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NEUROTECHNOLOGIES FOR UPPER LIMB REHABILITATION IN SUBACUTE AND CHRONIC STROKE PATIENTS

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Abstract

Introduction: There is a need for improved and optimized rehabilitation due to a group of stroke patients who do not achieve full function again both in the subacute and chronic phase after stroke. Neurorehabilitation technologies and predictions of functional outcome looks promising to overcome this challenge.

Aim: Sub-study 1 aimed to investigate the effect of the ArmeoSpring exoskeleton on improving upper limb function in subacute stroke patients as well as identifying underlying factors important for recovery and use these to create prediction models to predict the end outcome of the clinical scores Fugl-Meyer upper extremity (FM-UE) and action research arm test (ARAT). Sub-study 2 presents an intensive four-week combination therapy consisting of brain-computer interface (BCI), ArmeoSpring, and mirror therapy to improve upper limb function in chronic stroke patients.

Method: For sub-study 1, data from BCI-STAR project including 48 subacute stroke patients were used. To investigate the effect of ArmeoSpring, the patients were divided into an intervention group and a control group. Clinical scores FM-UE and ARAT were used to assess the upper limb function. Furthermore, an exploratory factor analysis (EFA) was performed on the same data to identify underlying factors. Lastly, a stacking model trained with data from BCI-STAR project was used to create the predictions models. For sub-study 2, a single group pre-post study with an intensive four-week training program consisting of BCI, ArmeoSpring, and mirror therapy is presented. 30 chronic stroke patients will be recruited. FM-UE, ARAT and transcranial magnetic stimulation (TMS) will be used to evaluate the effect of the combination therapy.

Results: Sub-study 1 showed that both the ArmeoSpring intervention and control groups significantly improved their clinical scores (p<.001), but there was no significant difference between the groups (p=0.673). The EFA showed three underlying factors identified as severity of stroke, quality of rehabilitation and age. The prediction models for FM-UE and ARAT received a MAE score of 3.47 and 3.86 points, respectively, meaning they are capable of predicting the outcome of the arm function accurately.

Conclusion: This thesis showed a tendency towards more intense training with ArmeoSpring can improve recovery following stroke. Furthermore, a rapid start of rehabilitation after stroke and motor evoked potential (MEP) status seems to be important factors for a greater recovery. The prediction models were able to predict clinical outcome precisely. Finally, it is proposed that a combination therapy consisting of BCI, ArmeoSpring and mirror therapy will significantly increase upper limb function in chronic stroke patients.

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Preface

This thesis explores upper limb rehabilitation in subacute and chronic stroke patients through neurorehabilitation technologies and prediction models. The thesis is built up with a general introduction following by two individual sub-studies and a general discussion.

This project was carried out at the Department of Health Science and Technology at Aalborg University in collaboration with Neuroenhed Nord at North Denmark Regional Hospital in Brønderslev and the Center for Neurotechnology and Rehabilitation during the period September 1st 2023 to May 31st 2024.

This project was written by group 9027 and supervised by Andrew James Thomas Stevenson and Benjamin Svejgaard.

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List of abbreviations

ARAT – Action research arm test
BCI -Brain-computer interface
CIMT – Constraint-induced movement therapy
CR – Clinical recovery
DNN – Deep neural network
EEG – Electroencephalography
EFA – Exploratory factor analysis
EMG – Electromyography
FES - Functional electric stimulation
FM-UE - Fugl-Meyer upper extremity assessment
GBR – Gradient boosting regression
ICH – Intracerebral hemorrhage
INF – Infarct
LR – Lasso regression
MAE – Mean absolute error
MCID – Minimum clinically important difference
MEP – Motor evoked potential
MIT - Motor imagery training
MRCP – Movement-related cortical potential
MSO – Maximum stimulator output
MT – Mirror therapy
MT – Motor threshold
NIHSS – National Institutes of Health stroke scale
PN – Peak negative
PPR - Proportional recovery rule
PR – Potential recovery
PREP2 - Predicting recovery potential 2
RFR – Random forest regression
rMT – Resting motor threshold
rTMS - Repetitive transcranial magnetic stimulation
SAFE - Shoulder abduction finger extension
TMS - Transcranial magnetic stimulation
XGB – Extreme gradient boosting

1. General Introduction

Stroke can be divided into ischemic stroke or hemorrhagic stroke, and it leads to loss of neurologic function[1,2] (Appendix 1, Pathophysiology of stroke). In Denmark one out of five above the age of 25 will be affected by a stroke during their life and it is estimated that around 12,000 are affected each year. Furthermore, 170,000 people in Denmark are living with the consequences of stroke where 25-30% need help on a daily basis[3–5]. Stroke can be divided into five phases where the first 24 hours are called the hyperacute phase, day one to seven is the acute phase, day seven to three months is the early subacute phase and the late subacute phase is from three to six months before going into the chronic phase[6]. Diverse risk factors have been associated with stroke, where the most significant are hypertension, diabetes, smoking, and elevated cholesterol levels as well as aging and anticoagulant medication[7,8]. Some of the common symptoms for stroke include loss of motor and sensory function in the limb contralateral to the lesion, aphasia, and neglect as well as cognitive impairment[1,9].

1.1 Rehabilitation

Limb weakness is a common stroke symptom. Specifically, up to 77% of stroke patients experience upper limb weakness, making it difficult for the patients to perform daily life activities[9–11]. Therefore, optimal rehabilitation therapies are crucial to regaining these functions and secure the patients' quality of life[12]. These therapies are based on neuroplasticity which is the brain's ability to reorganize its structure, connections, and function by responding to different stimuli[13] (Appendix 2, Neuroplasticity).

1.1.1 Rehabilitation therapies

Currently, multiple therapy forms are available to promote recovery of the upper limb post stroke and combination therapy is applied to induce the most efficient therapy for each individual patient. Which therapy to use for the individual is based on observations made by an interdisciplinary professional team, including neurologists, physiotherapists, occupational therapists, and the patient's goal for rehabilitation. Throughout the rehabilitation process, the patient´s functional ability and needs are considered, and the therapy is adjusted accordingly[14].

Physical and occupational therapy can be based on performing daily life activities[14] while other frequently used therapies include constraint-induced movement therapy (CIMT), mirror therapy (MT), motor imagery training (MIT), functional electric stimulation (FES), and repetitive

transcranial magnetic stimulation (rTMS)[11]. In CIMT, the unaffected arm is inhibited and thereby forces the patient to do task-oriented training with the affected arm to improve motor function[15]. This training requires some degree of voluntary movement; therefore, stroke patients with severe arm impairment can find it difficult. Patients with severe arm impairment could therefore have a greater benefit from MT where a mirror is placed between the arms and reflects the unaffected arm as if it was the affected arm. When the patient moves the unaffected arm, it will create the visual illusion that the affected arm is moving[16,17]. Another approach without voluntary movement is MIT where the patient is asked to think about moving the impaired arm without moving it[11]. FES is a method where electrical stimulation is applied to the nerves that generate muscle contraction in the affected arm to help generate movements to perform a certain task[18]. In rTMS, magnetic stimulation is applied to the motor cortex of the affected side to increase cortical excitability and thereby improve motor function in the affected arm[19]. rTMS can also be applied to the motor cortex contralesional to suppress cortical excitability[20]. Although the above-mentioned therapeutic methods have been proven to improve recovery in post stroke patients, especially when combined[11], each stroke case is different which can lead to unsatisfactory rehabilitation[21]. Furthermore, there is still up to 50% which do not achieve true recovery even though they receive therapy at a rehabilitations center[22–24]. Therefore, new rehabilitation methods are needed to secure an optimal and greater recovery for the individual patient.

1.1.2 Neurotechnology

In neurotechnology, methods and instruments are connected to the nervous system to record or manipulate neural activity[25], where FES and rTMS are well-used examples of neurotechnology used in rehabilitation settings[11,25,26]. Recently, neurotechnology such as robot-assisted therapy and brain-computer interface (BCI) have made an impact on rehabilitation methods[11,26]. These technologies have the potential to improve motor function and thereby quality of life for patients with stroke especially those where conventional therapies are lacking due to severe chronic stroke[26].

BCI systems detect brain activity which can be recorded by electroencephalography (EEG) directly from the user and send it to an external device which then produces the desired action to improve neuroplasticity. Robot-assisted therapies are exoskeletons that can support patients in improving motor function of upper and lower extremities[11,26]. An example of robot-assisted therapy is the

ArmeoSpring exoskeleton which can be used in rehabilitation of stroke patients. During training ArmeoSpring offers weight support while the patient plays various motivating games on a computer screen[27].

1.2 Prediction

Another way to optimize rehabilitation for post stroke patients than therapies is early prediction of recovery. By early prediction of the motor recovery of the arm, rehabilitation can be more efficient, and a more realistic goal setting can be fulfilled. Furthermore, it helps patients to manage their expectations for the process[28].

1.2.1 Proportional recovery rule

The proportional recovery rule (PRR) was formed by Krakauer et al. (2015) and describes the relationship between arm impairment and spontaneous recovery in stroke patients based on Fugl-Meyer upper extremity assessment (FM-UE) (Appendix 3, Clinical scores). More precisely, the PPR states that three months after stroke the patients will gain 70% of their maximum potential recovery back. An example could be a patient with an arm impairment of 46 measured by FM-UE will recover to 60 based on the calculation (66-46) * 0.7, where 66 is the maximum score of FM-UE. Even though the PRR fits patients with mild to moderate arm impairment and even with severe arm impairment, a small subgroup with severe arm impairment does not seem to follow the rule in their recovery and are called non-recovers. These non-recovers have an FM-UE under 20 and the proposed reason for failure to follow PRR is that the corticospinal tract is disrupted[29]. The PPR is based on spontaneous recovery and therefore optimal rehabilitation therapies are important to see if the PPR can be improved.

1.2.2 The PREP 2 algorithm

The predicting recovery potential 2 (PREP2) algorithm can be used to optimize rehabilitation by predicting the motor recovery of the upper limb by biomarkers. The PREP2 algorithm gives four possible outcomes “excellent”, “good”, “limited” and “poor” based on action research arm test (ARAT) clinical score at 3 months. If a patient receives the outcome “excellent” the patient has potential to make a complete or near-complete recovery, while in the outcome “good” the patient has the potential to use the arm for most activities of the daily life but with some weakness. For the “limited” outcome the patient will perform daily activities but with consequential changes. For the last outcome “poor” it is unlikely that the patient will regain useful function of the arm. To

obtain one of the four outcomes a shoulder abduction finger extension (SAFE) score should be made within 3 days of stroke symptom onset. If the patient scores a SAFE score of 5 or more and is younger than 80 years old the patient has the potential for an “excellent” outcome. The same outcome is obtained if the patient is older than 80 and has a SAFE score of 8 or more. If the patient is older than 80 years old but has a SAFE score less than 8 the patient has the potential to have a “good” outcome. Patients who receive a SAFE score less than 5 need a transcranial magnetic stimulation (TMS) assessment to evaluate the function of the corticospinal tract. The TMS is performed five to seven days post stroke. If TMS can elicit a motor evoked potential (MEP) it is said that the patient is MEP positive, and they have the potential for a “good” outcome. On the contrary, if TMS cannot elicit a MEP the patients are said to be MEP negative, and a National Institutes of Health stroke scale (NIHSS) score is necessary to determine the outcome. If a NIHSS score is over 7 the patient potential outcome is “limited” otherwise it is “poor”[30].

The PREP2 algorithm accuracy has been evaluated and it showed correct prediction for 75% of the patients at three months poststroke [30]. Most recovery of the motor functions after stroke happens in the acute and subacute phase, while little or no recovery is seen in the chronic phase[6]. Therefore, the PREP2 algorithm accuracy was also evaluated after two years. Here it was correct for 80% of the patients 2 years poststroke, where 83% was in the same category from three months to two years poststroke and indicates that the function of the arm is stable after three months poststroke[30,31].

1.3 Purpose

To summarize, there is still a need for improved rehabilitation methods due to a group of patients who do not achieve full function again. Novel rehabilitation methods such as BCI and ArmeoSpring look promising to overcome this challenge, but there is still a need for more research in this area to understand the full potential of these methods. Another way to optimize rehabilitation of stroke patients is through prediction of functional outcome for example by the PREP2 algorithm. However, the PREP2 algorithm only determines the patient's outcome and insight into the most optimal rehabilitation training to improve the patient's recovery is missing.

Therefore, the purpose of this thesis is to:

- Investigate the effect of ArmeoSpring in subacute stroke patients (Sub-study 1)

- Investigate which factors are most important for recovery of subacute stroke patients as well to use these factors to create a prediction model (Sub-study 1)
- Present a combination therapy consisting of BCI, ArmeoSpring, and mirror therapy in chronic stroke patients (Sub-study 2)

2. Sub-study 1 - An Optimization of Upper Limb Rehabilitation in Subacute Stroke Patients by ArmeoSpring and Prediction

2.1 Introduction

ArmeoSpring (Hocoma AG, Zurich, Switzerland) is a robot used for rehabilitation of patients with upper limb weakness due to a central or periphery injury to the nervous system such as stroke. The ArmeoSpring system consists of an exoskeleton which extends from the shoulder to the hand and can be adjusted to the individual patient. During training, the exoskeleton offers weight support for the impaired arm with built-in sensors that record movement. These movements are transferred to a computer, where the patient can play various motivating games which promote movements that are used in daily life such as reaching and grasping. These games can be adjusted to the individual's function of the arm and their cognitive state (Figure 1). During training with ArmeoSpring, the ArmeoControl software collects training data about duration, intensity, frequency and training domains, as well as any improvements (Figure 2)[27].

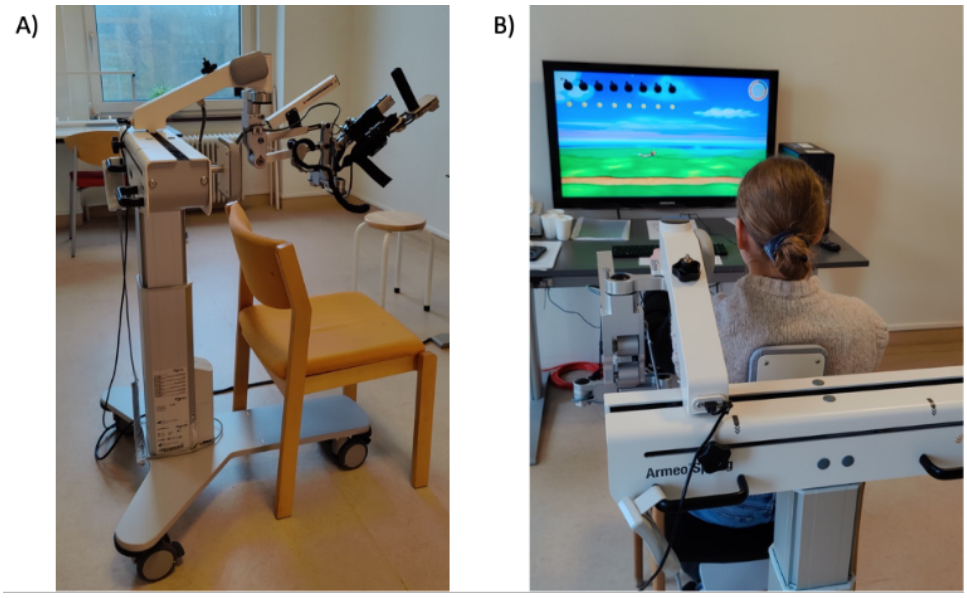


Figure 1: A) shows the ArmeoSpring exoskeleton. B) shows patient training with ArmeoSpring in a game. Here the patient controls an avatar in the game by the ArmeoSpring exoskeleton.

