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CHANGES IN POST-STROKE SKELETAL MUSCLES: AN ULTRASONOGRAPHIC EVALUATION

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ADL: activities of daily living

AF: Atrial fibrillation

AIS: Acute ischemic stroke

CBF: cerebral blood flow

CBV: cerebral blood volume

CCH: chronic cerebral hypoperfusion

CI: compressibility index

CNS: central nervous system

CPP: cerebral perfusion pressure

CSA: cross-sectional area

CTP: computed tomography perfusion

DM: diabetes mellitus

ECM: extra-cellular matrix

EI: echo intensity

FAC: Functional Ambulation Classification

fMRI: functional Magnetic Resonance Imaging

GM: Gastrocnemius medialis

GL: Gastrocnemius lateralis

SOL: Soleus

RF: Rectus femoris

ICMS: intracortical microstimulation

LACI Lacunar infarcts

MAS: Modified Ashworth scale

MFCV: muscle fiber conduction velocity

MI: motricity index

MT: muscle thickness

MTT: mean transit time

MU: motor units

OEF: Oxygen extraction fraction

PACI: Partial anterior circulation infarcts

PNNs: Perineuronal Nets

POCI Posterior circulation infarcts

SPECT: single Photon Emission Computed Tomography

TACI: Total anterior circulation infarcts

TMS: transcranial Magnetic Stimulation

tPA: plasminogen activator

UPS: ubiquitin–proteasome system

VEGF: Vascular Endothelial Growth Factor

Introduction

Stroke is the second leading cause of death worldwide, with chronic disability remaining in up to 50% of survivors. The improved acute care and the advances in early drug interventions after stroke, as the use of thrombolytic factors, have significantly increased the number of stroke survivors with a reduction of mortality. However, stroke survivors must cope with the long-term effects of stroke, suffering from persistent functional limitations that reduce autonomy in activities of daily life. Long-term disability caused by stroke is largely due to motor function impairment, and it is determined by primary and secondary changes to the acute event. The impairments can manifest progressively in the long-term, causing further modifications and adaptations: the lesions of descending neural pathways lead to altered neuromotor control and functional and structural changes of muscle tissue. Common symptoms in patients with stroke are weakness, hypotrophy, fatigability, and altered motor control, resulting from the combination of denervation, disuse, remodeling, and spasticity. Traditionally, the rehabilitation effort has been oriented toward the treatment of the central nervous system damage with a central approach, which exploits the plastic capacity of the neural cells to recover the best motor control. In this paradigm, muscle tissue is often overlooked. To ensure greater effectiveness of rehabilitation, new protocol approaches are needed, focused on muscle modifications over time.

Recent literature focuses attention on the knowledge of the cascade of transformation at different scales—genetic, molecular, histological, biomechanical, morphological, neurophysiological, and clinical changes—to understand the temporality of the occurrence of these changes and prevent them.

Ultrasonography studies to examine disruptions in the normative architecture of post-stroke muscles have documented changes in muscle thickness, fascicle length, pennation angle, and echo intensity (EI)in patients in chronic patients.

A better understanding of the association between instrumental and clinical features of muscle architecture in the early epoch of stroke may usefully inform clinicians in the appropriate selection of therapeutic approach and improve treatment functional impairment.

1 Stroke

1.1. Stroke definition, mechanism and pathophysiology

The definition of stroke in this study is consistent with the WHO definition as; 'rapidly developed clinical signs of focal, or global, disturbance of the cerebral function, lasting more than 24 hours or leading to death, with no apparent cause other than of vascular origin'.

Stroke is the second most common cause of death and a leading cause of disability worldwide. The incidence of stroke increases with age, leading to a dramatic increase of the global burden of stroke as the population ages. Further risk factors for stroke include hypertension, diabetes mellitus, obesity, atrial fibrillation (AF), smoking, chronic kidney disease, and obstructive sleep apnea. These are largely shared between ischemic and hemorrhagic stroke, which constitute two distinct types of stroke. Ischemic stroke is the most common type, accounting for 87% of all strokes. Ischemic stroke results from a decrease or interruption of focal cerebral blood flow leading to ischemic infarction. The ischemic cascade involves the accumulation of sodium, calcium, and water in the injured brain cells, which leads to the release of excitatory neurotransmitters causing further cell injury of the ischemic territory, where blood flow is most severely diminished, necrotic and excitotoxic cell death occurs within minutes. Thanks to collateral blood blow, cell death occurs less rapidly in the periphery of the ischemic area, which is termed ischemic penumbra.

Hemorrhagic stroke comprises intracerebral and subarachnoid hemorrhage, which account for 10% and 3% of all strokes,

respectively, and are caused by vessel rupture leading to bleeding into the brain or subarachnoid space.

Clinically, stroke manifests as an acute or rapidly evolving episode of focal neurological dysfunction, and further delineation between ischemia and hemorrhage can be achieved with neuroimaging (i.e., computed tomography or magnetic resonance imaging [MRI]). Brief transient episodes of neurological dysfunction attributable to focal ischemia but without neuroimaging evidence for infarction are termed transient ischemic attacks. Conversely, covert brain infarcts may frequently be detected on neuroimaging in patients without clinically manifest stroke as incidental findings, but their clinical importance is increasingly recognized. The clinical severity of stroke is commonly estimated on the National Institutes of Health Stroke Scale, with increasing scores indicating higher severity.

The modified Rankin Scale, a measure of global disability or dependence in daily activities, is commonly used to quantify the functional outcome of stroke.

The past few decades have witnessed major advances in stroke medicine. The introduction of intravenous thrombolysis in 1995 enabled the acute treatment of ischemic stroke, which was revolutionized in 2015 with the advent of mechanical thrombectomy. Advancements in neuroimaging included the widespread implementation of advanced imaging to guide acute reperfusion treatments and the introduction of several MRI modalities, expanding diagnostic capabilities. Stroke unit care and stroke rehabilitation reduce mortality and disability, and have been established as standard components of comprehensive stroke pathways in healthcare systems. Primary and secondary stroke prevention has continuously improved thanks to rigorous randomized clinical trials providing evidence for a vast array of effective interventions.

1.2. Stroke Epidemiology

Stroke is the second most common cause of death ranked after cardiac disease in 2020[1]. Stroke causes more disability than mortality and its social and economic impact is substantial. In 2018 there were reported 25.7 million stroke survivors worldwide (71% being ischemic stroke) and 6.5 million death from stroke (51% being ischemic stroke). In terms of stroke incidence there is some uncertainty as to whether stroke incidence trends are increasing, decreasing or remaining stable. This is because there are few ideal incidence studies where there are complete ascertainment of all cases in a geographically discrete population. Overall since 1990 there was an increase of global stroke burden with an absolute increase in disability-adjusted life years (DALY) more so in developing countries compared to high-income earning countries.

In regards to gender differences, females have an overall lower age-adjusted stroke incidence than males. In a study performed in Sweden the incidence of stroke for females in the age group between 55-64 years was 60% lower than males but higher incidence was found in the group of 75 years and older. The Oxford Vascular Study group investigators found similar trends in the differences between females and males in these age groups.

The risk of death at 5 years after the incident stroke ranges from 36 to 60%. This 5-year mortality rate increases with age. In general, the risk of death for stroke patient is about two to three times that of an age- and sex- matched general population. There are a number of predictors of death after ischemic stroke such as the initial stroke severity, size of stroke on imaging, posterior circulation stroke, advanced age, cardiac condition, atrial fibrillation, reduced conscious state, and hyperglycemia.

Ischemic strokes are caused by interruption of the blood supply to the brain through four mechanisms:

- Thrombosis (obstruction of a blood vessel by a blood clot forming locally)

- Embolism (obstruction of a blood vessel by an embolus formed elsewhere in the body)
- Systemic hypo-perfusion (systemic decrease in blood supply)
- Cerebral venous sinus thrombosis (blood clot in the cerebral veins or sinuses)

Ischemic strokes account for approximately 80% of all stroke episodes. There are various classification systems for ischemic stroke. The Bamford (or Oxford) classification system is commonly used and categorizes stroke based on initial presenting symptoms and clinical signs.

A classification of stroke is assigned to people based on the findings of a clinical neurological examination and a brain computed tomography scan. The following classifications are assigned to people:

- *Lacunar infarcts (LACI)* - A subcortical stroke (i.e. involving the basal ganglia or pons) occurring secondary to small vessel occlusion. Symptoms need to include one of the following:
  - Pure sensory impairment
  - Pure motor impairment
  - Sensory-motor impairment
  - Ataxia
- *Total anterior circulation infarcts (TACI)* – A cortical stroke affecting areas of the brain supplied by both the middle and anterior cerebral arteries. Symptoms need to include all three of the following:
  - Unilateral weakness (and/or sensory impairment) of the face, arm and leg
  - Homonymous hemianopia
  - Higher cerebral dysfunction (i.e. dysphasia or visuospatial disorder)

- *Partial anterior circulation infarcts (PACI)* – A cortical stroke affecting areas of the brain supplied by the anterior cerebral artery. Symptoms need to include two of the following:

  - Unilateral weakness (and/or sensory impairment) of the face, arm and leg
  - Homonymous hemianopia
  - Higher cerebral dysfunction (i.e. dysphasia or visuospatial disorder)

- *Posterior circulation infarcts (POCI)* – A stroke affecting areas of the brain supplied by the posterior cerebral artery. Symptoms need to include one of the following:

  - Cranial nerve palsy and contralateral motor sensory impairment
  - Bilateral motor/sensory impairment
  - Conjugate eye movement disorder
  - Cerebellar dysfunction (i.e. ataxia, nystagmus, vertigo)
  - Isolated homonymous hemianopia

Haemorrhagic strokes account for the remaining 20% of all stroke episodes and are most commonly caused by rupture of small aneurysms. These aneurysms are commonly caused by hypertensive small-vessel disease. Other causes of aneurysms are from intracranial vascular malformations, cerebral amyloid angiopathy, or infarcts into which secondary haemorrhages have occurred. Haemorrhages are divided into two sub-types: intracerebral (bleeding that occurs within the brain) and subarachnoid (bleeding that occurs outside the brain tissue, between the pia mater and arachnoid mater). An intracerebral haemorrhage can occur within the brain tissue (intraparenchymal) or within the ventricles (intraventricular).

