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TESI DI LAUREA

MACROSCALE IMAGING: A POTENTIAL BIOMARKER FOR POST-STROKE FUNCTIONAL OUTCOME?

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ABSTRACT

OBJECTIVE: Predicting clinical recovery in stroke using neuroimaging features is an emerging field of research. Previous studies have attempted to predict clinical recovery based on lesion volume and topology with disappointing results. Here, we investigated whether a multivariate clinical approach incorporating advanced lesion properties could improve functional outcomes prediction in stroke.

MATERIALS: We retrospectively enrolled stroke patients who had undergone T1-weighted imaging or CT scan and completed the stroke impact scale (SIS) at 6 to 12 months post-stroke. For each lesion we computed local measures (lesion shape, tract density index - number of white matter fibers affected by the lesion - and within-lesion diffusion, hindered diffusivity, and restricted diffusivity) and structural and functional network measures (percentage of networks disconnected by the lesion). Except for lesion shape, all other features were computed using a normative structural/functional connectome. A factorial analysis was performed on the SIS subitems. The resulting factors were used as dependent variables in a ridge regression model with a bagging procedure. Five models were tested: (1) local measures of the lesion, (2) structural disconnection patterns, (3) functional disconnection patterns, (4) structural disconnection network maps, (5) functional disconnection network maps. The models were compared based on R-square values.

RESULTS: A total of 60 stroke patients were included (age: 66±15). Three SIS factors were identified, explaining over 70% of the variance. The first factor was associated with motor items (renamed as Physical), the second factor with Cognitive items (renamed as Cognitive), and the third with emotional items (renamed as Emotion). The first model (local measures) showed a low variance explained (Cognitive: 14%; Physical: 11%; Emotion: 6%). The structural network disconnectivity model demonstrated a slight improvement, but still low (Cognitive: 17%; Physical: 13%; Emotion: 9%). The functional network disconnectivity model yielded higher prediction for the Physical factor (28%). When sociodemographic variables were included, the prediction accuracy

increased to 35% for the Physical factor. On the other hand, Cognitive and Emotion factors showed low prediction (7% and 10%, respectively).

DISCUSSION: The SIS scale comprises three factors (Physical, Cognitive, Emotion) that can be predicted differently based on lesion properties. The Cognitive and Emotion factors showed consistently low prediction accuracy based on both local and network measures. In contrast, the Physical factor showed better prediction when considering functional network disconnectivity. These results emphasize the potential of network disconnectivity as an important predictor of motor outcomes. Cognitive and emotional aspects may necessitate direct measures (e.g., diffusion-weighted imaging, resting-state fMRI) to better understand the complex alterations after a stroke.

CONCLUSION: We identified the factor structure of a functional outcome evaluation scale, the Stroke Impact scale, and its relationship with advanced lesion properties following stroke. Specifically, our results emphasize the role of network disconnectivity measures as potential predictors of motor outcomes.

RIASSUNTO

OBIETTIVO: Lo studio della predizione del recupero funzionale e clinico mediante tecniche di imaging neurologico rappresenta un campo di ricerca emergente. Studi precedenti in letteratura hanno analizzato la capacità di predire il recupero del paziente affetto da ictus da parte di misure basate sul volume della lesione e sulla sua localizzazione, anche se con risultati non univoci. In questo lavoro, abbiamo studiato quanto un approccio che includesse molteplici variabili (incluse misure avanzate di proprietà della lesione) possa migliorare la predizione del recupero funzionale dei pazienti affetti da ictus.

MATERIALI E METODI: Sono stati arruolati retrospettivamente pazienti che avessero effettuato in fase acuta una Risonanza Magnetica fase T1 o una scansione TC e che avessero completato la valutazione funzionale a 6 e 12 mesi mediante la Stroke Impact Scale (SIS) versione 3.0. Sono state quindi analizzate, per ciascuna lesione, misure locali (forma e volume della lesione, track density index, quantificazione delle fibre bianche colpite, indici di diffusione all'interno della lesione) così come misure di network strutturali e funzionali (tra cui la percentuale di network alterata dalla lesione). Tutte le misure (eccezion fatta per i modelli di forma e volume) sono state analizzate in maniera indiretta utilizzando atlanti ("connettomi") strutturali e funzionali ricavati da soggetti sani, e per questo definiti "atlanti normativi". Per quanto riguarda le diverse voci della SIS, normalmente raggruppate in 8 categorie, sono state sottoposte a un'analisi fattoriale, al fine di ridurne la dimensionalità.

Di seguito, è stata effettuata una analisi di regressione multipla chiamata "ridge regression", con una procedura d'apprendimento d'insieme denominata "bagging", considerando i fattori risultanti della SIS come variabili dipendenti. Sono stati testati 5 modelli: misure locali della lesione (1), misure di disconnessione di pattern strutturali (2), misure di disconnessione di pattern funzionali (3), infine misure di disconnessione di network strutturali (4) e funzionali (5). Questi modelli sono stati confrontati mediante l'analisi risultante dalla regressione ridge.

RISULTATI: La coorte studiata include 60 pazienti (età 66±15 anni). In relazione all'analisi fattoriale della SIS, sono stati individuati 3 fattori in grado di spiegare il 70% della varianza. Il primo fattore risulta correlato alla funzione motoria (quindi denominato "Physical"), il secondo alla funzione cognitiva (quindi denominato "Cognitive"), il terzo allo stato emotivo del paziente (quindi denominato "Emotion"). Fra i 5 modelli analizzati, il primo modello (misure locali) ha dimostrato una bassa capacità di predizione della varianza (11% per il fattore "Physical", 14% per "Cognitive", 6% per "Emotion"); le misure di disconnessione di network strutturali hanno dimostrato un miglioramento, seppur modesto, della performance predittiva (13% per il fattore "Physical", 17% per "Cognitive", 8% per "Emotion"). Le misure che hanno mostrato la migliore capacità di predizione sono quelle risultate dal modello di disconnessione di network funzionali, in particolare per il fattore "Physical" (28%). Tale dato aumentava a 35% se venivano incluse nell'analisi anche i dati socio-demografici (età, sesso, anni di istruzione). Per quanto riguarda invece i fattori "Cognitive" e "Emotion", queste hanno mantenuto valori di predizione bassi (rispettivamente 7% e 10%).

DISCUSSIONE: Sulla base delle caratteristiche della lesione, abbiamo rilevato 3 distinti fattori della SIS che possono venir predetti in diversi modi. In tutti i modelli analizzati, l'accuratezza della previsione dei fattori "Cognitive" e "Emotion" è risultata consistentemente bassa. Al contrario, la componente "Physical" ha dimostrato una discreta predicibilità considerando il modello di disconnessione funzionale. Tale risultato evidenzia la potenzialità dei modelli costruiti sui network di connessione come fattori predittivi del recupero motorio del paziente. Riguardo alle componenti emotive e Cognitive, si presume che siano meglio predicibili mediante misure dirette, come risonanza magnetica funzionale o metodiche di diffusion-weighted-imaging.

CONCLUSIONE: In questo lavoro, abbiamo identificato la struttura fattoriale di una riconosciuta scala di valutazione funzionale, quale la SIS, e la sua relazione con una caratterizzazione multifattoriale della lesione conseguente a ictus. In particolare, i nostri risultati evidenziano il ruolo delle misure di disconnessione dei network come potenziale fattore predittivo rispetto al recupero motorio del paziente.

PART I

What is stroke?

Definition

According to the classical definition, stroke is a neurological deficit due to an acute focal injury of the central nervous system (CNS) by a vascular cause, may it be a vessel occlusion, cerebral infarction, intracerebral haemorrhage (ICH), or subarachnoid haemorrhage (SAH). The clinical spectrum includes ICH, SAH, ischemic stroke and silent stroke and is commonly referred to as "central nervous system infarction".[1]

The WHO's original definition in 1970 mainly focused on clinical symptoms, defining stroke as "rapidly developed clinical signs of focal (or global) disturbance of cerebral function, lasting more than 24 hours or leading to death, with no apparent cause other than of vascular origin;[2] recent studies have now considered this definition outdated due to the important steps forward in "nature, timing, clinical recognition of stroke and its mimics, and imaging findings that require an updated definition'.[1]

Specifically, this update mainly refers to the clinical entity of the "Transient Ischemic Attack" definition, commonly referred to as TIA. The classical distinction between "stroke" and "TIA" was established on the duration of symptoms, which should resolve completely within 24 hours. However, several studies worldwide[3–5] have demonstrated that this arbitrary time threshold was too broad, since 30% to 50% of classically defined TIAs showed brain injury on diffusion-weighted magnetic resonance imaging (MRI DWI studies). Consequently, a newer, neuroimaging-informed, operational definition of TIA has been proposed, such as "a brief episode of neurological dysfunction caused by focal brain or retinal ischemia, with clinical symptoms typically lasting less than one hour, and without evidence of acute infarction".[6]

Epidemiology

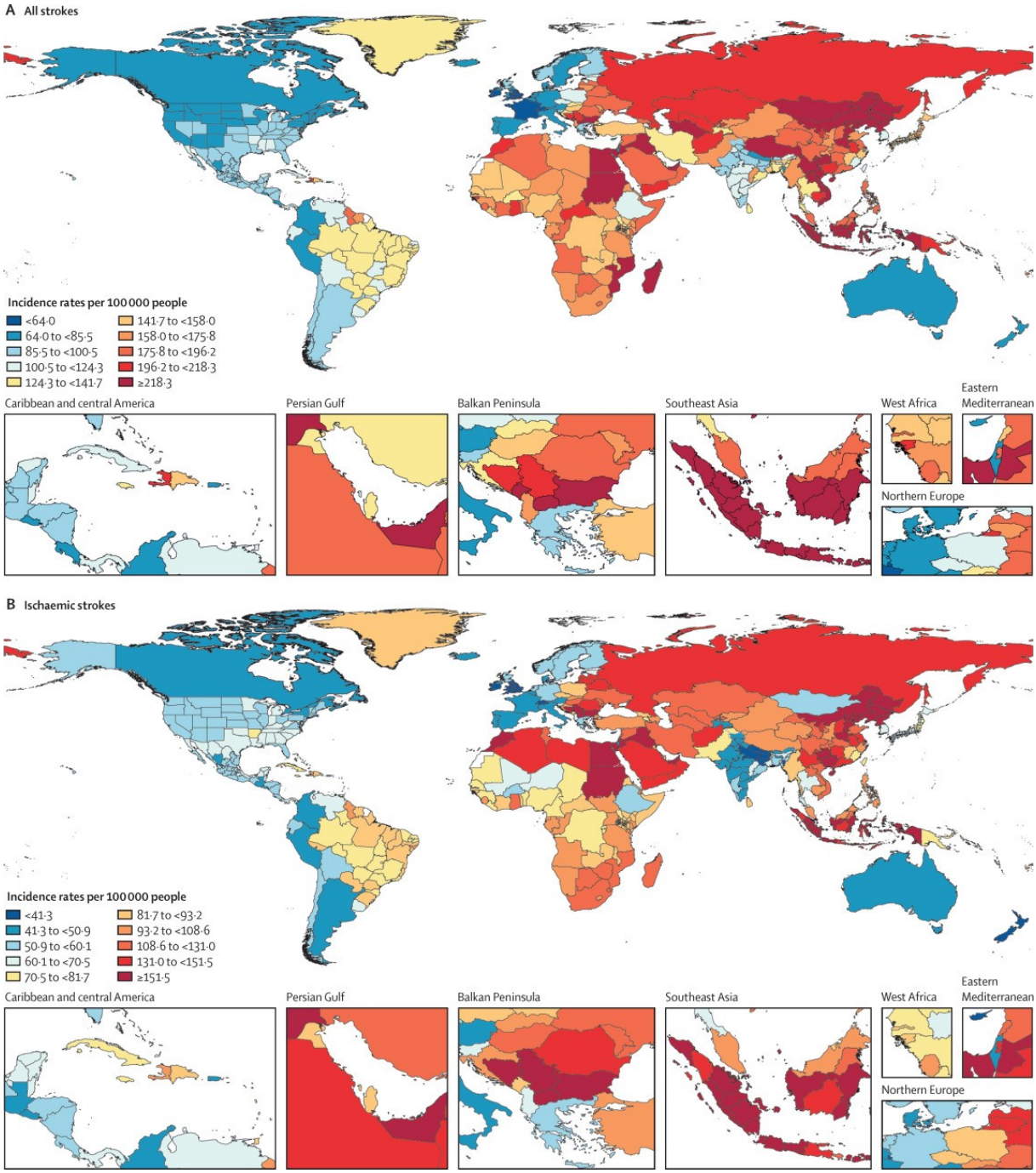
Stroke is the second leading cause of both disability and death worldwide, with the highest burden of the disease shared by low- and middle-income countries.[7] The lifetime risk of stroke has globally increased by 50% in the last 20 years, reaching nowadays the rate of one in four people (24.9% in men and 25.1% in women older than 25 years old).[8,9]

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, with stroke now the fifth leading cause of death in the United States.[10]. This may be firstly suggesting that, on average, global increases in stroke implications have been largely due to population growth and ageing; secondly, preventive measures are very effective at lowering the fatal risk for both ischemic and haemorrhagic stroke.[11]

Ischaemic stroke is the most prevalent type of CNS infarction, resulting in 62.4% of all new strokes (data from the "systematic analysis for the Global Burden of Disease Study in 2019"), while intracerebral haemorrhage constituted 27.9% and subarachnoid haemorrhage constituted the remaining 9.7%. Interestingly, intracerebral haemorrhage and subarachnoid haemorrhage showed larger reductions in age-standardised rates from 1990 to 2019 than ischaemic stroke.[12]

According to the Global Burden of Disease Study (GBD) the age of patients affected, their sex and their geographic location indicates that the socio-economic burden of stroke has increased over time.[13] The large increase in the global burden of stroke was probably not only due to population growth and ageing but also because of the substantial increase in exposure to several important risk factors such as high BMI, ambient particulate matter pollution, high fasting plasma glucose, high systolic blood pressure, alcohol consumption, low Physical activity, kidney dysfunction, and high temperature.[12]

The epidemiologic and financial impact of stroke is considerable and also relates to disability: stroke remains the third-leading cause of death and disability combined (as expressed by disability-adjusted life-years lost or DALYs) in the world, with an estimated global cost of stroke is over US$721 billion.


A All strokes
Incidence rates per 100 000 people
<64·0
64·0 to <85·5
85·5 to <100·5
100·5 to <124·3
124·3 to <141·7
141·7 to <158·0
158·0 to <175·8
175·8 to <196·2
196·2 to <218·3
≥218·3
Caribbean and central America
Persian Gulf
Balkan Peninsula
Southeast Asia
West Africa
Eastern Mediterranean
Northern Europe
B Ischaemic strokes
Incidence rates per 100 000 people
<41·3
41·3 to <50·9
50·9 to <60·1
60·1 to <70·5
70·5 to <81·7
81·7 to <93·2
93·2 to <108·6
108·6 to <131·0
131·0 to <151·5
≥151·5
Caribbean and central America
Persian Gulf
Balkan Peninsula
Southeast Asia
West Africa
Eastern Mediterranean
Northern Europe

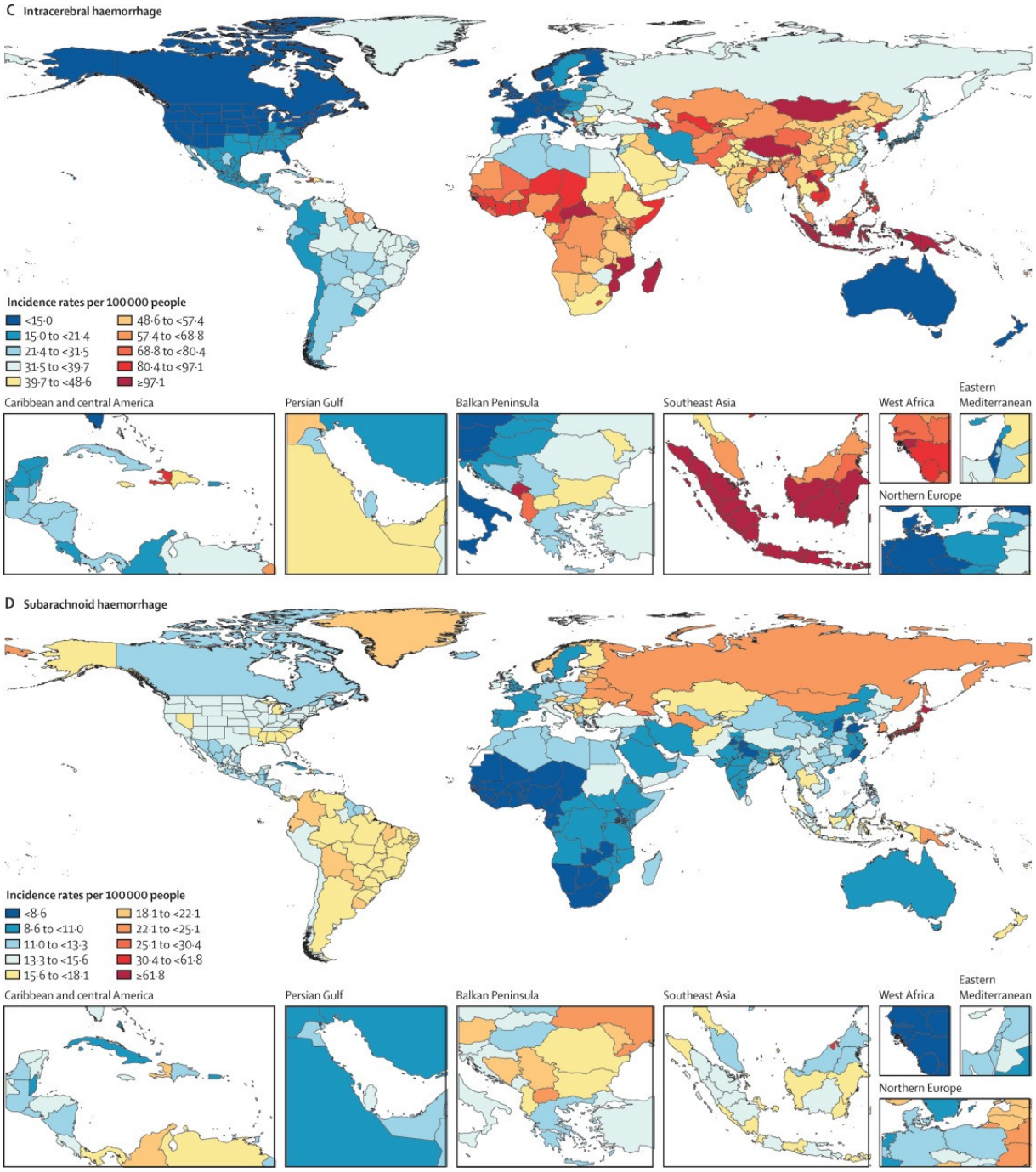


Figure 1 Age-standardised stroke incidence rates per 100 000 people by stroke type and country, for both sexes.

(A) All strokes. (B) Ischaemic stroke. (C) Intracerebral haemorrhage. (D) Subarachnoid haemorrhage. From "Global, regional, and national burden of stroke and its risk factors, 1990–2019: A systematic analysis for the Global Burden of Disease Study 2012"[12]

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Classification

The classification proposed by ICD-11in February 2022, divides cerebrovascular diseases into two main categories: intracranial haemorrhage and cerebral ischemia.

Intracranial haemorrhages can be classified according to their location in basal ganglia haemorrhage, pontine haemorrhage, lobar haemorrhage and cerebellar haemorrhage.

Intracerebral haemorrhages can be further distinguished based on their aetiology in primary haemorrhages (without underlying lesion) and secondary haemorrhages. Primary haemorrhages include hypertensive haemorrhages or haemorrhages due to cerebral amyloid angiopathy; on the other hand, secondary haemorrhages include cerebral venous thrombosis, vascular malformations, tumours, coagulopathies.

