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TESI DI LAUREA

BRAIN DYSCONNECTOME: A POTENTIAL BIOMARKER FOR FUNCTIONAL OUTCOME AFTER MECHANICAL THROMBECTOMY

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ABSTRACT

Rationale: Mechanical thrombectomy *(MT)* is a safe and effective procedure that has improved the prognosis of patients with large vessel obstruction *(LVO)* stroke. Even though we select patients based on various clinical and imaging criteria, more than half of those undergoing this procedure remain with severe long-term disability. Several factors, both pre-treatment (pre-stroke mRS score, NIHSS at presentation, time between event and recanalization, ASPECTS, core, and penumbra volumes) and post-treatment (TICI, NIHSS at 24h, final infarct volume, procedural complications, post-stroke complications) have been identified as predictors of outcome (i.e., mRS). Among these factors, the volume of the lesion and the size of the hypo-perfused region of the brain are used to make individual decisions about treatment. In retrospective studies, the volume of the lesion weakly correlates with clinical outcomes, while the location of the lesion is more predictive. In addition, more recently, measures of large-scale (brain networks) disruption have been developed based on the concept of structural and functional disconnection, i.e., the ensemble of structural and functional connections that are directly or indirectly damaged by the focal injury. These disconnection measures have been shown to be strongly predictive of acute impairment and recovery of function. Here we plan to measure in a group of patients with LVO who underwent MT the relationship between outcome (3 month-mRS) and the location of the lesion or its effect on structural and functional networks.

To examine whether the outcome is more related to the vascular distribution stroke or their effects on structural-functional brain networks, we mapped the lesions onto a vascular (blood vessels) atlas, a gray matter functional regions' atlas, or a white matter structural connections' atlas. Then using multi-variate statistical models, we tested which atlas was more predictive of clinical outcome. The same analysis was then applied to structural and functional disconnection patterns.

Materials and methods: A total of n=66 patients underwent MT at the Neurology Unit of Padua university hospital from January 2019 to June 2022. They were examined with the mRS and the National Health Institute Stroke Scale *(NIHSS)* both at admission and discharge. The mRS was also administered at three months post-stroke for measuring clinical outcomes. The location and volume of the lesions were measured from CT, and FLAIR MRI scans performed after MT and manually

segmented using the software ITK-SNAP. We computed voxel-wise maps of structural and functional disconnections that were significantly related to functional outcomes. We also investigated the relationship between lesion location computed on three different atlases (vascular, functional grey matter, and structural white matter atlas) and 3-month mRS.

Results: The mean pre-event mRS was 0.5±0.9, post-MT mRS was 3.1±1.9, at three-month follow-up mRS was 2.5±2.1. A voxel-wise analysis of the functional disconnection showed a significant involvement of the sensory-motor network *(SMN)* (R^2= 0.340), the visual network *(VIS)*(R^2= 0.379), and the dorsal attention network *(DAN)*(R^2=0.318). The voxel-wise structural disconnection analysis localized sensorimotor pathways and long-range association pathways. The prediction of lesion topography on clinical outcome was more robust for the functional atlas (R^2=0.382), followed by the structural atlas (R^2=0.338), while the vascular atlas provided the lowest prediction (R^2=0.146). Structural disconnection performed better than functional disconnection in predicting outcomes (respectively R^2=0.339 and R^2=0.205).

Conclusions: Stroke lesion topography is a strong prognostic factor of outcome at three months when computed on an atlas of functional and structural networks, as compared to a vascular-based atlas. These findings indicate that pre-treatment evaluations for MT shall take into consideration the network structure of the brain and less its vascular supply. Structural disconnection measures are of high prognostic value. Future studies will define the prognostic value of a network-based atlas in a pre-treatment setting.

PART I. WHAT IS STROKE? THE CLASSICAL FRAMEWORK

DEFINITION

A stroke occurs when blood flow is interrupted in different parts of the brain leading to the death of brain cells in the hypoperfused area, causing a sudden and focal neurological deficit. It develops when the involved blood vessel is either occluded -*ischemic stroke*- or ruptured- *hemorrhagic stroke*. If the interruption is transient and leads to a temporary neurological deficit that leaves no clinical or imaging trace, we can describe a TIA *(Transient Ischemic Attack)*.

According to the current world health organization *(WHO)* definition, stroke is characterized by "rapidly developing clinical signs of focal (or global) disturbance of cerebral function, with symptoms lasting 24 hours or longer or leading to death, with no apparent cause other than vascular origin."[1] However, ischemic lesions can be detected already after 45 minutes of hypoperfusion.[2]

EPIDEMIOLOGY

Stroke affects globally roughly 13.7 million people and is the second cause of death, killing around 5.5 million people annually. Ischemic infarctions represent 87% of all strokes, and their prevalence increased appreciably between 1990 and 2016, probably due to decreased mortality and improved clinical interventions.[3,4]
In 2020 the American Heart Association report on heart disease and stroke statistics estimated that 7 million Americans over 20 years of age had suffered a stroke, reporting nearly 800,000 stroke events and 150,000 deaths (prevalence 2,5%). The most important demographic factor was age, and although the incidence of stroke has diminished in recent years, its lifetime risk has increased due to population aging.[5]

GEOGRAPHICAL DIFFERENCES

Significant geographical differences in age-standardized incidence, prevalence, mortality, and disability-adjusted life years *(DALYs)* rates, with the highest rates in lower-middle income countries, were highlighted by the world stroke organization *(WSO).*[6] The most significant estimated risk of stroke was found in East Asia (38.8%) and Central and Eastern Europe (31.7% and 36.6%).

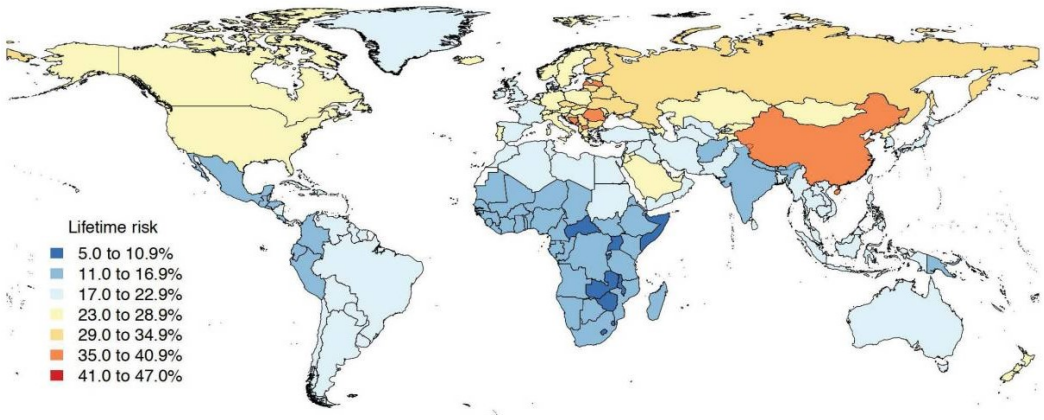


Figure 1. Worldwide stroke incidence: the lifetime risk of stroke (in %), for both sexes in 2016, from Feigin et al. (2018)

Furthermore, crude case fatality varied based by country: the greatest overall 28-day case fatalities were observed in India at approximately 37–42% at 28 days. The lowest case-fatality was observed in Dijon (2000–2004) at 10%.[7]

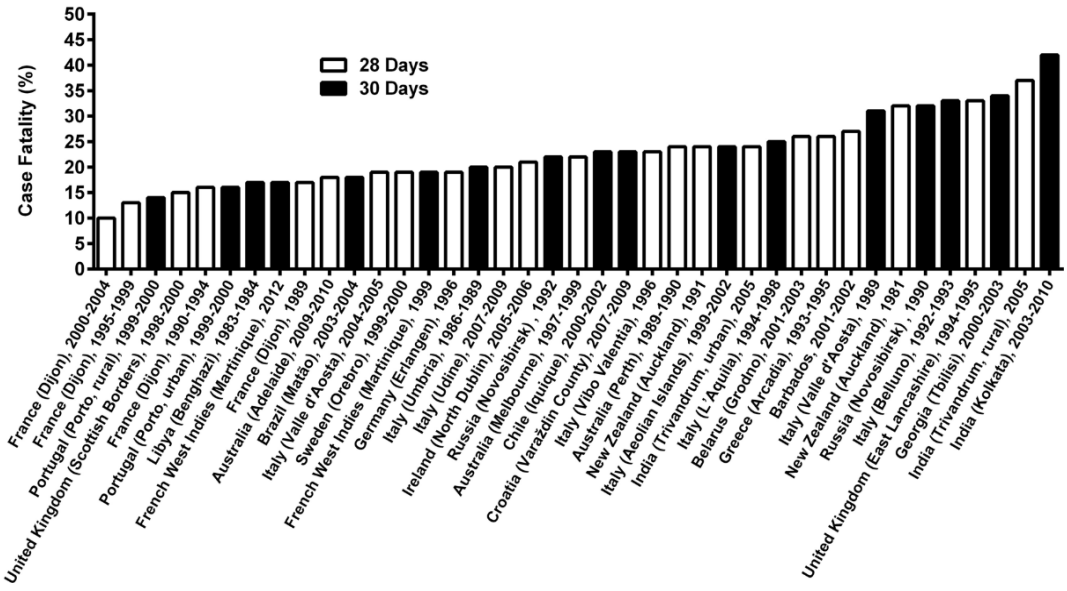


Figure 2. Stroke case fatality: state by state case-fatality at 28 and 30 days, from Thrift et al. (2017)

RISK FACTORS

The five leading risk factors for stroke are:

- high systolic blood pressure, accounting for 55% of total stroke DALYs
- high BMI (24.3%)
- high fasting plasma glucose (20.2%)
- ambient particulate matter pollution (20.1%)
- smoking (17.6%)

Hypertension, combined with smoking and hypercholesterolemia, is one of the leading causes of atherosclerosis, responsible for narrowing the arteries' lumen, artery-to-artery embolism, and superimposed thrombosis leading to vascular occlusion.

Metabolic risks such as high systolic blood pressure, high BMI, high fasting plasma glucose, high LDL cholesterol, and kidney dysfunction accounted for 70.3% of stroke-related DALYs. From 1990 to 2019, there was a significant reduction in the

contribution of behavioral (smoking and dietary risks) and environmental risks (air pollution cluster, low and high ambient temperature, and lead exposure) but a significant increase in the contribution of metabolic ones, primarily due to the rise in high BMI and high fasting plasma gluscose.[6]

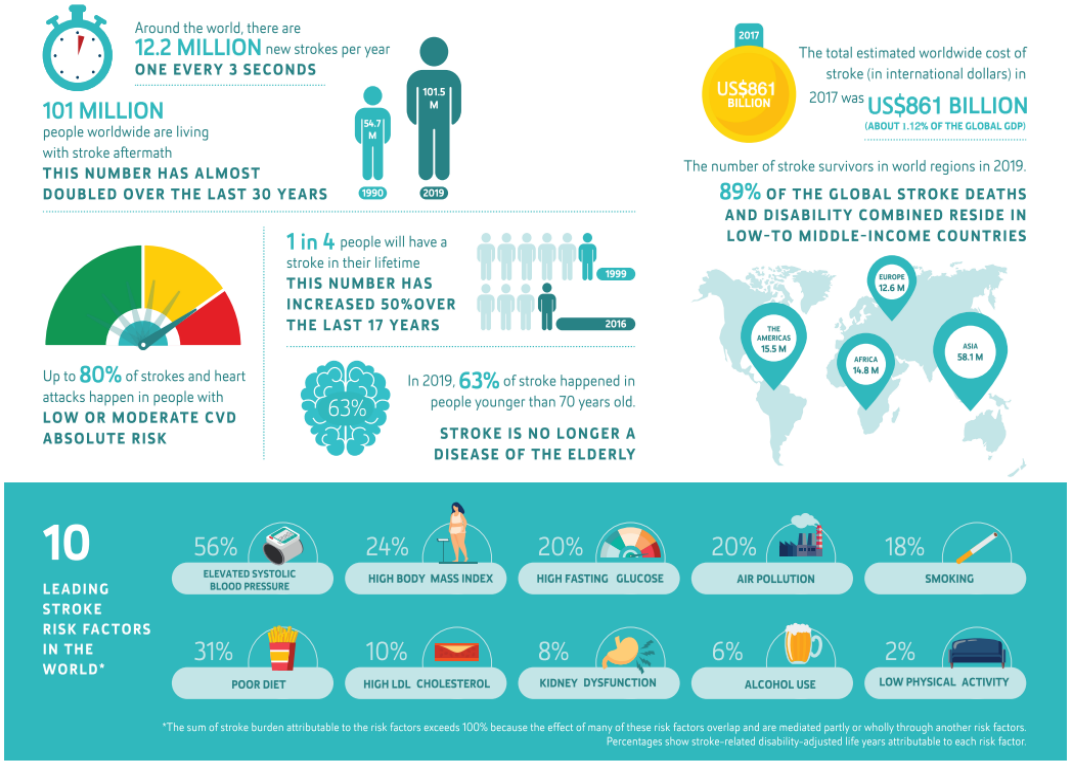


Figure 3. Stroke infographic: stroke incidence, prevalence, costs, geographical distribution, and risk factors, from Feigin et al. (2020)

ECONOMICAL BURDEN

The estimated global cost of stroke is over US 721$ billion, roughly as much as the 2021's GDP of the Netherlands.[8] In 2021 approximately 34% of the worldwide healthcare expenditure was spent on stroke. The average healthcare cost for stroke per person was estimated at US 140,048 $ in the United States, including inpatient care, rehabilitation, and follow-up care. The economic burden of stroke includes 1) direct or medical costs (86.2%) like hospital care, services of doctors and other medical professionals, equipment, medicines, and rehabilitation; and 2) indirect or non-medical costs (13.8%) such as informal caregiver costs and productivity lost.

The cost to manage stroke is high because of the extended hospital care required, after-discharge risk management, and long rehabilitation.[9]

Stroke is not only a major cause of death and disability but also results in a significant economic loss to the country.

CLASSIFICATION

Hemorrhagic strokes can occur within the parenchyma, *intracerebral hemorrhage*, or in the subarachnoid spaces, *subarachnoid hemorrhage* and are part of a broader collection of neurological disorders: intracranial hemorrhages, a collective term including many different conditions characterized by the extravascular accumulation of blood within different intracranial spaces.

Intracerebral hemorrhages can be further classified based on their etiology:

- Primary hemorrhages (without underlying lesion)
    - hypertensive hemorrhages
    - due to cerebral amyloid angiopathy
- Secondary hemorrhages
    - cerebral venous thrombosis
    - vascular malformation
    - tumor
    - coagulopathies

and their location:

- basal ganglia hemorrhage
- pontine hemorrhage
- lobar hemorrhage
- cerebellar hemorrhage

Ischemic stroke can be classified based on either lesion location or etiology.
TOAST is the most widely used etiological classification for ischemic stroke; it divides stroke into five subtypes:

- large artery atherosclerosis *(LAA)*
- cardioembolism *(CE)*
- small-vessel occlusion *(SAO)*
- stroke of other determined cause *(SOC)*
- stroke of undetermined cause *(SUC)*
    - two or more causes identified
    - negative evaluation
    - incomplete evaluation

Classification is based on clinical features and data collected by brain imaging (CT/MRI), arteriography, duplex imaging of extracranial arteries, cardiac imaging, and laboratory assessments for a prothrombotic state.

Depending on supplementary results, LAA, CE, SAO, and SOC can be sub-classified into “possible” and “probable”.[10]

TOAST classification has also been demonstrated to be effective in predicting the outcome of ischemic stroke. In fact, survival probabilities have proven to depend on stroke etiology: 5-year-survival probability was greatest in patients with stroke due to SAO and lowest in cardioembolic stroke. 28- and 30-day mortality varied Among the various subtypes of stroke.[11]

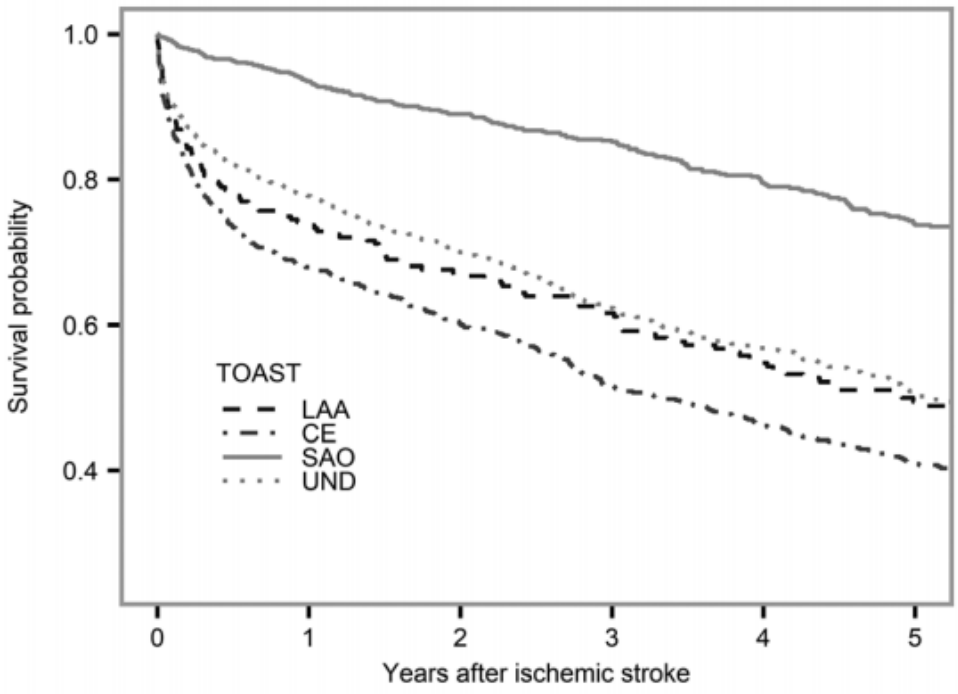


Figure 4. Stroke survival probability by etiology: Kaplan-Meier plot for stroke due to large artery atherosclerosis (LAA), cardioembolism (CE), small-vessel occlusion (SAO), and stroke of undetermined cause (UND) associated with patient survival, from Rücker et al. (2020)

TOAST classification depends on baseline CT scan findings, which are often unrevealing, and the patient's clinical features. To improve it, other classifications were implemented:

- Causative Classification System *(CCS)* relies on integrated results from diffusion-weighted MRI, CT angiography and magnetic resonance angiography, echocardiography, and Holter monitoring. The 2.0 version relies on clinical evaluation, brain imaging, extracranial and intracranial vascular survey, heart evaluation, and workup for common causes of stroke. It allows stratification of cardiac embolism into high and low-risk groups, revises the conventional definition of lacunes, and breaks "undetermined" TOAST's category into several subcategories (unknown, incomplete evaluation, unclassified stroke, and cryptogenic embolism).
- Chinese Ischemic Stroke Subclassification *(CISS)* introduces a classification system that considers both etiological and pathophysiological causes. It consists of two steps: 1) classifies stroke into five categories based on the concept of TOAST; 2) further classifies the underlying mechanism of ischemic stroke (previously divided into intracranial and extracranial large artery atherosclerosis) into four categories according to the modern imaging technology: patent artery, occluding penetrating artery, artery-to-artery embolism, hypoperfusion/impaired emboli clearance, and multiple mechanisms.[12]

Stroke can also be classified based on its clinical manifestations in:

- *lacunar infarcts (LACI)* that present with ataxic hemiparesis, pure sensory stroke, pure motor stroke, or sensory-motor stroke.
- *total anterior circulation infarcts (TACI)* that present with the combination of 1) new higher cerebral dysfunction (dysphasia, visuospatial disorder, dyscalculia), 2) ipsilateral motor or sensory deficit of at least two areas of the face, arm and leg; and 3) homonymous visual field defect. When upper brain function or the visual field cannot be assessed due to an impaired level of consciousness, they are considered affected.
- *partial anterior circulation infarcts (PACI)* that may present with milder motor or sensory deficits than those identified in TACI, with higher cerebral dysfunction alone, or with 2 of 3 components of the TACI syndrome.

- *posterior circulation infarcts (POCI)* that present with any of the following: bilateral motor and/or sensory deficit, ipsilateral cranial nerve palsy with contralateral motor and/or sensory deficit, a disorder of conjugate eye movement, cerebellar dysfunction without ipsilateral long-tract deficit or isolated homonymous visual field defect.[13]

PATHOGENESIS

VESSEL OCCLUSION

Ischemic stroke is caused by the occlusion of one of the vessels supplying the brain and it is mainly due to occlusion of small vessels, artery-to-artery embolism, cardioembolism, or atherothrombosis. To understand how the obstruction may impact clinical outcomes, it is necessary to consider neurovascular anatomy and physiology. The brain receives blood from two sources: the internal carotid arteries, from which anterior circulation originates, and the vertebral arteries, which merge at the bulb-pontine level, giving origin to the basilar artery. The vertebrobasilar system provides blood flow to the posterior circulation. The anterior circulation supplies 4/5 of the brain parenchyma, while the posterior perfuses the remaining 1/5.[14]

The internal carotid artery originates from the common carotid artery, travels in a superior direction along the neck, penetrates the skull, and reaches the cavernous sinus with an S-shaped course. The internal carotid artery originates five main branches:

- *Ophthalmic artery*
- *Anterior choroidal artery* that perfuses the caudate, the globus pallidus, the amygdala, the hippocampus, the hypothalamus, the substantia nigra, the red nucleus, the posterior limb of the internal capsule, and the optic radiation.
- A*nterior cerebral artery* that perfuses the head of the caudate, the anterior limb of the internal capsule, the medial portions of frontal lobes, the anterior part of the corpus callosum, and the superior and medial parts of parietal lobes.
- *Middle cerebral artery* that perfuses the lentiform nucleus, caudate, the internal capsule, the lateral portion of the parietal lobes, the superior and middle part of the temporal lobes, and the anterior and lateral portions of the frontal lobes.

