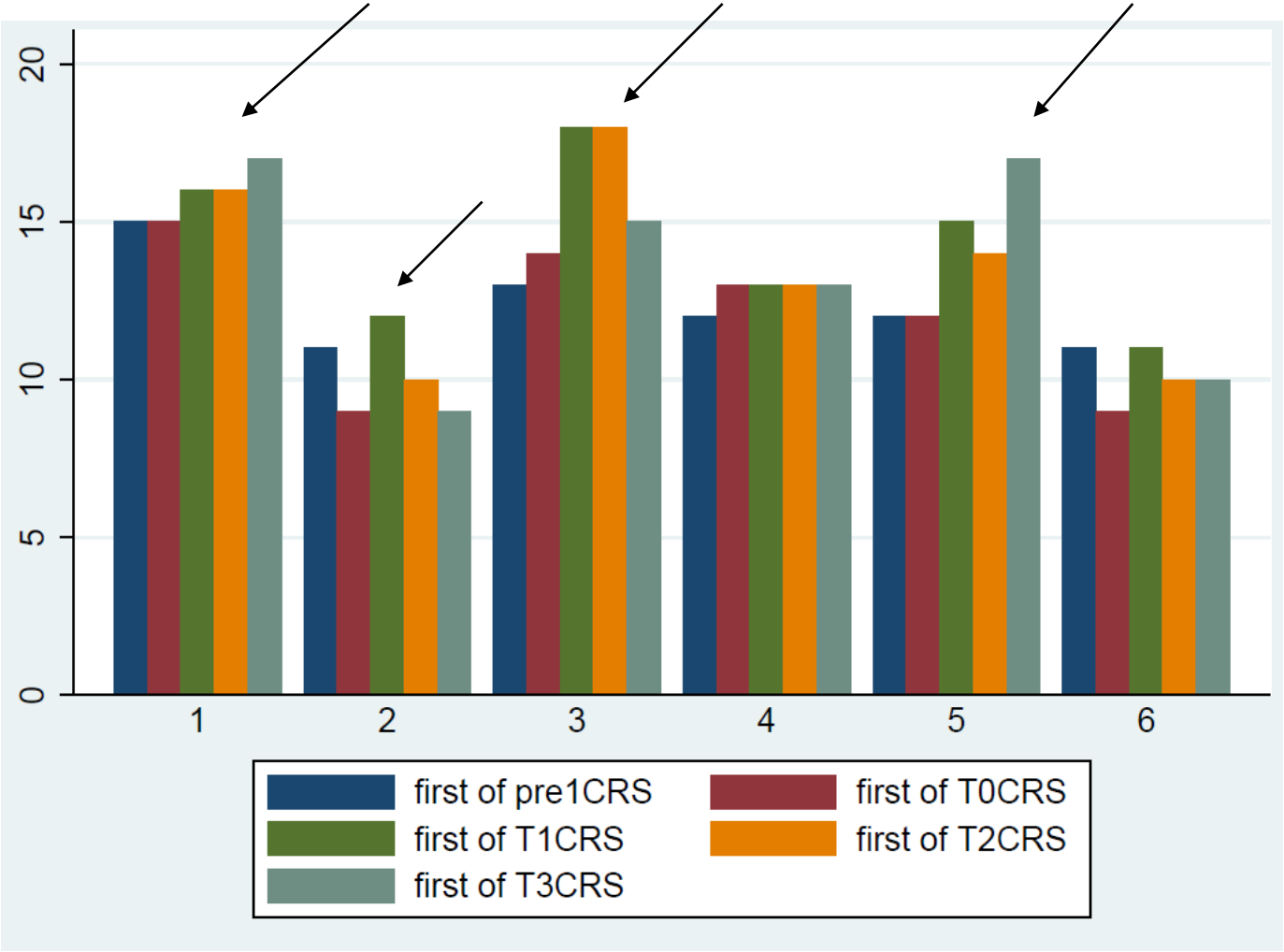
Patient	Sex	Age	Interval since trauma (y=years) (m=month)	CNS Drugs	Epilepsy	Devices intra and extra cranial	Setting
1	M	35	11 y				DH
2	M	36	8 y, 9 m			DVP	DH
3	M	47	4 y, 7 m		Levetiracetam	IB	UGC
4	M	34	19 y				DH
5	F	24	2 y	Amantadina	Levetiracetam		UGC
6	F	27	7 y, 6 m		Levetiracetam	IB	UGC

Safety

All patients tolerated well tDCS without any significant adverse effects related to the stimulation