Cerebral ischemia generally follows an etiological classification. The TOAST classification, the most commonly used from a clinical point of view, describes five subtypes of ischemic stroke: (1) large-artery atherosclerosis, (2) cardio-embolism, (3) small-vessel occlusion, (4) stroke of other determined aetiology, and (5) stroke of undetermined aetiology. The practical use of this system showed a high inter-physician agreement.[14]

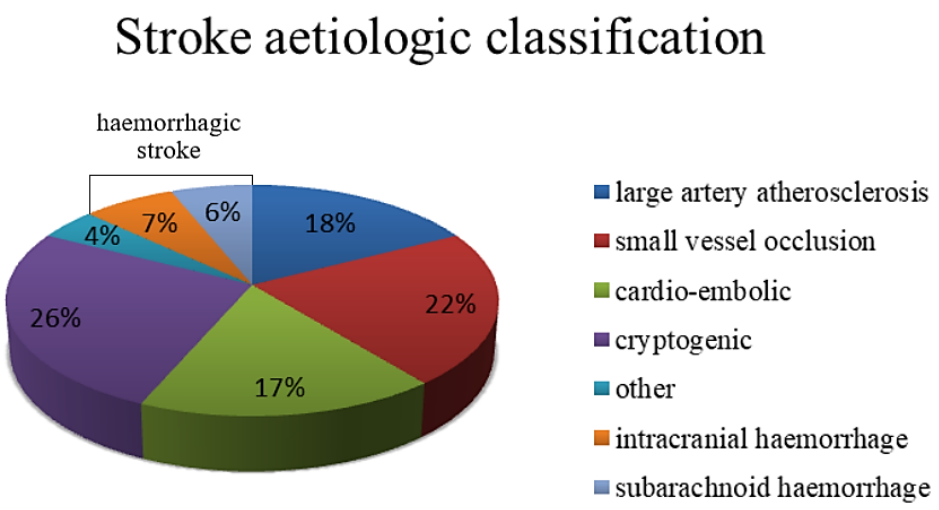


Figure 2 Stroke etiologic classification

Pathogenesis

Thromboembolic phenomena leading to vessel occlusion can produce ischemic conditions in the brain.[15]

When thrombosis occurs, the blood flow is limited by the narrowing of vessels due to atherosclerosis. The build-up of plaque will increasingly reduce the vascular lumen and lead to the formation of clots, causing a thrombotic stroke. In an embolic stroke, the cause of the occlusion resides in one or several embolic fragments that breaks off from a distant vessel and finally reach the cerebral circulation.[16]

As the blood flow to the brain decreases, ischemic stress triggers a cascade of events starting from loss of electrical function, to alterations of membrane function with increased calcium influx, finally leading to calcium-dependent excitotoxicity, generation of reactive oxygen species, and ultimately destruction of cell membranes and lysis of cells.[17] The consequent cell necrosis causes leaking of cellular contents into extracellular space,[18] and loss of neuronal function. Several other events have to be taken into account as factors of stroke pathology: inflammation, energy failure, loss of homeostasis, acidosis, increased intracellular calcium levels, excitotoxicity, free radical-mediated toxicity, cytokine-mediated cytotoxicity, complement activation, impairment of the blood–brain barrier, activation of glial cells, oxidative stress and infiltration of leukocytes.[19 20–23]

On the other hand, haemorrhagic stroke accounts for approximately 10–15% of all strokes and has a high mortality rate. In this condition, pathological stress in the brain tissue and internal injury cause blood vessels to rupture, resulting in infarction.[24] Haemorrhagic stroke can be classified into intracerebral and subarachnoid haemorrhage (ICH and SAH, respectively). In ICH, bleeding is usually derived from small, ruptured arterioles and causes abnormal accumulation of blood within the brain. Accumulation of blood occurs over minutes or hours; the hematoma gradually enlarges by adding blood at its periphery. The hematoma continues to grow until the pressure surrounding it increases enough to limit its spread or until the haemorrhage decompresses itself by emptying into the ventricular system or the cerebrospinal fluid (CSF) on the pial surface of the

brain. The most common causes of ICH are hypertension, trauma, bleeding diatheses, amyloid angiopathy, illicit drug use (amphetamines and cocaine), and vascular malformations;[25] less frequent causes include bleeding into tumours, aneurysmal rupture, and vasculitis.

In subarachnoid haemorrhage, blood accumulates in the subarachnoid space (between the arachnoid membrane and the pia mater surrounding the brain). Most common causes are head injury, or spontaneous rupture of an intracranial cerebral aneurysm.[26] From the ruptured aneurysm blood rapidly spreads within the CSF under arterial pressure, increasing intracranial pressure and resulting in death or deep coma if not arrested. The bleeding usually lasts only a few seconds but re-bleeding is very common.[27] Other rarer causes of SAH include: bleeding diatheses, trauma, amyloid angiopathy.

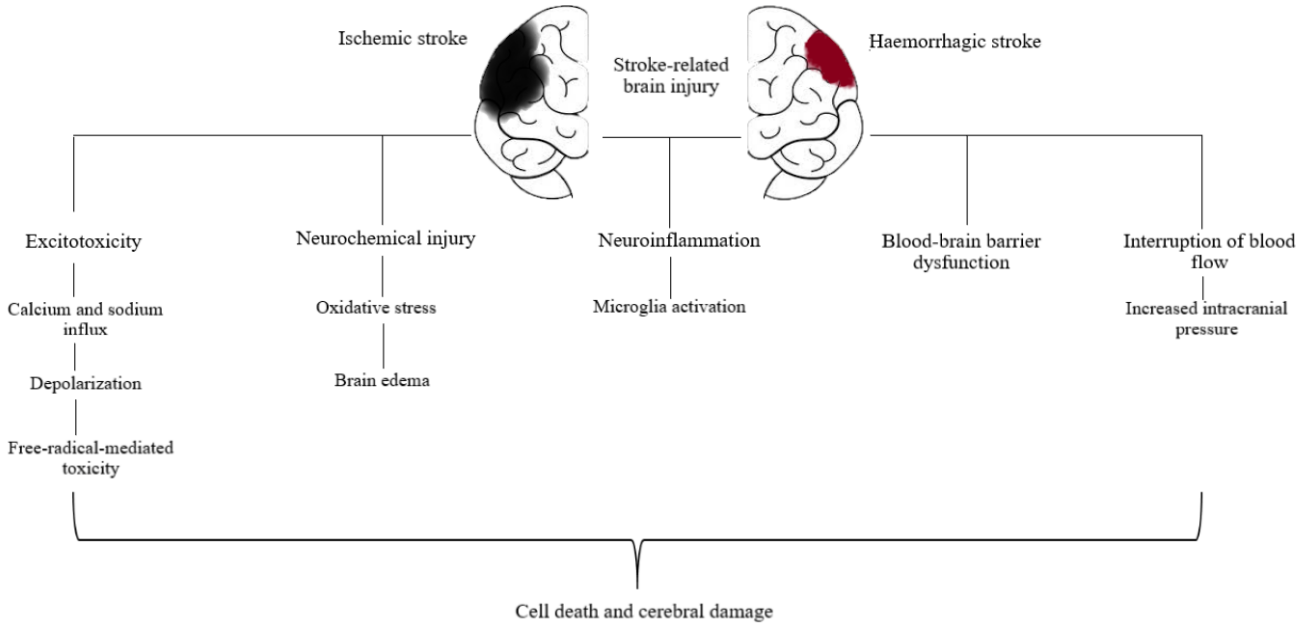


Figure 3 Pathological mechanisms of stroke-related brain injury

Clinical syndromes

The specific symptoms described in a haemorrhagic stroke rely on the precise location of the lesions (particularly in the case of lobar haemorrhages). Haemorrhagic stroke syndromes can cause sudden severe headache, decreased level of consciousness, vomiting, headache, seizures, very high blood pressure[28], as well as focal neurological symptoms like monolateral weakness or paralysis. Neck stiffness may be seen in cases with meningeal involvement. Finally the so-called "thunderclap headache", which often develops over seconds to minutes, makes subarachnoid haemorrhage easy to identify.[29]

Ischemic stroke syndromes depend on the location and extent of brain damage caused by the lesion. The most common ischemic stroke syndromes are classified, according to the affected vascular territory, as anterior cerebral artery (ACA) syndrome, middle cerebral artery (MCA) syndrome, posterior cerebral artery (PCA) syndrome.

Regarding the anterior circulation, patients with internal carotid and middle cerebral artery syndromes exhibit severe contralateral hemiparesis or hemiplegia, hemianesthesia, and lateral homonymous hemianopsia, as well as aphasia in lesions to the dominant hemisphere and apraxia, asomatosis, and anosognosia in lesions to the non-dominant hemisphere.[30] Leg weakness is a hallmark of anterior cerebral artery infarcts and is more severe in the leg than the arm.

On the other hand, with respect to the posterior circulation, vertebral arteries (VAs), basilar artery (BA) and the postero-inferior cerebellar arteries (PICAs) can be affected. In particular, if VAs or PICAs are involved, two main syndromes have been identified: the lateral medullary infarction syndrome (also known as the Wallenberg syndrome), and the medial medullary infarction syndrome, which is characterised by contralateral hemiplegia sparing the face, contralateral loss of deep sensation, and ipsilateral hypoglossal paralysis.[31]

Lastly, tetraparesis typically occurs when the basilar artery (BA) is affected.[32] In addition to dysarthria and dysphagia, ataxia or lack of coordination in the limbs are also common and severe. An significant indicator of abrupt BA obstruction is the alteration of consciousness: in particular, the so-called "locked-in" syndrome indicates the loss of all voluntary movements.

An elderly but used classification is the Bamford classification system, that divides cerebral infarction into groups based on the vascular region involved.[33] The size and location of the ischemia lesion in the brain are predicted using clinical characteristics. Total anterior circulation syndrome (TACS), partial anterior circulation syndrome (PACS), posterior circulation syndrome (POCS), and lacunar infarct syndrome (LACS) are the four classifications for lesions. The link between these stroke subtypes and cerebrovascular risk factors is largely unknown, despite the fact that this categorization method is beneficial for predicting the outcome and recurrence of strokes.[33]

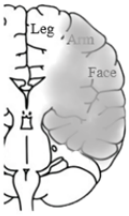
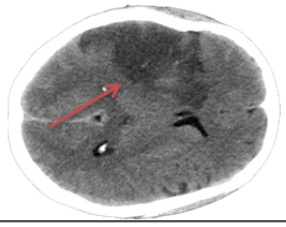
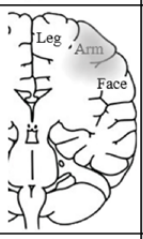
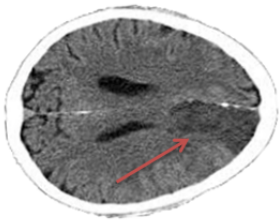
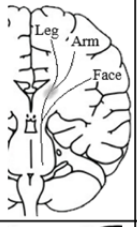
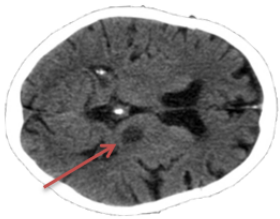
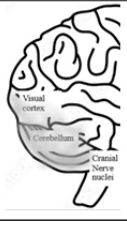
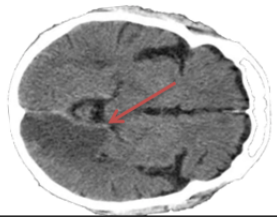
Clinical syndrome	Common symptoms	Common cause	CT scan features
Total anterior circulation syndrome (TACS) 	Combination of: - Hemiparesis - Higher cerebral dysfunction (e.g. aphasia) - Hemisensory loss - Homonymous hemianopia (damage to optic radiation)	Middle cerebral artery occlusion (Embolism from heart or major vessels)	
Partial anterior circulation syndrome (PACS) 	Isolated motor loss Isolated higher cerebral dysfunction (e.g. aphasia, neglect) Mixture of higher cerebral dysfunction and motor loss (e.g. aphasia with right hemiparesis)	Occlusion of a branch of the middle cerebral artery or anterior cerebral artery (Embolism from heart or major vessels)	
Lacunar syndrome (LACS) 	Pure motor strokes – affects 2 limbs Pure sensory stroke Sensory-motor stroke No higher cerebral dysfunction or hemianopia	Thrombotic occlusion of small perforating arteries (Thrombosis in situ)	
Posterior circulation stroke (POCS) 	Homonymous hemianopia (damage to visual cortex) Cerebellar syndrome Cranial nerve syndromes	Occlusion in vertebral, basilar or posterior cerebral artery territory (Cardiac embolism or thrombosis in situ)	

Figure 4 Bamford classification of cerebral infarction

Neuropsychology in stroke

Cognitive and mood impairments are a common consequence of stroke,[34,35] have a great impact on the quality of the patient's life and are significant predictors of long term functional outcomes.[36–38]

The main classification of Cognitive impairment after stroke separates Post-Stroke Cognitive impairment (PSCI) and Post-Stroke Dementia: the first (PSCI) is defined as failure in any Cognitive domain after stroke: executive function; memory; language; visuospatial ability; visuo-constructional ability; or global Cognitive function. The latter (PSD) is defined as any dementia occurring after stroke.[39] For the aim of this study, we will mainly focus on PSCI.

Post-stroke Cognitive impairment (PSCI) is a frequent sequel of ischaemic stroke and a leading cause of long-term disability and reduced quality of life.[40] From 30 to 50% of ischemic stroke survivors is likely to develop PSCI in the first year after stroke,[41,42] but providing an individualised Cognitive prognosis is still challenging. Specific stroke characteristics have been demonstrated to predict PSCI (independently from vascular and demographic risk factors), such as the presence of multiple acute infarcts, total infarct volume, and left cerebral hemispheric location.[41,42] In addition, the cause of stroke (small or large vessel disease) and lesion size and volume (lacunar or non-lacunar stroke) are also related to PSCI, and are unquestionably linked to lesion location.[42]

According to the comprehensive map of strategic infarct location predicting PSCI provided by Weaver et al., infarcts in the left frontotemporal lobes, right parietal lobe, and left thalamus were most strongly predictive of PSCI.[43]

Diagnosis

As JL. Saver reported in his article "Time is Brain" quantified the damage of neural tissue in 1.9 million neurons, 14 billion synapses, and 12 kilometres of myelinated fibres for every minute of cerebral ischemia.[44] Nonetheless, tissue damage and symptom severity are highly variable among individuals, depending mostly on the activation of collateral vessels and self-regulation of arteriolar vasodilation in response to ischemia.[30]

In this context, the role of a correct diagnosis in the therapeutic window is important to minimize brain injury, treat medical conditions, prevent neurological complications, and move towards uncovering the pathophysiologic basis of the patient's symptoms.

The correct combination of history, Physical examination, oxygen saturation, basic laboratory analysis (such as glycaemia and blood electrolytes, renal function tests, complete blood count, markers of cardiac ischemia, prothrombin time/ INR, activated partial thromboplastin time), ECG to rule out cardiac arrhythmias, and finally a non-contrast computed tomography (CT) scan are generally sufficient to guide acute therapy.[45]

The time dependent nature of stroke and the availability of acute interventions has also required a profound reorganization of services that involves emergency services, ER personnel for a streamlined admission, radiology and laboratory personnel, and in-hospital stroke teams of neurologists, neuroradiologists, and ancillary services.[46]

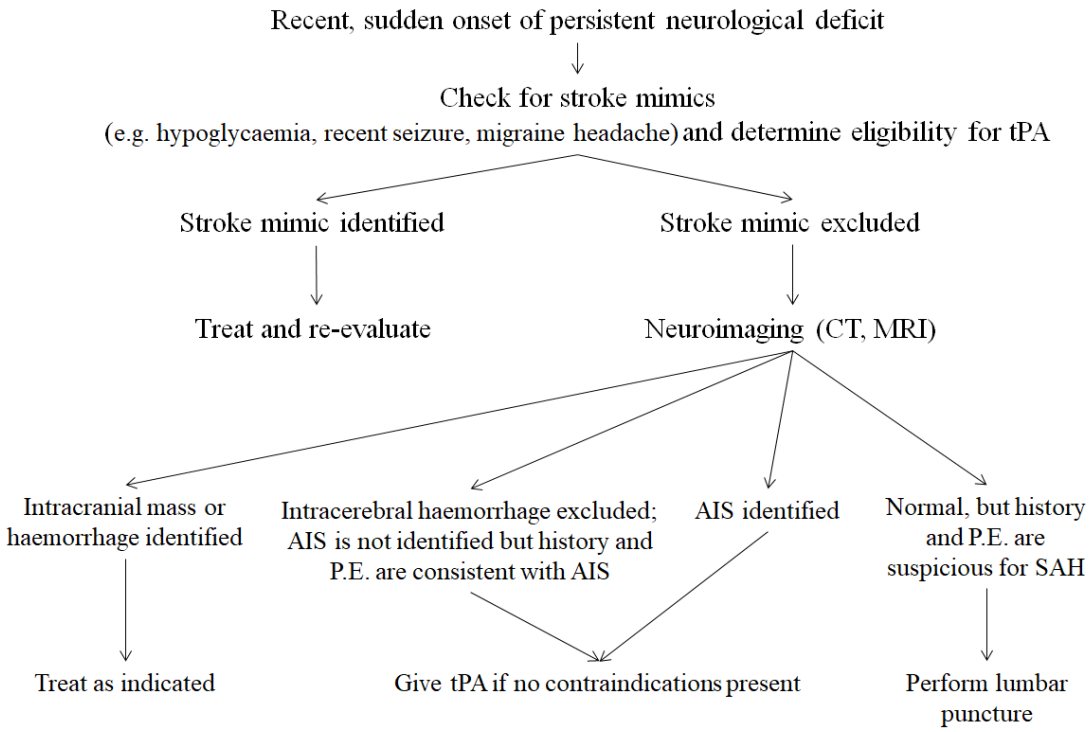


Figure 5 Diagnosis of acute stroke, modified from Yew et al.[47]

Neurological examination

Acute diagnosis of stroke involves a thorough medical assessment to determine the type and location of the stroke, as well as the severity of the symptoms. In particular, it has been demonstrated that clinical investigation based solely on history and examination had 92% sensitivity for diagnosing stroke and TIA in an emergency setting (studied among primary care physicians in a community-based study).[48]

Establishing the time of ischemic stroke symptom onset is critical since it represents the main determinant of eligibility for treatment with intravenous thrombolysis and endovascular thrombectomy.[49] For those patients unable to provide a reliable onset time, symptom onset is defined as the time the patient was last known to be normal or at baseline neurologic status (last known well or LKW).[50]

The history and physical examination should be also used to rule out other diagnoses: seizures, syncope, migraine, hypo or hyperglycaemia, movement disorders, or drug toxicity can mimic acute stroke.[46,50] Noticeably, the combination of focal signs and altered level of consciousness heavily affects the quality of the clinical interview. It is important to ask the patient or any reliable

informant whether the patient takes insulin or oral hypoglycaemic agents, or has a history of epilepsy, drug overdose or abuse, or recent trauma.

Finally, diagnosing an intracerebral haemorrhage (ICH) or subarachnoid haemorrhage (SAH) as soon as possible can be lifesaving.[51] The history may be helpful in this regard: the presence of acute onset headache, diastolic blood pressure higher than 110 mmHg and vomiting favour the diagnosis of ICH or SAH compared with a thromboembolic stroke,[52] while the abrupt onset of impaired cerebral function without focal symptoms favours the diagnosis of SAH.

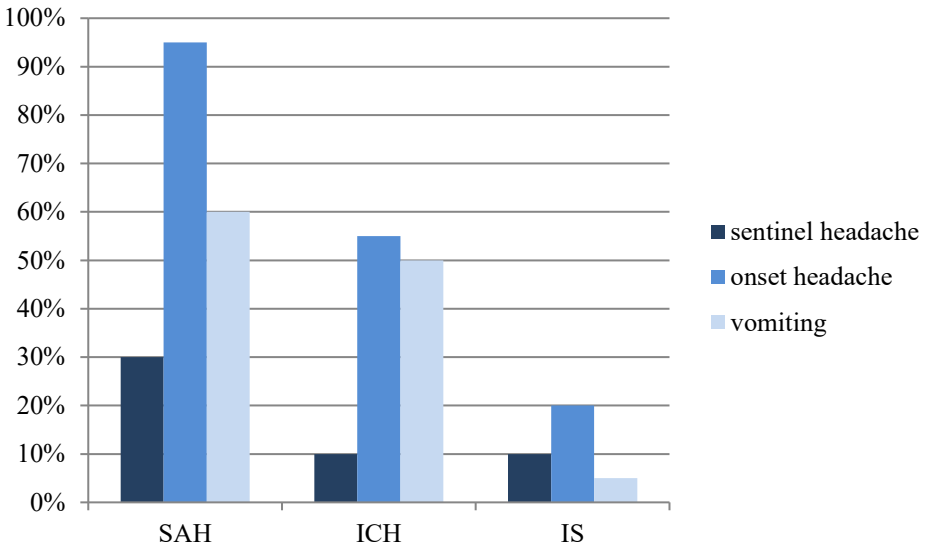


Figure 6 Headache in acute cerebrovascular disease, modified from Gorelick PB et al.[52]

Other symptoms of haemorrhagic stroke may include focal neurologic signs, photophobia, vomiting, meningismus, decreased level of consciousness, and seizures.[53,54] In these patients, fundoscopy should be performed to identify intraocular haemorrhages.

Neuroimaging

Neuroimaging plays a critical role in the diagnosis and management of stroke. It is used to differentiate haemorrhage from ischemic stroke, to assess the degree of brain injury, and to identify the vascular lesion responsible for the stroke. There are several neuroimaging techniques used in stroke diagnosis, including computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET). CT and MRI perfusion imaging can discriminate between irreversibly infarcted brain tissue and ischemic salvageable areas, thereby allowing selection of patients who are likely to benefit from reperfusion therapy. The choice of which technology to use is mainly dependent on availability.

CT imaging	MR imaging
Detection of haemorrhage	Detection of haemorrhage with SWI
Angiography offers better resolution	Doesn't require contrast
Faster, more available, less restrictive	Slower, limited availability, restrictive environment
Radiation	No radiation
Limited in posterior fossa	Better detection in posterior fossa
Limited detection of small lesions	Better detection of small lesions
Less specific in detection of «stroke mimics»	Better detection of «stroke mimics»
Lower sensitivity and contrast	Higher sensitivity, better contrast

Table 1 Comparison between CT and MR imaging

Perfusion imaging is a specialized imaging technique that can be used to assess blood flow to the brain in patients with stroke. It 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 where blood flow has reduced more than 70% in comparison to the normal contralateral hemisphere, while ischemic penumbra is

identified by a mismatch between cerebral blood flow and cerebral blood volume.[55] Importantly, the term “penumbra” refers to the reversibly injured brain tissue around ischemic core; which is the pharmacological target for acute ischemic stroke treatment.[56] It is critical to identify the ischemic penumbra and infarct core in patients with stroke, as this information can guide treatment decisions. In patients with a large penumbra and a small infarct core, aggressive interventions such as thrombectomy or thrombolysis may be considered to restore blood flow and salvage the potentially viable tissue. In patients with a large infarct core and a small penumbra, these interventions may be less effective and the focus may shift to supportive care and rehabilitation.

Revascularization treatment

Revascularization treatment is time-critical and aims to restore blood flow to the affected brain area and prevent further damage. The most common acute treatments include intravenous thrombolysis and endovascular treatment. they have shown to improve outcomes in AIS when applied to appropriate patients.[57–61]

Intravenous thrombolysis

In the past two decades, the pillar of acute ischemic stroke management has been attempted reperfusion of ischemic tissue with intravenous thrombolysis. The recommended eligible patients and time frame for treatment have evolved over time.[62] Alteplase and Tenecteplase are the two most common thrombolytic agents used in acute reperfusion therapy for stroke. They work by promoting the dissolution of blood clots (thrombi) that are causing the vessel obstruction.