The vertebral arteries originate from the subclavian artery and give rise to the posterior inferior cerebellar arteries *(PICA)* before merging into the basilar artery.

The basilar artery, along its way, originates collateral branches for the vascularization of the pons and then terminates, dividing into the posterior cerebral arteries.

The posterior cerebral artery *(PCA)* supplies blood to the midbrain and thalamus, the splenium of the corpus callosum, the inferior and medial parts of the temporal lobe, the occipital pole, and the visual cortex.

The anterior and posterior communicating arteries connect the anterior and the posterior circulation in the circle of Willis. This anastomosis provides collateral blood flow, protecting against ischemia in the event of vessel occlusion in one or more areas.

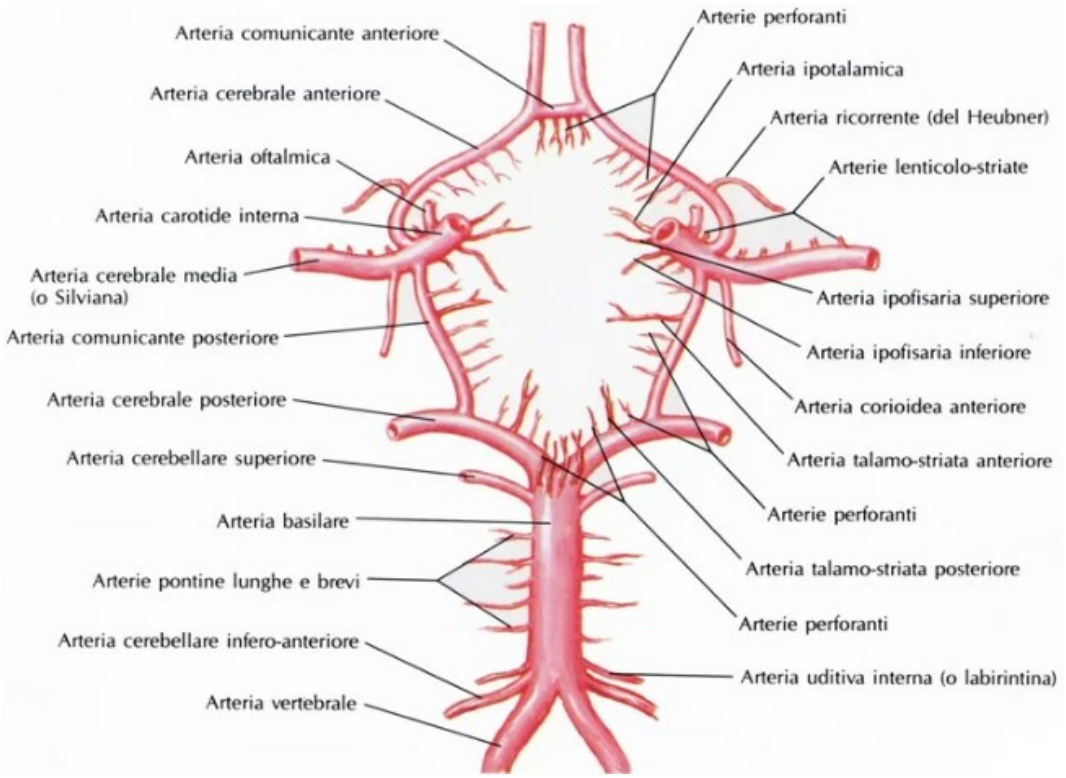


Figure 5. Circle of Willis, from Netter's Atlas of Human Anatomy by Frank Netter

MECHANISMS OF ISCHEMIA

To maintain proper brain function, a massive amount of glucose is required; to achieve so, 20% of the cardiac output is directed to the brain. The cerebral blood flow typically amounts to 800 ml/min, 50ml/ min for every 100gr of tissue. Ischemic stroke is caused by an inadequate blood supply to the cerebral tissue; while, at first, there is a reversible loss of tissue function, over time, the damage

becomes permanent, leading to the death of the cells involved.[15] Ischemia sets off a cascade of events that begins with the loss of electrical function, detectable by an EEG when the blood flow drops below 23 ml/100 g/min, while cell death develops below 10-12 ml/100 g/min. Between 12 ml/ 100g/ min and 23 ml/ 100g/ min, the function is abolished and the likelihood of complete infarction depends on the duration of ischemia.[16]

Cerebral flow self-regulates, maintaining a constant flow for arterial pressures between 60 and 150 mmHg. This process is activated by the variation of PaCO2 and mediated by myogenic factors, inducing constriction and dilation of the vessels and nervous modulation of vascular tone.[17] Other mechanisms concur in the preservation of neural oxygenation; in case of a drop in the O2 saturation, the brain extracts a higher percentage of oxygen from the blood.

Further decrease in blood flow leads to a dysregulation of the cell membrane homeostasis. Other biochemical changes in the marginal perfusion region include the increase of the K level and depletion of ATP and creatine. In the areas where the perfusion drops below 8 ml/ 100g/ min, the biochemical changes become irreversible: marked ATP depletion, Ca intracellular increase, K extracellular increase, and cellular acidosis. The activation of free fatty acid destroys the phospholipids of the cell membrane. The accumulation of prostaglandins, leukotrienes, and free radicals induces the denaturation of proteins and enzymes. The cell then swells in a process called cytotoxic edema.[16]

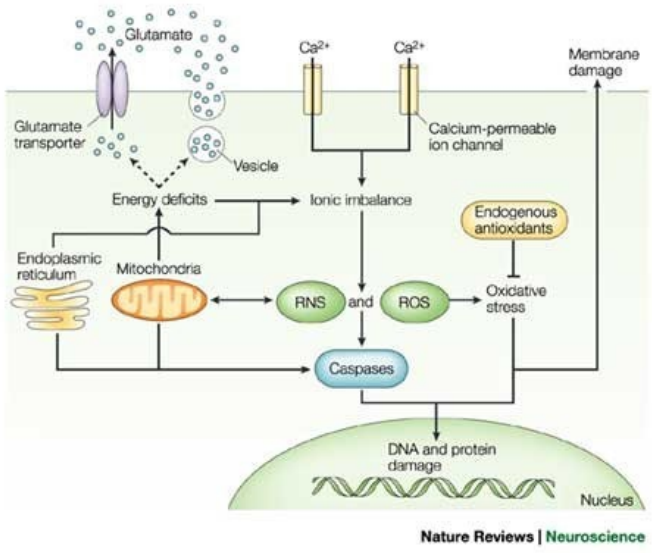


Figure 6. Most important pathways of ischemic cell death, from Lo et al. (2003)

In ischemic cells, excitatory neurotransmitters, particularly glutamate and aspartate, are formed from the Krebs cycle glycolytic intermediates. These neurotransmitters excite neurons, causing an intracellular influx of Na and Ca, partly responsible for irreversible cell injury. The increased free calcium gradient causes the activation of the NMDA receptor, responsible for the release of lipases, proteases, nucleases, and free radicals. However, studies have shown that cell death could not be prevented even with the blockade of NMDA, presumably because calcium continued to enter through several other types of calcium channels also affected by the ischemic changes. On the other hand, early activation of the AMPA receptor determines an accumulation of intracellular sodium and water, leading to cytotoxic edema.[16–18]

Other cells like microglia and oligodendrocytes also play an important role in ischemic stroke pathogenesis. Oligodendrocytes are highly vulnerable to oxidative stress due to their large size and low amounts of antioxidants, causing damage to the myelinated fibers and functional disconnections in regions far from ischemic stroke. Recent studies have shown that microglia activate almost immediately after the onset of brain ischemia, with both beneficial and detrimental effects. Microglial cells migrate towards the lesion site exacerbating tissue injury by producing cytotoxic substances and inflammatory cytokines; while also crucial for angiogenesis, neurogenesis, and synaptic remodelling, hence promoting functional recovery.[19]

Ischemia also activates programmed cell death pathways, favouring the expression of Bcl2, caspases, and HSPs gene.[18]

Around the core lesion, it is possible to identify a region of reversible ischemic damage, the “ischemic penumbra”, potentially salvageable by the quick restoration of adequate blood flow. Every minute of untreated stroke, 1.9 million neurons, 14 billion synapses, and 12 km of myelinated fibers are destroyed; the presence of the penumbra allows intervention to reduce these numbers.

However, the manifestations of stroke are much more complex than one could infer from the simple local effects of ischemia. A crucial concept in this context is that of diaschisis. According to this phenomenon, brain injuries can lead to distant alterations in areas not directly damaged by hypoperfusion.

DIASCHISIS

The remote effects of a focal lesion on brain function had already been suggested by Brown-Séquard more than 130 years ago during a debate at the French Society of Biology. Séquard's hypothesis was greeted with indifference, while far more respect was shown to von Monakow's masterpiece in 1914. In this publication were introduced the term "diaschisis" and four key aspects 1) the presence of a focal brain lesion; 2) a distant loss of excitability; 3) the interruption of the connections between the remote areas and the lesion; and 4) a dynamic and clinical nature of the progress that decreases over time. For the following 50 years, diaschisis has remained a phenomenon with an ill-defined clinical definition. Following the introduction of metabolic imaging techniques in the 1970s, it was discovered that some of the manifestations previously attributed to diaschisis were instead related to other phenomena, such as cerebral edema or ischemic penumbra. For the first time, good spatial accuracy could be achieved in measuring glucose metabolism in vivo. This led to a new definition of diaschisis based on the hypothesis that neurovascular coupling is preserved in areas of diaschisis. Since cortical diaschisis only partially correlates with behavioral changes, its clinical role has remained debated.[20]

There are two types of diaschisis: focal diaschisis, defined as the presence of isolated and remote neurophysiological changes, and non-focal diaschisis. Focal diaschisis can be further classified as diaschisis at rest and functional diaschisis. Diaschisis at rest is defined as "the focal decrease in energy metabolism at rest without stimulation of activation, in anatomically intact brain regions distant from the lesion"[20] The behavioral impact of diaschisis depends on lesion location. Cortical diaschisis, consequent to a subcortical lesion, produces clinical deficits that resemble isolated cortical lesions in the same areas. The impact of different diaschisis patterns on behavior following a cortical lesion is more controversial. However, the discovery of diaschisis shed new light on the possibility that new biomarkers can improve post-stroke behavioral predictions.

CLINICAL SYNDROMES

For centuries humankind has tried to predict the effect of a brain lesion. Many theories about the organization and function of the brain were proposed over the years that gradually led to the understanding we have these days. The fundamental concept was that the death of specific brain areas causes specific symptoms (i.e., "lesion-symptom mapping" technique). This method consisted of inferring information about the function of certain areas from the study of lesions associated with specific symptoms. This led to the development of the cortical atlas discussed in the second sub-chapter. These observations paved the foundations of the classical stroke vascular syndromes, currently used in everyday clinical practice with fundamental diagnostic and prognostic implications.

LESION-SYMPTOM MAPPING

Modern neuroanatomy and neurophysiology started in the XVI century with the anatomical dissections of Andreas Vesalius. Vesalius's work, together with Leonardo da Vinci's maps,[21] refuted previous Galen's theories sustaining that, at the base of the brain, there is a complex of arteries and veins lying close to each other, rete mirabile, where "animal spirits" are stored and flow through the nerves, and in the brain,[22] He paved the way for the anatomical studies of Thomas Willis and Joseph Gall (1758-1828). Gall described for the first time grey and white matter functions and developed "cranioscopy", later renamed "phrenology", a theory according to which the shape of the skull can be used to infer mental abilities and personality traits. According to Gall, the head's design changes with the development of human brains: where the skull protrudes represents excellent human qualities, whereas the location of depressions represents human defects.[23–25] This theory, then proven false, was based on the notion that mental functions are located in isolated regions of the cortex, a concept that was then taken up by Broca and became the leading principle in the development of modern neuroscience.[22] Furthermore, Gall distinguished six different memories: grammatical, verbal, colors, the relation of numbers, locality, and tonal harmony; a concept that inspired Jean-Baptiste Bouillaud's (1796-1875) theory of language lateralization.[24,26] Bouillaud, his son-in-law Ernst Auburtin (1825-1893), and, later, Paul Broca (1824-

1880) were the first ones to locate function, particularly language function, based on damage to the brain's cortex.[23,27–30] Broca planned his brain function localization theory based on three cardinal patients: Victor Laborgne, or "Tan" as he could only utter the syllable "Tan", Lazare Lelong, that had a language ability slightly better than Leborgne as he could pronounce five syllables, and Phineas Gage. Broca dissected their brains and located language function in the left frontal lobe, in an area now named after him.[31]

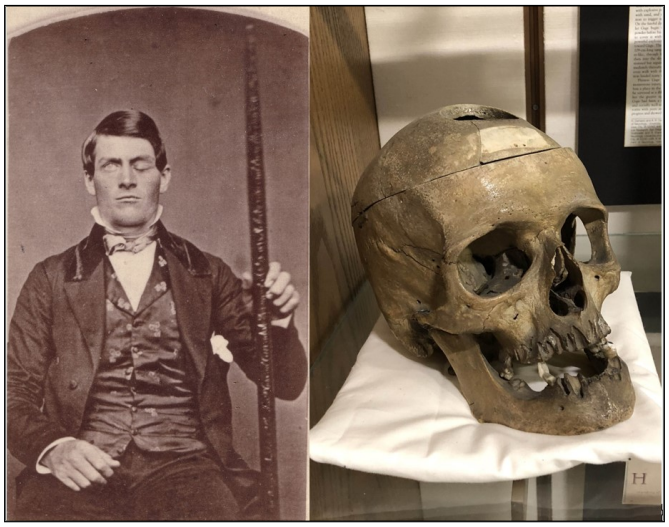


Figure 7. On the left, Phineas Gage (1823- 1869) holding the rod that penetrated his skull in 1849 while working on the construction of the railways. On the right, Phineas Gage's skull now on display at the Harvard Medical School in Cambridge, Massachusetts

The technological and methodological advancements of the XIX and XX centuries enabled a precise description of the nervous system structure. Thanks to Camillo Golgi's staining preparation and Ramon y Cajal's discoveries about the microscopic organization of the brain; Korbinian Brodmann (1868-1918) divided the cerebral cortex into 52 discrete regions based on cytoarchitecture.[32,33]

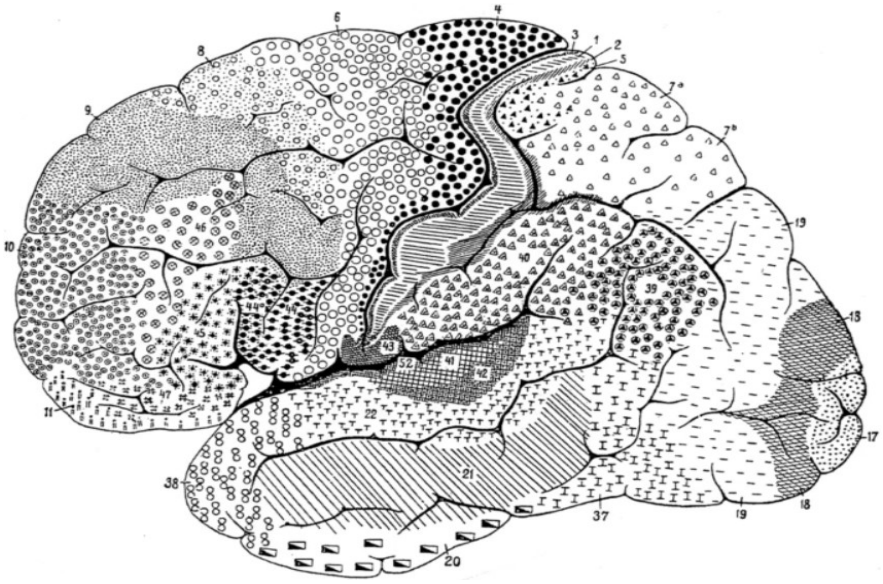


Figure 8. Brodmann's map of cerebral cortex, from Nieuwenhuys et al. (2020)

Numerous researchers then deepened the function-to-structure relationship through the identification of focal areas of damage after stroke. The earliest lesion studies were more correlative than they were causal because they were based on autopsy material and many years separated the symptoms and the lesion observed. Thanks to the introduction of magnetic resonance imaging *(MRI)* and computed tomography *(CT),* there was, finally, a temporal correspondence between the symptoms and the findings identified on imaging examinations. These techniques allowed scientists to infer causal relationships. The link between a lesion and the resulting symptom was strengthened when patients with the same symptom had overlapping lesions, see Figure 9.[34]

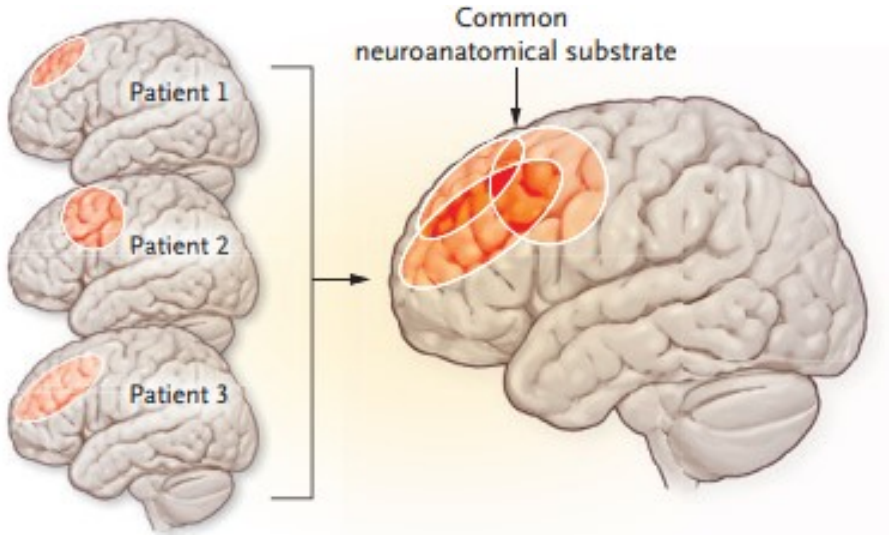


Figure 9. A common anatomical substrate can be identified in patients presenting the same symptom and with an overlap in lesion location, from Fox (2018)

Further evidence came from the use of electricity to directly stimulate cortical tissue to obtain a behavioral response; from the experiments of Wilder Penfield (1891-1976), who directly stimulated the cortex of his patients during brain surgery, to the Transcranial Magnetic Stimulation *(TMS)*, a less invasive jet still accurate procedure. In TMS, a coil is placed near the scalp, and a very brief but very large pulse of current is run through it, creating a strong, transient magnetic field. This abrupt change in the magnetic field induces a current flow in the nearby neurons. TMS can either activate the desired area or simulate a temporary lesion by disrupting regular activity in a localized region of the brain.[35]

Piece by piece, these findings allowed the creation of the cortex atlas that we still use today.

CEREBRAL CORTEX MAPS

The gyri and the sulci of the cerebral hemispheres form consistent patterns. The frontal lobes are in the frontal part of the brain and extend back to the *central sulcus of Rolando*; the gyrus running in front of the central sulcus is called *precentral gyrus*, and the remainder of the lateral frontal surface is divided into the *inferior, middle,* and *superior frontal gyri* by the *inferior* and the *superior frontal sulci.* The frontal lobes are divided from the temporal lobes by the *Sylvian fissure*. Similarly to the frontal lobe, the lateral temporal lobe is divided into *superior, middle*, *and inferior temporal gyri*.

The parietal lobes are defined anteriorly by the *central sulcus* but have no clear demarcation, on the lateral view, from the temporal or occipital lobes. The *parieto-occipital sulcus* can be noticed more easily from the medial aspect. The anterior portion of the parietal lobe consists of the *postcentral gyrus*, while the *superior* and *inferior parietal lobules* are divided by the intraparietal sulcus. The inferior parietal lobule is further divided into the *angular gyrus* and the *supramarginal gyrus*.

The *corpus callosum* becomes visible on the medial surface and is surrounded by the *cingulate gyrus*. The central sulcus does not usually extend onto the medial surface, but the region surrounding it is called the paracentral lobe. The *lingula* is a portion of the occipital lobe located below the *calcarine fissure*, while the portion above it is called the *cuneus.* The medial parietal lobe forms the *precuneus* just in front of the cuneus.