PRELIMINARY DATA CRS-R



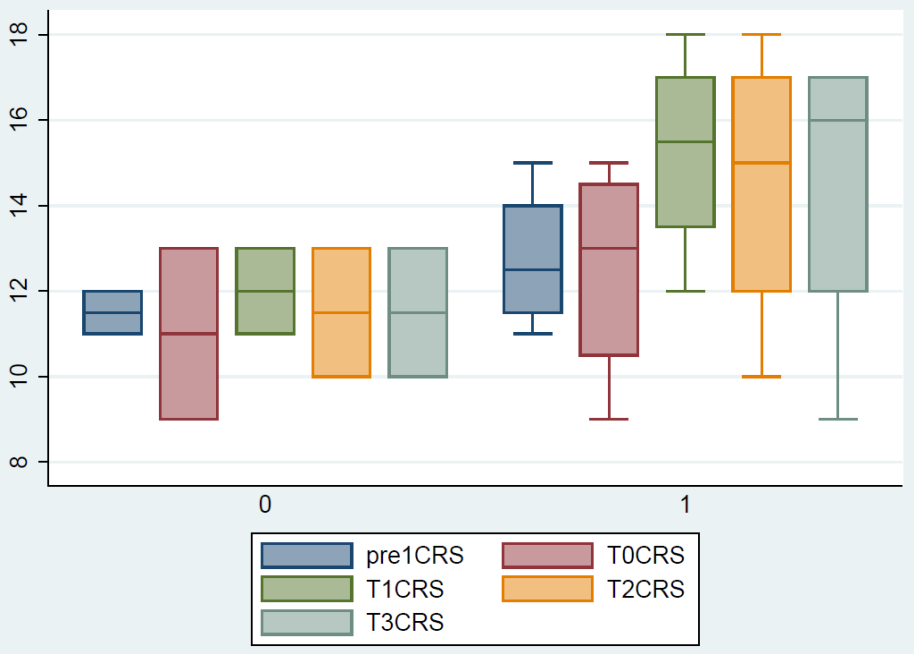
PRELIMINARY DATA CRS-R

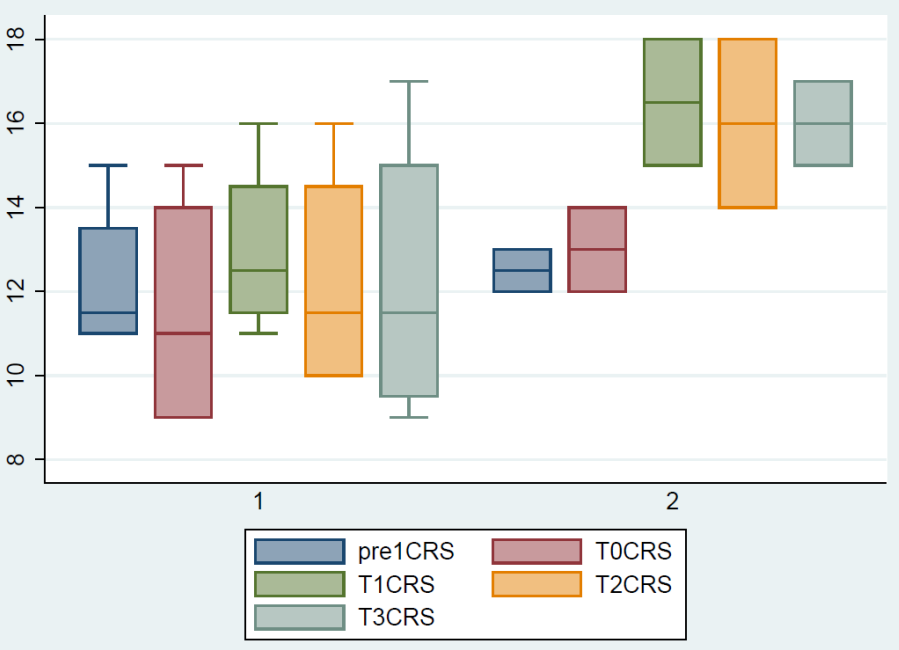
Patients non responder

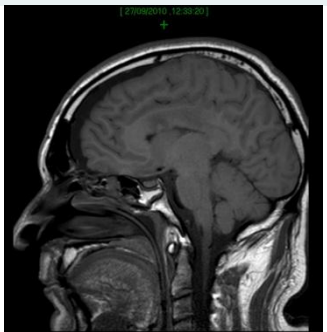
Patients responder

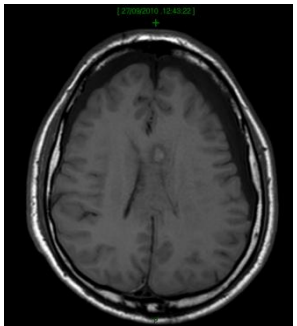
> 5 years

< 5 years



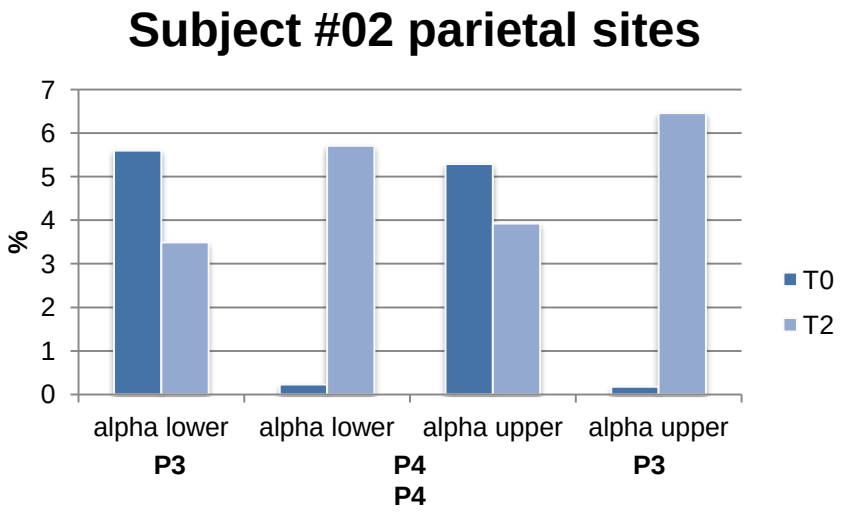


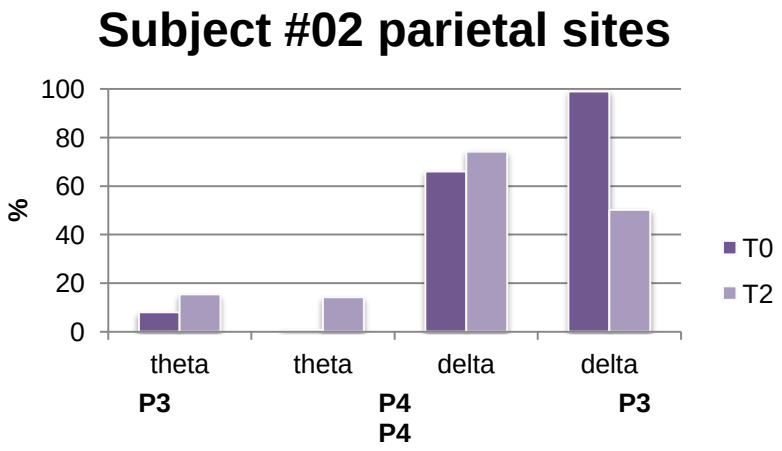




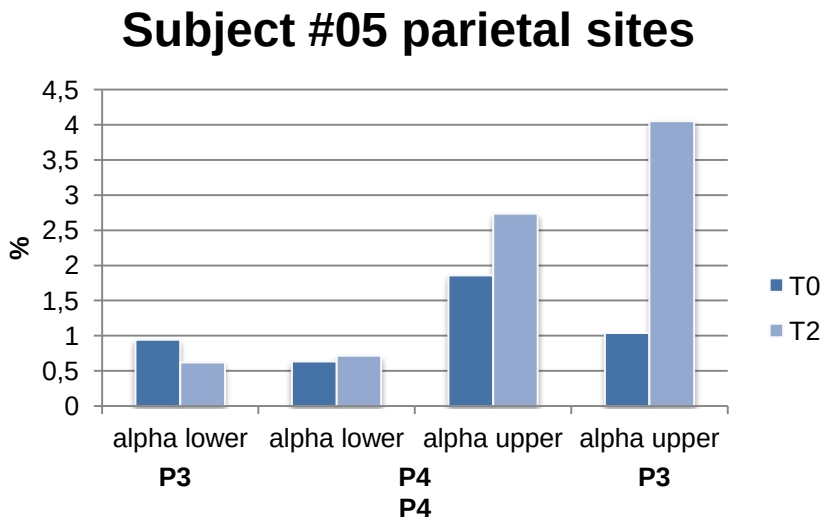
EEG – preliminary results

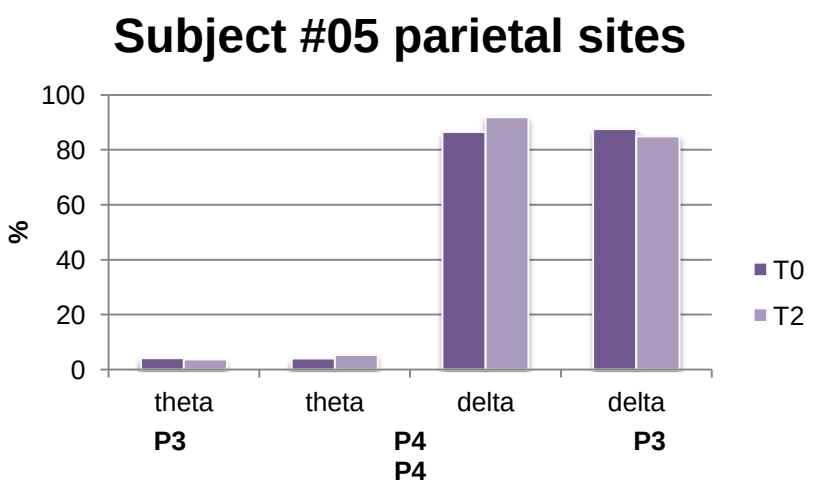
Patient 2





Patient 5



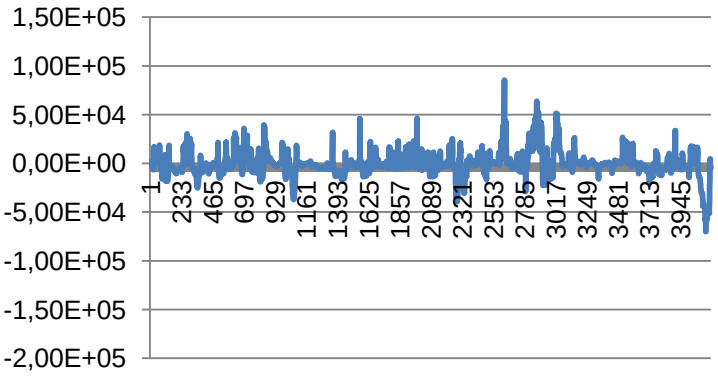


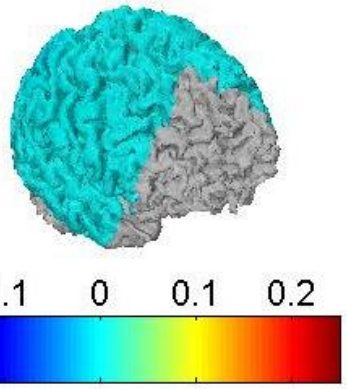
fNIRS – preliminary results

Data collected safely on 4 out of 6 patients

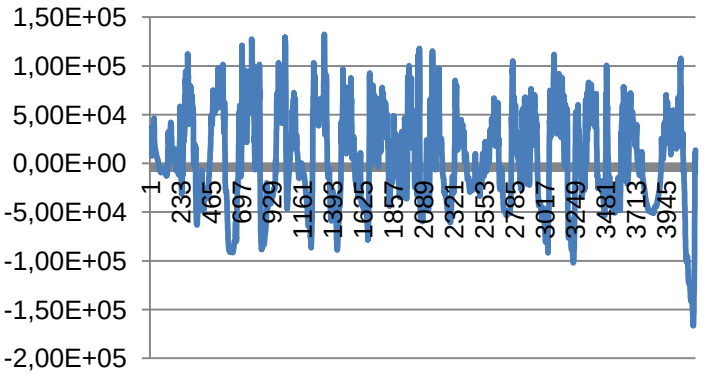
Preliminary data show that “Post-tDCS” Oxygenation appears greater than baseline oxygenation

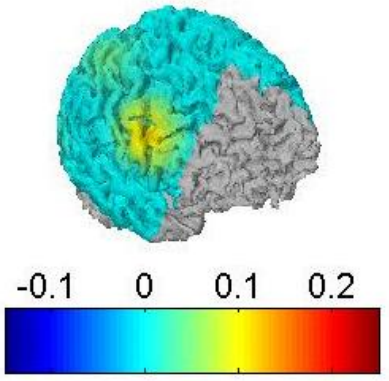
Pre-tDCS





Post-tDCS





Further analysis will be necessary for better study this interesting trend

Care givers reports, patient 3

"…. *Carmine sembra più attento rispetto a prima, ruota meglio il capo per guardare quello che sta intorno. Ascolta quando qualcuno parla con lui*… " (Moglie)

Care givers reports, patient 5

"…. *Eleonora, guarda di più quando è insieme ad altre persone, tiene su la testa da sola, la capisco di più quando parla, mi aiuta quando la sposto dalla sedia al letto*…" (Mamma)

Care givers reports, patient 2

"…*Oggi pomeriggio Cesare ha avuto un momento bellissimo, di estrema attenzione ascoltando un programma televisivo musicale. Ad una mia domanda su una canzone che stavamo ascoltando mi ha risposto sì per 3 volte con assoluta certezza, tenendo gli occhi chiusi stretti e sorridendo, un sorriso vero. È stato davvero emozionante vederlo così lucido, episodi rari ma che accadono sempre più spesso*." (Mamma)

LIMITS

- Subject’s rectuitment (criteria, organization and logistic issues)
- Awareness fluctiations (morning/evening)
- Fragility and clinical complexity
- Outcome measure (sensibility, physically demanting)

SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA
Azienda Ospedaliero - Universitaria di Ferrara

università di ferrara
DA SEICENTO ANNI GUARDIAMO AVANTI.

WP3: Scientific coordinator Fabio Manfredini, MD

Effectiveness of robot-assisted gait training versus conventional therapy on mobility in severely disabled multiple sclerosis patients.

Single center,single blinded, 2 arm-trial, conducted at PM&R Dept, Ferrara

Preliminary results

Roadmap

1. Introduction: the study

2. Response to rehabilitation (all patients - all treatments)

3. Efficacy of the experimental treatment
    a) Primary outcome measures
    b) Secondary outcome measures
    c) Clinical biomarkers

4. Individual response to the treatment

5. Conclusions

WP3:
Effectiveness of robot-assisted gait training versus conventional therapy on mobility in severely disabled multiple sclerosis patients.

1. Introduction: the study

prospective, randomized, single-blinded, controlled study

Primary objective:

to test **the efficacy** on **mobility** of the rehabilitation treatment

Robot-Assisted Gait Training (RAGT)

Conventional Therapy (CT)

Secondary objectives:

a) to test **the efficacy** on **walking endurance, balance, fatigue, QoL**

b) to associate circulating /metabolic **markers to clinical outcomes**;

c) to identify **biomarkers with a predictive value** to detect groups of patients who most likely will benefit from a particular rehabilitation program;

d) to improve the knowledge about the **recovery mechanisms**;

Study protocol

Assessment of elegibility

Randomization 1:1
(EDSS stratified)

Robot-Assisted Gait Training (RAGT)

Conventional Therapy (CT)

Inclusion criteria:
- males and females, 18 - 65 years
- EDSS 6-7
- lack of EDSS worsening in the last 3 months

Methods

Robot-Assisted Gait Training (RAGT)

12 Training sessions
3 sessions/week
Duration: 30’ real walking time
(30’ set up)

Walk on treadmill
with robotic-driven gait orthosis
Partial/Total Weight support
Speed: 0 to 3km/h

Conventional Therapy (CT)

12 Training sessions
3 sessions/week
Duration: 10’ stretching exercise
10’ muscle strenghtening
≈30’ walking time

Overground walking
with aid of physiotherapist

Outcome measures

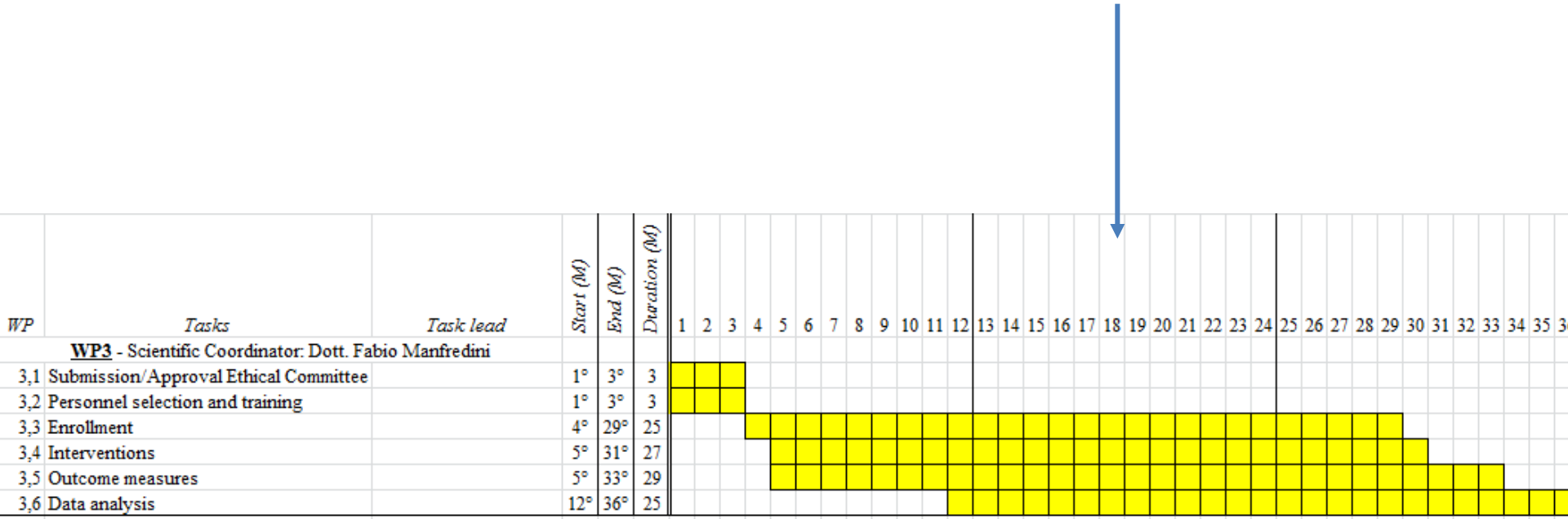
Clinical:

Primary outcome: **T25FW**

Secondary outcomes: 6MWT, UGT,
scales and questionnaries (BBS, FSS, MAS, SF-36)

Clinical biomarkers:

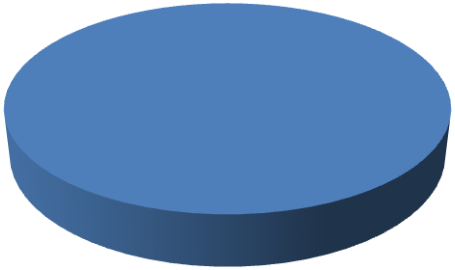
Metabolic measurements (NIRS: muscle, brain),
Circulating biomarkers (TReg, cytokines, EPC, CPC, MSC)



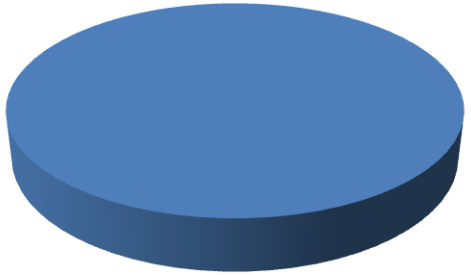
		Start (M)	End (M)	Duration (M)	Phase
3,1	Submission/Approval Ethical Committee	1°	3°	3	Completed
3,2	Personnel selection and training	1°	3°	3	Completed
3,3	Enrollment	4°	29°	25	In progress
3,4	Interventions	5°	31°	27	In progress (36/98)
3,5	Outcome measures	5°	33°	29	In progress
3,6	Data analysis	12°	36°	25	In progress