1.3. The ischemic penumbra

In 1981, Astrup and colleagues described the ischemic penumbra as functionally silent yet structurally intact brain tissue, lingering between the blood flow thresholds of electrical inactivity and irreversible damage. It was defined as "as: "*the region of reduced CBF with absent spontaneous or induced electrical potentials that still maintained ionic homeostasis and transmembrane electrical potentials*". From there, it was suggested that the penumbra was ischemic tissue that was potentially reversible with timely restoration of perfusion, with the key point that treatment of stroke should focus on salvaging this tissue. In acute ischemic stroke-associated health care, the expression "time is brain" (Figure 1) is used to emphasize the importance of rapid intervention and early restoration of blood flow (reperfusion) in order to salvage the penumbra. We now understand that the penumbra is kept viable in the short-term by metabolic adjustments such as increased oxygen extraction fraction (OEF), as well as by the recruitment of collateral vessels which provide compensatory blood flow from unaffected arteries in the brain.

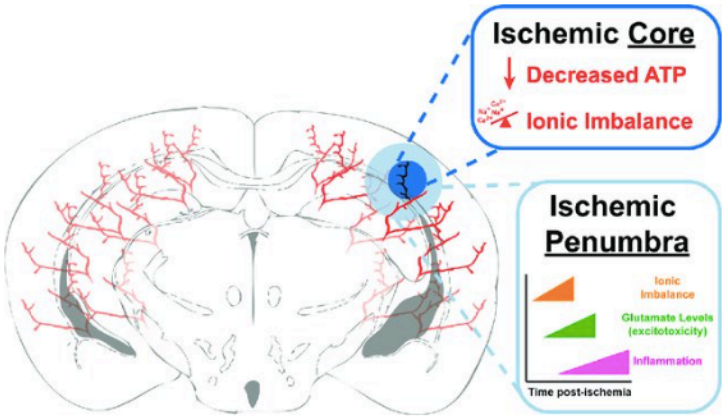

Fig.1 Ischemic Stroke. Schematic diagram of an ischemic stroke in a coronal section of a murine brain. The blocked blood vessel is shown as black and its immediate vicinity is categorized as the Ischemic Core (dark blue). Within the core, cell death occurs rapidly after the ischemia due initially to decreased ATP production causing an improper ionic balance and osmotic pressure. This in turn leads to edema and depolarization, which in neurons causes massive release of glutamate and excitotoxicity due to opening of calcium (Ca 2+) permeable NMDA

channels. The area surrounding the Ischemic Core is categorized as the Ischemic Penumbra and is considered "at-risk" tissue that, as the time post-ischemia increases, is at-risk for cell death due to cell lysis form edema, excitotoxicity from excess extracellular glutamate and eventually, inflammation.

1.3.1. Misery perfusion

Demands on the circulatory system to maintain a constant delivery of oxygenated blood to the brain are high, leaving neuronal tissue susceptible to injury and functional impairment in the case of a sudden drop in tissue perfusion. The healthy brain implements hemodynamic autoregulation in order to compensate for fluctuations in the mean arterial blood pressure. The autoregulatory mechanism is maintained by the continual dilation and constriction of the vascular bed, which allows cerebral regions to sustain a constant ratio between cerebral blood flow (CBF) and the metabolic Misery perfusion is a term used to describe the pathophysiological state during which metabolic and hemodynamic changes are employed to compensate for an acute loss in cerebral perfusion pressure (CPP), as in the case of acute ischemic stroke (AIS) or chronic cerebral hypoperfusion (CCH). The initial reaction following a sudden loss of CPP is a rapid dilation of the vascular bed within affected regions, as well as of the leptomeningeal anastomoses that connect the distal cortical branches of the major arteries of the brain. The dilation of small arteries and arterioles increases the local cerebral blood volume (CBV) within affected areas, thereby raising the CBV/CBF ratio. This is equivalent to a decrease in the rate of large-vessel cerebral circulation and a corresponding increase in the mean transit time (MTT) of red blood cells through tissue. A substantial decrease in CBF and an increase in MTT will result in inadequate amounts of oxygen being supplied to the brain tissue, a pathological condition known as ischemia.

1.3.2. Imaging penumbra

For identifying the salvageable brain tissue in acute stroke, the direct method is to image penumbra. In acute ischemic stroke, the viability and size of penumbra change dynamically [2] in response to regional cerebral blood flow, pathophysiological environment and treatment. Penumbra can be imaged using different technologies, such as MRI, CT [3], PET, and SPECT [4]. For targeting penumbra in stroke patients, imaging penumbra is necessary for monitoring treatment response as well as for patient screening. The "mismatch" of perfusion-weighted and diffusion-weighted images (PWI-DWI mismatch) is the most commonly used method for imaging penumbra and may serve for this purpose [5]. The diffusion-weighted image may represent reversibly injured tissue in the early hours after stroke whereas the perfusion-weighted image may include area of benign oligemia. The mismatched tissue represents "tissue-at-risk", not "tissue-doomed-to die"; therefore it does not identify lesion growth by itself [6]. For infarct expansion see the following paragraph.) Penumbra may resolve spontaneously [7], either by merging with the ischemic core, or becoming normal tissue. When re-canalizational therapy started early enough, the mismatched tissue, the penumbra, may be salvaged, which has been observed using both CT [8] and MRI [9] methods.

1.3.3. The ischemic penumbra: currently available treatments

The aim of ischemic stroke therapy is to reperfuse the penumbra and salvage as much brain tissue as possible to unsure a better clinical outcome. Currently, intravenous injection of tissue plasminogen activator (tPA) is the gold standard medical treatment for ischemic stroke. Another highly successful reperfusion treatment is

mechanical thrombectomy for large vessel occlusion. For both, earlier treatment increases the chance of benefit but it has become increasingly apparent that to focus on stroke onset time to guide decisions is over simplistic. Some patients have little to gain from reperfusion treatment early after stroke onset and others stand to gain many hours later. This relates to size of core and penumbra, and, is underpinned by the collateral supply to the tissue. The comparison of standard clinical predictors (i.e. onset-to-treatment time) with CTP imaging measurements, such as ischemic core and penumbral volumes, showed that imaging parameters (especially infarct core volume) were better predictors of good or bad clinical outcome following thrombolytic treatment than time to treatment, and, that CTP improves the identification of patients who can benefit from tPA from those who are less likely to. Several clinical trials have shown the clinical benefits of salvaging the penumbra in patients suffering from stroke and further identified imaging parameters that could predict the outcome of a given patient from thrombolytic therapies [10–12]. The comparison of standard clinical predictors, such as onset-to-treatment time, with CTP imaging measurements, such as ischemic core and penumbral volumes, showed that imaging parameters (especially infarct core volume) were better predictors of good or bad clinical outcome following thrombolytic treatment than time to treatment, and, that CTP improves the identification of patients who benefit from tPA from those who are less likely to [13,14].

Clinical trials have used the different imaging mentioned above to visualize the core and penumbra and to extend the time windows for reperfusion treatment. DEFUSE, an observational study involving 74 patients and EPITHET, a randomized controlled trial, used PWI/DWI mismatch to examine the time window for tPA to 6 h. They suggested (although not definitively due to too lenient perfusion thresholds) that tPA improved clinical outcome and salvaged the penumbra between 3 and 6 h of stroke onset [15,16].

2. Neuroplasticity after stroke

The term brain plasticity defines all the modifications in the organization of neural components occurring in the central nervous system during the entire life span of an individual. Such changes are thought to be highly involved in mechanisms of aging, adaptation to environment and learning. Moreover, neuronal plastic phenomena are likely to be at the basis of adaptive modifications in response to anatomical or functional deficit or brain damage. Ischemic damage causes a dramatic alteration of the entire complex neural network within the affected area. It has been amply demonstrated, by many studies, that the cerebral cortex exhibits spontaneous phenomena of brain plasticity in response to damage [17]. The destruction of neural networks indeed stimulates a reorganization of the connections and this rewiring is highly sensitive to the experience following the damage [18]. Such plastic phenomena involve particularly the perilesional tissue in the injured hemisphere, but also the contralateral hemisphere, subcortical and spinal regions.

New dendritic spines branch out and proliferate, and effectiveness of synaptic contacts is modulated through a complex network of intracortical and intercortical connections changes. These changes are detectable on different levels, starting from molecular mechanisms at the dendritic spines level continuing with axonal sprouting, involving, finally, the entire neural networks. Through these mechanisms, responsiveness and connectivity of each neuron is continuously modulated by processes of maturation, learning and experience.

In humans, the plastic potential of the brain is not constant throughout the individual life but seems to continue after birth until 18-20 years. However, during life there are periods of maximum plasticity, during which some crucial experiences reach the peak of their effectiveness in promoting proper behavioural development.

However, even if at the end of this time window, the plastic capacity of the brain progressively decreases, it does not disappear completely: in some cases, plastic events can occur also in the adult brain if appropriately stimulated. In fact, in the adult brain it is still possible to observe phenomena of structural plasticity: axonal elongation and pre-post synaptic modifications could be induced by learning and experience or in response to injury. The destruction of neural networks, in fact, usually stimulates a rewiring and a reorganization of the connections and this plastic environment is highly sensitive to the experience following the damage. The motor cortex (as the sensory cortex) shows a topographical organization. It means that it consists of a map of movement clustered for different body parts. These maps are quite stable, but they can change after experience-dependent plasticity or brain injury.