On the inferior surface, on top of the orbital crests of the eye, are located the *orbital frontal gyri*, separated medially from the *gyrus rectus* by the *olfactory sulcus*. On the inferior surface of the temporal lobe, the *occipitotemporal gyri* are separated, medially, from the *parahippocampal gyrus* by the *rhinal sulcus* and laterally from the *inferior temporal* gyrus by the *inferior temporal sulcus*.[36]

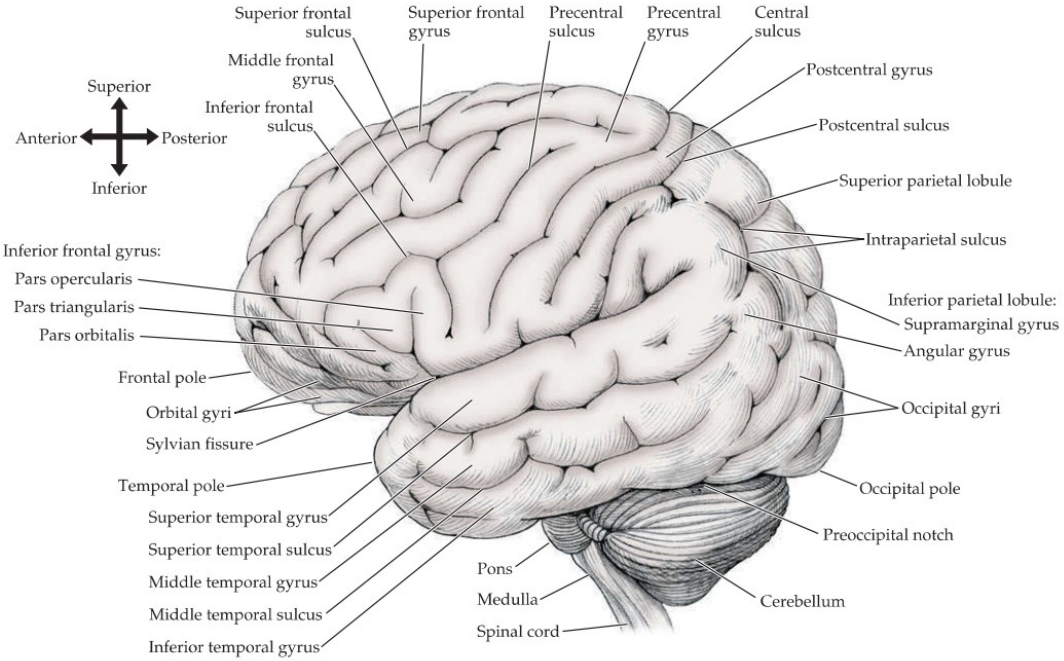


Figure 10. Cortical anatomy, from Blumenfeld "Neuroanatomy through clinical cases" (2010)

Primary sensory and motor areas

The primary sensory cortex and primary motor cortex, respectively located in the postcentral and precentral gyri, communicate with the opposite side of the body.
The primary visual cortex receives visual information from the opposite hemifield and is located along the banks of the calcarine fissure in the occipital lobe. The auditory cortex is in the scissure of Silvius on the superior surface of the temporal lobe and consists of two finger-like gyri *(Heschl's transverse gyri)*.
The sensory and motor pathways are topologically organized: adjacent areas of the sensorimotor cortex are mapped onto adjacent fibers in the white matter pathways and adjacent receptive or motor surfaces. These somatotopic maps on the cortex are called the motor or sensory homunculus. A cortical homunculus is a distorted representation of the human body that reflects the actual cortical surface dedicated to the sensation or the motion of that specific body area. It illustrates the concept of a representation of the body lying within the brain. Both sensory and motor homunculus are upside down, with the feet lying on the upper part of the cortex and the head and mouth on the bottom.[36]

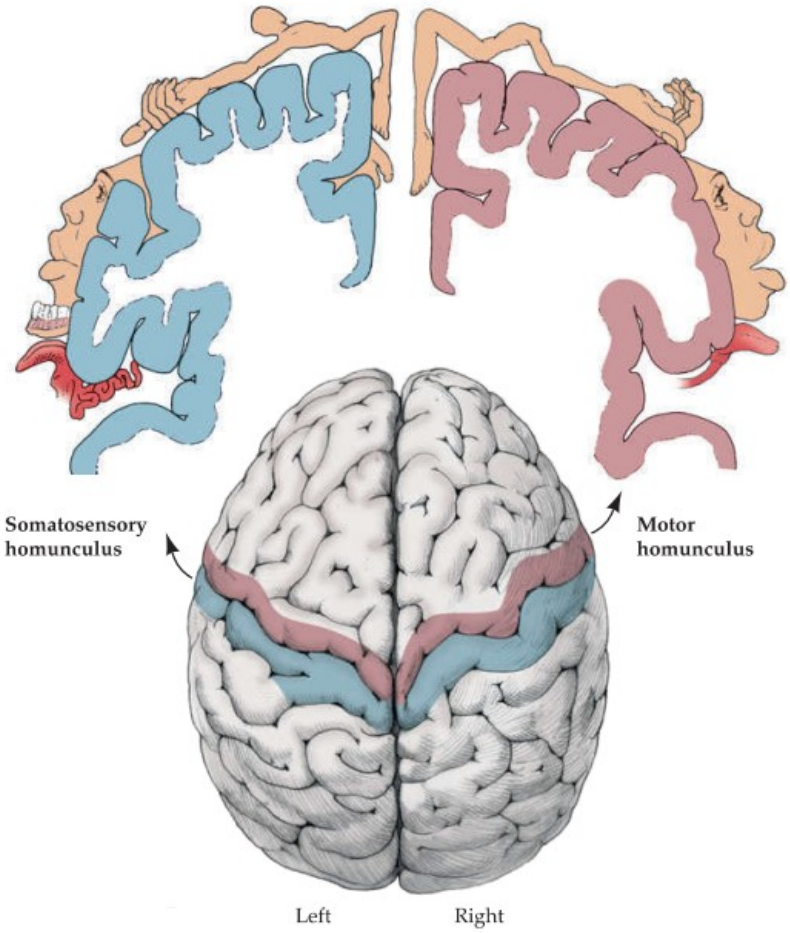


Figure 11. Somatosensory (blue) and motor (red) homunculi, from Neuroanatomy through clinical cases, Blumenfeld (2010)

Limbic System

The limbic system includes several structures: the inferior and medial frontal lobes, the medial and anterior temporal lobes, the anterior insula, the hippocampal formation and the amygdala, the cingulate gyri, several nuclei in the hypothalamus, medial thalamus, septal area, basal ganglia, and brainstem. It is involved in many functions, such as memory, regulation of emotions, appetitive drives, and autonomic and neuroendocrine control. Patients with lesions of the limbic system may present with deficits in the consolidation of immediate memories into long-term memories, i.e., they may have no problem recalling remote events but have difficulty forming new memories. In addition, they may present with behavioral alterations and a range of psychiatric disorders.[36]

Association cortex

The associative cortices cover a generous portion of the brain surface. These perform higher-order information processing. Association cortices can be divided into two main groups 1) unimodal association cortex that receives information from a cortical area, e.g., the visual associative cortex computes information from the primary visual cortex; and 2) heteromodal associative cortex, which integrates functions from multiple domains.

Linguistic information can reach different sensory systems: during reading, linguistic information reaches the primary visual cortex, while during listening, it reaches the primary auditory cortex. From there, it is transmitted to Wernicke's area in the left hemisphere. Lesions in this area cause deficits in language comprehension. The area responsible for language production, or Broca's area, is in the left frontal lobe near the areas of the primary motor cortex involved in laryngeal, tongue, lip, and facial movement. Lesions in this area cause deficits in language production.

Gerstmann syndrome presents with difficulties with written language and calculations, finger agnosia, and right-left confusion. It occurs in cases of lesions to the inferior parietal lobule in the left hemisphere.

Diffuse cortical lesions, or sometimes more focal lesions affecting the left frontal or parietal lobe, can produce abnormalities in motor conceptualization, planning, and execution, i.e., apraxia. Complex motor tasks require higher-order planning, a function that appears to be distributed across several areas of the cortex. The parietal lobes also play an essential role in spatial awareness. Lesions in the parietal lobe often cause contralateral hemispatial neglect and distortion of perceived space. Since attention is usually lateralized on the right side, lesions on the opposite hemisphere rarely cause this syndrome because of a compensatory mechanism from the nondominant hemisphere. With this syndrome, patients often ignore objects in the left visual field, even though they preserve their ability to see them. If a stimulus is presented in the affected visual field, the patient may still be able to localize it, even if unaware. They may also be completely unaware of the left side of their body, thinking, for example, that their left arm belongs to someone else, and they may be unaware of any weakness or other deficits on that side *(anosognosia)*. In addition, they may exhibit extinction, a phenomenon in which a stimulus is

perceived normally when presented to only one side but is not perceived on the side opposite to the lesion when administered simultaneously on both sides. Among the tests performed to assess visuospatial neglect, cancellation tests are most frequently used and slightly more sensitive. These require subjects to find targets on a centrally placed sheet of paper: patients with neglect tend to start at the edge of the page ipsilateral to the lesion, often failing to erase the more contralateral targets altogether.[37]

Figure 12. Star cancellation test, from Li and Malhotra (2015)

Frontal lobe lesions can cause altered cognitive impairments, personality changes and disinhibited behavior, and the reappearance of frontal release signs such as suck, grasp, snout, and root reflexes. These "primitive" reflexes are normal in infants but disappear in healthy adults. In addition, patients with frontal lobe lesions may present with perseverance, difficulty performing movement sequences, abulia, walking disorders, and urinary incontinence.

Lesions in the visual association cortex can produce various symptoms, including achromatopsia, prosopagnosia, and palinopsia. Seizures in the visual association cortex can cause elaborate visual hallucinations.[38]

VASCULAR SYNDROMES

Ischemic stroke is caused by the occlusion of a vessel, leading to the ischemic death of the downstream regions. The clinical manifestations of this event depend on the affected territory and can be inferred by the cortical maps previously described.
The vascular syndromes are the ensemble of symptoms resulting from an artery's occlusion and the necrosis of the territory supplied by it.

Internal Carotid Artery Syndromes

The territory affected by diminished blood flow in the brain in cases of carotid occlusion depends on the configuration of the circle of Willis, e.g., if the anterior communicating artery is tiny, the ipsilateral anterior cerebral territory is affected. The clinical manifestations of atherosclerotic thrombotic disease of this artery are highly variable because the internal carotid artery is not an end vessel, and no part of the brain is entirely dependent on it.
An occlusion that occurs immediately beyond the carotid bifurcation is usually silent. If the occlusion affects the distal intracranial portion of the internal carotid artery, the clinical picture is that of middle cerebral artery occlusion.
Suppose one internal carotid artery had been occluded at an earlier time. In that case, occlusion in the other may cause a bilateral infarction presenting with quadriplegia and continuous horizontal metronomic conjugate eye movement. When the anterior cerebral territory is affected, manifestations include leg paralysis and drowsiness or stupor.
When the circulation of the carotid artery has been incompletely compromised, the zone of maximal ischemia lies between the two vascular territories of the middle and anterior cerebral arteries. In this scenario, the weakness involves the shoulder and hip more than the hand and face.
Internal carotid artery syndrome is usually associated with a headache above the eyebrow. This vessel also supplies the optic nerve and retina: signs of carotid occlusion include transient monocular blindness that occurs prior to the onset of strkstroke10-25% of cases of symptomatic carotid occlusion.

Middle Cerebral Artery Syndromes

- *Stem Occlusion Syndrome* causes hemianesthesia and contralateral hemiplegia. It can also cause homonymous visual field deficit with a deviation of the head and eyes toward the side of the lesions. Partial syndromes are common. Drowsiness may be present at the onset.

- *Branch Syndrome:*
    - *Superior division:* ipsilateral deviation of the head and eyes and sensorimotor deficits in the contralateral face and arm and, to a lesser extent, in the legs.
    - *Inferior division:* superior quadrantanopia or homonymous hemianopia associated with lesions on either side and left visual neglect associated with right-sided strokes.

Anterior Cerebral Artery Syndromes

- *A1 segment*: proximal to its connection with the anterior communicating artery. The presence of good compensation by the circle of Willis makes the occlusion of this artery well tolerated. The presence of an anatomic variant characterized by a single stem from which both anterior cerebral arteries originate leads to a sizeable bilateral infarction. The patient may exhibit paraplegia, incontinence, frontal lobe personality changes, abulia, and non-fluent aphasic symptoms in this scenario.
- *A2 segment*: distal to the anterior communicating artery. It results in a sensorimotor deficit of the opposite foot and leg and, to a lesser extent, the shoulder and arm; the head and eyes may deviate to the side of the injury, while the hand and face are not involved.

Language disturbances may occur with anterior cerebral artery occlusions, particularly transcortical motor aphasia. Disorders of behavior such as muteness or a tendency to speak in whispers, distractibility, and abulia may also be present.

Anterior Choroidal Artery Syndrome

According to Foix et al.,[39] an obstruction of the anterior choroidal artery causes hemi-hyperesthesia, contralateral hemiplegia, and homonymous sectorial hemianopia or quadrantanopias in the upper and lower fields, with the sparing of a region that lies along the equator. Contrary to strokes involving the major cerebral arteries, cognition and language are well preserved in patients with anterior choroidal artery occlusion. Right-sided lesions are often accompanied by constructional apraxia and left spatial neglect, while speech and language disorders may be present in left-sided ones.
Hupperts et al.,[40] regarding anterior choroidal artery syndrome, came to a different conclusion, claiming that there is no uniform syndrome and that, in most cases, the territory of supply of this artery is overlapped by small surrounding vessels.

Posterior Cerebral Artery Syndromes

- Proximal PCA syndromes:
    - *Thalamic syndrome:* characterized by a severe deep cutaneous sensory loss of the opposite hemibody, including the face and the trunk, occasionally accompanied by transitory hemiparesis. Sometimes, dissociated sensory loss and homonymous hemianopia may be present.
    - *Central midbrain and subthalamic syndromes:* the result of the occlusion of the interpeduncular branches (*Table I*).

EPONYM[b]	SITE	CRANIAL NERVES INVOLVED	TRACTS INVOLVED	SIGNS	USUAL CAUSE
Weber syndrome	Base of midbrain	III	Corticospinal tract	Oculomotor palsy with crossed hemiplegia	Vascular occlusion, tumor, aneurysm
Claude syndrome	Tegmentum of midbrain	III	Red nucleus, superior cerebellar peduncles after decussation	Oculomotor palsy with contralateral cerebellar ataxia and tremor	Vascular occlusion, tumor, aneurysm
Benedikt syndrome	Tegmentum of midbrain	III	Red nucleus, corticospinal tract, and superior cerebellar peduncles after decussation	Oculomotor palsy with contralateral cerebellar ataxia, tremor, and corticospinal signs, may have choreoathetosis	Infarct, hemorrhage, tuberculoma, tumor
Nothnagel syndrome	Tectum of midbrain	Unilateral or bilateral III	Superior cerebellar peduncles	Ocular palsies (IV), paralysis of gaze, nystagmus, and ataxia	Tumor
Parinaud syndrome	Dorsal midbrain		Supranuclear mechanism for upward gaze and other structures in periaqueductal gray matter	Paralysis of upward gaze and accommodation; fixed pupils	Pinealoma and other lesions of dorsal midbrain, hydrocephalus
Millard-Gubler syndrome and Raymond-Foville syndrome	Base of pons	VII and often VI	Corticospinal tract	Facial and abducens palsy and contralateral hemiplegia; sometimes gaze palsy to side of lesion	Infarct or tumor
Avellis syndrome	Tegmentum of medulla	X	Spinothalamic tract; sometimes descending pupillary fibers, with Bernard-Horner syndrome	Paralysis of soft palate and vocal cord and contralateral hemianesthesia	Infarct or tumor
Jackson syndrome	Tegmentum of medulla	X, XII	Corticospinal tract	Avellis syndrome plus ipsilateral tongue paralysis	Infarct or tumor
Wallenberg syndrome	Lateral tegmentum of medulla	Spinal V, IX, X, XI	Lateral spinothalamic tract Descending pupillodilator fibers Spinocerebellar and olivocerebellar tracts	Ipsilateral V, IX, X, XI palsy, Horner syndrome, and cerebellar ataxia; contralateral loss of pain and temperature sense	Occlusion of vertebral or posterior-inferior cerebellar artery

Table I. Intramedullary brain syndromes, from Adams and Victor, "Principles of Neurology" (2019)

- *Anteromedial-inferior thalamic syndromes:* characterized by extrapyramidal movement disorders such as asterixis, hemichoreoathetosis, or hemiballismus. Hemiataxia, tremors, deep sensory loss, and Korsakoff syndrome may be present in various combinations.

- *Cortical syndromes:* if the occlusion affects the branches directed to temporal and occipital lobes, the patient presents with homonymous hemianopia involving especially the upper quadrants. Macular vision is usually spared. Other symptoms may include metamorphopsia, palinopsia, and visual hallucinations. Occipital infarcts in the dominant hemisphere may cause a range of visual agnosias, alexia without agraphia, anomia, and rarely some degree of impaired memory.

- *Bilateral stroke syndromes:* occur as a result of consecutive infarctions. Cortical blindness may result from large lesions, sometimes accompanied by visual hallucinations. In some cases, the patient neglects to be blind, even when it is pointed out to him. The pupillary reflexes are preserved. The lesions are often incomplete and spare a vision sector. The patient may present only central scotomas in bilateral lesions confined to the occipital pole. The patient central vision may be preserved if the occipital poles are spared. The patient has severe memory impairments when the inferomedial portions of the temporal lobes are involved bilaterally. If the infarction involves only the left side of the temporal lobe, retentive memory may be affected, while bilateral mesiotemporal-occipital lesions cause a lack of recognition of faces.

Vertebral Artery Syndromes

The presentations of vertebral occlusion are quite variable; it can also be asymptomatic if the artery occludes in its extracranial portion and there is an adequate flow on the opposite side.

- *Occlusion proximal to the origin of the PICA*: there may be no symptoms because the PICA is still perfused by retrograde flow through its vertebral artery.
- *Occlusion involving the PICA*: vertigo is the main symptom.
- *Medial medullary syndrome* causes contralateral loss of position and vibration sense, ipsilateral paralysis and lateral atrophy of the tongue, and contralateral paralysis of the leg and arm.
- *Lateral medullary syndrome* is relatively common and caused by infarction of a portion of the lateral medulla. Includes a variety of symptoms due to the numerous structures involved: 1) restiform body, inferior cerebellum, and olivocerebellar, spinocerebellar fibres (falling or toppling to the ipsilateral side, the sensation of lateropulsion and ipsilateral ataxia of limbs,); 2) nucleus and tractus solitarius (loss of taste); 3) vestibular nuceli (vertigo, vomiting, nystagmus, and oscillopsia); 4) descending tract and nucleus of the fifth nerve (altered sensation over ipsilateral half of the face, burning, and pain); 5) descending sympathetic tract (ipsilateral Horner

syndrome); 6) gracile and cuneate nuclei (numbness of ipsilateral limbs); 7) fibers of the ninth and tenth nerves (reduced gag reflex, ipsilateral paralysis of the palate and vocal cord, hoarseness, hiccough, dysphagia); 8) utricular nucleus (vertical diplopia and illusion of tilting of vision and rotation of the vertical meridian, rarely so severe as to produce upside down vision); and 9) spinothalamic tract (contralateral impairment of thermal and pain sense of the entire hemibody). The most common presentations are fragmentary syndromes.

Basilar Artery Syndromes

- *Complete syndrome* presents with coma due to the infarction of the high midbrain reticular activating system, quadriplegia, and ophthalmoplegia.
- *Occlusion of the distal end* is characterized by hemianopia, oculomotor disturbances, bilateral ptosis, transient loss of consciousness, and dilated pupils with preserved reaction to light.
- *Occlusion of the middle portion* gives rise to locked-in syndrome, characterized by quadriplegia due to the interruption of motor pathways and preserved state of consciousness due to the sparing of the ascending reticular substance. The patient retains some eye movement and can lift the eyelids and move the eyes vertically. The pupils are still partially reacting to light, although extremely narrow.
- *Occlusion of the proximal end:* occlusion of branches at the bifurcation that results in a various number of complex syndromes that include different combinations of akinetic mutism, somnolence or coma, an agitated confusional state, visual hallucinations, disorders of ocular movement memory defects, ptosis, and visual field defects. Usually, cranial and spinal nerves result in deficits on the opposite sides of the body in a “crossed” pattern
    - *Occlusion of the superior cerebellar artery* presents with slurred speech, loss of pain and sensation on the opposite side of the body, nausea and vomiting, and ipsilateral cerebellar ataxia of the limbs. Other symptoms may include ipsilateral palatal myoclonus, Horner syndrome, and tremors.