Milestones

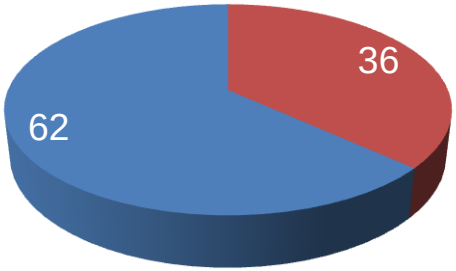
Trial registration



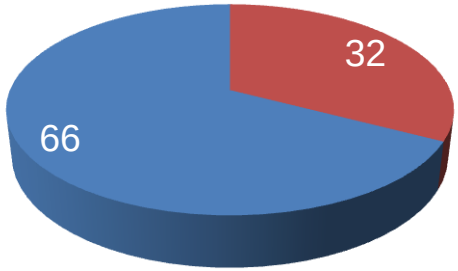
Personnel selection and training



Enrollment



Intervention / outcomes

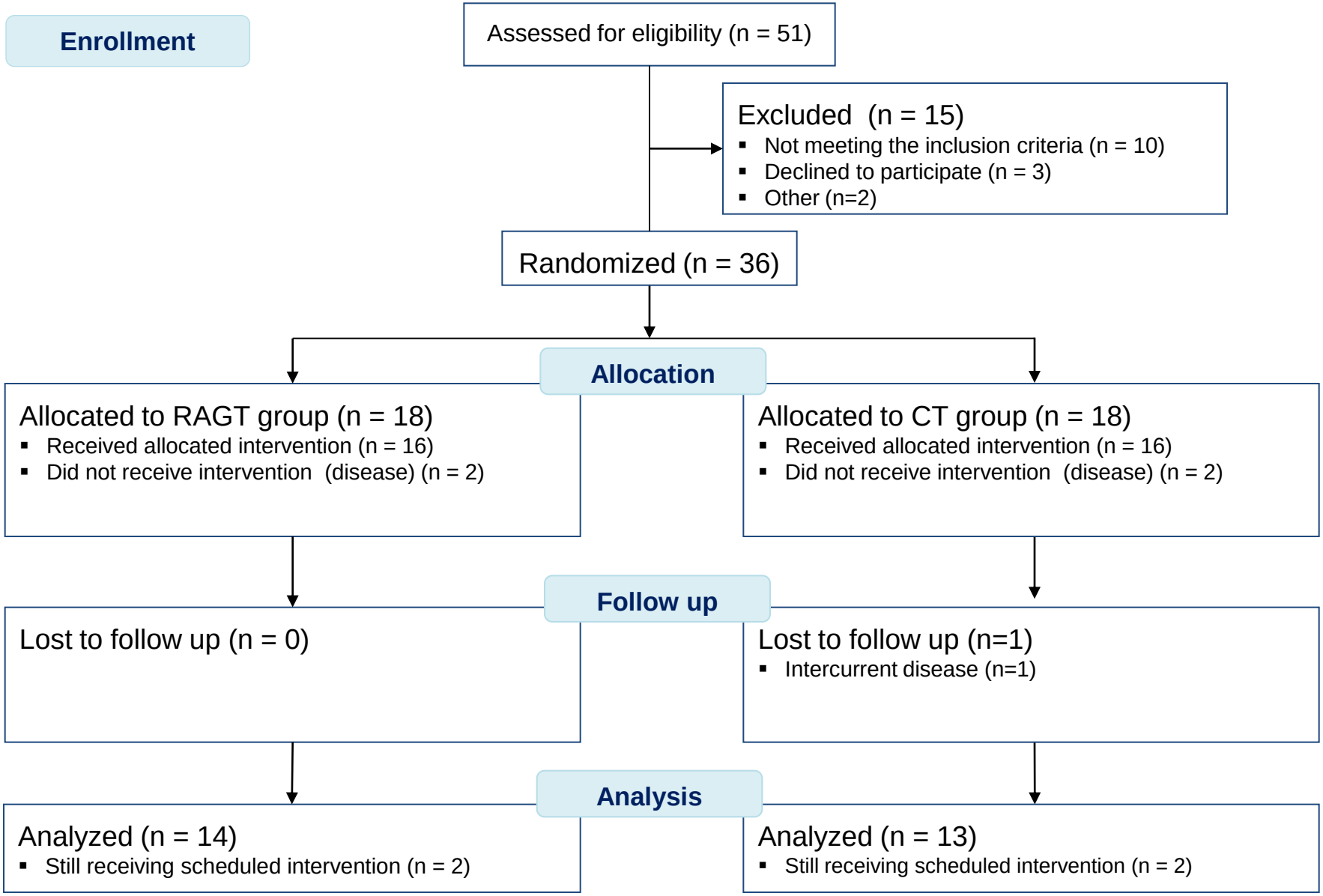


WP3:
Effectiveness of robot-assisted gait training versus conventional therapy on mobility in severely disabled multiple sclerosis patients.

PRELIMINARY RESULTS

2. Response to rehabilitation
(all patients - all treatments)

Intermediate results: Consort flow diagram



Waiting for enrollment: 26 people

Results: patients

N= 27

Beer, 2008 n=29
Vaney 2011 n= 49
Schwartz, 2011 n= 28
Lo , 2008 n= 13
Straudi, 2013 n =16
Gandolfi 2014 n=22

	RAGT (n= 14)	**CT (n=13)**
Age	55 ± 12	57 ± 9
Males; n(%)	4 (29)	6 (46)
Classification		
PP; n(%)	8 (57)	8 (62)
SP; n(%)	6 (43)	5 (38)
Disease duration	10.8 ± 7.7	18.1 ± 10.8
EDSS	6.3 ± 0.3	6.3 ± 0.3

Baseline values of the patients of the two study groups which completed the intervention phase

1. Primary outcome: Walking speed (Timed 25-Foot Walk)

Testing conditions:

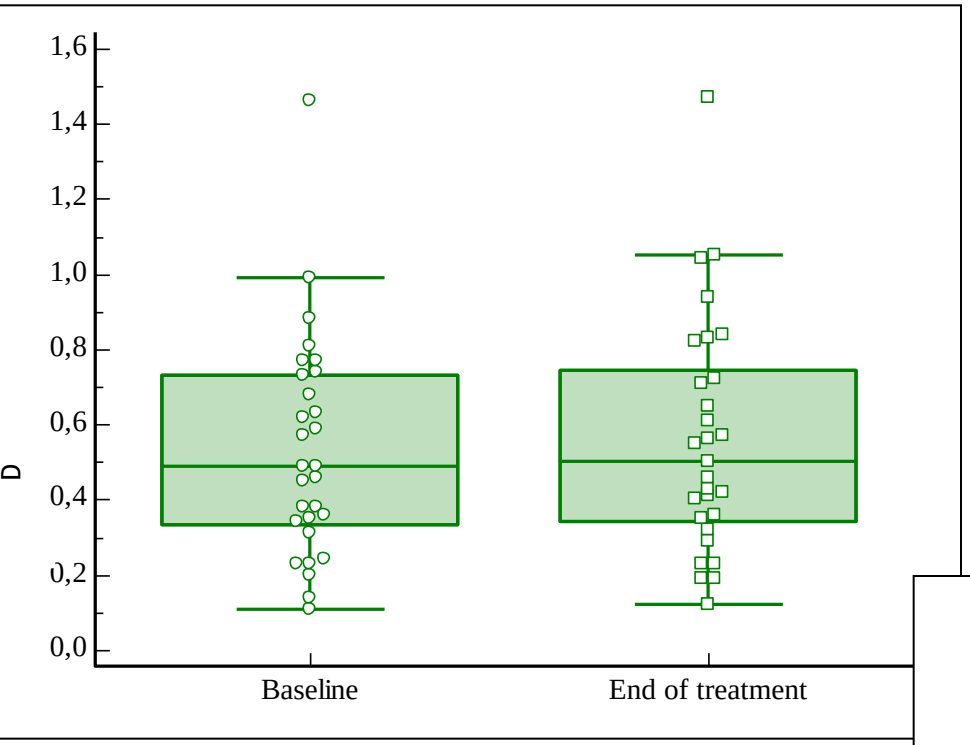
at the same time of day,

at the same conditions.

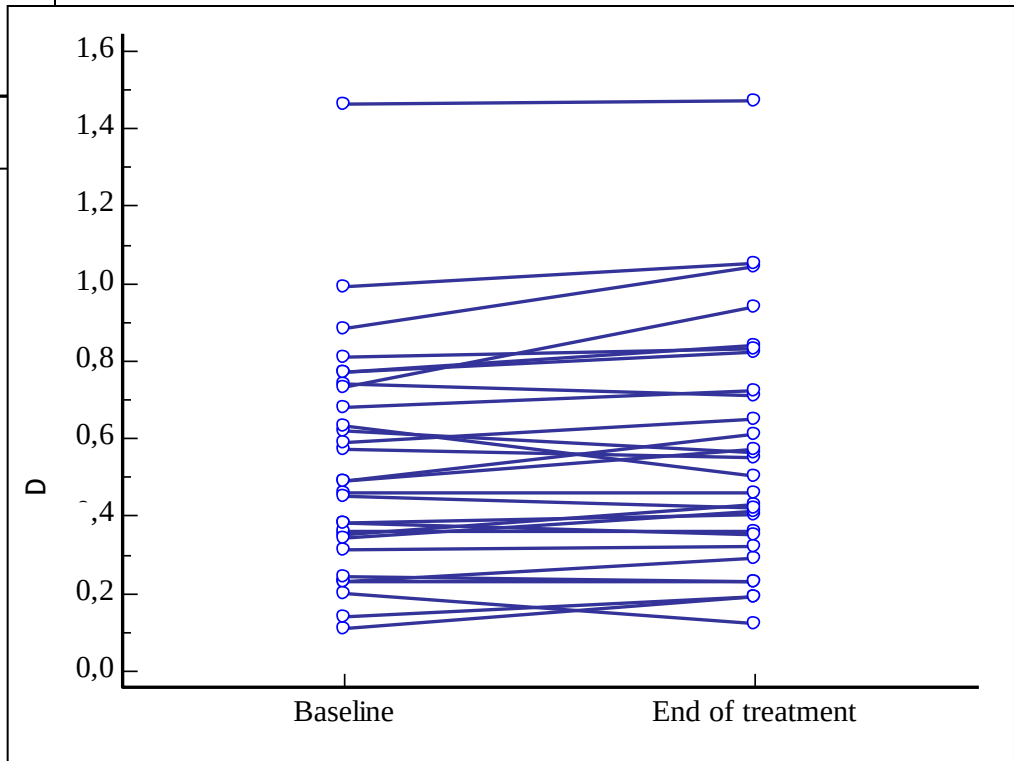
Patients picked up at entrance and brought to testing site on a wheelchair

Operators blinded to the treatment

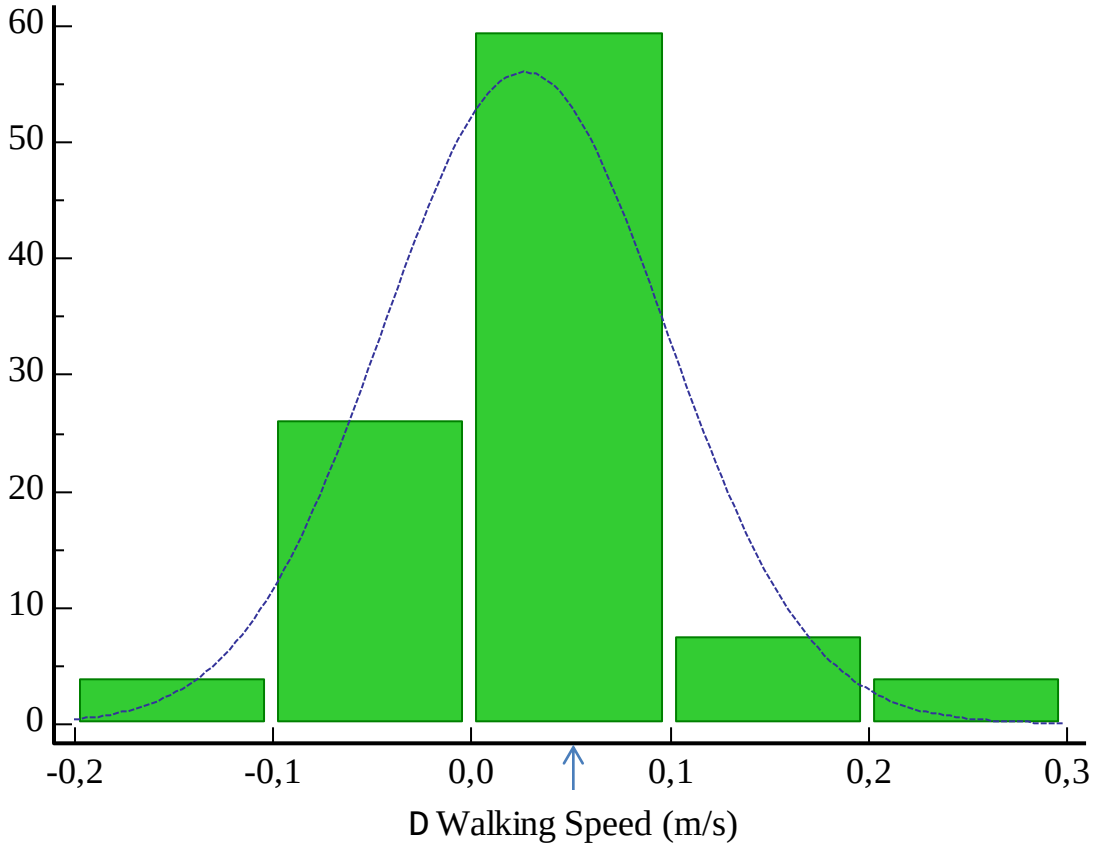
Primary outcome: all patients (n=27)



m/s 0.53 0.57 (+7.5%)