The most convincing evidence of post-stroke spontaneous plasticity in the perilesional area is the observation of topographical map reorganization. Motor cortices show in fact a topographical organization, so that sites evoking movements of specific body parts cluster together. Maps are shaped during early life and remain quite stable in adulthood. Interestingly, they can change even in the adult by experience-dependent plasticity (such as after an intensive training) or after brain injury. Remapping of the motor cortical areas has been observed in stroke patients via either functional Magnetic Resonance Imaging (fMRI) or Transcranial Magnetic Stimulation (TMS). In animal models, reorganization of motor maps has been observed using intracortical microstimulation (ICMS)[19] or optogenetic techniques [20]. Studies on primates have demonstrated that following an ischemic injury to the hand area of primary motor cortex (M1) there is a significant reduction of hand representation if no rehabilitative training is applied. Learning and post-stroke remapping seem to follow different mechanisms, even though they probably share many effectors [21,22].

It is well established that after a small subtotal cortical lesion, peri-infarct areas could actually vicariate lost or damaged functions. For example, following an ischemic injury in M1, premotor areas can remain functional and contribute to recovery. The ventral premotor area, which receives most of its inputs from M1, produces and releases Vascular Endothelial Growth Factor (VEGF), which has angiogenic and neuroprotective properties, in the early phase after the infarct. Indeed, it is proven that after a small subtotal cortical lesion, peri-infarct areas could actually vicariate lost or damaged functions. However, little is known about the cellular mechanisms that lead to these network reorganizations and regain of the lost motor function. It has been proposed that these processes are use-dependent and involve sprouting of new connections as well as the unmasking of pre-existing, normally subthreshold connectivity. The GABAergic system and the extracellular matrix could have an important role in controlling these plastic phenomena. For example, Perineuronal Nets (PNNs), specialized extracellular matrix structures made of condensed chondroitin sulfate proteoglycans, have been correlated with brain plasticity and repair, and preferentially surround the soma of GABAergic neurons, in particular fast-spiking parvalbumin-positive interneurons.

The role of PNNs has been extensively investigated during the maturation of the visual system in relation to the opening and closure of the critical period. PNNs are thought to stabilize mature connections and downregulate spine motility and functional plasticity. Following central nervous system (CNS) injury, the degradation of PNNs, by means of injections of the bacterial enzyme chondroitinase ABC, promotes sensory-motor recovery. The GABAergic system has also been studied in relation to the opening and closure of early “critical periods” in sensory cortices and in post-stroke motor recovery. In humans, a reduced GABAergic inhibition is associated with functional recovery [23]. Stroke induces the production of various inhibitors of neural regeneration, sprouting

and plasticity, such as myelin components (Nogo-A, myelin-associated glycoprotein), and guidance molecules (ephrins, semaphorins). The application of drugs able to neutralize the effect of anti-plastic agents, such as Nogo-A antibodies has been seen to encourage axon regeneration, sprouting and functional recovery in a variety of animal models of cortical and spinal injuries.

After stroke, it has been reported a consistent change in terms of "sprouting markers", for example, GAP43, CAP23, c-Jun, classical axonal growth markers increase in the peri-infarct region. On the other hand, ephrin-A5, chondroitin sulfate proteoglycans (CSPGs) and other growth inhibitory genes, are expressed in proximity of the lesion and at later time points. However, even if such spontaneous plastic changes stimulate cortical reorganization, this is insufficient to promote a full functional recovery.

The incapacity of the CNS to spontaneously recover completely from a brain injury, fully exploiting neuroplasticity, could be a protective mechanism: in fact, such regeneration, sprouting and synaptic plasticity, could be also maladaptive if the connections are not re-established in the adequate way and the complexity of the CNS of mammals makes it extremely subject to this kind of errors [24].

2.1. Neuroplasticity in contralesional hemisphere

Even in the contralateral hemisphere neuronal connections appear to be altered as a result of cortical damage; it has been seen in adult rat, for example, that after a cortical lesion, thanks to axonal sprouting, the healthy hemisphere contacts both the ipsilateral and contralateral (ipsilesional) striatum. The contralateral M1 normally inhibits the ipsilateral hemisphere during motor performance tasks [25]. However, patients with recent stroke commonly demonstrate increased M1 excitability on the contralesional hemisphere (equivalent to the ipsilateral hemisphere for healthy patients) for movements with the affected side [26–28]. Therefore, stroke

patients display an interhemispheric imbalance where the ipsilesional M1 no longer inhibits the contralesional hemisphere and the contralesional side appears to inhibit the ipsilesional [29] possibly through the transcallosal fibers (Figure 2).

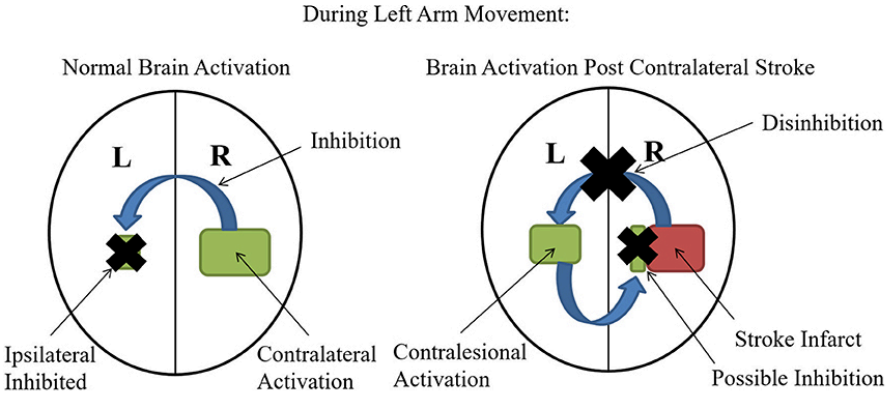

Fig 2. Interhemispheric Imbalance Post Stroke: Normally during unilateral motor performance tasks, activation of the contralateral hemisphere inhibits activation of the ipsilateral hemisphere (black X is showing inhibition of the ipsilateral side). However, when the contralateral M1 is impacted by stroke, this inhibition is lost (black X on the blue inhibitory arrow) and therefore the contralesional (analogous to ipsilateral for healthy controls) becomes more activated. Some studies suggest that this increased contralesional activation may also contribute to the interhemispheric imbalance by imposing increased inhibition on the ipsilesional (contralateral for healthy patients) hemisphere designated by the blue arrow and black X on the ipsilesional hemisphere

The magnitude of such an imbalance appears to positively correlate with the degree of motor impairment and the interhemispheric imbalance in other functional networks may also contribute toward other cortical functional disruptions including neglect and aphasia [30]. Although several studies demonstrate poorer motor recovery with increased contralesional hemispheric recruitment some evidence suggests that contralesional recruitment may play an integral role in post-stroke motor recovery. According to Hoyer et al., the importance and role of contralesional M1 neural activity is probably determined by variables including time since stroke, degree of damage to the motor system, and complexity of the motor task [31].

Therapies that offer repeated action of the impaired arm, including mirror therapy (MT) [32], virtual-reality therapy (VRT) [33], constraint-induced movement therapy (CIMT) [34], and brain-computer interface (BCI) therapy may reverse progressive down-regulation of sensorimotor activity and induce neural plasticity. However, most current rehabilitation methods that can target a specific M1 hemisphere, such as, transcranial magnetic stimulation [35](TMS), focus on ipsilesional hemispheric activation to facilitate motor recovery. Brain-computer interfaces are a newer modality that use reward-based neuromodulatory training to induce use-dependent plasticity and facilitate functional recovery for stroke rehabilitation.

Increased ipsilateral M1 activation has been shown for tasks with higher accuracy or complexity demands in healthy subjects. Additionally, well-recovered stroke patients with chronic striatocapsular motor strokes (≥2 months post-stroke) show notable contralesional motor activity and Schaechter and Perdue demonstrated that increased activation of the contralesional cortical network in chronic stroke (≥6 months post-stroke) patients is associated with good motor recovery [36]. These findings suggest that uncrossed corticospinal tracts, which include roughly 10% of the corticospinal neuronal fibers, play a role in motor movement and possibly in recovery. Touvykine et al. suggests that the contralesional premotor cortex may play a greater role in stroke recovery with larger lesions where damage on the ipsilesional side is more severe and where less ipsilesional neuronal recovery can develop [37]. This indicates that activation of the contralesional hemisphere provides recruitment of additional neural resources due to the increased demands of the damaged ipsilesional motor system [38]. While some research suggests that increased activation of the contralesional M1 may detract from motor recovery [39] recent evidence suggests continued supportive functions of the contralesional hemisphere in a subset of chronic stroke patients [40].

3. Muscle modification after stroke

Morphological changes may occur in parallel at a different level, including intrinsic fiber muscle phenomena (loss of muscle mass, muscle thickness, and decrease in the physiological cross-section area), but also at a more general tissue rearrangement, referring to sarcomere shortening and histological changes in the extracellular matrix. These phenomena may mutually influence each other's and be hardly individualized, but for a clearer and more identifiable discussion they will be treated differently in the next paragraphs. Furthermore, it should be mentioned that in several neurological disorders, characterized by spastic paresis, a dedicated term is used to qualify the specific muscle contracture (spastic myopathy), characterized by both increased muscle tension and stretch-sensitive evolution.

3.1. Stroke-Induced Sarcopenia

In healthy people, muscle tissue is gradually lost during aging, resulting in a decrease in mass and strength, a condition described as sarcopenia [41]. In specific pathological conditions, especially in those that may invoke inflammatory processes, disease-related immobility or malnutrition, sarcopenia can occur as secondary, defining a “specific sarcopenia”. Recently, the muscle atrophy, consequent to the stroke has been defined as a new condition called “stroke-induced sarcopenia” or “stroke-related sarcopenia”; it has also been associated with worse clinical outcomes and physical dysfunction [42].

