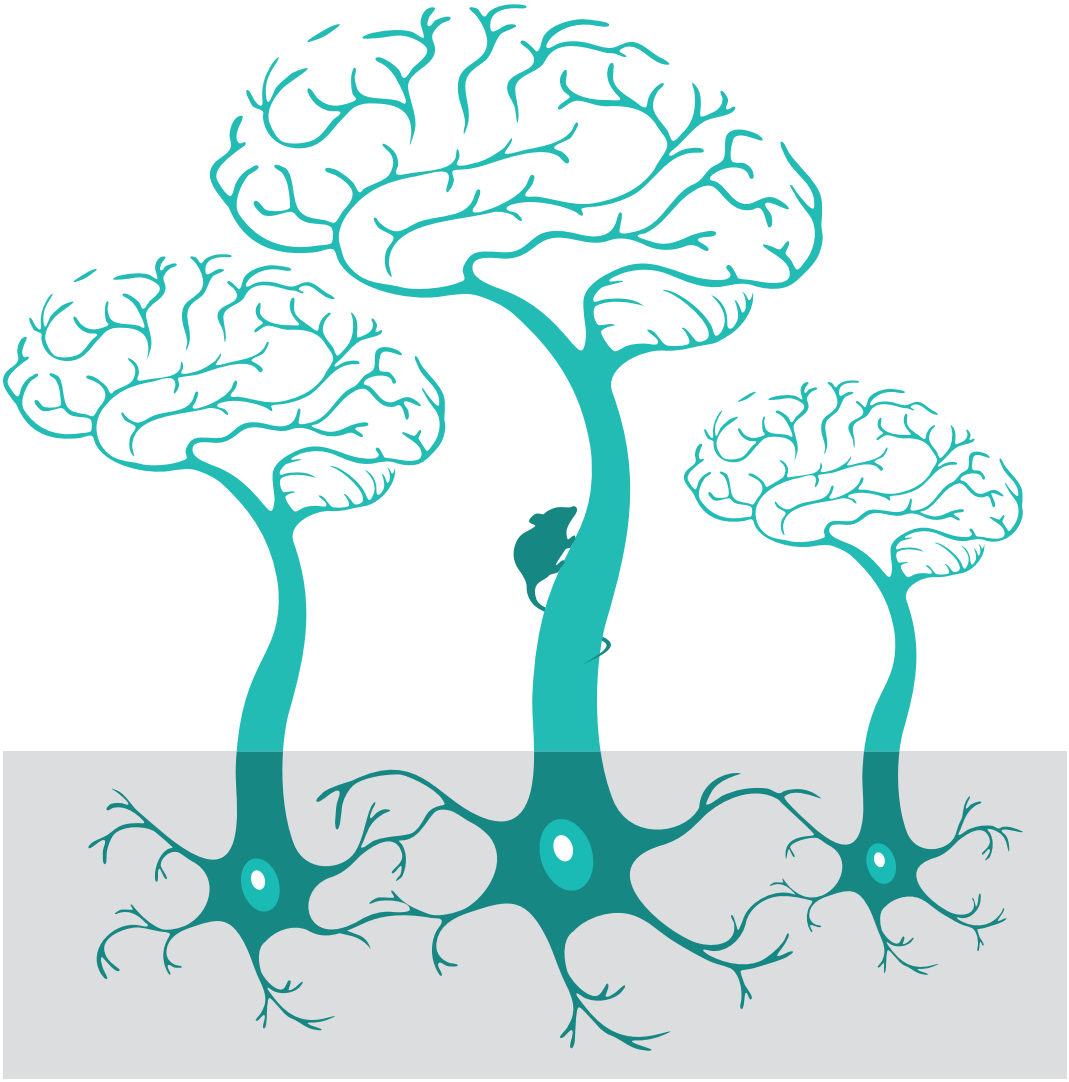

TREES Translational Research on Stroke

frontiers Event Abstracts

2ND MEETING TRANSLATIONAL RESEARCH ON STROKE (TREES)

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November 25, 2022, Aula Magna Careggi University Hospital, Florence, Italy

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Welcome to the 2nd Meeting TRANSLATIONAL RESEARCH on STROKE (TREES)

This meeting aimed at attracting the interest of the entire stroke research community highlighting both the challenges and the opportunities of recent advancements in preclinical and clinical knowledge of pathophysiology in stroke. In fact, translational stroke research occurs at the interface between basic science and clinical research, and encompasses contributors with varied backgrounds and areas of expertise.

The entire spatiotemporal evolution of stroke pathology needs to be better understood, both early after the ischemic event, when reperfusion and neuroprotection are key targets, as well as days-weeks-months post-stroke, when repair and regeneration are relevant targets.

Much information has been gathered about the microscopic anatomy and physiology of the brain, but more is still to be learned. We need to know more about how blood flow is regulated by various cells (neurons, astrocytes, and endothelial cells) and various chemical substances, and how the cells and substances interact. Most relevant from a translational medicine standpoint is how to apply in a clinical setting what is known about the molecular biology of the brain and vascular function.

Possible identification of druggable targets may pave the way to future neuroprotection strategies. Moreover, they might predict outcome before reperfusion treatments, and therefore support decision making with a precision-medicine approach.

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A brief history of reperfusion treatments

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It has been over 50 years since the first exploration of pathophysiology of cerebral ischemia. Animal research revealed very early the relationship between cerebral blood flow and tissue perfusion and damage. Since pivotal experimental studies it appeared clear that tissue damage occurred not so abruptly after arterial occlusion, but developed slowly for hours, during which in the so called "penumbral area" the tissue may remain viable, and potentially able to return vital and normal functioning. In 1995 the clinical NINDS Trial for the first time successfully applied the penumbral concept, showing the positive outcome of patients presenting with acute stroke and submitted to tPA intravenous infusion within 3 hours from stroke onset. This was followed by advanced research in brain imaging, confirming the largely variable time course of the so called "penumbral battleground". Endovascular treatments, introduced in clinical practice around 20 years ago, and thereafter submitted to successful clinical trials, have added a complementary strong instrument to reverse the progression of the penumbral sufference, surprisingly, in the last few years, even after many more hours from stroke onset in carefully selected patients. More experimental, strictly joined to clinical research, appear strongly needed for developing new drugs, that may concur slowing the progression of damage, thus ameliorating survival and quality of life in more and more patients.

Acute ischemic stroke, reperfusion, and the neurovascular unit

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With a limited repertoire of interventions to improve the outcome of ischemic stroke, recent clinical efforts have highlighted the value of acute recanalization strategies. Evolving and aggressive interventional arterial recanalization procedures provide a new perspective on the need to establish reperfusion. They also further emphasize our limited understanding of acute microvessel–neuron associated events following focal cerebral ischemia. Understanding fundamental events in the acute period of ischemic stroke, including cell-cell interactions, is still limited. This presentation will examine i) aspects of the territory-at-risk following ischemic onset, ii) the "neurovascular unit" in understanding post-ischemic events and tissue perfusion, iii) molecular aspects of microvessel injury and their prevention, and iv) reperfusion. An emphasis is that acutely following arterial occlusions that cause ischemic symptoms in the brain, neurovascular responses are distributed heterogeneously within the territory-at-risk.