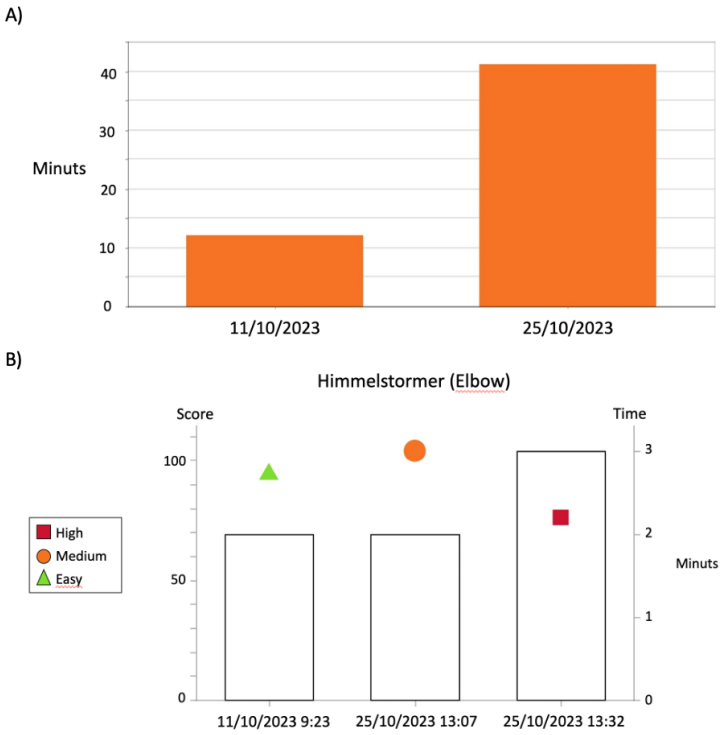


Figure 2: Examples of data extracted from ArmeoControl. A) shows which days and how many minutes the patient has trained with ArmeoSpring. B) shows data about the specific game Himmelstormer. Here the patient trained movement of the elbow. The graph shows how many minutes the patient has trained in this game and which dates as well as what level and which score the patient achieved.

2.1.1 Neurophysiological effect of ArmeoSpring

Rehabilitation is based on neuroplasticity and to induce neuroplasticity several factors are important to consider such as the number of repetitions and the intensity of the training[32]. An advantage of ArmeoSpring is the increase in repetitions and intensity of training exercises and sessions compared to conventional training due to specified and personalized therapy goals that encourage the patient to train with ArmeoSpring[33–35]. Furthermore, motivating exercises can increase patient engagement in the training through game-like augmented performance feedback and thereby further contribute to these factors[33–35].

Sehle et al. (2021) investigated the neurophysiological effects of ArmeoSpring in 30 subacute stroke patients by recording MEP peak-to-peak amplitudes from the deltoid muscles excited by TMS. The intervention group trained with ArmeoSpring for 45 minutes five times a week for three weeks, while the control group underwent conventional therapy. FM-UE were performed pre-intervention, three weeks after intervention and at a two-week follow-up, while TMS were performed pre-intervention and three weeks after the intervention. They found that the MEP peak-to-peak amplitudes recorded by TMS after the intervention were significantly larger in the

intervention group compared to the control group. These findings indicate that ArmeoSpring induces greater neuroplastic changes in corticospinal excitability compared to conventional therapy. Regarding FM-UE, the study found a significant improvement from pre-intervention to three weeks after intervention in both groups, but no significant difference in improvement between groups (Appendix 4, Table over articles)[36]. It could be suggested that the improvement in MEP amplitudes is too small to be measured as an improvement in the clinical score.

2.1.2 Clinical effect of ArmeoSpring

Several other studies have also investigated ArmeoSpring for the rehabilitation of stroke patients (Appendix 4, Table over articles)[33–39]. A study by Colomer et al. (2013) investigated the use of ArmeoSpring in 23 chronic stroke patients. The patients underwent three one-hour sessions per week in addition to their rehabilitation program. It should be noticed that there was no control group. Here they found significant improvement in all the function scales and activity scale in post-treatment compared to pretreatment. Furthermore, the improvement did not differ significantly at the four-month follow-up meaning it was stable over time[33].

Most recovery after stroke happens in the acute and subacute phases[6] and therefore, it could be interesting to investigate the effect of ArmeoSpring in these phases. A study by Esquenazi et al. (2021) investigated the ArmeoSpring in acute stroke patients divided into an intervention group and control group. The intervention group underwent one hour training session with ArmeoSpring four times a week throughout their rehabilitation stay, while the control group went through tabletop-assisted therapy exercises. The results showed significant improvements in motor function for the impaired arm within groups. Furthermore, a significant difference in improvement of active and passive range of motion for elbow flexion was seen between groups, with a greater improvement in the intervention group[34]. A study by Gueye et al. (2021) also investigated the ArmeoSpring intervention in the acute phase of stroke. Here 50 patients were enrolled in the study divided into two groups. The intervention group underwent twelve 45-min training sessions with ArmeoSpring over three weeks, while the control group underwent conventional therapy. The study found a significant improvement in clinical score within groups, which was significantly higher in the intervention group compared to the control group[38]. A study by Daunoraviciene et al. (2018) explored the use of ArmeoSpring in ten sessions divided into an intervention and control group in the subacute phase. Here, the control group underwent additional conventional therapy instead of training with

ArmeoSpring. This study showed that ArmeoSpring reduces impairment in the arm more effectively than conventional therapy but also enhanced cognitive abilities[39]. Based on these studies it is likely that the ArmeoSpring promotes recovery of the upper limb in both the acute, subacute and chronic phases.

2.1.2.1 ArmeoSpring compared to conventional therapy

Several studies have found significant improvements when acute and subacute stroke patients trained with ArmeoSpring but when it was compared to a control group undergoing conventional therapy, there was no difference between the groups[35–37]. In all three studies the control group underwent additional conventional therapy instead of training with ArmeoSpring and it could be an explanation for no difference between the groups because conventional therapy also improves motor recovery. Even though the conventional therapy seems to improve motor function as effectively as the ArmeoSpring intervention, the difference between the groups could be too small to be significant. As mentioned earlier, each case is different and maybe the population that benefits most from training with ArmeoSpring was not targeted. In addition, both the intervention and control groups received conventional therapy as a part of their rehabilitation training, which also could influence the outcome in terms of improving recovery. Furthermore, there is a difference between the studies regarding the number of training sessions and it could be suggested that the optimal amount of training sessions and training time was not performed or that the level of arm impairment could influence the result.

The level of arm impairment was investigated in a study by Chan et al. (2016) where 48 subacute stroke patients with different levels of arm impairment trained 45 minutes five days per week for three weeks with ArmeoSpring. The patients were divided into three groups: mild, moderate, and severe arm impairment. The study found that ArmeoSpring was beneficial for patients with moderate to severe arm impairments, while patients with mild arm impairment did not seem to benefit as much[40]. This could suggest that not all cases of stroke patients regarding level of arm impairment will benefit equally from training with ArmeoSpring. Another potential influencing factor is the size of the lesion. There has been found a significant correlation between the size of the lesion and motor recovery, indicating that a smaller lesion equals a better motor recovery[41,42]. This suggests that if the size of the lesion was different among the post stroke patients it could have affected the motor recovery and thereby a reason for no difference between groups. Another

reason for no difference between groups could be the MEP status. Based on the PREP2 algorithm MEP status is an important factor in determining outcome. A positive MEP status indicates a good outcome while a negative MEP status indicates a limited to poor outcome[30,31], which shows that the MEP status can affect motor recovery. It could be another explanation for no difference between groups if the studies did not account for the MEP status when the participants were assigned to the intervention or control group.

All together, these articles show that ArmeoSpring seems to induce neuroplasticity and to improve motor function in the upper extremity in both the acute, subacute, and chronic phase of stroke. However, more research in this area is still needed since several articles found no difference when it was compared to conventional therapy. Furthermore, research into which patient will benefit most from the training regarding arm impairment, severity of stroke and MEP status still needs to be explored. Finally, the proper number of sessions and amount of time training with ArmeoSpring also requires more investigation to find the most optimal training for stroke patients.

Therefore, the purpose of sub-study 1 was to investigate the effect of ArmeoSpring in subacute stroke patients. Furthermore, an exploratory factor analysis (EFA) was made to investigate which parameters are most important for recovery in subacute stroke patients. Finally, a prediction model based on factors from the EFA was made to predict the patient's clinical outcome.

These purposes lead to formulation of three hypotheses:

1. Patients in both the intervention and control groups will exhibit significant improvement in FM-UE and ARAT clinical scores from pre to post measurements. The intervention group will have a significantly greater improvement in FM-UE and ARAT clinical scores compared to the control group who did not receive training with ArmeoSpring.
2. With EFA it will be possible to investigate if there are unobserved factors that can explain the variance among the observed variables.
3. With two prediction models it will be possible to predict the end outcome of the clinical scores FM-UE and ARAT.

2.2 Method

2.2.1 BCI-STAR project

Data from the BCI-STAR project[43] was used for sub-study 1 and the method was used in sub-study 2. The BCI-STAR aimed to investigate the clinical and neurophysiological effect of a BCI system to improve arm function in subacute stroke patients. In this study electrical stimulation is timed with peak negative (PN) of a movement-related cortical potential (MRCP) when the patient performs or imagines a wrist extension of the impaired arm. Patients were randomized to one of two groups; patients in the associative group received electrical stimulation at motor threshold (MT), eliciting a motor response. The other, "sham"/control group received electrical stimulation below their perception threshold. The training consisted of 12 training sessions over four weeks with approximately three sessions per week. In each training session the patient performed 2x30 repetitions of a wrist extension of the impaired arm. The first 30 repetitions were performed without electrical stimulation to visualize and calculate the time of PN of the MRCP. The second part of the training, electrical stimulation was applied and times, so that sensory feedback from the elicited response arrived at the brain at the same time as the MRCP. Furthermore, the first, sixth and last training sessions also contained TMS immediately before, immediately after and 30 minutes after the training to evaluate the peak-to-peak MEP amplitude. FM-UE and ARAT clinical scores were performed at baseline, after four weeks of training and at 6- and 12-month follow-up to evaluate the function of the arm[43].

2.2.2 Participants

This study was conducted using data from 55 subacute stroke patients enrolled in the BCI-STAR project[43]. 7 patients were excluded since they dropped out of the BCI-STAR project, which leaves 48 subacute stroke patients that were enrolled in this current study. Enrollment of patients for the BCI-STAR project took place at the neurorehabilitation center Neuroenhed Nord in North Jutland in Brønderslev, Denmark between 22-04-2021 and 23-05-2023[43]. Data used from the BCI-STAR project included information about patient's birthday, sex, MEP status, group allocation, date for stroke, type and location of lesion, resting motor threshold (rMT), MT, PN, FM-UE and ARAT clinical scores for baseline (pre), after four weeks (post), 6-month and 12-month follow-up as well as date for each clinical score[43]. Sub-study 1 was approved by the Scientific Etichs Committee for Nordjylland, Denmark (reference no. N-20200004) and performed in accordance with the Declaration of Helsinki. Patient characteristics are presented in Table 1.

Table 1: Patient characteristics with ID, age, sex, type and location of lesion, days after stroke and motor evoked potential (MEP) status.

Patient ID	Age	Sex	Type of lesion	Location of lesion	Days after Stroke	MEP status
005	48	M	ICH	Left frontal lobe	25	POS
006	85	M	ICH	Left pons	8	POS
101	71	M	INF	Left medial cerebral artery	18	NEG
102	69	M	INF	Left corona radiata	12	NEG
103	64	M	INF	Right corona radiata + Basal ganglia	25	NEG
104	53	M	INF	Right posterior cerebral artery, P1	14	POS
105	69	F	INF	Basal ganglia + left pons	11	NEG
106	63	F	INF	Right basal ganglia	48	POS
107	36	F	INF	Right medial cerebral artery	14	POS
108	66	M	INF	Left medial cerebral artery	21	POS
109	73	M	INF	Left parietal lobe	20	POS
110	78	F	INF	Left medial cerebral artery, M2	17	POS
112	61	M	ICH	Right basal ganglia	21	POS
114	64	M	ICH	Right parietal lobe	23	POS
115	63	M	INF	Left pons	28	NEG
116	64	M	INF	Left medial cerebral artery	43	POS
117	57	M	INF	Left anterior cerebral artery	46	POS
118	57	F	ICH	Right corona radiata + mesencephalon	40	POS
119	55	M	INF	Right medial cerebral artery	49	NEG
121	71	M	INF	Right medial + posterior cerebral artery	40	POS
122	50	M	ICH	Right basal ganglia	30	NEG
123	76	M	ICH	Right parietal lobe	61	POS
125	56	M	INF	Right pons	17	NEG
126	70	F	ICH	Right basal ganglia	16	NEG
127	73	M	ICH	Right basal ganglia	19	POS
128	45	M	INF	Bilateral water shed areas	25	POS
129	62	F	INF	Bilateral water shed areas	18	NEG

130	71	F	INF	Right medial cerebral artery	70	NEG
131	66	F	INF	Right insula + frontoparietal lobe	43	NEG
132	79	M	INF	Right medial cerebral artery	27	NEG
133	75	M	INF	Right medial cerebral artery	57	POS
134	61	M	INF	Right corona radiata + parietal lobe	15	NEG
136	41	M	ICH	Basal ganglia + right corona radiata	28	POS
137	76	M	ICH	Right parietal + frontal lobe	25	POS
138	75	F	ICH	Right parietal + frontal lobe	35	NEG
139	61	M	ICH	Left basal ganglia	30	POS
140	25	M	INF	Left thalamus	35	NEG
141	72	M	INF	Right medial cerebral artery	33	NEG
142	53	M	ICH	Left basal ganglia	22	POS
143	53	M	INF	Right medial cerebral artery	78	NEG
144	79	F	INF	Right medial cerebral artery	53	POS
145	64	F	ICH	Left basal ganglia	64	NEG
147	59	F	ICH	Left pons	78	POS
148	68	F	INF	Right basal ganglia	44	POS
150	69	M	ICH	Right basal ganglia	13	POS
151	52	M	INF	Right capsula interna	43	NEG
152	39	F	INF	Right capsula interna/corona radiata	35	POS
153	57	M	INF	Right thalamus/temporal lobe	22	NEG
Mean	63				32	
SD	12				18	
M: male, F: female, ICH: intracerebral hemorrhage, INF: infarct, SD: standard deviation, MEP: motor evoked potential, POS: positive, NEG: negative.						