Alteplase is a genetically engineered form of tissue plasminogen activator (tPA), a protein naturally produced in the body. It functions by binding to fibrin, a protein component of blood clots, and converts plasminogen to plasmin. The effectiveness of Alteplase treatment has been shown in several trials, both with a dose of 1.1 mg/kg and 0.9 mg/kg.[63–66]

The contraindications listed by the FDA for the use of Activase (Alteplase) in AIS include current intracranial haemorrhage, subarachnoid haemorrhage, active internal bleeding, recent (within 3 months) intracranial or intraspinal surgery or serious head trauma, presence of intracranial conditions that may increase the risk of bleeding (e.g., neoplasms, arteriovenous malformations, aneurysms), bleeding diathesis, current severe uncontrolled hypertension.[67] Tenecteplase is another tissue plasminogen activator, which has been shown to have a higher affinity for fibrin and a longer half-life than alteplase.[68] It is widely used for acute coronary events and has a lower rate of systemic haemorrhage than Alteplase in that specific setting.[69]

According to the Italian stroke guidelines (ISO-SPREAD 2020) intravenous Alteplase is recommended in the first 4.5h after known stroke onset (even without advanced imaging), and up to 9h if suggested by advanced imaging plus specific criteria (explained in the EXTEND trial, that tested whether intravenous

Alteplase improved 3-month functional outcomes when given in the 4.5-9 hour window to patients with salvageable penumbra, as defined by a commercially available automated software package).[70]

Tenecteplase is also a recombinant form of tPA but has been modified to enhance its pharmacokinetic properties. It has a longer half-life than Alteplase and can be administered as a single bolus injection, simplifying the treatment regimen. Tenecteplase functions similarly to Alteplase by binding to fibrin and converting plasminogen to plasmin. It promotes the degradation of blood clots, restoring blood flow in the affected blood vessels of the brain. Tenecteplase has a longer duration of action compared to Alteplase, which may allow for a more sustained clot lysis effect.

While treating patients with thrombolytic therapy, healthcare professionals must be able to recognize and manage the two main potential complications of treatment: intracerebral haemorrhage and angioedema. Hospitals should have protocols for reversal of coagulopathy (typically with cryoprecipitate or protein complex concentrate).

In conclusion, the thrombolytic treatment substantially changed the management of this neurological emergency. However, the majority of stroke patients are not eligible to receive IV-tPA;[57] therefore, the introduction of MT has provided clinicians with a stronger therapeutic resource. The introduction of MT has provided clinicians with a stronger therapeutic resource.[71]

Mechanical thrombectomy (MT)

The introduction of MT (also referred to as “endovascular treatment” or “EVT”) has provided clinicians with an additional option.[71] This procedure consists in the Physical removal of the blood clot (typically performed in conjunction with imaging guidance, such as fluoroscopy or angiography) and, eventually, the placement of a stent in the narrowed segment of the artery. The success of this procedure is evaluated with the Thrombolysis in Cerebral Infarction (TICI) scale, a grading system used to assess the degree of recanalization or reperfusion achieved after mechanical thrombectomy in the treatment of acute ischemic

stroke. It provides a standardized measure of blood flow restoration in the affected cerebral vessels (from 0 = no perfusion to 3 = complete perfusion).[72]

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 of five pivotal trials: MR CLEAN,[73] EXTEND-IA,[74] ESCAPE,[75] SWIFTPRIME,[76] and REVASCAT.[77]

According to the AHA guidelines, mechanical thrombectomy should be performed in patients within 6 hours from the symptom onset. However, recent clinical trials have extended this window up to 24 hours if certain criteria of advanced neuroimaging are respected. In particular, DEFUSE 3 and DAWN demonstrated the effectiveness of thrombectomy (in a subset of patients who had proximal arteries in the anterior circulation occluded) up to 24 hours after the suspected onset of symptoms. Nevertheless, patient selection in these studies was exceedingly difficult, and it remained crucial to identify large-vessel occlusion as soon as possible.

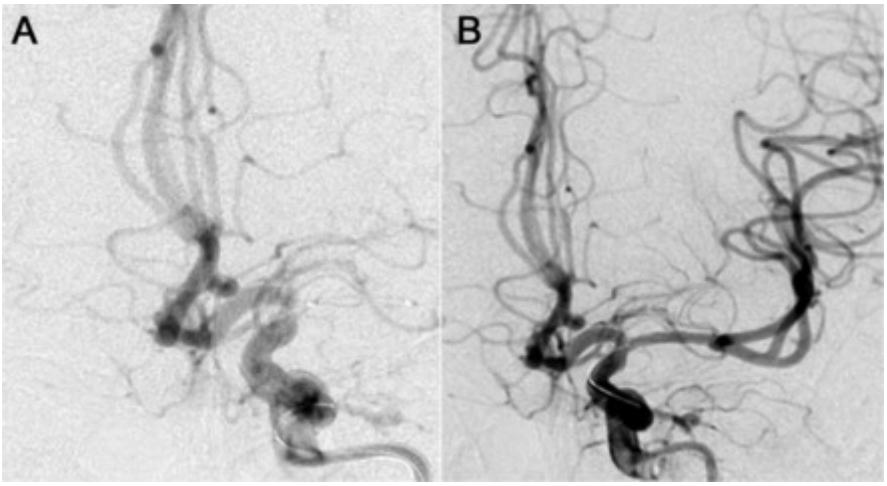


Figure 7 From Xavier et al. "Angioplasty and stenting for mechanical thrombectomy in acute ischemic stroke.[78]

In particular, in (A) the complete occlusion of left middle cerebral artery M1is visible; in (B) the post-stenting imaging revealed TICI 3 flow grade restoration (B).

Clinical scales

When considering chronic and variably progressive disorders with potential multisystem effects such as cerebrovascular disorders, the correct choice of assessment strategy is of uttermost importance. As stroke represents the leading global cause of adult disability, functional recovery is an important consideration for any study of stroke interventions.[79]

The scales used in stroke trials for functional assessment also have utility in clinical practice. Since these functional assessment scales evaluate quantitatively abstract concepts such as "disability," they can be used to objectively determine the patient's deficits, as well as recovery or worsening over time. Unmistakably, this can be helpful in the rehabilitation setting, as well as in clinical practice, to develop a common language between professionals for stroke recovery.

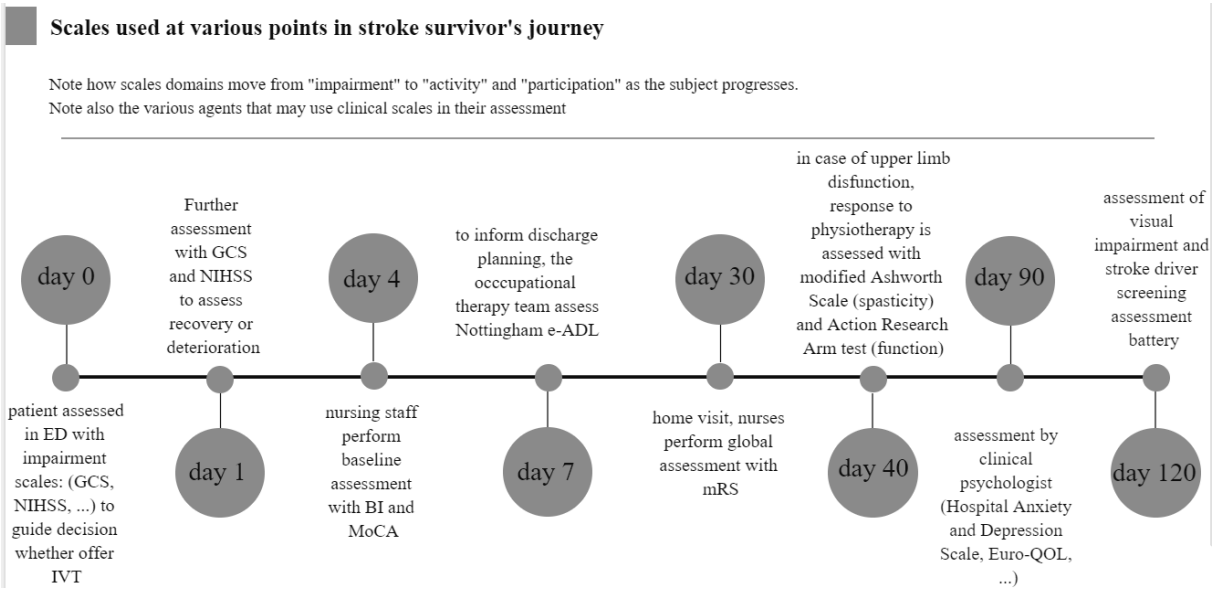


Figure 8 Example of the features of clinical assessment scales according to their clinimetric properties, derived from the theory of psychometrics.

Classically, the most important properties of a scale should be validity, reliability, acceptability (both to patient and to assessor), and responsiveness to change.[80] The archetypal scale would be easy and quick to administer, acceptable to patients and researchers, valid for its chosen purpose, reliable, and responsive to meaningful clinical change. Obviously, such a scale is not even likely to exist, so in the next paragraph we will briefly analyse advantages and disadvantages of the most used clinical scales in stroke assessment.

National Institutes of Health Stroke Scale (NIHSS): assessing stroke severity

The NIHSS is a 15-item scale that provides a quantitative measure of key components of a standard neurological examination.[81,82] The NIHSS provides an ordinal, nonlinear measure of acute stroke-related impairments by assigning numerical values to various aspects of neurological function: assessment of language, motor function, sensory loss, consciousness, visual fields, extraocular movements, coordination, neglect, and speech.[82] It is scored from 0 points (no impairment) to a maximum of 42 points, where scores of 21 or higher are described as "severe."

The NIHSS has several advantages as a stroke outcome-assessment tool: it is relatively easy and brief to perform, without need for additional equipment, making it a useful tool also for non-specialists. In the acute-stroke environment, it is well suited to serial measures of impairment in the evaluation of acute stroke patients, and importantly it has been suggested that a change in the NIHSS of more than 2 points represents clinically relevant early improvement or deterioration (see *Supplementary 1* for the detailed table and values).[83]

The NIHSS has predictive validity also in the long term, since the initial score is a robust predictor of in-hospital complication and outcome at 3 months.[84,85] It is also responsive to change and can measure impairment throughout the expected range of stroke severity.[86] Furthermore, several studies has demonstrated its correlations with objective measures of stroke severity (for instance size of infarct on imaging), providing further evidence of NIHSS validity.[87–89]

In comparison with BI and mRS, the NIHSS has the highest sensitivity in outcome scoring, requiring smaller sample sizes to detect appropriate therapeutic effects.[90,91]

The strongest criticism of the NIHSS relates to its validity in certain non-dominant-hemisphere stroke syndromes. In addition, it has been demonstrated that an individual can score 0 on the NIHSS, despite having evidence of ischemic stroke, especially in the posterior circulation territory.[92] The analysis of component subscales of the NIHSS reveals a focus on limb and speech impairments and relatively limited attention to cranial nerve lesions.

Barthel Index: assessing functional outcome

Since its origin, this scale was intended for use as "a simple index of independence, useful in scoring improvement in rehabilitation", whose particular utility was to improve discharge planning from long-term care wards.[93]

The BI rates ten functional tasks of daily living (activities of daily living or ADL), assessing the individual depending on independence in each task. Scores range from 0 and 100, with a higher score indicating greater independence (see *Supplementary 2* for the detailed table and values).[79]

The scale is recognized as a rational prognostic tool following stroke, particularly as predictor of recovery, level of care required,[94] and duration of rehabilitation required following stroke;[95] moreover, BI scores correlate with other stroke-assessment scales,[96] as well as with measures of infarct volumes.[97,98] An additional strength of BI scale is inter-observer reliability, even in non-stroke populations.[99]

Coming to the negative aspects of this scale, first of all it does not reflect the burden on the individual of communication and Cognitive deficits, due to its focus on Physical function.[100] Secondly, it has been shown that it doesn't ideally represent stroke mortality nor a good responsiveness to change in clinical trials. According to this aspect, also the "floor" and "ceiling" effects of the BI become apparent.[101]

The modified Rankin Scale (mRS): assessing functional outcome

The mRS is a ordinal hierarchical scale that attempts to measure functional independence, incorporating the WHO components of body function, activity, and participation. The scale is defined categorically with seven different grades:[102] 0 indicates no symptoms, 5 indicates severe disability, and 6 indicates death.[103] A 1-point shift on this scale is often deemed clinically significant because of the large category sizes (see *Supplementary 3* for the detailed table and values).

The mRS has many potential strengths. Firstly, the acceptability to patient and assessor and the short time required, with non-standardized interviews taking

around 5 minutes to complete.[104] Concurrent validity is demonstrated by strong correlation with measures of stroke pathology like infarct volumes, in addition to agreement with other stroke scales.[105,106]

It's important to notice that, having a limited number of scores, the mRS may be less responsive to change than some other scales; however, a single-point change on the mRS will always be clinically relevant.[79]

Several studies recommend the use of combination of these three core assessment scales: the NIHSS for studies looking at neurological impairment, the mRS as an outcome if the study is describing global disability, and the BI for studies looking at basic ADL.[79,107]

Stroke impact scale: assessing participation and recovery

The Stroke Impact Scale (SIS) is a disease-specific, self-report questionnaire that evaluates disability and health-related quality of life after stroke.[108] The SIS was developed in collaboration with stroke patients, informal caregivers and experienced healthcare professionals, to specifically measure changes in memory and thinking, emotion, communication and social role, primarily in mild-to-moderate strokes.[108]

In the past decades several studies have validated upgraded versions of SIS: version 2.0 includes 64 items in eight domains (strength, hand function, activities of daily living (ADL) / instrumental ADL, mobility, communication, emotion, memory and thinking, participation); based on the results of a Rasch analysis process, 5 items were removed from version 2.0 to create the current version 3.[102,109,110]

Lastly, 16 items from four of the eight domains of SIS 3.0 have been combined to produce a short composite Physical domain score, known as the SIS-16.[109]

Domains	**SIS 2.0**	**SIS 3.0**	**SIS-16**	Corresponding question in the **SF-SIS**
Physical	4 items	4 items	4 items	In the past week, how would you rate the strength of your leg that was most affected by your stroke?
Memory and thinking	8 items	7 items	7 items	In the past week, how difficult was it for you to think quickly?
Mood and emotions	9 items	9 items	9 items	In the past week, how often did you feel that you have nothing to look forward to?
Communication	7 items	7 items	7 items	In the past week, how difficult was it to understand what was being said to you in a conversation?
ADL/IADL	12 items	10 items	7 items	In the past 2 weeks, how difficult was it to do light household tasks/chores?
Mobility	10 items	9 items	8 items	In the past 2 weeks, how difficult was it to walk without losing balance?
Hand function	5 items	5 items	1 item	In the past 2 weeks, how difficult was it to use tour hand that was most affected by tour stroke, to pick up tour coin?
Participation	9 items	8 items	8 items	During the past 4 weeks, how much of your time have you been limited in your social activities?
Overall stroke recovery	Out of 100%	Out of 100%	-	-

References	*Duncan, Wallace et al.* *Stroke 1999*	*Duncan PW, Bode RK et al.* *Arch Phys Med Rehabil. 2003*	*Duncan PW, et al.* *Neurology. 2003*	*Jenkinson et al.,* *Stroke 2013*

Table 2 Comparison between different SIS versions

The SIS aims to measure several dimensions of health-related quality of life that are not specifically addressed in other scales. Nonetheless, its role in multicentre trials and clinical practice has yet to be established.

The major drawback of the SIS is the need for self-reporting or the use of a substitute. This requirement substantially limits its use in aphasic patients and in those with denial of their deficit or illness. Importantly, studies have reported that proxy responses tend to differ significantly from those of patients, often overstating the severity of the patients' conditions.[111]

Despite SIS has been validated in a multitude of languages (in particular, the Italian version has been validated by Vellone et al)[112] and has been thoroughly studied by its developers, it has not been applied more broadly to other centres and trials, so its generalisability remains to be verified.

The full assessment of SIS 3.0 is a thoroughly useful tool, nevertheless it can take considerable time to complete. This feature may limit the scale's use in practice, in particular with stroke survivors with persisting Physical and Cognitive impairments or in an emergency setting. [113] In a multidisciplinary, expert consensus statement, time required for SIS assessment was noted as a major limitation for stroke survivors.[114]

To overcome this limit, the Short Form or SIS (SF-SIS) was suggested and validated by using a multimodal approach, describing it a robust and broadly acceptable to stroke survivors and clinical/research staff.[113]

In comparison to other usual scales used in stroke clinical assessment, the SIS doesn't share the imperfect reliability of mRS, nor the floor and ceiling effects of

BI, nor the problem of poor validity for certain stroke syndromes (NIHSS).[79,115,116]

The WHO international classification of functioning, disability and health advocates a classification framework that outlines recovery in terms of: Physical impairment; functional activity (formerly disability); societal participation (formerly handicap).[117] Several assessment scales describing each of these domains are available: for example, the National Institutes of Health Stroke Scale (NIHSS) as a measure of impairment; the modified Rankin scale (mRS) as a measure of activity and finally the stroke impact scale (SIS) has been developed to measure participation. These scales also differ in their clinical purposes: NIHSS is mostly useful for early prognostication and serial assessment; the BI is useful for planning rehabilitative strategies; the mRS lays out summary measures of outcome and might be most relevant to clinicians and patients considering early intervention; finally the SIS was designed to measure the patient's perspective on the effect of stroke.[118]A combined measure, derived from several scales, would be considerably useful in measuring the multiple dimensions of outcomes after stroke.[118]

PART II

Stroke and the Human Connectome

Brain connectivity

The concept of brain connectivity is intrinsically associated with a network-model functioning of the brain. Behaviour, thoughts, and emotions depend on the distributed activation of brain regions that maintain over time functional interactions so that they can be conceptualized as brain networks.

As opposed to the XXth century framework, where neuroscience aimed to localize psychological processes to precise and discrete areas of the brain, recent literature has shown the brain can be conceptualized in a more complex framework, with regions of the brain that are interacting as networks that balance regional segregation and specialization of function with strong integration.[119,120] As a consequence, pathological perturbations will rarely be confined to a single locus affecting other interconnected regions.

The term “connectome” was suggested by Olaf Sporns to describe “the complete set of all neural connections of the human brain”.[121,122] It is considered a powerful tool to understand how functional brain states emerge from their underlying structural substrate, and allows new mechanistic insights into how brain function is affected if this structural substrate is disrupted.[122]

We can distinguish different general modalities of connectivity: anatomical, functional, and effective. Each mode refers to a specific pattern of links: structural links (such as synapses and fibre pathways) for the first, statistical dependencies (such as coherence and cross correlation) for the second, causal interaction for the third. The units between this links can be individual neurons, neuronal populations, or anatomically segregated brain regions.

Anatomical connectivity

Anatomical connectivity refers to a network of synaptic connections linking sets of neurons, together with their associated structural biophysical characteristics. This physical pattern is relatively stable at shorter time scales (seconds to minutes), while at longer time scales (hours to days), significant morphological changes and plasticity phenomena are most likely to happen.[123]

Neurons (both excitatory and inhibitory) and axonal pathways can be seen as nodes and edges of a brain network, respectively. Each of them can be analysed with different neuroimaging techniques. To identify structural "nodes", studies have used anatomical parcellation of the cerebral cortex using the Brodmann atlas; parcellation using structural magnetic resonance imaging (sMRI); automated cortical parcellation based on gyral folding patterns; quantitative cytoarchitectonic and neurochemical maps.[124]

On the other hand, the "edges" (meaning long-range axonal-fibre pathways), have been studied using diffusion weighted magnetic resonance imaging (DW-MRI) to map structural and functional properties of the human connectome. Particularly, diffusion tensor imaging (DTI) is the most used type of modelling of DWI datasets to compute structural brain properties.[125]

DTI is a modern modification in MRI data processing that can reveal non-invasively unique information of white matter microstructures within the central nervous system (CNS). DTI provides image contrast based on differences in the magnitude of diffusion of water molecules within the brain. It allows each voxel to produce a set of signal intensities data as well as directional data that result in the tensor (defined as 3D complex vector) in a three-dimensional space. Each voxel (represented by a single arrow) is then combined into an array of directional arrows in the orientation of neural tracts: these directional arrows are then tied together with graphic techniques and result into linear images of nerve tracts.[126]

In general, conventional MRI techniques are based on registering signal from a volume of tissue by repeated re-phasing and de-phasing of protons in the volume imaged. On the other hand, diffusion tensor imaging relies on different properties of the tissues: water molecules in most tissues tend to diffuse equally in all directions (phenomenon referred to as "isotropic diffusion") but in white matter tracts the diffusion is limited the direction of the tract ("anisotropic diffusion").[127]

By measuring the directional dependence of water molecule diffusion, DTI

generates tissue contrasts that can be used in the study of axonal organization in the CNS.[128]

In particular, DTI includes several techniques to compute structural brain models and create images reflecting the diffusion properties of the model. The elements processed are defined as eigenvalues (*λ1, λ2*, and *λ3*, that describe the shape and size of a tensor, independently of its orientation) or eigenvectors (*ε1, ε2*, and *ε3* that describe the orientation of a tensor, independently of its size and shape). Regular DWI produces images based on the sum or alternatively on the average of the eigenvalues: specifically, the sum of the eigenvalues is called "trace", while their average is called "mean diffusivity" (MD) or "apparent diffusion coefficient" (ADC).