The vascular compartment and the extravascular compartment (neuropil) can be viewed as at least five separate cell networks. The neurovascular unit is a structural and conceptual framework that recognizes functional interactions among microvessels (the endothelium, basal lamina, pericytes, and astrocyte end-feet), the intervening astrocytes, and the neurons and the axons they serve, and other supporting cells (e.g. microglia, oligodendroglia). While there is substantial evidence that neuronal stimulation can alter cerebral blood flow through the dependent cerebral microvascular bed, evidence that the microvessel endothelium–matrix–astrocyte complex communicates with the neuron is growing. Sensitivities of each compartment and network to ischemia and innate inflammation are central to these responses. The processes affected acutely in response to focal ischemia in the regions of neuron injury include i) microvessel patency, ii) barrier permeability, iii) generation of inflammatory mediators, iv) microvessel matrix alterations, and v) multi-layered responses of cell networks serving the neurons.

Ischemic brain injury targets the cerebral microvasculature. Temporal responses of the target microvasculature include i) loss of microvessel patency by activated peripheral inflammatory cells and local hemostasis, events that can be reversed in experimental situations, ii) structural alterations in the microvessels that coincide with increased permeability, edema, and hemorrhagic transformation, and iii) responses by the glial compartment that begin in the time frame when neuron function is altered. Current unknowns include how the structural and functional alterations occur, whether they are each reversible, and how microvessel and neuron behavior are coordinated.

Evidence that a tight capillary–neuron relationship exists stem from: i) *responses to focal cerebral ischemia.* Events within the microvasculature and adjacent neurons following the onset of focal ischemia occur simultaneously, rather than sequentially despite the relative resistance of the endothelium to ischemic injury. ii) *microvessel–neuron distance relationships.* In the primate striatum, the [microvessel–neuron distance] distribution is highly ordered and consistent. iii) *the generation of free radicals*. Signatures for free radical generation indicate that processes are set up in the space between the endothelium and astrocyte end-feet, although different cell networks (e.g. astrocytes) can be affected. iii) *matrix receptor responses and matrix proteolysis.* A significant loss in microvessel basal lamina matrix proteins occurs that corresponds to changes in endothelial– and astrocyte–matrix adhesion receptor expression following focal ischemia. This is accompanied by a significant up-regulation of matrix proteases including pro-MMP-2, its direct (MT1- and MT3-MMP) and indirect (u-PA/u-PAR) activation systems, cathepsin L, and heparanase that appear simultaneously within the microvessel and neighboring neurons. (pro)-MMP-9 is generated by activated microglia. iv) *leakage of the microvessel permeability barrier*. Loss of endothelial cell β1-integrin matrix receptors in this time frame coincides with increased permeability to plasma proteins, changes in F-actin, and in tight junction proteins. v) *hemorrhagic transformation*. Local thrombin generation is associated with PAR-1-receptor mediated increases in permeability to small molecules. However, thrombin distributed in the extravascular compartment can also promote hemorrhagic transformation. vi) *communication within the glial compartment*. Responses of astrocytes to ischemia (hypoxia) can include alterations in their mitochondrial function, such that mitochondria can be exchanged between astrocytes and microglia and between astrocytes and neurons.

Both ultrastructural and cellular responses to ischemia (hypoxia) in the neurovascular unit indicate that responses in the cell populations are somehow coordinated, and seem to relate to the regions of arterial supply. These observations support a number of implications about how stroke acutely injures both non-vascular and vascular tissues within the brain, how the cell components of the neurovascular unit communicate, the roles of the matrix environment in the function of the cells of the neurovascular unit, and how to improve the outcomes of potential treatments during ischemic stroke. These issues will be explored during the presentation.

Ischemic stroke and recanalization

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Endovascular treatment (EVT) of acute ischemic stroke has introduced several paradigm shifts in the therapy of cerebral artery occlusion. Until 2015, intravenous recombinant tissue-type plasminogen activator was the only evidence-based treatment option. After endovascular treatment has been demonstrated to be safe and effective by five large randomized trials[1-5], thrombectomy with stent-retrievers and/or thromboaspiration are recommended as the standard of care for acute ischemic strokes with a proximal cerebral artery occlusion according to specific selection criteria based on patient's characteristics, clinical presentation, timing and neuroimaging. Namely, good candidates to undergo EVT were previously self-sufficient patients, with a documented onset of symptoms within six hours and evidence of a small established stroke on NCCT (< 1/3 of the middle cerebral artery territory). DEFUSE III and DAWN trials have extended EVT up to 24 hours in case of a significant mismatch core/penumbra on CT or MRI perfusion;[6, 7] while trials to include patients with occlusions beyond the circle of Willis as well as patients with mild symptoms and large established stroke on NCCT are still running.

Concerning the approach, there has been a rapid evolution in techniques and devices for mechanical thrombectomy. Originally, stent-retrievers were used alone, while direct aspiration was limited by the availability of large, flexible and atraumatic catheter systems. These technical barriers have subsequently been overcome, and contact aspiration is now a valid alternative to stent-retrievers for LVO. In fact, the ASTER trial has been published on this topic and showed comparable results between the two techniques[8], while the COMPASS trial demonstrated that first-pass efficacy of primary aspiration is not inferior to stent-retrievers alone.[9] A combined approach with stent-retriever and adjunctive distal aspiration is a third commonly used endovascular option, but there is no evidence of superiority of this technique for occlusions in any specific location thus far. Figure

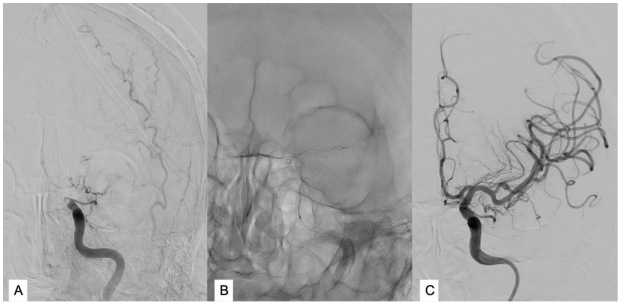

FIGURE 1: (**A**) occlusion of the internal carotid artery and its intracranial bifurcation (**B**) magnified view of distal aspiration catheter and stent-retriever in place (**C**) full recanalization of the artery.

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Ischemic stroke and recanalization

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BACKGROUND

Cerebral ischemic stroke is one of the main causes of death and disability worldwide. Over the past decades, several animal models of focal cerebral ischemia have been developed allowing us to investigate pathophysiological mechanisms underlying stroke progression. Despite intense preclinical research efforts, the need for non-invasive mouse models of vascular occlusion targeting the middle cerebral artery yet avoiding mechanical intervention is still pressing.

METHODS

To induce photothrombosis, we developed a custom-made setup to finely controlled the laser irradiation on the distal branch of the middle cerebral artery. To this aim, we employed a 532 nm laser focused with a 70 mm lens on the targeted blood vessel (laser intensity 128 mW/mm^2). The mouse was held by the side on a stage, allowing displacements in the x-y-z directions. Five minutes after the injection of the dye, the laser was focused before the MCA branch for 25 minutes in order to promote the formation of a stable clot and the consequent occlusion of the distal branch of the MCA. The green laser employed for the experiments focused on the blood vessel and did not heat the irradiated tissue near the MCA during photo-irradiation, as shown by the presence of perfused blood vessels near the illumination site.

RESULTS

Here, we developed and characterized a novel mouse model of stroke employing the photothrombotic occlusion of the middle cerebral artery, one of the most common injury sites in stroke patients. The light-mediated occlusion leads in the acute phase to a severe motor deficit accompanied

by the establishment of an inflammatory regime particularly pronounced in the periinfarct cortex.