2.2.3 ArmeoSpring

The objective of hypothesis 1 was to investigate the effect of ArmeoSpring on improving arm function in subacute stroke patients. For this sub-study patients were divided into an intervention group or control group. Group allocation was independent of the group allocation in the BCI-STAR project. The intervention group was defined as patients who had trained with the ArmeoSpring exoskeleton as a part of their rehabilitation training within the four-week period of their

participation in the BCI-STAR project, while the control group did not train with ArmeoSpring. Patients enrolled in the BCI-STAR project before Neuroenhed Nord acquired the ArmeoSpring was automatically part of the control group. If patients enrolled in the BCI-STAR project had trained with ArmeoSpring but not within the initial four-week period they were allocated to the control group.

2.2.3.1 Data analysis

Data from ArmeoControl (Version 2.2.5) were extracted to allocate the patients into intervention and control group. Furthermore, data about the training duration and frequency during the four-week period were extracted. For patients in the intervention group only training sessions within the four-week period of their participation in the BCI-STAR project were included, as ArmeoSpring training performed outside the period was assumed to have no effect on recovery within the period. Training sessions were included if the patients completed them on the same day as FM-UE and ARAT were performed.

2.2.3.2 Statistical analyses

Mann-Whitney U-tests was performed to evaluate the difference in sex, age and days after stroke between the intervention group and control group. Two Mann-Whitney U-tests were performed to evaluate the difference in FM-UE score and ARAT score between the intervention group and control group at baseline. Four Wilcoxon signed-rank tests were performed to evaluate the difference in FM-UE and ARAT score between baseline and after four-weeks of training within each group. Once again, two Mann-Whitney U-tests was performed to evaluate the absolute difference in FM-UE and ARAT between baseline and after four-weeks of training between the intervention group and control group. Four simple linear regressions were conducted for the intervention group to test the relationship between FM-UE and ARAT potential recovery, and time and sessions with ArmeoSpring. A simple linear regression was conducted to test the relationship between the FM-UE baseline score and FM-UE potential recovery, and ARAT baseline score and ARAT potential recovery across all patinets. A simple linear regression was conducted to test the relationship between days after stroke and FM-UE potential recovery, and days after stroke and ARAT potential recovery across all patients. Two unpaired t-tests were performed to evaluate the potential recovery for FM-UE and ARAT scores between the MEP positive and MEP negative patients. The

statistical analysis was conducted in Statistical Package for Social Science (SPSS, version 29; IBM, USA). The level of significance was set to 0.05.

2.2.4 Factor analysis

An EFA was made to identify underlying factors impacting variance between the observed variables. The EFA was performed according to the seven basic steps outlined in Decoster et al. (1998) (Appendix 5, Factor analysis)[44]. Observed variables chosen for the factor analysis were age, days after stroke, rMT from the first training session, mean MT of the radial nerve across all sessions, mean time of PN across all sessions, time in minutes and number of sessions in ArmeoSpring within the four-week period of the BCI-STAR project, FM-UE total pre, post, delta scores and potential recovery as well as ARAT total pre, post, delta scores and potential recovery.

Patients with missing data were excluded from the EFA. If a patient did not train with ArmeoSpring number of sessions and time in minutes were set to 0. If it was not possible to elicit a MEP at the highest intensity with TMS, rMT was set to 100%. Factor-analyzer toolkit version 0.5.1 in Python version 3.12 was used to perform the EFA.

Correlations matrix revealed that the mean MT of the radial nerve across all sessions did not correlate higher than 0.3 with any other variable and that time and sessions in ArmeoSpring unsurprisingly correlated with each other. Therefore, these three variables were removed from further analysis. Kaiser´s criterion and scree plot showed three eigenvalues above one, meaning the number of factors is three for this EFA (Appendix 5.1, Correlation matrix, Kaiser´s criterion and scree plot). To improve interpretability varimax rotation was used.

2.2.5 Prediction

Two separate prediction models were made: one for FM-UE and one for ARAT. Nine input variables for the FM-UE prediction model were obtained including age, sex, MEP status, days after stroke, FM-UE total pre score as well as the proximal, wrist and coordination FM-UE subcategories total pre scores. The output variable was FM-UE total post score.

Ten input variables for the ARAT prediction model were obtained including age, sex, MEP status, days after stroke, ARAT total pre score as well as the ARAT subcategories grasp, grip, pinch and gross movement total pre scores. The output variable was ARAT total post score.

A stacking model with the three base models random forest regression (RFR), lasso regression (LR), and gradient boosting regression (GBR) as well as a metamodel, which was a linear regression model, were used to create the prediction models (Appendix 6, Prediction). The base models were trained with all input variables. Patients with missing data was excluded. If a patient did not train with ArmeoSpring, sessions and time were set to 0. Regarding sex, female was assigned as 0 and male as 1. For MEP status negative was assigned as 0 and positive as 1. For the RFR and GBR 100 decision trees were used. For the LR alpha was set to 0.01. Data were split into training and testing sets with a test size of 20%. Cross validation was used to prevent overfitting[45]. The meta model was trained with the output from the base models. Mean absolute error (MAE) was used to evaluate the meta model performance on output variable data. Scikit-learn toolkit version 1.4.1.post1 in Python version 3.12 was used to train the stacking models.

2.3 Results

2.3.1 ArmeoSpring

2.3.1.1 Patient characteristics

Seventeen patients were included in the intervention group (5 females and 12 males; 62 ± 15 years old; 38 ± 21 days after stroke). 31 patients were included in the control group (10 females and 21 males; 63 ± 11 years old; 30 ± 15 days after stroke). The groups were matched for sex, age and days after stroke (p = 0.610, 0.923, 0.188, respectively).

Data for sex, age, days after stroke, training session and training time with ArmeoSpring are presented as mean ± SD while the clinical scores are presented as median [IQR].

2.3.1.2 Clinical scores

Fugl-Meyer Upper extremity

There was no statistically significant FM-UE difference between the two groups at baseline (p = 0.53). The intervention group significantly improved their median FM-UE score from baseline 15 [7 − 42] to 24 [20 − 41] after four weeks of training with ArmeoSpring (p < .001). The median in the control group improved significantly from 18.5 [7.25 − 33.25] to 34 [19 − 51] (p < .001) (Figure 3A). While both groups improved their FM-UE score during the four weeks, there was no significant difference in the pre-post difference between the groups (p = 0.673). The intervention group median for improvement was 9 [3 − 13] and 9 [4 − 13.5] for the control group (Figure 3B).

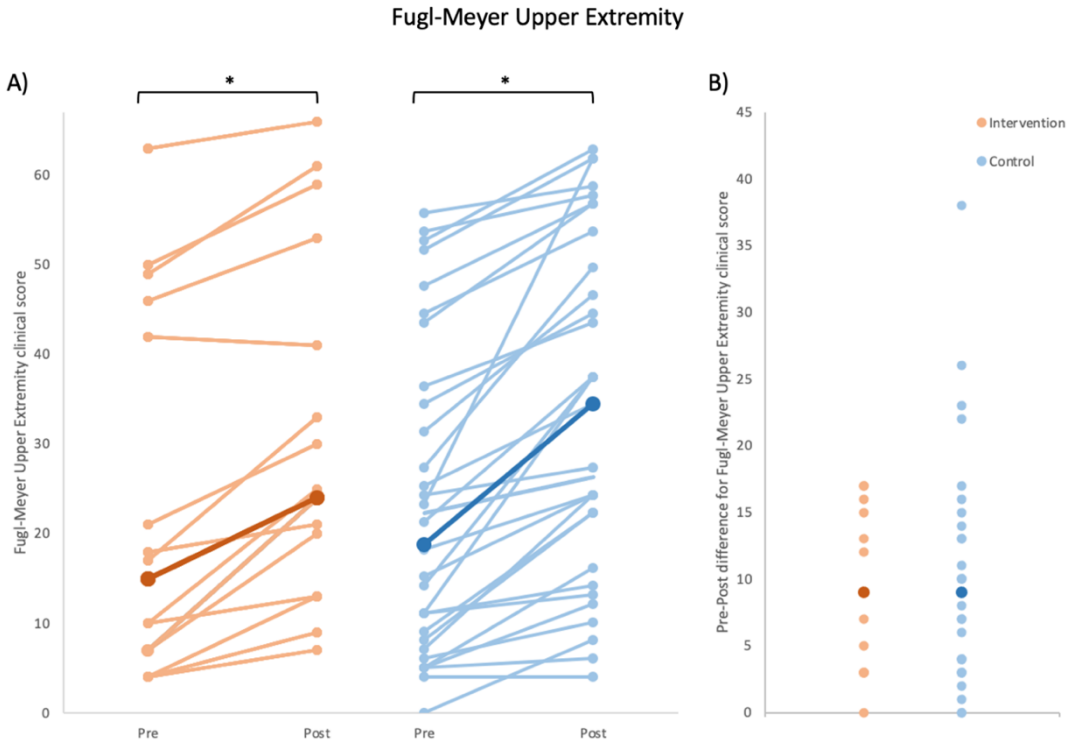


Figure 3: A) Fugl-Meyer upper extremity (FM-UE) clinical scores at baseline (Pre) and after four weeks of training (Post) for intervention group (Orange) and control group (Blue). The larger orange circles represent median pre and post assessment for the intervention group while the larger blue circles represent median pre and post assessment of the control group. The asterisk indicates significant difference. B) The difference between pre and post FM-UE for the intervention group (orange) and control group (blue). The larger orange circle represents median difference for the intervention group while the larger blue circle represents median difference for the control group.

Action Research Arm Test

There was no statistically significant ARAT difference between the two groups at baseline (p = 0.52). The intervention group significantly improved their median ARAT score from baseline 5 [0 − 23] to 14 [10 − 32] after four weeks of training with ArmeoSpring (p = .001). The median in the control group improved significantly from 10.5 [0 − 27] to 26 [8.5 − 38.5] (p < .001) (Figure 4A). While both groups improved their ARAT score during the four weeks, there was no significant difference in the pre-post difference between the groups (p = 0.627). The intervention group median for improvement was 6 [3 − 12] and 7 [3.5 − 17.5] for the control group (Figure 4B).

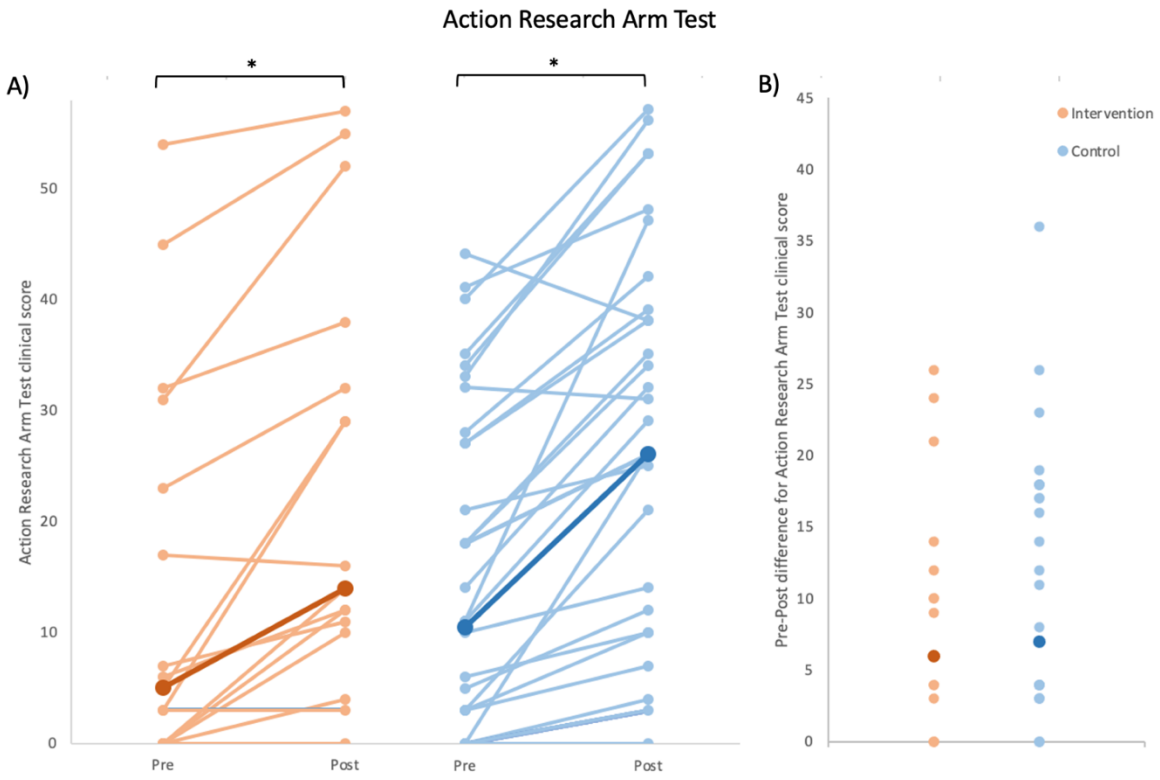


Figure 4: A) Action Research Arm Test (ARAT) clinical scores at baseline (Pre) and after four weeks of training (Post) for intervention group (Orange) and control group (Blue). The larger orange circles represent median pre and post assessment for the intervention group while the larger blue circles represent median pre and post assessment of the control group. The asterisk indicates significant difference. B) The difference between pre and post ARAT for the intervention group (orange) and control group (blue). The larger orange circle represents median difference for the intervention group while the larger blue circle represents median difference for the control group.

2.3.1.3 Training sessions and time in ArmeoSpring

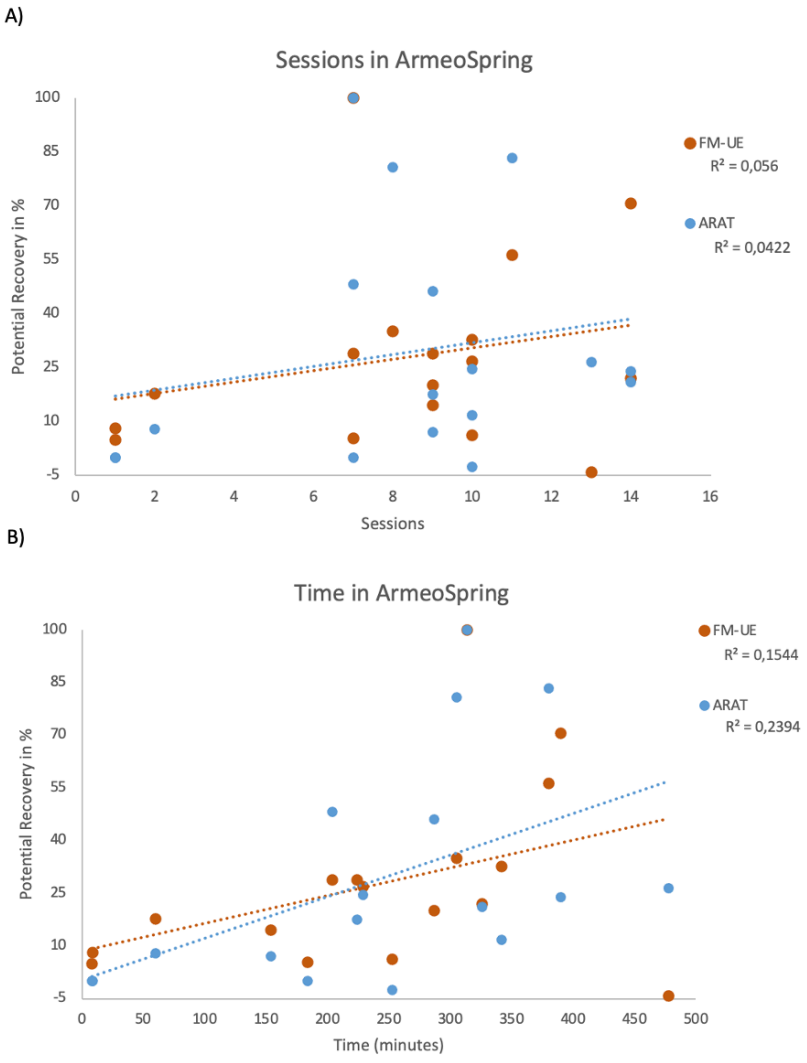
The intervention group received 8 ± 4 training sessions and trained for 244 ± 132 minutes with ArmeoSpring. Simple linear regression was used to test if training time and number of sessions in ArmeoSpring significantly predicted potential recovery for FM-UE and ARAT.