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. The term "anisotropy" refers to the restricted range of movement for the water particles in the brain, since they are limited by structures like white matter bundles (as opposed to "isotropy" or capability to diffuse in every direction, when water particles can move freely).

Consequently, in the brain the water particles tend to diffuse in specific directions (anisotropic diffusion); when the diffusion anisotropy increases, the eigenvalues become increasingly unequal, and FA values rise. FA is related to axonal integrity, but it can be affected by many factors (such as axonal loss, inflammation, cell death, gliosis, demyelination, increase in intracellular or extracellular liquid content); therefore it is not strictly specific to the type of damage.[129–131] For this reason, it is usually combined with MD (that represents the average of the 3 eigenvalues of the tensor, as mentioned before), which is able to identify increased extracellular spaces due to degeneration or shrinkage of axons and dendritic fibers.[132,133]

Ultimately, AD and RD are used to determine diffusivity direction: AD detects axonal degeneration, while RD is influenced by density, demyelination, and abnormal axonal diameter.[131,134]

Despite offering several details about microstructural details of the brain tissue (such as fiber organization, axonal direction coherence, tract integrity),[135] DTI can be affected by several confounding factors, for example tissue properties and its neuropathological alterations.[135] Additionally, DTI is considered not suitable to study grey matter diffusion, since it is nearly isotropic.[136]

Novel approaches have been developed that can be considered "biophysical models", in that they aim to parametrize the dMRI signal as a function of biophysically meaningful parameters (such as axon density).[137]

Among these, NODDI represents a new model of relating diffusion MRI signal with tissue biophysical properties, and its clinical properties represent a fervent setting for an increasing number of studies.[137]

NODDI discriminates three different tissue compartments (intra-neurite, extra-neurite, cerebral spinal fluid) that can be each modelled in a biologically informed fashion, resulting in the specific analysis and modeling for each compartment. This technique, being able to quantify specific neuron morphologies and compartment properties, can overcome the specificity problems of DTI as well as the adaptability to grey matter.[138]

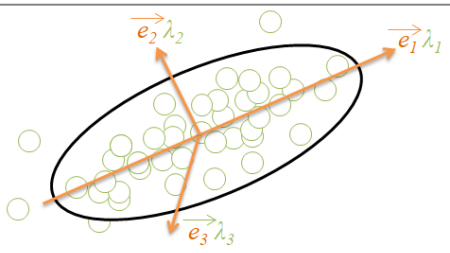
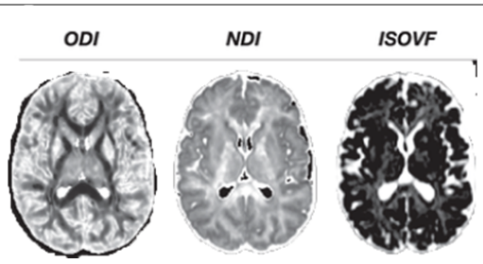
<table>
<tr><th colspan="3">DTI vs NODDI tissue modelling</th></tr>
<tr><td> 
</td><td colspan="2"> 
</td></tr>
<tr><td>3 orthogonal axes tissue model</td><td colspan="2">3 compartment tissue model</td></tr>
<tr><td>FA (fractional anisotropy): microstructural integrity;
V microstructural changes
X specific type of changes</td><td>NDI (neurite density index): intra-axonal compartment</td><td>Restricted distribution</td></tr>
<tr><td>MD (medial diffusivity): inverse measure of membrane density
V sensitive to cellularity, oedema, necrosis</td><td>ODI (orientation dispersion index)</td><td>Hindered distribution</td></tr>
<tr><td>AD (axial diffusivity): variable in WM changes, development and pathology</td><td>ISOVF (isotropic volume fraction)</td><td>Free water distribution</td></tr>
<tr><td>RD (radial diffusivity): variable in myelination changes and axonal diameter</td><td colspan="2"></td></tr>
</table>

Figure 9 Comparison between DTI (Diffusion Tensor Imaging) and NODDI (Neurite Orientation Dispersion and Density Imaging)

Another biophysical model that has been proposed to implement the microstructure analysis is SANDI. This compartment-based model distinguishes intra-cellular and extra-cellular non-exchanging compartments. The total signal results from the weighted sum of the signal from water molecules diffusing in each compartment, where *f(ec)* refers to the extracellular compartment and *1-f(ec)* to the intracellular compartment. Additionally, the intra-cellular compartment is itself divided into two non-exchanging sub-compartments: intra-neurite (f_{in}) and intra-soma ($1-f_{in}$). The intra-cellular MRI signal is then given by the weighted sum of the MRI signal from water molecules diffusing within the two sub-compartments; accordingly, this approach gives a more detailed and realistic model of water diffusion in different cellular types rather than considering the intracellular space altogether.

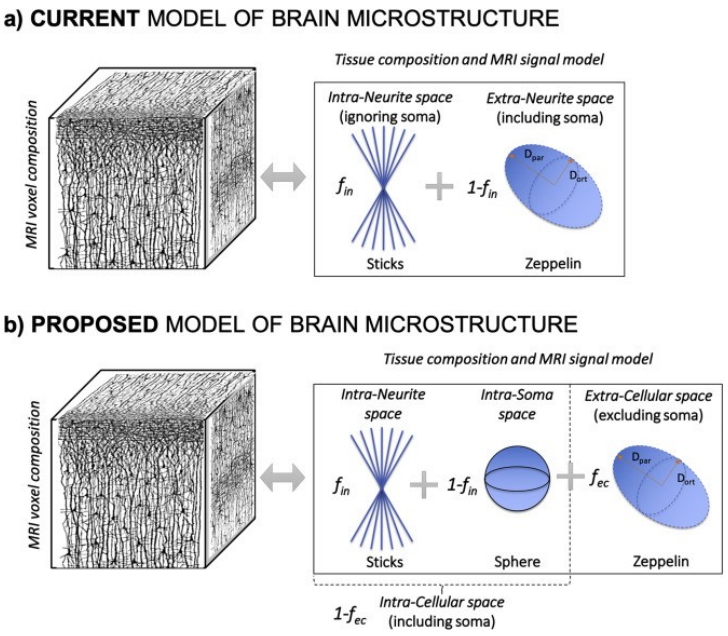


Figure 10 From Palombo M. et al "SANDI: A compartment-based model for non-invasive apparent soma and neurite imaging by diffusion MRI" [139]

The essential assumption for all tractography techniques is that when numerous axons are aligned along a common axis (as in white matter bundles), diffusion of water molecules will be easier along them than through them. Using this local information on orientation of the axons, tractography algorithms deduce long-range connections. This allows the non-invasive studies of white matter bundles in

vivo, both in normal and pathological conditions, resulting in maps of white matter bundles, representing the so-called "structural connectome".

Fibres extending superior-inferiorly are identified in blue; those extending left-right in red, and those extending anterior-superiorly in green. Other directions are represented by a combination of these colours.[140]

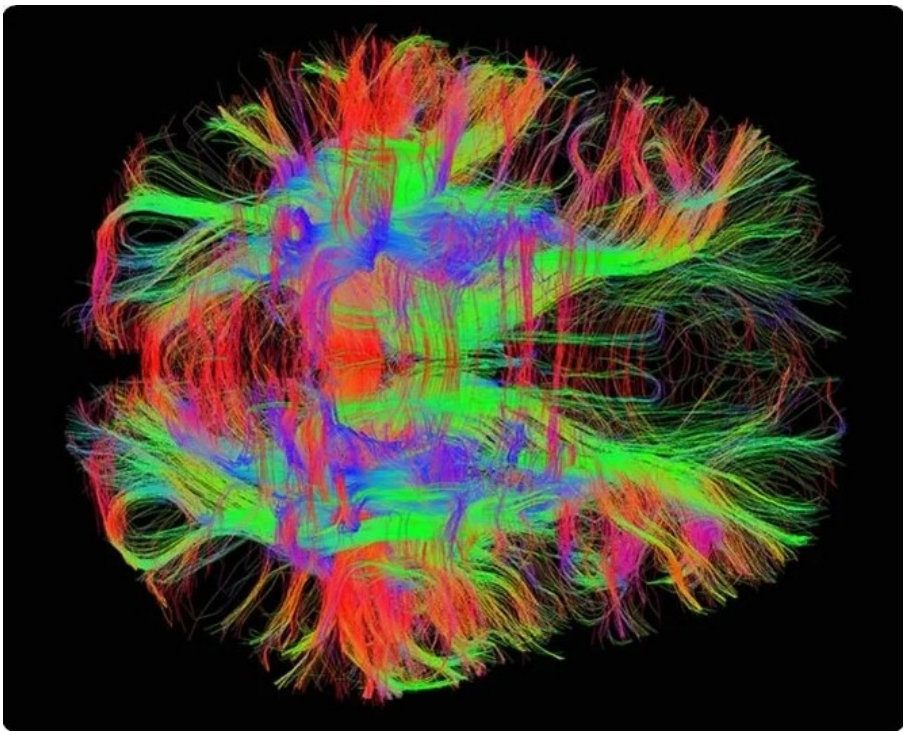


Figure 11 Conventional colour coding in tractography: Red: transverse fibres; Green: anteroposterior fibre; Blue: craniocaudal fibres.[141]

DTI-based tractography highlights both directionality and integrity of the axonal fibres, as well as their alterations in the event of neuronal injury for any aetiology.[127] As we mentioned above, the human brain network is prone to be defined a collection of nodes and edges,[142] where the nodes are identified as anatomically parcellated regions, while edges are the fibres linking those regions. According to the property of the edge, a network can be classified as weighted or binary: the former term is used if different weight values are assigned to the edges to characterize their connectivity strengths; the latter is used if only values 1 and 0 are used, indicating connected or not connected, respectively.[143]

Weighted networks have been broadly used the inter-cortical connectome, their weights being studied with tractography data.[144] In particular, weights values of the fibre pathways can be calculated with two different approaches: deterministic

tractography or probabilistic tractography. Deterministic tractography implies that any two voxels are either connected or not, while probabilistic tractography defines the likelihood by which any two voxels could be linked.[145]

Deterministic tractography, given it relative simplicity and computation speed, is commonly used to quantify structural networks, and the number of fibres can be directly counted (with approaches including DTI and NODDI, as we mentioned before). Importantly, the fibre density (defined as the number of fibres normalized by seed density) is a core measure in defining the strength (or the damage) of inter-regional connections, consequently the topology of a weighted structural network.[143] The tracking initiates in a seed region selected anywhere within a voxel, then a fibre is generated a propagated until it reaches its final destination. The outcome of the tracking is affected by several factors, including seeds location and signal-to-noise ratio (SNR). In particular, DTI data suffer from a limited SNR, resulting in the accumulation of uncertain fibre tracings and finally the dispersion of the fibre path.[146,147]

Functional connectivity

Functional connectivity can be defined as the statistical interdependence of signals between distinct brain regions. Functional connectivity presents important differences with anatomical connectivity: firstly, the statistical dependencies are often calculated between all elements of the brain, regardless of whether these elements are connected by direct structural links. Secondly, these statistical dependencies (and consequently functional connectivity itself) are highly time-dependent.[123]

Another definition of functional connectivity derives from neuroimaging studies: the temporal correlation in blood oxygen level-dependent signals in a resting-state or during specific tasks that can be measured by the time-series data of functional MRI (fMRI).[148]

Specifically, blood oxygen level-dependent (BOLD) contrast denotes signal differences in T2-weighted images as a function of the amount of deoxygenated haemoglobin present.[149] Blood flow in the brain is highly locally controlled in response to oxygen and carbon dioxide tension of cortical tissue. When a specific region of the cortex increases its activity in response to a task, the extraction fraction of oxygen from the local capillaries leads to an initial drop in oxygenated haemoglobin and a consequent increase in local carbon dioxide and deoxygenated haemoglobin. Following a period of 2-6 seconds, cerebral blood flow increases, delivering an oversupply of oxygenated haemoglobin, removing deoxyhaemoglobin. This large rebound in local tissue oxygenation is what is imaged: fMRI can detect this change due to a fundamental difference in the paramagnetic properties of oxygenated haemoglobin (not paramagnetic) and deoxygenated haemoglobin (paramagnetic). Consequently, the latter will cause local dephasing of protons, reducing the returning signal from the nearby tissues. Heavily T2- weighted sequences are used to detect this change, which is in the order of 1-5%.[150]

Functional connectivity has been studied in two main fMRI settings: task-related or resting state. The former has been broadly studied in neuropsychology, and many specific human behavioural functions have been localized by these studies. However, study designs for task-related fMRI are generally complicated, and many stroke survivors cannot complete the tasks required in these experiments.

Resting-state fMRI has the advantage that it does not require any specific tasks during imaging acquisition.[151] Resting state networks (RSN) can be defined as the result of temporal correlations of spontaneous activity between different areas. Using resting-state fMRI analysis, evidence of extensive changes in large-scale neural networks (i.e., default mode network, central executive network, dorsal attention network, and salience network), have been reported for various disease states, suggesting their possible role in characterizing neurological disorders.[152]

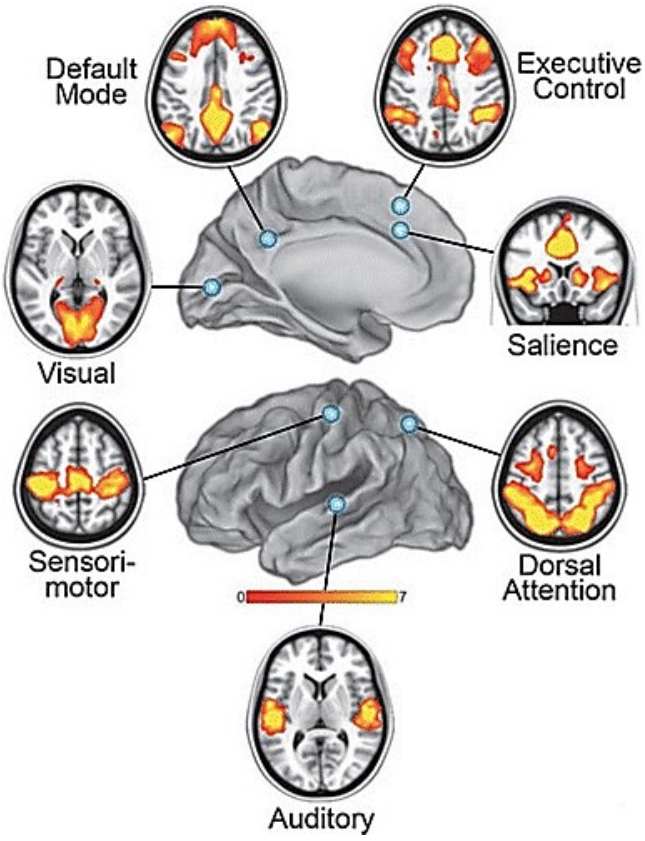


Figure 12 Distribution of resting-state networks, from "Resting-State Functional MR Imaging: A New Window to the Brain"[153]

Despite the capability in drawing increasingly detailed maps of brain connectivity at various resolutions in diverse species[154–157] and analysing their functional correlation with cutting-edge methods, the sheer scale of the data sets involved poses difficulties for analysis and interpretation. The human brain comprises about 8.6×10^{11} neurons and 10^{14} synapses,[158,159] representing a digital atlas of which would require more memory than is required to store all the written information present in the world today.[160]

Network science and graph theory offer powerful tools for overcoming these challenges to map and predict patterns of disease. As mentioned before, both structural and functional brain networks can be explored using graph theory, and the necessary steps can be summarized as follows. Firstly, the identification of the network nodes: they can be recognized using EEG or as anatomically defined regions under a histological or MR/DTI point of view. Secondly, the measure of association between nodes (network edges): this connection can be defined using inter-regional correlation in cortical thickness, or volume MRI measurements, or again it can be defined as the connectivity probability between two regions in a specific DTI dataset. Thirdly, the definition of network structure: generating an association matrix arranging all pairwise associations between nodes and applying a threshold to each element of this matrix to produce a network that could be binary, directed or undirected. Lastly, the study of the network parameters in this brain network model (such as path length, modularity, clustering coefficient) and their comparison with equivalent parameters of random population networks.[161]

Physiopathology of disconnections

Connectomics can track and predict patterns of disease in the brain. The biological impact of this pathological process strongly relates to the patient's behavioural symptoms and recovery.[162]

In the following section we describe the principal types of maladaptive responses and adaptation strategies that mediate the spread of pathology throughout the connectome. Maladaptive responses include: diaschisis, transneuronal degeneration and dedifferentiation. Adaptation strategies include compensation, degeneracy and reserve.

Maladaptive mechanisms

Diaschisis

Since the beginning of the last century, the relationship between focal brain lesions and clinical symptoms has undergone a radical change. In 1914, von Monakow, developed the concept of diaschisis as a hypothesis to explain functional recovery after a brain insult. Von Monakow described four main aspects of diaschisis: (1) damage to one brain area can, by loss of excitation, produce loss of function in regions adjacent to or remote from but connected to the primary site of damage and may be regarded as a "shock" confined to distinct nervous structures; (2) the presumptive mechanism is loss of excitation to intact regions (rather than inhibition of these); (3) diaschisis undergoes gradual regression in well-defined phases; this progressive resolution parallels recovery of function in areas of diaschisis; (4) the "wave of diaschisis" follows neuroanatomical pathways spreading from the site of injury.[163] With regard to these neuroanatomical pathways, three specific types were identified: corticospinalis diaschisis (defined as progression from a motor cortex injury to the spinal cord along the pyramidal tracts), commissuralis diaschisis (defined as functional contralateral cortical depression via axons of the corpus callosum following injury to the cortex of one hemisphere), and associative diaschisis (defined as intracortical fibre-mediated depression of function in intact cortical areas adjacent to the locus of injury).[164]

Modern neuroimaging studies have provided fundamental clues to the existence of diaschisis. Specifically, focal brain lesions are accompanied by widespread metabolic changes and these changes involve not only the affected cerebral hemisphere but also extend into brain areas supplied by different arteries. Furthermore, these remote metabolic changes are lesion-specific in terms of cerebral topography and clinical syndromes.[165,166]

Different mechanisms of diaschisis have been described as a result of the advancement of brain imaging tools and technology, progressively relating to clinical findings.[167]

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".[167] This reduction in neural activity is due to changes in connectivity and communication between brain regions caused by the lesion or injury.

Functional diaschisis, on the other hand, refers to a reduction in neural activity in a brain region that is directly affected by a task or stimulus. In this case, the reduction in neural activity is due to changes in the functioning of the neural network caused by the lesion or injury. The former definition by Di Piero et al.[168] originally referred to the alteration of functional responsiveness of a neural system remote from a lesion when challenged by physiological activation. In other words, functional diaschisis can be defined as the "focal abnormalities in metabolism or neuronal activity following activations or stimulations, in anatomically intact brain regions distant from the lesion".[169] To illustrate, several studies have shown that after a cortical lesion, an abnormal response to evoked potentials can be observed in the controlesional cortex, without concomitant alterations of metabolism 'at rest' in the same areas.[170,171]

Connectional diaschisis refers to the disruption or dysfunction of connections between different brain regions that are not directly affected by the initial brain injury. When a stroke or brain injury occurs in one region of the brain, it can lead to changes in the functioning of connected brain regions, even if they were not directly damaged. These changes can result in a reduction in activity or altered communication between brain regions, leading to functional deficits in those areas. Finally, connectomal diaschisis refers to the disruption or dysfunction of

the entire connectome or specific connectome pathways following a brain injury. It reflects the impact of the injury on the overall brain network, including both local and long-range connections, rather than focusing solely on individual brain regions.

In summary, both connectional diaschisis and connectomal diaschisis describe the functional and structural changes that occur in brain connectivity following a stroke or brain injury. They emphasize the impact of the initial injury on brain networks and the subsequent functional deficits that can arise in regions beyond the primary site of injury.