CONCLUSION

Here, by characterizing the photothrombotic occlusion of the distal branch of the MCA in mice, we observed the formation of a stable clot in the blood vessel after 25 minutes of laser irradiation that leads to reproducible extended damage in the mouse cortex one week after the lesion.

Circulating biomarkers-metabolomics

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Metabolomics is the comprehensive analysis of the metabolome, which is the complete set of metabolites in a biofluid or cell. Nuclear Magnetic Resonance (NMR) is an extremely powerful and highly reproducible technique able to provide the metabolic profile of a subject through the acquisition of spectra that require relatively short acquisition times and little sample handling. NMR-based metabolomics has been successfully exploited in different pathological contexts, providing significant information on a wide range of pathologies. In this talk, after a general introduction to the topic, examples of applications of NMR-metabolomics in biomedicine will be provided.

Neuroprotection in ischemic stroke

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Neuroprotection can be defined as the ability for a therapy to prevent neuronal cell death.

There are many neurological diseases where we can try to achieve neuroprotection such as degenerative diseases, infective/inflammatory disease, and even though they may share some common mechanisms of damage, the cause of each disease where we should mostly concentrate our efforts is different.

The acute interruption of arterial blood flow is the cause of ischemic stroke so revascularization is the best neuroprotector and the only therapy that was demonstrated to be able to change the natural history of the disease. As the therapeutic window for intravenous thrombolysis and intra-arterial thrombectomy is becoming wider, wider is the number of patients that can potentially profit of them. However revascularization is not always achievable, sometimes it can be futile, and it can also be a cause of damage. So it is important to have strategies to achieve neuroprotection that can be used with and without reperfusion therapies.

Some harmful factors such as hyper/hypotension, hyper/hypoglycemia, high body temperature, hypoxia can be controlled if we take care of the patient in a dedicated ward, so that stroke units can be considered a way to perform neuroprotection.

Ischemic penumbra is the target of neuroprotection and we now know that penumbra is not simply an area of "neuronal lethargy" but it is a dynamic and heterogeneous tissue in terms of cellular and molecular mechanism and their balance determines tissue fate. In the ischemic core, neurons rapidly die for necrosis while in ischemic penumbra the mechanisms of death are different and slower.

On one side apoptosis and neuroinflammation lead to neuronal death with a process that spreads and involves the periinfarct tissue, but on the other side inflammation and autophagy can save neurons. A critical role is performed by mitochondria. In condition of low glucose and low oxygen there is a reduction of ATP and increase of reactive oxygen species (ROS) that at the end lead to dysregulation of mitochondrial and cellular membrane, release of citocrom C, alteration of ionic homeostasis, increase of extracellular glutamate and cell death due to apoptosis, pyroptosis, excitotoxicity, inflammation. It is interesting to note that some molecules can exert an opposite effect on cells depending on their concentration; for example ROS play an important role for cellular homeostasis in physiological conditions but they became highly deleterious when their concentration increases. So again the balance and also the timing these molecules are in the cells can make a difference in their fate.

We know that in the last 30 years or more, experimental studies have shown promising results in terms of the efficacy of several molecules as neuroprotectants in the acute phase of stroke.

However the clinical translation of neuroprotective treatment strategies from bench to bedside has mostly failed.

Research and literature are flourishing in the very recent years.

Several reasons could be addressed for explaining the failure of neuroprotectants in clinical trials

1. Considering the complexity of ischemic cascade an emerging concept is that a single neuroprotectant could not to be enough to counteract the ischemic process so that a sort of cocktail of neuroprotectants could be hypothesized in association or not with reperfusion therapies. There are ongoing trials that are testing the efficacy of the administration of more than one compound and which kind of compounds can be safely administered together.

2. Administration time: the ischemic cascade is a process that begins very early after the vessel occlusion. Some molecules that have failed to demonstrate an efficacy could have been effective if administered earlier.

The pre-hospital administration of drugs have been proved to be feasible. This is a way the research could move towards

3. Administration route: neuroprotectants have to pass the blood brain barrier to be effective. There are trials ongoing that are testing the intranasal administration. An interesting field of research is the use of exosome, administered in the blood, as vehicle of potential neuroprotectants

4. Sample size of clinical trial The big variability of patients with stroke in terms of pathogenesis, comorbidities, time of evaluation could be overcome, at least in part, increasing the number of patients in clinical trials

5. The choice of the outcome measures should be rethought. The preclinical evidence are based on very precise measurements of infarct size. We should use new brain CT or cerebral MR post-processing analysis in clinical trials to achieve a more accurate assessment of brain infarct volume. From the clinical point of view the use of modified Rankin Scale should not be enough to unveil a benefit because we know that quality of life is not only related to walking ability. Tests like cognitive and language assessment should be of relevance.

Neuroprotection: Lost in translation? A short, critical, preclinical reappraisal

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In spite of the remarkable therapeutic advancements obtained in several areas of neurological disorders, stroke therapy is still in urgent need for drugs able to target ischemic neurodegeneration and afford neuroprotection. Hundreds of clinical trials have been conducted in stroke patients with the aim of translating in the clinical arena preclinical evidence of drug-induced ischemic neuroprotection. Unfortunately, none of them succeeded in the translation attempt. Besides the technical and conceptual mistakes that have been originally conducted, the current availability of efficacious mechanical and intravenous recanalization techniques has significantly changed the scenario, as well as the opportunities of drug-induced ischemic neuroprotection. In this light, we propose that stroke trials should now be specifically designed to provide the proof of concept that the ischemic human brain parenchyma can be pharmacologically protected. Theoretically, there should not be no obvious, gross anatomical or metabolic reasons that confer sensitivity to neuroprotective agents exclusively to the rodent brain. Further, considering that the multiple detrimental events contributing to cell death of the various component of the neurovascular units are all prompted by bioenergetic failure, it makes sense that the proof-of-concept trials should be conducted with drugs supporting energy homeostasis. Recent evidence that dexpramipexole, the first-in-class F1Fo-ATP synthase activator affords brain protection in multiple models of stroke and neonatal hypoxia-ischemia indicates that sustaining energy dynamics within the ischemic brain is of potential therapeutic relevance to stroke patients. The notion that dexpramipexole already showed and excellent tolerability and safety profile in ALS patients emphasizes its realistic translational potential. Repetitive failure in bench to bedside translation has dramatically dampened the enthusiasm in identifying stroke neuroprotection strategies. We believe that designing trials adopting drugs with a strong therapeutic/pathogenetic rationale, select a highly uniform patient population and conduct concomitant thrombectomy/lysis therapy may boost the chances of obtaining pharmacological stroke neuroprotection.

Neuroimaging markers of reperfusion and recovery after stroke: Clinical perspective

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The approach to the treatment of acute ischaemic stroke has been transformed over the last ~35 years with thousands of patients around the world benefitting from the clinical infrastructure changes that have allowed first the use of intravenous thrombolysis (IVT), and then, endovascular thrombectomy (EVT) to become embedded into care pathways. The assessment of patients both from a clinical and radiological perspective has become increasingly refined to facilitate this improvement. As hybrid trials of EVT and neuroprotection become more commonplace, there is a need to change again. IVT and EVT rely on a clot being identified on imaging to make a treatment decision. Investigating agents that offer neuroprotection at the time of stroke, or promote recovery following an event will need different clinical approaches and neuroimaging markers. These are required to explore whether that agent can deliver the expected improvements patient outcomes, and then be delivered at scale in health care systems that have increasing digital health care capabilities that may provide opportunities for artificial intelligence decision support.