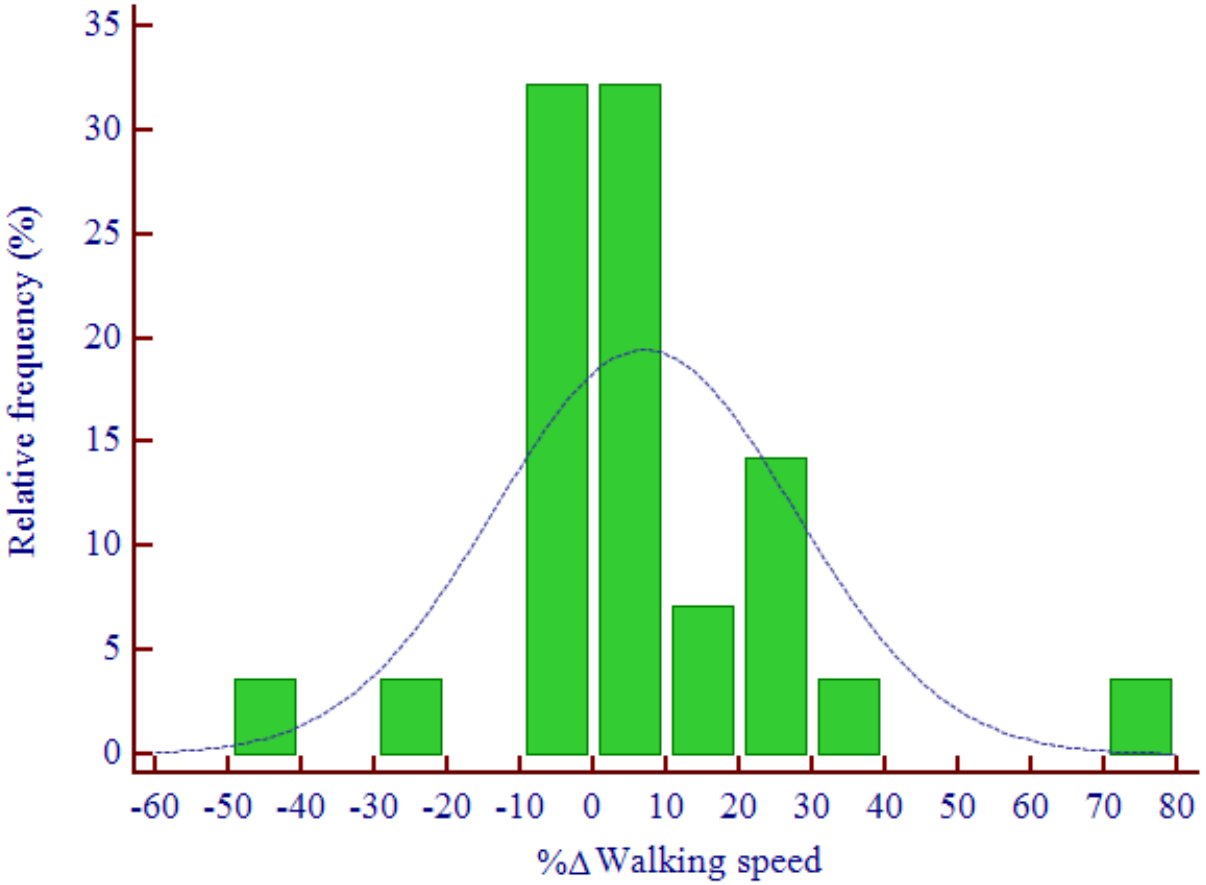
Primary outcome: all patients (n=27)



0.028 ± 0.072

Variations of walking speed at the end of treatment from baseline for all patients who completed the intervention phase.

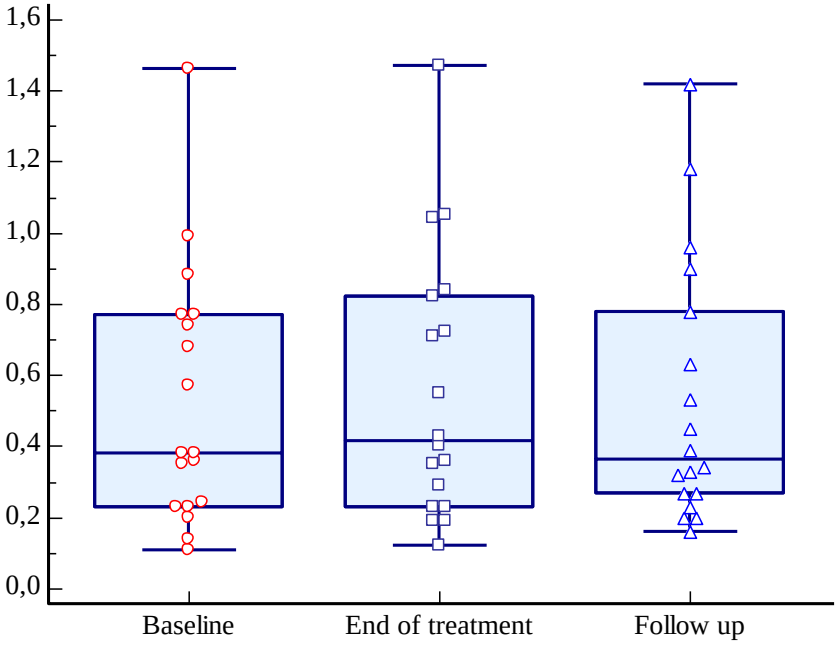
Primary outcome: all patients



n=27

%Variations of walking speed at the end of treatment from baseline for all patients who completed the intervention phase.

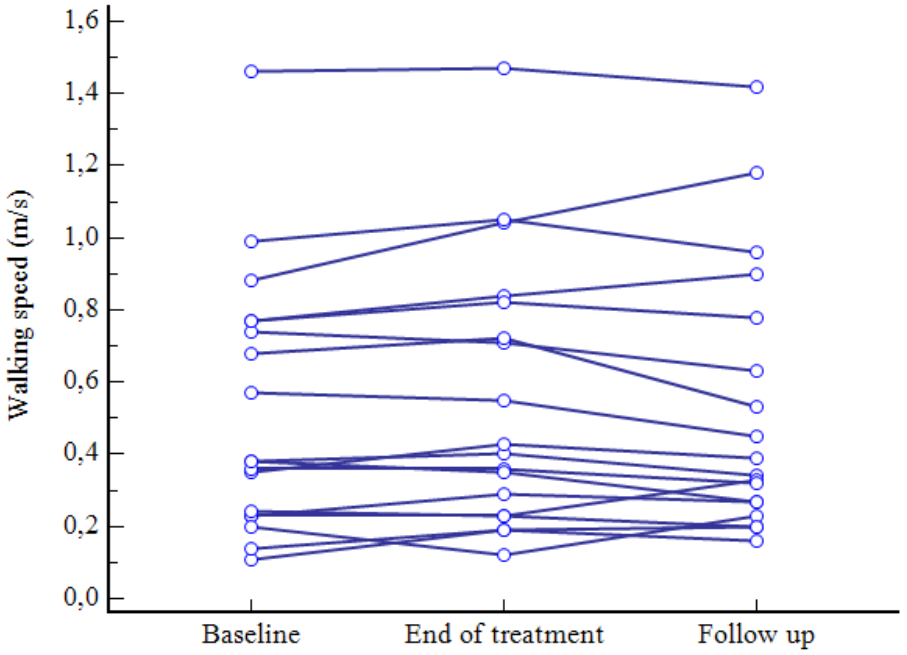
Persistence of treatment effects
Primary outcome (T25FW)



0.50

0.56

0.58



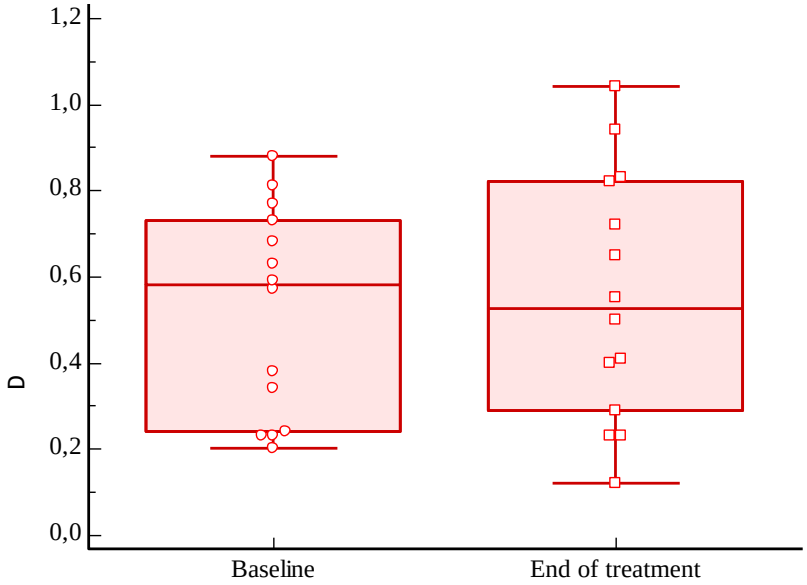
WP3:
Effectiveness of robot-assisted gait training versus conventional therapy on mobility in severely disabled multiple sclerosis patients.

PRELIMINARY RESULTS

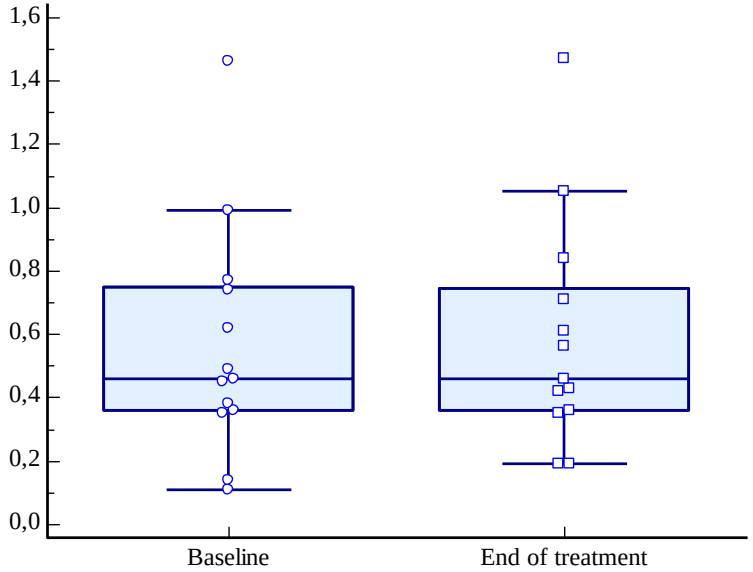
3. Efficacy of the experimental treatment
    a) Primary outcome measures
    b) Secondary outcome measures
    c) Clinical biomarkers

Primary outcome: walking speed (T25FW)

RAGT

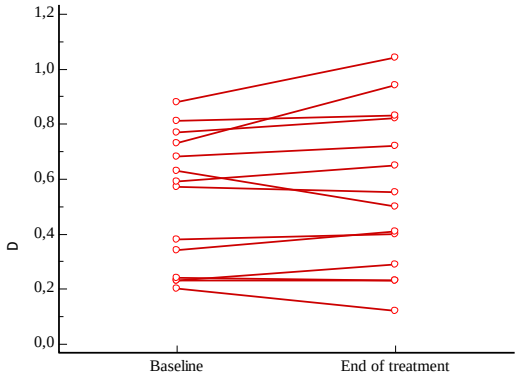


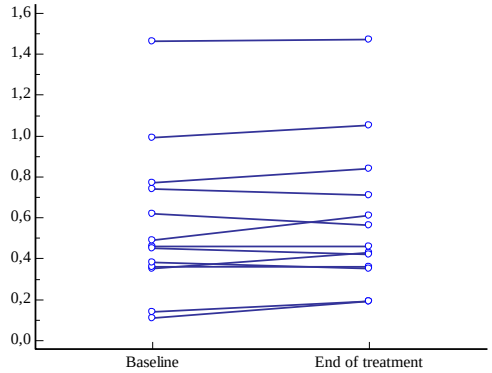
CT



(m/s) 0.52 0.55 (+5.8%) (+5.4%) 0.56 0.59

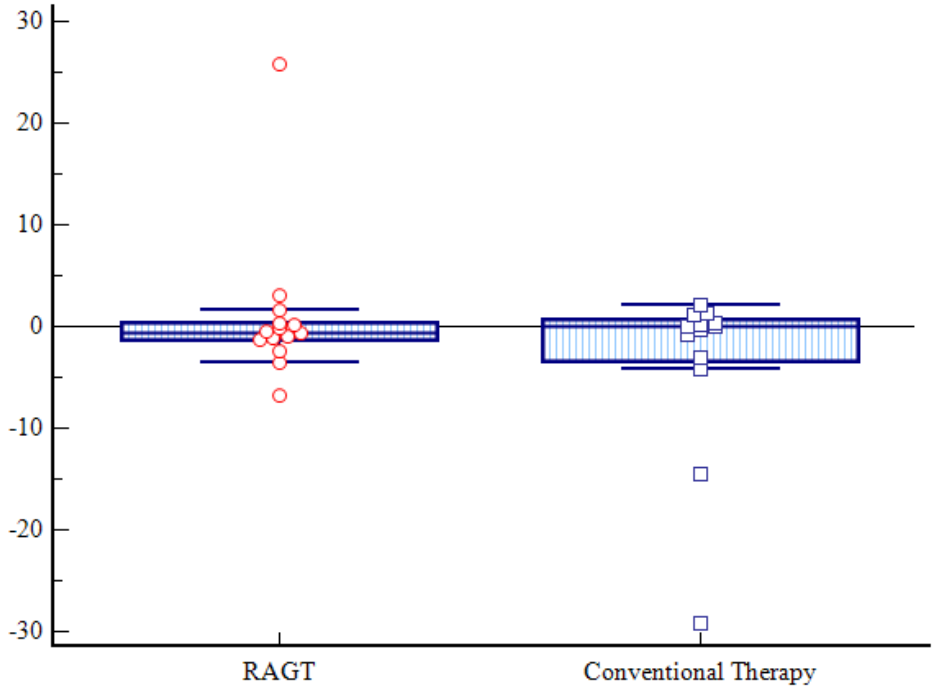
No intra & inter-group differences *(analysis as treated)*