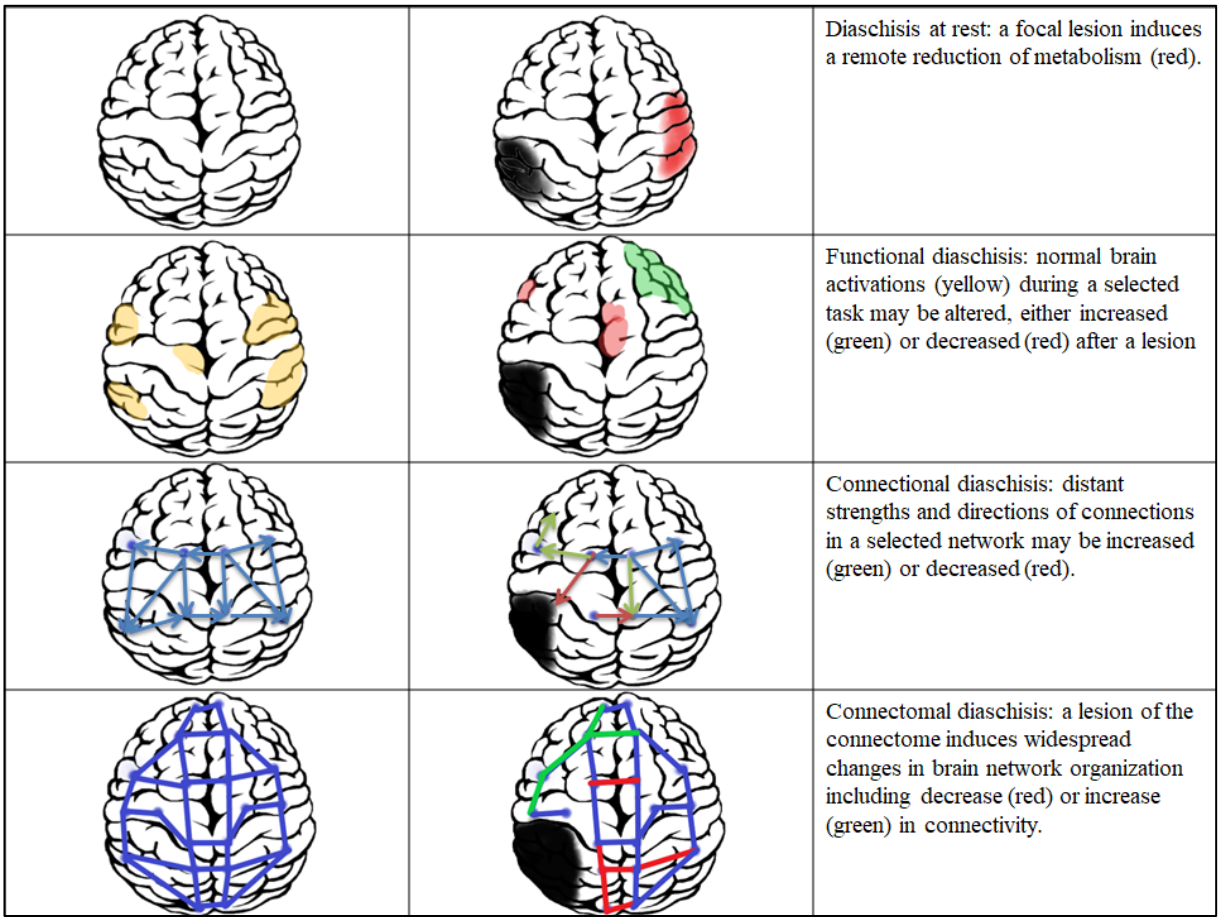


Figure 13 Types of diaschisis. Types of diaschisis before (left) and after (right) a focal brain lesion (black). From: Carrera et al "Diaschisis: Past, present, future"[169]

Transneuronal degeneration

Transneuronal degeneration refers to the structural deterioration of areas that are remote from the initial lesion. It is a process that occurs over time and can be either anterograde or retrograde: the former indicates that the damage/dysfunction of a neuron spreads to its postsynaptic target, the latter indicates that a presynaptic neuron degenerates as a result of reduced trophic support from the injured postsynaptic target.[172] The pathophysiology of degeneration can range from neuronal shrinkage, to reduction in synapses and dendrites, to alteration of axonal myelin and fibre number, and finally to neuronal death. It has been demonstrated that degeneration of remote regions (caused, for example, by the accumulation of amyloid in Alzheimer disease) might imply firstly reduced functional connectivity between the areas affected, and secondary also hypometabolism and structural damage in distant areas due to reduced excitatory input or loss of trophic support from the damaged presynaptic neuron.[173] Accordingly, diaschisis may precede transneuronal degeneration in some disorders.

In addition, also excessive neuronal stimulation due to a focal pathology in inhibitory areas can result in damage to remote sites: this form of excitotoxicity has been demonstrated to result in remote areas following focal cerebral ischemia.[174]

Axonal transport represents an important factor to transneuronal differentiation, both in anterograde and retrograde directions: pathological changes at the soma can alter the transport of factors that are essential for the maintenance and repair of the axonal cytoskeleton and the surrounding myelin. On the other hand, pathological changes affecting the white matter tract can inhibit the retrograde transport of trophic factors to the neuronal soma.[175]

Dedifferentiation

The last maladaptive mechanism relates to the diffuse and non-specific recruitment of brain regions to perform a specific function, and it likely results from the disruption of usually specialized and segregated neural activity.[176,177] Dedifferentiation could result on one hand from aberrant neural plasticity, on the other hand by a focal cortical pathology that alters the balance between excitatory and inhibitory signalling. The persistence of a dedifferentiated state corresponds to poorer recovery of function (for example, motor function after a stroke).[178]

Adaptive mechanisms

Compensation

Neural compensation describes the increase in activity or functional connectivity following a pathological insult, aimed to maintain behavioural output. It has been widely demonstrated to occur after stroke, where focal ischemic damage leads to the recruitment of unaffected and remote brain areas.[179–182] Overall, there is correlation between the extent of focal neural damage, the severity of the behavioural impairment and the compensatory recruitment with consequent functional reorganization.[180]Therefore, the functional recovery is affected proportionally to the damage to the axonal tracts that link damaged and preserved (and compensatory) areas.[183].

It is important to highlight that progressive recover to pre-injury conditions of the affected network predicts behavioural recovery.[178,179] This suggests that optimal recovery is likely to depend on the gradual return to baseline network dynamics.

Structural plasticity is a fundamental substrate for neural compensation.[184] Several studies have shown that focal ischemic injury leads to widespread depolarization of connected regions, that itself leads to the persistent hyper-excitability or disinhibition of networks that are functionally related but spatially distributed.[185] The consequent remodelling (including increased synaptogenesis and dendritic sprouting of the unaffected axons[186,187]) has several important features: it can occur over long distances, it is activity-dependant and can determine volumetric changes (visible at MRI). These plastic modifications are likely to result in greater flexibility and increased ability to adopt different strategies to maintain behavioural outcome as much unaltered as possible.

Importantly, the distinction between “dedifferentiation” and “compensation” is subtle, and can be considered related to behaviour. In detail, if in dedifferentiation the diffuse recruitment of distal brain regions is coupled with poor behavioural outcome, in compensation the increase of functional connectivity results in good behavioural outcome.

Degeneracy and reserve

Degeneracy is the ability of structurally distinct components of a system to perform the same function.[188]In the brain, it refers to the capacity of distinct neuronal system to overlap in their contribution to a specific output, resulting in higher functional adaptability and robustness to damage.[188] Degeneracy is evident from the microscale of individual neurons to large-scale systems and it represents a fundamental basis of Cognitive reserve.

On the other hand, Cognitive reserve defines the flexibility of the brain in engaging alternative compensatory strategies to cope with a Cognitive impairment due to a neural insult.[189,190] In addition, Cognitive reserve is influenced by neural reserve, described as the amount of remaining intact brain that is still able to perform the task. The concepts of degeneracy, compensation and reserve are strongly related, but it is important to highlight that degeneracy doesn't always imply that compensatory activity will follow; nevertheless, it has been demonstrated that the higher the neural reserve is, the greater will be the bearable damage before Cognitive or behavioural damage becomes evident.[189]

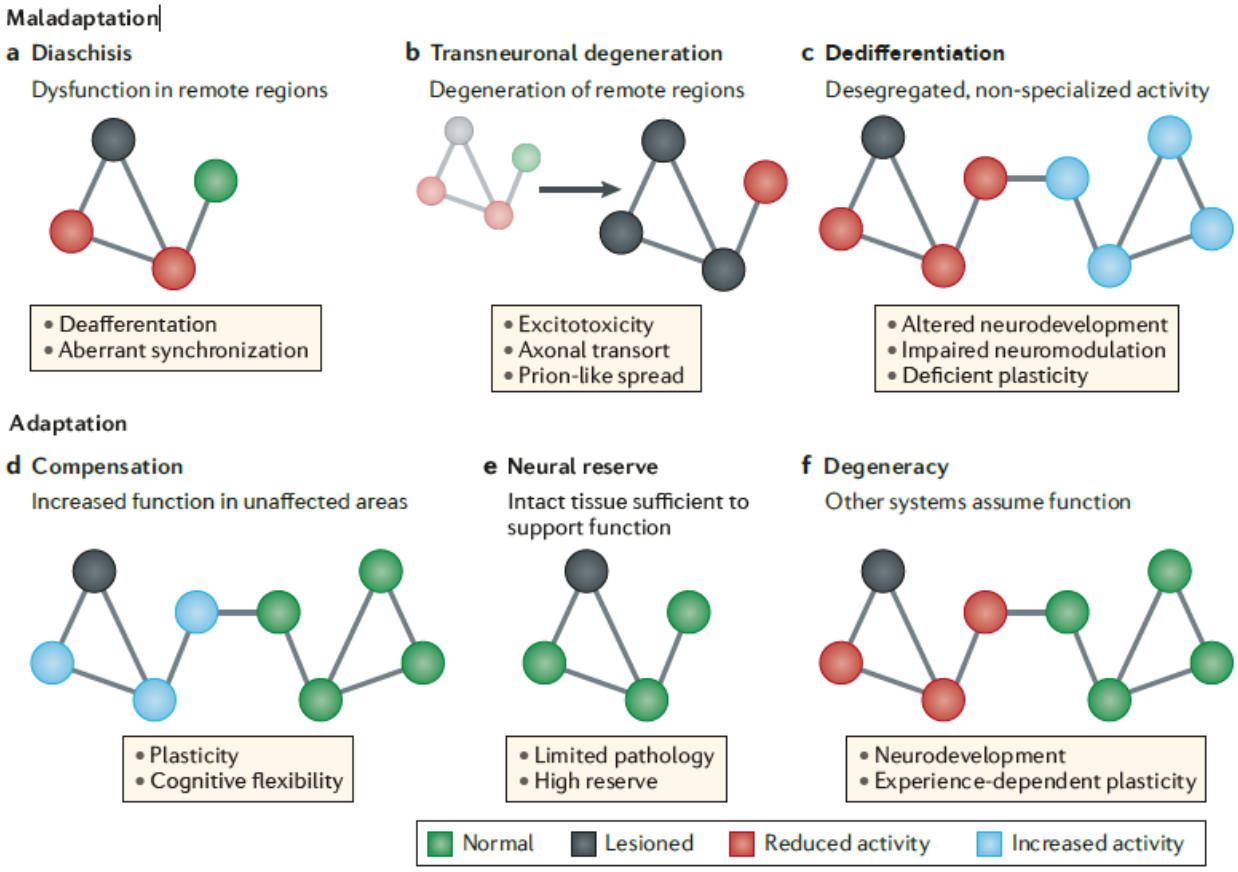


Figure 14 Dedifferentiating major classes of maladaptive (a-c) and adaptive (d-f) neural response to pathological perturbation. From Fornito et al "The connectomics of brain disorders"[191]

The impact of stroke on structural and functional connectivity: the new paradigm of stroke related syndromes

Focal lesion following stroke affect the large-scale functional and structural connectivity in the brain, resulting in variable behavioural outcomes.[162,192–194] Recent advances in neuroimaging have helped to correlate the "disconnectome" (defined as the computation of network disconnections) with behavioural impairment and finally functional recovery.[194–196]

Structural disconnections

The first hypothesis to correlate different deficits occurring after a focal lesion was historically the localization in the same vascular territory; nevertheless, it has been widely demonstrated that equal correlation of deficits exists also among areas without vascular overlap. [197] Even a minor lesion (but occurring in a strategically important connectivity hubs) can disrupt brain function on a large scale. Structural disconnection can be classified as direct or indirect: the former refers to the interruption of direct structural links connecting two distinct regions, while the latter refers to the increase of the minimum number of links necessary to connect two indirectly connected regions.

After stroke, neurodegenerative changes (like cortical thinning) can be found in cortical areas that have been directly disconnected by the lesion, as well as in the contralateral homologous. These modifications are caused by the long-term effects of the lesion-induced disconnection.[198]

Furthermore, structural disconnection outperforms region-level and voxel-level measures in explaining connectivity disruptions associated with stroke. [199]

Functional disconnections

Large-scale damage of inter-connectivity patterns following stroke, [200] is strongly related both to behavioral outcome and recovery.[179,197,201,202]

The pathological alterations of functional connectivity after a stroke vary in topography and severity, but are consistent in the features of connectivity.[192] Specifically, three particular shapes have been reported as the most common

impairment: the weakening of inter-hemispheric functional connectivity, the intensification of intra-hemispheric segregation, and finally the increase in functional connectivity between networks that are not correlated in healthy subjects. These alterations can be seen in a variety of Cognitive domains, from motor to visual, language, attention, and memory.[196,203,204]

Finally, stroke would cause a reduction in the modularity of functional connectivity. This hypothesis can be explained with the concept of entropy: in the healthy subject, where multiple segregated and interconnected systems are processing and integrating data concurrently, the system's entropy is at the most of its performance. A focal lesion leads to the decrease of the possible neural patterns generated by the brain. This reduction in entropy would limit the variety of possible behavioral responses.

The same correlation has been demonstrated during recovery when an increment in functional connectivity's modularity can be observed.[200]

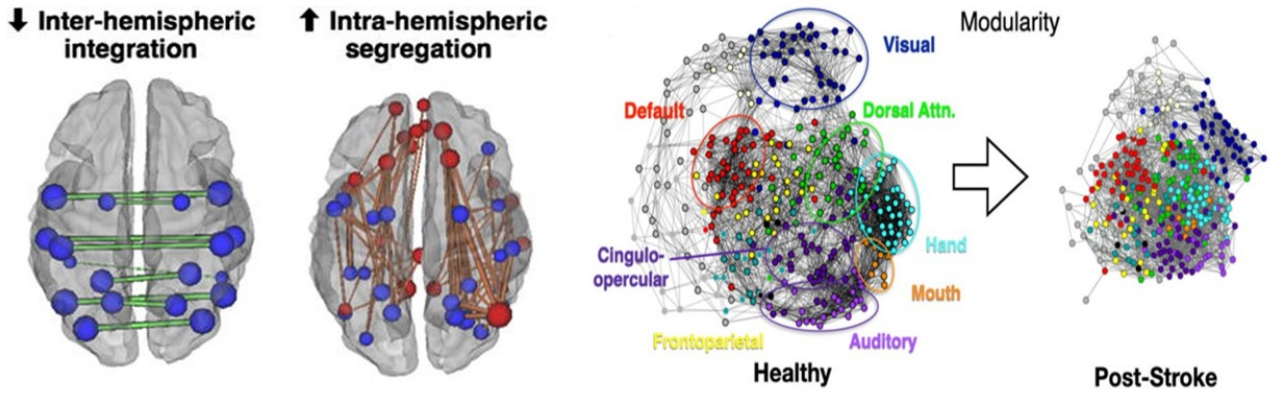


Figure 15 From Siegel et al. "Mapping correlated neurological deficits after stroke to distributed brain networks" 220

Consequently, three main parameters can be helpful in giving an organic interpretation of stroke consequences: firstly, structural damage, secondly functional abnormalities, lastly behavioral deficits. It is then evident that network analysis of the connectome in stroke patients is relevant to our understanding of the mechanisms underlying symptoms and functional recovery, including recovery from Cognitive dysfunction.[205,206]

To perform this kind of analysis, our group has pioneered connectomics in stroke to provide a network level explanation of neurological deficits, as well as

recovery of function.[192,200] Additionally, this approach may have implications for the development of novel interventions that can modulate maladaptive processes and facilitate neural circuit recovery. One example for all is the modulation of primary motor cortex function by repetitive transcranial magnetic stimulation.[207] Accordingly, the use of network perspective to describe stroke symptomatology and recovery mechanisms might open a new era of research and practice in clinical neurology and neurorehabilitation.[208]

Despite all the above-mentioned positive breakthrough offered by this connectome-approach and the cutting-edge models to define structural and functional connections, their effective clinical use is still limited due to their analytical complexity and the need of dedicated research MRI scans that often are not feasible in severe patients and not easily included in the busy time-dependent clinical workflows.

To overcome these issues, two indirect connectome-based methods have been proposed: a lesion network mapping approach advanced by Boes et al. and a tractography-based approach advanced by Foulon et al.

Lesion network mapping

The method suggested by Boes et al evaluates how to incorporate the functional network effects of brain lesions into traditional lesion mapping, without requiring specialized imaging for each patient.[209] This approach employs atlas of human functional connectome data to highlight the functional regions most connected to the area of injury. The lesion of interest is segmented and becomes a region of interest for a whole brain temporal correlation analysis using the atlas obtained from healthy data. Thus, the functional zones that are more related to the lesion are represented on the corresponding statistical map. This enables the localization of common networks of functional changes resulting from lesions in various places across a large number of patients. This method is especially useful for rare disorders when it is challenging to gather enough subjects. This method was actually created to analyse cases from the literature using published images of lesions that were then "embedded" in functional, healthy connectomes to extract their network functional impact and localise "common" networks across lesions that occur in different parts of the brain. The main advantage of this method is of using clinical scans of lesions to infer functional network effects.

There are also drawbacks. When the lesions are drawn from published pictures their volume is incomplete. Even when the lesion is directly measured, the analysis can extract functional maps of brain regions that are directly connected to the site of injury. However, the method cannot reproduce adjustments in functional connections caused by the lesion itself. Connections that are two or three steps away from the directly disconnected regions also change their level of synchronization, but their contribution cannot be measured with indirect methods. This weakness may be the reason why it is controversial whether lesion-symptom mapping is sensitive enough to correlate with behavioral deficits or recovery of function.[209,210]

DWI tractography

The innovative approach pursued by Foulon et al. focused on the combination of two important achievements of modern neuroimaging: on one hand, DWI tractography outlines how brain areas are structurally linked together;[211] on the other hand, functional MRI quantifies the interaction between brain areas.[212]

As discussed before, traditional lesion symptom mapping relates the patient's symptoms to the damaged areas, these latter providing the neural substrate for the behavioral and Cognitive function.[213–215] Nevertheless, Cognitive and behavioral deficits can be studied also in relation to structural connections between brain regions that are affected by the focal injury, and several fMRI studies have shown that networks can be disrupted even by distant lesion through disconnection and diaschisis mechanisms.[163,169,216] As discussed in the previous chapter, these phenomena show how white matter disconnections in a determinate area lead to both functional and anatomical changes (reduced elaboration of inputs and outputs, transneuronal degeneration, reduction of dendrites and synapses density, myelin sheets alterations).

Despite their focus on the fluency category, the package introduced by Foulon et al. can be used to analyze the pathophysiological mechanism underlying Cognitive deficits, and to determine the relationship between these mechanisms and the clinical outcome. This tractography-based approach was used to relate white matter tracts' disconnection with clinical performance (fluency, in this case). Additionally, using rs-fMRI they were able to identify large networks of

interconnected areas, and their disconnection in functional connectivity following the lesion.

The analysis of white matter tracts and of the disrupted cortical-subcortical areas highlighted the presence of “maps of disconnection”, adding further evidence to the relationship between disconnected areas and patients’ performance.[217]

Low dimensionality of stroke clinical syndromes

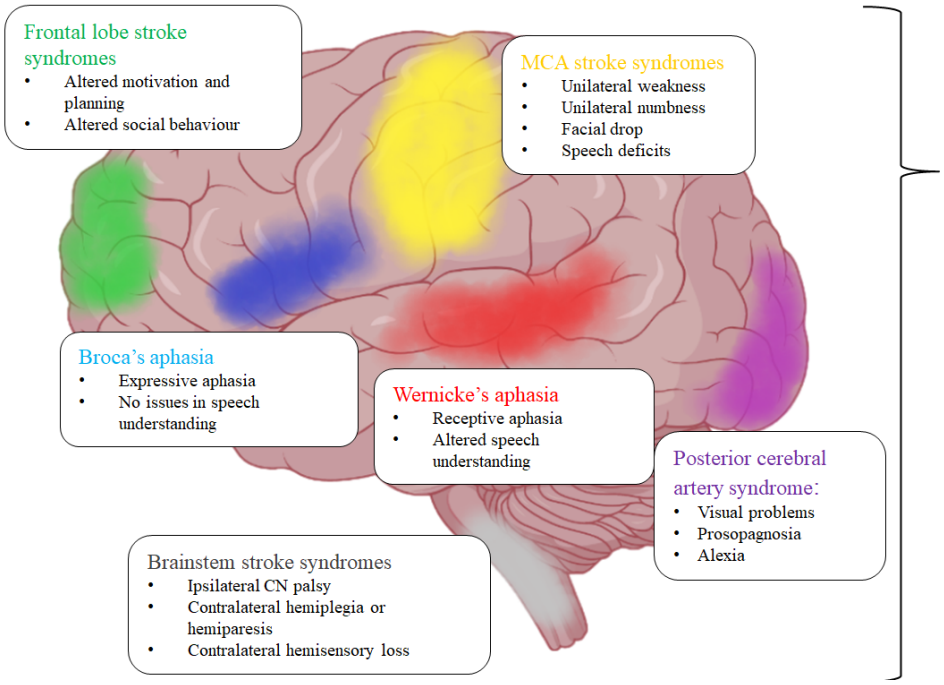


Figure 16 Traditional model of stroke syndromes

The classical view classifies behavioural syndromes based on the damage of specific brain regions (e.g., Broca aphasia) or the vascular distribution of stroke [e.g., middle cerebral artery (MCA)(see *Part I*, section "*clinical syndromes*"). Recent work offers evidence that these classical syndrome-based descriptions do not characterize behavioural deficits at the population level; conversely, a few factors account for the vast majority of inter-individual variability in behavioural performance. The examination of samples of stroke patients with the National Institutes of Health Stroke Scale (NIHSS) identifies two factors: one for left and one for right hemisphere lesions, which split respectively in a Cognitive and sensory-motor component, accounting for approximately 80% of behavioural variability across subjects.[87,218] A possible critique is that the NIHSS measures Cognitive deficits only coarsely.

However in a separate study, Corbetta et al. used principal component analysis (PCA) measured performance with an extensive neuropsychological battery and found that behavioural variability analysed with a principal component analysis can be summarized with three components that account for about 65% of variability.[219,220] The first factor encompassed language, including deficits of language expression and comprehension, and memory, both verbal and spatial.