- *Occlusion of anterior inferior cerebellar artery:* the size of the PICA greatly influences the extent of an infarct caused by occlusion of this vessel. The main symptoms include contralateral loss of pain and temperature sense of the leg, arm and trunk, an ipsilateral Horner syndrome and paresis of conjugate lateral gaze, nystagmus, tinnitus and sometimes unilateral deafness, vomiting, vertigo, ipsilateral cerebellar ataxia, and facial weakness.[16]

Lacunar stroke syndromes

Lacunar strokes are caused by the occlusion of small penetrating vessels such as the recurrent artery of Heubner, the pontine perforating, the thalamoperforating, and the lenticulostriate arteries. These lesions usually range from 3 to 15 mm in diameter. Lacunar stroke tends to develop rapidly but usually not as abruptly as an embolism.

- *Pure motor hemiplegia* (internal capsule or corona radiata): involving the opposite side of the face and the contralateral hand, arm, and leg. Motor disorders may present with fragmented patterns.
- *Pure sensory stroke* (ventral posterolateral nucleus of the thalamus): involving the opposite hemibody without motor or language impairments;
- *Ataxic hemiparesis* (corona radiata, posterior limb of the internal capsule or basilar part of the pons): is characterized by the simultaneous presence of contralateral ataxia and hemiparesis.
- *Dysarthria-clumsy hand* (basilar part of the pons or the genu of the internal capsule): presents with dysarthria and contralateral weakness of the hand.
- *Mixed sensorimotor* (thalamus and adjacent posterior limb of the internal capsule): presents with sensory impairment of the contralateral hemibody and hemiparesis.

DIAGNOSIS

Early examination of people with signs of an ischemic stroke can reduce their disability and help prevent a recurrence.

CLINICAL DIAGNOSIS

A study published in 2004 revealed that the diagnostical sensitivity of history and examination is 92% in an emergency setting. [41] The most common presentation is the “wake-up stroke”, in which the onset is unknown because the stroke occurred during sleep, and the patient acknowledges the symptoms only upon awakening. The most common manifestations are speech disorders and unilateral weakness. Upon taking medical history, it is crucial to define the time of onset to determine whether a patient meets the criteria for thrombolysis or not. Even in the case of nonspecific symptoms, the history and the physical examination for common stroke symptoms should uncover the stroke diagnosis. Only through neuroimaging is possible to distinguish between hemorrhagic and ischemic stroke, although patients with hemorrhagic stroke are more likely to have diastolic blood pressure greater than 110 mmHg42, vomiting, and headaches. Subarachnoid hemorrhage *(SAH)* presents in 80% of cases with a severe and abrupt headache; in up to 40% of patients, previous sentinel headaches are present. Other symptoms may include focal neurologic signs, photophobia, vomiting, meningismus, decreased level of consciousness, and seizures.[42,43] In these patients, fundoscopy should be performed to identify intraocular hemorrhages.

DIAGNOSTIC TESTS AND IMAGING

The evaluation of a patient with suspected acute ischemic stroke should include:

- non-contrast brain CT or brain MRI
- blood glucose
- oxygen saturation
- serum electrolytes
- renal function tests
- complete blood count
- markers of cardiac ischemia
- prothrombin time/ INR
- activated partial thromboplastin time
- ECG

These studies aim to identify contraindications to thrombolytic therapy, diagnose comorbidities, and detect mimics. To confirm the presence of a stroke and distinguish between ischemic and hemorrhagic stroke, CT and/or MRI are performed. It is essential to perform them in patients within the time window for thrombolytic therapy to assess the absence of intracerebral hemorrhage and evaluate ischemic changes.[44] MRI has higher sensitivity and better resolution than non-contrast CT, but CT is faster, cheaper, and more available.[45] Patients with suspected posterior infarction or acute vestibular syndrome should undergo diffusion-weighted MRI.[46] If SAH is suspected, the imaging test of choice is non-contrast CT which has a sensitivity of nearly 100% for detecting subarachnoid blood in the first 72 hours.[42] People with suspected SAH and a standard non-contrast CT should undergo a lumbar puncture to detect bilirubin.

Perfusion CT may be used to identify the penumbra and core areas in patients with large vessel occlusion to assess whether they are eligible for endovascular treatment *(EVT).* The infarct core is defined as an area of brain tissue with more than 70% reduction in cerebral blood flow compared to the normal contralateral hemisphere, while ischemic penumbra is identified by a mismatch between cerebral blood flow and cerebral blood volume.[47]

Other tests include cardiac imaging, duplex imaging of extracranial arteries, arteriography, and laboratory assessments for a prothrombotic state.

ACUTE TREATMENT

Ischemic stroke is a sudden and progressive event in which prompt treatment is crucial to prevent neurological and other clinical complications.

According to the AHA *(American Heart Association)* guidelines, it includes airway, breathing and Oxygen management, blood pressure, temperature, and blood glucose regulation:

- airway support and ventilatory assistance are recommended for patients with decreased consciousness or who have bulbar dysfunction that causes compromise of the airway;
- supplementation of oxygen should be provided to maintain oxygen saturation over 94%;
- to maintain systemic perfusion, hypotension and hypoperfusion should be corrected;
- patients eligible for treatment with IV alteplase should maintain systolic blood pressure <185mmH and diastolic blood pressure <100mmHg;
- if the patient presents with hyperthermia, body temperature over 38°C, antipyretic medications should be administered and sources of hyperthermia identified;
- hypoglycemia should be treated.[48]

According to the Italian guidelines for stroke management *(SPREAD guidelines),* released in 2016, the patient should also be admitted to a stroke unit where highly specialized personnel can assist him.[49]

The first step in stroke treatment consists of revascularization and limitation of secondary neuronal injury; two procedures are now available for this purpose: intravenous thrombolysis *(IVT)* and mechanical thrombectomy *(MT)*.

Thrombolysis is performed by injecting a thrombolytic agent *(recombinant tPA-streptokinase)* to rapidly dissolve the obstruction by converting plasminogen into plasmin, which can hydrolase fibrin and fibrinogen. IV- tPA has been shown to improve clinical outcomes by at least 30% if administered within the first 3 hours of symptom onset.[50] The thrombolysis substantially changed the management of this neurological emergency. However, 69% of stroke patients are not eligible to receive IV-tPA.

The introduction of MT has provided clinicians with a stronger therapeutic resource.[51] EVT is indicated for the treatment of patients with acute ischemic stroke with large vessel occlusion and has become the standard of care since the release, in 2005, of five pivotal trials: MR CLEAN,[52] EXTEND-IA,[53] ESCAPE,[54] SWIFTPRIME,[55] and REVASCAT.[56]

According to the AHA guidelines, patients eligible for IV alteplase should receive IVT, even if EVT is considered. MT should be performed in patients meeting the following criteria: 1) age ≥ 18 years; 2) pre-stroke mRS score of 0-1; 3) causative occlusion of the internal carotid artery or MCA segment 1; 4) ASPECTS of ≥6; 5) NIHSS score of ≥6; and 6) treatment should be initiated within 6 hours of symptom onset. Based on the results of the DAWN and DEFUSE-3 trials, new recommendations consider patients eligible for MT up to 16 hours after the event.[57] Even though the selection of patients was very complex, these trials demonstrated that MT has positive results up to 24 h after the onset of symptoms.[58]

PROGNOSIS

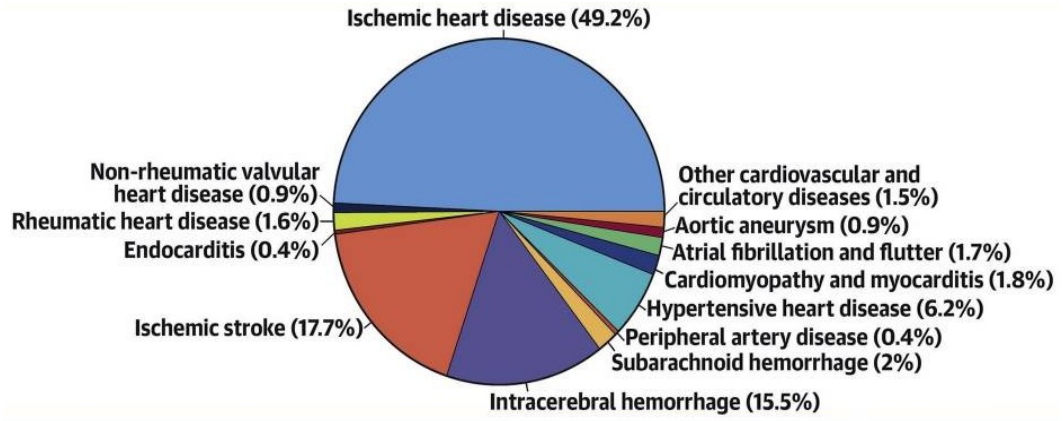


Figure 13. Proportion of cardiovascular disease deaths by cause in 2019, from Roth et al. (2020)

Stroke is one of the leading global causes of disability and death; according to the WSO *(World Stroke Organization),* it represents the second cause of death and the third cause of death and disability combined in the world.[6]

The GBD *(Global Burden of Diseases, Injuries, and Risk Factors Study)* estimated incidence, prevalence, mortality, YLLs, YLDs, and DALYs due to 369 diseases and injuries in 204 countries and territories, ranking stroke as the second cause of DALYs in people over the age of 50.[59]

Stroke (7.8 million people) is one of the three most burdensome neurological disorders in the US, together with Alzheimer's disease and other dementias (2.9 million people), and migraine (68.5 million people). In the US, stroke was the first cause of disability and the second cause of death from neurological disorders between 1990 and 2017. Because of the aging of the US population and population growth, the absolute number of people who are affected by non-communicable neurological disorders over that period has increased substantially and is likely to continue increasing.[60]

The lifetime risk of stroke has globally increased by 50% in the last 20 years and is now one in four people, 24.9% in men and 25.1% in women from age 25 onward.[6,61]The risk of having an ischemic stroke (18.3%) is much greater than that of having a hemorrhagic one (8.2%).[61]

Although the absolute number of DALYs due to stroke in males exceeded that in females in 2019, the point estimates of incident and prevalent strokes in females were higher than in males, and there were no noticeable sex differences in the number of stroke-related deaths. This phenomenon may be explained by greater age-standardized death rates and DALYs rates in males than in females.[6]

Ischemic stroke represents 17.7% of all deaths due to cardiovascular diseases, the major global cause of premature mortality. Although the total number of prevalent strokes increased steadily from 1990 to 2019, the age-standardized rates for deaths declined over the same period suggesting that: 1) preventive measures are very effective at lowering the risk for both ischemic and hemorrhagic stroke; 2) on average, global increases to stroke burden have been largely due to population growth and aging.[62]

TOAST Categories	Number at Risk	Survival Probability in % (95% CI)		
		3 Months	1 Year	5 Years
All	3346	87.0 (85.9–88.2)	79.0 (77.7–80.5)	54.4 (52.5–56.4)
Men	1563	90.7 (89.3–92.2)	82.8 (80.9–84.7)	59.2 (56.4–62.0)
Women	1780	83.8 (82.1–85.5)	75.9 (73.9–77.9)	50.4 (47.9–53.1)
LAA	272	82.4 (78.0–87.1)	73.7 (68.6–79.3)	49.4 (43.3–56.5)
Men	159	84.4 (78.8–90.3)	75.5 (68.9–82.7)	53.8 (45.7–63.3)
Women	113	79.6 (72.5–87.4)	71.3 (63.4–80.2)	43.7 (34.9–54.8)
CE	742	80.0 (77.2–83.0)	67.9 (64.6–71.4)	40.9 (37.2–45.0)
Men	321	86.1 (82.4–90.0)	74.0 (69.3–79.0)	47.8 (42.2–54.3)
Women	421	75.5 (71.5–79.7)	63.4 (58.9–68.2)	35.7 (31.0–41.1)
SAO	904	97.7 (96.8–98.7)	93.5 (91.9–95.2)	73.8 (70.4–77.3)
Men	451	98.4 (97.3–99.6)	94.0 (91.8–96.3)	75.9 (71.3–80.9)
Women	453	97.1 (95.5–98.7)	93.0 (90.6–95.4)	71.8 (67.1–76.9)
UND	1245	86.2 (84.3–88.1)	77.7 (75.4–80.1)	50.3 (47.2–53.7)
Men	554	90.8 (88.4–93.3)	81.8 (78.6–85.1)	54.3 (49.7–59.4)
Women	691	82.5 (79.7–85.4)	74.5 (71.2–77.8)	47.2 (43.1–51.7)

CE indicates cardioembolism; LAA, large-artery atherosclerosis; SAO, small-artery occlusion; TOAST, Trial of ORG 10172 in Acute Stroke Treatment; and UND, stroke of undetermined cause.

Table II. Ischemic Stroke survival Probabilities, from Rücker et al., (2020)

In 2020, the American Heart Association published a study investigating time trends in ischemic stroke case fatality and recurrence rate over 20 years. Data were collected within a population-based stroke register covering a source population of 105 164 German inhabitants. The five-year survival rate after ischemic stroke was estimated at 54.4%, while the cumulative risk of recurrent stroke was 20.1% over the same period. A significant decrease in stroke mortality and case-fatality rate

was observed over time across all stroke subtypes; these trends might be associated with improved acute treatment options. On the contrary, no significant trend for a decline in stroke recurrence was found, suggesting the need for improvement in second prevention measures in ischemic stroke survivors.[11]

Stroke prognosis is influenced by various factors that, after the advent of acute revascularization treatment, can be categorized into: 1) general prognostic factors, 2) pre-treatment prognostic factors, and 3) post-treatment prognostic factors.

GENERAL PROGNOSTIC FACTORS

The total number of stroke-related DALYs due to risk factors has increased substantially in the past three decades.[63] This phenomenon seems to be caused by a substantial increase in exposure to several important risk factors such as high systolic blood pressure, particulate matter pollution, high BMI, high fasting plasma glucose, low physical activity, smoke, and alcohol consumption.[64,65]

Socioeconomic factors may also influence the outcome: the burden of stroke is also greater in low-income countries than in high-income ones; this phenomenon may be explained by poorer acute health care for stroke[66] and poorer stroke awareness.[67]

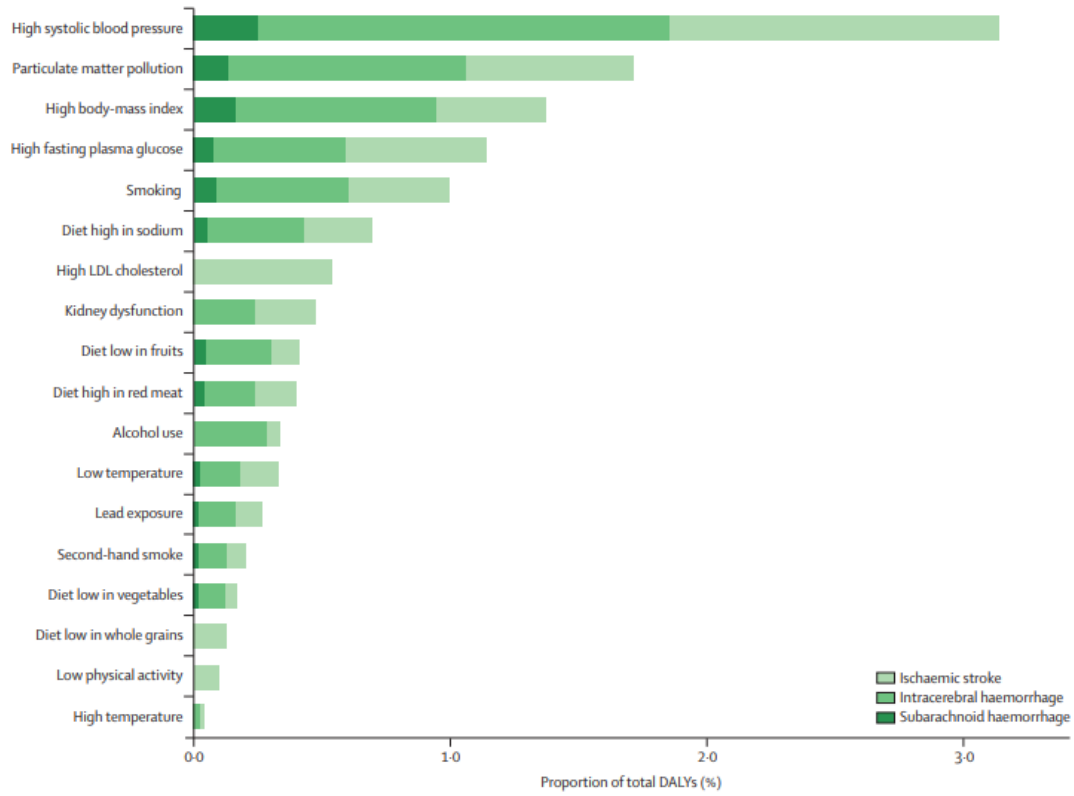


Figure 14. Proportion of DALYs attributable to risk factors by pathological type of stroke for both sexes combined, from GBD 2019 Stroke Collaborators, Lancet Neurology (2021)

PRE-TREATMENT PROGNOSTIC FACTORS

Since the primary purpose of this thesis is to investigate prognostic factors of functional outcome after MT, from now on, the focus will be shifted to this type of treatment.

The NIH Stroke Scale is one of the first examinations performed at admission. It consists of a 15-item neurologic examination that considers numerous clinical variables assessing the level of consciousness, space and time orientation, extraocular movement, visual-field loss, VII cranial nerve function, motor strength, ataxia, sensory, language, dysarthria, and neglect.[68] NIHSS at 24 hours predicts long-term outcomes in both anterior and posterior circulation ischemic stroke.[69,70]

1a—Level of consciousness	0 = Alert; keenly responsive 1 = Not alert, but arousable by minor stimulation 2 = Not alert; requires repeated stimulation 3 = Unresponsive or responds only with reflex
1b—Level of consciousness questions: What is your age? What is the month?	0 = Answers two questions correctly 1 = Answers one question correctly 2 = Answers neither questions correctly
1c—Level of consciousness commands: Open and close your eyes Grip and release your hand	0 = Performs both tasks correctly 1 = Performs one task correctly 2 = Performs neither task correctly
2—Best gaze	0 = Normal 1 = Partial gaze palsy 2 = Forced deviation
3—Visual	0 = No visual lost 1 = Partial hemianopia 2 = Complete hemianopia 3 = Bilateral hemianopia
4—Facial palsy	0 = Normal symmetric movements 1 = Minor paralysis 2 = Partial paralysis 3 = Complete paralysis of one or both sides
5—Motor arm Left arm Right arm	0 = No drift 1 = Drift 2 = Some effort against gravity 3 = No effort against gravity 4 = No movement
6—Motor leg Left leg Right leg	0 = No drift 1 = Drift 2 = Some effort against gravity 3 = No effort against gravity 4 = No movement
7—Limb ataxia	0 = Absent 1 = Present in one limb 2 = Present in two limbs
8—Sensory	0 = Normal; no sensory loss 1 = Mild-to-moderate sensory loss 2 = Severe-to-total sensory loss
9—Best language	0 = No aphasia; normal 1 = Mild-to-moderate aphasia 2 = Severe aphasia 3 = Mute; global aphasia
10—Dysarthria	0 = Normal 1 = Mild-to-moderate dysarthria 2 = Severe dysarthria
11—Extinction and inattention	0 = No abnormality 1 = Visual, tactile, auditory, spatial, or personal inattention 2 = Profound hemi-inattention or extinction
Score = 0–42	

Table III. NIHSS

Many prognostic scores have been developed to predict clinical outcomes after MT. A 3-month good outcome is typically defined as mRS 0-2, while an mRS 4-6 is generally associated with poor functional outcomes.[71,72] In Figure 15 are illustrated pre-intervention clinical variables that are usually relevant in decision making.

The ASPECTS[73] is a quantitative and topographic score used for patients with an occlusion of the middle cerebra artery. Starting from 10, a point is deducted for every region involved: M1 (anterior middle cerebral artery cortex), M2 (middle cerebral artery cortex lateral to insular ribbon); M3 (posterior middle cerebral artery cortex), M4 (anterior middle cerebral artery territory immediately superior to M1),

M5 (lateral middle cerebral artery territory immediately superior to M2), M6 (posterior middle cerebral artery territory immediately superior to M3).