Furthermore, the impaired function predisposes stroke survivors to inactivity that might contribute to deconditioning, fatigue, and further functional loss. Stroke-induced sarcopenia arises from the combination of multiple mechanisms, including immobilization and

dysfunctional atrophy, impaired feeding, inflammation, sympathetic overactivation, and denervation. The prevalence of stroke-related sarcopenia is higher than the one of general population, matching age, gender, and race of healthy individuals, indicating a specific pathway. Furthermore, this prevalence during the first month is 50% and it is ~34% after 6 months, suggesting that the adaptive responses in muscle tissue may be most pronounced early after stroke [43].

Moreover, the loss of muscle mass after stroke is commonly accompanied by fat deposition, often associated with a common stroke risk factor such as obesity, worsening the outcome [44].

These early changes in muscle, such as loss of muscle mass, reduced fiber cross-sectional area (CSA), and increased intramuscular fat deposition, occur between 3 weeks and 6 months after stroke in both paretic and non-paretic limbs [45]. In a recent study it was shown that the presence of sarcopenia, associated or not with obesity (sarcopenic-obesity), affects the improvement of activities of daily living (ADL), dysphagia, and discharge rates to home, so the researchers stated that the treatment of stroke-induced sarcopenia in a rehabilitative setting is crucial [46]. Some researchers suggested that sarcopenia could be due to the activation of catabolic pathways, especially the ubiquitin–proteasome system (UPS), autophagy, and apoptosis. These mechanisms seem to be in part due to the downregulation of the gene of Sirtuin1 (SIRT1), a key regulatory factor of the energetic status of the cell, counteracting metabolic and age-related disorders. Reduced expression of the SIRT1 gene and reduced SIRT1 activity, resulting in skeletal muscle atrophy, have been demonstrated in post-stroke animal models; similarly, attenuation of muscle atrophy was found when SIRT1 is overexpressed. Moreover, it was demonstrated that the expression levels of myostatin mRNA, which downregulate the skeletal muscle growth, are 40% higher in the paretic than non-paretic muscles in stroke survivors. This could be due to intramuscular fat accumulation, consequent to stroke, that may cause insulin

resistance, and the subsequent hyperinsulinemia has been shown to increase serum myostatin[44]. The knowledge of the molecular mechanisms of stroke-induced sarcopenia allows to identify specific therapeutic approaches. In fact, in a pre-clinical study, the use of resveratrol (RESV), an exercise mimetic drug, during the early acute phase of stroke, limited muscular atrophy, through the activation of SIRT1, and normalized the hypertrophy of slow-twitch muscle fibers (I, IIa), suggesting that RESV may improve oxidative metabolism in stroke-affected muscles [47]. Furthermore, it is known that a stroke patient could suffer from malnutrition, and its early recognition or misdiagnosis significantly affects the outcomes; in fact, malnutrition aggravates sarcopenia because muscle and adipose tissue wasting occurs. Finally, in a very recent study, it was highlighted that muscles of the trunk undergo atrophy in a later post-stroke phase, causing a worsening of the balance this suggests the need to accurately plan both the type of rehabilitation and the timing: patients should be treated pointing on different aims at different stroke phases, for example, in addition to continuous motor control training over time, strengthening and endurance training of trunk muscles could be helpful during the chronic phase [48].

3.2. Stiffness

Paresis induced by stroke leads to a reduced active voluntary movement and a reduced joint range of motion (ROM), often associated with an active or passive mobilization hyper-resistance or stiffness. Stiffness can be due to “neural” or “non-neural” phenomena, such as spasticity, spastic dystonia, or respectively, muscle contractures and soft-tissue fibrosis. Consequently, long-term secondary complications, such as soft tissue contractures, pain, pressure sores, decreased ADL, social isolation resulting in decreased quality of life, can occur. These phenomena seem to be linked to structural changes of intrinsic muscle properties over time, with an

increase of intramuscular connective tissue and fat content. It has been shown that these changes depend on the deposition of hyaluronan in the extra-cellular matrix (ECM), intramuscular fat, and lead to increased viscosity and could immobilize muscle in the shortened position. Indeed, fibrosis and abnormal accumulation of materials in the ECM leads to an increased collagen content and an altered orientation of collagen that likely contributes to a greater transverse tensile stiffness against radial expansion and fascicle shortening. Stiffness in the muscle will generate a constant stimulation of its spindles, triggering the activation of Ia fibers, further tension, failure to release contraction, and a muscle change as serious as the hyperactivity severity (spasticity). Gracies et al. found that muscle fibrosis and the other components of muscle contracture could increase spasticity through an overactivation of spindle afferents, and thus, increase spasticity [49].

3.3. Muscular Metabolism Changes

Stroke patients exhibit impaired metabolism compared to healthy subjects, with increased tissue lactate and glycerol production, delayed and impaired glucose utilization, and slightly increased energy expenditure. This contrasts with the “normal” age-related sarcopenia process, where there is a shift from fast-twitch type IIa/b to slow-twitch type I fibers, which is mainly attributed to the disuse of fast fibers. The paretic muscle of chronic stroke patients shows a smaller overall fiber cross-sectional area (CSA) with a shift toward a low oxidative type IIX fiber content and a reduced type I and type IIA fiber content. As a consequence, muscle resistance in affected limbs is likely decreased, because type IIX fibers are more prone to fatigue, leading to impaired muscle performance. Acute stroke patients rely on carbohydrate utilization during prolonged walking, while healthy individuals rely mostly on fatty acids oxidation; this carbohydrate utilization likely indicates preferentially anaerobic metabolism and

potentially limits the ability to walk for a long time. Conversely, in the chronic phase, other researchers did not find differences in skeletal muscle tissue substrate metabolism between paretic and not paretic leg, even if the energy consumption seems to be higher in the not paretic one; in these studies the increased glycolytic activity and reduced lipolytic activity in post-stroke skeletal muscle suggest a bilateral shift in fiber type.

Although these data seem to be in contrast, they actually highlight the importance of careful characterization of a patient's muscular metabolism through a specific evaluation over time. In a rehabilitative view, focusing on muscles could identify ways to reverse stroke-induced metabolic alteration; indeed, different therapeutic physical interventions (i.e., aerobic exercise or neuromuscular electrical stimulation) could exert different biological effects and can improve physical performance. High-intensity training, aimed to increase type IIA fiber percentages, might contribute to muscle power and endurance, crucial for functional capacity [50]. Aerobic exercise normalized the CSA of type I and IIb muscle fiber and increased peroxisome proliferator-activated receptor gamma coactivator 1-α (PGC1α) protein content, which is indicative of increased aerobic capacity.

3.4 Electromechanical Changes

Impaired voluntary muscle motion is also caused by a change in the motor unit (MU) activation. In the first phase post-stroke (in 4 h), there is an initial reduction of MU number with a larger amplitude of the outlier surface of MU action potential (MUAP) and a decrease in compound muscle action potential (CMAP) amplitude, that could continue for a long time. It may be related to the trans-synaptic inhibition of the spinal alpha-motor neurons, as a result of upper motor neuron involvement. Despite this, in the chronic phase, it was reported that the number of MUs increased, particularly in patients

with mild stroke; in the researchers opinion, this suggests that the initial decrease of MUs is due to functional inactivity and, therefore, its recovery is realistic over time. Regarding the larger MUAPs amplitude described in chronic stroke patients, it could indicate enlarged MU, possibly due to reinnervation (collateral sprouting).
Additionally, the threshold strength range for MU recruitment has been compressed to a lower level on the affected side, indicating a different type of MU fiber , with a hypertrophy of slow-twitch skeletal muscle fibers and an atrophy of fast-twitch fibers. It is confirmed by the study of muscle fiber conduction velocity (MFCV). The MFCV is inferior in the paretic than in the non-paretic muscles of stroke patients. Similarly, this might indicate an increase in the proportion of type I fibers compared to type II. The same pathophysiology observed in the paretic limb may be present in the non-paretic limb, although to a lesser degree, due to the presence of the ipsilateral corticospinal tract in humans; indeed, in some individuals, up to 30% of corticospinal axons may descend in the ipsilateral ventral tract.
Nevertheless, the reduction in MUs' number correlates with the reduced muscle mass in paretic limbs, but not in the non-paretic one, suggesting other factors for the reduced muscle mass in these patients as described above. sing conventional surface EMG, different parameters (such as power spectrum, spike distribution, clustering index) were described to be different in hemiparetic muscle and healthy ones, and for some of these different hypotheses were suggested, including central and peripheral process (increased motor unit synchronization, impairments in motor unit control properties, loss of large motor units, and atrophy of muscle fibers) [51].

4. Aims of the PhD thesis

The overall aim of this thesis was to provide a deeper understanding of the role of muscle modifications in the early post-stroke phase up to 6 months. To address this aim a sonographic and clinical assessment analysis was performed both on paretic side and non-paretic side. A 6-months followed the baseline assessment at defined intervals.

To address the aforementioned challenges and research gaps in the field of muscular modifications after stroke, this PhD thesis had the following aims:

1. Measure the difference of the following ultrasound parameters on both the paretic and non-paretic side in the immediate post-acute phase: muscle thickness (MT), ACSA (anatomical cross sectional area) in the following muscles: Biceps Brachii, Rectus Femoris, Gastrocnemius Medialis
2. Identify if there is a correlation between the "Compressibility Index" (CI) a new parameter of ultrasound assessment and the development of spasticity in the muscles examined.