The second and third factors loaded on the contralateral motor and visual attention deficits (i.e., left deficits for right lesions, and vice versa). Importantly, neither local damage nor vascular distributions could account for the observed correlation of deficits in different domains. These results were replicated by Bisogno et al. in an independent dataset using a shorter behavioural battery (NIHSS plus the Oxford Cognitive Screen, OCS) and multivariate ridge regression to examine the anatomy of these factors[221]. Interestingly, these factors tracked recovery at three and twelve months post-stroke .[220]

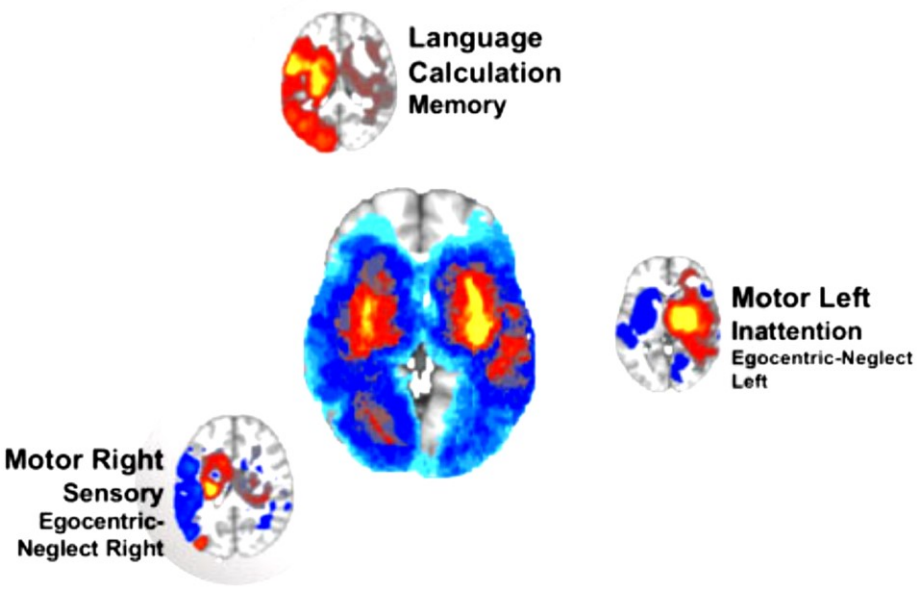


Figure 17 From Bisogno et al "A low dimensional structure of neurological impairment after stroke"[221]

Overall, these factors appear to be robust behavioural biomarkers for future stroke population studies for several reasons: consistency across different populations, consistency across neurobehavioral batteries, consistency at 3 and 12-months follow up, and finally can be described using a combination of clinically applicable batteries (National Institutes of Health Stroke Scale and Oxford Cognitive Screen). [88,89]

Most crucially, the entirely data-driven strategy offers topographical correlates for post-stroke multi-domain damage. High PC1 (language, memory, calculation, praxis) scores were primarily associated with damage to the left cortico-subcortical areas; high PC2 (left motor and visual attention) scores were associated with damage to the right cortico-subcortical regions; and high PC3 (right motor) scores were associated with damage to the left subcortical regions.

These findings raise several intriguing questions. Firstly, stroke lesions do not cause specific behavioural deficits but correlated deficit components across multiple domains of function. For instance, alterations of language (both expression and comprehension) co-vary with deficits of verbal and spatial memory. Moreover, changes in spatial memory over time bias changes in motor, attention, language, and verbal memory, as if spatial memory was a Cognitive 'hub'.[220] A possible interpretation is that the interdependence of behavioural deficits reflects the interdependence of physiological processes that are represented in a distributed network rather than in local modules.[221]

Secondly, these component deficits can be significantly associated with certain locations in the brain,[221] and this localization does not match vascular distributions.

Thirdly, while location explain some variance, more prediction can be derived by including also structural and functional connectivity information across the whole brain. Specifically, Cognitive impairments were more dependent on changes in multi-network functional connectivity, whereas sensorimotor abnormalities were more accurately predicted by structural factors.[222] Consequently, models that incorporate pathophysiology data (such as white matter disconnection and f-MRI analyses) were superior in predicting behavioural biomarkers consistent with a network view of behavioural deficits after focal injury.[217,223,224]

This acknowledgment represents a fundamental shift: from the traditional description of stroke syndrome related to a specific vascular territory, with highly specific dissociations, to a wider framework with interactions across highly integrated neural domains. This high integration justifies the clustering of post-stroke neurological impairments in only three sets of correlated deficits across different behavioural domains.

How can we explain the low dimensionality of behavioral deficits? The covariance of deficits is explained by principles of organization of the healthy brain. Billions of neurons and glia are organized in microcircuitry that give rise to circuitries of circuitries at the mesoscale level and functional brain regions. Brain regions in turn are connected by white matter tracts that preferentially connect certain systems or networks. At the functional level this anatomical segregation is

weighted by patterns of activity that give rise to a small number of functional networks (~7-15 networks at 3T in a healthy human). Finally, we know that a lesion produces a few canonical patterns of functional connectivity alterations that further decrease the dimensionality of structure-function relationships, and a decrease in the variability of neural states (entropy) that the brain can generate.[225,226]

The interaction between a normal brain architecture that organizes billions of neurons in a dozen of macroscale networks, and the effects of lesions on these networks explain qualitatively the observed relationships between lesion and/or network-based measures with behavioral deficits. Lesion location and volume are constrained by the vascular distribution of stroke syndromes and predict some variance. They are more sensitive for deficits whose functions are more localized. As an example, damage of the motor cortex or corticospinal tract is strongly associated with motor impairment.[180] The same for visual deficits in visual cortex and geniculo-calcarine tract.[227] Conversely, deficits for functions that are more distributed such as attention, working memory, or language will be more likely predicted by network measures that integrate across multiple locations. A lesion in the temporal cortex or frontal cortex will each cause some language impairment. This relationship will be averaged out in terms of location, but enhanced at the level of a network (language).[195]

PART III

How will I recover from stroke?

Stroke is the third most common cause of disability and second most common cause of death worldwide.[10] A broad variety of factors influence stroke prognosis, including age, stroke severity, stroke mechanism, infarct location, comorbid conditions, clinical findings, and related complications. Interventions such as thrombolysis, mechanical thrombectomy, stroke unit care, and rehabilitation can play a major role in the outcome of ischemic stroke. Clinicians usually face the challenge to predict the outcome of stroke patients. Prognosis is fundamental to provide a rational approach to patient management and to guide the patient and family understanding of the course of the disease.

The first 7 days after a stroke are of uttermost importance for stroke recovery: the majority of patients with no complications undergo moderate but steady improvement in neurological impairment.[228] Generally, the highest proportion of recovery occurs in the first 3-6 months after a stroke, with obvious variations about the degree of disability.[229,230]

The following paragraphs will discuss the main prognostic factors, with a specific focus on the acute stroke clinical setting .

Pre-hospitalization prognostic factors

In the acute management of stroke, patient's age and stroke severity are the strongest predictors of outcome. The latter can be classified clinically, according to the degree of Cognitive impairment and to size and location of the lesion on specific neuroimaging studies. Other meaningful effects on stroke outcome are determined by ischemic stroke mechanism, comorbidities, epidemiologic factors, stroke complications.

Stroke severity

The severity of stroke on neurological examination is likely to be the most important influence on both short- and long-term outcome.[231] The quantitative

assessment of neurological impairment is often defined using the National Institutes of Health Stroke Scale (that we discussed in the first part), and several studies have demonstrated its role as a good predictor of stroke outcome.[83,232,233]. In particular, an NIHSS score <6 usually predicts good recovery, while a score >16 is more often associated with high probability of death or disability.[234] Importantly, the NIHSS (and consequently the neurological impairment it mirrors) changes in relation to the time elapsed from the stroke onset,[235] partially for the intrinsic instability of early stroke-related deficits, and partially due to the gradual recovery that many patients undergo.

Age and other comorbidities

A multitude of studies have demonstrated that advanced age has a dramatic negative impact on stroke morbidity, mortality as well as long term outcome.[236–238] The effects of age in stroke outcome is visible independently from lesion size. It has been shown that age higher than 65 relates with and increase mortality rate in the two months following stroke.[239]

In addition to age, several comorbid conditions have to be taken into account when assessing the risk of poor outcome after an ischemic stroke: the most common include anemia, atrial fibrillation, cancer, coronary artery disease, dementia, dependency, diabetes mellitus or hyperglycaemia, heart failure, renal dysfunction, poor nutritional status.[240]

Epidemiologic factors

Stroke recovery can be affected also by variance in sex, race, socio-economic status. In particular, it has been demonstrated that men are more likely than women to have higher mortality but less disability after ischemic stroke;[241–243] nevertheless, the difference is mainly related to other factors such as age, stroke severity and pre-stroke dependency.[244]

Several American studies highlighted the differences of outcome from Black Americans and other minorities in comparison to White Americans: in particular, non-white ethnic group, lower education level and socio-economic status, and lesser degree of social support are correlated with poor outcome following stroke and worse health –related quality of life after 5 years. It remains unclear if these

can be considered independent prognostic factors, or if they can all be related to lower economic status itself (since it is associated with increased comorbidities and stroke severity).[245,246]

Ischemic stroke mechanism

The aetiology of ischemic stroke affects the outcome prognosis in a variety of ways: patients affected by a lacunar stroke show a better prognosis up to 1 year after the event, but in the long-term this difference tends to fade. Also, cryptogenic stroke tends to have better prognosis in the first year after the event, in comparison to stroke of determined aetiology. In particular, stroke due cardio-embolism or large artery disease are related to worse recovery.[247–249]

Early neurological recovery

Several studies have shown an 80% proportional recovery rule whereby most deficits, irrespectively of their initial level of impairment, will improve by a fixed amount.[250] This additive relationship determines that patients with relatively mild deficits early on will tend to normalize more likely than patients with severe deficits who will reach chronically only partial recovery.[251]

Post hospitalization prognostic factors

Neuroimaging: infarct volume, location, other findings

Neuroimaging studies represent a fundamental tool to match with clinical investigation in the process of prognosis assessment. Infarct size and location are both important aspects in this assessment: regarding the former, it has been validated to estimate stroke outcome in a variety of studies,[252] also combined with NIHSS score.[253] Nevertheless, in the majority of reports only supratentorial infarcts are analysed, and these results may not apply to posterior circulation or infratentorial infarcts where even a small volume is likely to result in severe disability.[254,255].

In addition, other imaging attributes may indicate poor outcome after a stroke. Diffusion-perfusion mismatch separates a core of infarcted tissue from the surrounding ischemic penumbra, where neural tissue can still be viable if re-perfused on time; if it doesn't happen, it represents a risk factor for lesion enlargement. Secondly, also poor collateral blood flow and development of cerebral oedema impact negatively on the outcome.[256,257]

Reperfusion therapy

The details of reperfusion therapy, both IVT and EVT, have been discussed in the *Part I*. In the following paragraph we will mention the impact of these procedures on the patient outcome.

In particular, full or partial recanalization up to 24 hours after onset of acute stroke is associated with a more positive outcome than persistent occlusion after thrombolysis.[258–260] As observed in several studies, factors associated with the response to thrombolytic therapy comprehend location of the symptomatic occlusive thrombus in the arterial tree, and clot-specific features such as size, composition, and source.

Regarding clot size and site, it has been shown that larger clots and more proximal clots (versus more distal location) are more resistant to thrombolysis;[261–263] this may be due partially to the larger size of clots that place in larger vessels.[264] In addition, in situ thromboses in large vessels associated with atherosclerotic lesions

may be more resistant to recanalization than fibrin rich embolic occlusions arising from the heart.[265]

Secondly, the age and composition of thromboembolic material likely affect its response to thrombolytic therapy.[266,267] The chance of recanalization in embolic stroke is related to the amount of red cells in the emboli and inversely related to the volume of emboli and to the fibrin content and density of the clots.[268] Thrombolytic drugs are unlikely to disrupt other types of embolic material, such as calcific plaque and fat.

With regards to mechanical thrombectomy, the 90 days prognosis is importantly affected by several clinical variables, including time to recanalization, NIHSS at 24h, final infarct volume, procedural complications, post-stroke complication (from acute sepsis to myocardial infarction), to end with rehabilitation.

In detail, 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).[269]

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 3 TICI scale, from Higashida et al.[270]

Finally, there are additional variables affecting outcome, such as age, sex, stroke severity, availability of collateral blood supply, and early ischemic change on CT or MRI. However, these factors do not necessarily predict which patients will or will not benefit from IVT; the only factor known to independently alter response to IVT is time to treatment. As an example, it has been demonstrated that each 15-minute reduction in the time to initiation of IVT treatment was associated with

an increase in the odds of walking independently at discharge (4 %) and being discharged to home rather than an institution (3 %) and a decrease in the odds of death before discharge (4 %) and symptomatic haemorrhagic transformation of infarction (4 %).[271]

Medical complications after stroke

Several conditions may affect stroke survivors during their hospitalization, influencing with various severity their recovery and functional prognosis. The most common clinical issues include falls (25% of patients), urinary tract infections (24%), chest infections (like aspiration pneumonia, 22%), pressure sores (21%); also depression has a role among these complications (16%).[272]

The mechanisms leading to the abovementioned complication may be different. Dysphagia is one of them and impacts greatly on general prognosis, since it is a common complication of stroke and is a major risk factor for developing aspiration pneumonia.[273] In addition, also venous thromboembolism (VTE) has to be mentioned: it encompasses deep vein thrombosis (DVT) and pulmonary embolism, which is potentially life threatening. VTE prophylaxis is indicated for all patients with acute stroke who have restricted mobility.

Stroke unit care

Several studies have highlighted the importance for patients with acute stroke of being early admitted to a specialized hospital unit, that can be reliable in treating all types of acute stroke, including ischemic, intracerebral haemorrhage, and subarachnoid haemorrhage.[274–276] Despite the precise components of an acute stroke unit may vary between centres and countries, they always generally include a hospital ward with dedicated telemetry beds and a permanent team of physicians, nurses and other personnel specialized in stroke care, emphasizing expertise in vascular neurology and neurosurgery.[277,278] In addition, prompt availability of neuroimaging (e.g., CT, MRI, various types of angiography, ultrasound, transcranial Doppler) and cardiac imaging would be recommended. An important component of stroke units as originally envisioned was the close proximity of acute stroke and rehabilitation beds.

Stroke rehabilitation

The aim of stroke rehabilitation is to ameliorate functional outcome and gain the highest possible level of independence, event taking into account the persistence of some stroke-related deficits.[279] Rehabilitation, early after stroke, is level 1 evidence, and it has an effect size that it is about 1/3 of thrombolysis. However, it can be given (theoretically) to the great majority of stroke patients in contrast to acute interventions that can be given to less than 30% of patients.

Rehabilitation should involve diverse professional figures, from the Physical to the occupational therapist, to the speech and language therapist. Health care systems in most Western countries propose inpatient rehabilitation services in the acute phase of hospitalization, typically starting one week after the event and lasting from two to six weeks depending on the stroke severity. As for what regards the exact dose and timing of the several rehabilitation techniques, further studies are needed, but as a main principle rehabilitation should be individualized according to the specific patient's needs and hospital resources.[279,280]

PART IV

Macroscale imaging: a potential biomarker for post-stroke functional outcome?

Introduction

Historically, stroke patients' outcome had been studied according to the specific syndromes related to the damaged vessels. Recent times have seen a paradigm change towards the examination of network dysfunction rather than just lesion volume, with a focus on the modification of connections between distal brain regions.

In fact, stroke causes alterations of functional connectivity (FC) measured with functional MRI (fMRI) in widespread parts of cortex that appear structurally normal.[183,205,281]

However, the evaluation of behaviour and MRI signals associated with network-level dysfunction in patients is rather difficult in a clinical setting. A possible approach to overcome this problem has come from the indirect measurement of these disconnections, through large databases of functional and diffusion MRI data and the generation of the so-called 'connectomes'.[282,283]

We have previously described (See chapter *"Clinical implication of the stroke disconnectome)* two different approaches. The first one, known as 'lesion network mapping',[209,284] computes whole-brain functional connectivity directed to or from the lesion, measuring the temporal correlation of the fMRI signal between the lesion and the rest of the brain. The second approach, known as the 'dis-connectome', evaluates structural disconnection from clinical structural MRI lesions.[217] This method estimates the probability of normal white matter tracts (measured with diffusion imaging in a population of healthy subjects) that pass through the lesion. In a structural disconnection (SDC) map, each voxel in the brain indicates the probability of structural disconnection caused by the lesion to healthy white matter tracts.[285,286]

These indirect measures certainly are useful in the evaluation of behaviour, but their prognostic role in functional outcome has not been yet determined. A correct

prediction od functional recovery is of uttermost importance for treatment planning, patient and family counselling, resource allocation, and finally to drive research and quality improvement efforts in stroke care.

Currently the most used prognostic factors (described in the Part III of this thesis) include lesion volume and vascular imaging (e.g. the degree of reperfusion).

The aim of this thesis was to analyse if advanced imaging analyses using readily available clinical scans can improve the prediction of long term outcome post-stroke as measured using and internationally accepted and worldwide used self-report assessment scale, the Stroke Impact Scale (SIS) 3.0.

We contrasted local measures of damage (location, volume, and local features on diffusion scans) with network measures of damage (structural and functional disconnection). Crucially all these measures were derived by embedding the stroke lesion, obtained through clinical scans, onto normative atlases of healthy human subjects. This approach, if successful, would be easily implemented through an automatic pipeline and could become part of the clinical assessment of stroke patients.

Materials and methods

Study sample

The patients recruited in this study were collected at the Neurology Clinic and Stroke Unit of the Padova Hospital and the Stroke Unit of the S. Antonio Hospital of Padova. They were prospectively recruited during a period of 11 months (from January 2018 to November 2018). Patients with a first-symptomatic stroke, either ischemic or haemorrhagic, were selected according to the following inclusion and exclusion criteria.

Inclusion criteria: (1) Age 18 or greater. No upper age limit; (2) First symptomatic stroke, ischemic or haemorrhagic; (3) Up to two ischemic lacunes, clinically silent, less than 15 mm in size on CT scan; (4) Time of enrolment: < 2 weeks from stroke onset; (5) Awake, alert and capable of participating in research

Exclusion criteria: (1) Previous stroke based on clinical imaging; (2) Multifocal strokes; (3) Inability to maintain wakefulness in the course of testing; (4) more than two asymptomatic lesions on CT scan; (5) Presence of central nervous system Neoplasms; (6) Presence of neurodegenerative diseases; (7) Previous central nervous system surgeries; (8) Presence of schizophrenia, bipolar disorder, major depression, or other severe psychiatric conditions; (9) Presence of other medical conditions that preclude active participation in research and/or may alter the interpretation of the behavioural/imaging studies; (10) Absence of the patients' consent.

To measure long-term functional outcome, from January 2019 to May 2019 a telephone assessment using the Stroke Impact Scale 3.0 (SIS 3.0) was given to all previously selected patients (N 114) whose stroke occurred either 6 or 12 months prior.

The sample described is a subgroup of a larger dataset (Padova Stroke Project) composed by N=237 patients with a first-symptomatic stroke, either ischemic or haemorrhagic, whose acute data have been previously published by our group.[221]

Each subject was evaluated with a neurobehavioral battery at the acute phase that included OCS and NIHSS within the first week after stroke symptoms onset.

Structural imaging (MRI scans and CT scans) was routinely collected for each subject at an average of 5 days post-stroke.

From January 2019 to June 2019, all patients were approached with a telephonic assessment by means of the Stroke Impact Scale 3.0 (SIS 3.0) to evaluate long-term functional outcome at either 6- or 12-months post stroke. Approximately half of the patients (n=58) were contacted by telephone at 6 months (187±20 days) after the first neurobehavioral assessment, 38 underwent the follow-up assessment. Patients (n=56) were contacted at 12 months post evaluation (378±15 days), with 36 undergoing the follow-up assessment. For 40 out of 114 it was impossible to collect the follow-up assessment for several reasons: the great majority didn't answer the calls, others had a second stroke or died, and a few did not accept evaluation. In six cases we were unable to administer the SIS directly to the patients for different reasons: incapability of using the phone, aphasia and poor general conditions. In such cases, we administered the test to proxy respondents (the proxy version of the SIS has already been validated).[111]

The final study sample consisted of patients who met post-enrollment inclusion criteria and underwent both acute and long-term assessment (n=74).

The mean age of the sample at enrolment was 66.8 years (ranging from 21 to 88 years old). Male subjects were the majority of patients (62%) over females, namely n=44 and n=30 respectively.

The length of education ranged from five to twenty years, with an average of 10.5 years.

Behavioural assessment

All recruited patients were assessed within the first week post-stroke after symptoms onset with an acute neurobehavioral battery including NIHSS and mRS scales. The NIHSS is a battery of 15 subtests: level of consciousness, gaze and visual field deficits, facial palsy, upper and lower motor deficits (right and left side), limb ataxia, sensory impairment, inattention, dysarthria, and language deficits. The total score was used as a measure of initial stroke severity. In particular, the NIHSS was evaluated both at hospitalization and at discharge of each patient. The mRS is a 6-point disability scale with possible scores ranging

from 0 to 5 (a separate category of 6 is usually added for patients who expire). The mRS was evaluated before stroke (namely pre-stroke mRS) and at discharge for each patient.

Outcome was measured either at six (n=38) or twelve months (n=36) using the SIS 3.0 during a telephone interview. The SIS 3.0 is a stroke-specific outcome measure that consists of items measuring eight domains of function (strength, hand function, activities of daily living/instrumental activities of daily living, mobility, communication, emotion, memory and thinking, and participation). When patients were not available to undergo the telephonic interview on their own (for incapability of using the phone, aphasia, or poor general health conditions; n=6), we administered the SIS 3.0 to their relatives or other proxies living with them.

SIS factorial analysis

We ran a Factorial analysis on the SIS domains as a data reduction strategy. The resulting principal factor were defined as Physical, Cognitive and Emotion (further details are discussed among the Results).