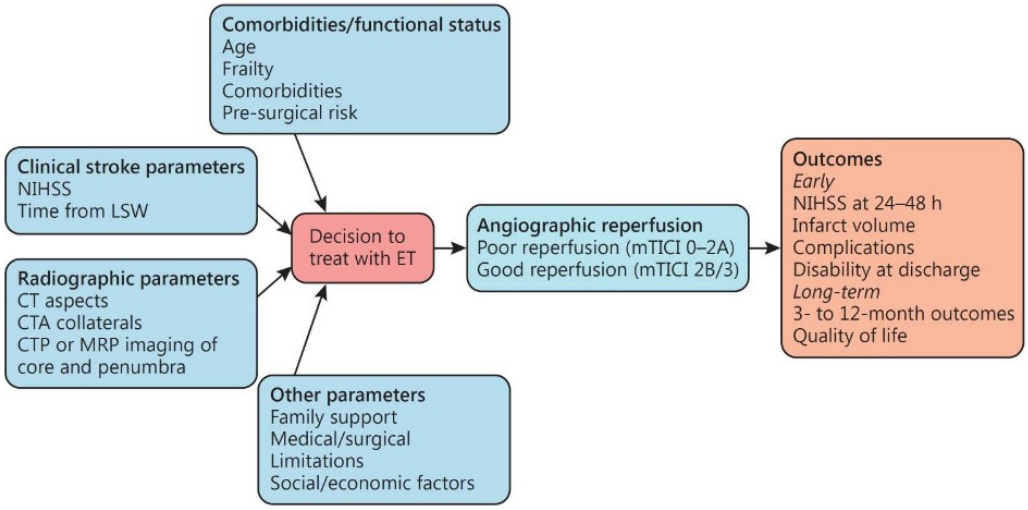


Figure 15. Parameters evaluated in decision-making by physicians while selecting large vessel occlusion patients for endovascular therapy, from Raza et al. (2018)

In a recent review by Raza et al., several pre-intervention prognostic scores were compared. These include 1) PRE *(Pittsburg Response to Endovascular therapy*) score, developed by taking into account patient age, NIHSS (Table III), and Alberta stroke program early CT score *(ASPECTS)*; 2) HIAT *(Houston Intrarterial Therapy)* score based on age, NIHSS, and glucose; 3) HIAT 2 score, adding CT ASPECTS to the parameters already taken into account by the HIAT score; 4) SPAN *(Stroke Prognostication using Age and NIHSS)* index that summates patient age and NIHSS;[74] 5) THRIVE *(Totaled Health Risks in Vascular Events)* score that incorporates age, initial NIHSS, and medical comorbidities;[75] 6) iSCORE that relies on multiple pre-treatment clinical variables;[76,77] and the 7) SAD *(Stanford Age and Diffusion-weighted imaging)* score that incorporated MRI DWI core volume.[78–83]

Clinical score	Variables, *n*	All ischemic strokes	IV tPA	Hemorrhage after tPA	Validation in LVOS cohorts	ET patient selection	AUC for good outcome
ASTRAL	6	+					0.7–0.9
iSCORE	11	+	+	+			0.7–0.8
DRAGON	5	+	+				0.8
HIAT	3	+	+	+	+		0.7
SPAN 100	2		+	+	+		0.68–0.73
SAD	2				+		0.69–0.82
SITS	9			+			0.60–0.70
THRIVE	5	+	+	+	+		0.60–075
HIAT-2	4				+	+	0.75
PRE	3				+	+	0.79

Table IV. Prognostic scores for acute ischemic stroke, from Raza et al. (2018)

The number of variables taken into account varies between scores (2-11), but the complexity of the score does not correlate with its predictive accuracy, e.g., the highly complex 11-variable iSCORE and the relatively simple 5-variable dragon score have very comparable discriminative power (AUC). However, the AUC values must be interpreted in the context of the studied population.

Other variables that influence stroke outcome but have not been considered in the scores include pre-ET core volume and pre-stroke functional status.

These scores should act only as adjunctive tools and not replacements for clinical judgement.[84]

POST-TREATMENT PROGNOSTIC FACTORS

The prognosis at 90 days after MT is strongly influenced by numerous clinical variables such as time to recanalization, degree of recanalization (TICI), follow-up neurological examination, particularly NIHSS at 24h, final infarct volume, procedural complications, post-stroke complications, (acute sepsis, urinary infection, pneumonia, and MI), and rehabilitation.

EVT outcome is measured by the Thrombolysis in Cerebral infarction *(TICI)* scale that standardizes the different degrees of reperfusion, ranging from complete perfusion (TICI 3) to no perfusion (TICI 0).[85]

Grade 0:	No Perfusion. No antegrade flow beyond the point of occlusion.
Grade 1:	Penetration With Minimal Perfusion. The contrast material passes beyond the area of obstruction but fails to opacify the entire cerebral bed distal to the obstruction for the duration of the angiographic run.
Grade 2:	Partial Perfusion. The contrast material passes beyond the obstruction and opacifies the arterial bed distal to the obstruction. However, the rate of entry of contrast into the vessel distal to the obstruction and/or its rate of clearance from the distal bed are perceptibly slower than its entry into and/or clearance from comparable areas not perfused by the previously occluded vessel, eg, the opposite cerebral artery or the arterial bed proximal to the obstruction.
Grade 2a:	Only partial filling (<2/3) of the entire vascular territory is visualized.
Grade 2b:	Complete filling of all of the expected vascular territory is visualized, but the filling is slower than normal.
Grade 3:	Complete Perfusion. Antegrade flow into the bed distal to the obstruction occurs as promptly as into the obstruction *and* clearance of contrast material from the involved bed is as rapid as from an uninvolved other bed of the same vessel or the opposite cerebral artery.

Table V. TICI, from Higashida et al. (2003)

Outcome measures

The modified Ranking Scale was conceived in 1857 to assess stroke outcomes and was later changed to improve its comprehensiveness.[86–88] The mRS assesses disability by measuring functional independence. The validity of this scale is documented, although its simplicity can affect its reliability; therefore, strict adherence to a series of rules is needed to ensure accuracy, e.g., with a structured interview.[89]

0	No symptoms
1	**No significant disability, despite symptoms** Able to perform all usual duties and activities
2	**Slight disability** Unable to perform all previous activities but able to look after own affairs without assistance
3	**Moderate disability** Requires some help, but able to walk without assistance
4	**Moderately severe disability** Unable to walk without assistance and unable to attend to own bodily needs without assistance
5	**Severe disability** Bedridden, incontinent, and requires constant nursing care and attention
6	**Dead**

Table VI. Modified Ranking Scale

The mRS is one of the most widely used scales to evaluate stroke outcomes. However, its coarse nature does not allow an accurate evaluation of clinical outcomes. The Stroke Impact Scale (SIS)[90] enables a more refined estimate. SIS considers numerous factors such as mobility, memory, psychological factors, language, daily activities, coordination, social activities, and quality of life. It assigns a score from 0 to 100, where 0 corresponds to no recovery and 100 to full recovery.

The Heidelberg Bleeding classification may be used to evaluate possible hemorrhagic infarction *(HI)* and parenchymatous hematoma *(PA)* after EVT.

Class	Type	Description
1	Hemorrhagic transformation of infarcted brain tissue	
1a	HI1	Scattered small petechiae, no mass effect
1b	HI2	Confluent petechiae, no mass effect
1c	PH1	Hematoma within infarcted tissue, occupying <30%, no substantive mass effect
2	Intracerebral hemorrhage within and beyond infarcted brain tissue	
	PH2	Hematoma occupying 30% or more of the infarcted tissue, with obvious mass effect
3	Intracerebral hemorrhage outside the infarcted brain tissue or intracranial-extracerebral hemorrhage	
3a	Parenchymal hematoma remote from infarcted brain tissue	
3b	Intraventricular hemorrhage	
3c	Subarachnoid hemorrhage	
3d	Subdural hemorrhage	

Table VII. Intracranial Hemorrhages, from von Kummer et al. (2015)

The prognostic accuracy of these pre and post-intervention scores is moderate, and any further improvement of prognostic accuracy requires the addition of radiographic and clinical variables that have not been considered previously.

PART II. WHAT IS A STROKE? A NETWORK FRAMEWORK

THE HUMAN CONNECTOME

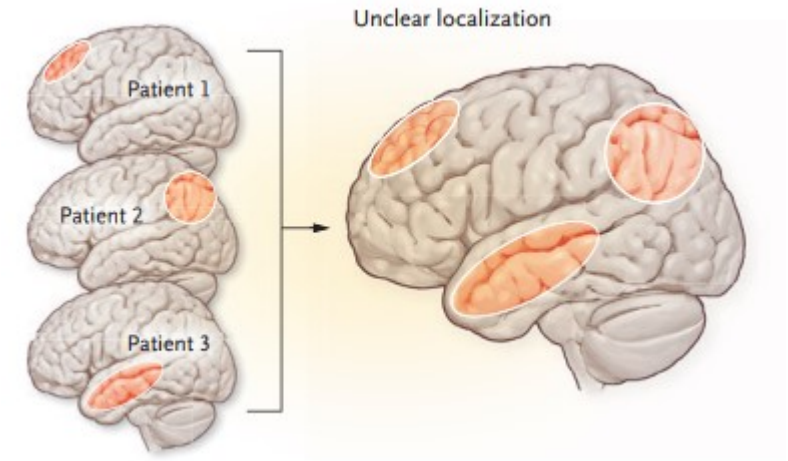


Figure 16. Lesions causing the same symptom (patient 1-2-3) often occur in different locations, from Fox (2018)

Although mapping stroke symptoms has enabled scientists to build a detailed atlas of the brain, the relationship between lesion location and symptoms is not straightforward. Over time, it became apparent that similar symptoms can result from lesions in different brain locations. Furthermore, lesion-based localization is also limited by the fact that many complex symptoms occur in patients without an apparent brain lesion, e.g., neuropsychiatric patients. A hint to the explanation for these phenomena came from the description of a new pathogenetic mechanism: diaschisis. According to this phenomenon, regions remote from a lesion may develop neurophysiological changes even though their neurovascular coupling is preserved. This finding is not explained by the classical neurophysiological interpretation of brain function.

With the advent of detailed structural and functional neuroimaging, researchers have been able to deepen their understanding of post-stroke dynamics.

These techniques have enabled the production of network-based models rather than anatomical models, in which specific regions are linked to specific cognitive and sensory abilities, and the creation of detailed maps of functional and structural connections: the connectome.

STRUCTURAL CONNECTOME

Connections play a fundamental role in neuroscience. Structural networks consist of anatomical links between neurons, represented by synapses, dendrites, axons, and gap junctions. Different approaches based on diffusion-weighted *(DWI)* MRI are available to determine structural network edges. Several models can be applied to the DWI data to compute structural brain properties. One of the most widely used models is diffusion tensor imaging *(DTI)* which includes a group of techniques processing eigenvectors (ε1, ε2, and ε3) and eigenvalues (λ1, λ2, and λ3). This technique aims to recognize the brain tissue's microstructural properties by identifying an ellipsoid representing an isosurface of diffusion probability in a voxel.[91–93] To quantify the shape of the tensors in each voxel, four DTI indices are used: fractional anisotropy *(FA)*, mean diffusivity *(MD)*, axial diffusivity *(AD)*, and radial diffusivity *(RD).* The most widely used anisotropy measure is the FA, which indicates the amount of diffusion asymmetry within a voxel. In this scenario, isotropy can be defined as the capability of water to diffuse equally in every direction. When it can move freely, water has isotropic diffusion. In the brain, water movements are limited by the presence of various structures, such as white matter bundles, the diffusion is therefore restricted, and the water diffuses preferably in some directions, anisotropic diffusion. When the diffusion anisotropy increases, the eigenvalues become increasingly unequal, and FA values rise. MD represents the average of the three eigenvalues of the tensor. Finally, AD and RD are used to determine diffusivity direction. FA is related to axonal integrity, but many factors can influence it (i.e., axonal loss, inflammation, cell death, gliosis, demyelination, and increase in intracellular or extracellular liquid content), making it nonspecific to the type of damage.[92–94] For this reason, it is usually paired with MD, which is able to identify increased extracellular spaces due to degeneration or shrinkage of axons and dendritic fibers.[95,96] AD detects axonal degeneration, while RD is influenced by density, demyelination., and abnormal axonal diameter.[94,97]

Figure 17. Three-dimensional reconstruction of DTI slices: DTI is an MRI technique that infers the axonal direction based on the anisotropic diffusion of water. In this figure, white matter bundles are depicted in different colors by a clustering algorithm, from O'Donnel and Westin (2011)[92]

Similarly, DWI can be used to assess the orientation of axonal tracts. All diffusion tractography techniques are based on the essential assumption that when numerous axons are aligned along a common axis, diffusion of water molecules will be easier along them than through them. Consequently, we expect to see diffusion preferentially in orientations that correspond to axonal fibers. Tractography algorithms infer long-range connections using local information on orientation. Magnetic resonance diffusion tractography identifies white matter bundles in vivo. White matter pathways connect distant brain regions and are, therefore, critical to understanding how normal and injured brains function. The only way to identify and measure these pathways in vivo is through tractography. Tractography measurements are error-prone, indirect, and difficult to interpret quantitatively. However, their non-invasive nature allows them to respond to clinical and scientific questions that would otherwise remain unanswered.[98] Animal studies confirmed results from DWI, suggesting that these in vivo techniques can identify real anatomical brain connections.[99,100] While intracortical connections are believed to

underlie the brain's computational capabilities; long-range white matter tracts are responsible for the distribution of information between brain systems. These macroscopic links account only for 10% of the total brain connections.[101]

These studies have resulted in maps of white matter tracts (structural connectome) that can be used to better understand brain injury's effects; this topic will be explored further in the following chapters.

FUNCTIONAL CONNECTOME

Functional neuroimaging can detect local variations in electrical activity, water diffusion, brain oxygenation, blood flow, and brain metabolism. Functional brain activity can be measured by PET, functional MRI *(fMRI)*, or EEG.

Historically, fMRI has been used to study the activation of brain areas during a task. It was later realized that the brain has fluctuations in the BOLD *(blood oxygenation level-dependent)* signal even at rest; these oscillations may be related to mental activity. Resting-state functional magnetic resonance imaging can be used to assess the brain's functional architecture at rest. This technique examines variations in the BOLD signal between brain regions. Two regions are considered functionally connected when their spontaneous activity is positively or negatively correlated.[34]

Functional neuroimaging is an emerging and promising tool to investigate neurodegenerative and psychiatric diseases such as Alzheimer's disease, Frontotemporal dementia, Parkinson's disease, depression, anxiety, and hallucinations.[34,102–107]

These studies suggest a close relationship between cognitive and psychiatric impairment and the breakdown of neural networks. Such findings indicate that functional connectivity might represent the biological substrate for cognitive and sensory impairment. Despite the vast number of possible connections that neurons can generate, several studies suggest that functional connectivity has a low spatio-temporal dimension. That is, few networks are responsible for performing cognitive, motor, sensory, social, and emotional skills.

Yeo et al. (2011) performed fMRI sequences on 1000 subjects and identified two solutions for functional cortical parcellation comprising 7 and 17 networks. The 17-network estimate consists of subnetworks of the 7-network one. While sensory

networks are more localized and are confined to sensory and motor cortices, association networks appear to be more distributed.

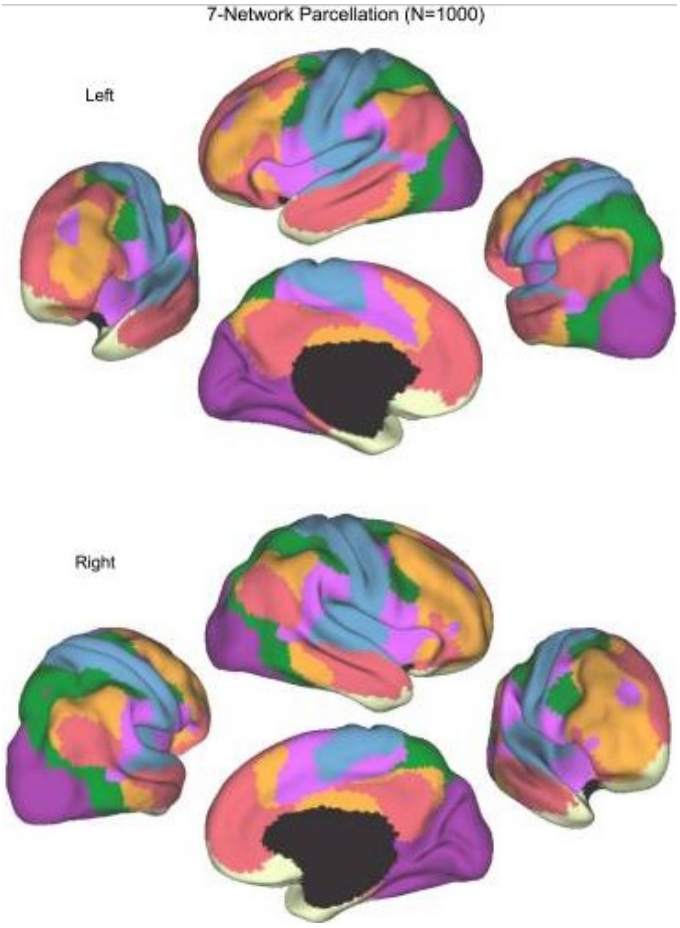


Figure 18. 7-network parcellation of functional connectivity: these networks were obtained by clustering fMRI data of n=1000 subjects. Each network is represented with a different color: purple (Visual), blue (Somatomotor), green (Dorsal Attention), violet (Ventral Attention), cream (Limbic), orange (Frontoparietal), and red (Default), from Yeo et al. (2011)

Based on Yeo's description, five cognitive and two sensory networks can be identified. This functional scaffold could play a role in representing cognitive abilities. Among the cognitive networks, the limbic *(LMB)* and the default mode network *(DMN)* play a crucial role in memory. Particularly the DMN is associated with episodic memory and autobiographic memory performance and shows a gradual shrinking with aging, in line with the natural decline of memory

performance in the elderly.[108] The LMB is more related to semantic information processing and retrieval.[109] These two networks are considered part of the same memory system but with a different specialization. The fronto-parietal network *(FPN)*, the dorsal attention network *(DAN)*, and the ventral attention network *(VAN)* are considered attentional networks. The DAN and the VAN act synergistically to orient attention to salient stimuli.[110] The FPN is important for attentional tasks and sustains executive functions.[111–113] Finally, the sensorimotor network *(SMN)* and the visual network *(VIS)* are linked with sensory abilities. The SMN is linked to motor skills, and it has been suggested that functional disruption of this network may be related to neurological conditions characterized by motor dysfunction.[104,114] The VIS is linked with visual abilities. According to the assumption that failure in networks underly cognitive dysfunctions, breakdown of VIS has been reported in clinical conditions characterized by visual impairment. An essential property of these circuits is inter-network connectivity. This means that brain disorders are not only characterized by disruption of a specific network, but inter-network connectivity can also be affected. The triple network theory states that a wide range of cognitive impairments may be explained by abnormal dynamic interactions among DMN, FPN, and VAN.[107,110] This theory posits that VAN integrates external information acting as an interface between DMN and FPN, regulating their competing inter-network activity and promoting appropriate behavioral response. Similarly, the VAN acts as a gate reorienting attention to relevant environmental stimuli interrupting the ongoing activity in the DAN, which in turn shifts attention to the new source of information.[110] Another important aspect of brain networks is the anti-correlation. For example, it has been assessed that the DMN and the DAN are anti-correlated in both healthy[115] and pathological conditions,[116] suggesting complex relationships between network inter-connectivity, which are linked with cognitive abilities. Similarly, changes in inter-connectivity patterns in stroke are one of the most common phenotypes observed, linked with behavioral disturbances.[117] After a stroke, the DMN and the DAN, which usually are anti-correlated, as stated above, reduce their segregation resulting in a lower anti-correlated pattern.

In the next section, we will describe structural and functional alterations following a stroke.

THE CONCEPT OF "BRAIN DYSCONNECTOME"

Behavioral outcomes highly depend on the impact of the focal lesion on large-scale functional and structural connectivity.[117–120] About 20% of all structural connections can be affected by a stroke. In the last decade, neuroimaging techniques have allowed the computation of network disconnections. Disconnectome is strongly correlated with behavioral impairment and the subsequent functional recovery.[120–122]

Conventionally the degree of structural and functional disconnection was measured using fMRI[122] and DWI-MRI,[121] but it is now possible to embed a patient's lesion into an atlas of functional and structural connections obtained from large datasets of healthy subjects and reproduce some of these maps. It is then possible to indirectly estimate the impact of the lesion on the connectome by analyzing the structural and functional connections passing through the lesion.[123–125] This allows clinical MRI sequences to be used to deduce structural and functional patterns of disconnection that would otherwise be difficult to measure. However, whether behavioral predictions using such indirect methods are as accurate as direct measurements is still controversial.[121,126]

Structural disconnections

Although the correlation of deficits can be partially explained by localization in the same vascular territory, it has been shown to be present even among areas without obvious vascular overlap. E.g., language and memory impairments covary even though these functions are localized in different hemispheres.[127] Because of the brain's high degree of interconnectivity, even a minor injury can disrupt multiple systems and severely alter large-scale brain function. Structural disconnections can be divided into two main groups direct disconnections, in which there is an interruption of direct structural connections between two regions, and indirect disconnections, characterized by an increase of the minimum number of links separating two indirectly connected regions.

