Clinical Findings and Outcome after Stroke. Including a Translational Stem Cell Therapy Perspective.

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Clinical Findings and Outcome after Stroke

Including a Translational Stem Cell Therapy Perspective

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Abstract

Background and Purpose: Stroke is one of the dominant causes of death and adult disability in the world. There is a need for novel therapeutic approaches to improve functional recovery and outcome after stroke, and experimental studies have shown that stem cell-based therapies (SCT) hold much potential in this regard. This thesis, comprising 5 papers, aims to explore and describe clinical symptoms, lesion appearance, and outcome after stroke to provide guidance and enhance possibilities for future clinical implementation of SCT.

Methods: In Paper I, a consecutive series of first-ever ischemic stroke patients (n=108) were examined ≤4 days of stroke onset regarding: (i) neuroradiological characteristics, and (ii) stroke severity measured with National Institutes of Health Stroke Scale (NIHSS). In Papers II and V, available survivors (n=84) from Paper I were assessed after 3-5 years regarding: (i) the frequency and recovery of upper extremity motor impairment (UEMI) measured with NIHSS arm and hand motor items; ii) the relation of UEMI to activity limitations measured with modifed Rankin Scale (mRS) and participation restrictions evaluated with Stroke Impact Scale (SIS); and iii) their knowledge and attitude about SCT using a questionnaire on SCT for stroke. In Papers III and IV, 10-year survivors (n=145) from a population-based group of 416 first-ever stroke patients in the Lund Stroke Register were assessed regarding: (i) functional status measured with mRS and Barthel Index (BI); (ii) patient-reported outcome using the European Quality of Life-5 Dimensions (EQ-5D) and Short-Form 36 Health Survey (SF-36), and iii) cognitive function using Mini-Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA), including comparisons with age- and sex-matched non-stroke control persons (n=354) from the population study Gott Åldrande i Skåne.

Results: In Paper I, the distance between the nearest margin of the infarct(s) to the subventricular zone (a known neurogenic area in the brain) was 0-2 mm in 51/102 patients with visible ischemic lesions on DW-MRI. Only 8 patients had infarcts predominantly confined to striatum (a commonly used lesion site in pre-clinical stroke studies with SCT), causing mild deficits with a median NIHSS of 3 (range 1-5). In Paper II, 56 (52%) of the stroke patients had UEMI (NIHSS arm/hand score ≥1) in the first days after stroke onset. Morever, 10/41 stroke survivors with UEMI at baseline and without recurrent stroke displayed residual UEMI after 3-5 years, whereas 31/41 individuals showed complete recovery. Post-stroke UEMI correlated to mRS (r_s=0.49, p<0.001) and the SIS participation domain (r_s=-0.38, p=0.001). In Paper III, 103 (71%) of the 10-year stroke survivors had mRS≤2, 106 (73%) had a BI score of 95-100, 105 (72%) reported no problems with self-care according to EQ-5D, and 90 (62%) had positive views about their general health status according to SF-36. In Paper IV, 75 (61%) out of 122 stroke survivors who completed the MoCA had a score of MoCA<25. The odds of having severe cognitive impairment (MMSE<23) were higher among stroke survivors than the controls (education-adjusted OR 2.48; 95% CI: 1.34-4.59; p=0.004). In Paper V, only 10 (12%) of the stroke survivors had prior knowledge of SCT, but 53 (63%) of the participants expressed positive attitudes towards SCT after having received standardized and neutral written information. Positive attitudes to SCT were associated with male gender (crude OR 3.74; 95% CI: 1.45-9.61; p=0.006) and higher degree of self-perceived stroke recovery according to the SIS (crude OR 1.02; 95% CI: 1.00-1.04; p=0.034).

Conclusions: Optimized endogenous neurogenesis may have a therapeutic potential, and striatum should probably not be the primary target for SCT aiming for neuronal replacement. SCT targeting post-stroke UEMI may be clinically valuable, and UEMI recovery may be a suitable outcome in later-phase pivotal stroke trials studying the efficacy of SCT. A majority of long-term stroke survivors have a relatively good prognosis accompanied by positive self-perceptions about their health, and would probably not have been in need of SCT to improve functional outcome. However, early prognostic assessments are needed to detect stroke patients with poor expected functional recovery and outcome where SCT may be beneficial. Cognitive impairment is common among long-term stroke survivors and should be taken into account both in pre-clinical studies and in future clinical trials with SCT. Targeted patient information on SCT for stroke may be valuable to facilitate recruitment to clinical trials and reduce risks of selection bias.

Key words: stroke, stem cell therapy, recovery,outcome, upper extremity motor impairment, cognition

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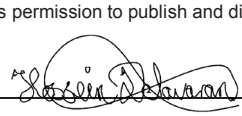
Signature  Date 2017-02-24

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To my family

Content

List of Papers

This thesis is based on the following five papers, henceforth referred to in the text by their Roman numerals. The papers are appended in the end of the thesis with due permission from the publishers.

I. Delavaran H, Sjunnesson H, Arvidsson A, Lindvall O, Norrving B, van Westen D, Kokaia Z*, Lindgren A* (*equal contribution). Proximity of brain infarcts to regions of endogenous neurogenesis and involvement of striatum in ischaemic stroke. *European Journal of Neurology*. 2013;20:473-479.

II. Delavaran H, Aked J, Sjunnesson H, Lindvall O, Norrving B, Kokaia Z, Lindgren A. Spontaneous Recovery of Upper Extremity Motor Impairment After Ischemic Stroke – Implications for Stem Cell-Based Therapeutic Approaches. *Translational Stroke Research* (2017). doi:10.1007/s12975-017-0523-9.

III. Jönsson AC, Delavaran H, Iwarsson S, Ståhl A, Norrving B, Lindgren A. Functional Status and Patient-Reported Outcome 10 Years after Stroke: The Lund Stroke Register. *Stroke*. 2014;45:1784-1790.

IV. Delavaran H, Jönsson AC, Lövkvist H, Iwarsson S, Elmståhl S, Norrving B, Lindgren A. Cognitive function in stroke survivors: A 10-year follow-up study. *Acta Neurologica Scandinavica* (2016). 00:1-8. doi:10.1111/ane.12709.

V. Aked J, Delavaran H, Lindvall O, Norrving B, Kokaia Z, Lindgren A. Attitudes to Stem Cell Therapy among Ischemic Stroke Survivors in the Lund Stroke Recovery Study. *Stem Cells and Development* (2017). Ahead of print. doi: 10.1089/scd.2016.0343.

Abbreviations

ADC	apparent diffusion coefficient
ADL	activities of daily living
ARAT	Action Research Arm Test
BI	Barthel Index
CCI	Charlson Comorbidity Index
CI	cerebral infarction
CI	confidence interval
CT	computed tomography
DALY	disability-adjusted life year
DW-MRI	diffusion-weighted magnetic resonance imaging
EQ-5D	European Quality of Life-5 Dimensions
FLAIR	fluid attenuated inversion recovery
FMA	Fugl-Meyer Assessment
FMA-UE	Fugl-Meyer Assessment of Upper Extremity
GBD	Global Burden of Disease
GRE	gradient echo
GÅS	Gott Åldrande i Skåne
HRQoL	health-related quality of life
IADL	instrumental activities of daily living
ICF	International Classification of Functioning, Disability and Health
ICH	intracerebral hemorrhage
LSR	Lund Stroke Register
LSRS	Lund Stroke Recovery Study

MMSE	Mini-Mental State Examination
MoCA	Montreal Cognitive Assessment
MRI	magnetic resonance imaging
mRS	modified Rankin Scale
NIHSS	National Institutes of Health Stroke Scale
PREP	Predicting Recovery Potential
PSCI	post-stroke cognitive impairment
SAH	subarachnoid hemorrhage
SCT	stem cell-based therapies
SF-36	Short Form 36 Health Survey
SIS	Stroke Impact Scale
SNAC	Swedish National Study on Aging and Care
SVZ	subventricular zone
TIA	transient ischemic attack
UEMI	upper extremity motor impairment
WHO	World Health Organization

Introduction

Stroke is a major worldwide health problem, constituting the second most common cause of death and one of the dominant causes of adult disability [1,2]. Despite the major advances that have been made in the stroke field over the past decades [3], still a large proportion of stroke survivors have lasting functional disabilities and dependency in daily activities [4], as well as lowered health-related quality of life (HRQoL) [5].

Multiple brain recovery mechanisms are initiated after stroke, and most patients exhibit some degree of spontaneous recovery [6,7]. Nevertheless, these brain recovery mechanisms are generally insufficient and recovery is often incomplete [6,7]. Consequently, there is a great need for not only effective rehabilitative interventions, but also new therapeutic approaches to improve functional recovery and outcome after stroke.

There are currently many recovery promoting therapeutic approaches being under study [3,7]. One such example is stem cell-based therapies (SCT), which hold much potential as a novel approach to improve functional recovery and outcome after stroke, and numerous pre-clinical studies in animal stroke models have showed promising results [8-11]. Moreover, several clinical stroke studies with SCT are ongoing, mostly testing safety in limited number of patients [8,9,12,13]. However, the efficacy of SCT for stroke remains to be demonstrated in later-phase pivotal trials, and several issues remain to be addressed before SCT can be translated into effective clinical treatments [9,10,14].

This thesis explores lesion appearance, clinical symptoms and outcome after stroke, both from a general perspective and with the specific aim to provide guidance and enhance possibilities for the clinical implementation of SCT intended to improve post-stroke functional recovery and outcome.

Stroke – Definitions and Terminology

Stroke is defined by the World Health Organization (WHO) criteria that were introduced in the 1970s, as: "rapidly developing clinical signs of focal (at times global) disturbance of cerebral function, lasting more than 24 h or leading to death, with no apparent cause other than of vascular origin" [15].

Accordingly, stroke is a clinical diagnosis based on the acute onset of neurological symptoms lasting longer than 24 hours (or leading to death) due to focal brain injury that can be ascribed to a vascular cause including cerebral infarction (CI), intracerebral hemorrhage (ICH), and subarachnoid hemorrhage (SAH). The term "at times global" was included to denote cases with deep coma or SAH without focal neurological symptoms [15,16]. Traumatic intracranial hemorrhage is not included in the definition, neither is transient ischemic attack (TIA). TIAs have classically been described as: "episodes of temporary and focal cerebral dysfunction of vascular origin, rapid in onset (no symptoms to maximal symptoms in less than five minutes and usually less than a minute), which are variable in duration, commonly lasting from 2 to 15 minutes but occasionally lasting as long as a day (24 hours)" [17].

By these definitions, the 24-hour limit of symptom duration distinguishes TIA from stroke. However, it has been suggested that the WHO criteria need to be revised and updated, as e.g. advances in neuroimaging have demonstrated that many patients with TIA (symptoms lasting less than 24 hours) indeed develop brain infarctions [18]. On the other hand, in some patients with stroke (symptoms lasting more than 24 hours) no infarctions can be visualized on neuroimaging [19]. The American Heart Association/American Stroke Association has recently published an expert consensus document suggesting an updated definition of stroke, based primarily on pathological and imaging findings, and secondarily on symptom duration and signs [20]. Nevertheless, the WHO stroke criteria are still widely in use today.

Stroke is commonly classified into two main pathological subtypes, comprising ischemic stroke (CI) and hemorrhagic stroke (ICH and SAH). Ischemic stroke is the most prevalent pathological subtype in the world, and constitutes approximately 85% of all stroke cases in developed countries [21]. Correspondingly, the proportional frequency of hemorrhagic stroke is roughly 15% in developed countries (10% ICH and 5% SAH) [21].

As ischemic stroke is the dominant main pathological subtype, the core focus of this thesis is on ischemic stroke.

Burden of Stroke

Incidence and Prevalence

The Global Burden of Disease (GBD) study estimated that there were 10.3 million individuals in the world with new strokes in 2013 (67% ischemic stroke) [22,23]. The age-adjusted global incidence rate of stroke has been estimated at 258 (95% CI: 234-284) cases per 100 000 person-years in individuals of all ages [24]. Moreover, the incidence rate of stroke increases sharply with advancing age. In developed countries, the age-adjusted stroke incidence rate among individuals younger than 75 years is 139 (95% CI: 131-148) cases per 100 000 person-years [24]. The corresponding rate in persons older than 75 years is 2724 (95% CI: 2554-2900) cases per 100 000 person-years [24]. There is also a considerable geographic variation in the world regarding the stroke incidence, as illustrated in Figure 1.

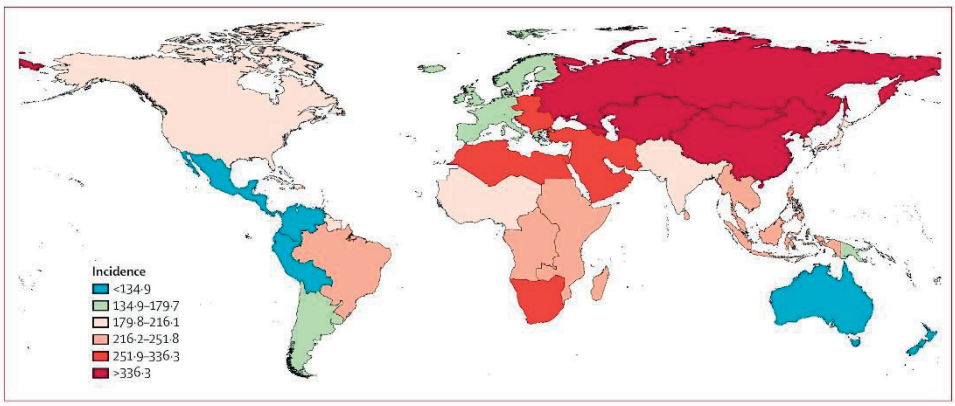

Figure 1.
Age-standardized incidence rates of stroke per 100 000 person-years in various regions of the world in 2010.
Reprinted from The Lancet, Vol. 383, Feigin et al., Global and regional burden of stroke during 1990-2010: findings from the Global Burden of Disease Study 2010, pp. 245-255, Copyright (2014), with permission from Elsevier.

According to the GBD study estimates, there are over 25 million stroke survivors in the world (71% ischemic stroke), and the global stroke prevalence is estimated at 502 (95% CI: 451-572) cases per 100 000 people of all ages [22,24].

In Sweden, there are roughly 30 000 first-ever stroke cases every year [25]. The age-adjusted (to the European population) stroke incidence rate in Sweden has been estimated between 144 (95% CI: 130-158) and 254 (95% CI: 227-284) cases per 100 000 person-years in individuals of all ages [26,27].

Mortality and Disability-Adjusted Life Years

Stroke is the second most common cause of death in the world, and one of the leading causes of disability in adults [1,2]. Nearly 6.5 million deaths (51% ischemic stroke) and 113 million disability-adjusted life years (DALYs) were caused by stroke in 2013 (58% ischemic stroke) [22]. The age-adjusted global mortality rate of stroke has been estimated at 88 (95% CI: 80-94) cases per 100 000 person-years among individuals of all ages [24], and stroke-related DALYs in the world amount to 1554 (95% CI: 1374-1642) per 100 000 people [24].

Epidemiological Trends

Although the worldwide incidence and mortality rates of stroke have decreased over the past decades (1990-2013), a substantial increase in the absolute number of incident and prevalent cases with stroke has been observed [22,23]. This increase of the global stroke burden in absolute terms has mainly been attributed to the aging and growth of populations [28].

Furthermore, the global burden of stroke-related deaths and DALYs as a proportion of the overall burden of all diseases has increased by 20-25% between 1990 and 2013 [23]. Figure 2 displays the proportional burden of stroke in relation to the burden of all health conditions.

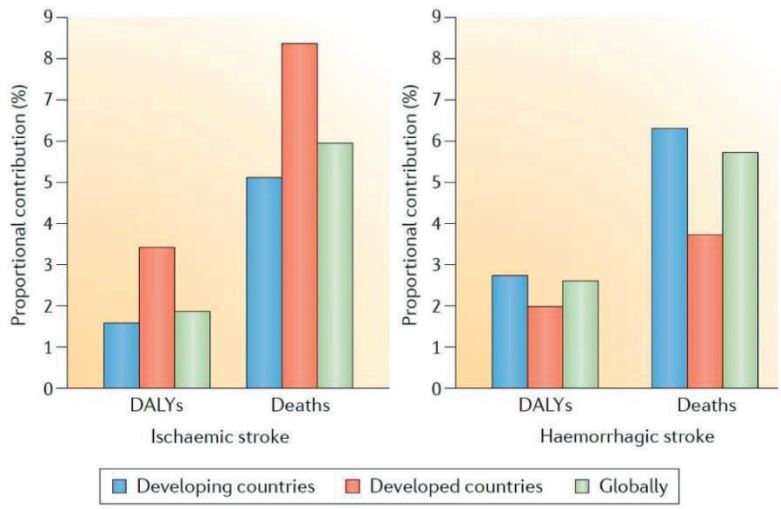

Figure 2.
Proportional contribution (in %) of ischemic and hemorrhagic stroke burden to the burden of all diseases in 2013.
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Societal Economic Costs

Stroke accounts for more than 4% of the direct healthcare expenses in developed countries [29]. The total annual cost (direct healthcare costs, direct non-medical costs and indirect costs) of incident stroke in Europe (all European Union member states as well as Norway, Iceland and Switzerland) has been estimated to be nearly 26.6 billion euros at 2010 prices [30]. The corresponding cost for prevalent stroke amounts to 37.4 billion euros [30].

In Sweden, the societal lifetime cost per person with first-ever stroke has been estimated to be 68 800 euros at 2009 prices [31]. Of this amount, hospitalization costs for the index stroke accounted for 14%, residential housing and home assistance accounted for 59%, and indirect costs due to productivity losses accounted for 21% [31]. By these estimates, the total societal lifetime cost for first-ever stroke in Sweden amounts to nearly 1.2 billion euros at 2009 prices [31].

To conclude, the global burden of stroke is massive and still growing, with considerable human and societal repercussions.

Pathogenetic Mechanisms

Large artery atherosclerotic disease is a major cause of ischemic stroke, accounting for about 10-15% of all cerebral infarctions [32-34]. The main underlying pathologies in large artery atherosclerotic disease include thrombosis superimposed on atherosclerosis (in situ thrombo-occlusion), atherosclerotic plaques causing embolism (artery-to-artery embolism), atherosclerotic plaques in an intracranial artery occluding the opening of a branch vessel (local branch occlusion), and distal hypoperfusion due to atherosclerotic stenoses (hemodynamic impairment) [35]. Multiple infarcts in the unilateral anterior circulation, as well as small scattered lesions in one vascular territory are associated with large artery atherosclerotic disease [36].

Small vessel disease is another major cause of ischemic stroke, and is estimated to be the underlying pathogenetic mechanism in about 25% of ischemic stroke patients [32]. The underlying vascular pathology in small vessel disease involves disorganized vessel walls, fibrinoid deposits and sometimes small hemorrhagic extravasation [37,38]. Small vessel disease is associated with small subcortical infarction (commonly called lacunar infarct), less than 20 mm in size (diameter in the axial plane) and located within the territory of a single perforating arteriole [39].

Cardiac embolism is the cause of roughly 25-30% of all ischemic strokes [32-34]. Atrial fibrillation is a common underlying heart condition causing cardio-embolic infarcts [40-42]. Other cardiac sources of embolism include e.g. recent myocardial infarction, infective endocarditis, bioprosthetic and mechanical heart valves, and patent foramen ovale [42]. Cardio-embolic infarcts are usually larger than those caused by large artery atherosclerotic disease [43]. Cardio-embolism is associated with cortico-subcortical infarcts, and multiple infarcts in the territories of multiple cerebral circulations [36].

Other uncommon causes of ischemic stroke include e.g. arterial dissection, vasculitis, cerebral venous thrombosis, coagulopathies, migraine, iatrogenic causes and drug-induced stroke [42]. These less common causes of stroke account for 2-3% of all ischemic stroke cases [32-34].

Stroke of undetermined origin refers to ischemic stroke with undetermined cause, either due to incomplete diagnostic evaluation, two or more concurrent possible pathogenetic mechanisms, or undetermined despite adequate diagnostic evaluation (cryptogenic stroke) [42]. In approximately 35% of all ischemic stroke cases the underlying pathogenetic mechanism is undetermined [32-34].

To conclude, ischemic stroke may be caused by different potentially underlying pathogenetic mechanisms, which are associated with different brain lesion patterns.

Different brain lesion patterns and lesion appearance among stroke patients may have important implications for SCT, such as e.g. which lesions to target.

Pathobiology

The abrupt occlusion of a brain vessel impairs blood flow and subsequent delivery of substrates, such as oxygen and glucose, to an ordinarily high-energy consuming tissue [44]. This initiates an intricate series of interacting pathobiological events and processes in the brain.

The cells in the center of the ischemic region (the ischemic core) undergo rapid energy depletion, followed by loss of membrane potential and anoxic depolarization [44]. This leads to a process called excitotoxicity, as excitatory amino acids such as glutamate are released and accumulated in the extracellular space, followed by the intracellular influx of calcium, sodium and chloride ions [8,44]. By way of osmotic gradients, there is a passive diffusion of water into the cells and cytotoxic edema follows [44]. The excessive intracellular influx of calcium also initiates several injurious cascades leading to degradation of cellular structural components,

activation of free radicals, and mitochondrial failure, leading to further damage and cell death [8,44,45].

In the ischemic core there is a rapid death of neural cells within minutes to hours [8,44]. However, the perfusion of tissue in the proximal surroundings of the ischemic core (the penumbra) is only partially compromised and some energy metabolism is preserved [44]. With prompt restoration of blood flow, the penumbra is potentially salvageable [44]. However, without reperfusion in due time (and sometimes even if reperfusion occurs - either spontaneously or therapeutically) the penumbra will also undergo infarction [44].

Focal brain ischemia not only affects neurons, but also astrocytes, oligodendrocytes, endothelial cells, pericytes, smooth muscles, basement membranes and extracellular matrix are all affected and involved in the pathobiological processes (Figure 3) [8,44,46-48].

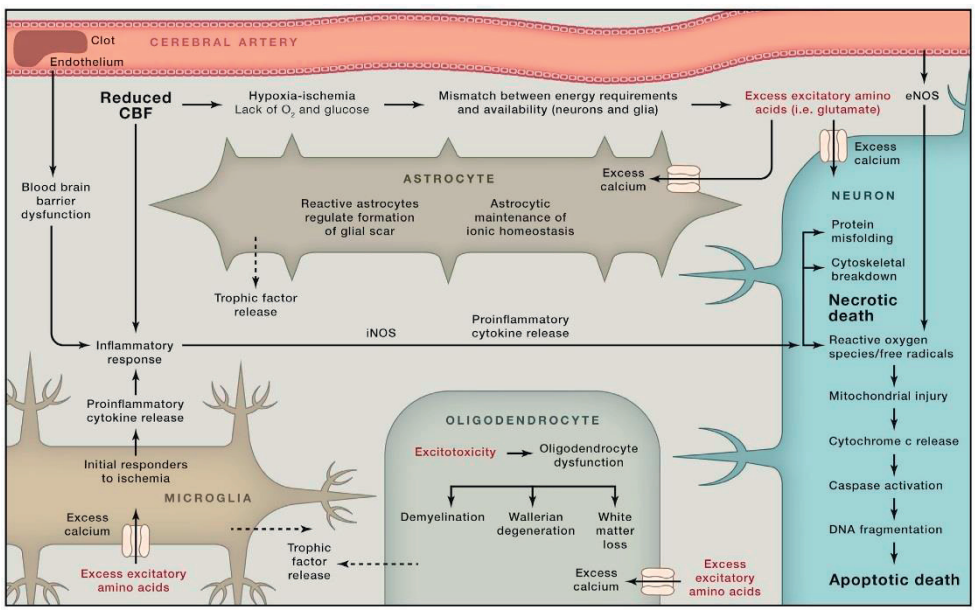

Figure 3.
The pathobiology of stroke. *Reprinted from Neuron, Vol. 87, George PM, Steinberg GK, Novel Stroke Therapeutics: Unraveling Stroke Pathophysiology and Its Impact on Clinical Treatments, pp. 297-309, Copyright (2015), with permission from Elsevier.*

Inflammation also plays an important role in ischemic stroke [45]. Focal brain ischemia activates local immune cells such as microglia and dendritic cells [8,44,45]. Due to the damage and death of astrocytes and the breakdown of the blood-brain barrier, there is also an infiltration of blood-borne immune cells to the injured area [8,44,45]. The immune cells in the stroke-damaged tissue release pro-

inflammatory cytokines and free radicals, thereby increasing the inflammatory response and contributing to cellular injury [8,44,45].

However, the inflammatory response also has several beneficial effects such as protection of neurons from excitotoxicity [8,49], and removal of damaged tissue enabling synaptic remodeling [8,50]. Immune cells also produce or modulate trophic factors that stimulate neural growth and recovery, axonal growth and remodeling [8,51-53], as well as augmenting endogenous neurogenesis in the subventricular zone (SVZ) - a known neurogenic area in the brain [8,54].

Likewise, glial cells form the glial scar that may impede neural plasticity, but they are also involved in regulating the blood-brain barrier, and stimulate angiogenesis and synaptogenesis [8].

Hence, many of the pathobiological events and processes that are induced by stroke also pave the way for the brain recovery mechanisms that are initiated the first days to weeks after stroke (Table 1).

Table 1.
Examples of brain recovery mechanisms after stroke at the molecular and cellular levels

Brain recovery mechanisms at the molecular and cellular levels
Raised levels of growth factors
Neurogenesis and migration of neural stem cells
Angiogenesis and vascular remodeling
Synaptogenesis, dendritic growth and axonal remodeling

Furthermore, advanced neuroimaging and electrophysiological studies in stroke patients have shown that several compensatory mechanisms may occur at the broader organ level (though often insufficient), such as: alterations in local cortical activity and thickness; increased activity in distant brain regions that are connected to the lesion area within a distributed network; augmented recruitment of the contralateral hemisphere (relative to the ipsilateral hemisphere); changes in cortical somatotopic representation; and alterations in brain network interactions [6,7].