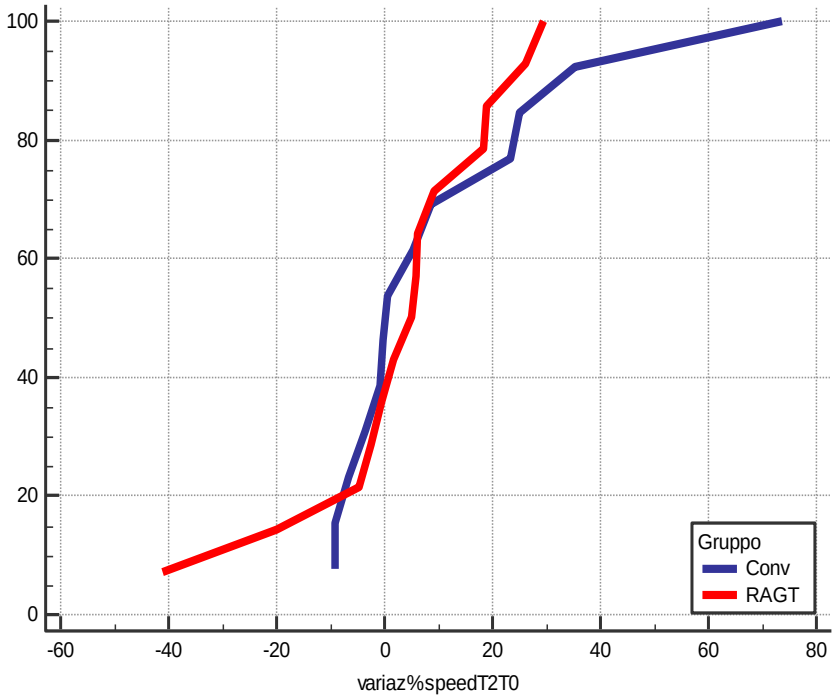




Primary outcome: walking speed (T25FW)

RAGT vs CT

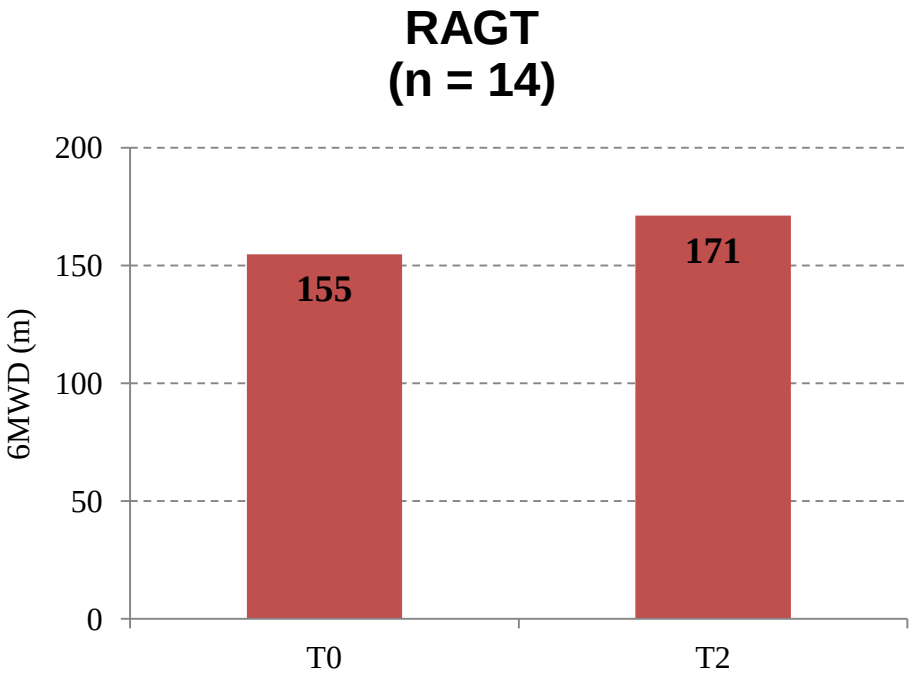




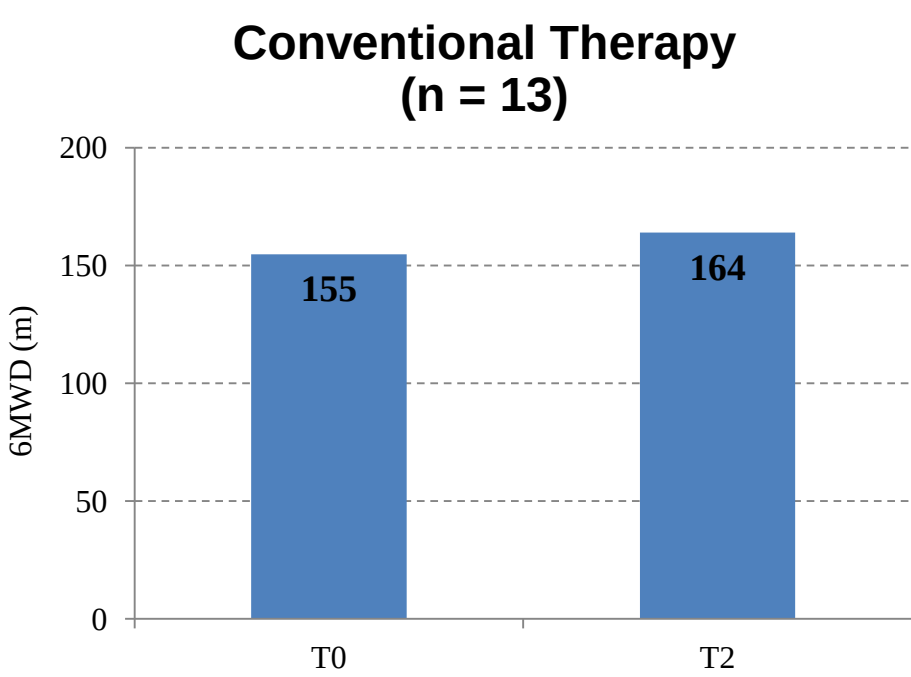
ES : 0.12 (trivial)

ES : 0.06 (trivial)

2. Secondary outcome measures: Walking endurance (6MWD)

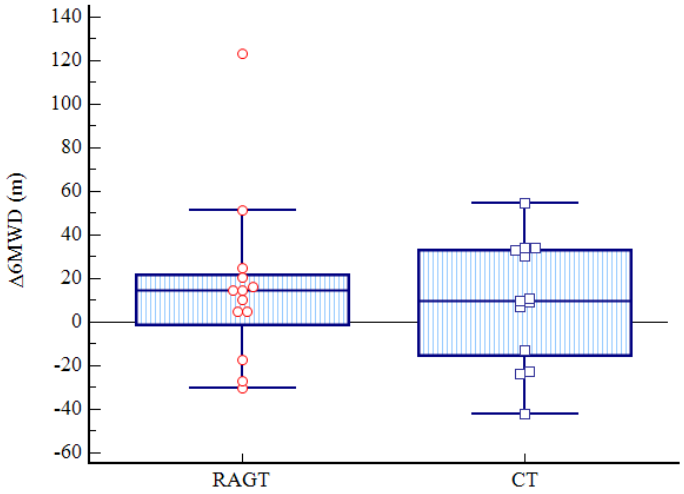


+10,3%



+5,8%

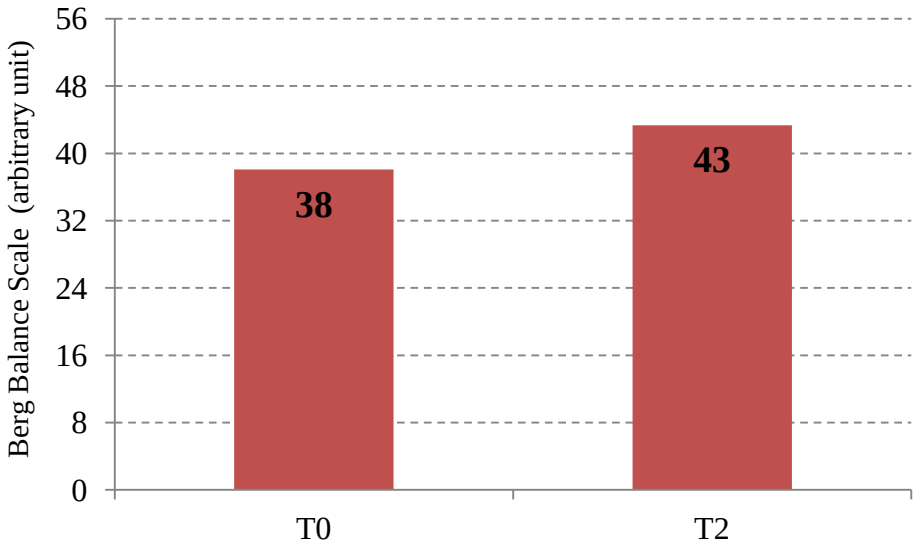
No intra & inter-group differences



p=0.98

Balance (Berg Balance Scale)

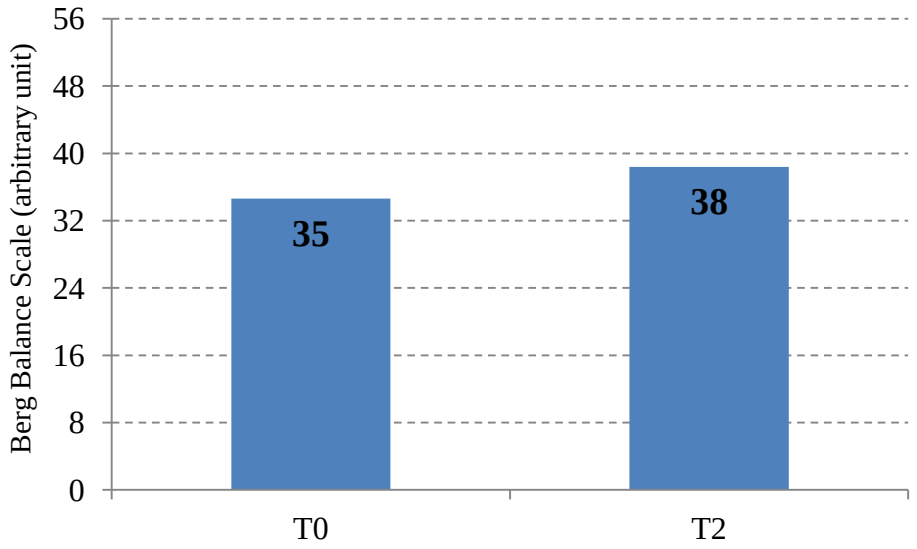
RAGT
(n = 14)



Baseline End of treatment

+13,1%

Conventional Therapy
(n = 13)