4.1 Material and Methods: inclusion criteria

All adults admitted in Physical Medicine and Rehabilitation with acute ischemic stroke from January 2019 to January 2022 were identified as the study population. Those fulfilling the inclusion criteria with expressed written consent for participation were recruited. Patients with past strokes or disabling neurological conditions were excluded. Socio-demographic factors (age, sex), risk factors, including past TIA, hypertension, diabetes mellitus (DM), dyslipidemia, ischemic heart disease (IHD), liver pathologies, body mass index (BMI), severity and outcomes were analyzed with a

specific tool. The study participants were assessed on admission, after 60, 90 days and at 6 months. The exclusion criteria were as follows: patients with double hemiplegia; patients with amputated lower limbs; patients with a fracture or surgical history in lower limbs that led to gait disturbance; patients with any surgeries that led to malnutrition; and patients with other systemic diseases that could affect the loss of muscle mass or malnutrition (hypogonadism, hypercortisolism, hyperthyroidism, growth hormone deficiency, chronic obstruction pulmonary disease, chronic kidney disease, liver cirrhosis, chronic heart failure, neurodegenerative diseases, inflammatory diseases, acquired immune deficiency syn- drome, and malignancy).

4.1.1. Anatomical site of ischemic stroke

Bamford et al., using data from the Oxford Community Stroke Project, defined four sub-categories of cerebral infarction on the basis of presenting symptoms and signs: lacunar infarcts (LACI); total anterior circulation infarcts (TACI); partial anterior circulation infarcts (PACI); and posterior circulation infarcts (POCI). While the classification is based upon bedside clinical features, the labels attached to each sub-category are anatomical, which reflects the close correlation between symptoms and signs and site of cerebral infarction. As shown in Table 4, this classification is of prognostic significance. A TACI is associated with high mortality, and significant disability in most survivors. A PACI is associated with the highest risk of early (i.e. within 3 months) recurrence of stroke. A patient with a POCI has the best chance of a good recovery, and patients with a LACI the best chance of survival. The advantage of this classification is that it uses relatively simple clinical criteria. The disadvantages are that it does not extend to sub-arachnoid hemorrhage or intracerebral hemorrhage, and that, for lacunar strokes, the relationship between clinical classification and anatomical site may not be very close.

4.1.2. Motricity Index

Before US testing, strength and impairment was evaluated using Motricity index [52].

The Motricity Index was used to measure strength in upper and lower extremities after stroke.

Tests for Each Arm:

(1) pinch grip: using a 2.5 cm cube between the thumb and forefinger

- 19 points are given if able to grip cube but not hold it against gravity
- 22 points are given if able to hold cube against gravity but not against a weak pull
- 26 points are given if able to hold the cube againt a weak pull but strength is weaker than normal

(2) elbow flexion from 90° so that the arm touches the shoulder

- 14 points are given if movement is seen with the elbow out and the arm horizontal

(3) shoulder abduction moving the flexed elbow from off the chest

- 19 points are given when the shoulder is abducted to more than 90° beyond the horizontal against gravity but not against resistance

Tests for Each Leg:

(1) ankle dorsiflexion with foot in a plantar flexed position

- 14 points are given if there is less than a full range of dorsiflexion

(2) knee extension with the foot unsupported and the knee at 90°

- 14 points are given for less than 50% of full extension
- 19 points are given for full extension yet it can be easily pushed down

(3) hip flexion with the hip bent at 90° moving the knee towards the chin

- 14 points are given if there is less than a full range of passive motion

• 19 points are given if the hip is fully flexed yet it can be easily pushed down

4.1.3 Modified Ashworth Scale

The Modified Ashworth Scale (MAS), modified from the Ashworth (AS) Scale, is the most commonly utilized clinical measure of quantifying spasticity. The original Ashworth Scale introduced in 1964 was constructed using a 5-point ordinal scale, with a Likert-like grade score of 0 (indicating no increase in muscle tone), 1, 2, 3, or 4 (affected part rigid in flexion or extension) to quantify spasticity. In 1987, Bohannon and Smith introduced the grade of "1+" and proposed slight changes in how each score was defined in order to increase the sensitivity of the measure and facilitate greater ease in scoring, which prompted the renaming of the Ashworth Scale as the "Modified Ashworth Scale". MAS scores were as follows: level 0 (no increase in muscle tone): 0 points; level 1 (slight increase in muscle tone, minimal resistance, or sudden release at the end of the joint range of motion): 1 point; level 1+ (slight increase in muscle tone, sudden stuck within 50% of joint motion and then minimal resistance at 50% of joint motion): 2 points; level 2 (a marked increase in muscle tone through most of the region of movement, but easily moved): 3 points; level 3 (considerable increase in muscle tone, passive movement dif cult): 4 points; and level 4 (affected part rigid in exion or extension): 5 points.21

4.1.4. Charlson Comorbidity Index

The Charlson Comorbidity Index is a method of categorizing comorbidities of patients based on the International Classification of Diseases (ICD) diagnosis codes found in administrative data, such as hospital abstracts data [53]. Each comorbidity category has an associated weight (from 1 to 6), based on the adjusted risk of mortality or resource use, and the sum of all the weights results in a single comorbidity score for a patient. A score of zero indicates that

no comorbidities were found. The higher the score, the more likely the predicted outcome will result in mortality or higher resource use. Since the publication of Charlson et al.'s original article in1987, the paper has been cited nearly 5,500 times, and the index has been validated for its ability to predict mortality in various disease subgroups, including cancer, renal disease, stroke, intensive care, and liver disease. In this study comorbidities were measured using the Modified Charlson Comorbidity Index, with 0 point indicating none, 1–2 points indicating moderate comorbidities and ≥3 points indicating severe comorbidities. The original Charlson Index includes cerebrovascular disease (weight 1) and hemiplegia (weight 2). Because these items are reflected in the condition being evaluated (ie, stroke), they are not included.

4.1.5 Muscle thickness assessment with ultrasound

Ultrasound images were acquired using the Samsung HS50 ultrasound system equipped the L3-12A linear array transducer. The gain was set to 50, the dynamic range at 66 dB, the frequency at 56 Hz and all of them were kept constant throughout the examination. Whenever required, the depth was increased so as to include the whole muscle in the image. The participants lay supine on a flatbed, the upper limbs were positioned at 45° relative to the body with the elbow at 0° flexion. Ultrasound images were assessed on both the paretic and non-paretic sides and were measured three times to obtain an average value for statistical analysis. The MT (muscle thickness) and EI (echo intensity) values of biceps brachii (BB) , rectus femoris (RF), gastrocnemius medialis (GM) were measured.

To obtain the images in a standard and uniform manner, the transducer was placed perpendicular to the skin and eventually slightly angled (in the elevational direction) so as to achieve the brightest echo from muscle fascia. In 2020, the SARCUS working group published a first article on the standardization of the use of

ultrasound to assess muscle [54]. Recommendations were made for patient positioning, system settings and components to be measured. The biceps brachii muscle was evaluated at 50% between the greater tubercle of the humeral head and the elbow crease, in a supine position. The rectus femori was measured halfway along the line from the anterior–superior iliac spine to the superior pole of the patella. The gastrocnemius medialis was evaluated at the medial head of the gastrocnemius at 30% proximal between the lateral malleolus of the fibula and the lateral condyle of the tibia

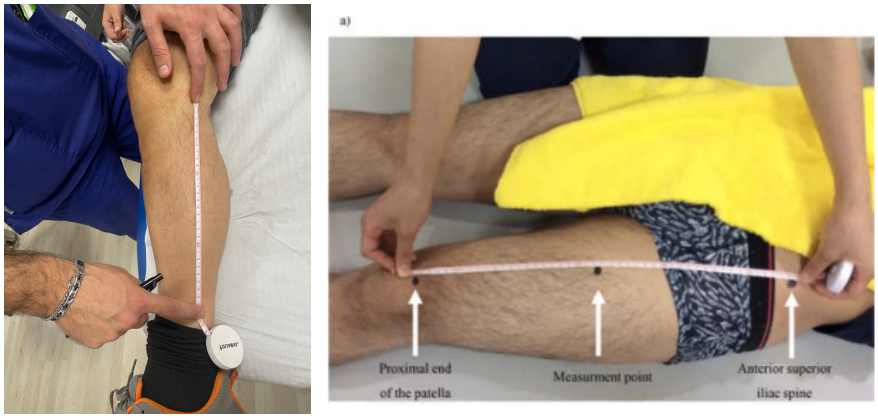

Figure 3: Anatomical landmarks for ultrasound, Gastrocnemius medialis (left) and Rectus Femoris (right) .

4.1.6. Cross sectional Area

Ultrasonography has been previously used for muscle Cross sectional area (CSA) measurements as it is more accessible and less costly than 'gold standard' methods, such as computed tomography (CT) or magnetic resonance imaging. The participants lay supine with both hip and knee fully extended and relaxed. CSA was obtained through the mark of internal edges of the RF muscle fascia.

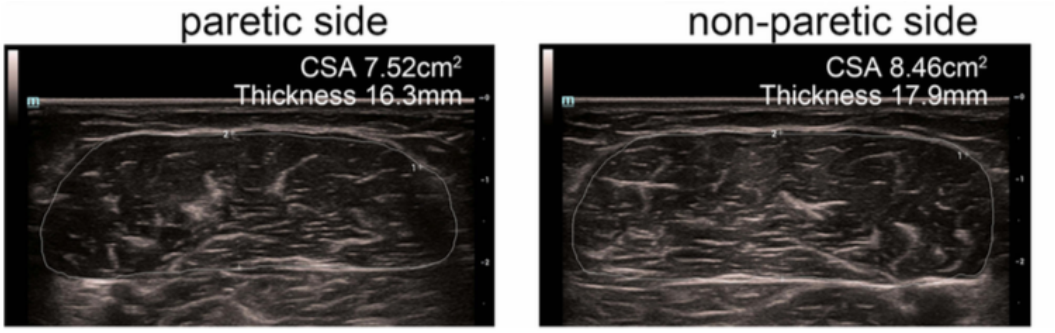

Figure 4: The area surrounded by the broken line was defined as the CSA of the rectus femoris muscle in paretic and non-paretic side.