Consequently, the alterations taking place in the brain following stroke can broadly be divided into 3 main time periods [7]:

(i) The first time period constitutes the acute phase and comprises the first hours after stroke when there is an accelerated death of cells in the ischemic core, but threatened brain tissue in the penumbra still has the potential to be saved [7].

(ii) The second time period begins the first days to weeks after stroke, and involves the start of endogenous brain recovery mechanisms [7].

(iii) The third time period comprises the chronic phase when the brain recovery processes are relatively stable, but changes of structure and function still may occur [7].

Therapeutic approaches to promote recovery and restore function, such as SCT, target the second and third time periods described above, and probably have a broader time window in which they can be delivered than treatments aiming to restrict the ischemic damage in the acute phase (e.g. reperfusion approaches).

To conclude, stroke induces multiple interacting pathobiological events and processes that are harmful and detrimental, but which are also protective and pave the way for brain recovery mechanisms.

SCT may contribute to functional recovery after stroke by modulating and/or enhancing many of these brain recovery mechanisms.

Clinical Presentation

The locations, sizes and number of brain lesions vary greatly among stroke patients, affecting diverse and heterogeneous neurological functions [36,55,56]. Consequently, the clinical symptoms and signs of stroke encompass a broad range of neurological deficits and various clinical manifestations [57].

Frequent stroke symptoms include sudden onset of unilateral weakness, numbness, visual field defects, language disturbances such as aphasia, gaze palsy, diplopia, ataxia and vertigo [3]. Other symptoms may be dysarthria, dysphagia, binocular blindness, headache, confusion, reduced consciousness and epileptic seizures [3].

Most stroke symptoms involve a reduction or loss of a particular neurological function (e.g. weakness, numbness and aphasia), whereas some more rare symptoms are excitatory (e.g. epileptic seizures, involuntary movements and tingling).

The neurological deficits following stroke may also vary in complexity, ranging from e.g. weakness (paresis) or reduced sensation for pain; followed by impaired planned movements or interpretation of sensory stimuli; to disturbances of higher cerebral functions such as aphasia, attention deficits, apraxia; and even more complex cognitive dysfunctions such as memory problems and executive dysfunctions; and psychological symptoms including e.g. affective disturbances.

Common clinical measures to assess stroke symptoms, and other aspects of stroke outcome, are described below in the section on “Assessment of Stroke Outcome”. However, it merits mention that the National Institutes of Health Stroke Scale

(NIHSS) is one of the most widely used stroke measures to assess the range and severity of stroke symptoms, especially in the setting of acute ischemic stroke [58,59]. Nonetheless, it may be difficult to detect all these types of neurological deficits and symptoms with a specific assessment measure.

Different Types of Neurological Deficits after Stroke

The major neurological domains that may be impaired after stroke include motor, sensory, vision, language, cognition and psychological [55].

Motor Deficits

The motor domain is the most frequently impaired neurological domain after stroke [6,60,61]. Impaired motor functions most commonly involve paresis of the face (facial palsy), arm/hand and leg [60,61]. Paresis of the face is observed in approximately 55% of acute stroke patients [61], while paresis in the upper and lower extremities affect roughly 80% and 70%, respectively [60,61].

Motor impairments caused by stroke also include gait abnormalities (about 11%) [61], ataxia (about 7%) [60], as well as cranial nerve motor deficits causing gaze palsy (about 18%) [60], diplopia (about 6%) [61], dysphagia (about 45%) [60] and dysarthria (about 42%) [60].

However, most of the previous community- and population-based studies reporting the frequencies of impaired motor functions in the acute phase of stroke were performed several years ago [60-62]. A more recent hospital-based study from Sweden reported that impaired motor function in the upper extremity was observed in 48% within 72 hours of stroke onset among a non-selected cohort of first-ever stroke patients [63].

Sensory Deficits

Sensory deficits following stroke are commonly manifested as reduced or loss of sensation to e.g. touch, temperature, pain, and proprioception [56,64,65]. Sensory impairments like motor deficits often involve the face and/or the upper and lower extremities [60,61]. The prevalence of sensory deficits after stroke ranges from 11-100% in previous reports [64]. This large variability in the reported frequencies has mainly been attributed to differences in the definition of sensory impairments as well as differences in assessments methods and study designs [64].

Visual Deficits

Visual deficits after stroke may be exhibited as partial or complete hemianopia, other visual field defects, monocular loss of vision, and sometimes cortical blindness [55,56,66]. In a previous population-based study, visual field defects were found among 26% of acute stroke patients [60].

Language Disturbances

Language disturbances referred to as aphasia may be exhibited as impaired language comprehension and/or expression, and may involve difficulties in verbal fluency, naming, repetition, reading or writing [55,56,67]. Previous studies with samples of stroke patients from acute settings have reported frequencies of aphasia ranging from 20-41% [68].

Cognitive Deficits

Cognitive deficits are frequently observed after stroke [69], and may involve e.g. disturbances in memory, attention, orientation, calculation, abstraction, visuospatial abilities and executive functions [55,56].

Post-stroke cognitive impairment (PSCI), ranging from mild cognitive impairment to dementia, can occur immediately after stroke, but usually there is some delay before it becomes apparent [69]. There is much variability in the previously reported prevalence rates of post-stroke cognitive impairment (PSCI), owing to different definitions and assessment methods [69]. Hence, previous studies have reported prevalence rates of mild cognitive impairment ranging from 17-92% at 3 months after stroke [69]. It has also been reported that 10% of first-ever stroke patients develop more severe cognitive decline leading to dementia shortly after stroke [70].

Psychological Disturbances

Following stroke, it is not uncommon that affective disturbances develop such as e.g. depression and anxiety disorders [71-73]. Estimates show that nearly one third of stroke patients experience depression at any time-point up to 5 years after stroke [71]. The symptoms of post-stroke depression, e.g. loss of joy and motivation, hopelessness, loss of appetite and sleeping problems, can appear already in the acute phase of stroke but may go unnoticed due to the concealing effect of other neurological impairments such as aphasia or cognitive deficits [73].

Many stroke patients also suffer from lack of physical and mental energy, which can be referred to as fatigue [74]. Fatigue may be a component of post-stroke depression, but some stroke patients have fatigue without displaying other symptoms of depression [74].

To conclude, stroke may cause impaired functions in several neurological domains, including motor, sensory, vision, language, cognition, and psychological, where motor dysfunctions are the most commonly observed in the acute phase.

To date, most pre-clinical studies with SCT in animal stroke models have focused on recovery of sensorimotor functions after stroke [75,76]. Whether SCT can

improve post-stroke recovery in patients with neurological deficits of different types and complexities, and how this should be assessed, remains to be explored.

Neuroimaging in Acute Stroke

There are several neuroimaging modalities that are commonly used in the acute stroke setting including computed tomography (CT), magnetic resonance imaging (MRI), diffusion-weighted (DW) MRI, fluid attenuated inversion recovery (FLAIR) MRI, gradient echo (GRE) MRI, perfusion imaging, non-invasive and invasive angiographic techniques and Doppler ultrasonography.

In this section, CT and DW-MRI will be described in more detail, whereas a more detailed account of the other modalities are beyond the scope of this thesis.

Computed Tomography (CT)

In the clinical setting of acute stroke, non-contrast CT of the brain is the most commonly used neuroimaging modality to distinguish ischemic from hemorrhagic stroke, as this distinction cannot be done on the basis of clinical findings only and because the distinction is crucial for the subsequent management of most stroke patients. Non-contrast CT has very high sensitivity for fresh hemorrhage, whereas it has relatively low sensitivity for ischemia that is in the very early stages, or small, or located in the vertebro-basilar territory [3].

The diagnosis of acute ICH is usually apparent with CT, visualized as hyperdensity in the brain tissue [77]. Acute SAH is also often apparent on CT and appears as hyperdensity in the subarachnoid spaces, whereas subacute SAH may be more difficult to detect [77].

Early ischemic changes in the brain may be visualized on CT as loss of grey and white matter differentiation, loss of sulci and focal swelling [77,78]. Manifest brain infarction appears as hypodensity [77]. After the resolution of the initial edema, the infarct volume decreases over the subsequent weeks, and the final infarct volume becomes established after about one month [79].

The use of CT in acute stroke also plays an important role in excluding stroke mimics, such as e.g. epidural and subdural hemorrhage, intracranial tumor, and abscess.

Diffusion-Weighted Magnetic Resonance Imaging (DW-MRI)

DW-MRI is the most sensitive neuroimaging technique to visualize ischemic stroke, and may detect ischemic brain damage within minutes of onset [79,80]. As previously mentioned, cytotoxic edema occurs in the first minutes after stroke due

to passive diffusion of water molecules from extracellular to intracellular spaces. This change in water diffusion results in a lowering of the apparent diffusion coefficient (ADC) in the injured area [77,81]. DW-MRI is partly related to the negative construction of the ADC map [77]. Therefore, ischemic lesions look strikingly bright on DW-MRI, but appear dark in the corresponding regions on the ADC map (Figure 4) [77].

Ischemic lesions may appear bright on DW-MRI for several days up to several weeks depending on the extent of infarction [82]. After this period, chronic infarcts become hypointense on DW-MRI due to the breakdown of brain tissue and increased diffusion of water molecules [77].

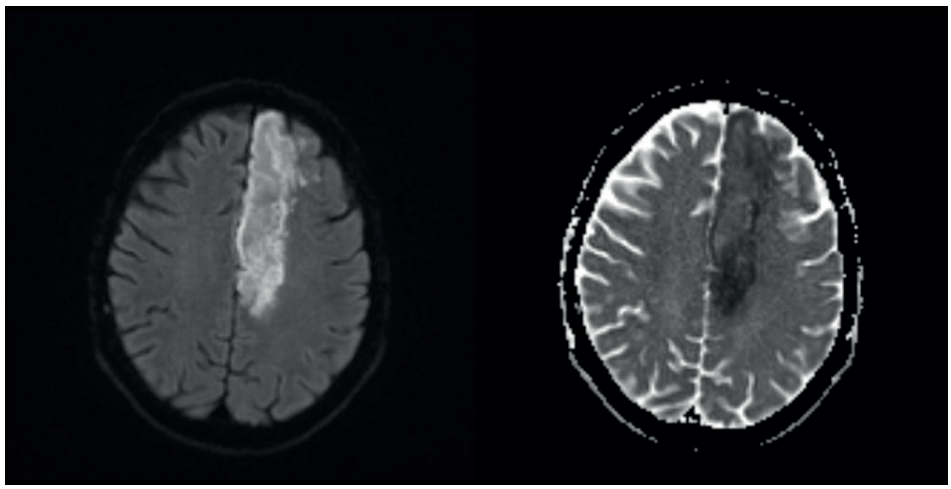

Figure 4.
Fresh ischemia in the territory of the left anterior cerebral artery, visualized as hyperintensity on transversal DW-MRI (image to the left) and reduced ADC in the corresponding region (image to the right). *Courtesy of Dr. Magnus Esbjörnsson, Clinic of Internal Medicine, Hässleholm's Hospital, Sweden.*

Treatment in the Acute Phase

Considerable advances have been made in the stroke field over the past decades with regard to the management and treatment of acute stroke patients.

One example is specialized stroke units that were introduced in the mid-1970s [83]. It has been shown that stroke unit care reduces mortality, improves functional outcome, and increases the prospects of discharge to ordinary home for stroke patients as compared to management and treatment in ordinary wards [84].

Moreover, the efficacy of intravenous thrombolysis for acute ischemic stroke patients was demonstrated during the 1990s [85]. Thrombolysis administered within 4.5 hours from ischemic stroke onset significantly raises the likelihood of good functional outcome at 3-6 months [86]. More recently, the benefit of endovascular thrombectomy was also demonstrated for acute ischemic stroke patients with proximal occlusion in the anterior circulation [87].

However, estimates show that no more than 5% of acute ischemic stroke patients receive thrombolysis in the US [88]. In Sweden, the proportion of acute ischemic stroke patients that are treated with thrombolysis and/or endovascular thrombectomy amounts to about 13% across all ages [40]. Besides, some stroke patients that have received acute reperfusion therapy still have lasting functional disabilities [85,89,90].

To conclude, major advances have been made regarding the management and treatment of acute stroke patients over last decades. Nevertheless, only a small fraction of acute ischemic stroke patients are treated with reperfusion approaches, and some patients still have remaining disabilities despite treatment.

This also reflects the need for other therapeutic approaches to improve functional recovery and outcome after stroke.

Assessment of Stroke Outcome

The International Classification of Functioning, Disability and Health

The International Classification of Functioning, Disability and Health (ICF) was developed by the WHO to provide a conceptual framework and a unified classification to describe health and health-related states [91].

The ICF model comprises two parts, each with two components, as follows [91]:

i) Functioning and Disability
   - Body functions and structures
   - Activities and participation

ii) Contextual Factors
   - Environmental factors
   - Personal factors

The components of the "Functioning and Disability" part can be described either positively or negatively [91]:

- functional and structural integrity vs. impairment
- activity vs. activity limitation
- participation vs. participation restriction

Within the ICF model, impairment refers to problems in body functions or structures (i.e. problems in physiological functions of the body, including psychological functions, or in anatomical parts of the body) [91]. Activity limitations refer to difficulties in performing activities (i.e. difficulties performing a certain task or action) [91]. Participation restrictions are difficulties that a person may experience when involved in different life situations [91].

Environmental factors include the physical, social, and attitudinal environment in which people live and conduct their lives, and personal factors refer to personal attributes that may influence functioning [91].

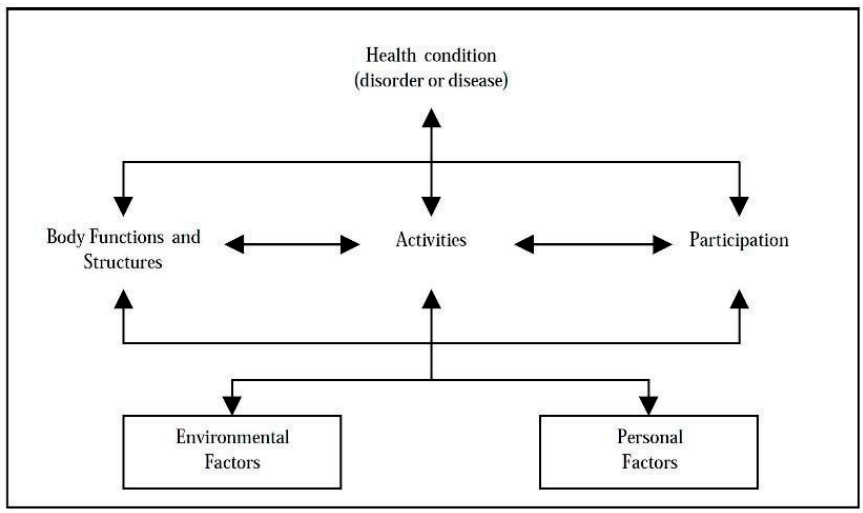

Figure 5.
The ICF model illustrating the interaction of the different components. *Reprinted from "International Classification of Functioning, Disability and Health", WHO Library Cataloguing-in-Publication Data, World Health Organization, Geneva, 2001, p. 18, Copyright (2001)*

Hence, the ICF provides a framework to evaluate the consequences of a particular health condition for an individual, taking into account the impact and interactions of the different components, and therefore offering a more holistic understanding.

Quality of Assessment Measures

One important aspect to consider in the choice of outcome measure(s) is the quality of the measure(s), described as psychometric properties. In this context, some of the important concepts include validity, reliability and responsiveness.

The validity of a particular assessment measure refers to the extent to which it measures what it is intended to measure, and it is evaluated in relation to a specific purpose or setting [92]. The reliability of an assessment measure concerns the internal consistency (homogeneity of scale items), as well as the reproducibility (whether the measure yields the same result after repeated measurements) of the instrument [92]. The responsiveness of a measure refers to its ability to detect important changes over time [92].

With regard to diagnostic tests, sensitivity refers to the accuracy of a test in correctly identifying subjects having a particular characteristic, whereas specificity refers to the accuracy in correctly identifying subjects not having the characteristic in question [93]. The probability of a test providing the correct diagnosis is described with the positive predictive value (the proportion of subjects with positive test results who are diagnosed correctly) and the negative predictive value (the proportion of subjects with negative test results who are diagnosed correctly) [93].

Assessment Measures in Stroke

There are numerous assessment measures that can be used to evaluate different aspects of stroke outcome [59,94,95], some examples of which are listed in Table 2.

Depending on what they aim to evaluate, stroke assessment measures can be categorized according to the ICF model. However, this categorization is not always precise as many assessment measures evaluate features that may belong to more than one of the ICF components [94]. In particular, several patient-reported outcome measures (commonly categorized as measures of participation) include self-perceived evaluations within some or all of the components of the ICF model, and have also been used to evaluate HRQoL after stroke [5,94].

Table 2.

Some examples of different stroke outcome measures, adjusted from Salter et al., 2013 [94]. The assessment measures used in this thesis are marked in bold.

Assessment of body functions and structures	Assessment of activities	Assessment of participation
Beck Depression Inventory (BDI)	**Action Research Arm Test (ARAT)**	Assessment of Life Habits (LIFE-H)
Behavioural Inattention Test (BIT)	**Barthel Index (BI)**	**European Quality of Life-5 Dimensions (EQ-5D)**
Canadian Neurological Scale (CNS)	Berg Balance Scale (BBS)	London Handicap Scale (LHS)
Clock Drawing Test (CDT)	Box and Block Test (BBT)	Nottingham Health Profile (NHP)
Frenchay Aphasia Screening Test (FAST)	Chedoke-McMaster Stroke Assessment Scale (CMSA)	Reintegration to Normal Living Index (RNLI)
Fugl-Meyer Assessment (FMA)	Chedoke Arm and Hand Activity Inventory (CAHAI)	**Short Form 36 Health Survey (SF-36)**
Geriatric Depression Scale (GDS)	Functional Ambulation Categories (FAC)	Stroke-Adapted Sickness Impact Profile (SA-SIP-30)
Hospital Anxiety and Depression Scale (HADS)	Functional Independence Measure (FIM)	**Stroke Impact Scale (SIS)**
Line Bisection Test (LBT)	Frenchay Activities Index (FAI)	Stroke Specific Quality of Life Scale (SSQOL)
Mini-Mental State Examination (MMSE)	**Modified Rankin Scale (mRS)**	
Modified Ashworth Scale (MAS)	Motor Assessment Scale (MAS)	
Montreal Cognitive Assessment (MoCA)	Nine-hole Peg Test (NHPT)	
National Institutes of Health Stroke Scale (NIHSS)	Rivermead Mobility Index (RMI)	
Scandinavian Stroke Scale (SSS)	Wolf Motor Function Test	

A description of the assessments measures used in this thesis are provided below.

Assessment of Body Functions and Structures

Fugl-Meyer Assessment (FMA)

The Fugl Meyer Assessment (FMA) is a widely used stroke-specific impairment measure, comprising 5 major sections as follows: (i) upper extremity motor function; (ii) lower extremity motor function; (iii) sensory qualities; (iv) balance; and (v) joint function [96]. The whole FMA scale has a total score from 0-226, with lower scores reflecting more severe impairment [96]. The different sections of the FMA may be administered independently [94].

The motor section concerning upper extremities (Fugl-Meyer Assessment of Upper Extremity, FMA-UE) comprises 33 items that are divided into the following subsections: shoulder-arm (score 0-36); wrist (score 0-10); hand (score 0-14); and upper limb coordination (score 0-6). Thus, the FMA-UE score ranges from 0-66 [96].

The FMA has strong psychometric properties, and the whole scale or its subsections are often used as a gold standard against which the validity of other impairment measures are evaluated [94,95].

Mini-Mental State Examination (MMSE)

The Mini Mental State Examination (MMSE) is one of the most commonly used cognitive screening tests in the world, originally developed to detect dementia [97].

The MMSE has a maximum score of 30 points, with questions and tasks grouped into the following subtests: orientation to time and place (score 0-10); word registration (score 0-3), attention and calculation (score 0-5); word recall (score 0-3); language/verbal tasks (score 0-8); and visuoconstruction (score 0-1).

A score of less than 24 on the MMSE has traditionally been used as an indication of cognitive impairment [97]. At this cutoff, the MMSE has high specificity and good sensitivity to detect moderate/severe cognitive impairment but low sensitivity for mild cognitive impairment [98,99]. Moreover, the MMSE has adequate validity, excellent test re-test reliability and adequate inter-observer reliability [94].

Montreal Cognitive Assessment (MoCA)

The Montreal Cognitive Assessment is also a frequently used cognitive screening test. The MoCA was more recently developed than MMSE to also detect milder degrees of cognitive impairment [100].

The MoCA also has a maximum score of 30 points, with questions and tasks divided into the following subtests: visuospatial/executive functions (score 0-5); naming (score 0-3); attention (score 0-6); repetition and verbal fluency (score 0-3); abstraction (score 0-2); short-term memory recall (0-5); and orientation (0-6). The original cutoff score used to indicate cognitive impairment is MoCA<26 [100].

The MoCA has high sensitivity for detecting cognitive impairment, including milder deficits, and acceptable specificity [99]. Furthermore, the MoCA has excellent validity and excellent test re-test reliability [94].

National Institutes of Health Stroke Scale (NIHSS)

The National Institutes of Health Stroke Scale (NIHSS) is a stroke-specific measure used to quantify the range and severity of stroke symptoms [58,59]. The NIHSS is widely used clinically and in stroke trials [40, 59].

The NIHSS comprises 11 items, measuring: the level of consciousness; visual fields; horizontal eye movements; facial palsy; motor arm and leg responses; limb ataxia; sensation, language, dysarthria and neglect [58,59,94]. The total score ranges from 0-42, with higher scores reflecting more severe symptoms. Some previous studies have also added an additional 12th item for motor response in the hand, yielding a composite total score of 0-46 [101].

The NIHSS has excellent validity, and adequate test re-test and inter-observer reliability [94].

Assessment of Activity

Action Research Arm Test (ARAT)

The Action Research Arm Test (ARAT) is a frequently utilized assessment measure of upper extremity functional capacity and dexterity [102,103].

The ARAT contains 19 items categorized into the following 4 subtests: grasp (score 0-18); pinch (score 0-12); grip (score 0-18); and gross movement (score 0-9) [94,103]. Hence, the total ARAT score ranges from 0-57, with lower scores representing poorer performance.

The ARAT has excellent validity, test re-test reliability and responsiveness [94].

Barthel Index (BI)

The Barthel Index (BI) is widely used as a measure of functional disability, assessing the degree of dependency in usual activities of daily living [104].

The BI evaluates independence/dependence with regard to usual activities of daily living including: feeding; bathing; grooming; dressing; bowels and bladder functions; toilet use; transfers (bed to chair and back); mobility (on level surfaces); and climbing stairs [94,104]. The measure yields a total score from 0-100, with higher scores reflecting higher degrees of functional independence [94,104].

The BI has excellent validity, and excellent test re-test and inter-observer reliability [94].

Modified Rankin Scale (mRS)

The modified Rankin Scale (mRS) is an extensively used outcome measure, evaluating the degree of post-stroke disability with reference to pre-stroke activities [105,106].

The mRS scores range from 0-5, with each point defined as follows: 0=no symptoms; 1=no significant disabilities despite symptoms (able to carry out all usual activities); 2=slight disability (able to look after own affairs without assistance, but unable to perform all previous activities); 3=moderate disability (requires some

help, but able to walk unassisted); 4=moderately severe disability (unable to walk without assistance, and unable to attend to own bodily needs); and 5=severe disability (requires constant nursing care and attention, bedridden, incontinent) [94,105].

The mRS has excellent validity, excellent test re-test reliability and adequate inter-observer reliability [94].

Assessment of Participation

European Quality of Life-5 Dimensions (EQ-5D)

The European Quality of Life-5 Dimensions (EQ-5D) is a self-administered questionnaire used to assess various dimensions of health and health-related states [94,107].

The EQ-5D is divided into two parts, where the first part describes 5 dimensions of health, as follows: mobility; self-care; usual activities; pain/discomfort; and anxiety/depression. Each of these dimensions are divided into three levels corresponding to: no problems, some problems, or severe problems. The second part of the EQ5D is comprised of a visual analogue scale rating overall health status from 0 (worst imaginable) to 100 (best possible) [94,107,108].

The EQ-5D has acceptable validity, and adequate test re-test and inter-observer reliability [94].

The Short Form 36 Health Survey (SF-36)

The Short Form 36 Health Survey (SF-36) is also a health status questionnaire, evaluating different dimensions of health and health-related states [94,109].

The SF-36 contains 36 items divided into 8 dimensions, including: physical functioning; role limitations - physical bodily pain; social functioning; general mental health; role limitations - emotional, vitality; general health perceptions, and two questions regarding change in health status over the last year [94,109]. With a specific scoring system the items of every dimension are scored and added to yield a total score for each dimension. Subsequently, each of the dimension scores are transformed through an algorithm to a scale from 0-100 [94].

The SF-36 has excellent validity and excellent test re-test reliability [94].

Stroke Impact Scale (SIS)

The SIS is a stroke-specific patient-reported outcome measure that assess the self-perceived impact of stroke on different domains of health and life [110,111].

The SIS version 2.0 has 64 items grouped into 8 domains, including strength, hand function, ADL/IADL, mobility, communication, emotion, memory and thinking, and participation.

The items of each domain are graded from 1-5, with lower grades reflecting greater self-reported difficulties. Using a specific algorithm, aggregate scores from 0-100 are generated for every domain. The last question (item) in the SIS considers the patients' self-perceived recovery after stroke, where the patients are asked to estimate their overall recovery from 0-100 [94,110,111].

The SIS has excellent validity and adequate test re-test reliability [94].

To conclude, the ICF model provides a conceptual framework to assess health conditions, considering different interacting components including body functions and structures, activity and participation. There are numerous assessment measures used in stroke to evaluate different aspects of outcome and they can be categorized according to the ICF model.

From the perspective of SCT, the choice of outcome measure(s) in later-phase clinical trials will depend on the choice of endpoint - an important point of consideration that will be discussed later on.

Rehabilitation and Therapeutic Options beyond the Acute Phase

Stroke rehabilitation refers to "a progressive, dynamic, goal-oriented, process aimed at enabling a person with impairment to reach their optimal physical, cognitive, emotional, communicative, social and functional activity level" [112].