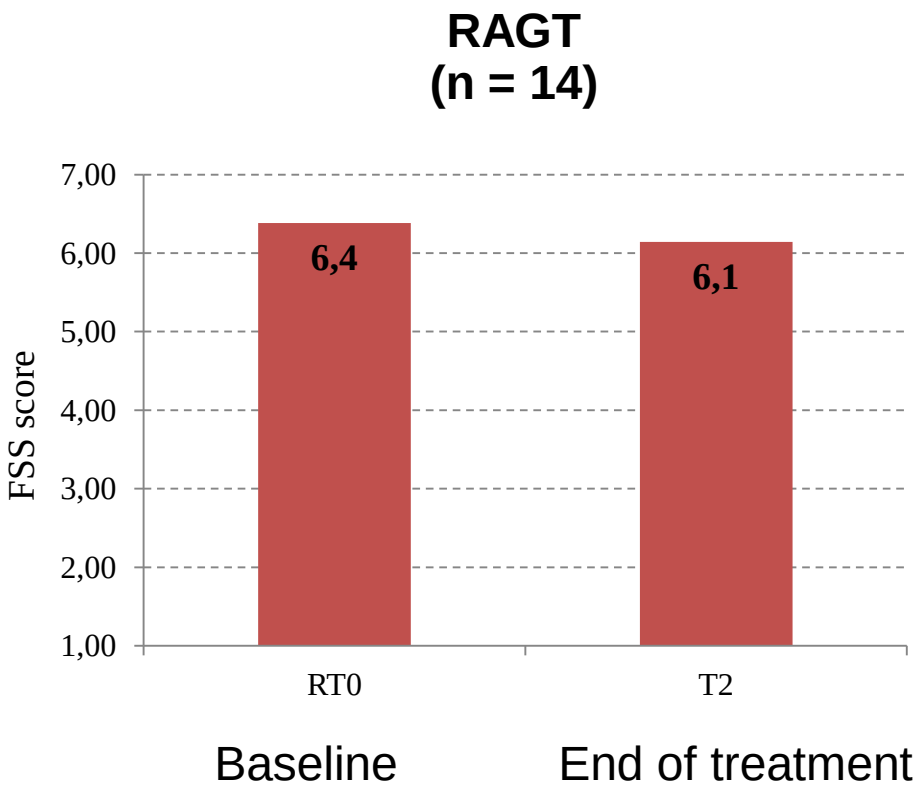


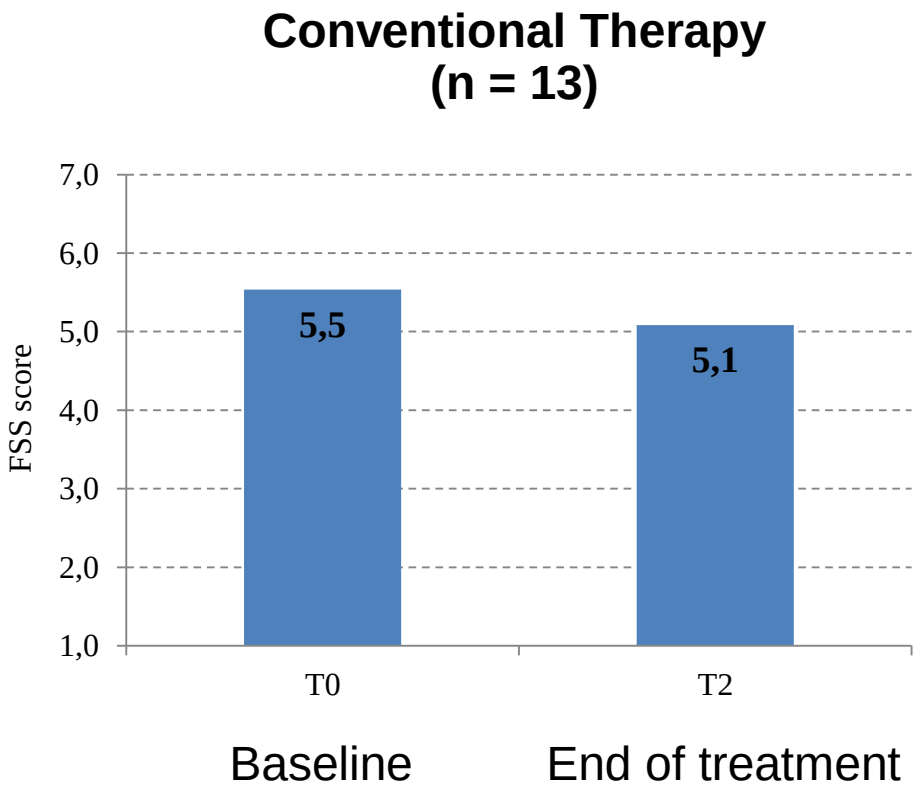
Baseline End of treatment

+8,6%

No intra & inter-group differences

Fatigue Severity Scale





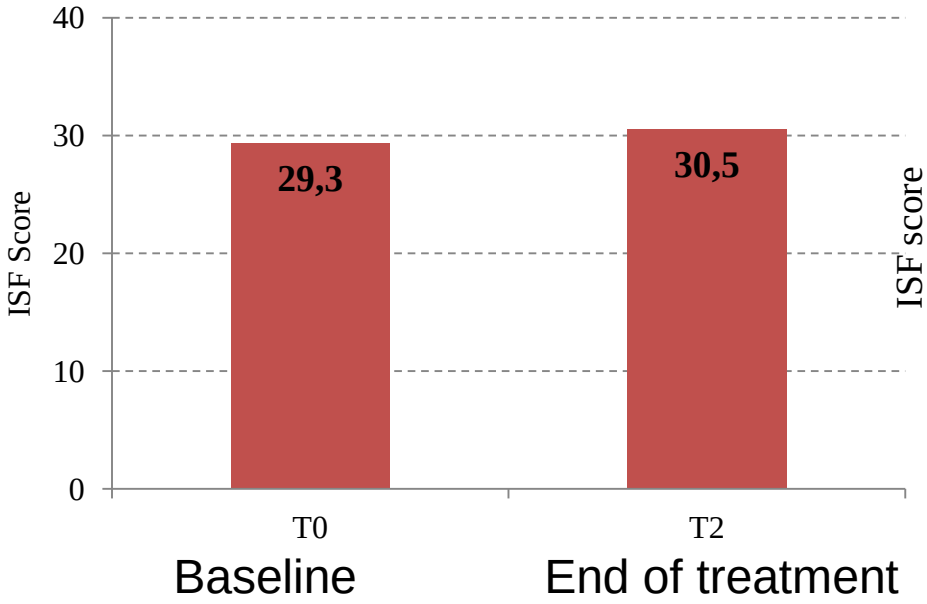
-4,7%

-7,3%

No intra & inter-group differences

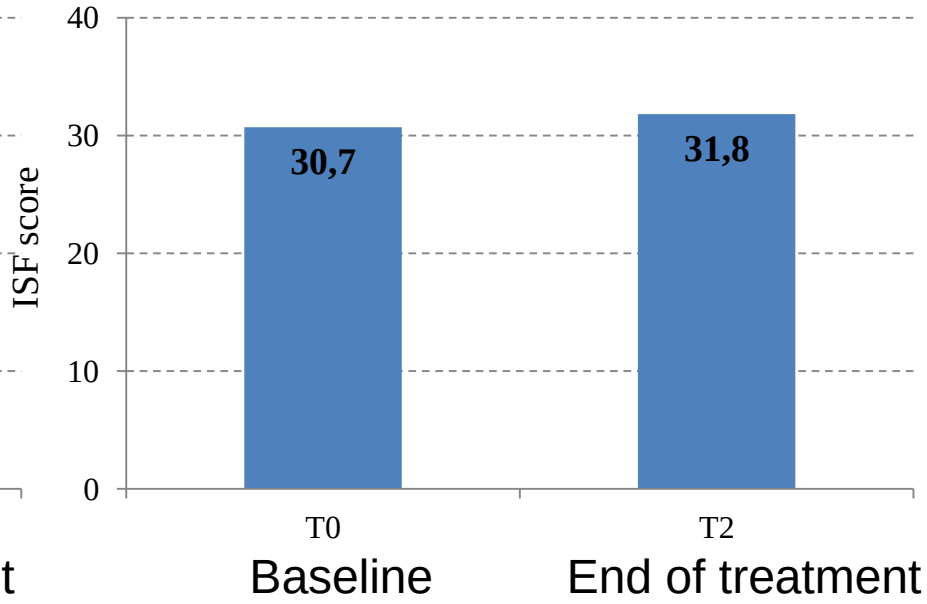
Quality of life
(Physical Component score of MOS SF-36 Questionnaire)

RAGT
(n = 14)



+3.8%

Conventional Therapy
(n = 13)



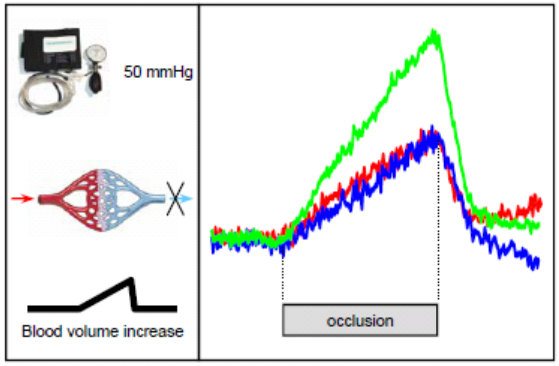
+3,6%

No intra & inter-group differences

Clinical biomarkers: Metabolic measurement by NIRS

Muscle oxygen consumption (mVO2) at gastrocnemius

Potential biomarker of muscle deconditioning



During venous occlusion, cuff pressure (50 mmHg) blocks venous ...erial inflow is not hindered. This results in an ...volume, seen in the NIRS signal as an increase

Resting Muscle Oxygen Consumption by Near-Infrared Spectroscopy in Peripheral Arterial Disease: A Parameter to be Considered in a Clinical Setting?

Anna Maria Malagoni, MD,[1] Michele Felisatti, DSc,[1] Simona Mandini, D...
Francesco Mascoli, MD,[1,2] Roberto Manfredini, MD,[1,3] Nino Basaglia, M...
Paolo Zamboni, MD[1] and Fabio Manfredini, MD[1,4,5]

Malagoni et al. BMC Neurology 2013, 13:52
http://www.biomedcentral.com/1471-2377/13/52

BMC Neurology

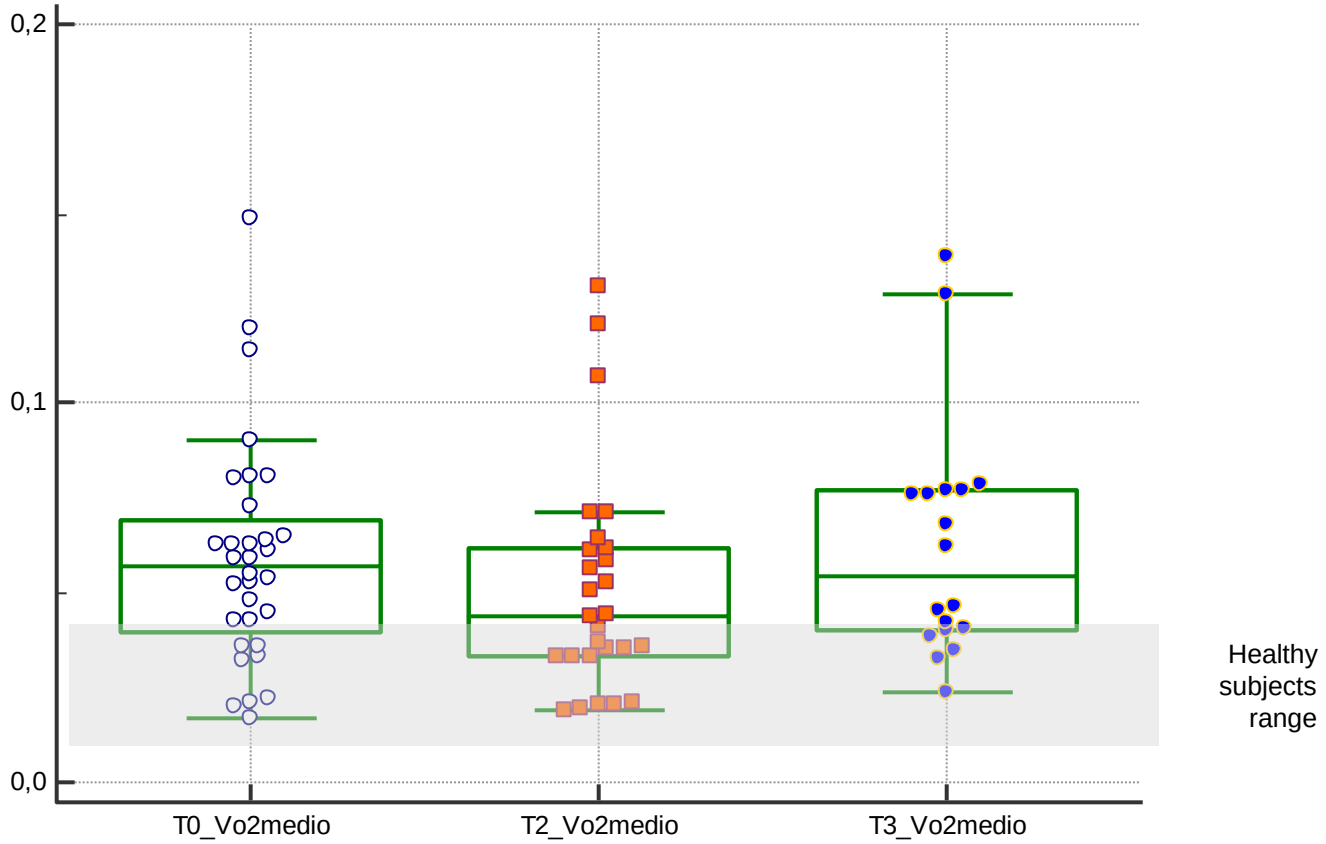
RESEARCH ARTICLE Open Access

Muscle oxygen consumption by NIRS and mobility in multiple sclerosis patients

Anna Maria Malagoni[1,2*], Michele Felisatti[2], Nicola Lamberti[2], Nino Basaglia[3], Roberto Manfredini[2,4], Fabrizio Salvi[5], Paolo Zamboni[1,2] and Fabio Manfredini[2,3]

Measurements of muscle oxygen consumption at rest by NIRS performed on all 36 patients randomized without adverse effects