4.1.7. Echo intensity

Echo intensity is the mean pixel intensity of a specific region of interest from an ultrasound image. This variable has been increasingly used in the literature as a physiological marker. Echo intensity relates to the pixel density of the image and was initially quantified by visual scoring (arbitrarily depicting if an image is darker or lighter) from ultrasound images. Although a few techniques exist in order to extract an arbitrary unit for echo intensity, most researchers now use image-processing programs, such as ImageJ (National Institute of Health, Bethesda, MD, United States) to determine echo intensity based on quantifying the pixel intensity of an ultrasound image (Figure 4).

Echo intensity is determined by drawing a region of interest on the ultrasound image without including subcutaneous fat or bone. This region of interest may be a pre-specified sized box, a box containing as much of the muscle as possible or free hand tracings between the adipose tissue and bone. The majority of studies on echo intensity use a scale between 0 (black) and 255 (white) or 0 (black) to 256 (white). When using pixel-intensity to compare healthy muscle to spastic muscle, spastic muscle appears more echogenic and have a higher pixel intensity.

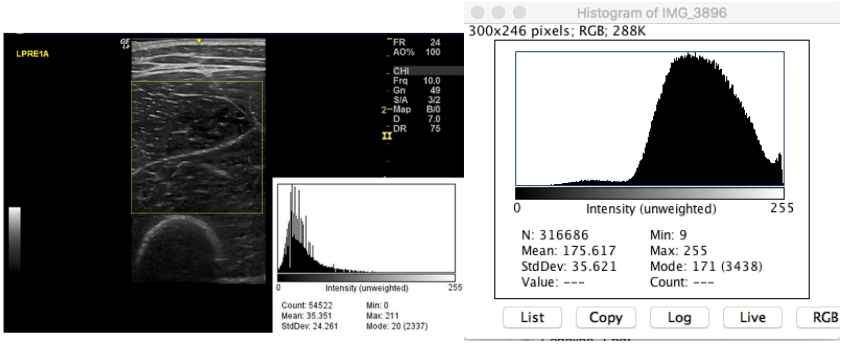

Fig. 4. Echo intensity of Biceps Brachii muscle and Rectus Femoris muscle

4.1.8. Compressibility Index

It is a new and innovative index, developed in this study. Muscle compressibility could be calculated with the following formula (α–ß)/α ×100.

where α=muscle thickness with minimum compression (mm); ß = muscle thickness with maximum compression (mm). Higher the value, higher the compressibility and lesser the hardness of muscles.

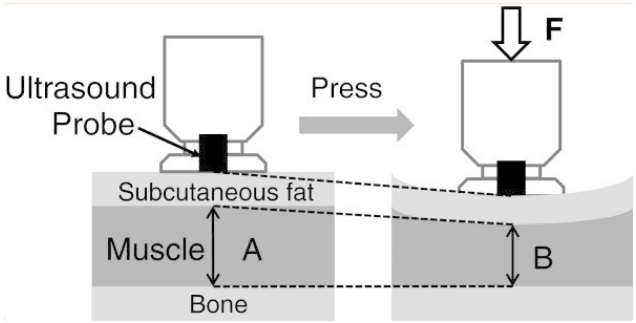

Figure 5: Mechanism for measuring hardness of muscle by elasticity measuring an ultrasound signal.

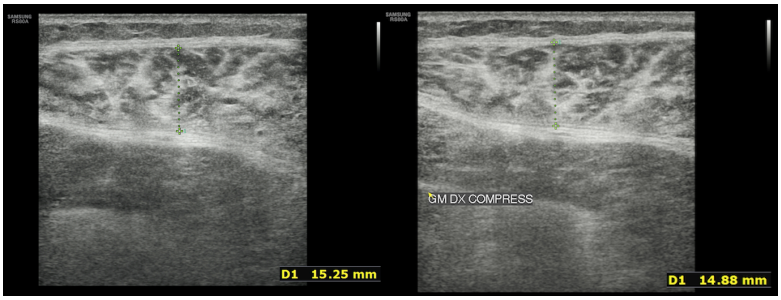

Figure 6: Gastrocnemius medialis muscle without any and with compression.

4.2.Statistical analysis

The distributions of the sociodemographic and clinical data were presented with appropriate descriptive statistics, e.g., standard deviation. For this purpose, the right and left lower limbs were considered as the affected and unaffected limbs, depending on hemiplegic side. To compare sonographic images between the tested and control groups and between the affected and unaffected lower limbs, paired Student's t-test was used. Data are presented in tables as mean, standard deviation, minimum, and maximum. The Student's t-test or analysis of variance (ANOVA) was used for the analysis of the continuous variables for the independent samples as appropriate when examining the effect of age and function on ultrasound measurements.

In all analyses, statistical significance was approved at $\alpha = 0.05$. Further, nonparametric rho Spearman correlations were performed. Only correlation rates with $\alpha = 0.05$ were considered statistically significant. The correlation between the muscle thickness and echo intensity of muscles with clinical characteristics as well as the outcome variables were determined using Spearman rank correlation coefficient. Poor, fair, moderately strong, and very strong correlation coefficients were defined as <0.3, 0.3–0.5, 0.60–0.80, and at least 0.80, respectively. Statistical analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). All statistical tests were performed at a two-sided 5% significance level.

4.3.Results

Study characteristics

Twenty-two patients were enrolled in the period between January 2019 and June 2022. Table 1 shows the demographic data referring to the sample under study.

Table 1 Demographic characteristics of patients

Variables	Discontinuers
No of patients	n = 22
Age (SD)	71 (7.49)
Variable	*Frequency*
Sex n (%)	
Male	12 (54.5%)
Female	10 (45.5%)
Stroke syndrome	
TACS	2 (9.1%)
PACS	9 (40.9%)
LACS	7 (31.8%)
POCS	4 (18.2%)
CCI	9.18
BMI (kg/m2)	22.9 ± 5.52

TACS: Total anterior circulation stroke syndrome; PACS: Partial anterior circulation stroke syndrome; POCS: Posterior circulation syndrome; LACS: Lacunar stroke; BMI: Body max index.

Most of patients were male (54,5%), the mean age was 71 (55-83). The commonest stroke etiology was PACS [9, (40.9 %)], followed by LACS [7, (31,8 %)], POCS and TACS accounted respectively 18.2 % and 9,1 % (Table 1).

The mean Charlson comorbidity index (CCI) score was 5.91 ± 2.15 (range, 3-10). Figure 7 shows the distribution of the CCI score in the 22 patients: 6 (27.27%) had a CCI score of 7, (22.73%) had a CCI score of 9, 3 (13.64%) had a CCI score of 8, 3 (13.64%) had a CCI score of 12, 4 (9.09%) had a CCI score respectively of 10 and 11, and 1 (4.55%) had a CCI score of 13. Thus, 1 patient had a low CCI score (< 3) and 21 had a high CCI score (≥3).

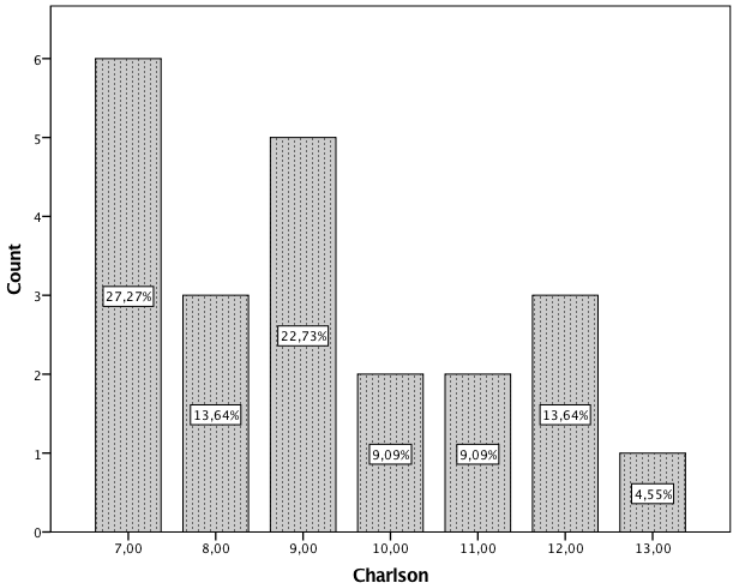

Figure 7: Distribution of Charlson Comorbidity Index sum scores across the entire intracerebral hemorrhage cohort (n=22).

As Table 2 and 3 is indicated, all scores of Motricity Index in upper and lower limbs of were recorded.

Table 2 Descriptive values for Motricity Index value .

MI UL	Minimum	Maximum	Mean	Std. Deviation
T0	0	73	47,36	18,64
T1	15	77	53,50	14,96
T2	15	77	56,36	15,41
T3	15	84	57,86	16,17
MI LL				
T0	18	63	41,86	15,62
T1	27	69	51,77	12,93
T2	37	77	60,00	12,03
T3	42	77	61,95	11,39

MI UL: Motricity index upper limb; MI LL: Motricity index lower limb. Data are reported as means.

Table 3: Means and significance at different intervals of MI.

	To-T1	*p*	T1-2	p	T2-T	p	To-T3	p
MI UL	47,36	,000	53,50	,000	56,36	,006	57,86	,000
MI LL	41,86	,015	51,77	,000	60,00	,000	61,95	,000

Table 4 shows the MT values of BB, RF, GM, in paretic and in non-paretic side.

Table 4: Means (and SD) of muscle thickness of Biceps Brachii, Rectus Femoris and Gastrocnemius Medialis from baseline to 6 months.