In clinical practice, the rehabilitation process begins immediately after stroke onset, and constitutes an essential part to promote functional recovery and improve various aspects of outcome. The stroke rehabilitation may occur in various settings, both acute and post-acute, including in stroke units, inpatient rehabilitation centers, outpatient clinics, primary health-care centers, and at the stroke patients' own places of residence [112]. A team of physicians, nurses, physiotherapists, occupational- and speech therapists, as well as psychologists and counselors, work to assist the stroke patients in the recovery of their impairments, using various rehabilitation interventions [56,112]. The specific rehabilitation interventions applied, and the duration of the targeted rehabilitation, is dependent on the particular individual stroke patient (impairments, disabilities, needs and goals), as well as the available resources [112].

A recent Cochrane review reported that physical rehabilitation improves post-stroke functional recovery, but no therapeutic approach to physical rehabilitation is clearly superior (or inferior) to another [113]. Physical rehabilitation may integrate several components of musculoskeletal, neurophysiological and cardiopulmonary interventions, functional task training, but also assistive devices and modalities [3,113]. However, the appropriate amount and optimum timing of the rehabilitation interventions are unclear [114]. Also, it was recently reported that very early, high-intensive and frequent mobilization after stroke actually lessened the odds of a favorable outcome at 3 months as compared to ordinary care [115].

Rehabilitation interventions in routine use could be categorized according to the different components of the ICF model (body functions and structures, activity, participation), but many interventions overlap between these components.

There are numerous rehabilitation interventions being used in routine practice, and several are under study. A detailed description of these is beyond the scope of this thesis, but some examples include [3,7]:

- Motor interventions
    - Constraint-induced movement therapy (training forced use of the paretic limb while restricting the other)
    - Sensory stimulation (e.g. transcutaneous electrical nerve stimulation and acupuncture)
    - Mirror therapy (methods using a mirror to create the illusion of the paretic limb performing movements or actions)
    - Mental practice (mental rehearsal of a movement or a task without actually performing it)
    - Virtual reality (observation of movements and actions, as well as performing tasks and activities, with virtual reality and interactive video gaming)
    - Robotic-assisted training devices
- Language interventions (e.g. intensive aphasia therapy)
- Cognitive interventions (e.g. cognitive training methods to improve attention deficits, memory and executive dysfunctions)
- Pharmacological interventions (e.g. treatment with selective serotonin re-uptake inhibitors)
- Brain stimulation (e.g. repetitive transcranial magnetic stimulation and transcranial direct current stimulation)

However, few large scale and high-quality clinical trials have been performed in the field of stroke rehabilitation [56], and a firm evidence base is lacking with regard to many of the abovementioned rehabilitation interventions [56,114]. More high-quality clinical trials are needed to establish their efficacy [3].

Other therapeutic approaches are also currently under consideration, such as biological therapies involving e.g. growth factors and monoclonal antibodies [116-118], and SCT described below.

To conclude, the stroke rehabilitation process is essential for promoting recovery and improving outcomes, but the appropriate amount and timing of rehabilitation interventions are unclear. There are many rehabilitation interventions and more studies are needed to establish their effectiveness.

Hence, beyond the acute phase of stroke, there are limited therapeutic options available with proven efficacy to promote functional recovery and improve outcome.

Recovery and Outcome after Stroke

Most stroke patients exhibit some degree of spontaneous recovery during the first period (weeks to months) after stroke onset [6,119]. However, the rate and degree of spontaneous recovery after stroke is highly variable among stroke patients [6,120].

General Patterns of Recovery

Some general patterns of post-stroke recovery have been described, as follows:

- There seems be an augmented rate of recovery during the first weeks which is then gradually followed by a plateau where the rate of recovery decreases or evens out [6,121,122].
- Most of the spontaneous recovery after stroke appears to take place during the first 3 months [6,122], even though it is not uncommon to encounter patients who report improvement also several years after the initial stroke onset.
- Stroke patients with milder impairments are more likely to recover than those with more severe deficits [6,121].
- Younger stroke patients are more likely to recover than older patients [123].

Nevertheless, there seem to be notable differences in the rate and extent of recovery of different types of neurological deficits [6,76]. For example, cognitive deficits have a higher likelihood of continuing to improve past the first 3 months after stroke as compared to motor deficits [6,124-127]. Significant improvements in cognitive function within 1 year after stroke have been observed in the areas of executive functions, language, and long-term memory, whereas disturbances in attention and short-term memory seem to remain [128]. However, others have reported that several aspects of memory may recover within 1 year after stroke [129].

Consequently, as the clinical manifestations of stroke are diverse, covering a broad range of neurological deficits, and since different types of deficits have variable courses of recovery, therapeutic approaches aiming to promote recovery may also have varying effects on different types of deficits [76]. Therefore, to demonstrate maximum potential treatment effect, it has been suggested that domain-specific outcomes might be better as end-points in clinical stroke trials with recovery promoting therapies, as compared to global outcomes (such as mRS or NIHSS) [76].

From the perspective of SCT, upper extremity motor impairment (UEMI) is of particular interest in this context since preclinical studies with SCT have largely focused on sensorimotor recovery after stroke, demonstrating that SCT may significantly improve forelimb motor function in rodent stroke models [130-133].

Recovery of Post-Stroke Upper Extremity Motor Impairment (UEMI)

Initial severity of UEMI is the strongest predictor of upper extremity functional capacity at 6 months after stroke [134]. However, previous studies have reported that irrespective of the initial severity, the most dramatic motor recovery tend to occur during the first 30 days post-stroke, but among stroke patients with severe impairments notable recovery can continue up to 90 days and sometimes later (Figure 6) [126,127].

In a previous study on UEMI recovery after stroke, the best possible function of the paretic upper extremity was achieved by 80% of the patients within 3 weeks, and by 95% within 9 weeks of stroke onset [62]. Significant recovery of post-stroke UEMI was unlikely beyond 11 weeks [62]. Although, more recent data from the hospital-based Stroke Arm Longitudinal Study at the University of Gothenburg (SALGOT) suggests that some stroke patients may exhibit continuous UEMI recovery up to 1 year after stroke [135]. In another study from the same group, it was also reported that there were discrepancies in objective assessments of upper extremity strength 10 days after stroke compared to the stroke patients' self-perceived strength [136].

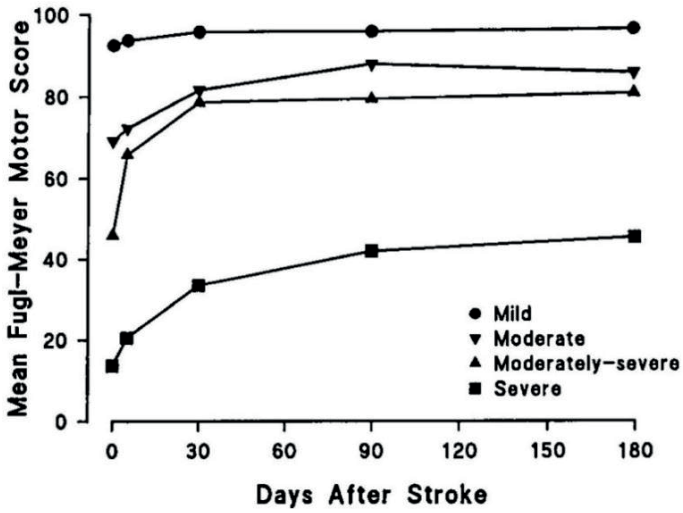

Figure 6.
Time course of motor recovery after stroke. *Reprinted from Duncan et al., Measurement of motor recovery after stroke. Outcome assessment and sample size requirements, Stroke, 1992, Vol. 23, Issue 8, pp. 1084-1089. http://stroke.ahajournals.org/content/23/8/1084.long*

To conclude, most spontaneous recovery seems to take place during the first weeks to months after stroke, but there is much variability in the rate and extent of recovery between individuals and between different types of neurological deficits. To demonstrate maximum potential treatment effect in clinical stroke trials with SCT, domain-specific outcomes, such as e.g. recovery of UEMI, might be better than global outcomes.

However, the feasibility of post-stroke UEMI as a target for SCT is unclear. More studies are needed to assess the frequency of post-stroke UEMI, the recovery of post-stroke UEMI in a longer-term perspective, as well as the relationship of post-stroke UEMI to activity limitations and self-perceived participation restrictions.

Case Fatality and Functional Outcome after Stroke

Population-based data on stroke outcome from 6 European stroke registries has demonstrated that more than 40% of first-ever stroke patients are either dead, dependent in ADL or institutionalized after 3 months from stroke onset, with some variations between populations [137].

The one-month mortality after stroke ranges from 5%-20%, and the one-year mortality has been reported around 20%-40% [89,138,139]. In the longer-term perspectives after stroke, roughly 60% die after 5 years, and 75-80% after 10-15 years [140-142].

A recent observational study based on data from the Swedish Stroke Registry recently reported that 16% of hospitalized stroke patients were dependent in ADL after 3 months, and 28% after one year [89].

Previous population-based studies on longer-term functional outcome after stroke have reported that roughly 30-40% of stroke survivors have moderate-severe disability after 5 years [4,143,144], and the corresponding figure is about 30% after 10-15 years [142]. Moreover, approximately 10-15% of stroke survivors are institutionalized in nursing or residential care homes after 5-10 years from stroke onset [4,143,145,146]. Yet, another recent population-based stroke study showed that 10-year stroke survivors have a high activity level in daily life, but there was considerable individual variation [147].

In the Oxford Vascular Study (OXVASC), it was reported that age, event severity, previous disability, recurrent stroke, and marital status were important predictors of disability or death 5 years after TIA and stroke [4].

To conclude, previous studies show that a large proportion of stroke survivors have lasting functional disabilities and dependency in daily activities after stroke.

However, there is a need for more up-to-date population-based studies covering long-term (≥10 years) outcomes after stroke that are not influenced by selection bias due to significant losses to follow-up, and this particularly applies for studies covering all the components of the ICF model (impairments, activity limitations and participation restrictions [141,142]. Reliable data on longer-term stroke outcomes are important for optimized planning of health-care services, but also for guidance of novel therapeutic approaches aiming to improve functional recovery and outcome after stroke, such as SCT.

Stem Cell-Based Therapies for Stroke

Stem cells have two important functional characteristics: an unlimited ability for self-renewal, and the capacity to differentiate into multiple types of cells [148,149].

Some stem cells are totipotent, meaning that they have the ability to differentiate into any type of cell (totipotent cells can form the embryo) [148]. Pluripotent stem cells have the capacity to differentiate into several different tissue cell lines (e.g. embryonic stem cells) [148]. Multipotent stem cells can only differentiate into more restricted number of cell lines, suitable for a specific tissue (e.g. neural stem cells) [148]. Unipotent stem cells can only form one particular cell type [148]. Progenitors

refer to proliferative cells that have a more restricted ability for self-renewal, and are mostly unipotent [149].

SCT aiming to improve recovery after stroke can be based on strategies focusing on promoting endogenous neurogenesis, or the application of either exogenous stem cells or induced pluripotent stem cells (somatic cells from a patient that have been reprogrammed into pluripotent stem cells) [8,10,150,151].

Endogenous Neurogenesis and SCT

There are two classically recognized neurogenic regions in the adult brain, the subventricular zone (SVZ), lining the lateral walls of the lateral ventricle [150-152], and the subgranular zone (SGZ) in the dentate gyrus [150,151].

Studies in animal models of stroke have shown that focal ischemic brain injury in the striatum or cortex induces proliferation of neural stem and progenitor cells in the SVZ, followed by migration of the newly formed neuroblasts toward the injured area, their differentiation into mature neurons and integration into the lesion area [150,151,153-157]. More recent studies indicate that new neurons might also be generated from astrocytes after stroke [158]. Experimental studies suggest that this process of endogenous neurogenesis following stroke may contribute to functional recovery [151,155,159-161].

Studies in humans have provided evidence of proliferating neural progenitor cells and an increase of neuroblasts in the SVZ, as well as in the brain parenchyma in close proximity to the ventricular wall after ischemic stroke [151,162,163]. In addition, proliferating cells and neuroblasts have been found in the ischemic penumbra surrounding cortical infarcts in stroke patients [164]. Findings of newly generated neurons from the SVZ has also been described in the striatum of adult humans under normal conditions revealed by ^{14}C dating techniques [165].

SCT aiming to promote endogenous neurogenesis following stroke have focused on approaches such as: (i) increasing neural stem cell proliferation by different growth factors, anti-inflammatory drugs and hormones; (ii) stimulating survival of newly formed neural stem cells through delivery of certain growth factors; and (iii) stimulating migration of endogenous stem cells through various chemokine receptors [8].

However, more studies are needed to ascertain the role and potential of endogenous neurogenesis for recovery after stroke, as well as the involvement of neurogenic areas in the brain such as the SVZ.

SCT with Exogenous or Induced Pluripotent Stem Cells

Exogenous stem cells can be derived from various sources such as an embryo or fetus (e.g. embryonic stem cells and neural stem cells), birth-related tissues (e.g.

umbilical cord and placenta), and adult sources (e.g. bone marrow, blood, adipose tissue and skin) [9]. There are also immortalized cell lines derived from tumor cells or developed through manipulation with oncogenes [8].

Induced pluripotent stem cells are of particular interest as they are derived from somatic cells that have been reprogrammed, through transcription factors, into patient-specific pluripotent stem cells, and may thereby circumvent many problems facing other stem cell sources, such as ethical issues, supply limitations and the potential necessity of immunosuppressive therapy [8,10].

The modes of delivery of the stem cells include systemic administration, either intravenously or intra-arterially, or intracranial transplantation through stereotactic injection in the brain [8,9].

Mechanisms of Actions of SCT

The precise mechanisms of action of SCT in stroke are not fully identified. However, possible mechanisms through which SCT may promote stroke recovery include:

- Neuronal replacement [10,166]
- Secretion of trophic factors [8,10]
- Immunomodulation and anti-inflammatory effects [8-10]
- Stimulation of angiogenesis [8-10,167]
- Promoting neural plasticity by stimulating synaptogenesis, dendritic growth and axonal remodeling [8,9,167]

There are two principal goals by which SCT aim to provide improved functional recovery after stroke: (i) intracerebral transplantation of stem cells (or stem cell-derived neuron precursors) with the aim to replace the lost neurons and restore the damaged circuitry, i.e. neuronal replacement; or (ii) the delivery of stem cells, either systemically or through intracerebral transplantation, to achieve the other mechanisms of action described above.

If achieved, replacement of lost neurons after stroke and the subsequent integration of the new stem-cell derived neurons into the damaged circuitry may provide optimal functional recovery. This represents a long-term scientific goal [9]. To date, most SCT under consideration for clinical applications aim for the other mechanisms of action [9].

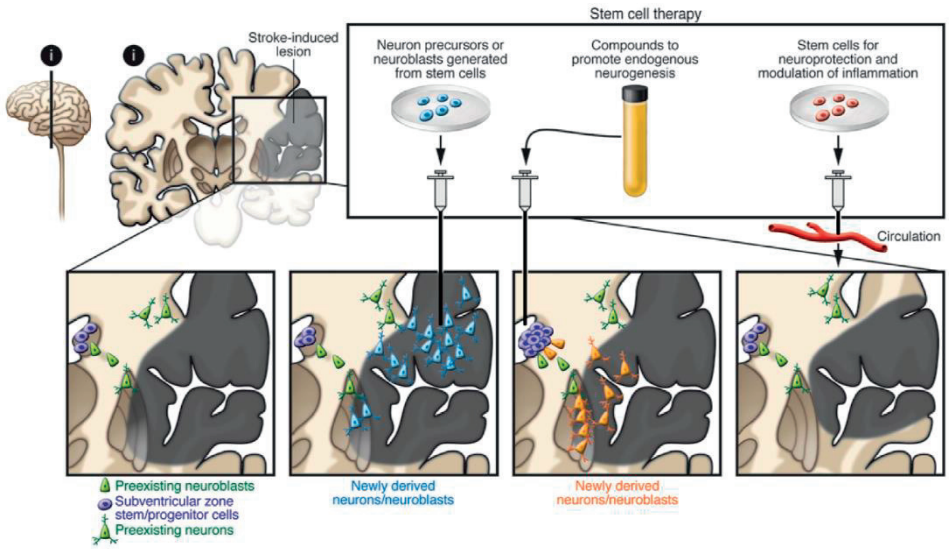

Figure 7.
SCT for stroke could be used to: transplant stem cells (or stem cell-derived neuron precursors) with the aim to replace the lost neurons and restore the damaged circuitry; or promote endogenous neurogenesis following stroke; or deliver stem cells, either systemically or through intracerebral transplantation, to achieve potentially beneficial paracrine and immunomodulatory effects. *Republished with permission from American Society for Clinical Investigation, from The Journal of Clinical Investigation, Stem cells in human neurodegenerative disorders - time for clinical translation?, Lindvall O, Kokaia Z, Vol. 120, Issue 1, Copyright (2010); permission conveyed through Copyright Clearance Center, Inc.*

SCT aiming for neuronal replacement through grafted neural stem cells, will probably require the generation of lesion specific neuronal cell types [168]. In this regard, ischemic lesions confined to striatum are of specific interest. Firstly, striatal neurons have been generated in vitro from neural stem cells [169,170]. Secondly, the striatum is an important lesion site in many animal stroke models [14]. Thirdly, experimental studies have reported improved functional recovery after transplantation of neural stem cells to stroke-damaged striatum, possibly providing benefit through neuronal replacement [11,14]. Fourthly, striatal astrocytes in the adult mouse brain parenchyma carry a latent neurogenic program that may be useful for stem-cell based neuronal replacement strategies [158]. Lastly, striatum is an important site for intracerebral cellular transplantation due to its surgical accessibility [11,75].

However, clinical studies on stroke patients with infarcts confined to striatum are scarce, and the frequency of striatal infarcts among ischemic stroke patients is unclear.

Translation to the Clinic

A plethora of studies with SCT in animal stroke models, performed over more than a decade and using various stem cell types and different modes of delivery, have provided pre-clinical evidence that SCT has a potential to improve recovery after stroke [8,10,13].

At present, several clinical stroke trials with SCT are ongoing, mostly testing safety in small patient samples [8,13]. For example, a phase-I study, with stereotactic injections of human neural stem cells in ipsilateral putamen of 11 chronic ischemic stroke patients, recently reported no adverse events with follow-ups between 9 and 24 months[75].

Although this field continues to evolve, still several crucial issues remain to be addressed. However, the following question is the most critical for the clinical application of SCT in stroke:

- *Is SCT feasible and beneficial for stroke patients?*

To increase the possibilities of translating pre-clinical findings to effective clinical treatments, given the fact that stroke is a heterogeneous disorder in many respects, some important points to consider include:

- What is the role and potential of endogenous neurogenesis for stroke recovery and what is the involvement of the SVZ in stroke patients?

- Which stroke lesions should be targeted by SCT, and which subtypes of neurons should be generated, especially if neuronal replacement strategies can develop to significantly contribute to stroke recovery?

- Which neurological impairments are feasible to target, taking into account the natural course of recovery, and which stroke measurements should be performed to assess the possible effect of SCT?

- What is the natural course of long-term outcomes after stroke as regards impairments, activity limitations and participation restrictions, and what are the implications for SCT?

- What are stroke patients' attitudes about SCT?

This thesis studies aspects of these questions, as described on the following page.

Aims

The overall aim of this thesis was to explore and describe clinical symptoms, lesion appearance and outcome after stroke to provide guidance and enhance possibilities for the clinical implementation of stem cell-based therapeutic approaches intended to improve recovery and functional outcome after stroke.

The specific aims were:

- To investigate the spatial relationship of brain infarcts to the SVZ in individuals with first-ever ischemic stroke potentially suitable for SCT (Paper I)
- To investigate the proportion of striatal infarcts in individuals with first-ever ischemic stroke potentially suitable for SCT (Paper I)
- To examine individuals with first-ever ischemic stroke potentially suitable for SCT regarding: the frequency of UEMI; the degree of spontaneous recovery of UEMI during a 3-5 years period after stroke; and the relation of post-stroke UEMI to activity limitations and participation restrictions (Paper II)
- To assess functional status and patient-reported outcome in 10-year survivors of a population-based group of individuals with first-ever stroke (Paper III)
- To investigate the prevalence and characteristics of cognitive impairment in 10-year survivors of a population-based group of individuals with first-ever stroke, compare the cognitive function of these long-term stroke survivors with matched non-stroke control persons, and to compare two common cognitive assessment instruments (Paper IV)
- To explore the knowledge and attitudes about SCT, as well as possible factors influencing this, among 3-5 year survivors of a selected group of individuals with first-ever ischemic stroke potentially suitable for SCT (Paper V)

Methods

Study Samples

Lund Stroke Recovery Study (Papers I, II and V)

Papers I, II and V are based on the inception cohort of the Lund Stroke Recovery Study (LSRS), which is a hospital-based prospective and longitudinal observational study on functional recovery and long-term effects after ischemic stroke. The study participants in LSRS were initially selected on the basis that they would have been potentially suitable for SCT if this treatment had been available, and they were selected as described below.

First-ever acute ischemic stroke patients admitted to Skåne University Hospital in Lund, Sweden, were included consecutively between July 7, 2009 and January 6, 2011. Stroke was defined according to the WHO criteria. Non-contrast CT of the brain was performed in all patients to rule out hemorrhagic stroke.

Inclusion criteria:

i) Age 20-75 years

ii) NIHSS score 1-18 on day 2-4 after stroke onset

iii) DW-MRI examination within 4 days of stroke onset

iv) Written informed consent

Patients who were treated with thrombolysis and/or endovascular intervention were allowed to be included. Initially, patients aged 71-75 years, with iatrogenic stroke, or living outside the local hospital uptake area were not included, but such patients were also included after 5 months from study onset in order to increase recruitment.

Exclusion criteria:

i) symptoms or CT findings indicating brainstem or cerebellar infarction

ii) severe comorbidity

iii) contraindications to MRI

To detect possible stroke patients admitted to the hospital during the study period, a study nurse performed daily screenings (Monday-Friday) of the hospital admission lists, inpatient lists of the Departments of Neurology, Neurosurgery and Internal Medicine, as well as daily screenings of the Emergency Department visits lists. The same multiple overlapping sources and prospective methods for case ascertainment were used in the LSRS as for the LSR, described below. The assessment of patients that could be appropriate for inclusion was performed by a NIHSS-certified study nurse and a physician. The decision to proceed for inclusion (or to exclude) was made by the physician.

In total, 466 patients were screened during the 18-month period of inclusion in the LSRS (Figure 8), of which 108 ischemic stroke patients were included and assessed in the acute/subacute phase for Paper 1. A clinical follow-up was aimed to be performed of these 108 patients after 3-5 years for Papers II and V (see "Follow-Up Procedures").

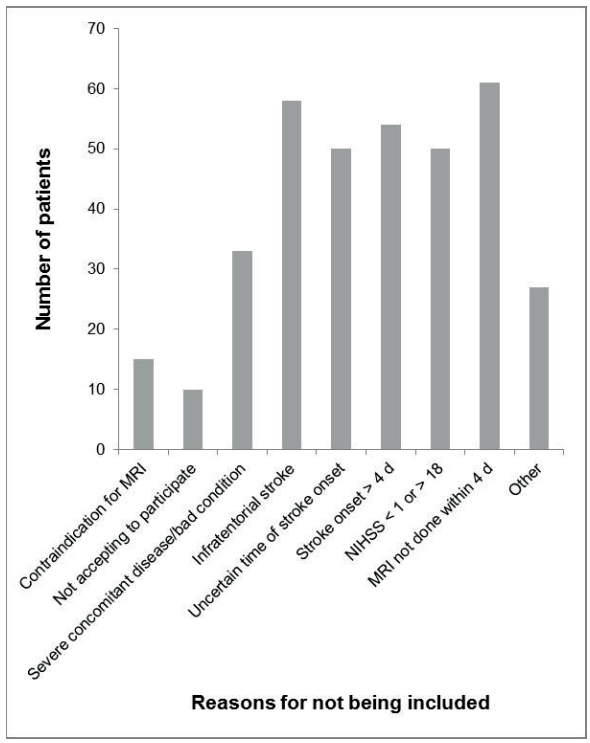

Figure 8.
Screened patients not included in LSRS inception cohort. *Reprinted from European Journal of Neurology, Vol. 20, Delavaran et al., Proximity of brain infarcts to regions of endogenous neurogenesis and involvement of striatum in ischaemic stroke, pp. 473-479, Copyright (2012), with permission from John Wiley and Sons.*

Lund Stroke Register (Papers III and IV)

Papers III and IV are based on the first year cohort, i.e. participants included between March 1, 2001 and February 28, 2002, of the Lund Stroke Register (LSR). The LSR is a population-based prospective and longitudinal observational stroke study in the south of Sweden.

First-ever stroke patients (defined by the WHO criteria) have been included consecutively in LSR since March 1, 2001. The LSR study area covers eight municipalities in the south of Sweden (Lund, Burlöv, Lomma, Staffanstorp, Kävlinge, Eslöv, Höör, and Hörby) with a total of 234 505 inhabitants (December 31, 2001).

During the first year of the LSR, multiple overlapping sources were used to identify possible stroke patients [171]. Both prospective and retrospective methods were used for case ascertainment. The prospective methods included daily week-day screenings of the Emergency Department visits list, admissions to the Department of Neurology and its outpatient clinic visits, as well as weekly screenings of admission lists of the Department of Neurosurgery. In addition, regular inquiries were made with the other clinics/departments of the hospital, and with general practitioners at the primary health care centers and nurses in skilled nursing facilities within the study area [171].

Searches were also performed retrospectively in the computerized medical records of the primary health care centers in the area, as well as screenings of the discharge diagnosis lists of the hospital, the autopsy registers of the Department of Pathology and the Department of Forensic Medicine. Finally, retrospective screenings were done in the Swedish Cause of Death Register. The screenings were performed for a range of diagnoses of a possible stroke or TIA according to the 10th revision of the International Classification of Diseases (ICD-10, including G45, G46, G81, G83 and I60-I69) [171].