Translational imaging of hemodynamics after acute ischemic stroke treatment

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Reperfusion therapy after acute ischemic stroke involves recanalization of the occluded vessel – pharmacologically with a thrombolytic agent or mechanically with a clot retrieval device – to restore cerebral blood flow (CBF). Although great progress in reperfusion treatments has been made, many patients still have relatively poor outcome despite successful recanalization.[1] This may be related to post-recanalization perfusion deficit, which can include cerebral hypoperfusion as well as hyperperfusion.[2] Yet, patterns, causes and consequences of post-recanalization perfusion deficit are largely unclear. In a recent literature review, we identified twenty clinical studies that reported post-recanalization perfusion deficit based on perfusion imaging measurements. Hyperperfusion after recanalization was reported in sixteen studies, whereas post-recanalization hypoperfusion was only found in three studies. However, hypoperfusion was in all cases associated with detrimental outcome, while various outcomes were observed after hyperperfusion.

To elucidate profiles and effects of perfusion changes after recanalization, we performed serial magnetic resonance imaging (MRI) of hemodynamics (with dynamic susceptibility contrast-enhanced MRI) and tissue injury (with diffusion- and T_2-weighted MRI) in rats during and after transient middle cerebral artery occlusion (tMCAO) with a filament or a thromboembolic clot. Recanalization was induced by withdrawal of the filament or by tissue plasminogen (tPA)-induced thrombolysis, respectively. The latter was executed in normotensive and spontaneously hypertensive rats.

Rapid recanalization after 45- or 90-min tMCAO with a filament led to normo- to hyperperfusion (i.e. CBF >115% of the contralateral median) in the post-ischemic lesion area within the first hours, further augmenting towards the subacute stage (day 4). Gradual tPA-induced recanalization after 2-h tMCAO with a thromboembolic clot resulted in a more heterogeneous reperfusion pattern with areas of hypo- and hyperperfusion. Incomplete reperfusion in

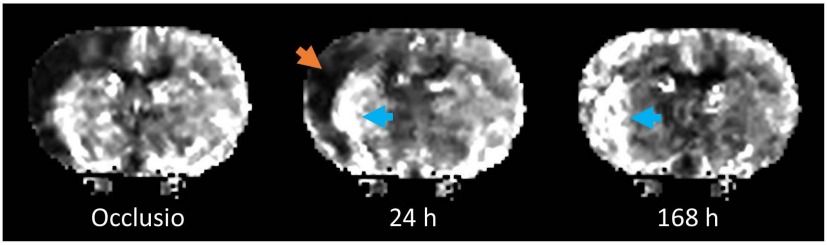

FIGURE 1: Maps of CBF in a coronal brain slice from a spontaneously hypertensive rat during tMCAO with a thromboembolic clot (left), and at 24 h (middle) and 168 h (right) after intravenous tPA injection (10 mg/kg, injected 2 h after MCAO). Areas with post-recanalization hypo- and hyperperfusion are indicated with orange and blue arrows, respectively.

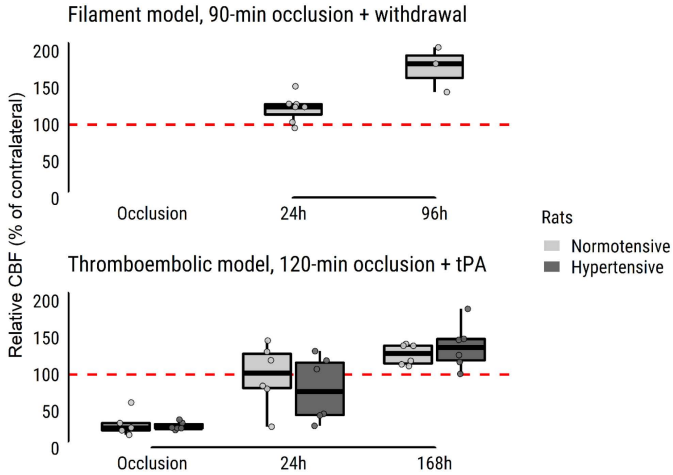

FIGURE 2: Relative CBF (% of contralateral) in the (post-)ischemic lesion area at different stages after tMCAO with a filament (top) or thromboembolic clot (bottom) in normo- or hypertensive rats. Recanalization was accomplished by filament withdrawal (filament model) or tPA-induced thrombolysis (thromboembolic model).

the post-ischemic lesion area after 24 h, followed by clear hyperperfusion after seven days, was particularly observed in spontaneously hypertensive rats after thrombolysis (Figure 1).

The relative CBF in the (post-)ischemic lesion area at different stages after tMCAO for the filament and thromboembolic clot model in normo- and hypertensive rats is shown in Figure 2.

Some post-ischemic tissue regions were salvaged after recanalization (reflected by normal T_2 and apparent diffusion coefficient (ADC) values following ADC reduction during MCAO), whereas other regions suffered delayed injury (reflected by T_2 prolongation). Linear regression of lesion volume change (between 0.5 h tMCAO and 4 days post-tMCAO) against hemodynamic parameters revealed that lesion growth correlated with increased regional CBF (β (standardized regression coefficient) = 15.5, $P<0.05$), while increased regional cerebral blood volume (CBV) predicted lesion reduction (β = -16.8, $P<0.05$) in the filament model of tMCAO.

In a parallel study, we applied an iron oxide-based contrast agent targeted at vascular cell adhesion molecule-1 (VCAM-1) for *in vivo* detection of vascular inflammation with MRI. We found that VCAM-1 expression was significantly enhanced at 6 h after 90-min tMCAO with a filament, coinciding with a transient decline in perfusion after initial hyperperfusion.[3]

In conclusion, post-ischemic cerebral reperfusion levels can be affected by different parameters, such as occlusion duration, recanalization speed and hypertensive state, and may co-depend on tissue fate. Post-ischemic hypoperfusion is typically associated with detoriation of tissue status and subsequent poor outcome. Post-ischemic hyperperfusion may be beneficial or detrimental to disease outcome, depending on which perfusion parameter is used as explanatory variable. Inflammatory processes may influence the degree reperfusion and could provide a target for post-recanalization therapy.

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Biomarkers and blood brain barrier leakage

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Focal cerebral hypoperfusion triggers a complex pathological ischemic cascade that involves not only neurons but all the components of the blood brain barrier (BBB) leading first to its dysfunction, then to irreversible cerebral damage[1]. Acute ischemic stroke - associated BBB leakage is thought to be responsible for the most feared complications (cerebral edema, infarct growth and hemorrhage) after reperfusion therapies[2]. Current acute therapeutic strategies aim to both recanalize vessel occlusion and to restore the reperfusion of the penumbra's downstream capillary bed. Unfortunately, recanalization may result clinically futile or even detrimental because of Reperfusion Injury (RI), a still incompletely understood complex pathological process, that takes place in the penumbra ischemic area[3]. Our group previously found that BBB leakage, assessed with CT-perfusion before reperfusion therapy, was associated with higher incidence of hemorrhagic transformation at 24 h[4]. We investigated the eventual association between a large panel of circulating biomarkers (inflammation, endothelial dysfunction and coagulopathy) and BBB leakage in acute settings. Our group prospectively enrolled 110 acute ischemic stroke patients treated with intravenous thrombolysis and/or endovascular treatment. Before treatment, each patient underwent a multimodal CT protocol (brain CT, CT angiography, CT-perfusion). Pre-treatment BBB leakage within the ischemic area was assessed using the volume transfer constant (K^{trans}) value on CT perfusion. Blood levels of circulating biomarkers were measured both before and 24 h after treatments. We found that higher 24-h levels of metalloproteinase (MMP) 9 and MMP-7 were associated with pre-treatment higher permeability on CT-perfusion.