Imaging acquisition and lesion masks

The full MRI protocol is described in Bisogno et al (2021).[221] Scanning was performed with a 3 Tesla MR scanner at the University Hospital of Padova in Neuroradiology Department, including structural, functional and diffusion imaging. For 60 out of 74 images, lesion segmentation was manually performed on individual structural MRI FLAIR images and CT scans using ITK-SNAP 3.6, a free and open-source software application for medical image segmentation, visualization, and analysis. DICOM files were transformed into NIFTI format using dcm2niix toolbox. Lesion normalization occurred with a non-linear transformation using the Advanced Normalization tool (ANTs) onto the MNI brain atlas.[287] This method used a cost function mask approach to improve the registration of the FLAIR or CT image. The normalization matrix was later applied to the lesion mask. Finally, the transformation matrix was resampled to a 2x2x2 mm space and applied to the lesion masks using nearest neighbour interpolation.

Advanced imaging lesion properties: local measures

Lesion shape and volume

As proposed by Cheng et al, several morphological features of the lesion can be measured using algorithms.[143] In particular, we studied the shape of the lesions employing the normalized shape factor of the ischemic lesion, mathematically calculated as:

$$S = (\sqrt{A}/\sqrt[3]{V})/2.199085233$$

Where A indicates the surface and V the volume of the lesion. This index yields a value of 1.0 for a sphere and rises as the form deviates from a perfect sphere; in other words it measures how closely the lesion resembles a sphere. As for what regards the volume of the lesion, it is measured by counting the number of voxels occupied by the lesion.

White matter tract density index

Tract Density Index (TDI) is used to quantify the density of neuronal fibres in a specific region of the brain. TDI is calculated by counting the number of tracts, or bundles of neuronal fibres, that pass-through a given voxel (a 3D unit of measurement in MRI imaging) of the brain. The TDI value of a voxel reflects the number of tracts that pass through that voxel, normalized by the volume of the voxel.

Data from healthy controls comes from the Human Connectome Project Team[288] . Each term is computed by calculating the difference between hemispheres for each pair of homologous voxels (in accordance with Karolis et al).[289] The resulting average streamline map was calculated from the HCP dataset to construct 180 HCP full brain tractographies registered to the MNI space. The mean number of white matter streamlines travelling through each voxel of the template is depicted by this average streamline map. The average streamline map was then overlaid with the normalised ischemic areas of each patient. These lesioned areas were subsequently employed as a mask to calculate a DI, which is equal to the total of the streamlines (per voxel) within the ischemic lesion mask. A

larger DI will be the result of an ischemic lesion that is situated in a part of the brain with more streamlines.

The complete description of this methodology has been described by Salvalaggio et al, and it has proven to have high accuracy prediction of "long" or "short" survival, better accuracy than classical prognostic factors, and has proven valid in two independent datasets.[290]

Diffusion metrics

The diffusion metrics analysis was performed using the Human Connectome Project dataset, using 1065 healthy individuals' diffusion tensor imaging (DTI). As explained by Pini et al,[291] diffusion weighted imaging (DWI) maps were preprocessed according to standard methods and used to compute DTI and NODDI outcomes, respectively FA, MD,AD, RD and ODI, ISOVF, ICVF.

Afterwards, a factorial analysis was performed independently for each subject. In this way, from 7 different maps (one for each DTI and NODDI outcome) they obtained 3 latent factors that were namely defined "Diffusion", "Hindered" and "Restricted".

In our study, we used these three latent factors to investigate the main microstructural brain properties (computed with different DWI models), how the ischemic lesions alter them and how these changes are related to the functional outcome after stroke.

Advanced imaging lesion properties: network measures

In our work we analysed both structural and functional disconnection maps (respectively referred to SDC and FDC), under two points of view: focusing first on the patterns of disconnection, and secondly on how these disconnections affect whole brain networks.

SDC patterns

We employed the BCB toolkit125 to assess structural disconnection, which indirectly calculates the structural disconnection caused by a lesion.[292] The damaged structural pathways are inferred by integrating the lesion in a normative

structural connectome derived from a healthy subject population ("Human Connectome Project", HCP7T).[1] The lesions were resampled to 1x1x1 mm in MNI space after being normalised. This area was designed to coordinate with one of the tracts found in the BCB toolset. Afterwards, we computed the probability that a white matter bundle directly linked to the lesion passes through each voxel. In other words, in a SDC map each voxel accounts for the inter-individual variation in tract reconstructions in controls and enables us to infer the likelihood that a lesion disconnects particular brain voxels (with the chance of disconnection ranging from 0 to 1 for each voxel).[293] Therefore, this approach indirectly estimates the degree of structural disconnection.

FDC patterns

Functional disconnection was quantified according to previous studies.[195,209] Each lesion was incorporated into a normative connectome of 173 HCP dataset individuals scanned with a 7T MRI scanner. The normalised lesions from our patients were binarized, resampled to 2x2x2 voxel space, and used as the seed region of interest for FC (functional connectivity) computation.

In seed-based FC analysis, functional disconnection has been typically calculated using patients' binarized lesions.[223] This method can present issues when seeding large lesions, which have numerous functionally distinct sections coupled to various networks.[37,210] Consequently, we used an approach suggested by Pini et al.[294], in which PCA is first performed on the connectivity of all voxels within each lesion in a sample of neurologically healthy people. The primary within-lesion connection axis was chosen to be the first principal component retrieved since it explains the greatest amount of variance.

By averaging the sign time-course over all voxels within the lesion, whole-brain temporal correlation maps were created utilising the entire lesion as the seed region of interest.

In other word, this approach produces a full brain map that expresses the strength of the connection between the lesion and the remainder of the brain. This method

parallels structural disconnection since this map is an indirect assessment of brain "disconnectivity" between the lesion and the brain.

In a successive analysis, we used a PCA to examine similarities in the SDC and FDC patterns. In particular, invidual SDC maps and FDC maps were separately analysed to derive the most indicative SDC and FDC spatial patterns.

To better exemplify the difference between structural and functional disconnection, we provide a comparison of the two different maps from a patient's subcortical lesion (black shape in the right panel).[295]

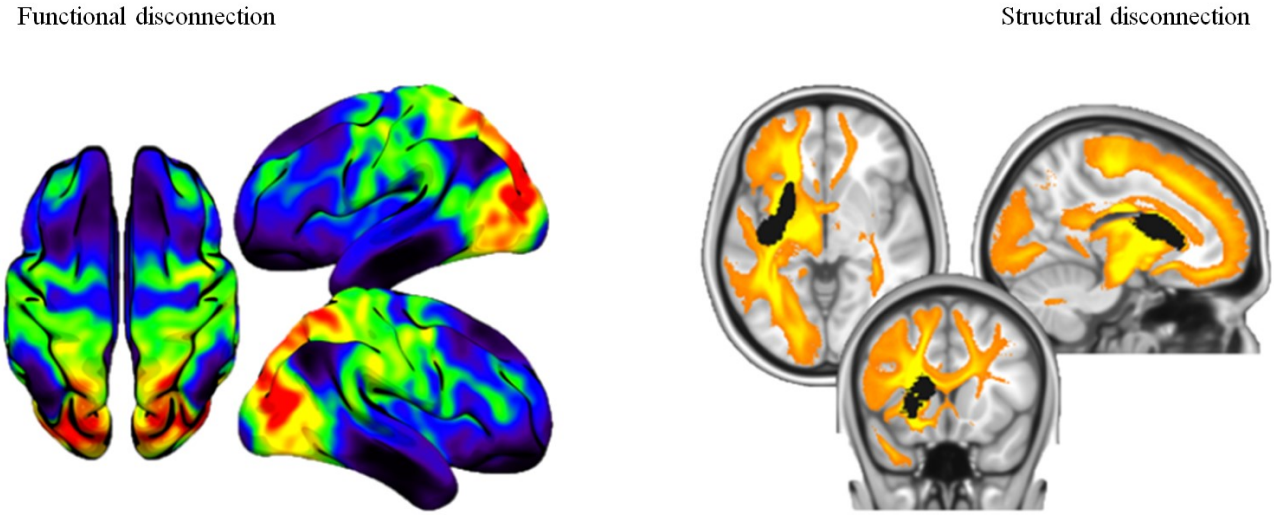


Figure 18 Exemplative maps of functional and structural disconnection from a patient with a subcortical lesion (black shape in the right panel

SDC and FDC networks

From a brain network perspective, we used the functional atlas suggested by Yeo et al,[296] that divides the brain into grey matter parcels belonging to the so-called "functional resting-state networks".

The functional atlas was generated from n=1000 fMRI scans. In short terms, a clustering method was used to identify functionally connected regions and to separate them into different networks.[297] In this study, this parcellation was employed to examine the impact of the prediction evaluated using various network dimensions. The default mode network (DMN), frontoparietal network (FPN), dorsal attention network (DAN), ventral attention

network (VAN), sensorimotor network (SMN), and visual network (VIS) are all included in the seven networks atlas.

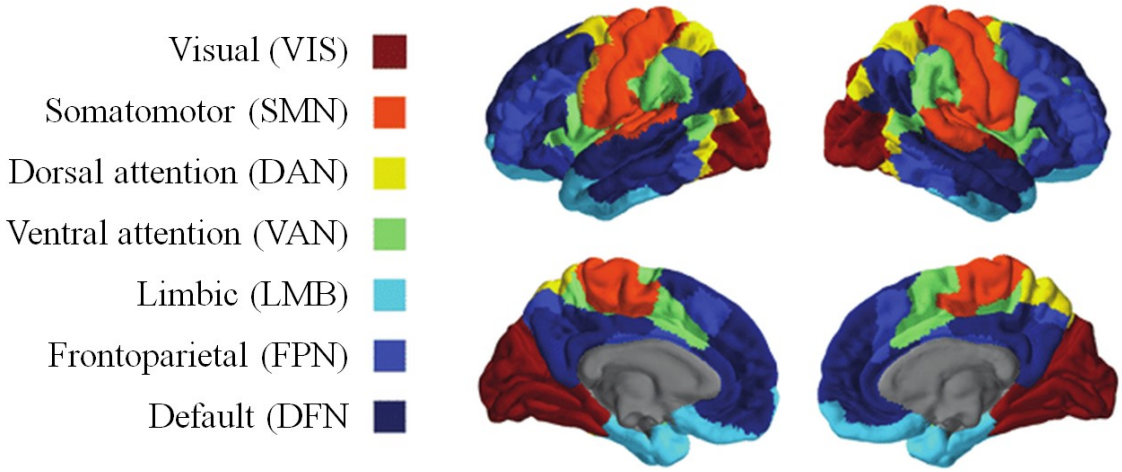


Figure 19 Example of Seven Yeo's template Networks[298]

In the first place, we intended to analyse the impact of SDC maps on these networks. In other words, we calculated how many voxels of each network were overlapping with the SDC maps, calculated as described above. Since there is a continuous probability that a functional network tract overlaps with a tract of the SDC map, we put a threshold of 0.5 (meaning that we kept voxels whose probability of belonging to a tract was more than 50%).

In the second place, we examined FDC disconnection measuring the average strength disconnectivity network. It can be explained as how much FDC of a single network is affected for every parcel included in the lesion.

In our study, data from 3 patients failed the FDC analysis procedure.

Statistical analysis

We performed a multivariate analysis with a ridge regression approach to enhance the prediction of function impairment (measured with the Stroke Impact Scale) though a procedure that lessens the error between real and predicted scores. This method provides an approximation of the highest possible explainable variance.

To evaluate the performance and generalization ability of our predictive model we employed the "5-fold cross-validation" technique. In this process the available

dataset is divided into five subsets or "folds" of approximately equal size. Consequently the model is trained and evaluated five times, with each iteration using a different fold as the validation set and the remaining four folds as the training set. By using cross-validation, the variability of the model's performance can be estimated, which helps in assessing how well the model is likely to generalize to unseen data.

In addition to this process, we used a bootstrap aggregating procedure (often known as “bagging”), for data augmentation. To explain briefly, with this procedure a specified number of subsets of a dataset are extracted multiple times with replacement ("bagging"). The outputs are then combined after each of these subgroups has been subjected to a machine learning algorithm.

Finally, we compared the R^2 (R squared) of each model to compare the fitness of every model to the given data and the ability to explain the variability in the target data. When comparing R^2 values, a higher value generally indicates a better fit or a higher proportion of the variance explained.

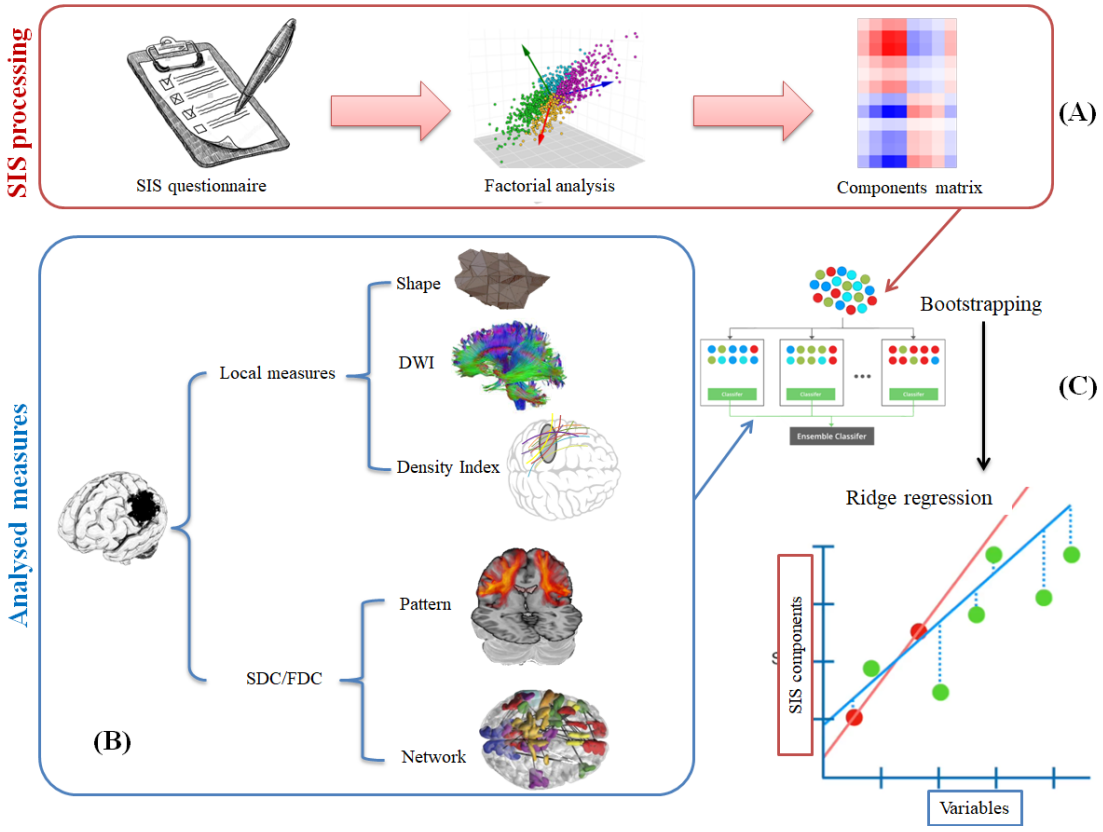


Figure 20 Workflow of our analysis:

(A) SIS processing via factorial analysis; (B) Identification of the 5 models to be compared; (C) Statistical analysis

Results

Participants

The final study sample included 74 patients. The mean age at enrolment was 66.8 years (ranging from 21 to 88 years old). Male subjects accounted for most patients (60%) over females, specifically n=44 and n=30 respectively.

The length of education ranged from five to twenty years, with an average of 10.5 years.

Anatomy of stroke

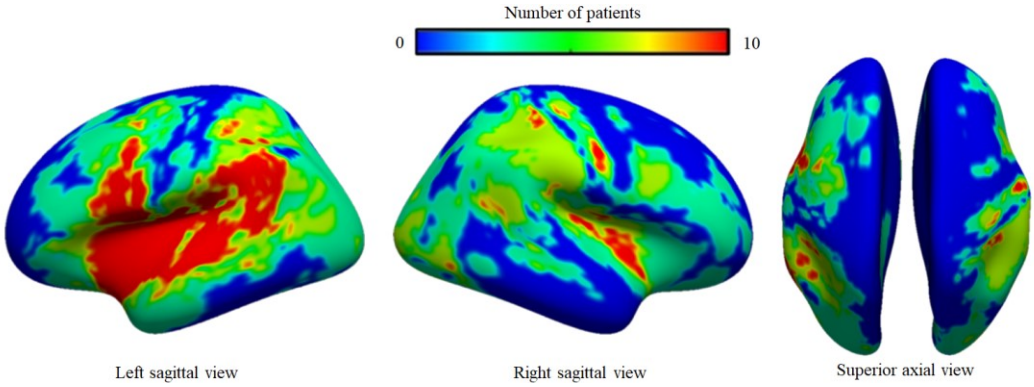


Figure 21 Lesion frequency map, where warmer colours indicate higher frequency of lesions

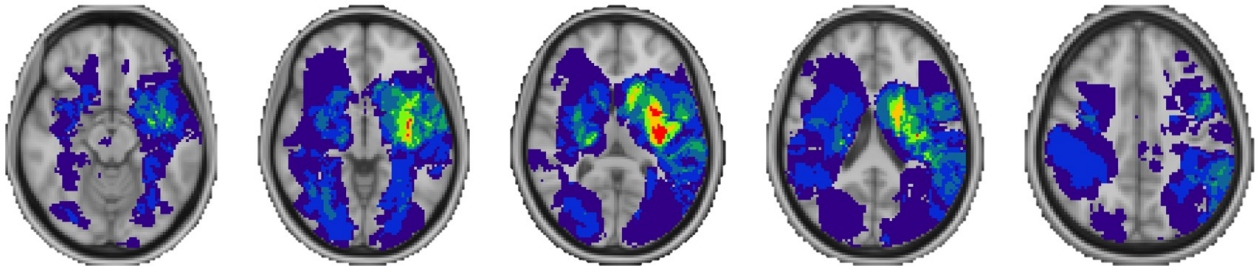


Figure 22 Lesion frequency maps: axial sections to evaluate the subcortical locations of damage.

The lesions were mainly located in the left hemisphere, particularly in the frontal, temporal and parietal hemispheres surrounding the Sylvian fissure (lateral sulcus). In Figures 21 and 22 the frequency maps of the lesions are reported.

SIS Factorial Analysis

The SIS factorial analysis identified 3 main factors. A Physical factor accounted for 32% of covariance, an Emotion factor for 20% variance and a Cognitive factor for 19% of covariance. Specifically, the Physical factor loaded on the sub items “Physical outcome, ADL, Mobility, Hand function”; the Emotion factor loaded on “Emotional status, Participation”; finally, the Cognitive factor loaded on “Memory and thinking, Communication”.

Considering these three factors altogether, we were able to explain 71% of the behavioural variance. The three components were relatively independent from each other, as can be seen in the loadings map.

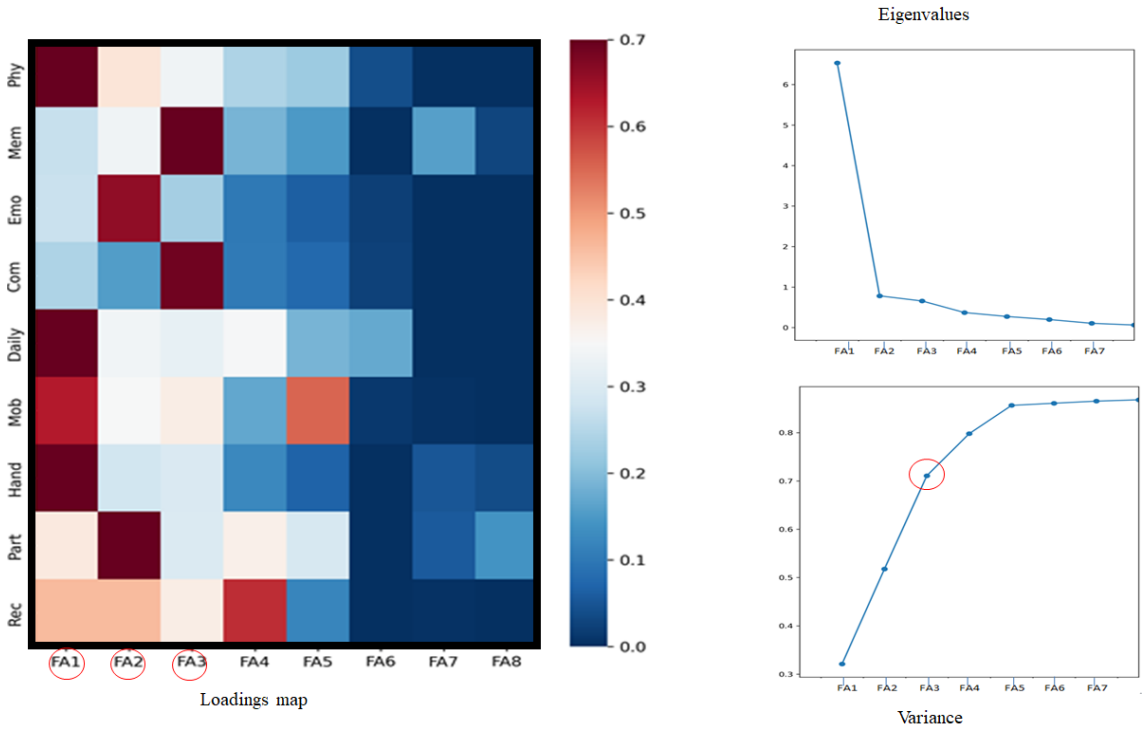


Figure 23 SIS factorial analysis showing loadings map, Eigenvalues, Variance

SDC patterns

We analysed SDC maps pattern performing a principal component analysis and the results highlighted 2 principal components (Figure 24 and Figure 25).

A two-component solution accounted for 40.1% variance of inter-individual variability (25.76% for the first component and 14.33 for the second component).