CT of the brain (or autopsy records in applicable cases) was used to identify the main pathological subtype of stroke (CI, ICH or SAH). In cases of suspected SAH and negative CT scan, lumbar puncture was performed to confirm or exclude the diagnosis. Undetermined stroke type (UND) was denoted to cases where neither CT nor autopsy had been done.

In total, 416 stroke patients were registered by the prospective methods in the first year of the LSR. The first-year LSR participants were followed-up after 4 and 16 months from stroke onset. Various features of stroke outcome was assessed at these follow-ups, including health-related quality of life [172], pain [173], shoulder pain [174], and weight loss [175]. Also, a 10-year follow-up was conducted of the first-year cohort in the LSR for Papers III and IV (see "Follow-Up Procedures").

Gott Åldrande i Skåne (Paper IV)

For Paper IV, we recruited a sample of non-stroke control persons from the "Gott Åldrande i Skåne" (GÅS). GÅS is a prospective and longitudinal observational population study, and constitutes one of four parts of the Swedish National Study on Aging and Care (SNAC) [176,177].

The GÅS study was initiated in 2001. Out of 4 893 eligible individuals invited by letter and residing in five municipalities in southern Sweden (Malmö, Eslöv, Ystad, Hässleholm and Osby) a total of 2 931 (60%) participated. The invited individuals were selected randomly (computerized random number generator) from the National Population Register. The only exclusion criteria was inability to speak Swedish. The invited individuals were of both genders and aged 60-93 years (belonging to the following age cohorts: 60, 66, 72, 78, 81, 84, 87, 90 and 93 years), and the target population was 3 000 individuals.

The investigation took place at a research center, but participants unable to attend were offered home visits in order to reduce selection bias. The investigation included a medical examination, functional and cognitive assessments, and questionnaires, and took place between February 2001 and July 2004. The cognitive tests including MMSE was performed by two trained test administrators with a Bachelor of Science in Behavioral Sciences according to a standardized routine. The medical examination performed by a physician also included medical history, ongoing medication, and medical diagnoses based on review of medical records.

For every 10-year stroke survivor from the LSR in Paper IV aged ≥60 years (n=118), 3 age- and sex-matched non-stroke control persons were selected from the GÅS database (in total 354 persons) by one of the co-authors, who was blinded to other details with regard to the stroke survivors. Control persons with a history of stroke according to medical examination, medical records or history were excluded before selection.

Follow-Up Procedures

Papers II and V

As previously mentioned, the LSRS inception cohort studied during the acute/subacute phase of ischemic stroke in Paper I, was followed-up after 3-5 years for Papers II and V.

We first performed a check in the National Population Register to examine whether any of the original LSRS participants were deceased at the time of follow-up. For the deceased ischemic stroke patients, the dates of death were registered. The

identified survivors from the LSRS were then invited (via telephone followed by regular mail) to participate in the follow-up. For all LSRS survivors who provided written informed consent to participate, a clinical follow-up examination was arranged 3-5 years after stroke onset (Figure 9).

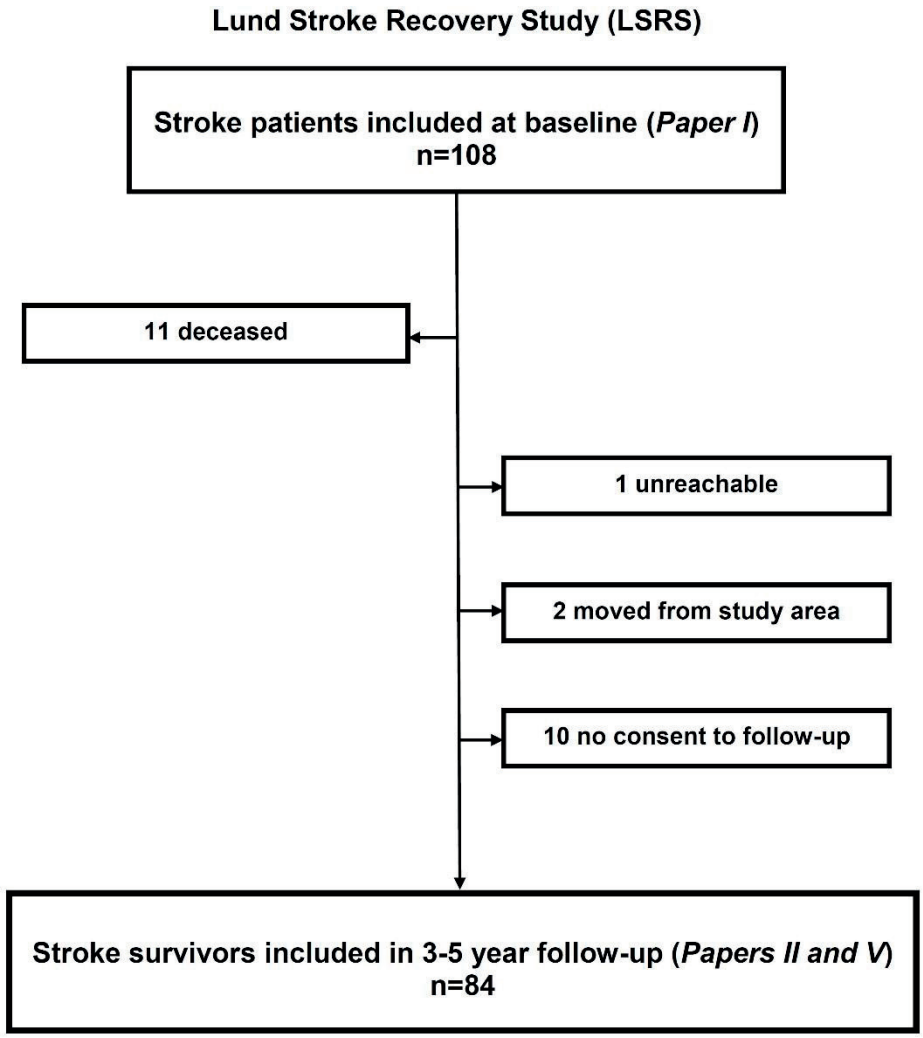

Figure 9.
Flow chart of the LSRS cohort

The clinical follow-up sessions were performed at the outpatient clinic of the Department of Neurology at Skåne University Hospital in Lund. Stroke survivors unable to come to the outpatient clinic were offered follow-up at their own homes. The LSRS survivors were examined according to a pre-specified clinical assessment

protocol including evaluations of neurological impairments (focusing on UEMI), activity limitations, and participation restrictions (see "Clinical Assessments and Outcome Measures"). The survivors were also presented an information sheet on stroke and SCT, and a subsequent multiple-choice questionnaire regarding their prior knowledge and attitudes about SCT (see "Clinical Assessments and Outcome Measures"). The duration of rehabilitation after index stroke, events of recurrent stroke, other medical diagnoses, and ongoing medication were also registered on the basis of information from the stroke survivors and review of medical records. The clinical LSRS follow-ups were performed by a physician.

Papers III and IV

As mentioned above, the survivors of the first-year cohort in the LSR were followed-up after 10 years from stroke onset for Papers III and IV.

Before follow-up, an inquiry was performed in the National Population Register to ascertain whether participants in the previous follow-up were deceased or had survived. The survivors were subsequently invited to the 10-year follow-up (via both telephone and regular mail). All survivors from the previous follow-up 16 months after stroke consented to also participate in the 10-year follow-up (Figure 10).

The 10-year follow-ups were arranged at the outpatient clinic of the Department of Neurology at Skåne University Hospital in Lund, or at the stroke survivors' own homes or in skilled nursing facilities. In some few cases the follow-ups were performed while the survivors were being hospitalized (n=9).

The 10-year follow-ups were performed following a pre-specified protocol including assessment of cognitive function (Paper IV), as well as evaluation of activity limitations and self-reported participation restrictions (Paper III) (see "Clinical Assessments and Outcome Measures"). Moreover, housing and work situation, education level, events of recurrent stroke, other diagnoses, vascular risk factors and ongoing medication were registered.

However, stroke survivors were excluded from cognitive testing if they were unwilling to perform the cognitive tests, if their overall health condition hindered cognitive testing, or if they could not perform the testing due to severe dysphasia/aphasia, poor vision, or hearing impairment.

The 10-year follow-ups were performed by a physician and a nurse.

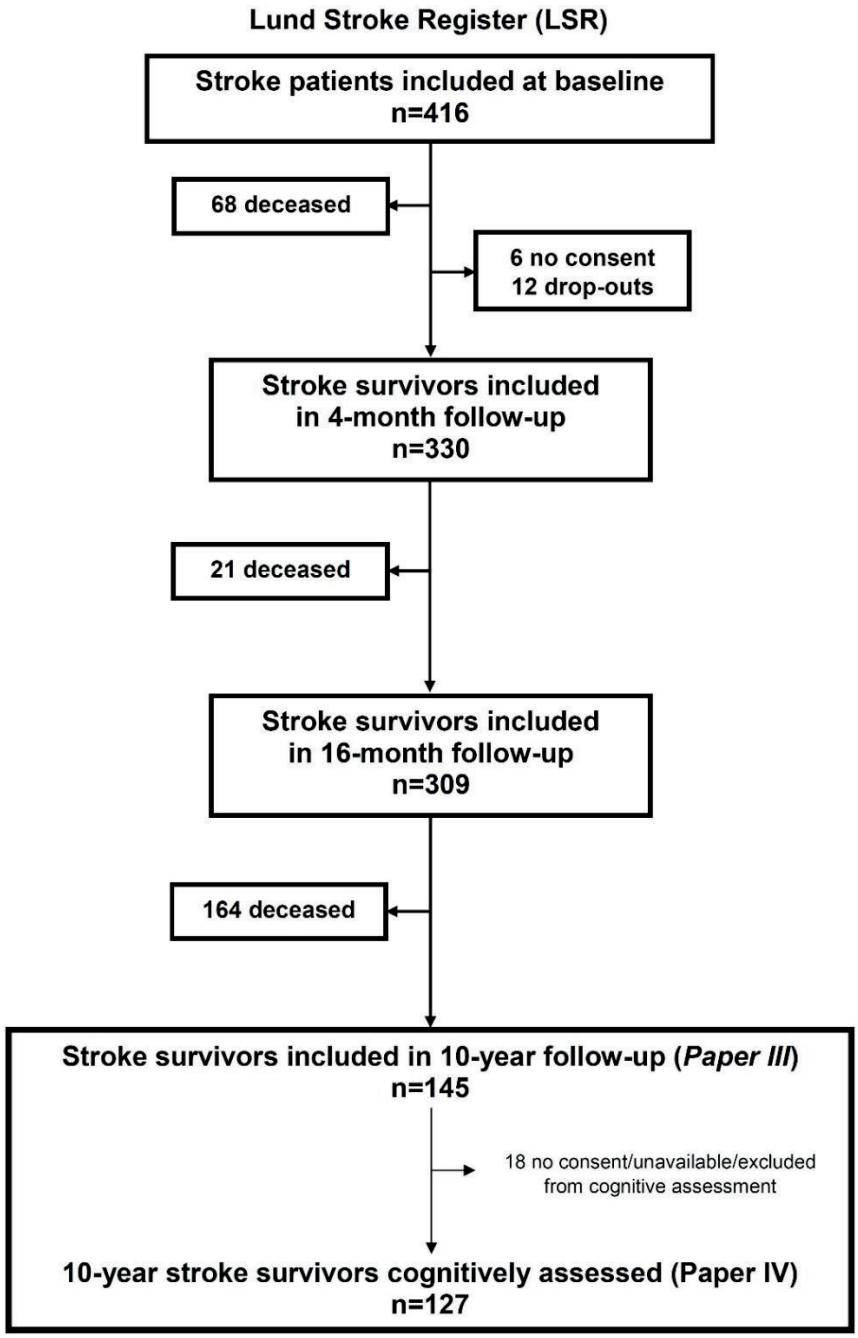

Figure 10.
Flow chart of the LSR cohort.

Neuroimaging

In paper I, the patients in the LSRS inception cohort were examined with transversal DW-MRI, as well as transversal FLAIR-, GRE- and sagittal T2-weighted sequences within 4 days from stroke onset. Acute focal ischemic abnormalities were defined as areas of restricted diffusion on diffusion-weighted sequences and with reduced ADC in the corresponding areas. The location, size and number of acute focal ischemic lesions were registered. Moreover, the acute ischemic lesions were manually demarcated on each slice, and the lesion volume was estimated as the sum of the delineated lesion areas multiplied with the sum of the slice thickness (5 mm) and the interslice gap (1 mm).

In addition, the spatial relationship of the ischemic lesions to the SVZ was evaluated by measuring the minimum distance from the nearest margin of the ischemic lesions to the lateral walls of the lateral ventricles. Striatum was defined as caudate nucleus and putamen.

In paper II, the baseline MR-images of the 3-5 year stroke survivors from the LSRS that had UEMI in the acute/subacute phase (including those that showed complete recovery of UEMI at follow-up and those that displayed residual impairment) were evaluated with regard to the ischemic lesions' possible involvement of the motor cortex (primary motor cortex, premotor areas and supplementary motor areas). In addition, the possible involvement of the corticospinal tract was assessed on the baseline MR-images, using previously established neuroradiological methods to estimate the location of the corticospinal tract on MRI [178].

The neuroimaging analysis was performed by an experienced neuroradiologist who was blinded to the clinical information.

Clinical Assessments and Outcome Measures

An overview of the clinical assessments and outcome measures used in Papers I-V are presented in Table 3.

Table 3.
Overview of the clinical assessments and outcome measures used in Papers I-V

	Paper I	Paper II	Paper III	Paper IV	Paper V
Study focus	Lesion appearance	Upper extremity motor impairment	Functional and patient-reported outcome	Cognitive function	Attitudes to stem cell therapy
Study cohort	LSRS	LSRS	LSR	LSR	LSRS
Assessment time after stroke onset	2-4 days	3-5 years	10 years	10 years	3-5 years
Assessment of impairments					
National Institutes of Health Stroke Scale (NIHSS)	X	X			X
Fugl-Meyer Assessment of Upper Extremity (FMA-UE)		X			
Mini-Mental State Examination (MMSE)				X	
Montreal Cognitive Assessment (MoCA)				X	X
Assessment of activity limitations					
Action Research Arm Test (ARAT)		X			
Barthel Index (BI)			X		
Modified Rankin Scale (mRS)		X	X	X	X
Assessment of participation restrictions					
Stroke Impact Scale (SIS)		X			X
European Quality of Life-5 Dimensions (EQ-5D)			X		
Short Form 36 Health Survey (SF-36), question 1		X	X		
Other assessments					
Ouestions on physical activitivy			X		
Stem cell therapy questionnaire					X
Charlson Comobidity Index (CCI)					X

Assessment of Impairments

National Institutes of Health Stroke Scale (NIHSS - Papers I, II and V)

The LSRS cohort was evaluated at baseline with the NIHSS including item 12 for hand motor function (composite score 0-46), on day 2-4 after ischemic stroke onset, to assess the severity of stroke symptoms (Paper I).

In the 3-5 year follow-up of the LSRS cohort (Papers II and V), the NIHSS was also used as a measure of stroke severity. In Paper II, the NIHSS data also includes item 12 (composite score 0-46), whereas in Paper V the NIHSS results are presented without item 12 (score 0-42).

In Paper II, the overall stroke severity was defined by the following NIHSS scores as follows: no symptoms=0; mild=1-4; moderately severe=5-14; and severe ≥15 [59]. Additionally, UEMI at both baseline and follow-up was defined as a score of ≥1 on the combined NIHSS arm and hand motor items (i.e. items 5 and 12; composite score 0-6). In case of bilateral UEMI, the mostly impaired side was assessed throughout the study. The combined score on the NIHSS arm and hand motor items was also used for the longitudinal evaluation of the recovery of UEMI between baseline and follow-up (ΔNIHSS arm/hand).

Fugl-Meyer Assessment of Upper Extremity (FMA-UE - Paper II)

In Paper II, we used the FMA-UE for a more comprehensive assessment of the 3-5 year survivors from the LSRS that had residual UEMI. Based on the FMA-UE scores, the degree of UEMI was defined as follows: severe=0-22; moderate=23-52; and mild=53-66 [179,180].

Mini-Mental State Examination (MMSE - Paper IV) and Montreal Cognitive Assessment (MoCA - Papers IV and V)

The cognitive assessment of the 10-year survivors from the LSR, presented in Paper IV, comprised of the MMSE and MoCA [100,181]. We used the MoCA version 7.0, which has a 1-point correction for ≤12 years of education [100]. The order of cognitive testing was pre-specified similar to previous studies [182], starting with MMSE and subsequently followed by MoCA. Identical tasks on the MMSE and MoCA (serial 7s subtraction and orientation questions) were only administered once.

We interpreted the MMSE and MoCA scores according to recently suggested cutoffs, based on validations in stable cerebrovascular patients [99,183,184]. For the MMSE, cognitive impairment was defined according to the following cutoff scores as: severe=0-22 (indicative of dementia); mild=23-26 (indicative of MCI); or none=27-30 (indicating normal cognitive function) [99,183,184]. With regard to the MoCA scores, cognitive impairment was defined as follows: severe=0-19

(indicative of dementia); mild 20-24 (indicative of MCI); or none=25-39 (indicating normal cognitive function) [99,183,184].

We particularly considered the items of MMSE and MoCA that involve visuoexecutive functioning, including the MMSE visuoconstruction subtest (score 0-1) and the MoCA visuospatial/executive functions subtest (score 0-5) [185].

The non-stroke control persons from GÅS included in Paper IV had performed the same MMSE version as the 10-year survivors from the LSR. However, the control persons had not been evaluated with MoCA.

In Paper V, describing the knowledge and attitudes about SCT among the 3-5 year survivors of the LSRS, we used the MoCA version 7.0, described above, to evaluate cognitive function.

Assessment of Activity Limitations

Action Research Arm Test (ARAT - Paper II)

The ARAT was used in Paper II, to examine the upper extremity functioning among the LSRS survivors with UEMI at the 3-5 year follow-up. The upper limb functional capacity was defined by the following ARAT cutoff scores as: none=0-10; poor=11-21; limited=22-42; notable=43-54; or full=55-57 [180].

Barthel Index (BI - Paper III)

We used the BI in Paper III, to evaluate the functional disability of the 10-year survivors from the LSR. The BI scores were graded as follows: major dependence (score 0-55); minor dependence (score 60-90); and independence (score 95-100) [186].

Modified Rankin Scale (mRS - Papers II, III, IV and V)

The mRS (score 0-5) was used at the 3-5 year follow-up in the LSRS (Papers II and V), as well as at the 10-year follow-up in the LSR (Papers III and IV), to assess the stroke survivors' degree of overall disability.

Assessment of Participation Restrictions

Stroke Impact Scale (SIS - Papers II and V)

In the 3-5 year follow-up of the LSRS cohort, we used the SIS version 2.0 to assess the patient-reported impact of stroke on different domains of health and life. For Paper II, we used the results of the specific SIS domains concerning ADL and social participation, and for Paper V we used the last SIS question with regard to overall stroke recovery.

European Quality of Life-5 Dimensions (EQ-5D - Paper III)

The first part of EQ-5D was used in Paper III to acquire self-reports from the 10-year survivors in the LRS with regard to mobility, self-care, usual activities, pain/discomfort, as well as anxiety and depression.

Short Form 36 Health Survey (SF-36 - Papers II and III)

We used the first question of the SF-36 ("In general, would you say your health is excellent, very good, good, fairly good, or poor?") in Papers II and III, to obtain the stroke survivors' self-perceived general health status.

Other Assessments

Questions on Physical Activity (Paper III)

In Paper III, the 10-year stroke survivors from the LSR were also asked questions concerning physical activity ("How often do you walk, bike, run, or practice physical activities in other ways – never, less than once weekly, once weekly, 2-3 times weekly, or ≥4 times weekly?").

Stem Cell Therapy Questionnaire (Paper V)

For Paper V, the 3-5 year survivors from the LSRS were presented a neutral information sheet on stroke and SCT, and thereafter they answered an 8-part multiple choice questionnaire about their prior knowledge of SCT and their attitudes towards it (the aforementioned information sheet and the SCT questionnaire are both appended in the end of Paper V).

The information sheet and the subsequent questionnaire were written by the authors of Paper V. The questionnaire contains 8 parts comprising questions regarding: prior knowledge about SCT in stroke; overall attitude towards SCT in stroke; willingness to undergo SCT through intracerebral transplantation; willingness to undergo SCT through systemic administration; ethical considerations about SCT; self-perceived need of a recovery promoting treatment such as SCT; impairments in most need of SCT for (if it had been available); and willingness to participate in a clinical stroke trial with SCT.

Charlson Comorbidity Index (CCI - Paper V)

In Paper V, the burden of comorbid health conditions among the 3-5 year survivors from the LSRS were evaluated using the Charlson Comorbidity Index (score 0-29), with higher scores reflecting higher degrees of comorbidity [187].

Statistical Methods

An overview of the statistical methods used in Papers I-V is presented in Table 4. In all statistical analyses performed, *p*-values <0.05 were considered statistically significant. The IBM SPSS Statistics for Windows software (versions 21 and 22) was used for all statistical calculations.

Table 4.
Overview of the statistical methods used in Papers I-V

Statistical Method	Paper I	Paper II	Paper III	Paper IV	Paper V
Mann-Whitney's two sample test		X	X	X	
Fisher's exact test		X		X	
Wilcoxon signed-rank test			X		
Spearman's rank order correlation		X	X		
McNemar's test				X	
Univariate logistic regression analysis				X	X
Multivariate logistic regression analysis				X	X

Paper I

In Paper I, averages were described using median values and variability of data was described with the range of values.

Paper II

In Paper II, we used Mann-Whitney's two sample test and Fisher's exact test for comparisons with continuous and categorical variables, respectively.

We also used the Spearman's rank order correlation to examine the relation of UEMI (as defined above) to: i) degree of overall disability as measured with mRS; ii) patient-reported activity limitations and participation restrictions as evaluated with the SIS domains concerning ADL and participation; and iii) self-perceived general health status as assessed with the first question of SF-36.

Paper III

In Paper III, we used Mann-Whitney's two sample test for comparisons with continuous variables. We also used Wilcoxon signed-rank test for comparisons of the same individuals between baseline and the follow-up after 10 years.

In addition, Spearman's rank order correlation was used to analyze the relation between outcome variables (as assessed with BI, mRS and the first question of SF-36) and age at the 10-year follow-up.

Paper IV

In Paper IV, we used Mann-Whitney's two sample test and Fisher's exact test for univariate case-control analyses with continuous and categorical variables, respectively.

Moreover, we used univariate and multivariate logistic regression analyses, adjusting for the possible confounding effect of education (number of years of formal education), to evaluate the odds of having severe cognitive impairment (MMSE<23) among the 10-year survivors from the LSR vs. the controls.

We also used McNemar's test to assess possible discrepancies between MMSE and MoCA at corresponding cutoff scores (MMSE<27 vs. MoCA<25; and MMSE<23 vs. MoCA<20).

Paper V

In Paper V, we evaluated possible factors that may influence the 3-5 year LSRS survivors' attitudes to SCT, using pre-specified univariate and multivariate logistic regression analyses. Independent variables in the analyses were chosen as follows: age, gender, education (number of years of formal education), cognitive function (MoCA score), comorbid burden (CCI score), stroke severity (NIHSS score), overall recovery of stroke symptoms between baseline and follow-up (ΔNIHSS), self-perceived overall stroke recovery (as assessed with SIS), and prior knowledge of SCT. The dependent variables were the 3-5 year LSRS survivors' responses to the SCT questionnaire with regard to: i) attitude to SCT ("positive" vs. combined "negative" or "do not know/do not wish to answer"); ii) willingness to undergo SCT via intracerebral transplantation ("yes" vs. combined "no" or "do not know/do not wish to answer"); and iii) willingness to take part in a clinical SCT trial ("yes" vs. combined "no" or "do not know/do not wish to answer").

In this paper, we also did post-hoc analyses with univariate and multivariate logistic regression models to assess possible factors associated with the tendency to respond "do not know/do not wish to answer" in the SCT questionnaire.

Ethical Approval

The studies described in Papers I-V were approved by the Regional Ethical Review Board in Lund, Sweden, with the following registration numbers: 2009/156 (Paper I); 2014/298 (Papers II and V); and 2011/278 (Papers III and IV)

Informed consent was obtained from the individual study participants or their relatives.

Results

Table 5 presents an overview of the demographic characteristics and main pathological subtypes of the study participants in Papers I-V.

Table 5.
Overview of demographic characteristics and main stroke subtypes in Papers I-V

Variable	Paper I	Paper II	Paper III	Paper IV		Paper V
Study cohort	LSRS	LSRS	LSR	LSR	GÅS	LSRS
Selection	All eligible	3-5 year survivors	10-year survivors	10-year survivors	Control persons	3-5-year survivors
Sample, n	108	84	145	127	354	84
Sex, n (%)						
Female	37 (34)	30 (36)	59 (41)	54 (43)	156 (44)	30 (36)
Age, median (range)	64 (28-75)	68 (33-80)	78 (27-97)	77 (27-93)	78 (60-93)	68 (33-80)
Stroke types, n (%)						
CI	108 (100)	84 (100)	126 (87)	111 (87)	-	84 (100)
ICH	-	-	10 (7)	8 (6)	-	-
SAH	-	-	8 (6)	8 (6)	-	-

CI=cerebral infarction; ICH=Intracerebral hemorrhage; SAH=subarachnoid hemorrhage

Paper I

Among the 108 ischemic stroke patients that were included in the LSRS inception cohort, brain DW-MRI performed within 4 days of stroke onset visualized acute ischemic lesion(s) in 102 (94%) patients, while 6 (6%) of the patients were considered to have MRI-negative strokes. Of the 102 patients with visible acute brain infarct(s), 60 (59%) had single brain infarcts (7 of these had brainstem or cerebellar infarction without clear localizing symptoms from these areas), whereas multiple brain infarcts were observed in 42 (41%) individuals.

The median NIHSS score was 3 (range 1-18) among the 108 included patients. A total of 17 (16%) patients had received acute reperfusion therapy (thrombolysis and/or endovascular intervention).