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Anatomical distortion application in a retrospective cohort and preliminary analysis

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BACKGROUND

It has been widely discussed that cerebral edema and haemorrhagic transformation are the most feared manifestations of reperfusion injury in stroke patients, especially if undergoing recanalizing therapies.

Therefore, we aimed to measure edema and haemorrhagic transformation in a quantitative and objective manner using the Anatomical Distortion (AD) method. In addiction we have planned to investigate the relationship between the measure of AD and the clinical outcome.

METHODS

Starting from the Magic study data[1], we carried out a retrospective analysis of all patients with stroke of the anterior circulation, treated with systemic thrombolysis within 4.5 hours. We included patients whose brain CT scans were available in an adequate format (DICOM), at baseline and at follow-up (performed within a time range between 18 and 36 hours), in order to have sufficient homogeneity in the images to be analysed.

Applying the method described in detail by Harston et al.[2], we then calculated the volume of anatomical distortion (which, as mentioned reflects the presence of edema and blood in the ischemic tissue).

The AD measure is therefore derived from the difference between the volume of the lesion obtained with a linear method and that obtained with a non-linear method, volumes defined by the masks shown in yellow and blue in Fig.1.

Starting from the 327 patients initially present in the cohort, by applying the inclusion criteria described before, we managed to apply the AD method in 108 patients, 6 of whom were excluded for technical reasons (movement artifacts and incomplete brain image acquisition).

RESULTS

The baseline characteristics of the 102 patients included are substantially in line with the homologous data present in the literature, as regards comorbidities (e.g. hypertension and diabetes) as well as for the median age of 72.7 years, for the average baseline NIHSS equal to 12 and time elapsed between the onset of symptoms and thrombolytic treatment (160 minutes on average).

We therefore obtained the average volume values of the total and corrected ischemic lesion, where the difference between the two lies precisely in the AD value. In practice, the total lesion corresponds to the mask originating with linear registration (in yellow), while the volume of the corrected lesion (i.e. subtracted from edema and blood) corresponds to the mask resulting from the non-linear method coloured in blue (Fig.1).

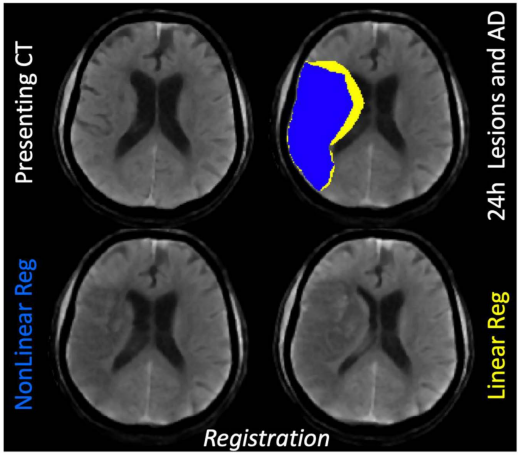

FIGURE 1: Example of two masks of the same Ischaemic lesion, obtained from linear (yellow) and non-linear (blue) registration approach.

Table 1: Logistic regression preliminary analysis

	mRS (3-6) UNIVARIATE			mRS (3-6) MULTIVARIATE		
	OR	**95% CI**	**P value**	**OR**	**95% CI**	**P value**
Age	1.01	0.98 – 1.04	0.641	1.00	0.96 – 1.04	0.939
Blood glucose baseline	0.79	0.18 – 3.40	0.751	0.99	0.21 – 4.66	0.996
Baseline NIHSS	1.27	1.17 – 1.39	**<0.001**	1.27	1.17 – 1.39	**<0.001**
Onset to treatment time	1.00	0.99 – 1.01	0.612	1.00	0.99 – 1.01	0.457
«Effective» lesion volume	1.82	1.47 – 2.24	**<0.001**	1.39	1.13 – 1.71	**0.002**
Anatomical Distortion volume	1.89	1.56 – 2.30	**<0.001**	1.43	1.12 – 1.83	**0.004**

We therefore carried out a logistic regression analysis in which the dependent variable was the unfavourable functional outcome at 90 days measured as mRS ≥3 which showed that high values of NIHSS, corrected lesion volume and AD are associated with worse outcomes in both univariate and multivariate analysis (Table 1).

CONCLUSION

In this preliminary analysis we found that increased AD is associated with poor outcome in patients with ischemic stroke. The potential clinical and therapeutic implications of an accurate and objective quantification of anatomical distortion are: AD could have a predictive role in estimating post-stroke outcome; It could enable to identify patients at risk and consequently to start an early treatment of cerebral edema in those patients; finally, the measurement of AD could allow a better evaluation of the efficacy of new experimental anti-edema therapies.

The next steps foreseen in our group work plan of are to apply the measure of AD in the patient cohorts of the RISK and NIMBLE studies and to evaluate the relationship of AD with blood and neuroimaging biomarkers.

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Transcriptomics: An enticing approach to understanding the pathophysiology and clinical outcomes of acute ischemic stroke

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BACKGROUND

Acute ischemic stroke (AIS) represents one of the principal causes of neurological morbidity and mortality worldwide and is characterized by a multifactorial etiology, caused by interactions among blood vessel and environmental and genetic factors. For a prompt and efficient cerebral blood restoration, intravenous thrombolysis with rt-PA is often combined with mechanical thrombectomy (MT). MT represents a golden ticket from a research perspective, providing cerebral thrombi (CT) as new study material, enabling in-depth studies concerning their cellular composition and etiology correlation. In addition, it represents a key pillar for the creation of virtual predictive models of different gene expression based on annotated histopathological evidence. The trend towards a medicine based on personalized and individualized prevention and treatment strategies has led to the need to investigate the genetic aspect of the disease, due to its significant contribution to the genesis of the ischemic event. For that, focus of the research is the highlighting and exploration of profiles in which peripheral blood (PB) mirrors CT through the analysis of global gene expression profiles, and the identification of promising markers that can serve as sentinels for different pathophysiological mechanisms and/or determinants of clinical outcomes, such as haemorrhagic transformation, 24h edema, modified 3 months Rankin scale-mRS, death. This approach could allow to gain deeper insights into the pathogenesis of the disease through investigation of the relationship between gene expression and phenotypic differences.

METHODS

We performed gene expression profiles of RNA samples obtained from 40 CT and 37 PB of 52 patients. The CT obtained during MT were stored in RNA

later, while PB, collected before and 24 hours after MT, in tubes containing a reagent that protects RNA from degradation and minimizes ex vivo changes in gene expression. RNA was extracted by PAX gene blood miRNA kit; the global gene expression profile was assessed by Affymetrix technology using GeneChip Human Transcriptome Array 2.0, allowing the analysis of 44,699 genes, with more than 285,000 full-length transcripts coverage. Data analysis was performed in R environment with dedicated pipelines.