For sessions and FM-UE the fitted regression model was $y = 1.569x + 14.840$. The overall regression was not statistically significant (R^2=0.056, $F(1,15)$=0.891, p=0.360). It was found that the number of training sessions in ArmeoSpring did not significantly predict FM-UE potential recovery (B=1.569, p=0.360) (Figure 5A).

For time in minutes and FM-UE the fitted regression model was $y = 0.079x + 8.790$. The overall regression was not statistically significant (R^2=0.154, $F(1,15)$=2.726, p=0.120). It was found that training time in ArmeoSpring did not significantly predict FM-UE potential recovery (B=0.079, p=0.120) (Figure 5B).

For sessions and ARAT the fitted regression model was $y = 1.629x + 15.566$. The overall regression was not statistically significant (R^2=0.042, F(1,15)=0.652, p=0.432). It was found that the number of training sessions in ArmeoSpring did not significantly predict ARAT potential recovery (B=1.629, p=0.432) (Figure 5A).

For time in minutes and ARAT the fitted regression model was $y = 0.118x + 0.495$. The overall regression was statistically significant (R^2=0.237, F(1,15)=4.672, p=0.047). It was found that training time in ArmeoSpring significantly predicts ARAT potential recovery (B=0.118, p=0.047) (Figure 5B).



Figuer 5: A) A scatterplot illustrating the relationship between sessions with ArmeoSpring, Fugl-Meyer upper extremity (FM-UE) and action research arm test (ARAT) potential recovery. On the y-axis the potential recovery in % for FM-UE and ARAT is presented as the dependent variable and on the x-axis training sessions with ArmeoSpring is presented as the independent variable. B) A scatterplot illustrating the relationship between time with ArmeoSpring, Fugl-Meyer upper extremity (FM-UE) and action research arm test (ARAT) potential recovery. On the y-axis the potential recovery in % for FM-UE and ARAT is presented as the dependent variable and on the x-axis time in minutes with ArmeoSpring is presented as the independent variable.

2.3.2 Relationship between baseline and potential recovery across all patients

Simple linear regression was used to test if baseline score significantly predicted potential recovery for clinical scores: FM-UE and ARAT across all patients.

For FM-UE baseline and potential recovery the fitted regression model was $y = 0.844x + 10.086$. The overall regression was statistically significant (R^2=0.404, *F*(1,46)=31.210, *p*=<0.001). It was found that FM-UE baseline score significantly predicts FM-UE potential recovery (B=0.844, *p*=<0.001) (Figure 6A).

For ARAT baseline and potential recovery the fitted regression model was $y = 1.156x + 11.654$. The overall regression was statistically significant (R^2=0.307, *F*(1,46)=20.358, *p*=<0.001). It was found that ARAT baseline score significantly predicts ARAT potential recovery (B=1.156, *p*=<0.001) (Figure 6B).

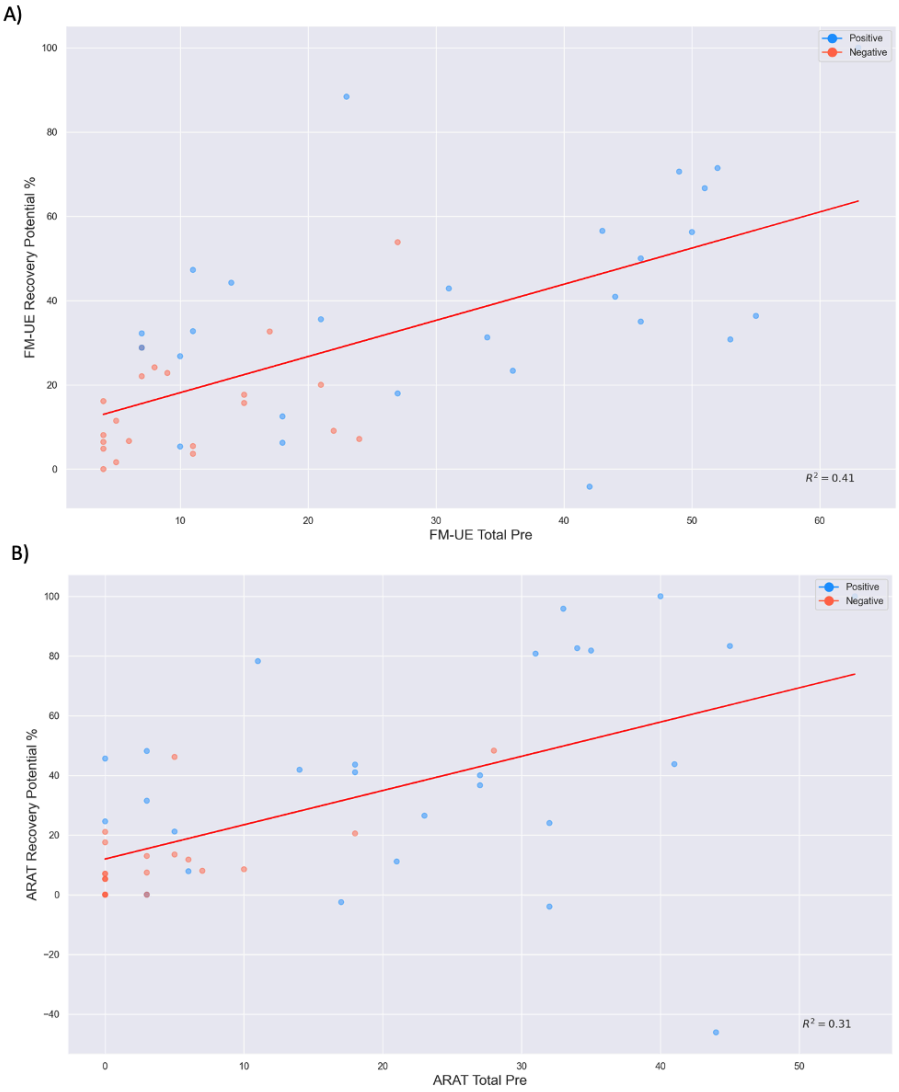


Figure 6: A) shows the relationship between Fugl-Meyer upper extremity (FM-UE) total pre score and FM-UE potential recovery in %. On the x-axis is FM-UE total pre score presented as the independent variable and on the y-axis is FM-UE potential recovery in % presented as the dependent variable. B) shows the relationship between action research arm test (ARAT) total pre score and ARAT potential recovery in %. On the x-axis is ARAT total pre score presented as the independent variable and on the y-axis is ARAT potential recovery in % presented as the dependent variable. The blue dots represent the positive motor evoked potential (MEP) status, and the red dots represent the negative MEP status.

2.3.3 Relationship between days after stroke and potential recovery across all patients

Simple linear regression was used to test if days after stroke significantly predicted potential recovery for clinical scores: FM-UE and ARAT across all patients.

For days after stroke and FM-UE potential recovery the fitted regression model was $y = -0.377x + 41.525$. The overall regression was not statistically significant (R^2=0.080, $F(1,46)$=4.019, p=0.051). It was found that days after stroke did not significantly predicts FM-UE potential recovery (B=-0.377, p=0.051) (Figure 7A).

For days after stroke and ARAT potential recovery the fitted regression model was $y = -0.680x + 50.841$. The overall regression was statistically significant (R^2=0.139, $F(1,46)$=7.455, p=0.009). It was found that days after stroke significantly predicts ARAT potential recovery (B=-0.680, p=0.009) (Figure 7B).

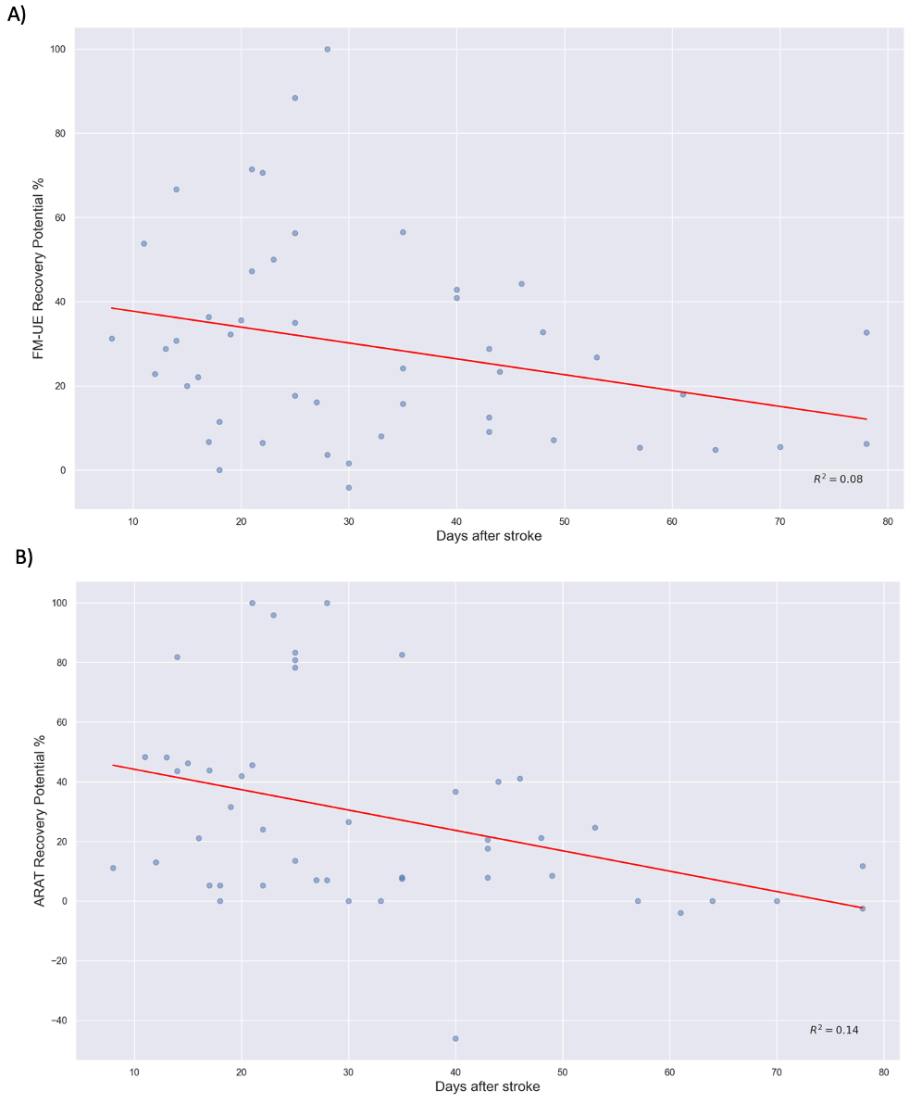


Figure 7: A) shows the relationship between days after stroke and Fugl-Meyer upper extremity (FM-UE) potential recovery in %. On the x-axis is days after stroke presented as the independent variable and on the y-axis is FM-UE potential recovery in % presented as the dependent variable. B) shows the relationship between days after stroke and action research arm test (ARAT) potential recovery in %. On the x-axis is days after stroke presented as the independent variable and on the y-axis is ARAT potential recovery in % presented as the dependent variable.

2.3.4 Motor evoked potential status across all patients

An independent samples t-test was conducted to compare FM-UE potential recovery in MEP positive and MEP negative subacute stroke patients. There was a significant difference in the scores for MEP positive (N=27, M=40.2 points, SD=24.6 points) and MEP negative (N=21, M=15.2 points,

SD=12.8 points); *t*(46)=4.232, *p*=<0.001 (two tailed). The mean score for MEP positive was significantly higher than for MEP negative (mean difference=25.0 points, 95% CI:13.1, 36.9 points) (Figure 8A).

An independent samples t-test was conducted to compare ARAT potential recovery in MEP positive and MEP negative subacute stroke patients. There was a significant difference in the scores for MEP positive (N=27, M=42.2 points, SD=36.5 points) and MEP negative (N=21, M=11.6 points, SD=13.4 points); *t*(46)=3.641, *p*=<0.001 (two tailed). The mean score for MEP positive was significantly higher than for MEP negative (mean difference=30.6 points, 95% CI:13.7, 47.5 points) (Figure 8B).

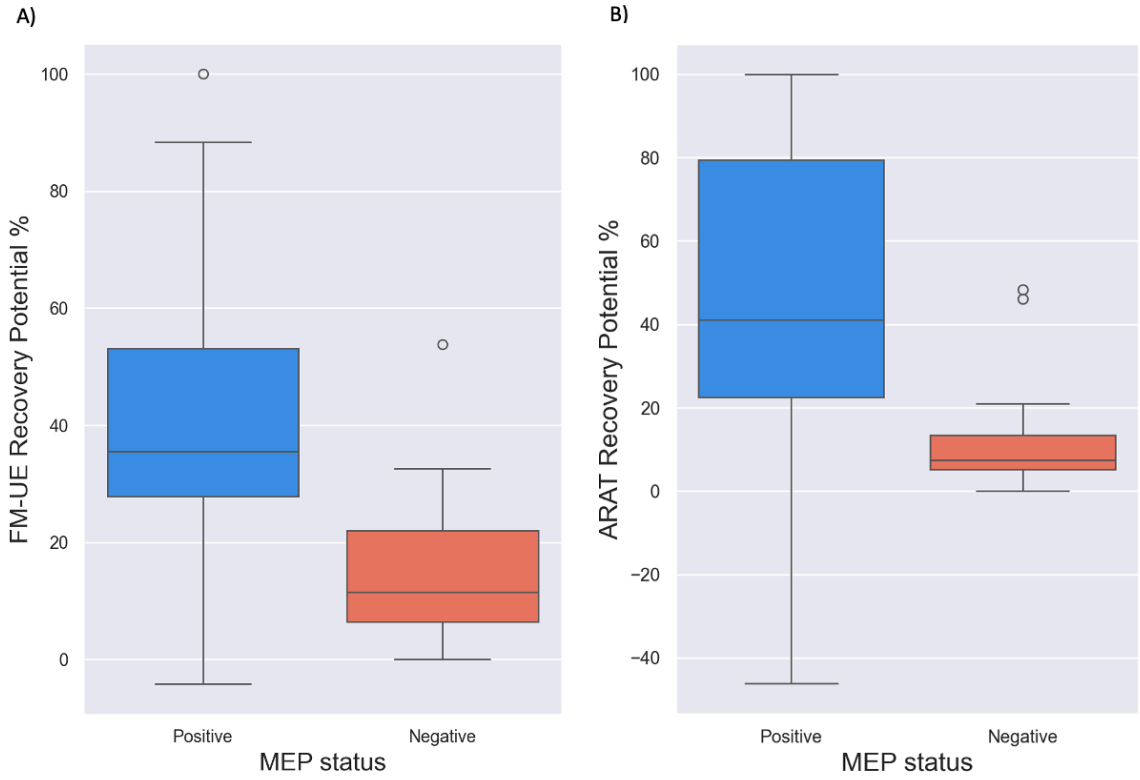


Figure 8: A) Boxplots for Fugl-Meyer upper extremity (FM-UE) recovery potential in percentage and motor evoked potential (MEP) status. B) Boxplots for action research arm test (ARAT) recovery potential in percentage and MEP status. The blue boxplot illustrates MEP positive while the red boxplot illustrates MEP negative subacute stroke patients. A circle indicates an outlier.