Table 6 demonstrates the neuroradiological characteristics and stroke severity among the patients with single and multiple acute brain infarcts.

Table 6.
Characteristics of patients with single supratentorial infarcts and multiple infarcts

			Striatal involvement[c]			
Lesion pattern[a]	***n***	**Lesion volume[b]**	**0%**	**≤50%**	**>50%**	**NIHSS[d]**
Single infarcts						
Cortico-subcortical	9	19.0 (0.3-155.3)	7	2	0	6 (1-18)
Cortical	6	2.5 (0.4-103.0)	6	-	-	2.5 (1-17)
Subcortical						
≥15 mm	10	3.9 (0.9-15.0)	3	1	6	2.5 (1-11)
<15 mm	28	0.5 (0.1-1.7)	27	1	0	2 (1-7)
Total	53	1.1 (0.1-155.3)	43	4	6	2 (1-18)
Multiple infarcts						
Scattered lesions in one vascular territory						
Small scattered	2	1.0 (0.2-1.8)	0	1	1	2.5 (1-4)
Confluent and an additional lesion	4	10.7 (2.6-39.2)	4	0	0	4.5 (1-9)
Multiple lesions in multiple vascular territories						
In unilateral AC	19	11.8 (1.1-77.0)	10	8	1	3 (1-7)
In the PC	2	15.0 (8.3-21.8)	2	0	0	3.5 (1-6)
In bilateral ACs	7	2.4 (0.4-36.7)	5	2	0	6 (1-8)
In the AC and PC	8	9.6 (3.8-37.4)	7	1	0	4 (2-16)
Total	42	8.6 (0.2-77.0)	28	12	2	3 (1-16)

a) Lesion pattern according to Kang et al., 2003 [36] (AC=anterior circulation, PC=posterior circulation) b) Median supratentorial lesion volume in mL (range); c) number of patients with striatal involvement (percent of total lesion volume located in striatum); d) median National Institutes of Health Stroke Scale (NIHSS) score at day 2-4 after stroke onset (range)

Spatial Relationship of Brain Infarcts to the Subventricular Zone

In the 102 patients with visible acute brain infarct(s), the minimum distance from the nearest margin of the infarct(s) to the SVZ was 0-2 mm in 51 (50%) patients, and 0-5 mm in 63 (62%). The median minimum distance was 2.5 mm (Figure 11).

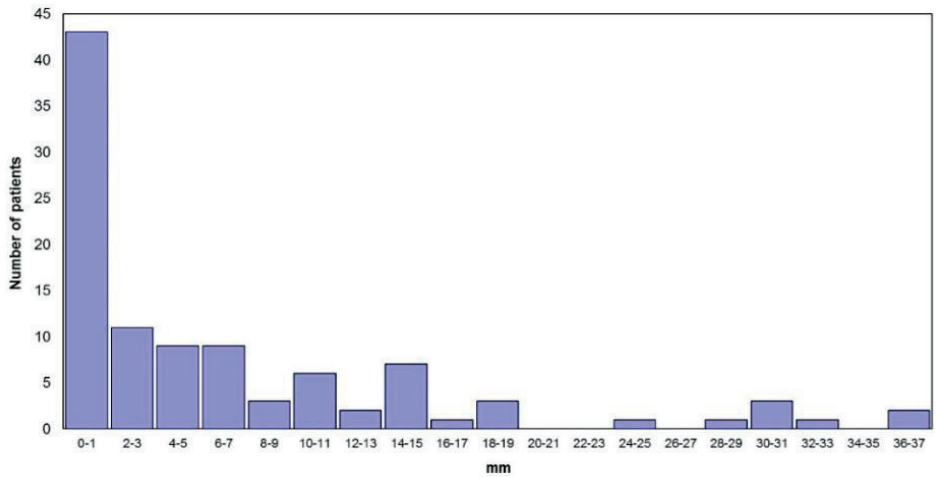

Figure 11.
Bar chart displaying the minimum distance from the nearest margin of the brain infarct(s) to the subventricular zone in 102 stroke patients with visible acute ischemic lesions. *Reprinted from European Journal of Neurology, Vol. 20, Delavaran et al., Proximity of brain infarcts to regions of endogenous neurogenesis and involvement of striatum in ischaemic stroke, pp. 473-479, Copyright (2012), with permission from John Wiley and Sons*.

Involvement of Striatum in Ischemic Stroke

The striatum was involved in 24 (24%) of the 102 patients with visible acute brain infarcts. In these 24 cases with striatal involvement, 10 individuals had a single infarct and 14 had multiple infarcts. The median NIHSS score was 4 (range 1-18) for these patients.

Only 8 of the 24 patients displayed a predominantly striatal infarction, i.e. >50% of the total ischemic lesion volume confined to striatum (Figure 12). No patient had 100% of the total ischemic lesion burden confined to striatum.

The 8 patients with predominantly striatal infarcts had a median age of 60 years (range 38-67), and they displayed a median NIHSS score of 3 (range 1-5) at day 2-4 after stroke onset. However, 3 of these 8 patients had more severe initial stroke symptoms at admission, and they were treated with acute reperfusion therapy (thrombolysis and/or endovascular intervention) with virtually instant improvement. At discharge, the 8 patients with predominantly striatal infarcts displayed mild residual symptoms with a median NIHSS of 1 (range 0-3).

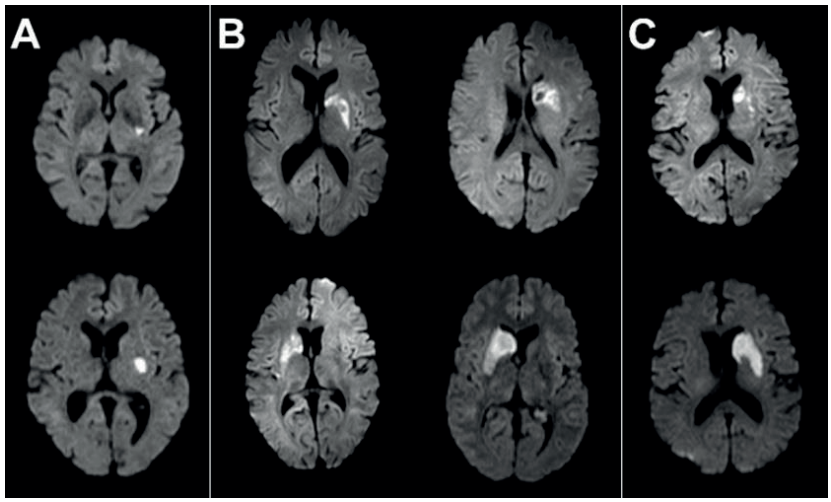

Figure 12.
Brain DW-MRIs of the patients with predominantly striatal infarcts, from Delavaran et al., 2013. A) Two individuals with a single infarct within the territory of a single perforator of the MCA. B) Four individuals with a single infarct within the territory of multiple perforators of the MCA. C) Two individuals with multiple infarcts but with >50% of the total ischemic lesion volume located in striatum. *Reprinted from European Journal of Neurology, Vol. 20, Delavaran et al., Proximity of brain infarcts to regions of endogenous neurogenesis and involvement of striatum in ischaemic stroke, pp. 473-479, Copyright (2012), with permission from John Wiley and Sons.*

Paper II

In total, 84 stroke survivors from the LSRS inception cohort were included at the 3-5 year follow-up. A detailed sample-flow chart, with baseline information about the non-participating stroke survivors, as well as baseline characteristics of the included stroke survivors, are presented in Figure 1 and Table 1 in Paper II, respectively, appended in the end of the thesis.

The median age at follow-up was 68 years (range 33-80), and 30 (36%) stroke survivors were female. The median time from onset of index stroke to follow-up was 4.6 years (range 3.5-5.7). In total, 41 (49%) of the 84 stroke survivors at follow-up had performed some form of rehabilitation intervention after the index stroke, with a median time of rehabilitation amounting to 5 weeks (range 1-109). Recurrent stroke(s) was reported for 8 (10%) of the 84 stroke survivors.

The severity of stroke symptoms and degree of disability for the stroke survivors at follow-up are presented in Figures 13 and 14.

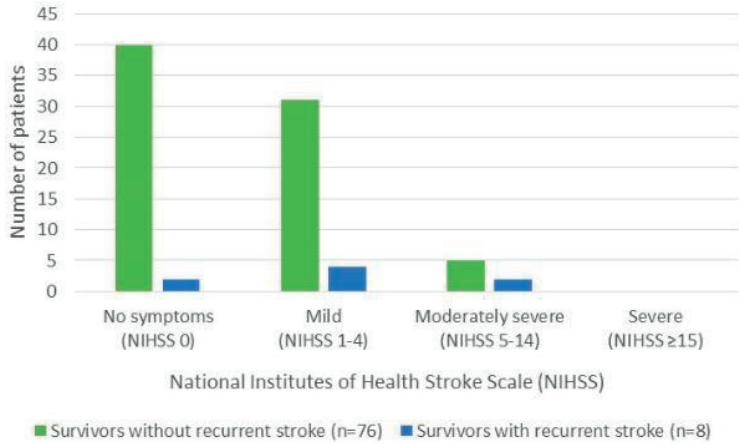

Figure 13.
Stroke severity according to the National Institutes of Health Stroke Scale (NIHSS) including item 12 for hand motor function (composite score 0-46).

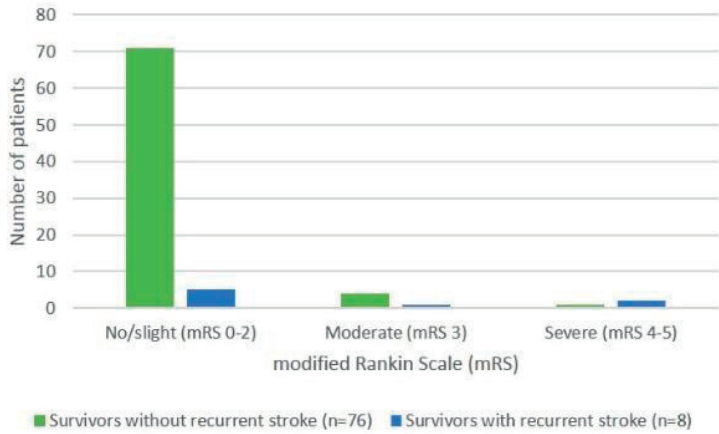

Figure 14.
Degree of disability according to the modified Rankin Scale (mRS, score 0-5).

Frequency and Severity of Post-Stroke Upper Extremity Motor Impairment

Among the 76 stroke survivors without recurrent stroke at the 3-5 year follow-up, 41/76 had UEMI at baseline of which 10/41 had residual UEMI at follow-up.

The degree of UEMI for the 10 individuals with residual impairment was classified as mild in 3/10 cases (FMA-UE=53-66), moderate in 4/10 cases (FMA-UE 23-52) and severe in 3/10 (FMA-UE=0-22) cases.

Spontaneous Recovery of Post-Stroke Upper Extremity Motor Impairment

In 31 of the 41 stroke survivors with UEMI at baseline, a complete recovery was observed at the 3-5 year follow-up. However, among the 10/41 stroke survivors with residual UEMI at follow-up, 5/10 showed only partial recovery whereas the other 5/10 displayed no recovery at all. The spontaneous recovery of post-stroke UEMI among the survivors from the LSRS is illustrated in Figure 15.

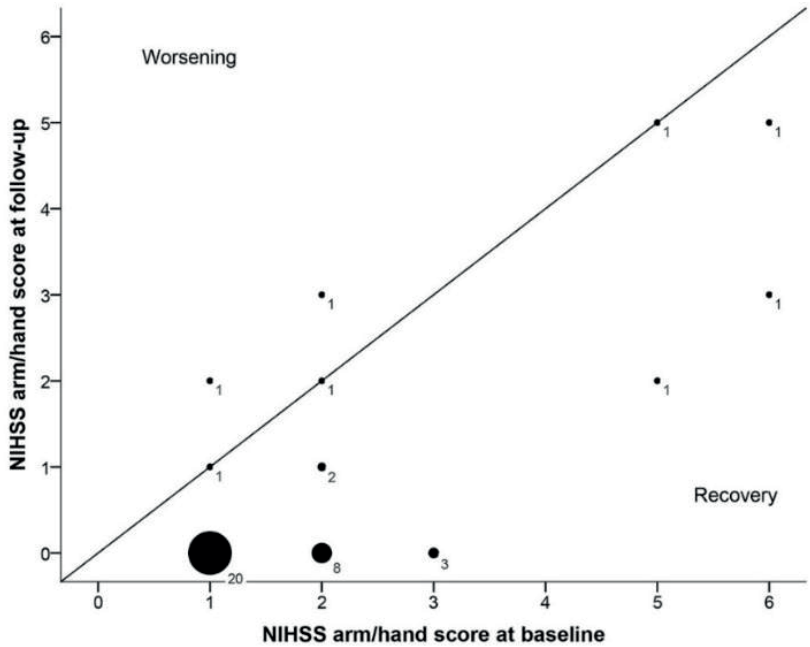

Figure 15.
Bubble plot of the spontaneous recovery of UEMI (n=41 stroke surviovrs w/o recurrent stroke). UEMI was defined as change in scores on the combined NIHSS arm and hand motor items (composite score 0-6) between baseline and 3-5 year follow-up (ΔNIHSS arm/hand). The bubble sizes indicate number of stroke survivors. The median ΔNIHSS arm/hand was -1 (range -3 to 1).

Stroke survivors with partial or no UEMI recovery had higher initial NIHSS scores at baseline (p<0.001), larger lesion volumes (p=0.003), and more frequent involvement of the motor cortex (p=0.03) as compared to the survivors with complete recovery (Table 7).

Table 7.
Characteristics of stroke survivors with no/partial UEMI recovery versus those with full recovery

Variable	No or partial UEMI recovery (n=10)	Complete UEMI recovery (n=31)	*p*
Sex, n (%)			
Female	5 (50%)	10 (32%)	ns
Age at stroke onset, median (range)	65 (36-74)	64 (28-75)	ns
NIHSS at baseline, median (range)	7 (2-18)	3 (1-8)	<0.001
NIHSS arm/hand at baseline, median (range)	2 (1-6)	1 (1-3)	0.004
Lesion volume in mL, median (range)	26.5 (0.4-155.3)	1.0 (0.1-23.3)	0.003
Lesion location, n (%)			
Cortico-subcortical	7 (70)	7 (25)	0.02
Cortical only	0	3 (11)	ns
Subcortical only	3 (30)	16 (57)	ns
Motor cortex involvement, n (%)	7 (70)	8 (29)	0.03
Corticospinal tract involvement, n (%)	8 (80)	18 (64)	ns

NIHSS = National Institutes of Health Stroke Scale including item 12 for hand motor function (composite score 0-46); UEMI = upper extremity motor impaiment, defined as of score ≥1 on the NIHSS arm and hand motor items

Relation of Post-Stroke Upper Extremity Motor Impairment to Activity Limitations and Participation Restrictions

Among the 10 stroke survivors with residual UEMI, a total of 6/10 exhibited either limited upper limb capacity (ARAT 22-42), poor capacity (ARAT score=11-21) or no functional upper limb capacity at all (ARAT score=0-10) (Figure 16).

With regard to the degree of overall disability, 3/10 stroke survivors with residual UEMI exhibited moderate-severe disability (mRS>2) (Figure 17).

Moreover, 9/10 individuals with residual UEMI described difficulties in ADL (as assessed with the SIS ADL domain), and all 10 also reported problems in social participation (as evaluated by the SIS participation domain). Nonetheless, the self-perceived general health status (as assessed with the first question in SF-36) was rated as “very good” by 1/10, as “good” by 3/10, and “fairly good” by 4/10, whereas it was described as “poor” by 2/10.

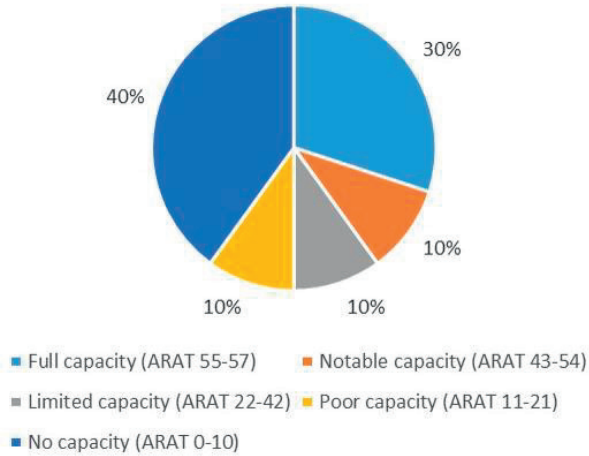

Figure 16.
Upper limb functional capacity according to the Action Research Arm Test (ARAT) among the stroke survivors with residual UEMI (n=10).

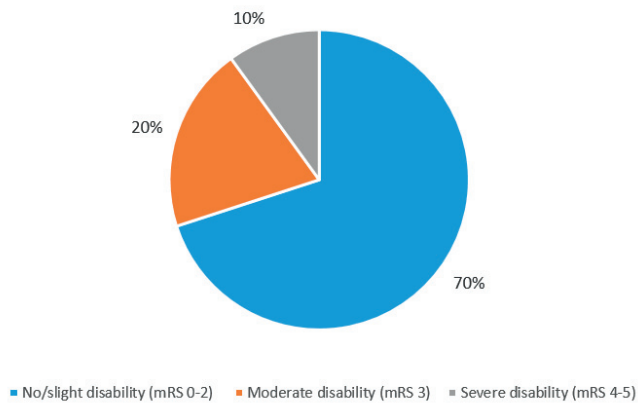

Figure 17.
Degree of disability according to the modified Rankin Scale among the stroke survivors with residual UEMI (n=10).

As illustrated in Figure 18, post-stroke UEMI correlated strongly to dependency in ADL (as measured with mRS), as well as to self-perceived difficulties in ADL and social participation (as assessed with SIS) and self-perceived general health status (as evaluated by SF-36).

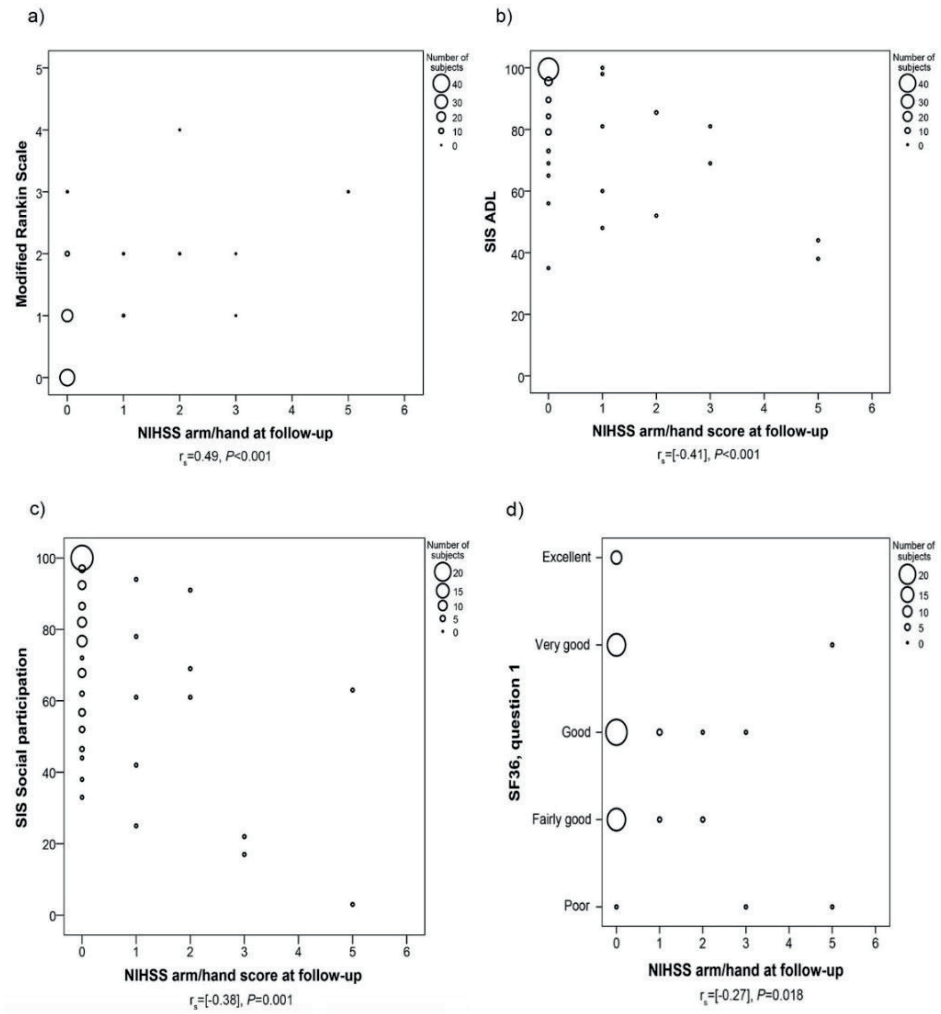

Figure 18.
Correlation of post-stroke UEMI to activity limitations and paricipation restrictions. Scatter plots showing the relation of post-stroke UEMI, as measured with the NIHSS arm and hand motor items (score 0-6) at the LSRS 3-5 year follow-up (n=76 stroke survivors w/o recurrent stroke) to: a) overall disability according to the mRS (score 0-5); b) self-reported activity limitations according to SIS item 5; c) self-reported participation restrictions according to SIS item 8; and d) self-perceived general health status according to SF-36 question 1.).

Paper III

The baseline characteristics of the initial 416 LSR participants and the 145 stroke survivors at the 10-year follow-up are presented in Table 1 in Paper III appended in the end of the thesis.

At the 10-year follow-up, 99 (68%) of the 145 stroke survivors lived in ordinary housing without home care, whereas 31 (21%) lived in ordinary housing with home care and 15 (10%) in skilled nursing facilities. Moreover, 62 (43%) of the 10-year stroke survivors lived alone.

With regard to work situation, 10 (7%) LSR survivors were occupied with full-time jobs and 2 (1%) worked part time 10 years after stroke. The others were either retired (n=123; 85%) or received disability pension (n=10; 7%).

Functional Status 10 Years after Stroke

The assessment of activity limitations showed that 106 (73%) of the 10-year stroke survivors were independent in ADL. Correspondingly, 103 (71%) of the stroke survivors were categorized as having no/slight disability according to the mRS (score 0-2). Table 8 shows the functional status of the 10-year survivors.

No significant gender differences were found in functional outcome after 10 years from stroke onset. However, higher age at follow-up was associated with worse functional status as assessed with both BI (r_s=0.48, p<0.001) and mRS (r_s=0.43, p<0.001).

Table 8.
Functional status 10 years after stroke

Functional status	*n* (%)
Barthel Index (BI, score 0-100))	
Independence (95-100)	106 (73%)
Minor dependence (60-90)	19 (13%)
Major dependence (0-55)	20 (14%)
Modified Rankin Scale (mRS, 0-5)	
No symptoms (0)	39 (27%)
No significant disability (1)	39 (27%)
Slight disability (2)	25 (17%)
Moderate disability (3)	20 (14%)
Moderately severe disability (4)	7 (5%)
Severe disability (5)	15 (10%)

Patient-Reported Outcome 10 Years after Stroke

In total, 79 (49%) stroke survivors reported that they were physically active ≥4 times per week. Furthermore, most of the 10-year stroke survivors reported that they had no problems regarding mobility, self-care, usual activities, pain/discomfort or anxiety/depression as evaluated by the EQ-5D (Figure 19)

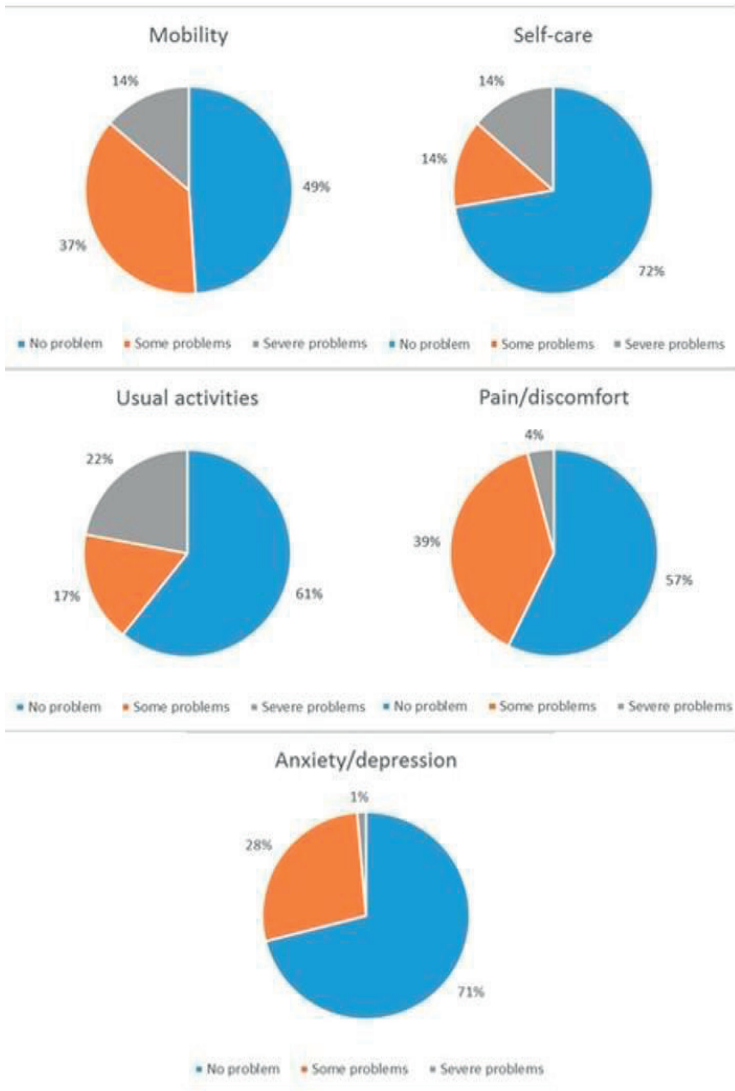

Figure 19.
Patient-reported outcome among the 10-year stroke survivors (n=145) as evaluated with the first part of the European Quality of Life-5 Dimensions (EQ-5D).