Muscle oxygen consumption (mVO2) at gastrocnemius

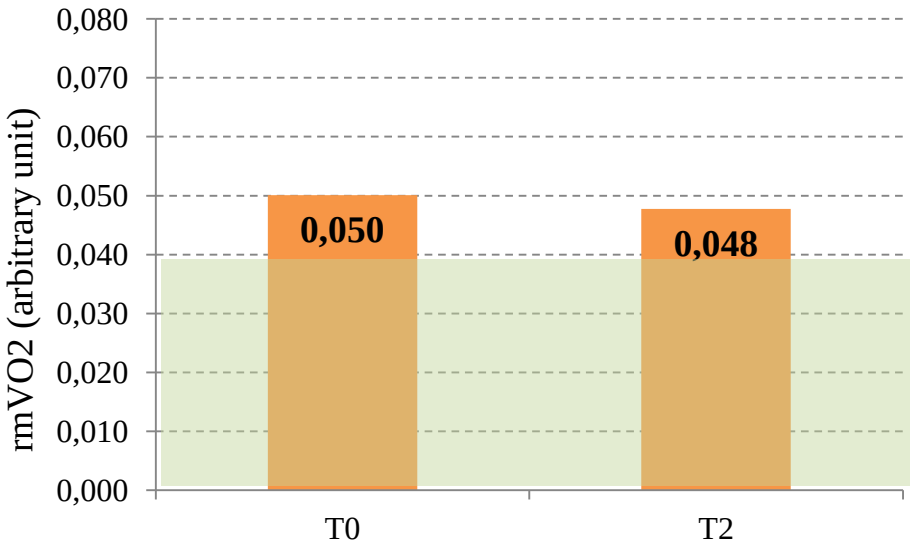


0,05700 0,04400

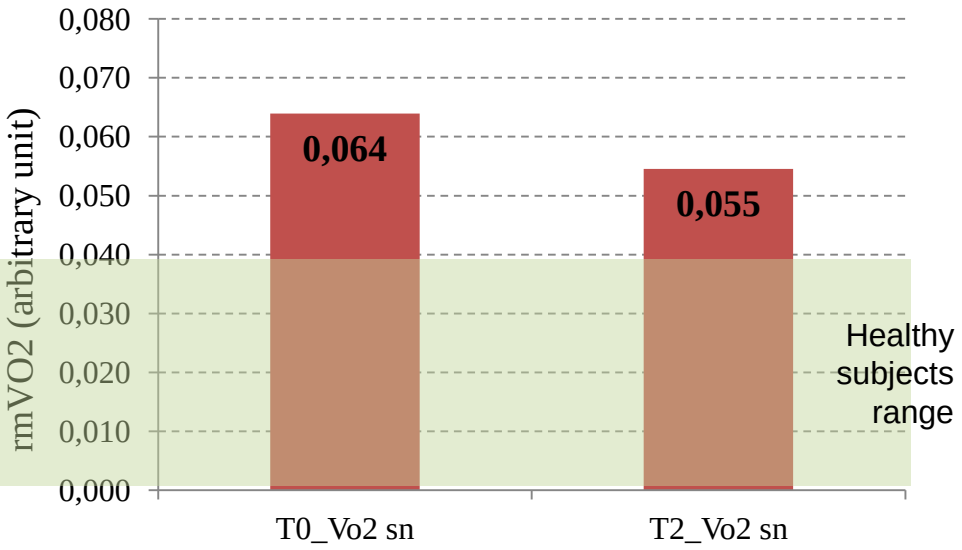
P = 0,1548

Clinical biomarkers: Metabolic measurment by NIRS

RAGT
(n = 14)

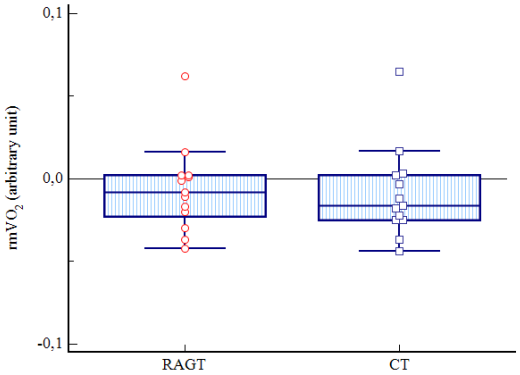


Conventional Therapy
(n = 13)



-4%

-22%



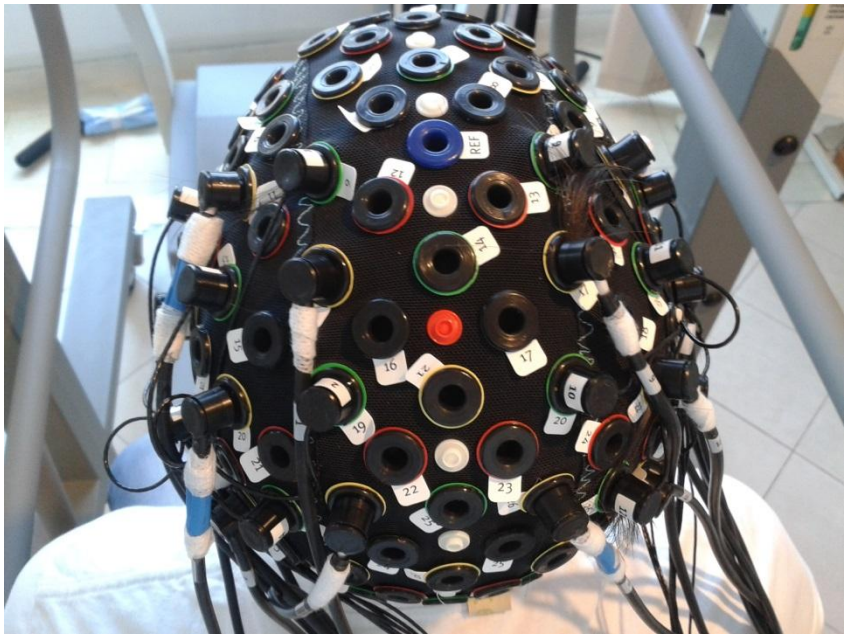
p=0.54

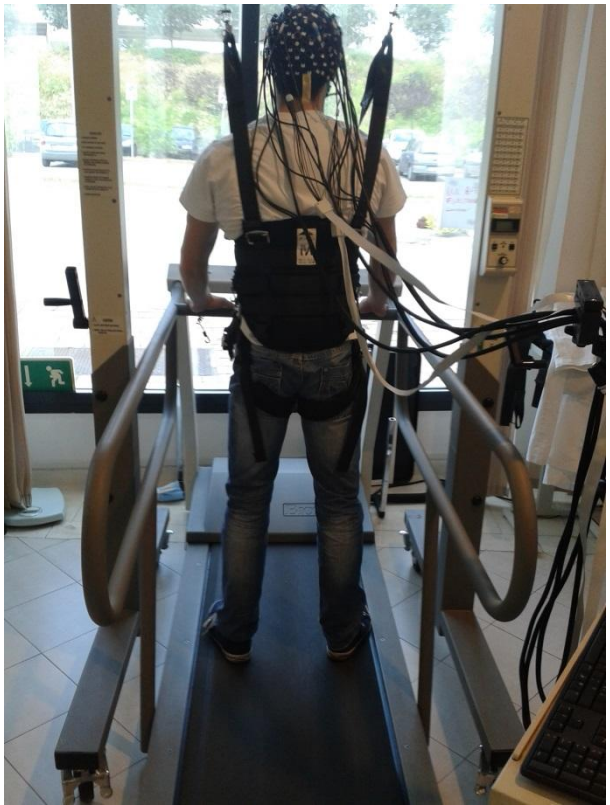
Clinical biomarkers: Metabolic measurement by NIRS
Brain perfusion

walking task on a treadmill (30” walking at 0,2km/h – 30” resting; 4 times)
Data collected on 21 out of 36 patients

Limits:
poor infrared light penetration towards scalp in people with long hairs.

Data stored, under evaluation





Circulating biomarkers

Data collected in all patients

Data analysis performed by 3 Research Units

Preliminary results available only for some parameters

CEC
EPC
Treg
MSC-Like cells

WP3:
Effectiveness of robot-assisted gait training versus conventional therapy on mobility in severely disabled multiple sclerosis patients.

PRELIMINARY RESULTS

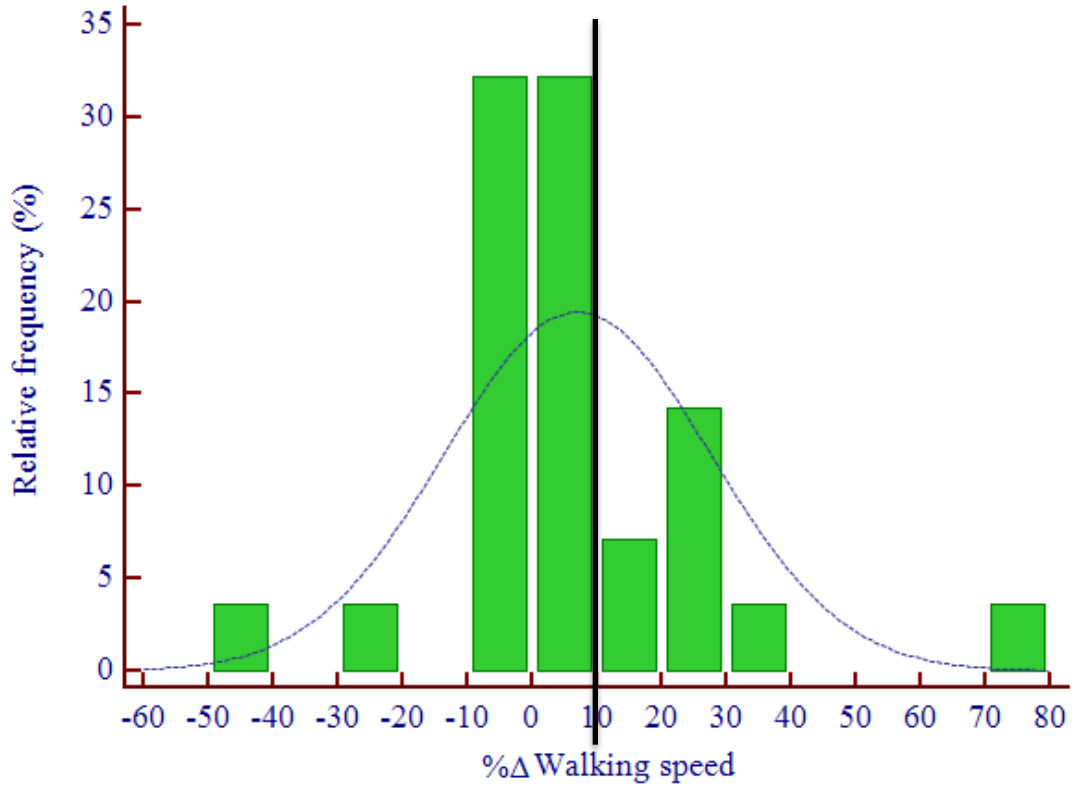
4. Individual response to the treatment

   Identification of the potential responders to any treatment
   or early Identification of response to a single treatment

Among 27 patients who completed the treatment for both groups (RAGT and CT), the number of the patients who respond positively at the rehabilitation has been arbitrarily identified.