2.3.5 Factor analysis

2.3.5.1 Patient characteristics

Data for sex, age and days after stroke are presented as mean ± SD. 48 subacute stroke patients were included in the EFA (15 females and 33 males; 63 ± 12 years old; 32 ± 18 days after stroke.

2.3.5.2 Factor analysis

The factor loadings for each variable are seen in Table 2 and loading plots is seen in Figure 9. A loading above 0.5 is said to contribute to a factor. An explanation of each factor is described below.

Factor 1 is based on rMT, FM-UE Pre, FM-UE Post, ARAT Pre, ARAT Post as well as FM-UE and ARAT potential recovery. The factor can therefore be interpreted as severity of stroke.

Factor 2 is based on ARAT and FM-UE clinical recovery and potential recovery. The factor can be therefore interpreted as quality of rehabilitation.

Factor 3 is based on Age therefore the factor can be interpreted as age.

The factor scores can be seen in Figure 10.

Table 2: Factor loadings. A factor loading above 0.5 is said to contribute to a factor and marked as bold. Each factors variance is written in the parentheses.

	Factor 1 (37.9%)	Factor 2 (22.1%)	Factor 3 (10.8%)
Age	-0.062	-0.003	**-0.835**
Days after stroke	-0.144	-0.345	-0.029
rMT	**-0.756**	-0.119	0.153
PN	-0.185	-0.255	-0.366
FM-UE Pre	**0.938**	0.037	0.281
FM-UE Post	**0.850**	0.405	0.249
ARAT Pre	**0.973**	-0.025	0.170
ARAT Post	**0.864**	0.435	0.195
ARAT CR	0.125	**0.915**	0.119
FM-UE CR	-0.065	**0.756**	-0.002
ARAT PR	**0.529**	**0.596**	0.332
FM-UE PR	**0.567**	**0.581**	0.329

rMT: resting motor threshold, MT: motor threshold, PN: peak negative, FM-UE: Fugl-Meyer Upper Extremity, ARAT: Action Research Arm Test, CR: clinical recovery, PR: potential recovery.

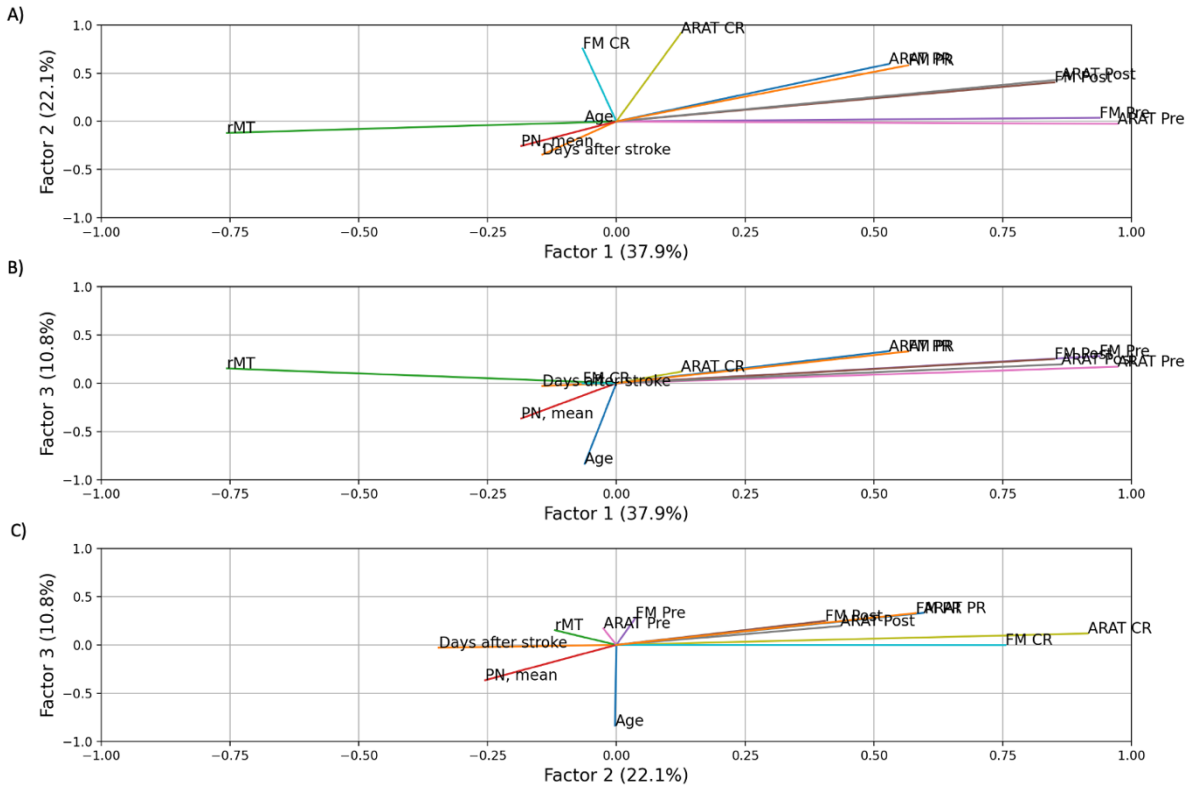


Figure 9: A) Loading plot for factor 1 and factor 2 with each variable. B) Loading plot for factor 1 and factor 3 with each variable. C) Loading plot for factor 2 and factor 3 with each variable. Factor 1 is interpreted as severity of stroke, factor 2 is interpreted as quality of rehabilitation while factor 3 is age. The variance for each factor is written in parentheses. rMT: resting motor threshold, PN: peak negative, FM: Fugl-Meyer Upper Extremity, ARAT: Action Research Arm Test, CR: clinical recovery, PR: potential recovery.

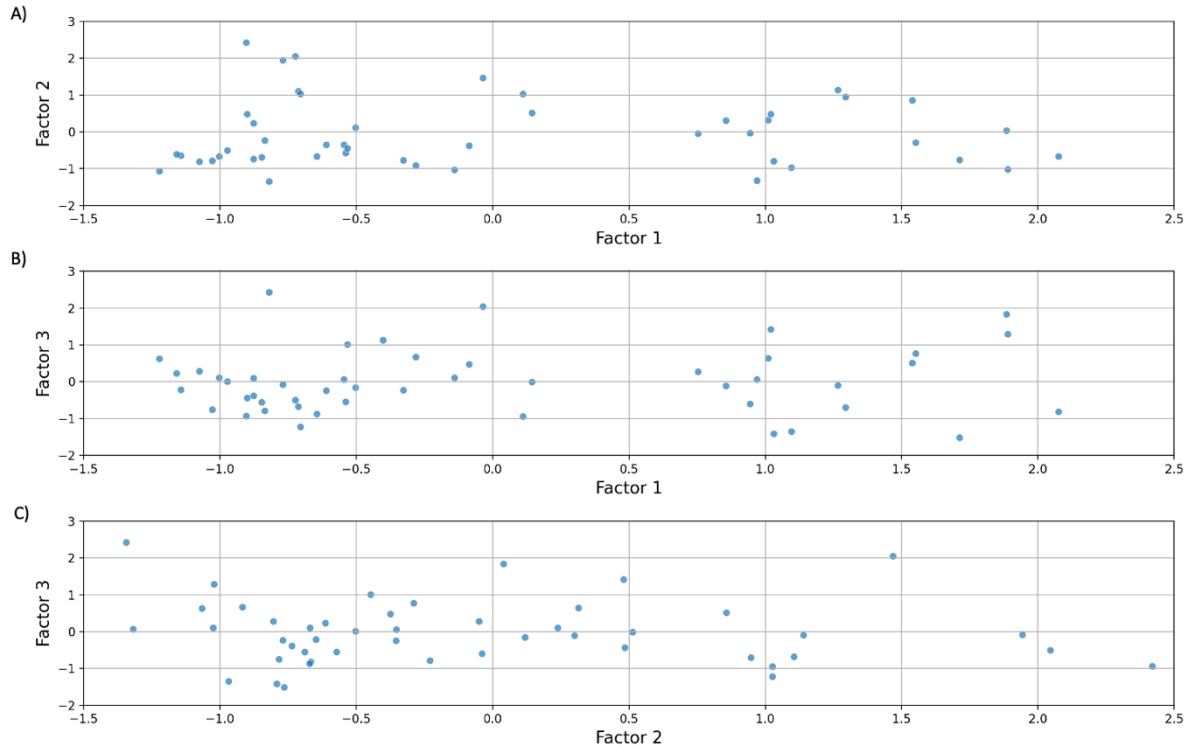


Figure 10: A) score plot for factor 1 and factor 2 with individual factor scores. B) score plot for factor 1 and factor 3 with individual factor scores. C) score plot for factor 2 and factor 3 with individual factor scores. Factor 1 is interpreted as severity of stroke, factor 2 is interpreted as quality of rehabilitation while factor 3 is age.

2.3.6 Prediction

2.3.6.1 Patient characteristics

Data for sex, age and days after stroke are presented as mean ± SD. 48 subacute stroke patients were included for each prediction model (15 females and 33 males; 63 ± 12 years old; 32 ± 18 days after stroke).

2.3.6.2 Evaluation of models

The prediction model for FM-UE received a MAE of 3.47 points. The distribution of base models and final predictions compared to y test data is seen in Figure 11A. Comparison between final predictions and y test data is seen in Figure 12A.

The prediction model for ARAT received a MAE of 3.86 points. The distribution of base models and final predictions compared to y test data is seen in Figure 11B. Comparison between final predictions and y test data is seen in Figure 12B.

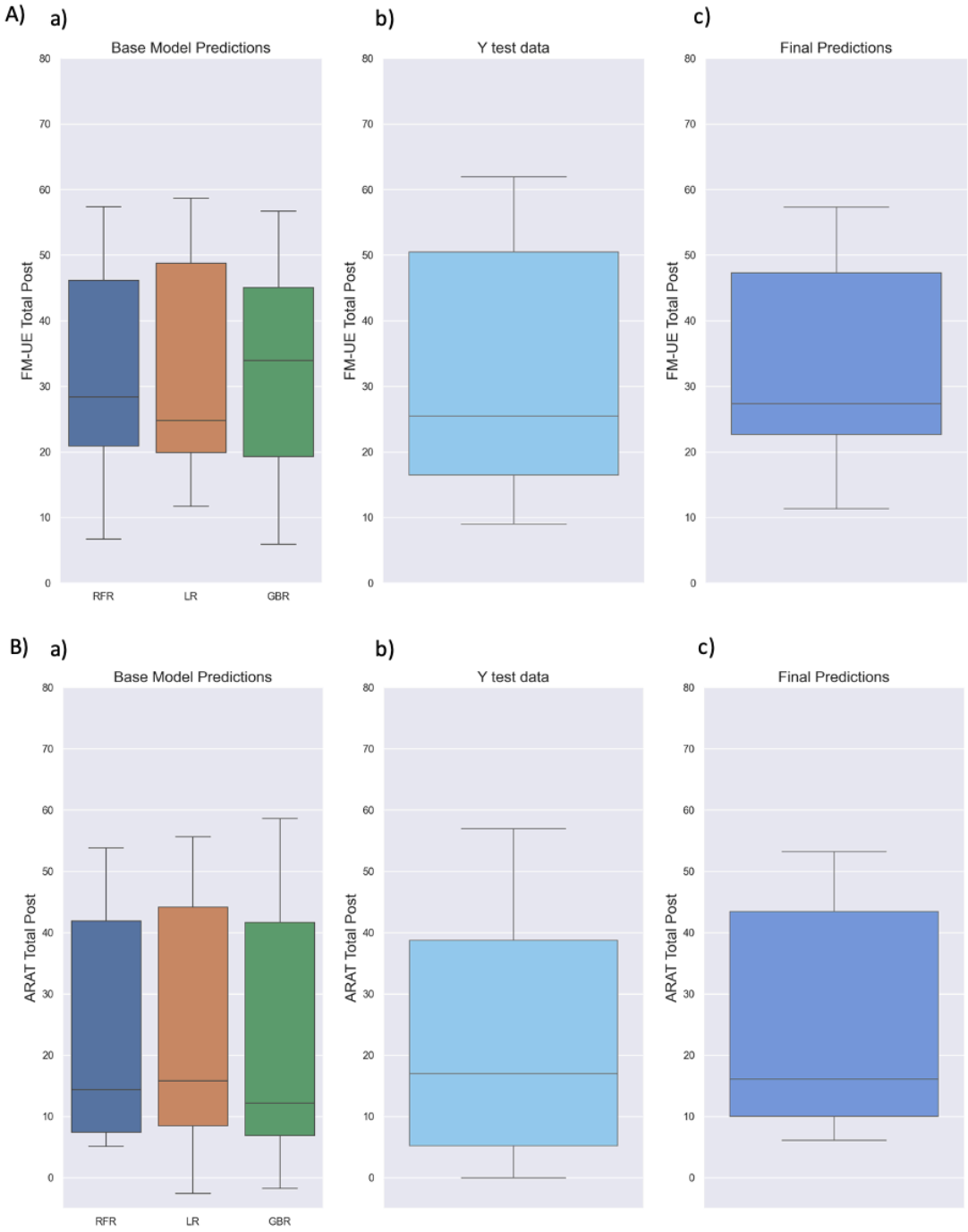


Figure 11: Aa) shows the distribution of each base model prediction for the Fugl-Meyer upper extremity (FM-UE) prediction model. Ab) shows the distribution of Y test data for FM-UE prediction model. Ac) shows the distribution of final predictions for FM-UE prediction model. Ba) shows the distribution of each base model prediction for the action research arm test (ARAT) prediction model. Bb) shows the distribution of Y test data for ARAT prediction model. Bc) shows the distribution of final predictions for ARAT prediction model. RFR: Random forest regression, LR: Lasso regression, GBR: Gradient boosting regression.