Also, the majority of the 10-year survivors from the LSR (62%) appraised their overall health status in positive terms (Figure 20).

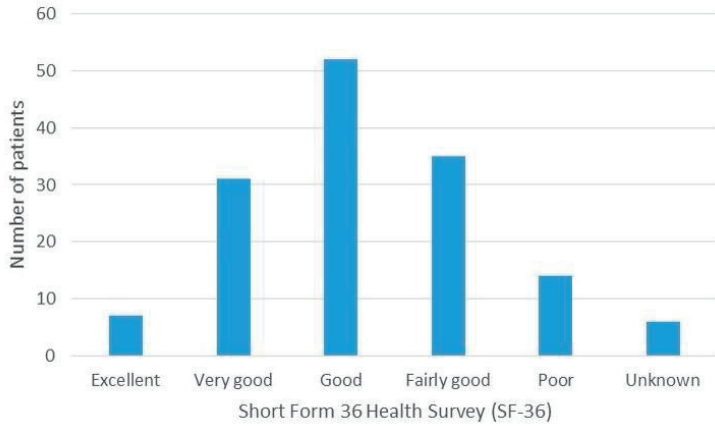

Figure 20:
Patient-reported outcome among the 10-year stroke survivors (n=145) as evaluated with the first question of SF-36 ("In general would you say your health is: excellent, very good, good, fairly good, or poor?").

Notably, 13 out of 36 individuals with moderate overall disability or worse (mRS>2) reported their self-perceived general health status as good or even better (Figure 21).

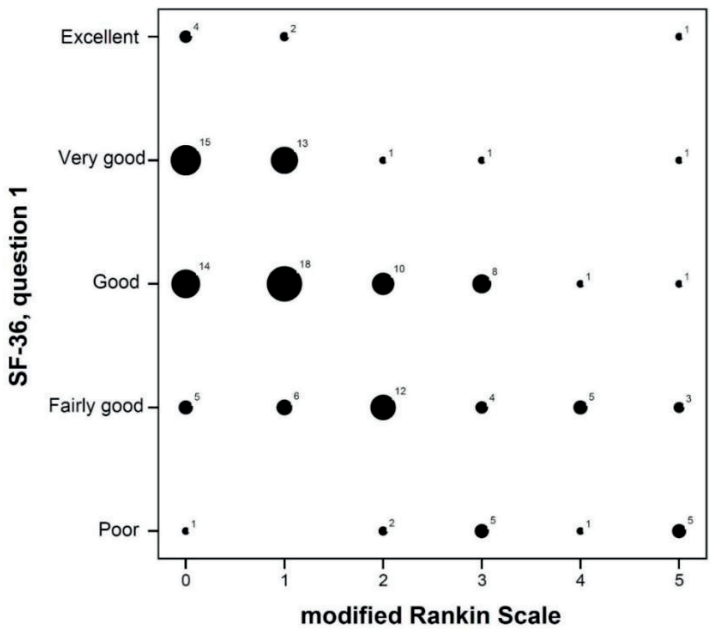

Figure 21.
Responses of the 10-year stroke survivors on the first question of Short Form 36 Health Survey (SF-36) according to disability level as measured by modified Rankin Scale. The bubble sizes indicate number of subjects.

Paper IV

Of the 145 LSR survivors after 10 years, a total of 127 (88%) performed cognitive assessment. A detailed flow chart for Paper IV, with an account of the non-participating stroke survivors, is presented in Figure 1 in Paper IV appended in the end of the thesis.

All the 127 stroke survivors performed cognitive assessment with MMSE, and 122 (96%) also completed subsequent testing with MoCA (5 stroke survivors declined to do the MoCA). In total, 354 age- and sex-matched non-stroke controls were included. Overall, the stroke survivors had a higher education level (median 8.5 years, range 5-20) compared to the controls (median 7 years, range 4-20; p=0.015). Detailed demographic characteristics for the study participants are presented in Table 1 in Paper IV appended in the end of the thesis.

The included 127 stroke survivors had a median NIHSS of 3 (range 0-27) at baseline. Recurrent stroke(s) was reported for 17 (13%) of them during the time period from baseline to 10-year follow-up. With regard to functional status at the 10-year follow-up, 96 (76%) of the 127 stroke survivors had no/slight disability (mRS=0-2), 17 (13%) had moderate disability (mRS=3), whereas 14 (11%) had severe disability (mRS=4-5).

Cognitive Function of 10-Year Stroke Survivors

Among the 127 stroke survivors, the median MMSE score was 27 (range 10-30). In total, 58 (46%) of the stroke survivors displayed some degree of cognitive impairment on the MMSE (MMSE<27). With regard to impaired visuoexecutive functioning, 51 (40%) out of the 127 stroke survivors failed the MMSE visuoconstruction subtest.

The median MoCA score was 23 (range 4-30) among the 122 stroke survivors that did the test. With MoCA, cognitive impairment was observed in 75 (61%) stroke survivors. Notably, 94 (77%) of the stroke survivors had inaccuracies in the MoCA visuospatial/executive functions subtest. The cognitive testing results of the study participants are presented in Table 9.

Table 9.
Cognitive assessment of study participants

Cognitive assessment	10 –year stroke survivors		Control persons
Mini-Mental State Examination (MMSE)	n=127[a]	n=118[b]	n=354
Cognitive Impairment, n (%)			
None (score 27-30)	69 (54)	63 (53)	179 (51)
Mild (score 23-26)	34 (27)	32 (27)	132 (37)
Severe (score 0-22)	24 (19)	23 (20)	43 (12)
Montreal Cognitive Assessment (MoCA)	n=122[c]		-
Cognitive Impairment, n (%)			
None (score 25-30)	47 (39)		-
Mild (score 20-24)	40 (33)		-
Severe (score 0-19)	35 (29)		-

a) All 10-year stroke survivors included in Paper IV; b) All 10-year stroke survivors aged ≥60 years in Paper IV with available non-stroke control persons; c) All 10-year stroke survivors who completed the MoCA

Cognitive Function of 10-Year Stroke Survivors vs. Non-Stroke Persons

Univariate regression analysis showed that the odds of having severe cognitive impairment, as defined by MMSE<23, was higher among 10-year stroke survivors compared to the controls (crude OR=1.75; 95% CI 1.00-3.05; p=0.048). When adjusting for number of years of education with multivariate regression analysis, the odds of having severe cognitive impairment (MMSE<23) were even higher among the stroke survivors (OR=2.48; 95% CI: 1.34-4.59; p=0.004) (Table 10).

Table 10.
Odds of having severe cognitive impairment, as evaluated by the Mini-Mental State Examination (MMSE), among the 10-year stroke survivors compared to the non-stroke controls

	Severe cognitive impairment (MMSE <23)		
	OR	95% CI	*p*
Logistic regression model			
Simple (stroke only)	1.75	1.00-3.05	0.048
Multiple (stroke+education)	2.48	1.34-4.59	0.004

Moreover, univariate analysis showed that stroke survivors more frequently failed the MMSE visuoconstruction subtest compared to the controls (42% versus 16% respectively; p<0.001).

Comparison of MMSE with MoCA

When comparing the MMSE and MoCA results among the stroke survivors, the overall MMSE scores were skewed towards higher values (median 27, interquartile range 25-29) than the corresponding MoCA scores (median 23, interquartile range

19-26). Figure 22 illustrates the comparison of MMSE with MoCA among the stroke survivors.

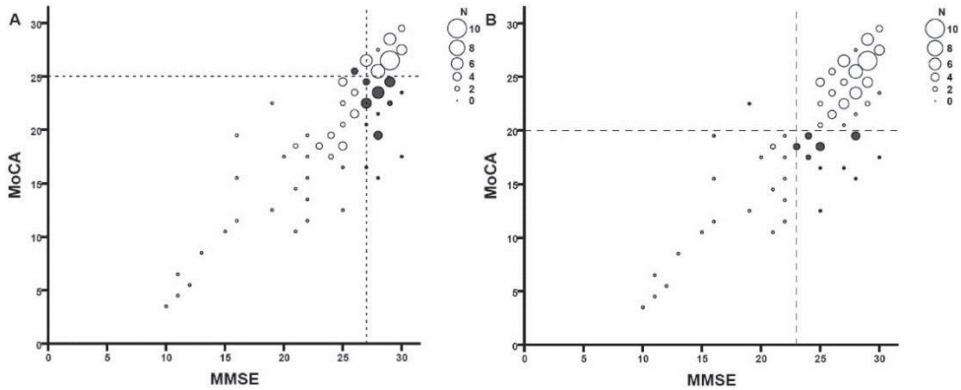

Figure 22.
Comparison of MMSE versus MoCA among 10-year stroke survivors from the LSR (n=122). The number of stroke survivors with divergent classifications according to MMSE and MoCA, at corresponding cutoff scores, are illustrated by the dark-shaded bubbles. A. Among the 75 stroke survivors with any cognitive impairment according to MoCA (MoCA<25; horizontal dashed line), 26 had normal MMSE (MMSE≥27; vertical dashed line). On the other hand, only 4 stroke survivors with normal MoCA (MoCA≥25) were classified as cognitively impaired by MMSE (MMSE<27). These discrepancies were statistically significant (p<0.001). B. Of 35 stroke survivors with severe cognitive impairment according to MoCA (MoCA<20; horizontal dashed line), 16 scored MMSE≥23 (vertical dashed line). Conversely, only 2 survivors with severe cognitive impairment according to MMSE (MMSE<23) scored MoCA>20. These discrepancies were also statistically significant (p=0.001).

Paper V

All of the 84 stroke survivors from the LSRS that participated in the 3-5 year follow-up completed the SCT questionnaire. The demographic and follow-up characteristics of the stroke survivors have been described in table 1 in Paper V appended in the end of the thesis.

Stroke Survivors' Knowledge and Attitudes about SCT

Of the 84 stroke survivors, only 10 (12%) reported that they had previously heard of SCT in stroke. However, 53 (63%) expressed a positive attitude towards it after having read the preceding information sheet on stroke and SCT.

With regard to the administration mode of SCT, 26 (31%) stroke survivors answered that they could consider receiving SCT through intracerebral transplantation, and 40 (48%) responded that they could consider SCT through systemic administration.

Furthermore, 5 (6%) stroke survivors had ethical considerations/moral doubts about SCT with adult stem cells, 6 (7%) about embryonic stem cells, 7 (8%) about SCT with fetal stem cells, and 4 (5%) expressed ethical considerations/moral doubts about SCT with induced pluripotent stem cells.

Impairments of motor and cognitive functions were the major domains that the stroke survivors most frequently responded that they wanted SCT to improve (38% and 32%, respectively), if SCT had been available (see Figure 23).

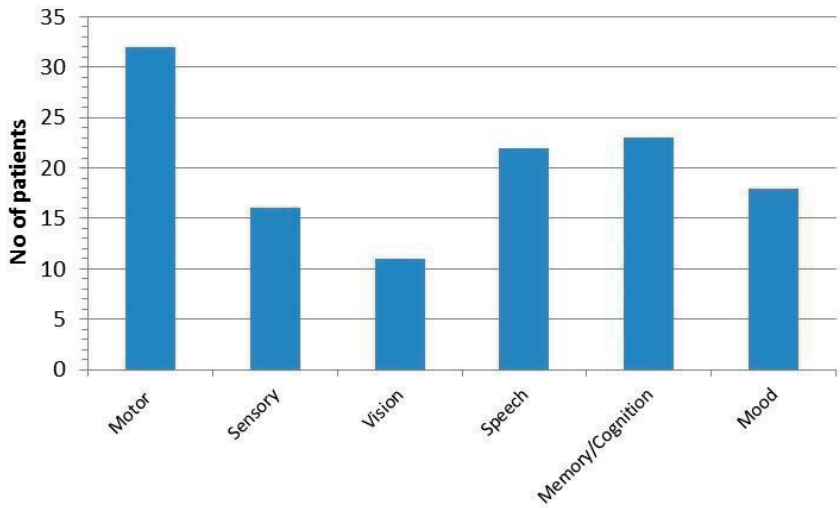

Figure 23.
Neurological dysfunctions that the stroke survivors reported that they were in most need for stem cell therapy to improve, if it had been available.

The stroke survivors' responses to the SCT questionnaire are presented in Table 11.

Table 11.
Stroke survivors' responses to the SCT questionnaire

Question	SCT questionnaire respondents (n=84)		
	Yes/ Positive	No/ Negative	Do not know/ do not wish to answer
Knowledge of SCT			
Previously heard of SCT for stroke	10 (12%)	61 (73%)	13 (16%)
Attitudes to SCT			
Attitude towards SCT for stroke	53 (63%)	3 (4%)	28 (33%)
Willing to undergo SCT via intracerebral transplantation	26 (31%)	24 (29%)	34 (41%)
Willing to undergo SCT via systemic administration	40 (48%)	10 (12%)	34 (41%)
Willing to participate in a clinical stroke trial with SCT	33 (39%)	21 (25%)	30 (36%)
Ethical considerations/moral doubts			
Attitude towards stem cell research/SCT with:			
Adult stem cells	44 (52%)	5 (6%)	35 (42%)
Fetal stem cells	40 (48%)	7 (8%)	37 (44%)
Embryonic stem cells	41 (49%)	6 (7%)	37 (44%)
Induced pluripotent stem cells	44 (52%)	4 (5%)	36 (43%)
Perceived need of SCT			
In need of SCT	20 (24%)	17 (20%)	47 (56%)
Impairments in most need of SCT for			
Paralysis/weakness	32 (38%)	19 (23%)	33 (39%)
Loss of sensation/numbness	16 (19%)	35 (42%)	33 (39%)
Speech difficulties	22 (26%)	29 (35%)	33 (39%)
Vision problems	11 (13%)	40 (48%)	33 (39%)
Difficulties with memory and thinking	27 (32%)	24 (29%)	33 (39%)
Mood problems	18 (21%)	33 (39%)	33 (39%)

Factors Influencing Stroke Survivors' Attitudes to SCT

Positive attitude towards SCT was associated with male gender (crude OR: 3.74; 95% CI: 1.45-9.61; p=0.006), and higher self-perceived stroke recovery as assessed with the SIS recovery question (crude OR: 1.02; 95% CI 1.00-1.04; p=0.034). There was also a possible trend towards a higher degree of stroke recovery, objectively measured as the change in overall NIHSS score between baseline and follow-up

(ΔNIHSS), being associated with positive attitude to SCT (crude OR: 1.19; 95% CI: 0.99-1.43; p=0.058).

Additionally, willingness to undergo SCT through intracerebral transplantation was associated with male gender (crude OR: 6.68; 95% CI: 1.80-24.73; *p*=0.004), as well as higher degree of stroke recovery as measured with ΔNIHSS (crude OR: 1.26; 95% CI: 1.03-1.54; *p*=0.023). Likewise, the willingness to participate in clinical SCT trials was also associated with male gender (crude OR: 5.39; 95% CI: 1.80-16.15; *p*=0.003) and higher degree of stroke recovery (crude OR: 1.32; 95% CI: 1.07-1.62; *p*=0.009).

All the aforementioned associations were confirmed with multivariate logistic regression analyses adjusting for age, gender, educational attainment (number of years of formal education), cognitive function (MoCA score), burden of comorbid diseases (CCI score), severity of neurological impairments (NIHSS at follow-up), objectively assessed stroke recovery (ΔNIHSS), self-perceived stroke recovery (according to SIS), and prior knowledge of SCT.

With regard to the ethical considerations about stem cell sources, there was a possible trend towards female gender and lower MoCA scores being associated with responding "do not know/do not wish to answer" (see Supplement Table S4 in Paper V appended in the end of the thesis).

Discussion

Methodological Aspects

There are several methodological problems that may influence the reliability of the study results in medical research, and methodological issues may occur during study design, data collection, data analyses, and in data presentation.

Major methodological aspects in observational studies include selection bias, imprecision of measurements and confounding. The degree to which the findings of this thesis may have been influenced by these issues are discussed below.

Patient Selection and Potential Bias

Lund Stroke Recovery Study

The primary patient selection objective for Papers I, II and V was to detect stroke patients potentially suitable for SCT. Therefore, the inclusion and exclusion criteria for LSRS were designed to identify patients that would be potentially eligible for SCT regarding age, comorbidity, main pathological stroke type, stroke severity, and MRI lesion appearance. This selection reduces the studies' generalizability to all stroke patients.

Hence, given the strict eligibility criteria as well as the hospital-based case ascertainment method, our findings with regard to the proximity of brain infarcts to the SVZ and the frequency of striatal infarcts (Paper I), post-stroke UEMI (Paper II), and the knowledge and attitudes about SCT (Paper V), may not be representative of the overall population of stroke patients. However, our aim was not to study these questions in the overall stroke population but rather among stroke patients potentially eligible for SCT.

The eligibility criteria for LSRS might have influenced the selection towards patients with lower degrees of stroke severity as the median NIHSS score of the LSRS inception cohort was 3 (range 1-18). However, only 11% of the initially screened patients were excluded due to not fulfilling the stroke severity criteria (NIHSS 1-18), most of which exhibited NIHSS 0 when assessed at day 2-4 after stroke onset. Additionally, among stroke patients registered in the Swedish Stroke

Register in 2015 the median NIHSS was also 3, and 63% of those patients had mild strokes with NIHSS 0-5 [40], which is in line with the findings in our cohort. It is currently unclear if patients with mild strokes are suitable for SCT, but even low overall NIHSS scores may represent significant disability and lowered HRQoL.

Another reason for selection bias is that 19% of the totally 466 screened patients were not included in the LSRS due to reasons other than not fulfilling the eligibility criteria (such as e.g. MRI not done within 4 days because of logistic reasons).

Nevertheless, the same multiple overlapping sources and prospective methods for case ascertainment were used in the LSRS as for the LSR, which detects nearly 90% of all stroke patients compared to population-based screening methods [171], as described below.

Lund Stroke Register

The case ascertainment for the first-year cohort of the LSR (n=416), that were followed up in Papers III and IV, was based on multiple sources and prospective methods as described above in the Methods section. However, the LSR does not completely fulfil the golden standard for ideal population-based stroke studies [188]. By adding the cases detected by the retrospective screening methods described in the Methods section, it has been shown that the total number of first-ever stroke patients during the first year of LSR increased to 456 cases [171].

Taken this into account, the prospective case ascertainment methods used for the first-year LSR cohort followed up in Papers III and IV cover 88% of all stroke patients detected by screening methods adhering to the more strict criteria for an ideal epidemiological stroke study. Therefore, the first-year LSR cohort is representative of hospitalized stroke patients, but may have reduced generalizability for stroke patients managed merely within the primary care or in skilled nursing facilities.

Follow-ups

For the LSRS follow-up after 3-5 years (Papers II and V), a total of 84 stroke survivors were included from the 108 initial stroke patients at baseline. Of these 108 patients, 11 were deceased at the time of follow-up and 13 were lost to follow-up. The results in Papers II and V are therefore limited by a relatively small study sample, and potential effects of selective attrition due to death before the follow-up. Also, the 12% loss to follow-up may have resulted in an underestimation of the proportion of patients with residual UEMI (Paper II), and influenced the findings on attitudes towards SCT (Paper V). The patients who died before follow-up and those who dropped out or were lost to follow-up are accounted for in Paper II.

In the LSR, only 18 out of the 416 initial stroke patients were lost to follow-up between baseline and the first 4-month follow-up. Thereafter, there were no

dropouts or losses to follow-up other than death, which is a strength of the LSR follow-up (Paper III).

In Papers III and IV, the 10-year stroke survivors probably represent a selected profile of younger patients with less severe strokes and less comorbidity at their index stroke onset. In this regard, it would have been valuable to also have studied the stroke patients who died (n=253 out of the initial 416 patients at baseline). No comparative analyses were performed between the stroke survivors and the stroke patients who died concerning baseline characteristics. Also, regular interim assessments between the early LSR follow-ups and the 10-year follow-up could have provided additional valuable information about the stroke survivors who died during this time span, and how risk factor profiles changed among the survivors. Consequently, the results in Papers III and IV may have been influenced by selective attrition due to death, causing an underestimation of the proportion of stroke survivors with poor functional and patient-reported outcome (Paper III), and an underestimation of the prevalence of PSCI (Paper IV)[189].

In Paper IV, some stroke survivors were not available for cognitive testing (n=3 no consent; n=4 moved from the study area), and some were excluded from testing (n=11), which may have introduced a selection bias leading to further underestimation of PSCI [190-192]. Nonetheless, the 10-year stroke survivors who did not take part in the cognitive assessment have been accounted for in Paper IV.

Measurements

Paper I

DW-MRI is a very sensitive neuroimaging technique to visualize acute ischemic stroke [77]. Whether ischemic lesions visualized on early DW-MRI represent reversible injury or infarcted brain tissue has been debated [77,193-197], even though, more recent studies in stroke patients have reported that early DWI lesions generally do not reverse to normal [198,199].

The spatial relationship of the ischemic lesions to the SVZ was evaluated by measuring the minimum distance from the nearest margin of the ischemic lesions to the lateral walls of the lateral ventricles. For infarcts that there located close to the lateral walls of the lateral ventricle the intra-observer agreement is likely to be high, but may be lower for infarcts with longer distances to the SVZ. The inter-observer agreement for these measurements was not assessed.

Paper II

While the NIHSS has excellent validity and adequate inter-observer reliability, the responsiveness may be limited by ceiling effects [94]. A use of a more

comprehensive assessment of UEMI at both baseline and follow-up with FMA-UE, which has excellent validity, test re-test and inter-observer reliability as well as excellent responsiveness, might have yielded different results [94]. Still, NIHSS is widely used, more time efficient and logistically easier to administer, and possibly poses less burden on the patients compared to FMA-UE [94].

Paper III

Functional outcome was assessed using the mRS and BI. The degrees of mRS have been criticized for being broad, and susceptible to subjective interpretation [94]. Nonetheless, mRS is widely used and has excellent validity, excellent test re-test reliability and adequate inter-observer reliability [94]. The BI has been reported to have large ceiling and floor effects, but has excellent validity and reliability (both test re-test and inter-observer reliability) and is also extensively used to measure dependency in daily activities [94].

The patient-reported outcomes measures used included the first part of the EQ-5D and the first question of the SF-36. The EQ-5D has adequate validity and adequate test-re test and inter-observer reliability, while the SF-36 has excellent validity and reliability [94]. However, there may be discrepancies between the patient and the proxy responses [94].

Paper IV

The estimates of PSCI were based on cognitive screening with MMSE and MoCA, and not on formal diagnostic criteria or detailed neuropsychological assessments, which may have influenced the findings. Nevertheless, both MMSE and MOCA are valid and frequently used cognitive assessment measures in stroke and have shown acceptable accuracy in detecting PSCI [98,99,183,200].

Paper V

The information sheet and the subsequent SCT questionnaire that was used in Paper V is not a validated instrument to assess knowledge and attitudes about SCT, and was created by the authors for this specific study. In addition, some of the questions were hypothetical. Therefore the stroke survivors' expressed responses should be interpreted with caution as some of their answers might have been different in the setting of a real clinical situation and if SCT had been available as a therapeutic option.

Confounding

Paper I

The subacute time point for performing brain MRI may have confounded the estimation of infarct volumes because of infarct swelling. This may have caused an overestimation of the infarct volumes in striatum and in turn the number of predominantly striatal infarcts (defined as >50% of the total ischemic lesion volume confined to striatum). Similarly, infarct swelling might also have caused an overestimation of the proximity of infarcts to the SVZ.

Paper II

Nearly half of the stroke survivors in Paper II underwent some form of rehabilitation treatment after the index stroke, and this may have confounded our findings regarding post-stroke UEMI, since this was not assessed in detail or taken into account. Moreover, advanced age and other concurrent comorbidities may also have influenced the results. However, we excluded stroke survivors' with recurrent strokes in our analyses to avoid confounding effects of stroke recurrence on the natural course of UEMI recovery from the index stroke.

Papers III and IV

The effects of advanced age, other comorbidities and recurrent stroke may have confounded the outcomes in the LSR 10-year follow-up. The effects of the index stroke on the outcomes in Papers III and IV were especially difficult to ascertain as we did not evaluate pre-stroke disability (Paper II) and pre-stroke cognitive impairment (Paper III), or no corresponding data at baseline, nor were there any interim assessments between the 16-month and 10-year follow-up.

The cognitive testing in Paper III may also have been confounded by depression, as this was not assessed. As described above, post-stroke depression is common after stroke [71], even in longer term perspectives [142], and might therefore have caused an overestimation of PSCI in Paper III. Other possible confounders that may have influenced the performance on MMSE and MoCA include higher cerebral dysfunctions such as neglect, apraxia and mild to moderate dysphasia. The MoCA scores may also have been negatively impacted by the order of testing due to fatigue because MoCA was always performed after MMSE.

Nevertheless, the education-adjusted comparisons to non-stroke control persons in Paper III suggest that the prevalence and characteristics of cognitive deficits differ between long-term stroke survivors and age- and sex-matched non-stroke persons. Similar comparisons to non-stroke controls would also have been valuable for the outcomes in Paper II.

Paper V

The concurrent presence of cognitive impairment may possibly have influenced the stroke survivors' responses to the questions of the SCT questionnaire, and the tendency to answer "do not know/do not wish to answer" to some of the questions seemed to be associated with lower MoCA scores.

General Discussion

Lesion Appearance after Ischemic Stroke

Proximity of Brain Infarcts to Regions of Endogenous Neurogenesis

One of the major findings in Paper I was that brain infarcts were localized in close proximity (0-2 mm) to the SVZ in a large fraction (50%) of the ischemic stroke patients. To the best of our knowledge, this is the first study to describe the spatial relationship of brain infarcts to the neurogenic area in the SVZ among ischemic stroke patients.