RESULTS

Data processing and the application of appropriate filtering criteria showed an average of analyzable probe sets of 440,085 in CT and 602,874 in PB. In the two different type of specimens 20,341 were found to be common features, whereas 3 and 562 symbols were unique in CT and PB, respectively. The Gene Ontology (GO) enrichment analysis allowed the identification of the biological processes, common and peculiar, in CT and PB, indicating that peripheral and local mechanisms of damage and response to damage are present in both. The significance analysis of microarrays, according to different outcomes and GO analysis, brought into focus 221 significant biological processes associated with poor outcome according to mRS in CT, and 27 terms associated with 24h edema in PB. Among significant terms in CT, those associated with regulation of neutrophil mediated immunity and activation play a crucial role. Concerning PB, particularly significant enriched terms were associated with regulation and activation of transcriptomes of cells.

CONCLUSIONS

Our results provided interesting insights into the mechanisms underlying the AIS and the response to treatments. In particular, the analysis of CT and PB gene expression profiles, differentially expressed probe sets and their biological processes alterations according to stroke outcomes, has not only confirmed and extended several known pathophysiological mechanisms, but also suggested novel pathways to be explored that may provide an important starting point for expanding knowledge on this cryptic disease.

Integrating novel neuroimaging measurements and circulating biomarkers for the prediction of secondary injury following stroke: From bench to bedside. Protocol presentation and interim analysis of clinical section from the NIMBLE study

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BACKGROUND AND AIMS

Brain Edema (BE), Haemorrhagic Transformation (HT) and Infarct Growth (IG) represents the most feared complications of acute ischemic stroke (IS)[1] acute ischemic stroke patients treated by endovascular thrombectomy in a tertiary university hospital were included. Early neurological deterioration (END. Investigating interactions between circulating[2] and neuroimaging biomarkers[3] the Imaging Working Group of StrokeNet, the American Society of Neuroradiology, and the Foundation of the American Society of Neuroradiology sponsored an imaging session and workshop during the Stroke Treatment Academy Industry Roundtable (STAIR, in relation to stroke outcomes, and understanding the reorganization of neurons at cellular and subcellular level during ischemia may provide information to prevent or contrast clinical deterioration after IS. To achieve this goal, we constituted a research group composed by clinical (neurologists, neuroradiologists, interventional-neuroradiologists, biologists, nurses, statisticians) and preclinical researchers and we planned to perform joint research on humans and animal stroke-model. In this text we describe the clinical protocol and preliminary data of this study. The preclinical section concerning the mouse model of photothrombotic occlusion and recanalization will be discussed in a separate section.

METHODS

This is a monocentric prospective observational study conducted in Careggi University Hospital. We plan to enrol at least two-hundred and fifty patients with acute IS of anterior circulation, admitted to our centre within 12 hours from onset, either treated or not with reperfusion therapies.

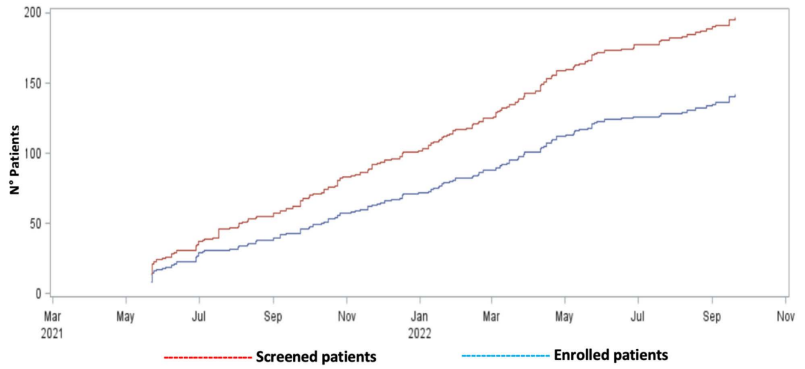

FIGURE 1: Patient recruitment trend in the NIMBLE study.

The neuroradiological assessments at baseline will be performed with brain CT, plus CT Angiogram and CT Perfusion (CTP) when clinically appropriate according to guidelines[4,5]; with brain CT and brain MRI will be carried out 24 hours and seven days after stroke onset, respectively. CE and HT will be objectively measured according to the Anatomical Distortion (AD) methods[6]: lesion masks will be defined on brain CT performed at 24 hours by two clinicians with a radiologist resolving any discrepancy. The images will co-registered for each patient on baseline CT to enable the quantification of AD at 24h from stroke onset, reflecting the volumes of edema and blood present in brain tissue. We will compare core lesion volume derived from CTP on presentation and Final lesion on MRI in order to assess IG.

Circulating biomarkers will be collected at baseline and after 24 hours, to analyse serum levels of multiple molecules, including, metalloproteases and their inhibitors, early indicators of neuronal damage (i.e. S100 proteins and Neuron-Specific Enolase) and inflammation (such as interleukins, Aptoglobin, Serum Amyloid A, Alfa-2 macroglobulin, and ultrasensitive CRP), and in addition to metabolomics panels.

Clinical status will be evaluated at baseline, after 24hours and after 7days from stroke with NIHSS while functional outcome will be assessed at 3 months with the mRankin Scale.

Appropriate statistical tests will be used to explore the correlation between circulating biomarkers values and variation in time, AD, IG and clinical status. Potential predictors of BE and HT will also be sought among the blood biomarkers.

Preliminary descriptive analysis

The recruitment officially started on April 24, 2021. 197 patients were screened, of which 55 were excluded by applying the exclusion criteria (haemorrhagic stroke, posterior circulation, hospital arrival later than 12 hours from onset and refusal to participate). We have therefore so far enrolled 142 patients with a mean age: 76.20 $\pm$ 6.9, of which 62 women and 80 men. The mean baseline NIHSS is 11.8. 99 patients underwent recanalization therapies, of which 21 treated with combined therapy, 21 with thrombectomy only and 57 with iv thrombolysis only. As shown in the graph (Fig.1), recruitment maintains a regular trend and in line with the goal of reaching the target of 250 patients by October 2023.

CONCLUSIONS

Through this study we will try to understand the role and the interaction that seems to be present between the "Wet" and "dry" biomarkers (i.e. circulating and neuroimaging), in order to identify possible prediction tools, with the final goal of preventing or limiting the occurrence of harmful phenomena such as the BE, the HT and IG.

We will also verify to what extent the animal model can reliably reproduce and explain events occurring in the brain tissue of stroke patients. Once validated, the information obtained at the tissue level in animal models will be used to try to understand the mechanisms determining the clinical deterioration due to BE, HT and IG. We will the animal model can reliably reproduce significant parameters that are evaluated in stroke patients.

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Biomarkers in stroke: When the laboratory serves the clinic. New perspectives in patient's management and care

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Stroke is one of the most important cause of disability and mortality worldwide. The aim of the NIMBLE Study is to gain a broad understanding of the pathophysiology. In particular, laboratory's goal is to identify serum biomarkers for rapid and accurate diagnosis, useful for prognosis and patients' monitoring. Serum from patients with first-in-life stroke were analyzed before any kind of treatment (basal) and after 24h from the onset of symptoms, with the aim to assess the concentration of inflammation biomarker, Serum Amyloid A (SAA) and alfa-2 macroglobuline, as well as marker of blood brain barrier damage (S100, NSE) and of cardiovascular risk, like ultrasensitive CRP (hsCRP). For each patient, we further considered any comorbidities or ongoing therapies. Regarding marker analysis, we considered the status of the patients on the first day of stroke and after 24h, according to NIHSS score, and the presence of intra cranial haemorragy (ICH). Among the 150 patients analyzed to date, a statistically significant correlation was proven between SAA and haemorragic trasformation (HT) ($p = 0.02$), both basal and after 24h, and between hCRP and HT ($p=0.05$). Same results emerged when an univariate logistic regression analysis was performed, for both the marker above mentioned

when related to the NIHSS score. Since only 15 out of 105 patients underwent haemorragic event 24h after symptom's onset, data needs to be further expanded to be confirmed. The study of correlations between circulating biomarkers and neuroimaging, related to unfavorable clinical, neurological, and functional outcomes after ischemic stroke, could provide information to prevent or counteract early clinical worsening after acute ischemic stroke. Given the complexity of the disease, due to its pathophysiology but also to the patients' heterogenity, it is important that all the professionnels involved, from bench to the bedside, cooperate to ensure a fast and accurate diagnosis: "time is brain".