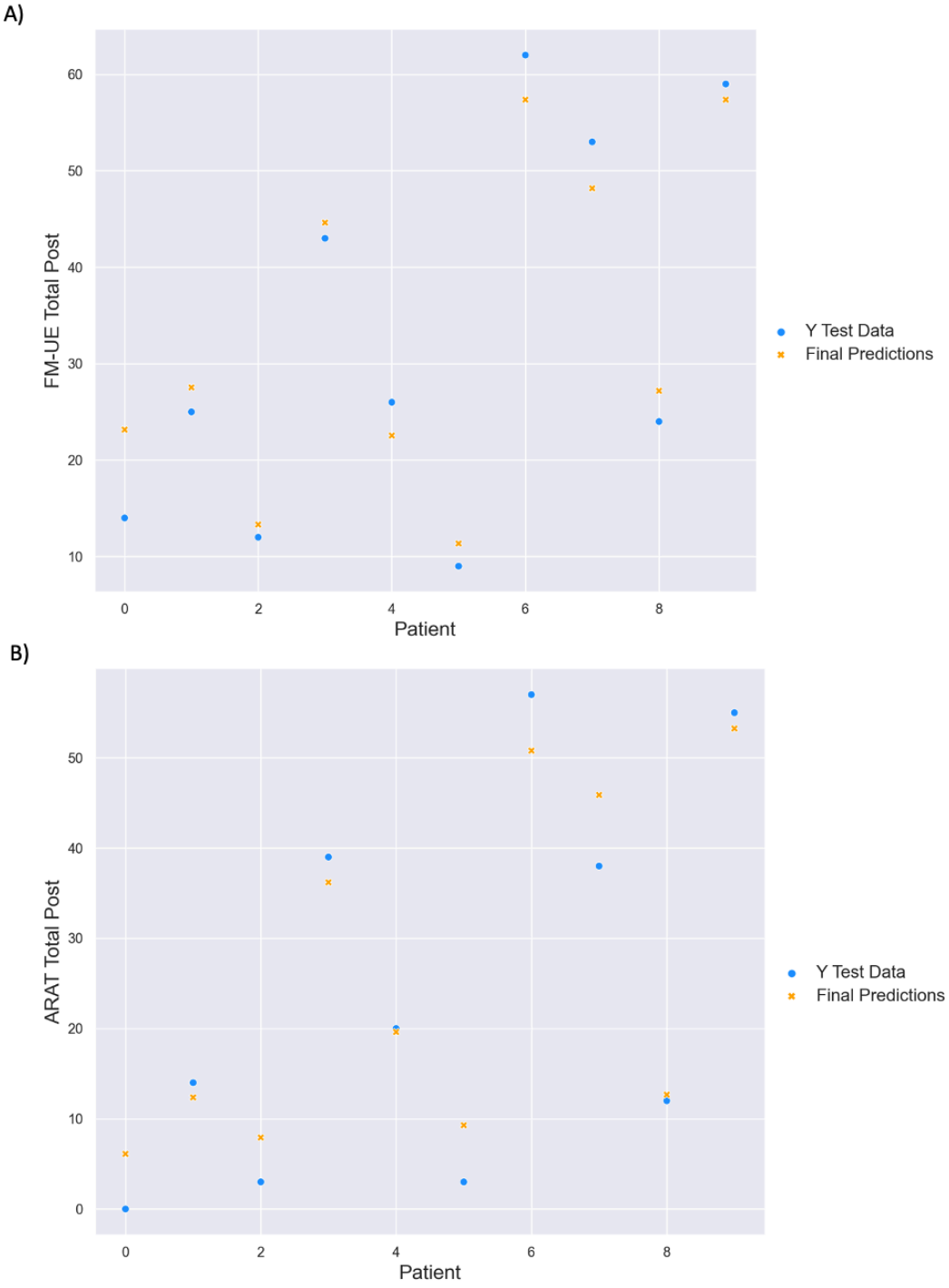


Figure 12: A) Comparison between final predictions (orange) and y test data (blue) for each patient for the Fugl-Meyer upper extremity (FM-UE) prediction model. The x-axis shows the patients, and the y-axis shows the FM-UE total post score. B) Comparison between final predictions (orange) and y test data (blue) for each patient for the action research arm test (ARAT) prediction model. The x-axis shows the patients, and the y-axis shows the ARAT total post score.

2.4 Discussion

The aim of this study was to investigate the effect of ArmeoSpring on subacute stroke patients. Furthermore, it aimed to identify underlying factors that are most important for recovery and use them to create two prediction models.

The presented results showed that both the ArmeoSpring intervention and control groups significantly improved their clinical scores over four weeks, but there was no significant difference between the groups. Furthermore, a positive relationship was found between training time and sessions in ArmeoSpring and potential recovery which might indicate that more training time and sessions in ArmeoSpring is associated with a better recovery. This relationship was statistically significant between ARAT potential recovery and time spent in ArmeoSpring but was not found significant for the other relationships.

For the EFA across all patients three underlying factors were identified to explain the variance between the observed variables. Factor 1 is based on rMT, FM-UE pre, post, potential recovery and ARAT pre, post potential recovery and can therefore be interpreted as severity of stroke and explained 37.9% of the variance. Factor 2 is based FM-UE and ARAT clinical recovery and potential recovery and can therefore be interpreted as quality of rehabilitation and explained 22.1% of the variance. Lastly, factor 3 is based on age and explained 10.8% of the variance. Taken together, this suggests that three underlying factors can explain the variance among the observed variables, where severity of stroke and quality of rehabilitation explains the most variance.

Two prediction models were created to predict the FM-UE and ARAT outcome. The prediction model for FM-UE received a MAE score of 3.47 points and the prediction model for ARAT received a MAE score of 3.86 points.

2.4.1 The effect of ArmeoSpring

Based on the results from ArmeoSpring there was no significant difference in the clinical improvements between the intervention and control groups, indicating that ArmeoSpring did not influence improving arm function in subacute stroke patients. But it should be noted that the sample size consisted of 48 patients and thereby the sample size might not be large enough to give a significant difference and a small sample size does not representant the whole population. A larger sample size could be a better representation of the population and might lead to a statistically

significant difference. A study by Rémy-Néris et al. (2021) included 215 subacute stroke patients for training with ArmeoSpring. They found no significant difference between the groups even though the training intensity was increased compared to this present study[37]. This indicates that even though the sample size and training intensity would be increased it won't lead to significant difference between the groups.

Furthermore, there were only 17 patients in the intervention group compared to the control group that consisted of 31 patients. This give an inequality between the groups and thereby there may not been enough patients in the intervention group to give a significant difference between the groups, though there was no significant difference between the intervention group and control group regarding sex, age and days after stroke.

Another reason for no difference between the groups may be due to other training therapies the patients received during their rehabilitation. This also explains the significant improvements observed in the control group and has been observed in several other studies where the control group received other training during the study. However, these studies achieved different results[34–39] . In the studies by Rémy-Néris et al. (2021), Sehle et al. (2021) and Bartolo et al. (2014) no difference between the groups were found[35–37] while in Esquenazi et al. (2021), Gueye et al. (2021) and Daunoraviciene et al. (2018) there was found a significant difference in arm function between the groups[34,38,39]. Comparing this literature to this present study it can therefore not be concluded that other training therapies in the control group were the reason for no significant difference between the groups.

Patients in the intervention group improved their arm function from pre to post, but session number and time spent using ArmeoSpring differed within the group. With a SD of 4 training sessions and 132 minutes there was a relatively large span between the patients training in ArmeoSpring. But even though the patients did not train equally with ArmeoSpring the regression analysis shows a significant relationship between time spent in ArmeoSpring and ARAT potential recovery. Together with the other results from the regression analysis it seems like there is tendency towards that more training with ArmeoSpring leads to better recovery due to positive unstandardized beta coefficients and positive slopes on Figure 5. It might have influenced the effect of ArmeoSpring if the patients in the intervention group had undergone the exact same amount of sessions and time

in the ArmeoSpring or an increase in training with ArmeoSpring. On average, the patients trained four hours with ArmeoSpring, compared to other studies where the patients trained 14 hours in average with ArmeoSpring[33–40]. Here, the studies by Rémy-Néris et al. (2021) and Colomer et al. (2013) should be highlighted since the patients received in average 32 hours of training with ArmeoSpring[33,37]. As mentioned, Rémy-Néris et al. (2021) found no difference between groups in improving arm function[37], while Colomer et al. (2013) found significant improvement in arm function from onset to end of training[33]. It could be discussed what the correct amount of training with ArmeoSpring is necessary to achieve an effect for each individual patient. It was reported in a study by Nielsen et al. (2015) that approximately 10,000 hours or 3 hours of training every day for 10 years is necessary to induce neuroplasticity and thereby improve motor function[46]. But it is almost impossible to reach this amount of training at a rehabilitation center. Therefore, it can be discussed for this present study if the correct amount of time spent in ArmeoSpring was reached. Based on this it would be of interest to conduct a study where the intervention and control group are matched for training intensity and duration. Even then, it will be difficult to reach this amount of training time each week, since the patients also undergo several other therapies during their rehabilitation, and some of these patients might experience cognitive difficulties that compromise this goal[1,9,14].

2.4.1.1 Factors affecting recovery

It is well-known that MEP status can influence clinical recovery[47–49], which is seen in the PREP2 algorithm where patients who are MEP negative are more likely to have a limited to poor outcome compared to MEP positive who are more likely to have a good outcome[30,31]. Our findings concur with these studies as we found a significantly higher recovery potential in the MEP positive compared to the MEP negative patients, which is also illustrated in Figure 8. Furthermore, in Figure 6 there was a tendency towards that those patients with a lower pre score had a lower potential recovery. The patients with a low pre score and a low potential recovery seems more likely to be MEP negative while the patients with a low pre score and a higher potential recovery are MEP positive. In the EFA factor 1 consists of rMT and the pre, post and potential recovery clinical scores which are interpreted as the severity of stroke. rMT is related to the MEP, so a higher rMT might indicate that a patient is closer to or are MEP negative. In Figure 9 there is a tendency towards a higher severity of stroke being associated with a higher rMT and a lower clinical score both in pre,

post and potential recovery. This means that a high severity of stroke leads to a lower recovery of the arm function for stroke patients.

Results from this study showed that a rapid start of rehabilitation following stroke is crucial to regain lost function, which is consistent with other studies[6]. This is seen in the regression analysis where a significant relationship was found between days after stroke and potential recovery for ARAT. This is also supported in Figure 7 where negative beta coefficients indicate that patients who start rehabilitation early have a better recovery. The relationship between days after stroke and potential recovery for FM-UM are near-significant. Although this finding is insignificant, it can be surmised that early commencement of rehabilitation can contribute to recovery of arm function in stroke patients. In addition, in the EFA it is seen that days after stroke has the highest factor loading towards factor 2 which is interpreted as quality of rehabilitation. This is also seen in Figure 9 where a longer period after stroke can mean a lower quality of rehabilitation and thereby less recovery.

The PRR states that stroke patients will regain 70% of their maximum FM-UE potential recovery in the first three months based on FM-UE[29]. As described earlier Figure 6A shows that patients with a lower pre score have a lower potential recovery and that only four out of 48 patients regained 70% or more of their maximum potential recovery. But the average days after stroke for patients in this present study was 32 days which mean that the remaining 44 patients would still have time to reach their maximum potential recovery. Furthermore, PRR call a group of patients for non-recovers since they do not follow the rule, and these patients will have a FM-UE score under 20 points[29]. Figure 6A shows that 26 patients have a FM-UE pre score under 20 points which mean that these patients might not regain 70% of their potential recovery. In addition, 16 of these patients are MEP negative status which as mentioned earlier is also a factor for a lower recovery. Since the PRR is based on spontaneous recovery optimal rehabilitation is important for these patients to reach or go beyond this goal.

Figure 4A shows that the median baseline score is lower in the intervention group compared to the control group and the slope is steeper for the control group. This indicates that the control group had improved clinical recovery compared to the intervention group which both MEP status, days after stroke and the influence of PRR could be a cause for. A study by Sehle et al. (2021)

showed that stroke patients who trained with ArmeoSpring significantly improved their MEP amplitude compared to the control group, but non-clinical difference was seen between the groups[36]. This could also be the case for this present study, where the intervention group improved their MEP compared to the control group but not enough to impact the clinical outcome. Thereby, this could be an explanation for the lack of difference between the groups, because even though the intervention group significantly improved their clinical scores it is possible that the improvement was too small to be significantly different from the control group. In addition, an article by Chan et al. (2016) showed that ArmeoSpring is most beneficial for stroke patients with moderate to severe arm impairments[40] and since the intervention group seemed to have the most severe arm function compared to the control group in this present study it could lead to the conclusion that the intervention group would have had a lower recovery if they did not train with ArmeoSpring and thereby had led to an even larger difference between the groups.

While the lack of significant differences between the groups was seen, it is of note that a positive relationship between clinical scores and training time in ArmeoSpring with a significant impact between time spent in ArmeoSpring and ARAT was found, but also a significant relationship between days after stroke and potential recovery was seen. This suggests that early and time intense ArmeoSpring training could enhance clinical recovery, which should be investigated in a later study. When conducting studies for rehabilitation of stroke patient it is important to take different factors, such as the MEP status, baseline score and days after stroke into consideration. If the patients are too different in their severity of stroke it can give yield an aberrant result of the rehabilitation method that are investigated. Other factors that can impact on the motor function are engagement and motivation[46], and thereby also valuable to take into account when designing rehabilitation studies and training for stroke patients. Together with the result of this study this also supports the importance of individualized training at the rehabilitation centers, to engage the patients most in their rehabilitation, which eventually could lead to a better recovery.

2.4.2 The performance of the prediction models

The prediction model for FM-UE received a MAE of 3.47 points and for ARAT the MAE were 3.86. The minimum clinically important difference (MCID) for FM-UE and ARAT are 4 to 12.4 and 12 to 17 points, respectively[50–52]. Both prediction models scored a MAE score below the MCID, which means they are capable of predicting the outcome of the arm function accurately. This is

supported in Figure 11 where it is seen that the span for final prediction is inside the span for the Y test data and Figure 12 where the closer the final predictions are to the Y test data the better det model are.

A limitation for the models in this current study can be found in the sample size, which is relatively low, and it might not represent the whole stroke population in order for the prediction model to be accurate enough to predict the outcome. Due to the small sample size, the models might not have been trained with enough data to predict the correct outcome. Several studies have tried to make models that can predict the functional outcome of stroke patients. These studies used data from 132 up to over 4000 patients to train their models with[30,53–61] . Though these models cannot be directly compared to the models of this current study, since they used different models, variables and outcome variables it can still give insight into the sample size that might be required to obtain a more accurate model.

When choosing the variables for the prediction model it should be relevant variables but also easy to collect and relevant for the clinic. In this study age, sex, MEP status, days after stroke as well as the clinical scores total pre value and their subcategories were used as input variables, while the clinical scores total post scores were used as outcome variables. The choice of variables is based on the EFA and clinical relevance. In comparison, the PREP2 algorithm uses SAFE score within three days after stroke, age, MEP status and NIHSS score to obtain an outcome of either “excellent”, “good”, “limited” or “poor”[30,31]. It is seen that the PREP2 algorithm uses similar variables for predicting outcome as this present study like clinical scores, days after stroke, age and MEP status. Furthermore, several other studies have also used input variables like age, sex and clinical scores to predict the clinical outcome[53–61], which further supports the choice of variables for this present study. These studies did not use MEP status as an input variable but results from this present study and several other studies[30,31,47–49] concludes that MEP status is import for a clinical recovery. Therefore, MEP status should be an important factor to take into account when building future predictions models.