It is unclear whether the distance between brain infarcts and the SVZ affects the potential of endogenous neurogenesis from the SVZ to promote recovery after stroke. In rodent stroke models, the neuroblasts formed in the SVZ can migrate several millimeters towards the ischemic lesion site [154,155,157]. However, the migrating cells are distributed along a gradient in relation to the SVZ, which points to the possible relevance of the distance of infarcts to the SVZ [154]. The migratory capability of neuroblasts derived from the SVZ in humans is unknown. It is also unknown whether the proliferating cells detected around cortical infarcts in stroke patients were derived from the SVZ or from local parenchymal progenitor cells. However, it has recently been shown that ischemic stroke does not induce neurogenesis in the human neocortex [201].

Altogether, the findings in Paper I of a close proximity of brain infarcts to the SVZ in a high proportion of ischemic stroke patients provide some support that strategies with SCT to promote endogenous neurogenesis, such as e.g. stimulating the survival of newly formed neurons from the SVZ, may have therapeutic potential to improve functional recovery after stroke.

Involvement of Striatum in Ischemic Stroke

Paper I also demonstrated that infarcts predominantly confined to striatum were rare (<10%) and resulted in mild neurological deficits.

Clinical studies on stroke patients with predominantly striatal infarcts are scarce. Among the ischemic stroke patients potentially suitable for SCT in our study, brain infarcts involving striatum were visualized in roughly one fifth of all patients. Brain infarcts predominantly confined to striatum were only observed in less than 10% of the patients. Besides, the patients with predominantly striatal infarcts exhibited mild neurological deficits at discharge from hospital (median 1, range 0-3).

The findings of a low frequency of ischemic stroke patients with infarcts predominantly confined to the striatum, displaying mild neurological deficits,

suggest that pre-clinical studies with SCT should consider animal stroke models involving also other brain areas. The generation of striatal neurons should probably not be the primary goal of SCT strategies aiming for neuronal replacement. Rather, the findings indicate that stem cell-based neuronal replacement studies in stroke should emphasize the substitution of other types of neurons, e.g. cortical neurons. Interestingly, the feasibility of neuronal replacement and restoration of damaged cortical network was further supported by a recent pre-clinical study [166]. This study reported that human induced pluripotent stem cell derived cortical neurons, transplanted into stroke-damaged rat cerebral cortex, received direct functional synaptic input from the stroke-damaged brain and responded adequately to sensory stimulation, providing evidence that these neurons can be integrated into the injured cortical circuitry [166].

Variety of Ischemic Brain Lesion Patterns

The findings in Paper I further illustrate the large variety of brain lesion patterns that are observed among stroke patients in the clinical setting. For example, more than 40% of the stroke patients with visible lesions on DW-MRI had multiple brain infarcts, and many patients had multiple infarcts in multiple vascular territories.

This heterogeneity in lesion appearance and the frequency of multiple infarcts among stroke patients should be taken into account in pre-clinical models of stroke, as it may have implications for which lesions to focus on, the source of the stem cells, the intended mechanism of action and the mode of stem cell delivery.

Post-Stroke Impairments and Measurements to Perform

Upper Extremity Motor Impairment after Ischemic Stroke

Paper II demonstrated that approximately 50% of the stroke patients had UEMI in the first days after stroke, which is in accord with recent studies [63], and nearly one quarter of these had residual UEMI after 3-5 years. Of the stroke survivors with residual UEMI, 50% exhibited either poor or no functional capacity in the paretic upper extremity.

The findings in Paper II demonstrate the significance of post-stroke UEMI as it correlates strongly to activity limitations, as well as to self-perceived participation restrictions and subjectively assessed general health status. The significance of UEMI was also corroborated by our findings in Paper V, showing that impaired motor functions was the category of impairments that the stroke survivors most frequently reported they would want SCT to improve, if SCT had been available.

Taken together, the findings of a high proportion of patients with UEMI in the first days after stroke, the non-negligible proportion of patients with significant residual

UEMI 3-5 years after stroke, the strong correlation of post-stroke UEMI to activity limitations and participation restrictions, as well as the patients' own views about motor impairments, suggest that SCT targeting UEMI may be clinically valuable with potentially significant and meaningful benefits for patients.

The results also suggest that post-stroke UEMI is a clinically relevant and feasible domain-specific outcome potentially suitable as primary end-point in clinical stroke trials aiming to study the efficacy of SCT. An approach that could be considered in such trials would be to use multiple outcome measures of UEMI as a composite domain-specific endpoint [202], assessing the effects on body structures and function (e.g. with Fugl-Meyer Assessment of Upper Extremity) as well as activity (e.g. with Action Research Arm Test) and participation (e.g. with SIS). With this approach, a more complete UEMI evaluation would be obtained, covering all the components of the ICF model. As described below, this may particularly be important as there may be discrepancies between objective measurements compared to what is perceived as important by the patients themselves [203].

Long-Term Cognitive Function after Stroke

Up-to-date and population-based studies on the prevalence of PSCI in a long-term perspective (≥10 years) are scarce [69]. Paper IV demonstrated a high prevalence of PSCI (46% according to the MMSE and 61% according to the MoCA) among the 10-year stroke survivors. By including the whole spectrum from mild to severe cognitive impairment, the findings in Paper IV suggest that the prevalence of long-term PSCI might be higher than previously estimated [142].

Moreover, the odds (after adjustment for education) of having severe cognitive impairment defined as MMSE<23 (indicative of dementia) were 2.5 times higher among the stroke survivors as compared to the non-stroke control persons, which supports previous studies reporting an increased long-term risk of dementia after stroke compared to non-stroke individuals [204,205]. Paper IV also showed that visuoexecutive deficits were more prevalent among the stroke survivors than the control persons, which is also in line with previous studies with shorter-term follow-ups, reporting a high frequency of visuoexecutive dysfunctioning among cerebrovascular patients [185].

Paper IV also highlights the differences between two commonly used cognitive screening tests, i.e. MMSE and MoCA. The results indicate a ceiling effect with MMSE, as many stroke survivors with normal MMSE were classified as cognitively impaired by MoCA. These findings support previous reports of a higher sensitivity of MoCA over MMSE for milder cognitive deficits and visuoexecutive dysfunctioning [99,182,184,185]. Hence, our findings suggest that MoCA may be more suitable than MMSE to assess PSCI long-term after stroke, which has previously also been proposed in more general descriptions of PSCI evaluation.

Nevertheless, the choice of which cognitive screening test to use also depends on the type and degree of cognitive impairment aimed to be detected, as both tests are valid cognitive screening instruments in stroke and have shown to perform similarly in detecting more severe degrees of cognitive impairment (corresponding to dementia and multidomain MCI) [98,99,183,200].

Notably, cognitive deficits seem to be of great subjective importance for the stroke patients. This is illustrated by the observation that cognitive deficits constituted the second major category of neurological dysfunctions (after impaired motor functions) which the stroke survivors in Paper V reported that they would want SCT to improve, had it been available.

Taken together, our findings on PSCI raise some important considerations from the perspective of SCT in stroke. Firstly, SCT in stroke should focus on targeting impairments of clinical significance, and PSCI falls into that category as it is prevalent and seems to be of much subjective importance for the stroke patients themselves. Whether SCT can enhance the recovery of cognitive deficits after stroke is unclear, since data from pre-clinical studies is scarce and elusive on this matter [206,207], and needs to be further studied in pre-clinical models of stroke. Secondly, PSCI should be taken into account in the stratification and selection of stroke patients for clinical trials with SCT, since it may have implications for e.g. obtaining consent to treatment, ensuring that the patients have understood the therapeutic procedure, and possible benefits and risks, as well as ensuring compliance for potential adjunct medications and follow-ups [208]. Thirdly, cognitive assessment measures such as the MMSE or MoCA may be feasible and suitable cognitive screenings tests in this context, and have shown acceptable accuracy in detecting PSCI as well as being widely used [98,99,183,200]. If higher sensitivity might be preferred compared to specificity in order to detect all potential cases of cognitive impairment, MoCA might be an appropriate option due to its higher sensitivity for milder cognitive deficits and visuoexecutive dysfunctions after stroke than MMSE.

Post-Stroke Recovery and Outcome

Recovery of Post-Stroke Upper Extremity Motor Impairment, and Functional and Patient-Reported Outcome Long-Term after Stroke

Paper II detected a substantial variability in the spontaneous recovery of post-stroke UEMI, as 75% of the stroke survivors showed complete recovery whereas 25% displayed only partial or no recovery after 3-5 years. These findings are consistent with previous studies reporting significant inter-individual variability in post-stroke UEMI recovery [120,135]. In addition, the findings in Paper III demonstrate that a large majority of the long-term stroke survivors (70%) have a relatively good

prognosis, accompanied by positive self-perceptions about their overall health status.

Hence, most of these chronic stroke patients would probably not have been in need of a recovery promoting treatment, had it been available, to improve function with regard to daily activities after stroke. However, a non-negligible proportion of patients in Paper II had residual UEMI, many of which had poor or no functional capacity. Likewise, in Paper III around 15% of the 10-year stroke survivors had moderately severe/severe disability and major dependency in daily activities.

These findings emphasize the necessity for early prognostication of stroke patients with poor expected functional recovery and outcome, and who will be in need for recovery promoting treatments such as SCT.

In Paper II, there seemed to be a trend towards a higher degree of initial paresis in the arm/hand, larger lesion volumes and a higher frequency of motor cortex involvement among the stroke patients with partial or no UEMI recovery compared to those with complete recovery. Among the stroke patients in Paper III, the degree of disability and dependency in daily activities increased with age. Similarly, previous studies have reported that important determinants of recovery and functional outcome include age, initial degree of severity of impairment, lesion size and lesion location, but also comorbid medical conditions such as depression and cognitive impairment [4,6].

Nevertheless, predicting stroke recovery and outcome in individual cases is difficult. In general, there are limitations to applying findings from prognostic studies - which are based on average outcomes in samples of patients under certain settings - to an individual patient in a real clinical situation. However, prognostic models can be useful in this context [209]. For example, one model for predicting individual patients' potential for functional recovery of post-stroke UEMI within 3 months is the Predicting Recovery Potential (PREP) algorithm [210]. The PREP algorithm combines information from clinical evaluation (shoulder abduction and finger extension at 72 hours after stroke onset), neurophysiological examination (transcranial magnetic stimulation) and neuroimaging (DW-MRI) [210]. With regard to overall functional outcome, one proposed model is based on age and NIHSS in ischemic stroke patients admitted within the first hours of onset, and predicts mortality and dependency in daily activities at 100 days after ictus with relatively high accuracy [211]. This prognostic model of functional outcome has been suggested to be used for stratification of treatment groups in clinical trials and to guide inclusion criteria [211]. Whether these models could be feasible and useful in the context of stratifying and selecting patients for SCT is unclear.

Taken together, our findings in Papers II and III call attention to the significance of early stratification of stroke patients on the basis of predicted recovery and functional outcome, as this will be crucial for patient selection for SCT.

Self-Perceptions about Health and Health-Related Domains

Although 70% of the individuals with residual UEMI in Paper II exhibited moderate-severe impairment and 60% of them displayed either limited, poor, or no upper extremity functional capacity, some of these persons expressed positive perceptions regarding their general health status. Similar findings were observed in Paper III where more than one third of the long-term stroke survivors, in spite of poor functional outcome (mRS>2), expressed positive views regarding their general health status.

While the degree of disability and functional status are important determinants of HRQoL after stroke [5], these findings emphasize that aspects other than impairments and disability may also influence the self-perceptions of health. Such other aspects may include e.g. social factors, mood, coping mechanisms, and response shift phenomena such as altered internal standards, values and conceptualization [5,172,212].

From the perspective of SCT in stroke, these findings highlight the importance of also considering the patients' own perceptions concerning the impact of stroke on their health and life. This is important to take into account in the context of patient selection for SCT, since patients without negative perceptions of their impairments and disabilities and with an overall good HRQoL might perhaps not be suitable for (or want) advanced treatments such as SCT (despite objective assessments showing considerable degrees of impairment and disability). On the other hand, patients with objectively mild impairments might still experience a huge impact on their health and life with significantly lowered HRQoL. These aspects may also be important to consider in the assessment of outcomes in later-phase pivotal trials. In the end, the main goal of SCT is to improve functional recovery and outcome to ultimately augment HRQoL after stroke.

Knowledge and Attitudes on Stem Cell-Based Therapies for Stroke

Paper V showed that only a minor proportion of the stroke survivors (12%) had prior knowledge of SCT and stem cell research in stroke. Yet, a majority of the stroke survivors (63%) in Paper V expressed positive attitudes towards SCT after having received standardized and neutral information. These findings suggest that there might be a need for improved strategies by researchers and medical professionals to raise awareness about SCT among stroke patients, especially if SCT can be

translated into effective clinical treatments to improve recovery and functional outcome after stroke.

Moreover, we found that male gender and higher degree of stroke recovery were associated with positive attitudes towards SCT, as well as willingness to participate in clinical trials with SCT. The finding that a higher degree of stroke recovery was associated with positive attitudes to SCT is somewhat surprising, and contradicts previous reports that poor functional status (measured with mRS) after stroke is associated with more positive attitudes to SCT [213]. However, there might be differences in static assessments of functional outcome compared to evaluations of recovery over time as described in our study, and one possible explanation why the stroke patients with better recovery in Paper V had more positive attitudes towards SCT could be that better recovery invokes trust and more positive views about healthcare in general. Also, previous studies have shown that female gender is associated with poor functional recovery and self-perceived unmet rehabilitation needs after stroke [214,215], and this may partly explain our findings regarding the gender differences.

Altogether, our findings in Paper V indicate that improved strategies with targeted information may be of value to raise awareness and knowledge about SCT among stroke patients and possibly facilitate recruitment to clinical trials and reduce risks of selection bias.

Conclusions

- Many ischemic stroke patients have brain infarcts located in close proximity to the SVZ, providing some support that strategies with SCT to optimize endogenous neurogenesis may have a therapeutic potential.
- Brain infarcts confined to striatum are rare among ischemic stroke patients (<10% of the patients), and tend to cause mild neurological deficits. Consequently, striatal infarcts and the generation of striatal neurons should probably not be the primary goal for SCT aiming for neuronal replacement, but rather the substitution of neurons at other stroke lesion sites, e.g. cortical neurons.
- UEMI is frequent among acute/subacute ischemic stroke patients (52%), and a non-negligible proportion of the patients have residual UEMI after 3-5 years. Moreover, post-stroke UEMI correlates strongly to activity limitations and participation restrictions. Thus, post-stroke UEMI may be a clinically valuable target for SCT, and a suitable outcome in later-phase clinical trials studying the efficacy of SCT. However, there is a large inter-individual variability in post-stroke UEMI recovery which emphasizes the necessity for early prognostication of patients that are likely to have a poor recovery and who will be in need of a recovery promoting therapy.
- The majority of 10-year stroke survivors have a relatively good prognosis with good functional outcome accompanied by positive self-perceptions about their general health status. Hence, most of these long-term stroke survivors would probably not have been in need of SCT (if SCT had been available) to improve function with regard to daily activities. This further highlights the importance of early prognostication to detect patients with poor expected functional outcome where SCT might be beneficial.
- PSCI with visuoexecutive dysfunctioning is prevalent among 10-year stroke survivors, and the odds of having severe cognitive impairment is higher among long-term stroke survivors compared to non-stroke persons. Furthermore, MoCA may be more suitable than MMSE to assess long-term PSCI. These findings indicate that pre-clinical stroke studies with SCT should also focus on recovery of cognitive deficits, and PSCI should be taken into account in clinical stroke trials with SCT.

- Targeted information to improve knowledge about SCT among stroke patients may be valuable to facilitate recruitment to clinical trials and reduce risks of selection bias, as attitudes to SCT might be influenced by gender and degree of stroke recovery, and since most stroke patients have limited knowledge about SCT but express positive attitudes towards it after having received standardized and neutral information.

Future Perspectives

Observational studies in stroke are important for the guidance of novel research areas and implementation of new therapies. Data obtained from observational studies may provide important clues for the design of pre-clinical studies in animal models, as well as the design of clinical trials. Moreover, the generalizability of findings from clinical trials may be evaluated by comparisons to outcomes in observational studies.

The field of SCT for stroke is evolving, and early-phase clinical trials are indicating the safety of SCT in stroke patients [12]. As we are moving towards later-phase trials to further evaluate safety and assess efficacy, several issues should be considered, some of which are highlighted in this thesis.

Firstly, stroke is a heterogeneous disorder affecting people of all ages, and may cause numerous brain lesion patterns which can be clinically manifested as various neurological deficits with variable courses of recovery. The findings in Paper I regarding striatal infarcts, and the findings in Paper IV regarding PSCI, illustrate the importance of pre-clinical studies with SCT adapting their stroke models to what is actually being observed in the clinical setting to enhance possibilities of developing therapeutic approaches that are clinically meaningful.

The findings in Paper II concerning post-stroke UEMI recovery, and in Paper III regarding long-term functional and patient-reported outcome after stroke, highlight the importance of stratifying suitable stroke patients on the basis of predicted recovery and outcome in later-phase clinical trials, because many patients display a good recovery and functional outcome without a recovery promoting treatment. However, predicting prognosis and outcome in individual cases is difficult, and validated prognostic scores with high accuracy may be helpful in this regard. Not only prognostic factors associated with poor recovery and functional outcome should be considered, but also markers of differential treatment response [216]. Therefore, more studies are needed to establish the mechanisms of action of SCT, and identify factors that might facilitate successful recovery with SCT.

An interesting approach in later-phase stroke trials with SCT would be to use domain-specific outcomes as previously mentioned (e.g. motor, speech, cognitive impairments) [76], and our findings in Paper II indicate that recovery of post-stroke UEMI may be suitable in this regard. In such a setting, a prognostic score e.g. the

PREP algorithm could be used to stratify stroke patients on the basis of predicted functional recovery of UEMI [210]. An appropriate outcome measure that could be utilized is the FMA-UE, which is well studied and has strong psychometric properties. To increase possibilities of demonstrating proof of concept for SCT in stroke patients, it may be valuable to initially use surrogate outcomes (such as the FMA-UE), and in later studies more clinically meaningful outcomes could be used including outcome measures of activity and participation (in accordance with the ICF model). Still, several important aspects remain to optimize the design of randomized clinical trials of SCT, such as the most suitable source of the stem cells, optimal timing and mode of delivery, suitable lesion characteristics, and risk-benefit assessments.

Furthermore, an important aspect that emerged in several of the papers in this thesis is the patients' perspectives and self-perceptions concerning their impairments and disabilities. As described in Papers II and III, stroke patients with severe disabilities may express positive self-evaluations about their health and vice versa, and in Paper V the patients reported which type of neurological dysfunctions they most wanted to be improved. These dimensions should serve as guidance for SCT in stroke, and be taken into account in the patient selection to clinical stroke trials with SCT, as well as in the assessment of outcomes in later trials.

Populärvetenskaplig sammanfattning

Stroke är en av våra stora folksjukdomar, och innefattar såväl hjärnblödning som hjärninfarkt. Många strokepatienter får kvarstående funktionshinder, och i dagsläget finns få effektiva behandlingsmetoder för att förbättra återhämtningen av nedsatta funktioner efter genomgången stroke. De senaste åren har experimentell forskning på försöksdjur visat att stamcellsbehandling kan förbättra återhämtningen efter stroke. Någon etablerad klinisk stamcellsbehandling för stroke finns emellertid inte i nuläget, och fortsatt forskning är nödvändigt för att stamcellsbehandling ska kunna bli ett alternativ i den kliniska behandlingen av strokepatienter.

I detta avhandlingsarbete, bestående av fem delarbeten, studeras kliniska symptom och radiologisk bild vid stroke, återhämtning och långtidseffekter efter stroke, samt strokepatienters kännedom om och inställning till stamcellsbehandling. Det övergripande syftet med avhandlingen är att bidra med utökad klinisk kunskap för att understödja utvecklingen av stamcellsbehandling som ett kliniskt behandlingsalternativ för att förbättra funktionell återhämtning efter stroke.

I det första delarbetet undersöktes 108 förstagångs-strokepatienter med hjärninfarkt, vilka initialt bedömdes vara potentiella kandidater för stamcellsbehandling. Patienterna undersöktes kliniskt och genomgick magnetkamera-undersökning av hjärnan inom 4 dagar efter strokeinsjuknandet. Majoriteten av strokepatienterna hade hjärninfarkter som var belägna i närheten av den subventrikulära zonen (område i hjärnan där det förekommer kroppsegen nervcellsnybildning). Detta tyder på att stamcellsbehandling för att stimulera hjärnans egen nervcellsnybildning efter stroke kan ha en klinisk behandlingspotential. Delarbetet visade också att hjärninfarkter som var belägna i striatum (område i hjärnan som använts i många experimentella djurförsöksmodeller med stamcellsbehandling vid stroke) var ovanliga bland patienterna och resulterade i milda symptom. Detta tyder vidare på att experimentell stamcellsforskning också bör fokusera på andra områden i hjärnan som kan påverkas vid stroke.

I delarbete II gjordes en 3-5 års uppföljning av 84 överlevande strokepatienter som deltog i det första delarbetet. Deltagarna undersöktes med avseende på förekomst av kvarvarande förlamning i armen, grad av återhämtning av motorisk funktion i den förlamade armen, samt relationen mellan armförlamning och aktivitetsbegränsningar samt delaktighetsinskränkningar i vardagssituationer. Delarbetet visade att förlamning i armen drabbade ungefär hälften av deltagarna i

det akuta skedet efter strokeinsjuknandet, och att ungefär en fjärdedel av dessa uppvisade kvarstående förlamning i armen efter 3-5 år. Graden av återhämtning varierade avsevärt mellan individerna. Vidare visades att armförlamning var starkt korrelerat till aktivitetsbegränsningar och delaktighetsinskränkningar i vardagslivet. Sammantaget tyder dessa fynd på att armförlamning är ett kliniskt betydelsefullt symptom att satsa på med stamcellsbehandling, och att armförlamning möjligen kan utgöra ett lämpligt utfallsmått i framtida kliniska prövningar som studerar effekt av stamcellsbehandling vid stroke. Den stora variationen som noterades avseende återhämtningen av den motoriska funktionen i armen efter stroke visar att man tidigt bör identifiera de patienter som bedöms ha en sämre återhämtning och därmed vara i större behov av stamcellsbehandling.

I delarbete III undersöktes 145 strokepatienter från Lund Stroke Register 10 år efter insjuknandet. Deltagarna undersöktes avseende livssituation, funktionsstatus samt självupplevd syn på hälsa. En majoritet av deltagarna uppvisade en god funktionsnivå, var inte hjälpberoende i livets dagliga aktiviteter och uttryckte ett gott självupplevt allmänt hälsotillstånd. Resultaten visar att många överlevande strokepatienter har en relativt god prognos på lång sikt, och hade sannolikt inte varit i behov av stamcellsbehandling. Detta understryker än mer betydelsen av att tidigt identifiera patienter som bedöms ha en dålig prognos och i större behov av stamcellsbehandling för att förbättra nedsatta funktioner efter stroke.

I delarbete IV undersöktes kognitiv funktion hos 127 strokepatienter från Lunds Stroke Register 10 år efter insjuknandet, och jämfördes med 354 personer i studien ”Gott Åldrande i Skåne” med motsvarande ålder och kön som inte drabbats av stroke. En majoritet av strokepatienterna uppvisade tecken till kognitiv nedsättning efter 10 år, och strokepatienterna hade också en ökad sannolikhet att drabbas av svår kognitiv nedsättning jämfört med personer som inte drabbats av stroke. Fynden visar att kognitiva besvär är vanligt förekommande bland överlevande strokepatienter, och att stamcellsbehandling vid stroke bör ta detta i beaktande både i experimentella modeller på försöksdjur och i framtida kliniska studier.

I delarbete V undersöktes de 84 deltagare som deltog i det andra delarbetet, med avseende på kännedom om och inställning till stamcellsbehandling vid stroke. Enbart ett fåtal deltagare hade tidigare kännedom om stamcellsbehandling vid stroke, men majoriteten av deltagarna uttryckte en positiv inställning efter att ha erhållit standardiserad och neutral skriftlig information om stamcellsbehandling vid stroke. Manligt kön och bättre grad av återhämtning efter strokeinsjuknandet var associerat med positiv inställning och intresse för att delta i framtida kliniska prövningar med stamcellsbehandling. Resultaten tyder på att förbättrad och inriktad information om stamcellsbehandling till strokepatienter kan vara värdefullt för att öka rekryteringen till framtida kliniska prövningar med stamcellsbehandling och för att motverka skevhet i deltagarurvalet till dessa studier.

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References

1. Mortality GBD, Causes of Death C. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet 2016;388:1459-1544.
2. DALYs GBD, Collaborators H. Global, regional, and national disability-adjusted life-years (DALYs) for 315 diseases and injuries and healthy life expectancy (HALE), 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet 2016;388:1603-1658.
3. Hankey GJ. Stroke. Lancet 2016;389:641-654.
4. Luengo-Fernandez R, Paul NL, Gray AM, et al. Population-based study of disability and institutionalization after transient ischemic attack and stroke: 10-year results of the Oxford Vascular Study. Stroke 2013;44:2854-2861.
5. Carod-Artal FJ, Egido JA. Quality of life after stroke: the importance of a good recovery. Cerebrovasc Dis 2009;27 Suppl 1:204-214.
6. Cramer SC. Repairing the human brain after stroke: I. Mechanisms of spontaneous recovery. Ann Neurol 2008;63:272-287.
7. Cassidy JM, Cramer SC. Spontaneous and Therapeutic-Induced Mechanisms of Functional Recovery After Stroke. Transl Stroke Res 2017;8:33-46.
8. George PM, Steinberg GK. Novel Stroke Therapeutics: Unraveling Stroke Pathophysiology and Its Impact on Clinical Treatments. Neuron 2015;87:297-309.
9. Savitz SI. Developing Cellular Therapies for Stroke. Stroke 2015;46:2026-2031.
10. Lindvall O, Kokaia Z. Stem cell research in stroke: how far from the clinic? Stroke 2011;42:2369-2375.
11. Bliss T, Guzman R, Daadi M, Steinberg GK. Cell transplantation therapy for stroke. Stroke 2007;38:817-826.
12. Kalladka D, Sinden J, Pollock K, et al. Human neural stem cells in patients with chronic ischaemic stroke (PISCES): a phase 1, first-in-man study. Lancet 2016;388:787-796.
13. Savitz SI, Cramer SC, Wechsler L, Consortium S. Stem cells as an emerging paradigm in stroke 3: enhancing the development of clinical trials. Stroke 2014;45:634-639.
14. Bliss TM, Andres RH, Steinberg GK. Optimizing the success of cell transplantation therapy for stroke. Neurobiol Dis 2010;37:275-283.
15. Hatano S. Experience from a multicentre stroke register: a preliminary report. Bull World Health Organ 1976;54:541-553.