The lesions most frequently associated with extensive disruption of the structural connectivity are located deep in the white matter of the frontal, parietal, and temporal lobes.[128]

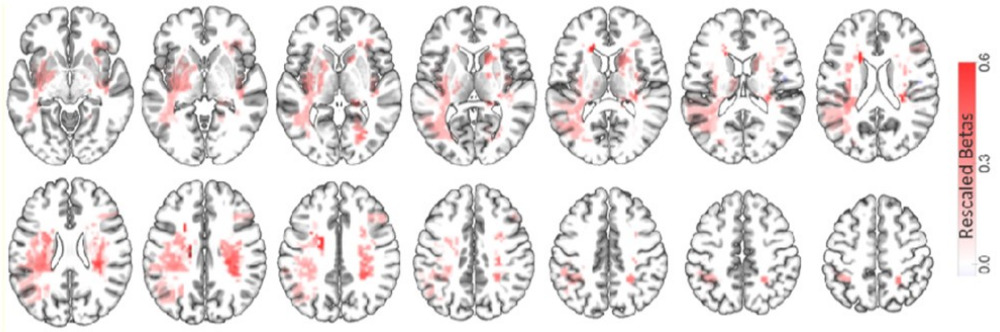


Figure 19. Stroke topography of white matter disconnection, from Griffis et al. (2020)

Consequent to a lesion, directly disconnected cortical areas may present neurodegenerative changes such as cortical thinning in both the directly disconnected areas and their contralateral homologous. These changes could be long-term consequences of the functional disruptions caused by the disconnection itself.[128]

Functional disconnections

Traditionally it was thought that a lesion in an area contributing to function (e.g., ability to understand words) caused very specific deficits and did not alter entire domains of function (e.g., language). As early as 1975, this assumption was challenged by Howes and Boller, who observed that a focal lesion causes a reduction in accuracy and speed of processing.[129] When a stroke occurs, it causes a perturbance in the functional network in which it is embedded and alters the communication with the networks connected to it. These changings result in large-scale disruption of functional connectivity, particularly of the inter-connectivity patterns.[130] These functional abnormalities correlate with short-term behavioral outcomes; in parallel, their normalization correlates with recovery.[127,131–133]
E.g., various lesions in the temporal, parietal and frontal lobes may lead to common disruptions of functional connectivity in the frontal eye field and the posterior intraparietal sulcus. These changes have been demonstrated to predict short-term severity and recovery.[134]

Baldassarre and colleagues, studying the relationship between neglect and functional connectivity, discovered that more than 50% of the cortical patterns of functional connectivity were affected, including numerous attention-related areas. These findings suggest that stroke involves multiple systems through large-scale disruption in functional connectivity and inter-connectivity patterns, leading to behavioral deficits that are correlated within and across domains.[135] E.g., defects in the dorsal attention network correlate with spatial neglect and motor deficits.[136] The presence of large-scale impairments is not inconsistent with the evidence that the effects of an injury are more severe in the directly affected system.[137] In fact, the prediction of sensory and motor impairments mostly depends on direct damage to these brain systems, while language, attention, spatial and verbal memory impairments are deeply related to functional inter-network connectivity alterations. Thus, patterns of widespread functional disconnection co-exist with more network and behavior-specific patterns.[130]

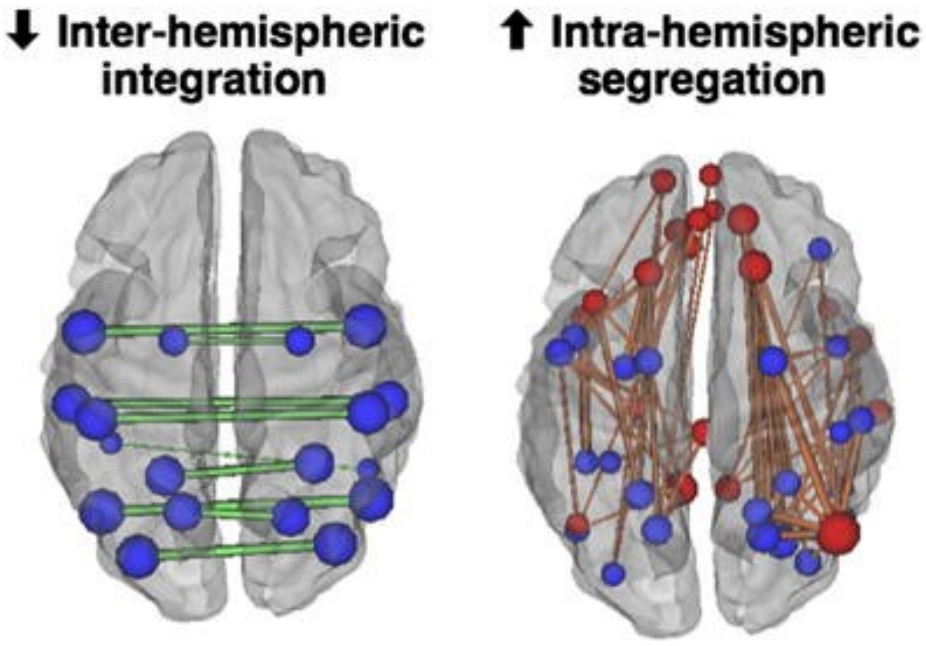


Figure 20. Inter-hemispheric integration and intra-hemispheric segregation after stroke, form Corbetta et al. (2018)

Functional connectivity abnormalities tend to differ in severity and topography but not in shape.[117] The weakening of inter-hemispheric functional connectivity, the intensification of intra-hemispheric segregation, and the increase in functional

connectivity between networks that are typically not correlated are common impairments found in multiple behavioral domains such as motor, visual, language, attention, and memory domains.[122,135,138] Furthermore, stroke causes a significant reduction in the modularity of functional connectivity.

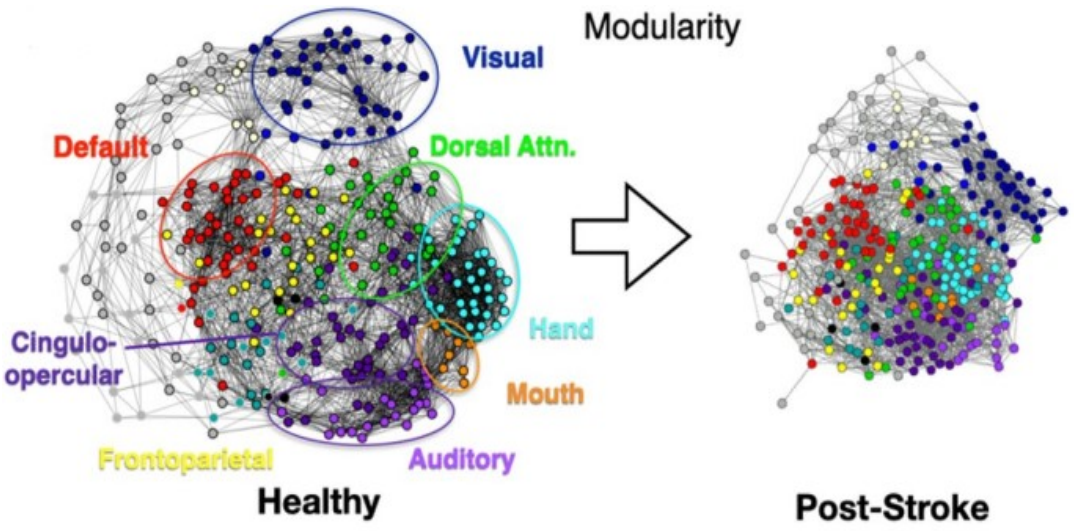


Figure 21. In healthy subjects, brain networks form segregated clusters (left), after stroke is evident a reduction in inter-network segregation and integration (right), from Siegel et al. (2022)

In a healthy brain, multiple segregated and interconnected systems process data simultaneously, exchanging and integrating information, thus maximizing the system's entropy. The reduction in modularity may reflect a reduction in the range of possible neural patterns that can be generated, hence a decrease in its entropy. A reduction in the distinct states that the brain can display causes a reduction in the number of behaviors that can be generated. Thus, entropy reduction is related to the amplitude of behavioral impairments; these concepts will be further explored in the following section.[130]

Behavioral recovery is associated with a significant increase in functional connectivity's modularity and interhemispheric connectivity's normalization.[130]

Structural disconnections may explain the impairment of functional connectivity

Stroke causes large-scale destruction of the functional connectome. In order to thoroughly understand the pathophysiology of these changes, it is essential to link them to the underlying structural lesion. The strength of normal functional connectivity varies with the length of the shortest structural pathway for both inter- and interhemispheric functional connections. Functional connectivity tends to be stronger between directly connected regions and weaker with increasing structural path length. Lesion-induced disruption of functional connectivity is mediated not only by disconnections of direct connections but also by damage to indirect connections. In other words, functional connectivity in the resting state is altered between both directly and indirectly disconnected regions. Moreover, direct structural disconnections produce a more severe alteration of functional connectivity, implying that the extent of impairment of functional connectivity depends directly on the nature of the affected connections.[128]

Structural disconnections often involve interhemispheric communications and influence functional connectivity on a large-scale; stable interhemispheric integration is indeed essential for functional connectivity organization. Interhemispheric structural disconnections increase intrahemispheric functional connectivity and reduce interhemispheric functional connectivity. Reduction in the modularity of functional connectivity is strongly related to the structural disconnection that occurs because of the injury. Furthermore, structural disconnection outperforms region-level and voxel-level measures in explaining functional connectivity disruptions associated with stroke. White matter damage also outperforms focal damage measures. Even though region-based SCD performs the best, tractography shows a comparable performance.[139]

The relationship between structural disconnection and behavior may be mediated by functional connectivity disorders, which, in turn, are precipitated by structural disconnection itself.

An organic interpretation of stroke effects, based on three parameters, i.e., structural damage, functional abnormalities, and behavioral deficits, has enabled a broader comprehension of the phenomenon and paved the way for a different approach to stroke rehabilitation.

THE LOW DIMENSIONALITY OF CLINICAL SYNDROMES

The brain may use synergies to reduce the dimensionality of neural states and lower its computational load. For example, in the motor domain, synergies have been proven to reduce the degrees of freedom that characterize hand movement.[140,141] Similar strategies may be adopted by other functional domains. Recent studies have shown that the behavioral impairments following a stroke can be described by a few factors accounting for most of the variability across subjects. This evidence contrasts with the classical neuropsychological interpretation, according to which lesions in distinct brain areas cause very specific deficits. These results are, however, in line with clinical findings in patients who present with similar sets of deficits even when the lesion causing them is located in very different areas.[117] The correlation of behavioral deficits reflects the brain's highly interconnected organization of functional networks. It is not difficult to understand how, in a background of flexible interactions among many brain regions, the disturbance introduced by stroke can resonate in different and dislocated areas, leading to large clusters of deficits.

In a recent study, Corbetta et al. identified three factors that together explained 69% of the variance across behavioral domains: C1) language, verbal and spatial memory; C2) left body strength, right visual field bias, and average performance based on accuracy and reaction times; C3) right body strength, left visual field bias, and attention shifting.[127] This result was also replicated on a larger and independent dataset (Bisogno et al., 2021).[142]

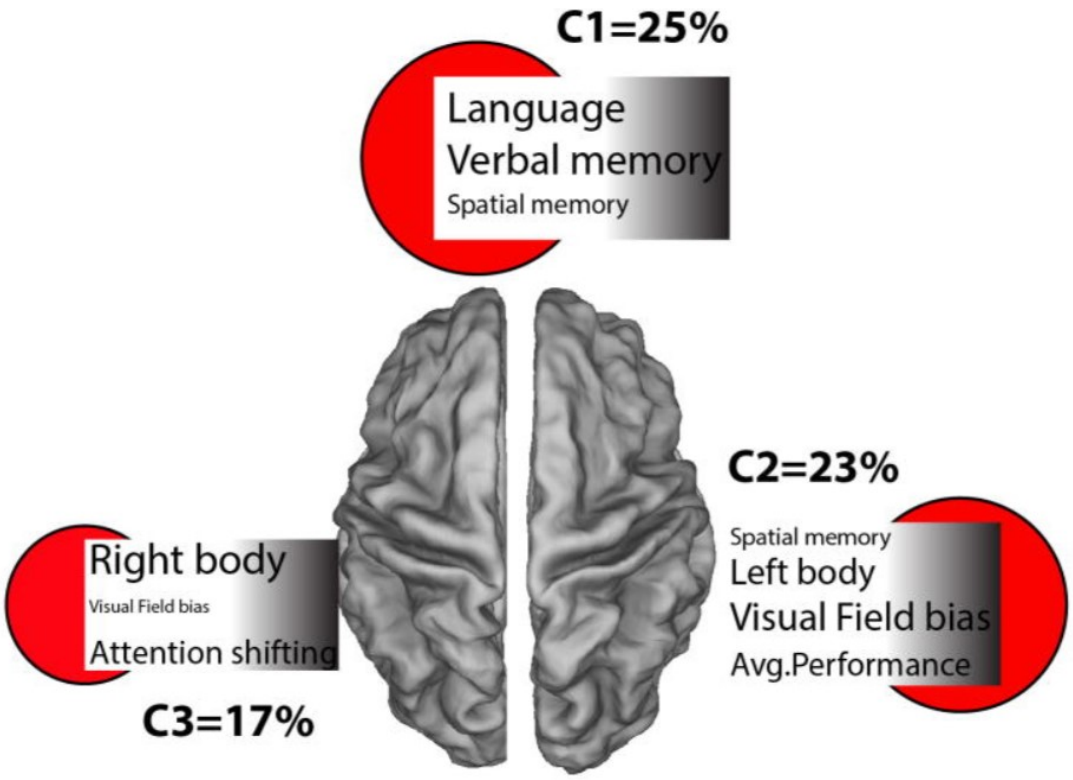


Figure 22. C1, C2 and C3, from Corbetta et al. (2015)

The covariance of deficits is mainly explained by broad functional connectivity abnormalities that involve not only the ipsilateral but also the contralateral hemisphere, leading to a reduction in entropy and neural states that the brain can generate.[143,144]

There are three possible neurological factors that could be the bases of the low-dimensional presentation of stroke 1) deficits co-occur because they are embedded within the same vascular territory; this hypothesis has been rebutted by the presence of correlated deficits in widely separate regions, even in different hemispheres; 2) stroke affects white matter regions more frequently than grey matter regions; in fact, lesions affecting a large number of pathways are correlated with more extensive deficits; 3) stroke has physiological effects on regions far from the injury, as shown by functional connectivity studies.[117]

As will be explained more thoroughly in the next section, the structural disconnections that develop consequently to an injury may explain the functional alterations we have discussed. Since white matter pathways are spatially clustered in large bundles, lesions in these locations may produce similar structural disconnections.[99] Structural disconnections tend to overlap more than lesion

localization among different patients. Therefore, measures of structural disconnection may reveal common structural disturbances among patients with heterogeneous lesions. The low dimensional pattern of structural disconnection is correlated with the low dimensionality of functional connectivity disruptions, which are, in turn, associated with the low dimensionality of stroke behavioral outcome.[139] For each patient, it is, therefore, possible to derive information regarding direct and indirect structural disconnections that provide essential knowledge about structural and functional connectome. This information could be used to 1) identify possible targets for neuromodulation techniques such as TMS; 2) determine how the post-stroke structural connectome may influence the outcome of these therapies.[128]

The low dimensionality of stroke presentations is also preserved in the long term, and the variance accounted for remains stable regardless of recovery. Furthermore, interactions between deficits influence recovery; that is, recovery of one function may positively correlate with the recovery of another function. For example, attention scores are bidirectionally correlated with language recovery, and spatial memory deficit covary with all other domains.[117]

PART III. BRAIN DYSCONNECTOME: A POTENTIAL BIOMARKER FOR FUNCTIONAL OUTCOME AFTER MECHANICAL THROMBECTOMY

INTRODUCTION

Mechanical thrombectomy *(MT)* restores blood flow after the acute occlusion of a brain vessel, causing ischemic stroke. This revascularization treatment prevents damage to the so-called 'penumbra' brain regions suffering from reversible ischemia. While this technique has led to significant improvement of functional outcomes in patients, as demonstrated by several randomized control trials, only 40% of patients recover completely after a stroke, while the remaining 60% experience significant disabilities.[52–56]

Several prognostic factors have been identified to predict a good or poor outcome, e.g., in most randomized control trials, mRS 0-2 corresponds to a good outcome, while mRS 3-6 corresponds to a poor outcome.[145] Pre-treatment prognostic factors are essential to decide whether a patient with large vessel occlusion could benefit from MT. These include clinical parameters (i.e., age, pre-stroke mRS score, NIHSS at presentation, time between event and revascularization) and imaging parameters such as ASPECTS and core and penumbra volumes. Core and penumbra volumes are volumetric parameters that have been demonstrated to help identify patients who might benefit from MT even in a 'late window' setting, thus significantly widening the eligible population for treatment.[146,147] The ASPECT score instead is a semi-quantitative measure, providing coarse topographical data according to an intracerebral vascular distribution, in addition to volumetric information.[148]

Post-treatment prognostic factors include numerous clinical variables such as time to recanalization, TICI, NIHSS at 24h, final infarct volume, procedural complications, post-stroke complications, and rehabilitation. Among these features, neuroimaging data, especially when collected in an early setting, has helped define personalized treatment for stroke patients. However, volumetric data only

moderately correlate with stroke severity, while several studies have shown that qualitative data on lesion location is crucial to determine post-stroke impairment. For example, Rosso et al. observed that the perfusion-diffusion weighted imaging mismatch poorly predicts outcome, whereas lesion location might provide a better prediction.[149] Weaver et al. (2021) confirmed these results and identified the right parietal lobe, left frontal and temporal lobes, and left thalamus as the regions most predictive of impairment.[150] Also, focal lesions cause remote functional changes that reflect extensive network disruption. Functional connectivity abnormalities include the weakening of inter-hemispheric functional connectivity, the intensification of intra-hemispheric segregation, and the increase in functional connectivity between networks that are typically not correlated. Furthermore, stroke causes a significant reduction in its modularity.[122,130,135,138]

The metabolic patterns in the human connectome (i.e., indirect measures of functional and structural disconnections) correlate with behavior and provide a better understanding of recovery after stroke.

Therefore, while literature recognizes the predictive value of topographical data (including lesion location and structural/functional disconnection), this information has yet to be efficiently integrated into clinical practice, where volumetric or vascular data guides the neurological evaluation and revascularization protocols entirely.

To explore this issue, we conducted this thesis with two main objectives. Firstly, we describe the anatomy of structural and functional disconnection related to a clinically applicable functional outcome measure (i.e., the mRS at three months) in stroke patients. Secondly, we compare the prognostic ability of topographical and disconnection measures and test whether they provide a better prediction when applied in a large-scale network framework compared to an ASPECTS-like vascular framework.

MATERIALS AND METHODS

Study sample

Our sample included 66 patients enrolled in the Stroke and Neurology Unit of the Padua University Hospital who underwent MT from January 2018 to June 2022. The inclusion criteria were as follows:

1. First symptomatic ischemic stroke.
2. LVO eligible for EVT.
3. Anterior circulation stroke.

Exclusion criteria were as follows: (i) Previous stroke based on clinical imaging; (ii) No LVO or not eligible for MT; (iii) Not available pCT imaging; (iv) undergoing rtPA; (v) lack of follow-up clinical data.

Clinical data

On admission, all patients were evaluated using the modified Ranking Scale *(mRS)* and the National Institutes of Health Stroke Scale *(NIHSS),* and pre-event mRS data were collected. Before performing MT, they underwent perfusion-CT and CT examinations. They also underwent CT scans and MRI examinations after MT. At discharge, they were re-evaluated using NIHSS and mRS; the latter scale was also administered at three months to verify clinical outcomes.

Other data were collected. Anamnestic data included age at the event, gender, high blood pressure, atrial fibrillation, coronary artery disease, and diabetes. Pre-treatment prognostic factors comprised ASPECTS, a score assessing lesion location and extension, and TOAST, an etiologic classification for ischemic stroke. Post-treatment prognostic factors included TICI, a recanalization index, Heidelberg bleeding classification, assessing post-treatment hemorrhagic infarctions and parenchymatous hematomas, and systemic complications.

Imaging acquisition

We performed pre-treatment (CT and CT perfusion) and post-treatment (CT or FLAIR MRI) imaging. From pre-treatment CT perfusion images, we obtained the core and penumbra volumes. Final lesions were obtained from FLAIR MRI, and CT scans performed after MT. FLAIR images were preferred when both were present due to the higher resolution. These acquisitions were performed using MRI/CT facilities from the Neuroradiology unit of Padua's Hospital.

Lesion maps

Patient's lesions were manually segmented by three MD students (GA, SR, CB) using the ITK-SNAP tool and then verified by a neurology resident (AB). DICOM files were transformed into NIFTI format using dcm2niix toolbox. Lesions were normalized with a non-linear transformation using the Advanced Normalization tool (ANTs) onto the MNI brain atlas.[151] This procedure used a cost function mask approach aimed at improving the registration of the FLAIR or CT image. The normalization matrix was then applied to the lesion mask. Values were transformed through a nearest neighbour interpolation function as suggested for binarized images.

Structural disconnection

To evaluate structural disconnection, we used the BCB toolkit,[125] that indirectly estimates the structural disconnection caused by a lesion. The inference about the affected structural pathways is made by embedding the lesion into a normative structural connectome obtained from a sample of healthy subjects (human connectome project). The lesions were normalized to MNI space resampled to 1x1x1 mm (182, 218, 182 voxel space). This space was used to match the one of the tracts included in the BCB toolkit. In contrast to the toolkit available online, which had only 10 controls, we used an advanced approach, including 172 healthy subjects for structural connectome computation. For each voxel, the likelihood that a white matter bundle directly connected with the lesion passes through it is

calculated. In other words, this method allows us to indirectly estimate the probability that a lesion disconnects specific brain voxels.