2.4.2.1 Choice of model

For this current study stacking method was used to create prediction models. The stacking model uses the advantages from each base model to create the meta model which improves the overall prediction performance, and the model is more robust and accurate compared to any single base

model. However, this tends to increase complexity due to use of several models, thereby increasing the programming level of the model[62]. In a review by Mahajan et al. (2023) stacking, boosting, bagging and voting methods were compared in relation to diabetes, skin cancer, kidney disease, liver disease and heart condition. It was demonstrated that boosting and bagging was the most frequently used but stacking method showed the highest accuracy in prediction[63]. The study was not conducted for stroke and therefore, it could be interesting to conduct a study that evaluates the performance of the stacking method on stroke to see if similar results occur.

In this study, three base models were chosen: RFR, LR and GBR. All three models have been used to predict functional outcome in stroke patients[54–61]. Here, the LR proved to be the most inaccurate model for predicting functional outcome[59] and it could be discussed if this model should have been replaced. LR was used since one of its advantages is selection of irrelevant variables especially if the number of variables is relatively large compared to the sample size[64,65]. If the number of variables is relatively large compared to sample size it can result in overfitting of the model. For the FM-UE model ten variables were used compared to a sample size of 45 and for the ARAT model nine variables were used. By using LR it can reduce the number of variables and exclude irrelevant variables and thereby prevent overfitting. But LR can also be too aggressive if important variables are excluded due to high correlation with irrelevant variables and then selection bias occurs[66]. This can be overcome by elastic net regularization. The elastic net uses the regularization L1 from LR but also the regularization L2 from ridge regression. In the L2 the coefficients shrink towards zero but never go to zero because the squared value of the coefficients is used instead of the absolute value like in L1. By combining L1 and L2 in elastic net regularization the advantages from each regularization can be used and controlled through a self-determined weight of each regularization[64–66].

GBR was invented by Jerome Friedman in 1999 and modified in 2001[67]. Since then, an updated version of GBR called extreme gradient boosting (XGB) been released. XGB was invented in 2016 by Tianqi Chen and Carlos Guestrin and it optimize GBR in both effectivity and speed. Furthermore, XGB has several build-in functions that reduces overfitting[68,69]. In a study by Xie et al. (2018) they evaluated both GBR and XGB to predict acute stroke patient outcome in modified ranking scale scores at 90 days based on CT scans, demographic and clinical information. The results showed that both models can make accurate predictions, but XGB have a better performance[61].

This indicates that XGB might be better at predicting clinical outcome than GBR and it could be interesting to use XGB for one of the base models for future studies to increase potential performance of the stacking model.

Recently, deep learning such as the deep neural network (DNN) has been used for prediction models. DNN is inspired by the human brain neural network and it is composed of an input layer, a complex network with several hidden layers and an output layer. Each layer in the hidden layers gets weighted input from the previous layer to improve the performance of the model until it reaches the output layer with final predictions. In an article by Heo et al. (2019) they compared the performance of DNN, RFR and logistic regression for prediction of long-term outcome for acute stroke patients. Here they found that DNN was better at predicting outcome in stroke patients than RFR and logistic regression[57].Another article by Hung et al. (2017) compared DNN with GBR, logistic regression and support vector machine to predict 5-year occurrence of stroke. Here DNN and GBR showed the best prediction accuracy, but DNN was most optimal by using a smaller amount of patient data[70].

2.5 Conclusion

To conclude, this study cannot fully support the effect of ArmeoSpring in subacute stroke patients, but a tendency towards more intense training with ArmeoSpring and a rapid start of rehabilitation following stroke was seen. Together with severity of stroke, MEP status and baseline score these are important factors for a greater recovery. This calls for larger and more controlled studies to fully conclude on the tendency in this present study. The prediction models were able to predict clinical outcome precisely which can lead to a more efficient rehabilitation, but a limitation was the small sample size. Studies with larger sample sizes and different choice of base models like elastic net regression, XGB and DNN are needed to improve the performance of the stacking model.

3. Sub-study 2: Combination of Neurotechnological Interventions to Improve Upper Limb Function in Chronic Stroke Patients: Protocol for a Single Group Pre-Post Study

3.1 Introduction

Most recovery after stroke happens in the acute and subacute phases and little to no recovery in the chronic phase[6]. In the chronic phase, up to 50% of patients still experience upper limb impairments[24] and are quiescent in their recovery[6]. This group of patients is also in need of optimal rehabilitations therapies to regain their lost function and ensure their quality of life[6,12].

Combination therapy is well-used in rehabilitation of stroke patients to improve upper limb function[14]. Recently, neurotechnology has made an impact on rehabilitation therapies for stroke patients and can be used in combination with other often used therapies[11,26].

MT is a well-established therapy where most patients, even those with severe arm impairment can benefit since it does not require movement of the affected arm. In MT, a mirror is placed between the arm and reflects the unaffected arm as if it was the affected arm. When the patient moves the unaffected arm, it will create the visual illusion to the patient that the affected arm is moving[16,17]. The effect of MT has already been proven in several studies. Here chronic stroke patients improved their function in the affected arm[71] and patients training with MT also seems to improve more when compared to a group who did not receive MT[17,72–80].

A new approach of brain state dependent peripheral stimulation based BCI has been developed over the last years which is based on the principle of Hebbian plasticity (Appendix 2, Neuroplasticity). In this BCI method Hebbian plasticity is hypothesized to be induced when sensory feedback is timed to arrive at the brain when the cortical activity is associated with the intention to move is greatest[81]. In a proof-of-concept study by Mrachacz-Kersting et al. (2016), 22 chronic stroke patients received three sessions with the BCI intervention. Thirteen patients underwent the intervention where peripheral electrical nerve stimulation of the common peroneal nerve was timed to reach the motor cortex at PN during actual or imagined ankle dorsiflexion, while the remaining nine patients served as a control group and received peripheral electrical nerve stimulation timed randomly in relation to PN. One session consisted of 30 to 50 dorsiflexions of the ankle. The study showed that the intervention group had significant improvements in both neurophysiological and clinical outcomes compared to the control group[82].

Another study by Mrachacz-Kersting et al. (2019) investigated the same method but in 24 subacute stroke patients. Here the patients underwent three sessions per week for four weeks. A session was composed of 2x30 dorsiflexions of the affected ankle. The patients were randomly allocated to either an intervention group or a control group. Both groups received peripheral electrical nerve stimulation of the common peroneal nerve timed to reach the motor cortex at PN during actual or imagined ankle dorsiflexion. The timing of the electrical stimulation is calculated by the time of PN minus 25 ms, where 25 ms represents the delay for stimulation to reach the patients motor cortex. The intervention group received electrical stimulation equivalent to their motor threshold, while the control group received electrical stimulation just below the patient perception threshold. The intervention group showed significant improvements in clinical scores compared to the control group. Furthermore, only the intervention group showed significant greater MEP amplitudes measured by TMS indicating that the intervention group had a significant increase in excitability of the corticospinal tract[83].

Both studies by Mrachacz-Kersting were performed in the affected foot and thereby it is of interest to examine the effect of the brain state dependent peripheral stimulation method in the upper limb in subacute stroke patients. This was done in the BCI-STAR project by Svejgaard (unpublished). The method was similar to the methods in Mrachacz-Kersting et al. 2019 but the sessions were composed of wrist extension of the affected arm in subacute stroke patients and the radial nerve was stimulated. The method is described in detail under sub-study 1. The results for this study showed that there was no significant improvement in clinical scores between the intervention and control group, but it showed significant improvement in neurophysiological outcomes measured by a significant greater MEP amplitude excited by TMS in the intervention group compared to the control group[43].

To summarize, brain state dependent peripheral stimulation based BCI has the potential to be a novel rehabilitation therapy in subacute and chronic stroke patients but results regarding clinical effects for the upper limb are lacking. Furthermore, results from sub-study 1 showed that there was no significant improvement between groups when training with ArmeoSpring. Since combination therapy is often used in rehabilitation of stroke patients[14] it would be of interest to investigate the effect of BCI together with ArmeoSpring and a well-established therapy like MT on improving arm function in chronic stroke patients. This led to two hypotheses that:

1. Chronic stroke patients would not have significant improvement in FM-UE and ARAT clinical scores from baseline 0 to baseline 1 (measured 4 weeks later).
2. Chronic patients would have a significant improvement from baseline 1 to post FM-UE and ARAT clinical scores when they undergo an intensive four-week training program consisting of BCI, ArmeoSpring and MT.

3.2 Method

3.2.1 Study design and setting

The proposed study is a single group pre-post study and consists of an intensive four-week training program consisting of BCI-training, ArmeoSpring training, and MT. The study will be performed at Neuroenhed Nord, North Denmark Regional Hospital, Brønderslev, Denmark. The study is submitted for approval in the Scientific Ethichs Committee for North Denmark (reference no. N-20240006) and will be performed in accordance with the Declaration of Helsinki. This study protocol follows the Standard Protocol Items: Recommendations for Interventional Trials guidelines[84] and will be registered on ClinicalTrials.gov.

3.2.2 Participants

We aim to recruit 30 chronic stroke patients by advertisements in forsøg.dk, Facebook, public news outlets, and relevant patient associations. Patients are eligible for inclusion if they have sustained a clinical stroke more than six months prior to first examination, have an MRI or CT scan confirming a cerebrovascular lesion, are able to make informed decisions, minimum 18 years old, and experience continued upper limb weakness. Patients will be excluded if they are pregnant, have a current or former drug addiction, have verbal or cognitive difficulties resulting in them not being able to understand the experiment, have a general weakness including severe cardiac and pulmonary disease, have any comorbidity inhibiting motor function, and if they have epilepsy or a history of seizures. Potential participants will receive written information about the study followed by verbal information from a research staff member at a physical meeting. At the meeting written consent will be obtained as well as a time and place for the study to be held will be agreed.

3.2.3 Interventions

The experiment consists of a four-week training program consisting of BCI-training, ArmeoSpring training, and MT. During training the patient will start with BCI-training followed by ArmeoSpring and then MT. The overall experimental design can be seen in Figure 13. Clinical scores will be obtained four weeks prior to training (baseline 0), immediately prior to the start of training (baseline 1) and immediately following four weeks of training (outcome) to evaluate upper extremity function. The clinical scores will be performed by the physiotherapists at Neuroenhed Nord. By examining clinical scores four weeks prior to the start of training it can be verified that no spontaneous improvement has occurred prior to training and thereby the included patients will form their own

control. Furthermore, TMS will be performed at the same time as the clinical scores to determine MEP status. In addition, TMS will be also be performed at session 1, 6, and 12 of the BCI training to examine the excitability of the corticospinal tract before (Pre), after (Post), and 30 min (Post30) after training.

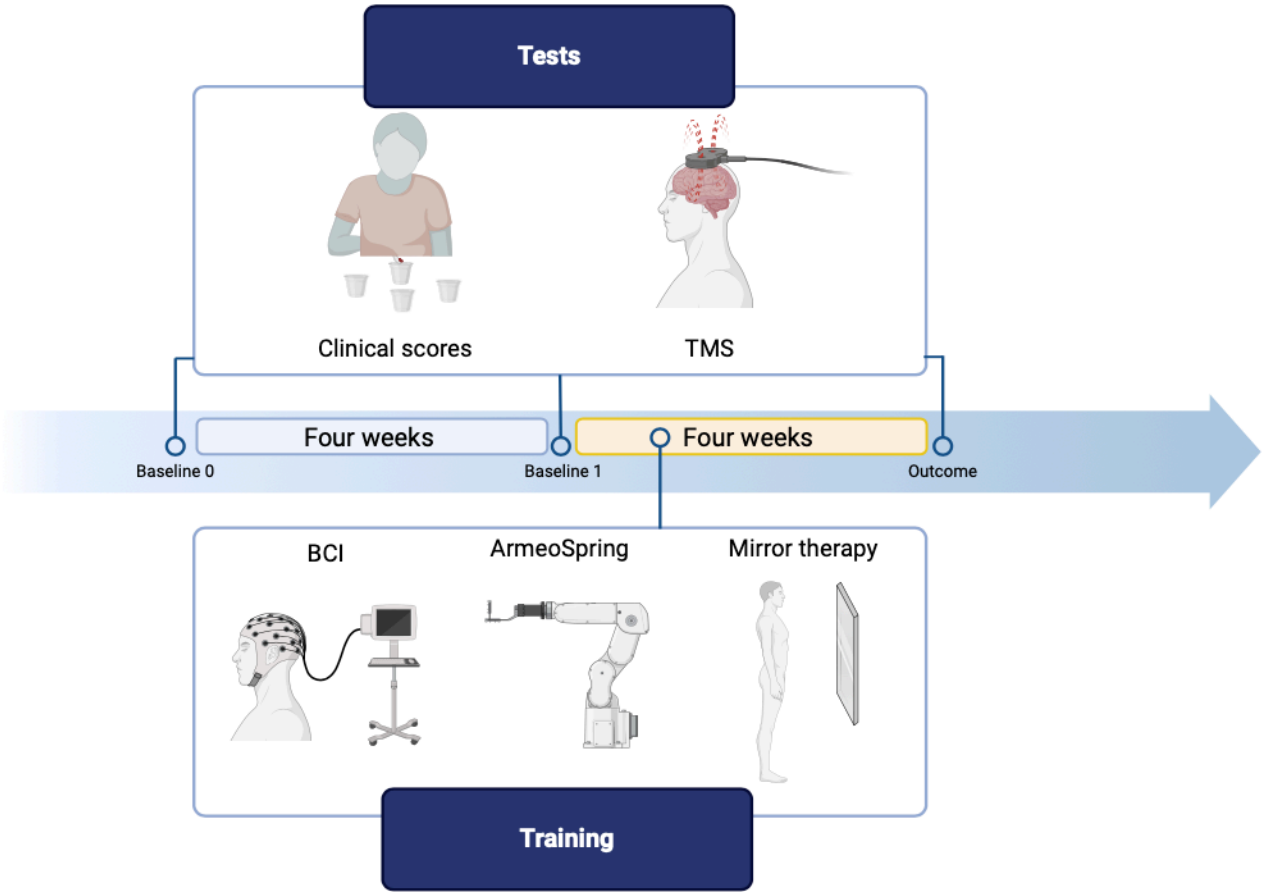


Figure 13: Overall experimental design. Four weeks prior to the start of training (Baseline 0), Immediately prior to training (Baseline 1), and after the four-week training period (outcome), Fugl-Meyer upper extremity (FM-UE) and action research arm test (ARAT) clinical scores and transcranial magnetic stimulation (TMS) will be performed to assess function of the patients' arm and their motor-evoked potential (MEP) status. The four-week training program consists of brain-computer interface (BCI) training, ArmeoSpring training and mirror therapy.

3.2.3.1 Transcranial Magnetic Stimulation

Each patient will undergo TMS together with the clinical scores; one four weeks before the start of training (baseline 0), immediately before the start of training (baseline 1), and immediately after the four weeks of training regime (outcome). In addition, they will receive TMS during session 1, 6, and 12 of the BCI training before (Pre), after (Post), and 30 min (Post30) after the BCI training.

MEPs evoked by TMS will be recorded by electromyography (EMG) surface Ag/AgCl electrodes (20 mm Ambu Neuroline 720, Ambu A/S, Denmark) placed over the extensor carpi radial longus

muscle of the affected arm. Surface EMGs will be pre-amplified and sampled at 2 kHz using scientific software Mr. Kick II 2.3 (Knud Larsen, SMI®, Aalborg University, Denmark).