Modeling stroke-related cerebral edema

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BACKGROUND

After a stroke, the disruption of the neurovascular unit is responsible for main of the clinical complications of ischemia, namely cerebral edema and hemorrhagic transformation. Preclinical research is making a great effort to understand the fine mechanisms underlying stroke clinical complications. Here by we investigate the insurgence of cerebral edema in the acute phase after the insult characterizing a newly developed mouse model of photo-thrombosis of the distal middle cerebral artery.

METHODS

In order to evaluate the presence of blood-brain barrier permeability we injected in the mouse tail vein Evans Blue dye right after the surgery. 24 hours after the injection the animal was perfused with 100 mL of PBS in order to remove the blood from the brain tissue. Then the brain was sectioned with a brain matrix producing approximately 10 slices 1 mm thick. We then performed the evaluation of brain water content as an indirect measure of cerebral edema. 24 hours after stroke, mice were sacrificed with an overdose of anesthetic. The brain was divided along the midline and the contralateral and ipsilateral tissue was weighed right after removal to obtain wet weight. The tissue was then dried and weighted to obtain dry weight.

RESULTS

The diffusion of Evans Blue dye affects a large portion of the ipsilesional hemisphere, extending both in the rostral direction up to the olfactory bulbs and in the caudal regions. Moreover, the evaluation of brain water content, as a measure of cerebral edema, highlighted an increase of brain water content in the ipsilesional hemisphere of stroke mice revealed 24 hours after stroke induction through ex vivo experiments.

CONCLUSION

The photothrombotic occlusion of the distal middle cerebral artery induces acute alterations of blood-brain barrier permeability and an increase of brain water content in the ipsilesional hemisphere.

Clinical and biological markers of functional outcome in stroke

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Stroke is the main cause of disability, the second of dementia and the third of death in most countries and more than a third of stroke survivors are dependent in one or more activities of daily living. These limitations are associated with decreased quality of life and increased use of hospital and nursing home care. Ischemic stroke is the most prevalent stroke type representing about eighty percent of all stroke events . In the last two decades thrombolytic treatments, such as intravenous tissue plasminogen activator and endovascular therapy, have improved functional outcome after ischemic stroke but are received by only a small minority of stroke patients and nonthrombolyzed stroke survivors has been largely unchanged. Similarly acute inpatient rehabilitation which reduces longterm disability and enhances functional outcome is received by only a minority of stroke patients after the acute hospitalization. The ability to predict functional outcome after stroke may help to improve the selection of the most appropriate therapy already in the acute phase in the individual patient. Many clinical prediction models for mortality and functional outcome following ischemic stroke have been employed (Fahey M et al, 2018). Stroke severity represents the main clinical predictor both of early and late functional outcome (Rost NS et al,2016) and NIH Stroke Scale score was widely validated to measure neurological impairment for this aim (Saver JL et al,2012). Demographic factors which especially the age , premorbid functional state including cognitive impairment and multiple chronic conditions evaluable by Charlson Comorbidity Index are further functional outcome predictors (Jiang X et al,2020). Moreover functional outcome may depend on the presence of dysphagia, seizures ,fever, metabolic impairment such as hyperglicemia and hyponatremia occurring during the acute phase. In particular high systolic blood pressure values and coagulopathies worsen the prognosis in haemorragic stroke.

In contrary to the cardiovascular disease, no single blood biomarker is available for the ischemic stroke. Probably the reason depends in whether ischemic stroke is a dynamic and heterogeneous process related to the coexistence of several phenomena such as oxidative stress, immunological response,

reactive gliosis and dysfunction of hemostatic system. Since the coagulation system plays an important role in stroke pathogenesis, blood biomarkers of coagulation have been studied to identify patients at risk of poor clinical outcome. Blood cell composition and protein biomarkers such as C-reactive protein or interleukins in serum dosed in the first few days following stroke are frequently considered as biomarkers of outcome (Donkel S et al 2019). Protein biomarkers are classified as immune-inflammatory, coagulation-related and adhesion-related biomarkers. Some of these biomarkers are related to cellular senescence and in particular to the inflammatory processes that can be triggered by senescent cells. Moreover the processes that underlie inflammation, hypercoagulation and cellular senescence connect stroke to cancer and biomarkers of cancer-associated thromboembolism overlap strongly with stroke biomarkers.

Therefore protein biomarkers form a close-meshed functional interaction network so the outcome after stroke is determined by an interplay of molecular processes relating to infammation, coagulation, cell adhesion and cellular senescence. To date the most documented molecular blood markers panel for long-term outcome after stroke consists of tumor necrosis factor alpha (TNF), interleukin 6 (IL6), fibrinogen (FGA) and plasminogen activator inhibitor-1 (SERPINE1) (Fuellen G et al,2022). The neurovascular unit (NVU) is considered a source of ischemic stroke biomarkers (Wang L et al,2020). An important role of NVU is attributed to its characteristic morphological structures, such as tripartite synapses, astrocytic perivascular end-feet and vascular tight junctions. In accordance with the NVU concept, ischemic stroke biomarkers can be categorized as the representatives of either individual cell type or its several components. Moreover this concept enables division of ischemic stroke biomarkers into groups characterized by similar structure and functions representing different categories such as neuroglial and neuronal structural proteins, amino acid neurotransmitters and enzymes,inflammatory mediators and neurotrophic and growth factors. Many of the NVU-derived markers could be used for assessment of the risk of stroke related complications. These include hemorrhagic transformation of the ischemic stroke (S-100β, vWF and MMP-9), development of malignant stroke (S100β), increasing infarct volume (S-100β, NSE, MMP-9, IL-6, TNFα, and Glu), early neurological deterioration and progressive stroke or death (IL-6, S100β, TNFα, MMP-9; NSE, Glu, and GABA). Potential benefits resulting from the ischemic biomarkers assessment in the later phase of stroke can be related with information about its progression (GFAP, S100β, IL-6, MMP-9), monitoring of the results of therapy (IL-6, S100β, MMP-9) and functional outcome estimation

(IL-6, S100β, vWF, BDNF) (Steliga A et al,2020). Despite activation of numerous markers their prognostic value is not yet satisfactory and requires further investigations searching for new components of functional outcome panels representing various elements of NVU.