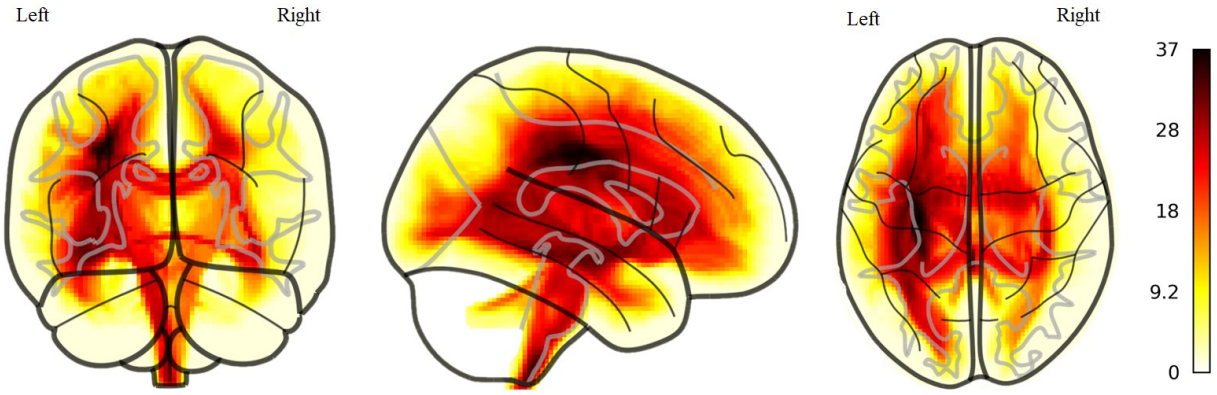


Figure 24 First component of PCA performed on SDC patterns.

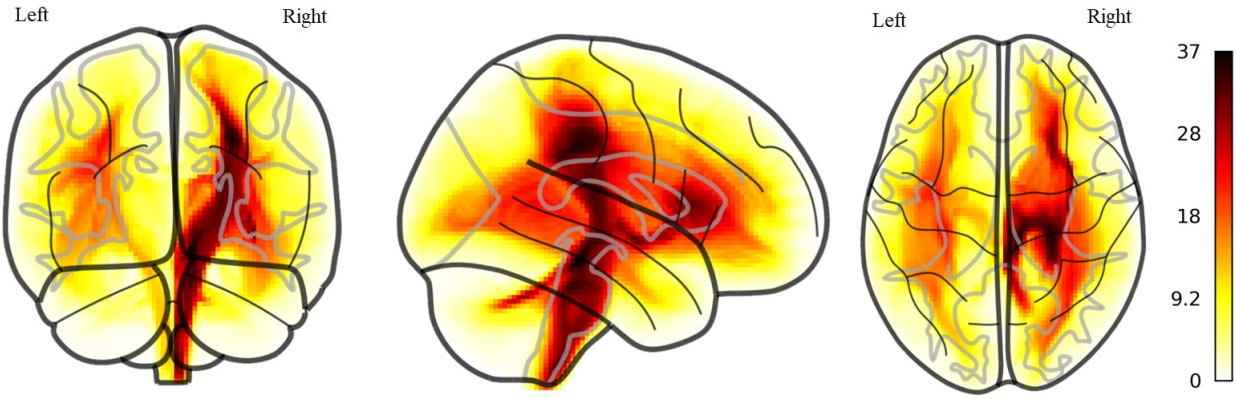


Figure 25 Second component of PCA performed on SDC patterns

The first component of SDC (Figure 24) showed a general involvement of the whole tractography map, and may represent the average map of our sample.
The second component (Figure 25) emphasized the right corticospinal tract.

FDC patterns

Next, we applied the same approach to FDC patterns. The resulting two components solution explained 89.21% of variability (82.18% for the first component and 7.03 for the second component).

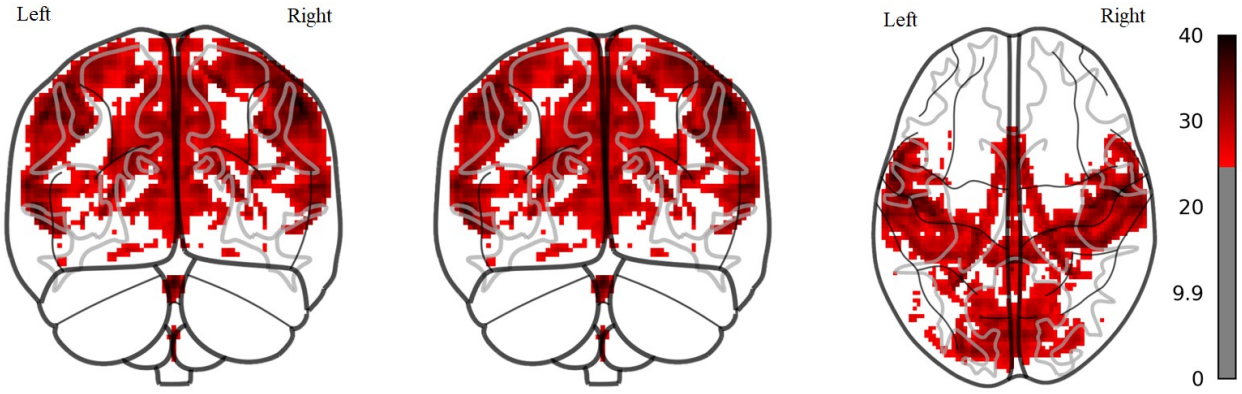


Figure 26 First component of PCA performed on FDC patterns

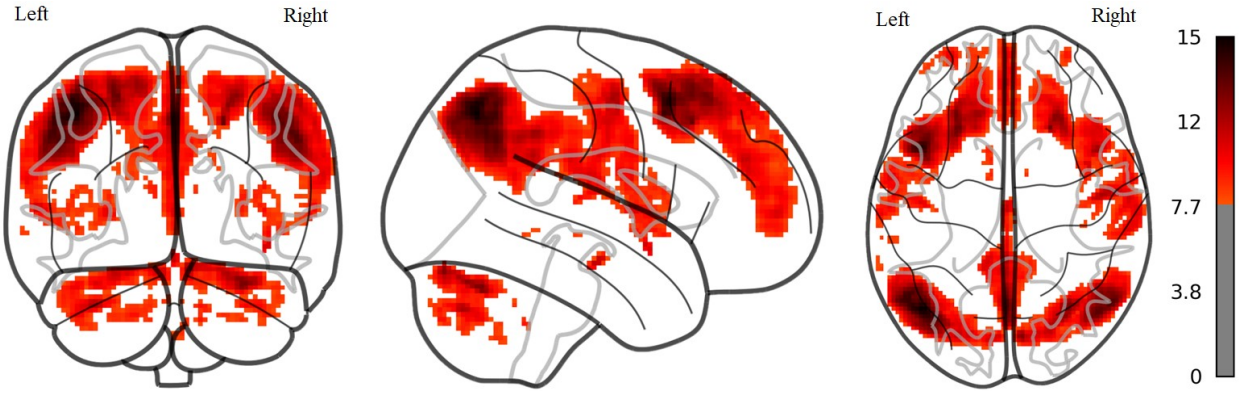


Figure 27 Second component of PCA performed on SDC patterns

Comparing these FDC maps with the Yeo's Seven Network Atlas,[296] it resulted that the first PC localised to the DAN and VIS networks. The second PC localised to the FPN network.

SDC and FDC networks

Furthermore, we studied the impact of SDC and FDC network disconnection on each of the seven Yeo's networks. We described the percentage of voxel overlapping between SDC maps and Yeo's functional network (Figure 28), and secondly the percentage of strength reduction due to FDC disconnection on Yeo's functional networks (Figure 29).

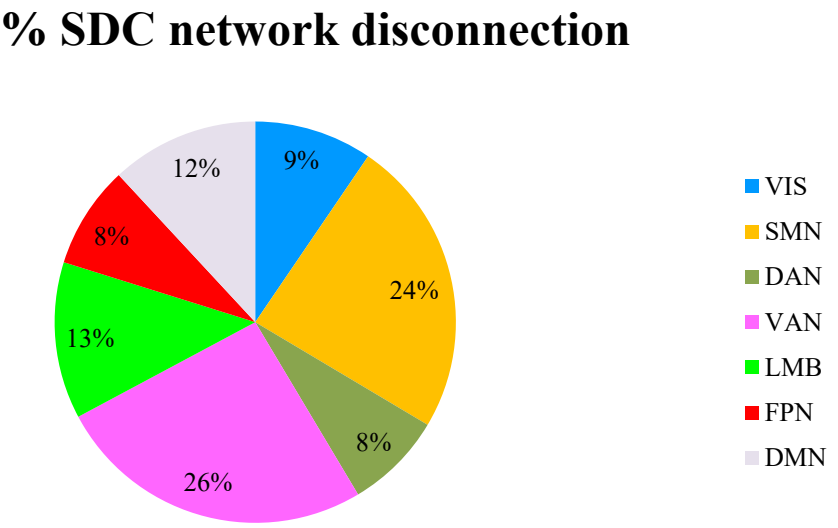


Figure 28 Pie chart of SDC network disconnection

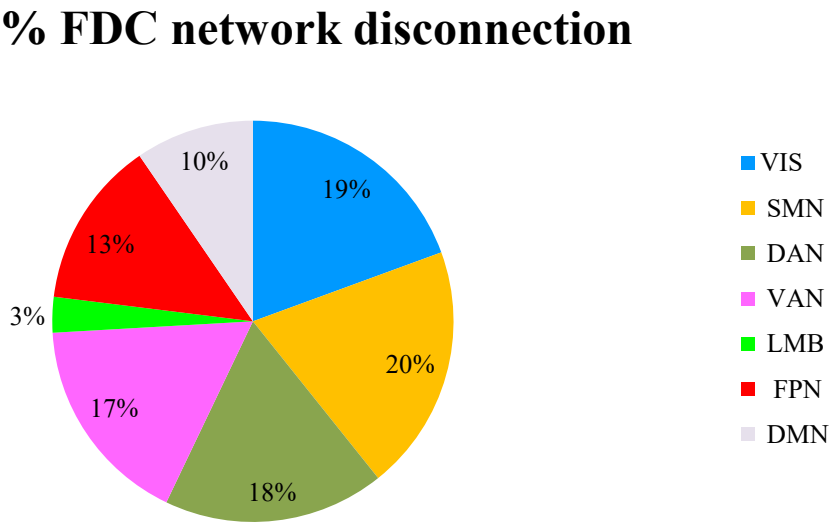


Figure 29 Pie chart of FDC network disconnection

Statistical analysis

We performed a multivariate analysis using a ridge regression model with bootstrapping procedure. Five models were analysed: shape and volume (1), TDI (2), microstructural diffusion metrics (3), SDC and FDC patterns (4), SDC and FDC networks (5).

Separately, we analysed the impact of demographic factors (age, sex, education) alone or in combination with each of the other models.

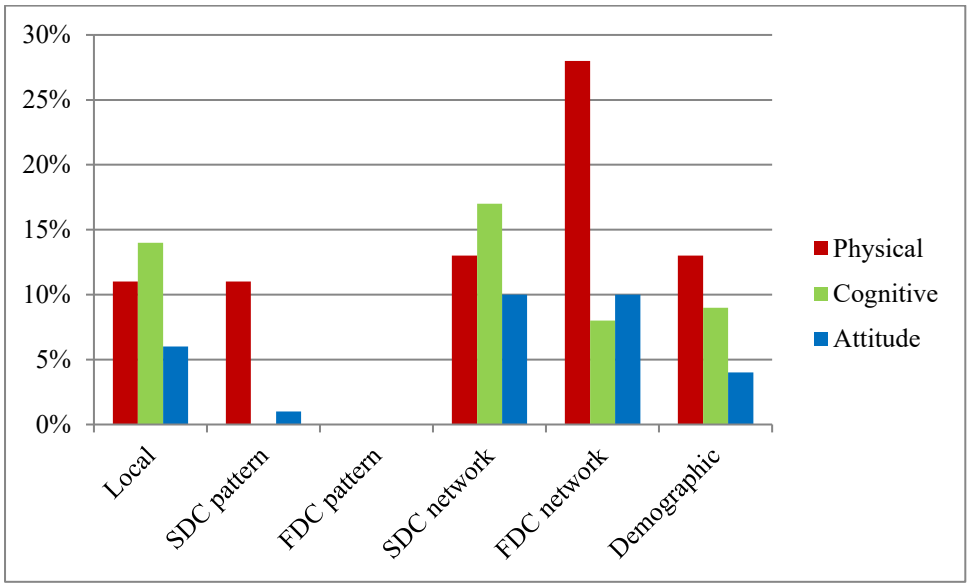


Figure 30 Comparison of the RR^2 of the five models and, separately, demographic factors

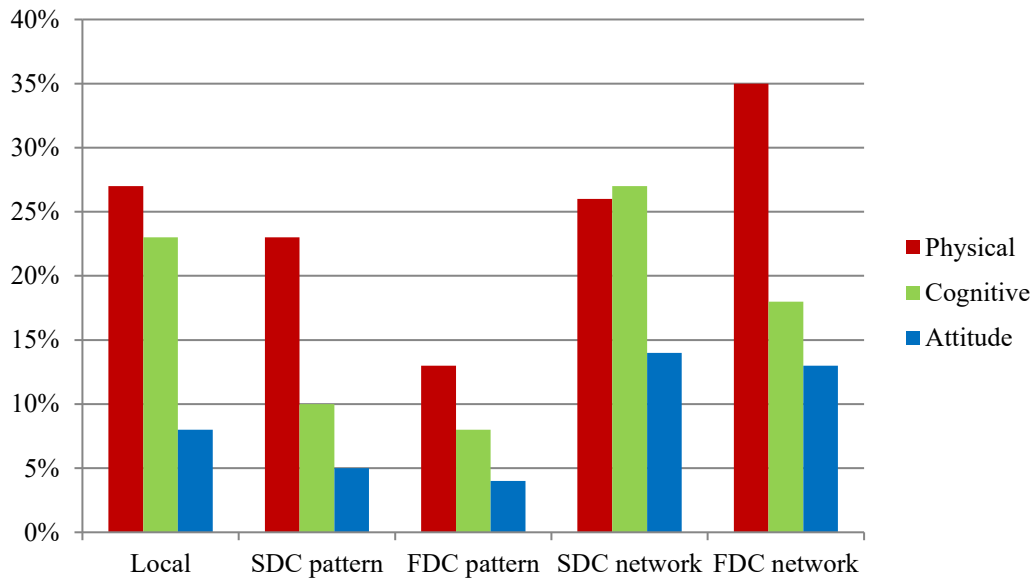


Figure 31 Comparison of the RR^2 of the five models including demographic factors

Local measures

The first model, that included shape and volume analysis, TDI and diffusion metrics, performed with low predictability if demographic factors were not considered (Physical 11%, Cognitive 14%, Emotion 6%). These values were slightly improved by the implementation of demographic factors, reaching a predictability of 27% for the Physical factor, 23% for the Cognitive factor, lastly 8% for the Emotion one.

SDC patterns

With reference to Cognitive and Emotion factors, the predictable covariance remained low, regardless the addition of socio-demographic factors. Only for the Physical factor the results showed a mild predictability (11%), equal to that of local measures.

FDC patterns

Ridge regression results were low if these measures were considered alone, and the addition of socio-demographic characteristics implemented only slightly the predictability of all three SIS components.

SDC and FDC networks

FDC network maps revealed the best result in predicting 28% of covariance of the Physical factor, reaching up to 35% with the inclusion of sociodemographic factors. If we consider demographic measures alone, it is evident an important increase (+22%) including FDC network measures. As for what regards the Cognitive and the Emotion factors, FDC measures didn't show better qualities than SDC in prediction, remaining statistically low.

On the other hand, results of SDC network analysis were slightly more favourable: they showed a prediction of 17% covariance for the Cognitive factor and of 13% for the Physical factor; if we include also sociodemographic measures in the analysis, the prediction rises to 27% and 26% respectively.

The results showed that the Physical SIS factor was the most predictable. FDC network measures performed best, when considered alone (28%) and with socio-demographic factors. FDC measures improved outcome prediction from 13% when considered alone to 35% when considered with demographics. The SDC maps related to this component showed higher disconnection frequency in the corticospinal tract.

Regarding the Cognitive factor, the level of prediction was only moderate (up to 27%) when considering SDC maps plus sociodemographic factors). Despite the evident size limitation of our study, we consider these data a possible implication that direct measure could allow higher values and more specific prediction, despite being more difficult to perform.

Finally, the Emotion factor consistently showed lower predictability, regardless of the measure used.

Discussion

This study examined whether advanced lesion properties implemented on clinical scans can improve functional outcome predictions in stroke patients evaluated with the Stroke Impact Scale at 6 and 12 months. We considered demographic variables, local lesion characteristics and network measurements of structural and functional disconnection. In addition, we described the factor structure of a commonly used functional outcome assessment following stroke (i.e. the SIS).

The factor structure of the Stroke Impact Scale

We identified three factors that explained the majority (71%) of variance. To our knowledge this is the first study to examine the correlation of SIS scores across subjects. It provides an interesting data reduction to the analysis of the SIS. The low dimensionality resembles the low dimensionality of the behavioural data in the acute phase.[192] For instance, acutely, multiple measures of motor strength, coordination, dexterity and function all correlate within a motor component [ref to Corbetta 2015]. Here, similarly, measures of Physical outcome, ADL, Mobility, and Hand function all collapse in a Physical component.

Functional outcome prediction: local measures vs. network measures

In this study we found that network measures (both SDC and FDC) performed better than local measures: a modest difference (+2%) for SDC network but a stronger level of prediction (+17%) for FDC network measures. The stronger predictive power of network over local measures is in line with the literature on impairment, and relates to the distributed functional effect (diaschisis) produced by local damage.[192,200,220]

As to our knowledge, it is the first time these network measures has been evaluated the SIS, with possible future clinical implications.

Functional outcome prediction: Physical vs Cognitive and Emotion factor predictability

Another important result of this study is the modest predictability of "Cognition" and "Emotion", in comparison to the "Physical" factor with both local and indirect FDC (and SDC) methods. The inherent organisation of functional connections may be a factor in the increased prediction of sensory and motor

impairments. Comparing sensory and motor cortices with associative ones, it is evident that we face a unimodal organization in the former, and a trans-modal organization in the latter.[299] The structure of the trans-modal areas might enable a more adaptable and integrated response to various stimuli.[300] As a result, changes in these regions spread upstream and downstream through connectors (regions of integration across modules), affecting Cognitive functions in a widespread manner that wouldn't show up with these indirect methods. [294,301] In fact, lesions result in a decline in both between-network segregation and within-network integration, which reduces modularity overall. [199,225]

Sensory and motor networks, on the other hand, exhibit a higher degree of synchronisation within the same circuit, and their activity is highly reliant on inputs.[302] Therefore, lesions within these circuits may result in more definite functional disconnection effects that are simpler to anticipate using indirect methods.[199,225]

In conclusion, we suppose that direct measurements (such DWI and resting state fMRI) may be necessary for a more detailed prediction of Cognitive and affective components.

The impact of topography measures

We performed a post hoc analysis to investigate whether the topography of stroke lesions were associated with SIS outcomes. To this aim, a voxel-wise analysis was run considering the three SIS factors independently (we employed a non-parametric approach with n = 1000 and a p-value <0.05 FWE-corrected). The results showed no effects of lesion topology over the SIS factors, suggesting that network results were not linked with the localisation of brain lesions but rather to the degree of disconnectivity.

Conclusion

We identified the factor structure of a functional outcome evaluation, the Stroke Impact scale, and its relationship with advanced lesion properties following stroke. Specifically, our results emphasize the role of network disconnectivity measures as potential predictors of motor outcomes.

As to our knowledge, it was the first study where several innovative measures (ranging from microstructural aspect to structural and functional network properties) were evaluated in their ability to predict items of the Stroke Impact Scale; surely further research is needed to improve the quality of these predictors and the role of the SIS 3.0 in the clinical setting.

To answer the question at the title of this thesis, we could not to define these network disconnectivity measures as a proper “potential biomarker”. However, our study suggests a novel framework of analysis, where these measures, clinically available, could be encompassed alongside the commonly used stroke functional predictors, in order to improve clinical management, rehabilitation programs and overall outcome prognosis.

Supplementary materials

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	

Supplementary 1: The National Institutes of Health Stroke Scale (NIHSS)

THE BARTHEL INDEX

Patient Name: _____
Rater Name: _____
Date: _____

Activity	Score
FEEDING 0 = unable 5 = needs help cutting, spreading butter, etc., or requires modified diet 10 = independent	_____
BATHING 0 = dependent 5 = independent (or in shower)	_____
GROOMING 0 = needs to help with personal care 5 = independent face/hair/teeth/shaving (implements provided)	_____
DRESSING 0 = dependent 5 = needs help but can do about half unaided 10 = independent (including buttons, zips, laces, etc.)	_____
BOWELS 0 = incontinent (or needs to be given enemas) 5 = occasional accident 10 = continent	_____
BLADDER 0 = incontinent, or catheterized and unable to manage alone 5 = occasional accident 10 = continent	_____
TOILET USE 0 = dependent 5 = needs some help, but can do something alone 10 = independent (on and off, dressing, wiping)	_____
TRANSFERS (BED TO CHAIR AND BACK) 0 = unable, no sitting balance 5 = major help (one or two people, physical), can sit 10 = minor help (verbal or physical) 15 = independent	_____
MOBILITY (ON LEVEL SURFACES) 0 = immobile or < 50 yards 5 = wheelchair independent, including corners, > 50 yards 10 = walks with help of one person (verbal or physical) > 50 yards 15 = independent (but may use any aid; for example, stick) > 50 yards	_____
STAIRS 0 = unable 5 = needs help (verbal, physical, carrying aid) 10 = independent	_____
TOTAL (0–100):	_____

Supplementary 2 Exemplative Barthel Index form [93]

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**

Supplementary 3 modified Rankin Scale (mRS)

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