To determine a patients MEP status a monophasic Magstim 200 TMS stimulator (Magstim Company, Dyfed, UK) with a focal figure of eight coil (70 mm diameter) will be used to apply single TMS pulses to evoke a MEP in extensor carpi radial longus muscle of the affected arm. The coil will be positioned to produce posterior-to-anterior current flow in the ipsilesional primary motor cortex. A patient will have a MEP positive status if amplitude of MEPs is seen at a consistent latency (±3 ms) in at least eight stimulations in the recorded muscle.

To examine the excitability of the corticospinal tract before (Pre, after (Post), and 30 min (Post30) after training in session 1, 6, and 12 of the BCI training a monophasic Magstim 200 TMS stimulator (Magstim Company, Dyfed, UK) with a focal figure of eight coil (70 mm diameter) will be used to apply single TMS pulses to evoke a MEP in extensor carpi radial longus muscle of the affected arm. First the hotspot on the head corresponding to the contralateral motor cortex controlling the desired muscle will be found. This will be done by stimulating at a 5-7s interval at different sites by moving the coil in approximately 1 cm steps anteriorly and laterally from vertex. The hotspot will be marked with a felt pen as the location with the highest peak-to-peak amplitude of the MEP elicited at approximately 50% of maximal stimulator output. After location of the hotspot the rMT, where 50% of the stimulations leading to a peak-to-peak amplitude of the MEP of more than 50 µV will be found. The rMT will be found using the software TMS motor Threshold Assessment Tool (MTAT 2.1). Hereafter, ten stimulations of six different intensities (90, 100, 110, 120, 130 and 140% of rMT) will be given in a random order for a total of 60 stimulations. If a patient is MEP negative 10 stimulations at 100% of maximum stimulator output (MSO) will be performed.

For the data management for TMS conducted at the BCI training sessions, the mean of the 10 stimulations for each of the six intensities at Pre, Post and Post30 will be determined for each patient. MEP max will be found in the Pre TMS conduction for all three TMS sessions. Then the mean for each TMS conduction will be found as a fraction of the Pre MEP max for Pre, Post, Post30 measurements. The MEP data will be converted to fractions of the maximum MEP (MEP max)

from the pre-measurements of the session in question. Across the six different intensities, the mean of the fraction of pre-MEP max for PRE, POST, and POST30 will be determined for each patient. Lastly, the mean of pre-MEP max across intensities for the three TMS sessions will be calculated for each patient and the data will be processed as such. In MEP data, MEP negative patients will be excluded.

3.2.3.2 Brain Computer Interface

The procedure for the BCI training is similar to the BCI-STAR project[43]. Each patient will receive up to three weekly BCI training sessions and up to 12 sessions overall with BCI training. During a BCI training session the patient will be seated comfortably with their affected arm resting on the armrest. Then the patient will be equipped with an EEG cap (g.GAMMAcap^2, gTec Medical Engineering GmbH, Austria) to record monopolar EEG signal from FP1, Fz, FC1, FC2, C3, Cz, C4, CP1, CP2 and Pz according to the standard international 10-20 system. The EEG cap will be connected to a g.USBamp amplifier (gTec Medical Engineering GmbH. Austria) and the channel selection will be based on the large Laplacian filter, with Cz as the central channel. The reference electrode will be placed on the left or right earlobe and the ground electrode on Fz. Single channel surface EMG electrodes will be placed over the extensor carpi radial longus muscle of the affected arm to record muscle activity. All EEG signals will be sampled at a frequency of 256 Hz and hardware filtered from 0 to 100 Hz. EMG data will be collected with a g.USBamp amplifier (gTec Medical Engineering GmbH, Austria) at a sampling frequency of 256 Hz.

Stimulation electrodes (32mm, PALS! Plantinum, Patented Conductive Neurostimulation Electrodes, Axelgaard Manufacturing Co., Ltd. USA) will be placed over the radial nerve of the affected arm and connected to a Noxitest isolated peripheral stimulator (IES 230, Aalborg, Denmark). Then the motor threshold will be found by stimulation in 1 ms pulses increasing the amplitude until an activation of the tendon of the extensor carpi radial longus muscle will be felt over the wrist. In the first part of the training, the patient will be asked to perform 30 wrist extensions of the affected arm in relation to a visual cue without electrical stimulation. The cue will be provided by a custom-made Matlab script (R2017b, Matworks®) on a screen placed 1.5 m in front of the patient. The instructions will be to relax for 3 seconds, focus for three seconds, prepare for two seconds, and perform or imagine a rapid wrist extension retaining the tension for two seconds. This will be used to visualize and calculate the MRCP to assess the time of PN.

Continuous EEG signals will be filtered by Matlab software (R2017b, Mathworks®) using a second order band-pass filter from 0.05 to 10 Hz. Then EEG data will be divided into four second epochs with data two seconds before and two seconds after the cue for each movement. Hereafter data from 500 ms before and 500 ms after the movement will be chosen and PN outside this window will be discarded.

Furthermore, epochs with recorded activity greater than 400 µV will be also discarded. The average of the remaining epochs will be used to define the time of mean PN as the lowest value in relation to the visual cue. The time of PN will be used to calculate the timing of the electrical stimulation. The timing of the electrical stimulation will be calculated by the time of PN minus 25 ms, where 25 ms represents the delay for stimulation to reach the patients motor cortex. In the second part of the training the patients will be asked to perform 30 wrist extensions of the affected arm in relation to visual cue, but with electrical stimulation.

3.2.3.3 ArmeoSpring

For this procedure the patient will be placed in a chair and the ArmeoSpring exoskeleton will be adjusted to the individual patient by a physiotherapist. For the first session the patient will perform a series of initial tests for the ArmeoControl to evaluate the patient's arm function. Hereafter the patient will train with the ArmeoSpring in various games that will be adjusted to the individual patient by a physiotherapist based on arm function and cognitive state. A physiotherapist can find a game unacceptable for a patient if the game is too difficult for the patient to perform or if the game requires a high cognitive state. Instructions from physiotherapist about which games are acceptable for each patient will be followed. The patients will receive up to three weekly training sessions and up to 12 sessions overall with ArmeoSpring. One training session will last 30 minutes.

3.2.3.4 Mirror therapy

For this procedure the patient will be placed in a chair in front of a table and instructed to remove any rings/watches from the non-impaired arm. The patient's arms will be placed on the table with a mirror box between the arms. The impaired arm will be placed and obscured behind the mirror, while the unimpaired arm will be placed in front of the mirror. The patient will then be instructed by a physiotherapist to perform gross, grasp and grip movements with the unimpaired arm while looking in the mirror. The patients will receive up to three weekly sessions and up to 12 sessions overall with MT. One training session will last 20 minutes.

3.2.4 Outcomes

The participants descriptive data includes age, sex, location and type of stroke, date of stroke, MEP status as well as FM-UE and ARAT clinical scores. The data will be collected through self-reporting and medical records.

3.2.4.1 Primary outcome

The primary outcome will be improvements in the clinical scores FM-UE and ARAT. The scores will be obtained four weeks prior to the start of training, immediately prior to the start of training and immediately following four weeks of training to evaluate upper extremity function.

3.2.4.2 Secondary outcome

The secondary outcome will be MEP status which is determined by TMS. MEP status will be obtained four weeks prior to the start of training, immediately prior to the start of training and immediately following four weeks of training to evaluate upper extremity function. In addition, TMS will also be performed at session 1, 6, and 12 of the BCI training to examine the excitability of the corticospinalis tract before (Pre), after (Post), and 30 min (Post30) after training in MEP positive patients.

3.2.5 Harms

Due to risk of epilepsy seizure in patients with former or unknown epilepsy when using TMS the patient's medical history will be evaluated and patients with known epilepsy will be excluded. To address the risk of unknown epilepsy in patients, precautions will be taken even when there is no medical history of epilepsy. Before a patient is exposed to TMS they will be asked to fill out a written questionnaire to evaluate the risk for TMS complications. The questionnaire will be designed to avoid inclusion of persons with head injury, pregnant women or women who might be pregnant as well as patients with a family history of epilepsy. Furthermore, the research staff will be trained in recognizing symptoms of epilepsy seizures and how to provide first aid in the case of seizure. The patient will be encouraged to report any minor or major adverse events during the study and will be advised that they at any time can withdraw from the study at any time with no explanation required.

3.2.6 Data management

All data will be collected and managed in the secure, web-based software platform REDCap hosted at the Region of Northern Denmark[85,86]. Paper documents, such as written consent forms, will be stored in a locked cabinet in an area of limited access.

3.2.7 Sample size estimation

A power calculation was conducted in G*Power (V.3.1.) to determine the sample size and based on former published meta-analysis about the effect of mirror therapy, where the smallest effect size reported was 0.5[87]. We expect the effect of combinations therapy with BCI, ArmeoSpring and MT will increase by 20% for this study. Therefore, the effect size was set to 0.6. The power calculation based on Wilcoxon signed-rank test (parried data) showed 25 participants is required to achieve a power of 80% with a significant level of 0.05. To allow for a 20% dropout risk, 30 subjects will be recruited.

3.2.8 Statistical analysis

The data analysis will be conducted in Statistical Package for Social Science (SPSS, version 29; IBM, USA). The level of significance will be set to 0.05. Descriptive data will be presented as mean and SD or median and IQR where appropriate.

3.2.8.1 Clinical scores

To investigate the primary outcome Wilcoxon signed-rank test will be used to investigate difference in FM-UE and ARAT from four weeks before training to immediately before start of training. Furthermore, a Wilcoxon signed-rank test will be used to investigate FM-UE and ARAT from immediately before start of training to after four weeks of training.

3.2.8.2 TMS sessions

To investigate the secondary outcome a two-way repeated measures ANOVA with time (Pre, Post and Post30) and intensity (90-140% rMT) as factors will be performed on data from each TMS-session.

3.3 Discussion

Up to 50% of chronic stroke patients still experience upper limb impairments. Therefore, they require optimal rehabilitation to regain their lost function and ensure their quality of life[6,12,24]. MT is a well-established therapy in rehabilitation of stroke patients and its effect has already been proven in several studies[16,17,71–80]. Recently, a new BCI method has been developed. This method has proven both clinical and neurophysiological effect for the lower limb in both subacute and chronic stroke patients, both results are lacking in the upper limb[43,81–83]. Based on result from sub-study 1 and that combination therapy is often used in stroke rehabilitation[14] an intensive four-week training program consisting of BCI, ArmeoSpring and MT for chronic stroke patients is presented. The effect of the combination therapy will be evaluated by clinical scores. It is believed that a combination therapy consisting of BCI, ArmeoSpring and MT delivering in an intensive program will increase upper limb function in chronic stroke patients who normally experience quiescent in their recovery[6]. An improvement in their recovery will also lead to insight into optimal rehabilitation therapies for future stroke patients.

Recruitment of participants is expected to begin in 2024 and end in 2026 with results to be accessible in 2027.

3.3.1 Ethics and dissemination

The study is submitted for approval in the Scientific Etichs Committee for North Denmark (reference no. N-20240006) and will be performed in accordance with the Declaration of Helsinki. Each patient will fill out a written consent form prior to any study activities. Danish legislation for secure data storage and use will be complied. Personal data will be deleted after the end of study, while data from the study will be anonymized and saved. Participants will remain anonymous and pseudonymized. None of the researchers or funds involved have any economic interest in this study. All results will be published regardless of the outcome.

3.3.2 Funding

The study is funded by Melsen Fonden, Sundhedsinnovationspuljen at Region Northern Denmark, Grosserer L.F.Foghts fond, Neurological Department at Aalborg University Hospital and Neuroenhed Nord, North Denmark Regional Hospital, Brønderslev, Denmark.

4. General discussion

The general purpose of this master's thesis was to explore upper limb rehabilitation in subacute and chronic stroke patients through neurotechnologies and optimization of rehabilitation through prediction models. In sub-study 1 the effect of the upper limb exoskeleton ArmeoSpring was investigated in subacute stroke patients, where the primary results showed that ArmeoSpring did not influence improving arm function. Furthermore, sub-study 1 showed that factors such as MEP status, baseline score, and days after stroke are important for recovery after stroke regardless of whether patients trained with the ArmeoSpring. Lastly, a prediction model for FM-UE and ARAT was made which were able to predict clinical outcome for subacute stroke patients with a MAE score of 3.47 and 3.86, respectively. In sub-study 2, a proposed study for an intensive combination therapy consisting of BCI, ArmeoSpring training, and MT was presented for chronic stroke patients, and it is expected that this combined neurotechnology-based therapy can significantly improve arm function.

Even though ArmeoSpring did not show an effect in improving upper limb function in subacute stroke patients with the small intensity of training, there was a tendency indicating that more training with ArmeoSpring leads to a better recovery. It is also important to take the individual patients into account when planning out their rehabilitation[14]. Because if a patient enjoys training with ArmeoSpring, it could lead to a better recovery and thereby ArmeoSpring could be implemented to make the most optimal rehabilitation for the individual patient. Furthermore, limitations regarding the effect of ArmeoSpring were outlined and it is proposed that more research is needed. A larger and more controlled study with the specific objective of investigating the effect of ArmeoSpring with a higher training intensity might lead to greater significant results. This can give more insight into the effect of ArmeoSpring for the use of upper limb rehabilitation.

The baseline score, MEP status, and days after stroke showed they are important for recovery in subacute stroke patients. This calls for an early start of rehabilitation after occurrence of stroke to increase the chances of greater upper limb recovery. Both the baseline score and days after stroke are relatively easy to collect for each patient, while MEP status requires specialized equipment and staff education to determine by TMS. According to the PREP2 algorithm the prediction accuracy was increased when MEP status was implemented[30], but the prediction accuracy for PREP2 significantly decreased if SAFE score and MEP status first were obtained on day 13 instead of on

day 3-6[88]. This calls for the importance of implementing MEP status in the early settings following stroke to accurately predict the functional outcome of the patients. It can therefore be discussed whether TMS should be more widely used in the clinic as a part of the assessment of each patient when they are admitted at the hospital or rehabilitation centers after a stroke to optimize the rehabilitation despite the aforementioned limitations of TMS.

The results for the prediction models showed that they can predict an accurate clinical outcome for subacute stroke patients. However, more research with larger training data is still needed to completely evaluate the performance of the model. If a prediction model based on larger dataset can predict the clinical outcome, it can help the patients to set a more realistic goal and handle their expectations which eventually lead to a more efficient rehabilitation[28]. Furthermore, it can help to find the most optimal interventions for the individual patient.

Sub-study 2 focused on chronic stroke patients since this group of stroke patients often experience quiescent in their recovery. This can be due to a decrease in intensity of training after discharge from the rehabilitation centers, which is known to be an important factor for recovery[46]. To increase this intensity, the intensive combination therapy including neurotechnological interventions over four-weeks is presented to improve arm function in chronic stroke patients. An improvement in arm function for chronic patients is believed to still be possible and it is assumed that the same or greater improvement will be seen in both acute and subacute stroke patients since their capacity for neuroplasticity is larger[6]. Based on this, an intensive combination neurotechnology-based therapy could in the future be implemented at the rehabilitation centers both for acute, subacute and chronic stroke patients.

Altogether, this master thesis offers insight into the importance of rehabilitation with an early start and determination of MEP status to improve upper limb rehabilitation, but there is still a need for larger and more controlled studies in this area to fully understand what each patient needs to regain their lost function.

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