Functional disconnection

Functional disconnection was computed according to previous studies (Boes et al., 2015, Salvalaggio et al., 2020).[121,124] Specifically, each lesion was embedded into a normative connectome of 173 subjects from the HCP dataset scanned with a 7T MRI machine. Specifically, normalized lesions were resampled to 2x2x2 voxel space, binarized, and used as seed-region of interest for FC computation. We used the same functional data described in Pini et al., 2021.[152] Whole-brain temporal correlation maps were calculated using the entire lesion as a seed region of interest by averaging the sign time-course across all voxels within the lesion. Then, for each voxel, we computed the Pearson's correlation between the voxel time series and lesion averaged signal. This procedure resulted in a whole brain map expressing the strength of the association between the lesion and the rest of the brain. It is worth noting that this procedure parallels the structural disconnection since this map is an indirect measure of brain "dysconnectivity" between the lesion and the brain. Exemplative maps reporting both structural and functional disconnection are shown in Figure 23.

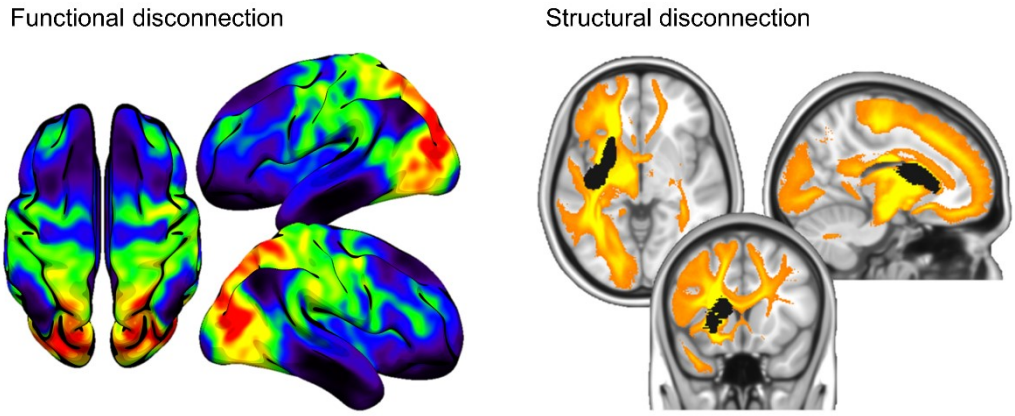


Figure 23. Exemplative maps of functional and structural disconnection from a patient with a subcortical lesion (black shape in the right panel)

Normative Atlases

Tree different atlases were used to investigate the prediction of the lesion on functional outcome (mRS at three months). The first atlas was used to investigate the prediction of outcome based on vascular territories. The other atlases explored outcome prediction based on a brain (structural, functional) "network" perspective. Below, the three different atlases are described in detail.

The vascular atlas[153] represents arterial territories in a three-dimensional space based on stroke lesion distributions in n= 1,298 acute patients. The atlas encloses supra- and infra-tentorial regions created by a mixture of anatomical and vascular criteria. Specifically, this atlas defines four major supra- and infra-tentorial arterial territories: Vertebro-Basilar, Anterior, Middle, Posterior Cerebral Arteries, and sub-territories (cerebellar arterial territories, basilar, thalamo-perforating, and lenticulostriate), distributed in two hierarchical levels. Level 2 represents the major vascular territories (ACA; MCA; PCA; VB), while Level 1 includes 32 subdivisions. We utilized the latter since this atlas provides better anatomical resolution of the vascular territories. This atlas encompasses the following regions: anterior cerebral artery left, anterior cerebral artery right, medial lenticulostriate left, medial lenticulostriate right, lateral lenticulostriate left, lateral lenticulostriate right, frontal pars of middle cerebral artery left, frontal pars of middle cerebral artery right, parietal pars of middle cerebral artery left, parietal pars of middle cerebral artery right, temporal pars of middle cerebral artery left, temporal pars of middle cerebral artery right, occipital pars of middle cerebral artery left, occipital pars of middle cerebral artery left, insular pars of middle cerebral artery left, insular pars of middle cerebral artery right, temporal pars of posterior cerebral artery left, temporal pars of posterior cerebral artery right, occipital pars of posterior cerebral artery left, occipital pars of posterior cerebral artery right, posterior choroidal and thalamo-perforators left, posterior choroidal and thalamo-perforators right, anterior choroidal and thalamo-perforators left, anterior choroidal and thalamo-perforators right, basilar left, basilar right, superior cerebellar left, superior cerebellar right, inferior cerebellar left, inferior cerebellar right, lateral ventricle left, lateral ventricle right.

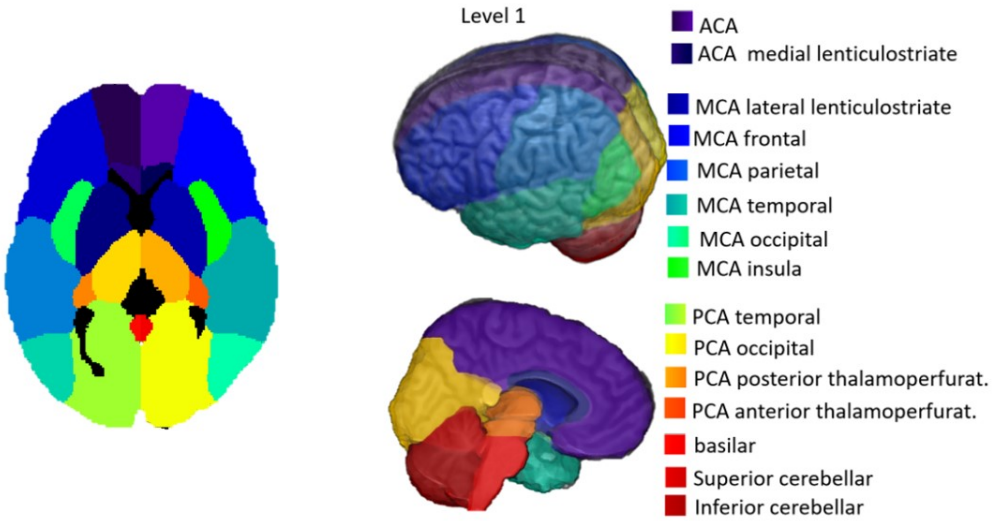


Figure 24. The arterial atlas, each vascular parcel is represented with a different colour, from Liu et al. (2021)

From a brain networks perspective, we used two different atlases. The first template divides the brain into grey matter parcels belonging to the so-called "functional resting-state networks". The second atlas included white matter bundles connecting these resting-state networks.

The functional atlas was obtained from fMRI sequences in n=1000 subjects. The whole approach was described by Yeo et al. Briefly, functionally coupled regions were identified using seed-based analyses and divided into distinct networks using a clustering algorithm.[111] This atlas has two different subdivisions: one in 7 networks, the other in 17 sub-networks that are subdivisions of the main networks. Both parcellations were used in this study to investigate the impact of the prediction assessed with different network dimensions. The 7-network atlas includes: limbic (LMB), default mode network (DMN), frontoparietal network (FPN), dorsal-attention network (DAN), ventral attention network (VAN), sensorimotor network (SMN), visual network (VIS). The 17 networks parcellation includes: Peripheral-VIS, central-VIS, SMN-A, SMN-B, DAN-A, DAN-B, VAN-A, VAN-B, LMB-A, LMB-B, control network A, control network B, control network C, DMN-A, DMN-B, DMN-C, temporo-parietal.

In addition, we included in this functional parcellation the subcortical structures not considered in the original Yeo's parcellation. Specifically, we enclosed the Harvard-Oxford atlas:[154] amygdala, hippocampus, thalamus, caudate, putamen,

nucleus accumbens, and pallidum. These structures were grouped as unique parcellation (basal ganglia network) for both 7 and 17 network templates.
The functional white matter atlas is obtained from fMRI-guided DTI tractography in *n*=32 healthy subjects and individuates white matter bundles that connect the regions of 13 functionally-defined brain networks: the dorsal DMN, the ventral DMN, the left executive control network, the right executive control network, the anterior salience network, the posterior salience network, the arcuate network, the basal ganglia network, the higher visual Network, the language network, the precuneus network, the sensorimotor network, and the visuospatial network.
These white matter networks connect the main grey matter networks corresponding to Yeo's space through anatomical pathways.

Lasso regression

We derived two different metrics to assess outcome prediction.
Lesion overlap: to estimate the prediction value of the lesion on the outcome, we calculated the percentage of the overlap for each lesion and each parcel belonging to the vascular, functional, and structural atlases. To this aim, two measures were computed. The first metric reflects the spatial distribution of the lesion as the lesion percentage encapsulated in each parcel. The second metric represents a volumetric parameter defined as the percentage of each parcel overlapping with the lesion mask. This latter measure was used jointly with the first to compute an exclusion score to identify the parcels with a low lesion loading. Specifically, we excluded parcels with a lesion/parcel percentage overlap lower than 5%. In other words, we just considered overlaps involving at least 5% of the lesion or 5% of the parcel (see Figure 25 for a visual example). This procedure was repeated for the vascular, Yeo's (7 and 17 networks), and Figley's parcels.

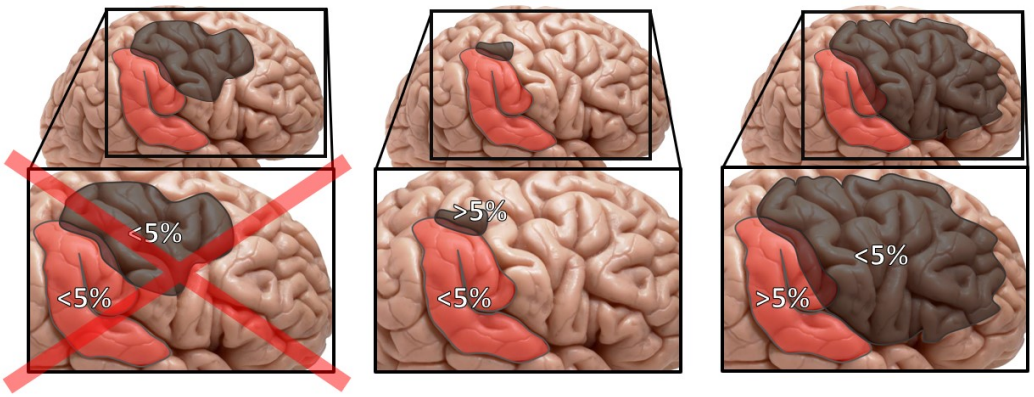


Figure 25. Cut-off for vascular parcels.

Disconnections outcomes: we computed the average value of functional and structural disconnection in each parcel of the correspondent atlas. Specifically, the mean functional and structural disconnection was estimated for each parcel of Yeo's and Figley's atlases. These values correspond to the functional dysconnectivity strength and the structural dysconnectivity probability for each network considered. These metrics investigated the relationship between lesion features and mRS at three months.

We used the lasso function to prevent overfitting. Overfitting occurs when a statistic model comprises a disproportionate number of parameters compared to the sample size. The model adapts excessively well to the population on which it was developed and cannot perform accurately against new data. Using a regularized approach, we insert a penalty coefficient to prevent this overfitting on the model. Lasso regression is a linear regression that shrinks the coefficients toward zero. Compared to other regularized regressions (e.g., ridge, elastic net), lasso allows the coefficients to reach the value of zero, hence, excluding parameters from the equation which do not contribute to the prediction.

In the lasso regression, we tested 5 different models corresponding to the two frameworks described above (topology vs. network). Specifically, we run the analysis considering as the independent set of variables: 1) lesion in the vascular atlas, 2) lesion in the grey matter networks (7 and 17 parcellations), 3) lesion in the white matter networks, 4) functional disconnection within the grey matter networks (7 and 17), and 5) structural disconnection in the white matter networks.

The regularization parameter for the lasso regression (L1) was assessed through a leave-one-out cross-validation *(LOOCV)* procedure between a set of 100 values

ranging from 1e-05 to 1e+05. The best L1 value resulting from the LOOCV was then used to assess the prediction in the whole cohort. Prediction was assessed using the R^2 score. The entire procedure was run on Python v3.0 and the scikit-learn library designed for machine learning analysis. Imaging data were manipulated through the Nibabel library on python 3.0.

Voxel-wise relationship with the functional outcome: the relationship between the functional outcome and brain features was further investigated at a voxel-wise level. For this analysis, we included the structural and functional disconnectivity maps computed for each patient. We then implemented a linear correlation through nonparametric inference using fsl randomise (n=1000 permutations FWE-corrected at TFCE with a stringent p value < 0.01). Before running the analysis, functional dysconnectivity maps were threshold at a value of r=0.2, as in previous studies,[152] while structural disconnection was considered with a value higher than 0.5.

RESULTS

Participants

A total of *n*=135 patients were screened, with *n*=66 meeting the inclusion criteria. The mean age of the sample was 73 years ± 12, the distribution by age group is shown in Supplementary 1. The patients were 56% females and 44% males. The clinical data revealed that 67% of them had high blood pressure, 11% suffered a heart attack, 36% had atrial fibrillation, and 20% had diabetes (Supplementary 2). On admission, they were evaluated by stroke neurologists. The mRS pre-event was 0.5±0.9 and the mean NIHSS at presentation was 13±6.8. The success of the recanalization was measured by the TICI scale (Supplementary 4). After treatment, 33% of the patients developed intraparenchymal bleeding that was assessed by the Heidelberg Bleeding Classification (Supplementary 5), 50% presented a systemic complication (Supplementary 6), and 6 patients died before discharge. At discharge, they were re-evaluated with NIHSS (average score 5±5.9) and mRS (2.5±2.1). At three months the mRS was 2.5±2.1 and 56% of patients had a poor outcome (mRS 3-6).

Anatomy

For each patient, we collected perfusion CT and CT scans. Core volume measured on average 14216±20659mm^3, while penumbra volume measured 71249±34880mm^3. ASPECTS had a mean value of 8.4±1,7. We then achieved a total of 40 post-EVT CTs and 19 post-EVT FLAIR MRIs. Of the 66 patients enrolled, 7 were excluded during data analysis: 4 patients were excluded because the final lesion was not detectable, and 3 more were excluded due to image corruption.

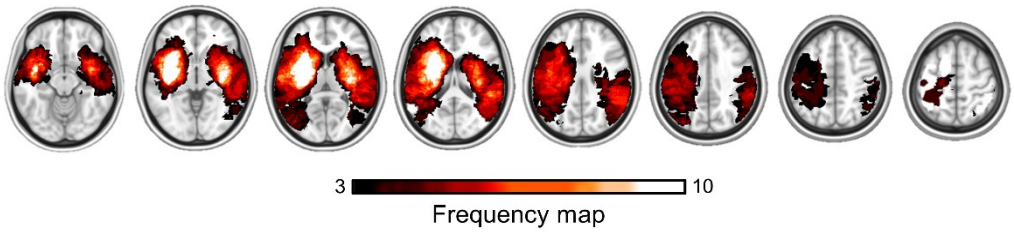


Figure 26. Frequency map of the lesions. Lesions map predominantly in the subcortical nuclei and the temporal/parietal cortices

The lesions were mainly located deep in the parietal and frontal lobe affecting the central white matter, the basal ganglia, and the thalamus, in line with previous studies[127,142,155,156]. In Figure 26 is reported the frequency map of the lesion.

Stroke Dysconnectome

The statistically significant voxel-wise functional dysconnectivity map showed regions positively associated with mRS score at three months. The regions of functional disconnection across the entire group localized to the frontal eye fields, the precentral/postcentral gyri, the supplementary motor area, the superior and inferior parietal lobe, the cuneus and precuneus, the calcarine cortex, the inferior occipital lobe, the middle temporal gyrus, the right inferior temporal lobe, the cingulum, and the cerebellum. From a network perspective, these regions overlapped with the VIS (R^2=0.379), the SMN (R^2=0.340), and the DAN (R^2=0.318) (see Figure 27). The relationship between connectivity and mRS was positive, i.e,. stronger functional disconnection higher (poorer outcome) mRS. No significant voxel survived for the oppositive relationship ($p>0.05$).

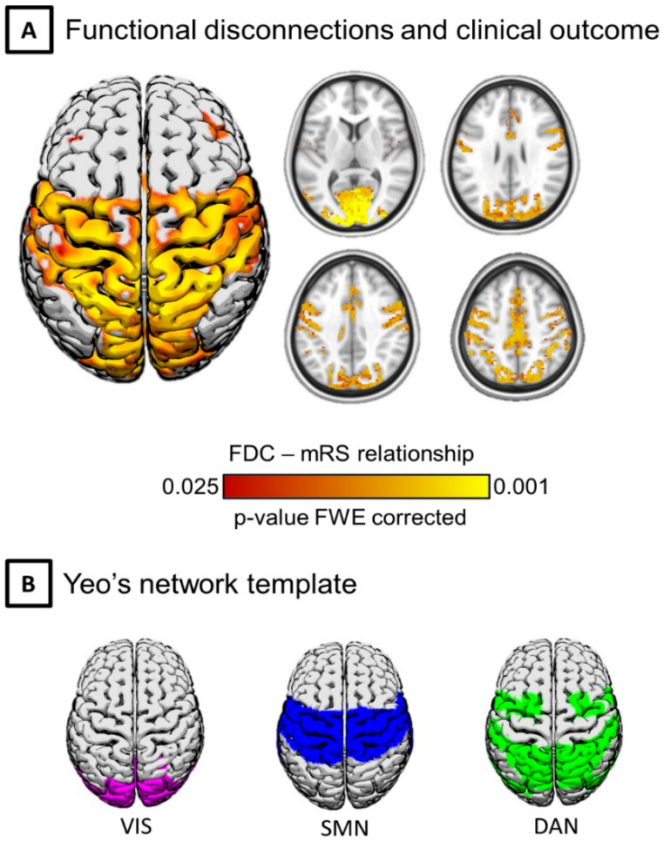


Figure 27. Panel A. functional voxels surviving the statistically significant relationship between mRS at three months and disconnectivity. Panel B: vis-à-vis comparison with the visual (VIS), sensorimotor (SMN), and the dorsal-attention (DAN) from Yeo's 7 network atlas

The voxel-wise structural disconnection analysis showed that several white matter bundles significantly correlated with the three month-mRS, including corticospinal tract, corpus callosum, corona radiata, thalamic radiation, and left inferior and superior longitudinal fasciculus (Figure 28). Also, in this case, more structural disconnection corresponded to higher mRS. As for the functional disconnection, no significant voxel survived for the negative relationship between structural disconnection and mRS ($p>0.05$).

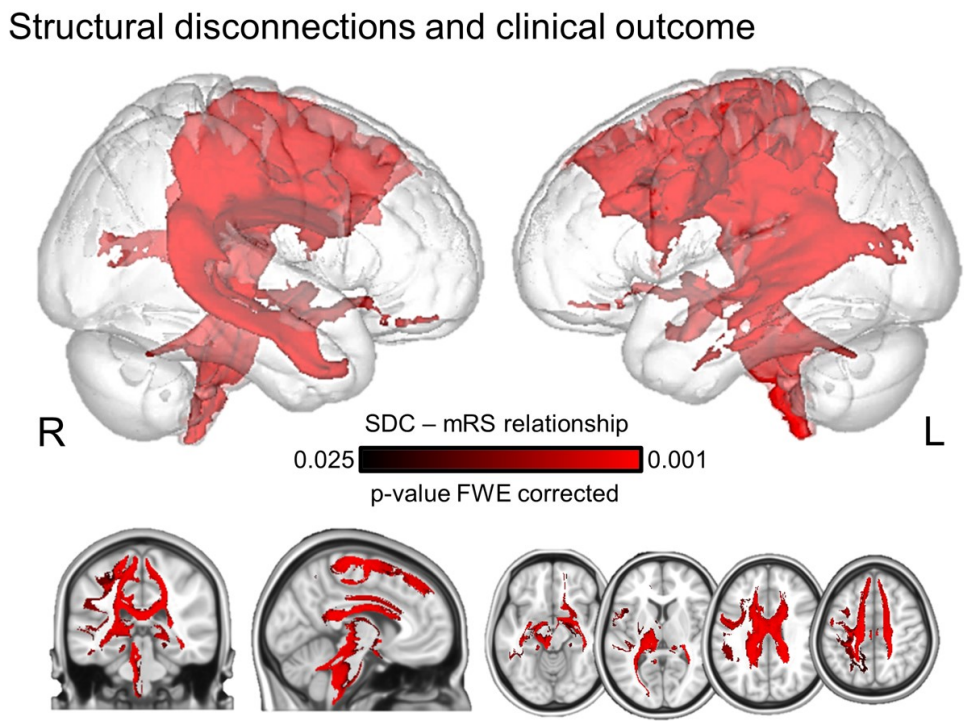


Figure 28. Structural connectivity map of white matter bundles that presented a statistically significant correlation with mRS at three months

Lasso regression

Lasso regression was computed for lesions mapped onto a: i) vascular atlas, ii) Yeo's functional networks (7 and 17 templates), and iii) Figley's structural connection networks. We also compared two different frameworks: a lesion framework and a functional/structural disconnection framework.