	To	T1	T2	T3
MT BB				
Paretic	18,49(1,19)	18,13 (1,22)	17,03(1.04)	16,15(0,87)
Not paretic	18,29(0,98)	18,18(0,97)	18,04(1,12)	18.05(0,96)
MT RF				
Paretic	15,95(0,96)	15,55(1,12)	15,32(0,98)	14,75(0.98)
Not paretic	15,55(0,95)	15,79(0,97)	15,65(0,96)	15,53(0,96)
MT GM				
Paretic	15,86 (0.95)	15,74 (1,01)	15,39 (0.92)	14,39(0,88)
Not paretic	15,80 (0,87)	15,70 (0,89)	15,53 (0,97)	15,63(0,90)

BB Biceps brachii; RF Rectus femoris, GM Gastrocnemius medialis

Table 5: Change in and biceps brachii thickness and comparison between means at baseline, after 1 months, 2 months and 6 months in paretic side.

Paretic side								
	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Muscle thickness								
BB T0-T1	,359	,4283	,091	,169	,549	3,932	21	,001*
BB T1-T2	1,009	1,404	,299	,386	1,631	3,369	21	,003*

BB T2-T3	1,040	,994	,211	,600	1,481	4,911	21	,000*
BB T0-T3	2,409	1,476	,314	1,754	3,063	7,653	21	,000*

BB: Biceps Brachii. P <0.05

Table 6: Change in and BB thickness and comparison between means at baseline, after 1 months, 2 months and 6 months in non-paretic side.

Non paretic								
	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Muscle thickness		’			’			
BB T0-T1	,104	309	,065	-,032	24170	1,585	21	,128
BB T1-T2	,004	,158	,033	-,065	,074	,134	21	,894
BB T2-T3	,04	,206	,043	-,050	,132	,930	21	,363
BB T0-T3	2,20	1,428	,304	1,571	2,837	7,240	21	,000*

BB thickness decreased during the first 30 days and in the following time intervals. Mean thickness at admission was 18,49 mm [95% CI, 0.169; 0.549] and decreased to 18,13 at T1 [95% CI, 1.24; 1.665], corresponding to a 1.95% variation in muscle thickness. At 6 months, mean thickness decreased with a variation of 12.66%. BB in non-paretic side, did not significantly decreased after 30 and 60 days.

Figure 8 depicts the evolution of biceps brachii muscle thickness between T0 and T3.

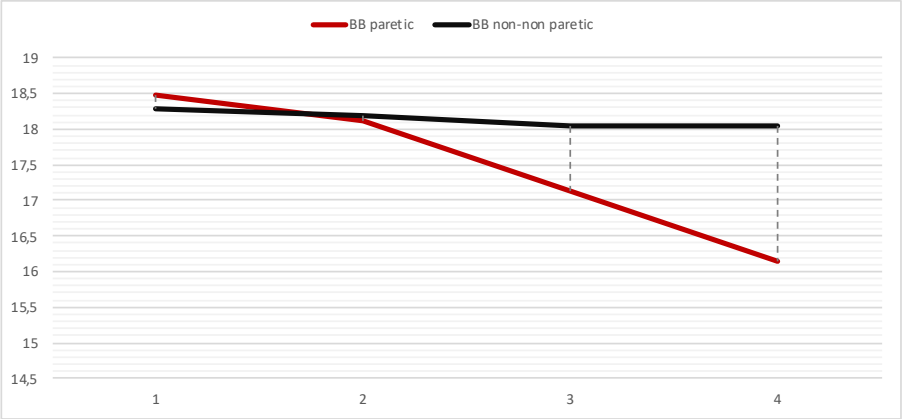

Figure 8: BB thickness from T0 to T3.

RF thickness decreased from 15.95 to 14.75, with a difference of 7.52% from baseline to T3 [95% CI, .0272; 0.817], $p < 0.001$. No significant different was found in T0-T1 and T1-T2. In non-paretic side, MT decreased in interval T0-T1 [95% CI, .049; 0.214], and T1-T2 [95% CI, .0079; 0.817],

Table 7: Change in and rectus femoris thickness and comparison between means at baseline, after 1 months, 2 months and 6 months in non-paretic side.

Non paretic								
	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Muscle thickness	’							
RF T0-T1	150	,162	,034	,0779	,222	4,328	21	,000*
RF T1-T2	,131	,186	,039	,049	,214	3,321	21	,003*
RF T2-T3	-,16	1,468	,313	-,814	,487	-,523	21	,607
RF T0-T3	,118	1,50	,321	-,55	,787	,367	21	,717

RF: Rectus Femoris, p <0.05

Table 8: Change in rectus femoris thickness and comparison between means at baseline, after 1 months, 2 months and 6 months in paretic side.

Paretic side								
	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Muscle thickness		’						
RF T0-T1	,404	324	,069	,260	,548	5,85	21	,008
RF T1-T2	,250	,268	,057	,130	,369	4,36	21	,012
RF T2-T3	,5454	,614	,131	,272	,817	4,16	21	,004*
RF T0-T3	1,200	,73095	,15584	,87592	1,52408	7,70	21	,000*

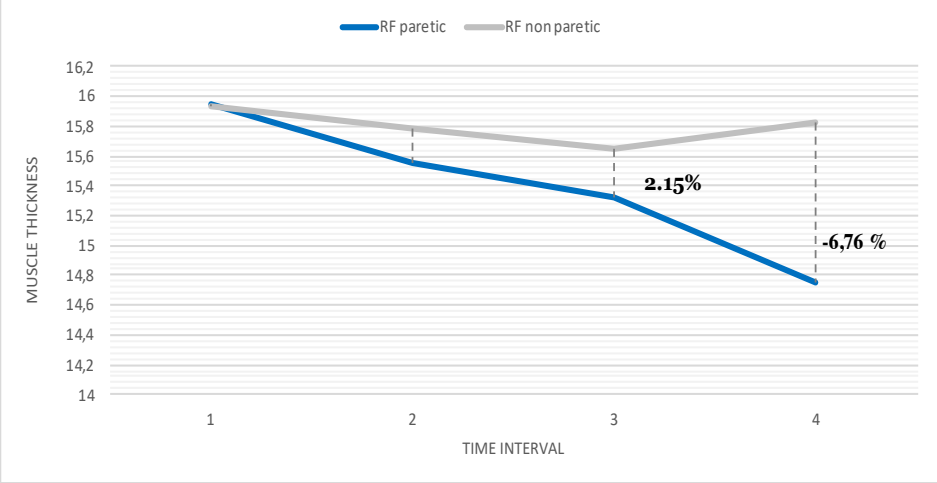

Figure 9: RF thickness from T0 to T3.

GM thickness decreased from 15.86 to 14,39, with a difference of 9.27% from baseline to T3 [95% CI, . ,033; 0. ,058], p = 0.001. No significant different was found in T2-T3 and T0-T3. In non-paretic side, MT decreased in interval T0-T1 [95% CI, .123; ,203], and T1-T2 [95% CI, .083; 0.265]. Globally MT GM decreased with a difference 6.76%.

Table 9: Change in gastrocnemius medialis thickness and comparison between means at baseline, after 1 months, 2 months and 6 months in non-paretic side.

Paretic side								
	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Muscle thickness GM T0-T1	,127	,154	,033	,058	,195	3,855	21	,001*
GM T1-T2	,345	,236	,050	,240	,450	6,851	21	,000*
GM T2-T3	1,00	2,358	,502	-,041	2,05	1,998	21	,059
GM T0-T3	1,47	2,37	,507	,422	2,53	2,913	21	,008

Table 10: : Change in gastrocnemius medialis thickness and comparison between means at baseline, after 1 months, 2 months and 6 months in non-paretic side.

Non paretic								
	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Muscle thickness GM T0-T1	,163	,090	,019	,123	,203	8,50	21	,000*
GM T1-T2	,172	,200	,042	,083	,261	4,042	21	,001*
GM T2-T3	-,090	,211	,045	-,184	,002	-2,017	21	,057
GM T0-T3	,245	,351	,074	,089	,401	3,275	21	,004*

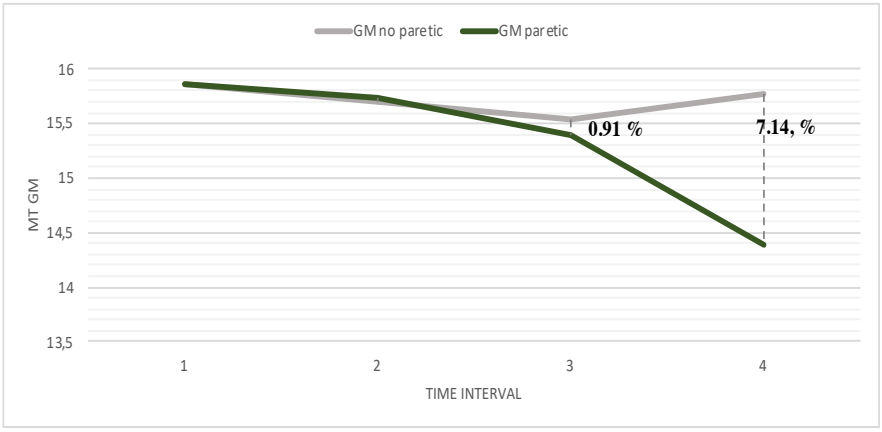

Figure 10: GM, Gastrocnemius medialis thicknes from To to T3.

The Pearson's correlation coefficient test showed the following results. The age had a significant negative correlations with MT RF and ACSA RF. Charlson index had a negative correlation with MT GM. MI LL and FAC had a significant positive correlation with MT RF and ACSA RF. EI had negative correlation with MT RF and ACSA RF.