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Neurorehabilitation: Clinical research

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Stroke is the epidemic disease of the 21st century, as strongly evident by its high incidence (1.5 Million cases /year in Europe and 15 Million /year worldwide). Besides its incidence rate, stroke is the main cause of long-term disability, allowing currently less than 15% of the patients to fully recover and get back to normal life. The fact that up to 20% of the stroke patients are below 55 years, thus fully in their professional life, makes it even more relevant. Efforts in improving neuro-rehabilitative therapy remain unsatisfactory, leaving many patients significantly impaired with a lack of independence and need of continuous assistance from health care providers. The reasons for this unsatisfactory recovery after stroke are manifold, but can be summarized into two main groups, which are strongly interrelated: (i) insufficient understanding of the processes relevant for recovery and (ii) limited and non-personalized usage of the available, innovative rehabilitative treatment strategies. To bridge this gap remain still imperative to identify biomarkers of stroke recovery in a way to: (i) to identify the intrinsic recovery potential of single patient; (ii) to be able to predict the answer to standardized treatment. To reach these goals in the last years neurophysiological (i.e High Density EEG) approaches have been applied. It has been proposed that alterations in EEG after stroke could correlate with recovery both in animals and in humans, and some neurophysiological measurements have been proved to possess a certain predictive power (Grefkes et al., 2011, Assenza et al., 2013).

Our group conducted clinical trials aiming at describing EEG characteristics in subjects affected by cortico-subcortical lesion respect to those affected by subcortical lesion in the early subacute phase post-stroke. We described a higher and more symmetrical Delta power in patients with cortical involvement. Conversely we found a higher alfa power spectrum in patients with a subcortical lesion (Chiara Fanciullacci et al., 2017). These results confirmed that stratifying patients on the basis of the lesion location is mandatory to design clinical trials able to give reliable and univocal results.

Successively we studied the "spontaneous" functional recovery by following patients from the early to the late subacute phase of the disease. In particolar

we tried to find predictive biomarkers of recovery and again we found that patients with cortical involvement presents specific findings: we observed a negative correlation between the delta power at T0 (10-30 days after stroke) and the motor recovery 3 months later (Chiara Fanciullacci et al., 2021).

More recently we attempted to predict the motor recovery due to a standardized robotic treatment by means of neurophysiological parameters. We obtained clear indication about the predictivity of Power Spectrum Density and Connectivity measures extracted by qEEG (data unpublished).

A very interesting field of interest in stroke rehabilitation is the finding of plasticizing drugs able to maximize and guide the functional recovery. For sure the unbalance between the serotoninergic and the gabaergic patways plays a crucial role for the spontaneous and guided recovery. Our group showed that the recovery is paralleled by a reduction of the gabaergic tone in the unaffected hemiphere. We used an indirect way to study this phenomenon: the length of the silent period evoked by a Transcranic Magnetic Stimulation during a voluntary contraction (Giuseppe Lamola et al., 2016). These data are extremely interesting as advice of avoiding the use of gabaergic drugs (i.e. benzodiazepine) in the early and late subacute phase of stroke. Very interestingly the administration of 5HT1a agonists in animals showed an improvement of the motor performance paralleled by a reduction of gabaergic tone (Conti S. et al., 2021).

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Novel experimental strategies to promote brain repair and predict motor function after stroke in mice

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Novel experimental strategies to promote brain repair and predict motor function after stroke in mice Text (max 1,000 words, references included) Stroke is a devastating pathology and the main cause of long-term disability, allowing currently less than 15% of the stroke survivors to fully recover and get back to normal life. Many cognitive domains result affected by strokes and motor functions are impaired in 80-85% of subjects. Efforts in improving rehabilitative therapy remain unsatisfactory, leaving many stroke survivors significantly impaired and with a need of continuous assistance from health care providers. Therefore, nowadays it is not possible to predict the final degree of recovery, making difficult to stratify stroke survivors and address them towards their optimal therapeutic protocol in order to maximize its own restoration potentiality. Here, we longitudinally collected Local Field Potentials (LFP) from stroke and healthy mice to define acute post-stroke electrophysiological alterations to be used as biomarkers to predict the final degree of recovery for each subject. Mice were implanted in their left and right Caudal Forelimb Areas (CFA) and electrophysiological signals were acquired during a forelimb retraction movement, before and after Middle Cerebral Artery Occlusion (MCAO). In particular, we analysed post-stroke alterations in the Event Related Potential (ERP), in the Perievent Spectograms and in the Event Related Synchronization/Desynchronization (ERS/ERD). These measures were also correlated with the individual degree of spontaneous recovery. However, be able to predict spontaneous recovery would not be enough if a rehabilitative protocol is not available. Cell-based approaches have emerged as an intriguing and promising strategy to promote brain repair. Recently, we developed protocols to obtain cortical or hippocampal neurons derived from embryonic stem cells (ESCs). These two types of cells showed different degrees of axonal outgrowth and targeted different regions when co-transplanted in vivo. In hippocampus, only precursor cells with hippocampal molecular identity were able to extend projections towards CA3

neurons. Conversely, cortical-like cells were capable of extending long-range axonal projections only when transplanted in motor cortex. A cortical stroke greatly enhanced the capability of corticallike cells to extend far-reaching projections. Our results indicate that neural precursors generated by ESCs carry intrinsic signals specifying axonal extension in different environments. As second approach to promote neural repair, we exploited direct reprogramming of endogenous reactive astrocytes into neurons, with the major advantages of obtaining neurons with the correct positional identity and immunogenic profile. To this aim, we forced the expression of pro-neural transcription factors through flexed AAVs injection in GFAP-Cre transgenic mice. Two months later, we observed successful reprogramming in the perilesional tissue. Moreover, motor tests were used to evaluate the therapeutic effect of this approach in promoting motor function after stroke, alone and in combination with motor rehabilitation. TREES conference – II Edition Florence, Italy Careggi University Hospital November 25th, 2022 Findings from this study will contribute to the Neurorehabilitation quest in identifying early, reliable neurophysiological biomarkers of recovery, which would allow a better tailoring of rehabilitation pathways and allocation of resources in an ageing world destined to a sharp increase in stroke disability. Moreover, our results are important to move the field forward and to bridge the gap between pre-clinical studies and clinical developing of new combined therapeutic strategies for stroke patients.

Rehabilitation and neuro-modulation after stroke: Novel therapeutic strategies in murine models

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Motor recovery after brain damage induced by an ischemic event is always challenging and often unsuccessful. Despite several innovative approaches have emerged to treat ischemic patients acutely, for most of them the treatment can be applied only in the subacute phase after injury. This time window is extremely precious for its plastic potential and offers the possibility to recover motor function by guiding peri-lesional areas to vicariate what was lost. Unfortunately, this plastic potential, if not properly guided, could led to maladaptive rearrangements and unwanted movement patterns. Here we use a mouse model of stroke in forelimb motor cortex to test novel and highly translational neurorehabilitative approaches in subacute phase by combining robotic rehabilitation with plasticizing treatments. We first engaged the serotoninergic system and demonstrated that a selective chemogenetic boosting of the serotoninergic system can increase perilesional plasticity and lead to a significant forelimb recovery without maladaptive movement detected by kinematic analysis. These results were confirmed by using an FDA approved drug to increase the serotoninergic tone. We are now focusing on non-invasive neurostimulation approaches and on the role of Parvalbumin Interneurons (PV-IN) in post-stroke recovery. We assessed the consequences of the ischemic lesion onto PV-IN activity by electrophysiological recordings and Wide Field Imaging in awake head restrained mice before and after stroke. Based on the results obtained we successfully tested non-invasive brain stimulation approaches, first targeting PV-IN by optogenetics and then using a more translational approach with Non-Invasive Transcranial Alternating Current Stimulation. Both of the approaches led to a significant improvement of forelimb motor function and pave the way for successful combined approaches in clinical practice.

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