In the lesion framework, we measured whether outcome was more related to the vascular distribution of the lesion or the underlying functional-structural organization. The prediction with mRS was the most robust for the Yeo's functional atlas (R^2=0.382), followed by Figley's structural atlas (R^2=0.338), while, as hypothesized, the vascular atlas (ASPECTS-like) provided the lowest prediction for the functional outcome (R^2=0.146). The results for the functional template were confirmed using the 17 network parcellation (R^2=0.363).

In the second framework, we compared whether indirect maps (functional and structural disconnection) within the functional/structural atlases would better predict the functional outcome. Mean functional disconnection maps values within

Yeo's networks provided a higher prediction compared to the lesion-vascular approach (framework 1) but lower than the lesion-connectivity atlases (R^2=0.205, R^2=0.281, respectively, for the 7 and 17 network parcels). Finally, structural disconnection maps explained 3-month MRS to a similar level than the lesion prediction (R^2=0.339). This white matter model was superior to the functional disconnection model in the prediction of mRS at three months. See Table VIII for a direct comparison between the different R^2.

	VASCULAR ATLAS	GREY MATTER ATLAS (7 NETWORKS)	WHITE MATTER ATLAS
LESION	14,6%	38,2%	33,8%
FDC	-	20,5%	-
SDC	-	-	33,9%

Table VIII. Percentage of variance explained by each model

DISCUSSION

This study aimed to describe the anatomy of structural and functional disconnection patterns related to long-term functional outcomes in stroke patients undergoing MT. We compared the outcome prediction ability of lesion location and indirect structural and functional disconnection assessed in three brain spaces: a vascular (ASPECTS-like) atlas, a functional grey matter atlas, and a structural white matter atlas. Indirect structural and functional disconnection measures are inferred from clinical MRI and CT scans.

Structural and functional disconnection from stroke

The lesions were segmented from post-treatment MRI and CT scans. Their location was mainly subcortical and affected the central white matter, the basal ganglia, and the thalamus. These results are in line with those reported in previous studies.[127,142,155,156] The most affected areas correspond to the middle cerebral artery territory. This vessel was the most affected, with 61/66 patients presenting an obstruction of its lumen. Moreover, the middle cerebral artery is the most easily accessed vessel for revascularization procedures. These lesions were embedded onto maps of structural and functional dysconnectivity.

We then performed voxel-wise analysis of functional and structural disconnection. Functional disconnection at the group level involved the sensorimotor system; in fact, the SMN explained 34% of the variance. Additional networks included the DAN (31.8%) and the VIS (37.9%). Interestingly, the mRS, a coarse scale that globally measures disability, loads not only on the sensory-motor network, which is most affected by MCA lesions but also on visuospatial, oculomotor, and visual networks. It suggests that disability involves visuomotor and visuospatial functions. The voxel-wise disconnection analysis of the white matter confirmed the involvement of sensorimotor pathways like the corticospinal tract; but also callosal fibers connecting the two hemispheres; thalamo-cortical pathways; and, finally, left inferior and superior longitudinal fasciculus, association pathways that connect anterior and posterior regions. The importance of white matter damage and disconnection in stroke has already been reported in recent studies, [117,127,142,157] particularly Rosso et al. discovered that the extent of ischemic damage to the

corticospinal tract was the best predictor of the mRS at three months.[149] The degree of damage in this network is associated with motor impairment, an important cause of disability detected by the mRS.[158] The disconnection of long association tracts is the likely bases of the functional disconnection abnormalities involving the DAN and the VIS. Several recent studies have shown that alterations in functional and structural networks correlate well with behavioral outcomes. These patterns have been evaluated on detailed scales that are difficult to apply to clinical practice.[126,127]

Outcome prediction: vascular atlas versus functional and structural large-scale network atlas

We compared the predictive ability of lesion location computed onto three different atlases: a vascular (ASPECTS-like) atlas, Yeo's functional grey matter atlas, and Figley's functional white matter atlas.

The lesion prediction on the mRS at three months was most robust for the network atlases, particularly when embedded onto Yeo's functional atlas (R^2=0.382, R^2=0.363, respectively, for the 7 and 17 network parcels). The lesion-vascular model gave the worst prediction for functional outcome (R^2=0.146).

This is an important result because it shows directly that damage to specific vascular territories is not the critical variable to assess behavioral deficits. Rather the structural and functional organization of the brain supported metabolically by the vascular supply is the critical level of organization that ought to be considered both for prognostic and even prospective studies.

Specifically, here we proposed that studies of pre-treatment factors for MT shall specifically use information about gray and white matter networks to decide whether to perform MT. Vascular-based assessments like the commonly used ASPECT are likely to be less sensitive for behavior. This hypothesis would require a prospective study in which the decision to treat shall be based on either ASPECT or some network-based atlas. A more accurate selection could detect patients who, based on current methods, are not eligible for EVT but might benefit from this treatment. It could also help identify subjects who, although eligible for EVT according to classical methods, would not benefit from this procedure and might have an unfavorable risk/benefit ratio.

Our findings indicate that the specific location information, i.e., the affected vascular territory, has little prognostic value on clinical outcome. Previous studies have already shown that behavioral symptoms following a stroke do not fit classic vascular syndromes.[117,127,142] Rather behavioral deficits across people correlate across 3 variables that account for the majority of behavioral variability. These variables represent clusters of associated deficits that covary at a population level, e, g., patients with left visual field neglect are likely to present also left motor deficits and spatial memory impairments. Corbetta et al. attributed this phenomenon to the disruption of functional connectivity patterns.

The high predictive value of lesion location applied to a functional-network framework on a widely used clinical outcome such as mRS constitutes promising evidence. Because the mRS is a very crude scale, even one-point changes correspond to significant variations in the quality of life. The fact that lesion location may have high prognostic value for this type of outcome makes it particularly appealing for possible future applications in the clinical setting. Particularly, lesion location provides promising results when computed within the grey matter framework. These findings may help develop a new network-based atlas that could be applied in the pre-treatment framework in order to improve the prediction of outcomes provided by current models and refine the selection of subjects eligible for thrombectomy.

Future studies are therefore needed to assess the prognostic value of a network-based atlas in the pre-treatment framework.

Outcome prediction: stroke disconnection measures versus topographical data

Functional disconnection maps performed worse than structural ones (R^2=0.205 vs. R^2=0.339). This result is in line with Salvalaggio et al., 2020 who showed that lesion location and structural disconnection maps were accurate predictors of behavioral impairment acutely, but that functional disconnection maps indirectly estimated based on healthy atlases were not. According to Salvalaggio et al., functional disconnection maps perform worse because of their lower dimensionality, i.e., a limited number of functional networks irrespective of the location in the brain from which they are computed. Functional connectivity networks that are already low dimensional become even fewer when a lesion

reduces their modularity, or in other words, it diminishes the range of possible neural patterns that the brain generates. In contrast, structural disconnection maps provide a level of prediction that is like that conveyed by lesion location information (33.9% vs. 38.2% of the variance). This is explained by the fact that structural disconnection maps closely relate to the site of the lesion. However, the lower prediction is related to the observation that independent white matter pathways can pass through the same region in the white matter. In other words, the dimensionality of lesion location is higher than that of white matter pathways. Aside from lower accuracy, the clinical implementation of structural disconnection maps could be more challenging, given the complexity of the procedure. Overall, we think that either a lesion computed in functional/structural atlas space or a functional disconnection map could be the most applicable methods to clinical practice.

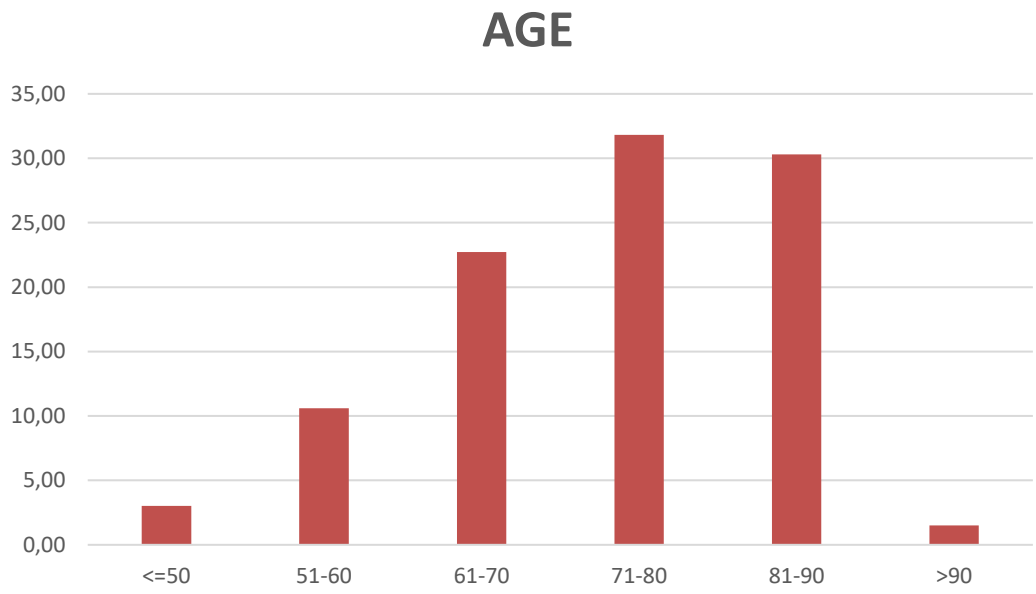
We reinforce the idea that the vascular framework, typically used in clinical practice, had low prognostic value even though the dimensionality of the parcels used was high (n=32) as compared to the number of networks (n=7, n=17) or structural connectivity systems (n=13).

CONCLUSION

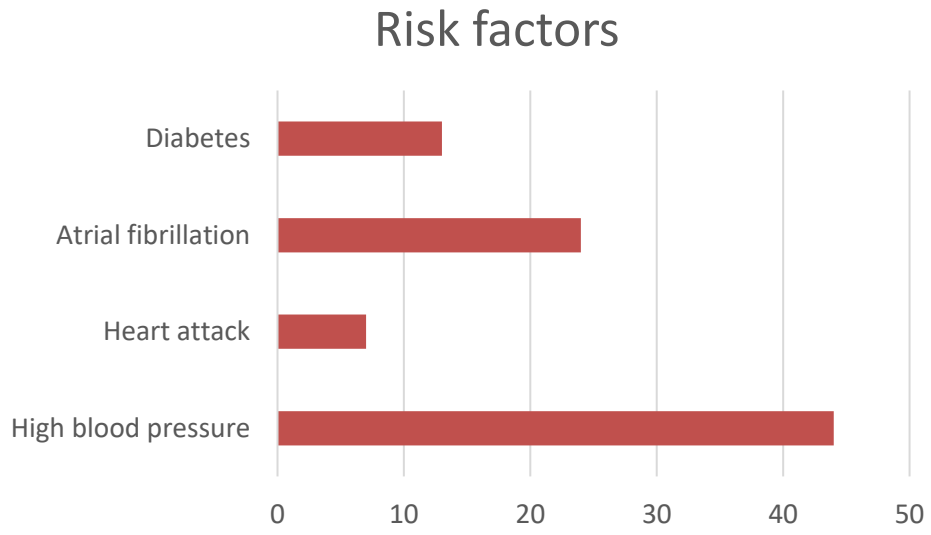
The introduction of MT in stroke treatment has provided clinicians with a robust therapeutic resource.[51] However, approximately half of the patients that undergo this procedure have poor long-term clinical outcomes. Thanks to the description of valuable predictive factors, recent literature has modified the pool of eligible patients, improved treatment effects, and provided useful prognostic information. In addition, over the last few years, large-scale network disruption measures have shown consistent post-stroke prognostic validity on many behavioral scales.[126,127] In line with this work, we investigated the structural and functional disconnection patterns related to a clinically applicable functional outcome measure (i.e., the mRS at three months). We observed that these measures, together with topographical data, provided robust outcome predictions only when considered in a functional-network framework (i.e., grey matter and white matter atlas) compared to the vascular framework typically used in clinical practice.

These results provide significant clinical and theoretical implications. In particular, they define post-stroke dysconnectome measures as clinically applicable prognostic factors. Most importantly, they suggest a possible application in pre-treatment acute stroke management, at present driven by neuroimaging features including solely volumetric (core and penumbra volume and ratio) and vascular (i.e., ASPECTs) scores.

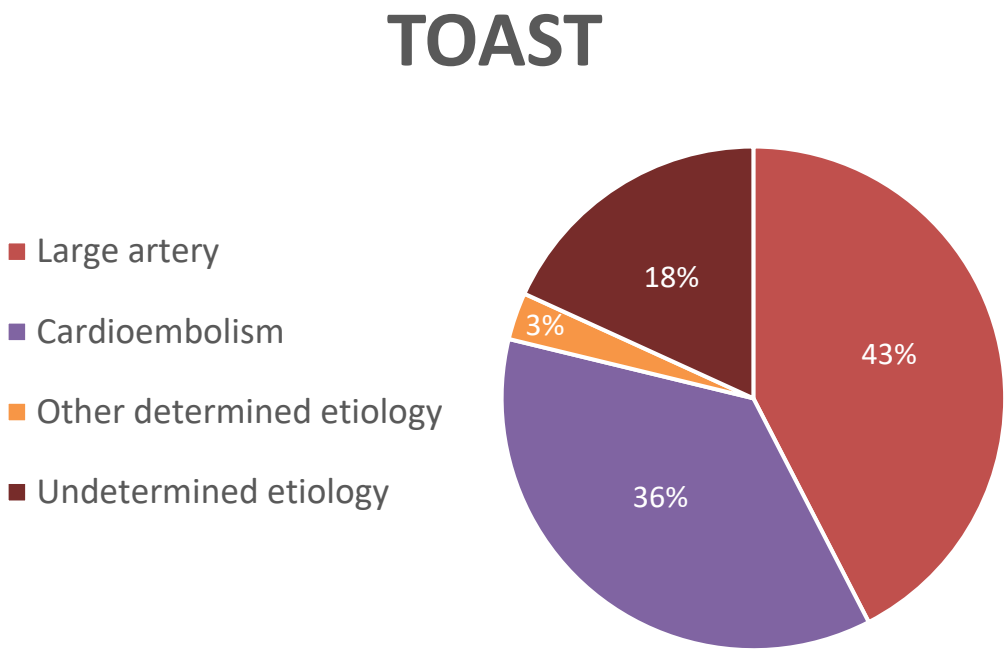
SUPPLEMENYARY



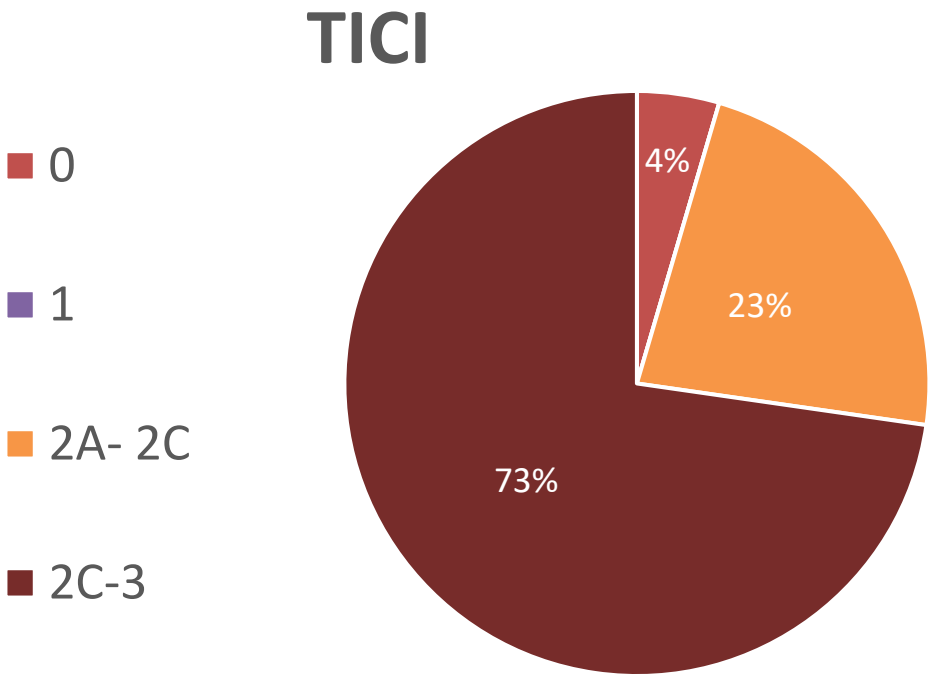
Supplementary 1. Bar chart showing sample distribution by age group



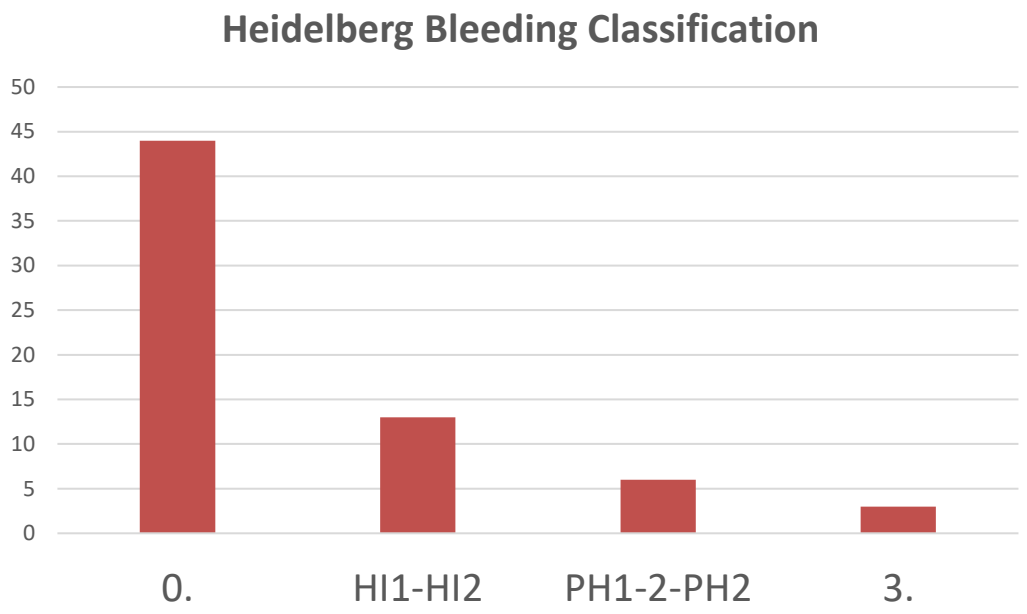
Supplementary 2. Bar chart showing the incidence of risk factors (sample n=66)



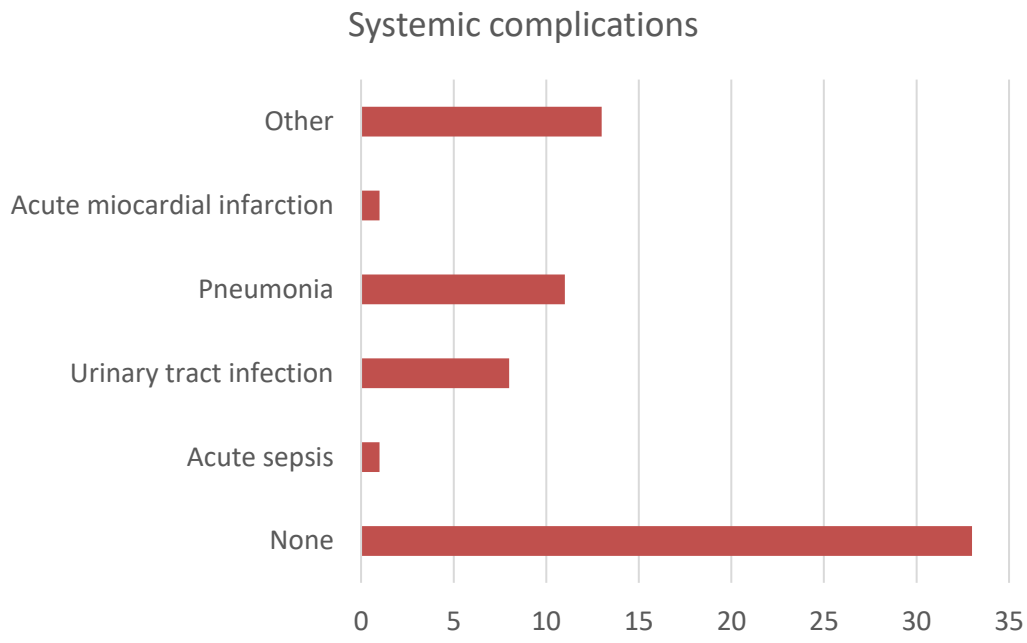
Supplementary 3. Pie chart showing the percentage distribution of stroke etiology (TOAST) in the sample (n=66)



Supplementary 4. Pie chart showing the distribution of TICI (%) in the sample (n=66)



Supplementary 5. Bar chart showing the number of hemorrhagic events after MT (Heidelberg Bleeding Classification) in the sample (n=66)



Supplementary 6. Bar chart showing the number of systemic complications in the sample (n=66)

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