Table 11: Pearson's correlation coefficient of various factors

	MT BB	MT GM	MT RF	ACSA RF
Age	-0.072	0.033	-0.206*	-0.206*
Gender	0.045	0.027	0.010	0.024*
Charlson	-0.072	-0.723*	0.041	0.017
MI UL	0.024	-0.006	0.045	0.045
MI LL	0.041	0.072	0.234*	0.233*
FAC	-0.035	0.056	0.691*	0.427*
EI	-0.071	-0.233*	-0.245*	-0.207*

Table 12: EI values of GM and RF on the paretic and nonparetic sides in stroke patients

		Paretic (SD)	Non-paretic (SD)	*p*
To				
	RF	67.85 (10.7)	68.1 (9.28)	
	GM	72.28 (12.4)	72.16 (12.7)	
T1				

	RF	69.23(7.4)	67.4(8.12)	
	GM	79.61(12.1)	71.83 (9.2)	
T2				
	RF	69.35 (8.41)	65. 4(6.2)	
	GM	81.34(6.7)*	73.12 (9.2)	< 0.001
T3				
	RF	71.23 (6.17)	66.1 (4.6)	
	GM	98.45(12.4)*	73.25 (4,12)	< 0.001

Table 13: CI values of GM muscle at To and T3.

	Mean Difference	Sig. (2-tailed)	95% Confidence Interval of the Difference	
			Lower	Upper
To	8,700	,000	8,310	9,091
T3	5,095	,000	4,487	5,702

CI: Compressibility index.

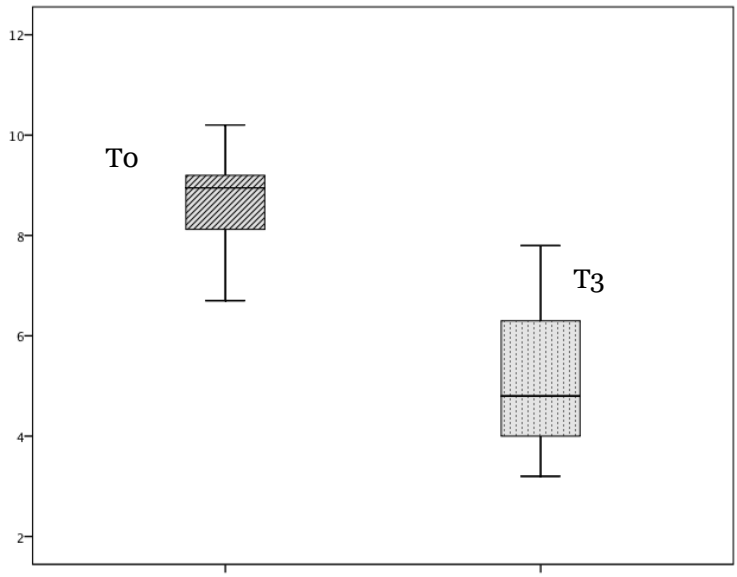

Figure 11: Compressibility index of GM at baseline and after 6 months.

4.4. Discussion

Stroke is one of the leading causes of acquired physical disability in the adult population. Rehabilitation is the most effective way to limit the amount of disability and improve patients' functionality. However, despite continuously improving and innovating interventions, distinct trajectories of recovery are seen. For example, two-third of patients after stroke have residual hemiparesis, resulting in chronic, long-term disability. This emphasizes the complex stroke recovery process and highlights the possibility of distinct underlying recovery mechanisms that support the functional ability of stroke survivors. Gaining insight into the mechanisms that contribute to the heterogeneous treatment response in stroke survivors could help us to better understand the complex recovery process and to optimize functional rehabilitation. Skeletal muscle is suggested to be the main effector organ accountable for physical disability in the stroke population. This disability traditionally contributed to the neurological insult itself, primarily causing motor symptoms, with muscle weakness, altered muscle tone and decreased muscle control being the most common motor manifestations post-stroke. More recently, the functional aspects, structural adaptations and metabolic integrity of peripheral muscle tissue, which remained unrecognized until the past decades, have become an important focus in the evaluation and treatment of the stroke population. Previous studies have shown that chronic stroke survivors (> 6 months post-stroke) experience a loss in muscle mass and a decrease in muscle strength in both the paretic and non-paretic limbs [55,56]. According to research by Miller and colleagues, a decreased central activation can only partly explain bilateral muscle weakness, which might implicate that skeletal muscle changes after stroke can be considered as a multifactorial syndrome depending on various underlying mechanisms besides the brain lesion itself (e.g. neurodegeneration, loss of motor neurons, local muscle metabolic

alterations)[57]. In addition, the muscle's architecture, defined as the geometric arrangements of muscle fibers, appears to be altered in chronic stroke survivors with a shorter fascicle length on the paretic side. Reports about pennation angle measurements (i.e. fibre orientation) have been less consistent.

From a clinical point of view, these (mal-)adaptive skeletal muscle changes have an impact on the recovery process. Muscular atrophy is moderately correlated with decreased gait speed and reduced fitness levels in individuals following stroke in the chronic stage[55]. Also, increased severity of gait impairments have been observed as a result of hemiparetic muscle phenotype changes, a decline in muscle mass and muscle strength. Furthermore, the muscle's architecture has a profound influence on muscle function, and therefore, structural changes might negatively affect the force-generating process and impact functional recovery.

Despite the growing body of evidence about the contribution of underlying skeletal muscle changes to physical disability after stroke, none of the clinical stroke guidelines address these peripheral muscle changes.

One way to improve our understanding of how stroke recovery is achieved in the first 3 months following stroke onset is to evaluate changes in the skeletal muscle over time. That's where this project comes from. Although clinical assessment scales are an essential tool in the measurement and evaluation of motor impairments they often have limitations, such as ceiling effects and a lack of sensitivity to detect small, but relevant, changes. Therefore, to accurately evaluate peripheral muscle changes, measurements are preferably performed by objective, quantitative assessment methods. Medical imaging techniques, such as ultrasound, medical resonance imaging and computed tomography, but also hand-held dynamometers are just a number of the widely used objective tools in research and clinical practice to address changes in function, structure and consistency of the peripheral muscles.

Ultrasound can distinguish muscle from other tissues, but it requires skill and experience to properly measure the muscle mass. Ultrasound is available at the bedside. Thus, it is a promising tool for monitoring muscle mass after stroke. First of all, we noticed a strict correlation between motor impairment at limbs and Charlson Index: this may be explained by the nature of stroke itself and its most common risk factors. In terms of function, we found a very significant We found a significant reduction of MT of BB, GM, RF after 2 and 6 months. RF thickness did not significantly decrease during the first 2 months, this is may be explained because the concomitant activation of Vastus medialis and lateralis. We also found an increase muscle mass in non-paretic limb at 6 months both in RF and GM. These results are in line with previous studies. Muscle hypertrophy of the RF and GM on the non-paretic side may be associated with the long-term compensatory strategy on the non-paretic limb during daily activities.

The EI values of GM was significantly higher in the paretic limb at T1, after 30 days. Previous studies demonstrated that EI is strongly correlated with the amount of fibrous and fat content measured by muscle biopsy. These results may be associated with muscle phenotypic abnormalities after stroke. Therefore, the decrease in the MT values and increase in the EI values of lower-leg muscles may represent a specific loss of muscle fibers and relative increase in intramuscular noncontractile tissues after stroke. The ACSA were obviously smaller on the paretic side, when compared with non-paretic side. The paretic side exhibited significantly smaller ACSA and thickness than the non-paretic side (both $P<0.05$). We also found a negative correlation between age and rectus femoris muscle thickness and ACSA. In patients with stroke the protein breakdown in the legs is increased, and the cross-sectional area of the rectus femoris is decreased due to decreased protein synthesis [58]. In healthy older adults, protein synthesis decreases, the lean mass of the

leg is reduced, and the muscle power is weakened after only 10 days of bed rest [59].

We did not find any correlation between function in upper limb and muscle thickness, on the contrary we found a significant correlation between MI LL and rectus femoris MT and ACSA. This could be explained because ACSA is an indirect parameter of motricity and muscular strength. We found an increased EI, but not significantly, in early epoch of stroke, in T1 in GM muscle. Lateral Gastrocnemius was not considered as it has a minor role in comparison to GM and SOL in determining plantar flexion due to a smaller muscle mass. Moreover, its biomechanical and histological characteristics are similar to GM. EI is significantly increased in the paretic side after 6 months after stroke and showed a negative correlation with MT and ACSA of rectus femoris. Echo intensity, as supposed, is strictly correlated with development of spasticity at 6 months. We also found reduced echo intensity, which indicates reduced intramuscular fat, to be correlated with superior muscle function and ambulatory outcomes. Our findings, therefore, suggest that ultrasound-derived measurements of muscle size, as a surrogate of muscle strength, is potentially a marker of ambulatory performance.The assessment of muscle compressibility, and so muscle hardness in the early epoch of stroke is a strong indicator of severe spasticity at 6 months, especially in GM and it is associated with increased fibrosis.

Data from literature suggest that altered muscle morphology of the paretic muscle may contribute to abnormal muscle elastic property during passive stretch.

4.5 CONCLUSION

This data are in line with the new emerging neurophysiological approach which considers a down-top model. The exiguity number of patients, actually, reflects a reduction in follow-up visits in the first two years during the pandemic. We found interesting data related the possibility to early detect, in the first thirty days, ultrasound signs of muscle modifications that may impact on recovery or the development of pathological hypertonia. As known, muscle hypo-mobilization in short position in the context of paresis, in the hours and days after paresis onset evolves in myopathy, defined spastic myopathy. From a rehabilitative point of view, early modifications in muscle properties may be better exploited to further optimize neurorehabilitation program in favor to avoid effect of maladaptive plasticity. This research opens the door to continue this approach: early research at the patient's bedside for insights that can improve the outcome and prevent irreversible and useless muscle modification in paretic side in post-stroke patients.

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