Criteria for identification of respondents on 2 outcome measures:

T25FW: End of treatment walking speed ≥ 10% of baseline

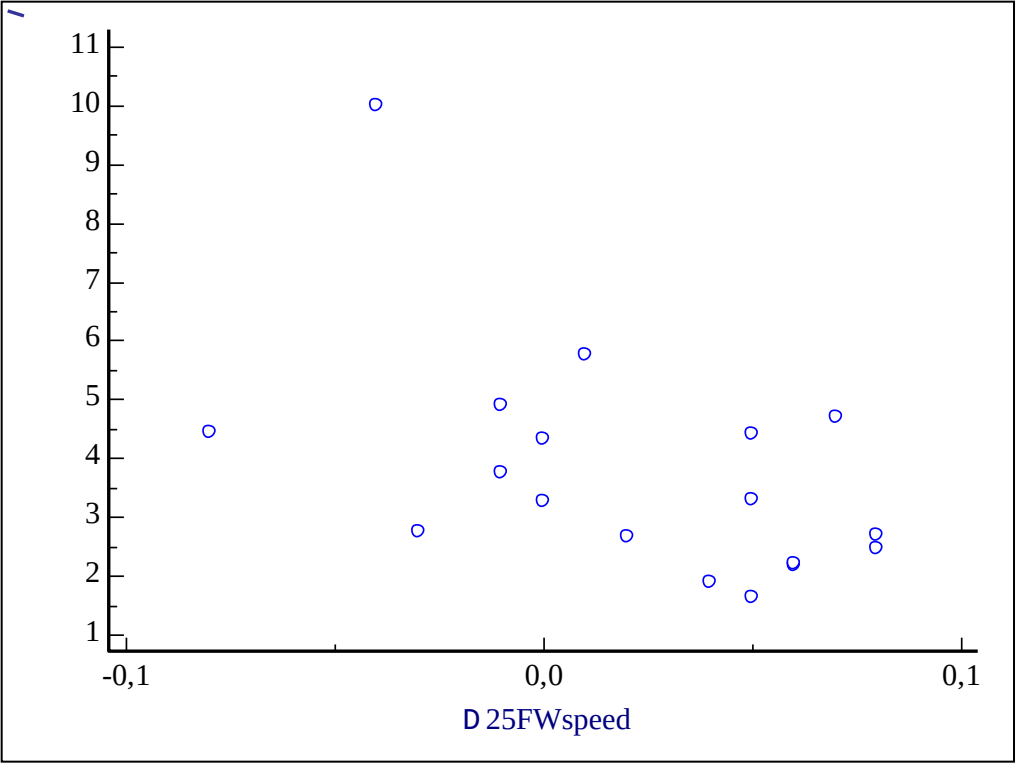


Factors and response to the treatments

Possible correlation between baseline biomarkers and response to treatment

All patients

Baseline values	**Respondents (n = 9)**	**Non-respondents (n = 18)**	**Statistics**
Age	57	56	n.s.
M/F (n)	1/8	9/9	0.09
EDSS	6.4	6.3	n.s.
Mean rmVO2	0.056	0.057	n.s.
CEC	0.33	0.40	n.s.
EPC	0.02	0.03	n.s.
MSC_73	0.10	0.08	n.s.
MSC_146	0.19	0.18	n.s.
Treg	2.65	4.32	0.07

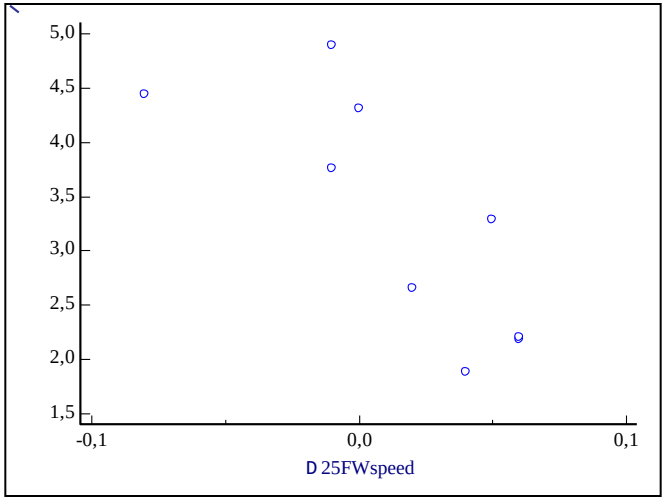


Low T reg levels correlate with a more positive final outcome independentely from the treatment

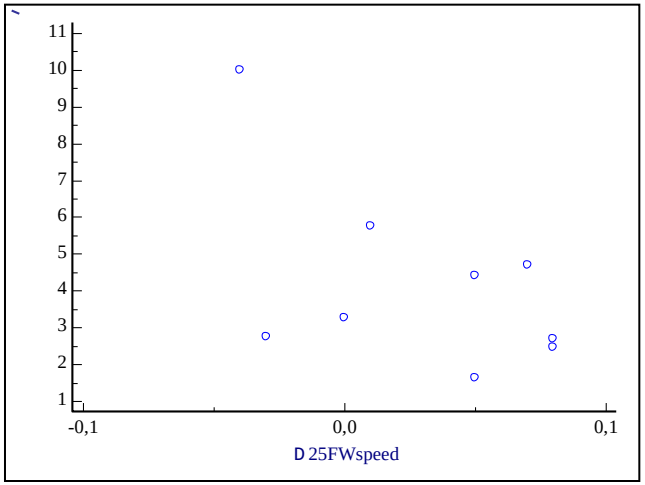
r = -0.50
p = 0.03

Whole population

RAGT



CT



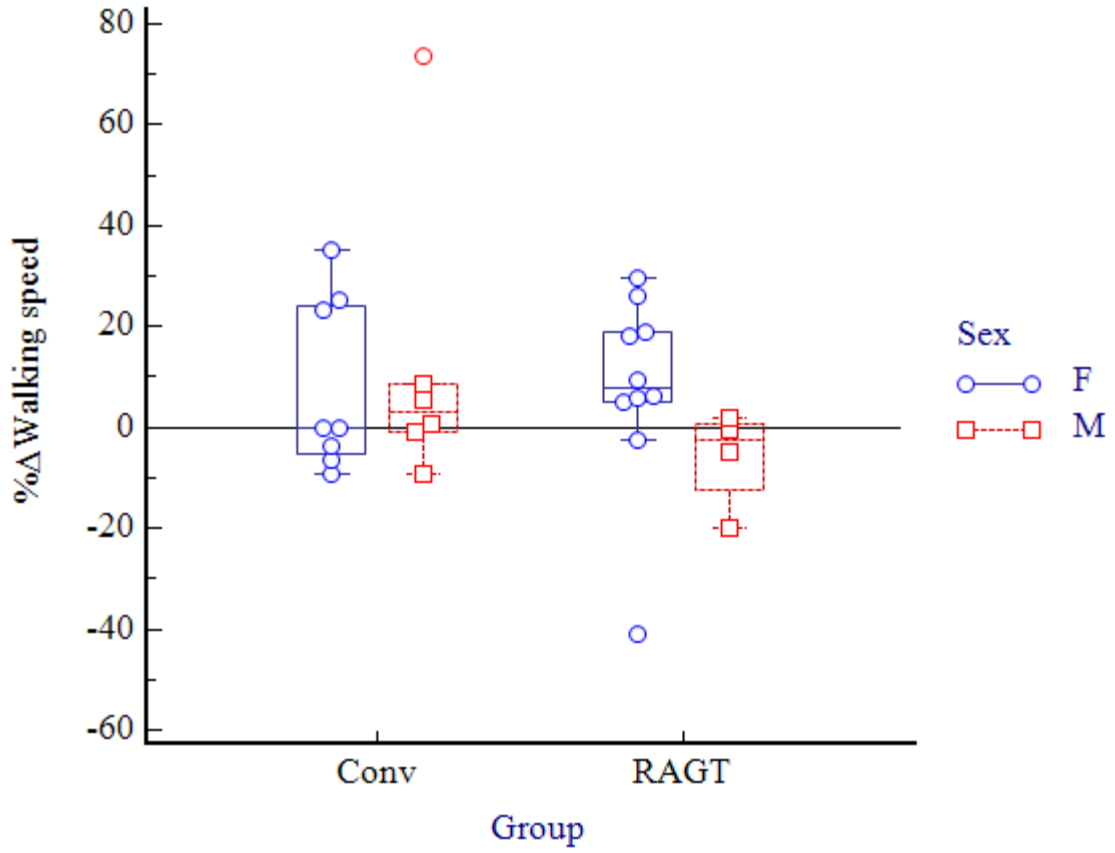
Factors and response to the treatments

Possible correlation between baseline biomarkers and response to treatment

RAGT

Baseline values	**Respondents (n = 5)**	**Non-respondents (n = 9)**	**Statistics**
Age	55	57	n.s.
M/F (n)	0/5	4/5	0.10
EDSS	6.3	6.3	n.s.
Mean rmVO2	*0.057*	*0.046*	n.s.
CEC	0.44	0.38	n.s.
EPC	0.02	0.02	n.s.
MSC_73	0.17	0.10	n.s.
MSC_146	0.35	0.28	n.s.
Treg	3.05	3.60	n.s.

Identification of the potential responders to the 2 treatments : gender



Males are poorly responsive to the robotic treatment

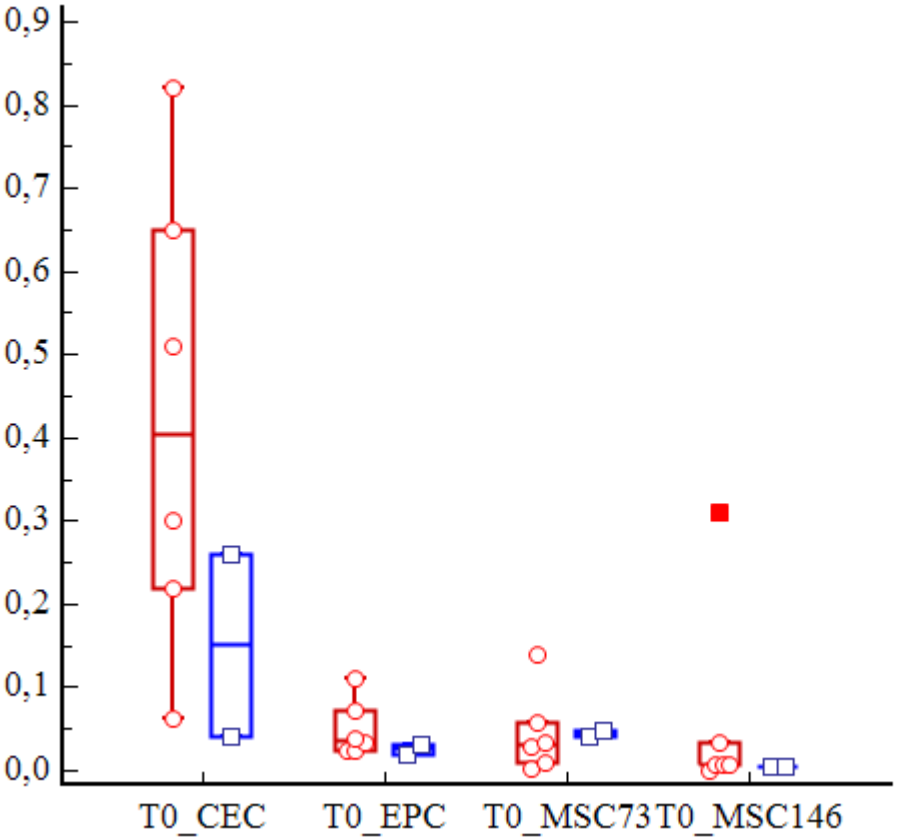
Factors and response to the treatments

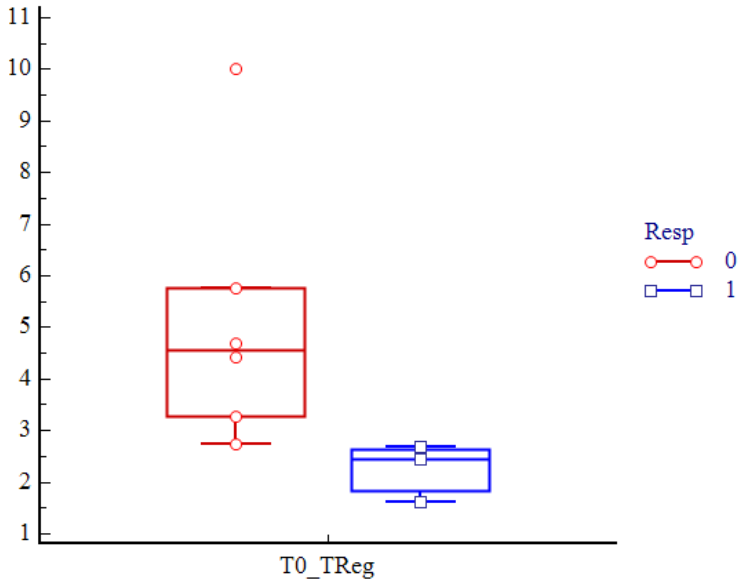
Possible correlation between baseline biomarkers and response to treatment

CT

Baseline values	**Respondents (n = 4)**	**Non-respondents (n = 9)**	**Statistics**
Age	56	62	n.s.
M/F (n)	1/3	5/4	n.s.
EDSS	6.3	6.5	n.s.
Mean rmVO2	0.055	0.068	n.s.
CEC	0.15	0.43	n.s.
EPC	0.03	0.05	n.s.
MSC_73	0.03	0.45	n.s.
MSC_146	0.04	0.06	n.s.
Treg	2.25	5.15	n.s.

Conventional therapy





Respondents have circulating biomarkers level slightly lower than non respondents for some biomarkers, especially for Treg cells.

Mobility – vascular protection

EPC levels at baseline correlate with

T025 FW	-0.634	p=0.0035
T0 TUG	-0.523	p= 0.026
T0 FSS	-0.553	p= 0.014

Exercise Capacity and Circulating Endothelial Progenitor Cells in Hemodialysis Patients

F. Manfredini[1]
G. M. Rigolin[2]
A. M. Malagoni[1]
S. Soffritti[3]
B. Boari[4]
F. Conconi[1]
G. L. Castoldi[2]
L. Catizone[3]
P. Zamboni[5]
R. Manfredini[4]

WP3:
Effectiveness of robot-assisted gait training versus conventional therapy on mobility in severely disabled multiple sclerosis patients.

PRELIMINARY RESULTS

5. Conclusions

Conclusions

Preliminary results

No significant differences
of walking speed and/or endurance for both
treatments (RAGT vs Conventional Therapy)

nor for the intragroup analysis
neither for the inter-group comparison
for the primary and secondary outcome
measures

Beer, 2008	walking speed	intragroup
Vaney 2011		
Schwartz, 2011		
Lo , 2008		

Interesting preliminary observations on the
relationship between outcomes and biomarkers.