16. Aho K, Harmsen P, Hatano S, Marquardsen J, Smirnov VE, Strasser T. Cerebrovascular disease in the community: results of a WHO collaborative study. Bull World Health Organ 1980;58:113-130.
17. Clark. A classification and outline of cerebrovascular diseases II. Stroke 1975;6:564-616.
18. Ovbiagele B, Kidwell CS, Saver JL. Epidemiological impact in the United States of a tissue-based definition of transient ischemic attack. Stroke 2003;34:919-924.
19. Doubal FN, Dennis MS, Wardlaw JM. Characteristics of patients with minor ischaemic strokes and negative MRI: a cross-sectional study. J Neurol Neurosurg Psychiatry 2011;82:540-542.
20. Sacco RL, Kasner SE, Broderick JP, et al. An updated definition of stroke for the 21st century: a statement for healthcare professionals from the American Heart Association/American Stroke Association. Stroke 2013;44:2064-2089.
21. Feigin VL, Lawes CM, Bennett DA, Barker-Collo SL, Parag V. Worldwide stroke incidence and early case fatality reported in 56 population-based studies: a systematic review. Lancet Neurol 2009;8:355-369.
22. Feigin VL, Krishnamurthi RV, Parmar P, et al. Update on the Global Burden of Ischemic and Hemorrhagic Stroke in 1990-2013: The GBD 2013 Study. Neuroepidemiology 2015;45:161-176.
23. Feigin VL, Norrving B, George MG, Foltz JL, Roth GA, Mensah GA. Prevention of stroke: a strategic global imperative. Nat Rev Neurol 2016;12:501-512.
24. Feigin VL, Forouzanfar MH, Krishnamurthi R, et al. Global and regional burden of stroke during 1990-2010: findings from the Global Burden of Disease Study 2010. Lancet 2014;383:245-254.
25. The Swedish National Board of Health and Welfare. Statistical Database for Stroke (in Swedish). Available at: http://www.socialstyrelsen.se/statistik/statistikdatabas/stroke. Accessed 13 February 2017.
26. Hallstrom B, Jonsson AC, Nerbrand C, Norrving B, Lindgren A. Stroke incidence and survival in the beginning of the 21st century in southern Sweden: comparisons with the late 20th century and projections into the future. Stroke 2008;39:10-15.
27. Appelros P, Nydevik I, Seiger A, Terent A. High incidence rates of stroke in Orebro, Sweden: Further support for regional incidence differences within Scandinavia. Cerebrovasc Dis 2002;14:161-168.
28. Roth GA, Forouzanfar MH, Moran AE, et al. Demographic and epidemiologic drivers of global cardiovascular mortality. N Engl J Med 2015;372:1333-1341.
29. Rothwell PM, Algra A, Amarenco P. Medical treatment in acute and long-term secondary prevention after transient ischaemic attack and ischaemic stroke. Lancet 2011;377:1681-1692.
30. Olesen J, Gustavsson A, Svensson M, et al. The economic cost of brain disorders in Europe. Eur J Neurol 2012;19:155-162.
31. Ghatnekar O, Persson U, Asplund K, Glader EL. Costs for stroke in Sweden 2009 and developments since 1997. Int J Technol Assess Health Care 2014;30:203-209.

32. Kolominsky-Rabas PL, Wiedmann S, Weingartner M, et al. Time trends in incidence of pathological and etiological stroke subtypes during 16 years: the Erlangen Stroke Project. Neuroepidemiology 2015;44:24-29.
33. Kolominsky-Rabas PL, Weber M, Gefeller O, Neundoerfer B, Heuschmann PU. Epidemiology of ischemic stroke subtypes according to TOAST criteria: incidence, recurrence, and long-term survival in ischemic stroke subtypes: a population-based study. Stroke 2001;32:2735-2740.
34. Petty GW, Brown RD, Jr., Whisnant JP, Sicks JD, O'Fallon WM, Wiebers DO. Ischemic stroke subtypes: a population-based study of incidence and risk factors. Stroke 1999;30:2513-2516.
35. Kim JS, Nah HW, Park SM, et al. Risk factors and stroke mechanisms in atherosclerotic stroke: intracranial compared with extracranial and anterior compared with posterior circulation disease. Stroke 2012;43:3313-3318.
36. Kang DW, Chalela JA, Ezzeddine MA, Warach S. Association of ischemic lesion patterns on early diffusion-weighted imaging with TOAST stroke subtypes. Arch Neurol 2003;60:1730-1734.
37. Caplan LR. Lacunar infarction and small vessel disease: pathology and pathophysiology. J Stroke 2015;17:2-6.
38. Fisher CM. The arterial lesions underlying lacunes. Acta Neuropathol 1968;12:1-15.
39. Wardlaw JM, Smith EE, Biessels GJ, et al. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. Lancet Neurol 2013;12:822-838.
40. Riksstroke. Årsrapport Stroke och TIA 20152016.
41. Baturova MA, Lindgren A, Shubik YV, Olsson SB, Platonov PG. Documentation of atrial fibrillation prior to first-ever ischemic stroke. Acta Neurol Scand 2014;129:412-419.
42. Ay H, Benner T, Arsava EM, et al. A computerized algorithm for etiologic classification of ischemic stroke: the Causative Classification of Stroke System. Stroke 2007;38:2979-2984.
43. Kim HJ, Yun SC, Cho KH, et al. Differential patterns of evolution in acute middle cerebral artery infarction with perfusion-diffusion mismatch: atherosclerotic vs. cardioembolic occlusion. J Neurol Sci 2008;273:93-98.
44. Dirnagl U, Iadecola C, Moskowitz MA. Pathobiology of ischaemic stroke: an integrated view. Trends Neurosci 1999;22:391-397.
45. Anrather J, Iadecola C. Inflammation and Stroke: An Overview. Neurotherapeutics 2016;13:661-670.
46. del Zoppo GJ. Inflammation and the neurovascular unit in the setting of focal cerebral ischemia. Neuroscience 2009;158:972-982.
47. Dirnagl U. Pathobiology of injury after stroke: the neurovascular unit and beyond. Ann N Y Acad Sci 2012;1268:21-25.
48. Ozen I, Deierborg T, Miharada K, et al. Brain pericytes acquire a microglial phenotype after stroke. Acta Neuropathol 2014;128:381-396.

49. Mukherjee P, Thomas S, Pasinetti GM. Complement anaphylatoxin C5a neuroprotects through regulation of glutamate receptor subunit 2 in vitro and in vivo. J Neuroinflammation 2008;5:5.
50. Stephan AH, Barres BA, Stevens B. The complement system: an unexpected role in synaptic pruning during development and disease. Annu Rev Neurosci 2012;35:369-389.
51. Foster EL, Simpson EL, Fredrikson LJ, et al. Eosinophils increase neuron branching in human and murine skin and in vitro. PLoS One 2011;6:e22029.
52. Wang J, Yang Z, Liu C, Zhao Y, Chen Y. Activated microglia provide a neuroprotective role by balancing glial cell-line derived neurotrophic factor and tumor necrosis factor-alpha secretion after subacute cerebral ischemia. Int J Mol Med 2013;31:172-178.
53. Yang H, Feng GD, Liang Z, et al. In vitro beneficial activation of microglial cells by mechanically-injured astrocytes enhances the synthesis and secretion of BDNF through p38MAPK. Neurochem Int 2012;61:175-186.
54. Butovsky O, Ziv Y, Schwartz A, et al. Microglia activated by IL-4 or IFN-gamma differentially induce neurogenesis and oligodendrogenesis from adult stem/progenitor cells. Mol Cell Neurosci 2006;31:149-160.
55. Kelly-Hayes M, Robertson JT, Broderick JP, et al. The American Heart Association Stroke Outcome Classification. Stroke 1998;29:1274-1280.
56. Winstein CJ, Stein J, Arena R, et al. Guidelines for Adult Stroke Rehabilitation and Recovery: A Guideline for Healthcare Professionals From the American Heart Association/American Stroke Association. Stroke 2016;47:e98-e169.
57. Caplan LR, van Gijn J. Stroke Syndromes, 3 ed. Cambridge: Cambridge University Press, 2012.
58. Brott T, Adams HP, Jr., Olinger CP, et al. Measurements of acute cerebral infarction: a clinical examination scale. Stroke 1989;20:864-870.
59. Anamaet W. Using Standardized Measures to Meet the Challenge of Stroke Assessment. Top Geriatr Rehabil 2002;18:47-62.
60. Lawrence ES, Coshall C, Dundas R, et al. Estimates of the prevalence of acute stroke impairments and disability in a multiethnic population. Stroke 2001;32:1279-1284.
61. Rathore SS, Hinn AR, Cooper LS, Tyroler HA, Rosamond WD. Characterization of incident stroke signs and symptoms: findings from the atherosclerosis risk in communities study. Stroke 2002;33:2718-2721.
62. Nakayama H, Jorgensen HS, Raaschou HO, Olsen TS. Recovery of upper extremity function in stroke patients: the Copenhagen Stroke Study. Arch Phys Med Rehabil 1994;75:394-398.
63. Persson HC, Parziali M, Danielsson A, Sunnerhagen KS. Outcome and upper extremity function within 72 hours after first occasion of stroke in an unselected population at a stroke unit. A part of the SALGOT study. BMC Neurol 2012;12:162.
64. Doyle S, Bennett S, Fasoli SE, McKenna KT. Interventions for sensory impairment in the upper limb after stroke. Cochrane Database Syst Rev 2010:CD006331.

65. Tyson SF, Hanley M, Chillala J, Selley AB, Tallis RC. Sensory loss in hospital-admitted people with stroke: characteristics, associated factors, and relationship with function. Neurorehabil Neural Repair 2008;22:166-172.
66. Gray CS, French JM, Bates D, Cartlidge NE, Venables GS, James OF. Recovery of visual fields in acute stroke: homonymous hemianopia associated with adverse prognosis. Age Ageing 1989;18:419-421.
67. Gronholm EO, Roll MC, Horne MA, Sundgren PC, Lindgren AG. Predominance of caudate nucleus lesions in acute ischaemic stroke patients with impairment in language and speech. Eur J Neurol 2016;23:148-153.
68. Flowers HL, Skoretz SA, Silver FL, et al. Poststroke Aphasia Frequency, Recovery, and Outcomes: A Systematic Review and Meta-Analysis. Arch Phys Med Rehabil 2016;97:2188-2201 e2188.
69. Brainin M, Tuomilehto J, Heiss WD, et al. Post-stroke cognitive decline: an update and perspectives for clinical research. Eur J Neurol 2015;22:229-238, e213-226.
70. Pendlebury ST, Rothwell PM. Prevalence, incidence, and factors associated with pre-stroke and post-stroke dementia: a systematic review and meta-analysis. Lancet Neurol 2009;8:1006-1018.
71. Hackett ML, Pickles K. Part I: frequency of depression after stroke: an updated systematic review and meta-analysis of observational studies. Int J Stroke 2014;9:1017-1025.
72. Cumming TB, Blomstrand C, Skoog I, Linden T. The High Prevalence of Anxiety Disorders After Stroke. Am J Geriatr Psychiatry 2016;24:154-160.
73. Nakase T, Tobisawa M, Sasaki M, Suzuki A. Outstanding Symptoms of Poststroke Depression during the Acute Phase of Stroke. PLoS One 2016;11:e0163038.
74. Glader EL, Stegmayr B, Asplund K. Poststroke fatigue: a 2-year follow-up study of stroke patients in Sweden. Stroke 2002;33:1327-1333.
75. Kalladka D, Muir KW. Brain repair: cell therapy in stroke. Stem Cells Cloning 2014;7:31-44.
76. Cramer SC, Koroshetz WJ, Finklestein SP. The case for modality-specific outcome measures in clinical trials of stroke recovery-promoting agents. Stroke 2007;38:1393-1395.
77. Xavier AR, Qureshi AI, Kirmani JF, Yahia AM, Bakshi R. Neuroimaging of stroke: a review. South Med J 2003;96:367-379.
78. Parsons MW, Pepper EM, Bateman GA, Wang Y, Levi CR. Identification of the penumbra and infarct core on hyperacute noncontrast and perfusion CT. Neurology 2007;68:730-736.
79. Gaudinski MR, Henning EC, Miracle A, Luby M, Warach S, Latour LL. Establishing final infarct volume: stroke lesion evolution past 30 days is insignificant. Stroke 2008;39:2765-2768.
80. Hjort N, Christensen S, Solling C, et al. Ischemic injury detected by diffusion imaging 11 minutes after stroke. Ann Neurol 2005;58:462-465.
81. van Gelderen P, de Vleeschouwer MH, DesPres D, Pekar J, van Zijl PC, Moonen CT. Water diffusion and acute stroke. Magn Reson Med 1994;31:154-163.

82. Schaefer PW, Grant PE, Gonzalez RG. Diffusion-weighted MR imaging of the brain. Radiology 2000;217:331-345.
83. Norris JW, Hachinski VC. Intensive care management of stroke patients. Stroke 1976;7:573-577.
84. Stroke Unit Trialists C. Organised inpatient (stroke unit) care for stroke. Cochrane Database Syst Rev 2013:CD000197.
85. Tissue plasminogen activator for acute ischemic stroke. The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. N Engl J Med 1995;333:1581-1587.
86. Emberson J, Lees KR, Lyden P, et al. Effect of treatment delay, age, and stroke severity on the effects of intravenous thrombolysis with alteplase for acute ischaemic stroke: a meta-analysis of individual patient data from randomised trials. Lancet 2014;384:1929-1935.
87. Goyal M, Menon BK, van Zwam WH, et al. Endovascular thrombectomy after large-vessel ischaemic stroke: a meta-analysis of individual patient data from five randomised trials. Lancet 2016;387:1723-1731.
88. Adeoye O, Hornung R, Khatri P, Kleindorfer D. Recombinant tissue-type plasminogen activator use for ischemic stroke in the United States: a doubling of treatment rates over the course of 5 years. Stroke 2011;42:1952-1955.
89. Ullberg T, Zia E, Petersson J, Norrving B. Changes in functional outcome over the first year after stroke: an observational study from the Swedish stroke register. Stroke 2015;46:389-394.
90. Hacke W, Kaste M, Bluhmki E, et al. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. N Engl J Med 2008;359:1317-1329.
91. WHO. International Classification of functioning disability, and health: ICF Geneva. WHO Library Cataloguing-in-Publication Data 2001.
92. Fitzpatrick R, Davey C, Buxton MJ, Jones DR. Evaluating patient-based outcome measures for use in clinical trials. Health Technol Assess 1998;2:i-iv, 1-74.
93. Altman DG. Practical statistics for medical research. 1st ed. London ; New York: Chapman and Hall, 1991: 409-411.
94. Salter K CN, Richardson M, Mehta S, Jutai J, Zettler L, Moses M, McClure A, Mays R, Foley N, Teasell R. ESRSR. 2013.
95. Bushnell C, Bettger JP, Cockroft KM, et al. Chronic Stroke Outcome Measures for Motor Function Intervention Trials: Expert Panel Recommendations. Circ Cardiovasc Qual Outcomes 2015;8:S163-169.
96. Fugl-Meyer AR, Jaasko L, Leyman I, Olsson S, Steglind S. The post-stroke hemiplegic patient. 1. a method for evaluation of physical performance. Scand J Rehabil Med 1975;7:13-31.
97. Tombaugh TN, McIntyre NJ. The mini-mental state examination: a comprehensive review. J Am Geriatr Soc 1992;40:922-935.
98. Lees R, Selvarajah J, Fenton C, et al. Test accuracy of cognitive screening tests for diagnosis of dementia and multidomain cognitive impairment in stroke. Stroke 2014;45:3008-3018.

99. Pendlebury ST, Mariz J, Bull L, Mehta Z, Rothwell PM. MoCA, ACE-R, and MMSE versus the National Institute of Neurological Disorders and Stroke-Canadian Stroke Network Vascular Cognitive Impairment Harmonization Standards Neuropsychological Battery after TIA and stroke. Stroke 2012;43:464-469.
100. Nasreddine ZS, Phillips NA, Bedirian V, et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. J Am Geriatr Soc 2005;53:695-699.
101. Wityk RJ, Pessin MS, Kaplan RF, Caplan LR. Serial assessment of acute stroke using the NIH Stroke Scale. Stroke 1994;25:362-365.
102. Lyle RC. A performance test for assessment of upper limb function in physical rehabilitation treatment and research. Int J Rehabil Res 1981;4:483-492.
103. Yozbatiran N, Der-Yeghiaian L, Cramer SC. A standardized approach to performing the action research arm test. Neurorehabil Neural Repair 2008;22:78-90.
104. Mahoney FI, Barthel DW. Functional Evaluation: The Barthel Index. Md State Med J 1965;14:61-65.
105. van Swieten JC, Koudstaal PJ, Visser MC, Schouten HJ, van Gijn J. Interobserver agreement for the assessment of handicap in stroke patients. Stroke 1988;19:604-607.
106. Rankin J. Cerebral vascular accidents in patients over the age of 60. II. Prognosis. Scott Med J 1957;2:200-215.
107. EuroQol G. EuroQol--a new facility for the measurement of health-related quality of life. Health Policy 1990;16:199-208.
108. Burstrom K, Johannesson M, Rehnberg C. Deteriorating health status in Stockholm 1998-2002: results from repeated population surveys using the EQ-5D. Qual Life Res 2007;16:1547-1553.
109. Ware JE, Jr., Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care 1992;30:473-483.
110. Duncan PW, Wallace D, Lai SM, Johnson D, Embretson S, Laster LJ. The stroke impact scale version 2.0. Evaluation of reliability, validity, and sensitivity to change. Stroke 1999;30:2131-2140.
111. Duncan PW, Lai SM, Keighley J. Defining post-stroke recovery: implications for design and interpretation of drug trials. Neuropharmacology 2000;39:835-841.
112. Hebert D, Lindsay MP, McIntyre A, et al. Canadian stroke best practice recommendations: Stroke rehabilitation practice guidelines, update 2015. Int J Stroke 2016;11:459-484.
113. Pollock A, Baer G, Campbell P, et al. Physical rehabilitation approaches for the recovery of function and mobility following stroke. Cochrane Database Syst Rev 2014:CD001920.
114. Ward NS, Kitago T. Getting the right prescription for rehabilitation after stroke. Neurology 2016;86:2120-2121.
115. Bernhardt J, Langhorne P, Lindley RI, et al. Efficacy and safety of very early mobilisation within 24 h of stroke onset (AVERT): a randomised controlled trial. Lancet 2015;386:46-55.

116. Mizuma A, Yamashita T, Kono S, et al. Phase II Trial of Intravenous Low-Dose Granulocyte Colony-Stimulating Factor in Acute Ischemic Stroke. J Stroke Cerebrovasc Dis 2016;25:1451-1457.
117. Schabitz WR, Laage R, Vogt G, et al. AXIS: a trial of intravenous granulocyte colony-stimulating factor in acute ischemic stroke. Stroke 2010;41:2545-2551.
118. Cramer SC. An overview of therapies to promote repair of the brain after stroke. Head Neck 2011;33 Suppl 1:S5-7.
119. Kwakkel G, Kollen B, Lindeman E. Understanding the pattern of functional recovery after stroke: facts and theories. Restor Neurol Neurosci 2004;22:281-299.
120. Prabhakaran S, Zarahn E, Riley C, et al. Inter-individual variability in the capacity for motor recovery after ischemic stroke. Neurorehabil Neural Repair 2008;22:64-71.
121. Jorgensen HS, Nakayama H, Raaschou HO, Vive-Larsen J, Stoier M, Olsen TS. Outcome and time course of recovery in stroke. Part I: Outcome. The Copenhagen Stroke Study. Arch Phys Med Rehabil 1995;76:399-405.
122. Jorgensen HS, Nakayama H, Raaschou HO, Vive-Larsen J, Stoier M, Olsen TS. Outcome and time course of recovery in stroke. Part II: Time course of recovery. The Copenhagen Stroke Study. Arch Phys Med Rehabil 1995;76:406-412.
123. Knoflach M, Matosevic B, Rucker M, et al. Functional recovery after ischemic stroke--a matter of age: data from the Austrian Stroke Unit Registry. Neurology 2012;78:279-285.
124. Rasquin SM, Lodder J, Verhey FR. Predictors of reversible mild cognitive impairment after stroke: a 2-year follow-up study. J Neurol Sci 2005;229-230:21-25.
125. Desmond DW, Moroney JT, Sano M, Stern Y. Recovery of cognitive function after stroke. Stroke 1996;27:1798-1803.
126. Duncan PW, Goldstein LB, Matchar D, Divine GW, Feussner J. Measurement of motor recovery after stroke. Outcome assessment and sample size requirements. Stroke 1992;23:1084-1089.
127. Duncan PW, Goldstein LB, Horner RD, Landsman PB, Samsa GP, Matchar DB. Similar motor recovery of upper and lower extremities after stroke. Stroke 1994;25:1181-1188.
128. Lesniak M, Bak T, Czepiel W, Seniow J, Czlonkowska A. Frequency and prognostic value of cognitive disorders in stroke patients. Dement Geriatr Cogn Disord 2008;26:356-363.
129. Snaphaan L, de Leeuw FE. Poststroke memory function in nondemented patients: a systematic review on frequency and neuroimaging correlates. Stroke 2007;38:198-203.
130. Oki K, Tatarishvili J, Wood J, et al. Human-induced pluripotent stem cells form functional neurons and improve recovery after grafting in stroke-damaged brain. Stem Cells 2012;30:1120-1133.
131. Tornero D, Wattananit S, Gronning Madsen M, et al. Human induced pluripotent stem cell-derived cortical neurons integrate in stroke-injured cortex and improve functional recovery. Brain 2013;136:3561-3577.

132. Hicks AU, Lappalainen RS, Narkilahti S, et al. Transplantation of human embryonic stem cell-derived neural precursor cells and enriched environment after cortical stroke in rats: cell survival and functional recovery. Eur J Neurosci 2009;29:562-574.
133. Daadi MM, Maag AL, Steinberg GK. Adherent self-renewable human embryonic stem cell-derived neural stem cell line: functional engraftment in experimental stroke model. PLoS One 2008;3:e1644.
134. Kwakkel G, Kollen BJ, van der Grond J, Prevo AJ. Probability of regaining dexterity in the flaccid upper limb: impact of severity of paresis and time since onset in acute stroke. Stroke 2003;34:2181-2186.
135. Persson HC. Upper extremity functioning during the first year after stroke. Gothenburg, Sweden: Sahlgrenska Academy at University of Gothenbutg, 2015.
136. Persson HC, Alt Murphy M, Danielsson A, Lundgren-Nilsson A, Sunnerhagen KS. A cohort study investigating a simple, early assessment to predict upper extremity function after stroke - a part of the SALGOT study. BMC Neurol 2015;15:92.
137. Heuschmann PU, Wiedmann S, Wellwood I, et al. Three-month stroke outcome: the European Registers of Stroke (EROS) investigators. Neurology 2011;76:159-165.
138. Appelros P, Nydevik I, Viitanen M. Poor outcome after first-ever stroke: predictors for death, dependency, and recurrent stroke within the first year. Stroke 2003;34:122-126.
139. Hankey GJ, Jamrozik K, Broadhurst RJ, et al. Five-year survival after first-ever stroke and related prognostic factors in the Perth Community Stroke Study. Stroke 2000;31:2080-2086.
140. Hardie K, Hankey GJ, Jamrozik K, Broadhurst RJ, Anderson C. Ten-year survival after first-ever stroke in the perth community stroke study. Stroke 2003;34:1842-1846.
141. Wolfe CD, Crichton SL, Heuschmann PU, et al. Estimates of outcomes up to ten years after stroke: analysis from the prospective South London Stroke Register. PLoS Med 2011;8:e1001033.
142. Crichton SL, Bray BD, McKevitt C, Rudd AG, Wolfe CD. Patient outcomes up to 15 years after stroke: survival, disability, quality of life, cognition and mental health. J Neurol Neurosurg Psychiatry 2016;87:1091-1098.
143. Feigin VL, Barker-Collo S, Parag V, et al. Auckland Stroke Outcomes Study. Part 1: Gender, stroke types, ethnicity, and functional outcomes 5 years poststroke. Neurology 2010;75:1597-1607.
144. Hankey GJ, Jamrozik K, Broadhurst RJ, Forbes S, Anderson CS. Long-term disability after first-ever stroke and related prognostic factors in the Perth Community Stroke Study, 1989-1990. Stroke 2002;33:1034-1040.
145. Hardie K, Hankey GJ, Jamrozik K, Broadhurst RJ, Anderson C. Ten-year risk of first recurrent stroke and disability after first-ever stroke in the Perth Community Stroke Study. Stroke 2004;35:731-735.
146. Liman TG, Heuschmann PU, Endres M, Floel A, Schwab S, Kolominsky-Rabas PL. Impact of low mini-mental status on health outcome up to 5 years after stroke: the Erlangen Stroke Project. J Neurol 2012;259:1125-1130.
147. Norlander A, Carlstedt E, Jonsson AC, et al. Long-Term Predictors of Social and Leisure Activity 10 Years after Stroke. PLoS One 2016;11:e0149395.

148. Savitz SI, Rosenbaum DM. Stroke recovery with cellular therapies. Current clinical neurology. Totowa, N. J.: Humana Press, 2008: 1-9.
149. Seaberg RM, van der Kooy D. Stem and progenitor cells: the premature desertion of rigorous definitions. Trends Neurosci 2003;26:125-131.
150. Marlier Q, Verteneuil S, Vandenbosch R, Malgrange B. Mechanisms and Functional Significance of Stroke-Induced Neurogenesis. Front Neurosci 2015;9:458.
151. Lindvall O, Kokaia Z. Neurogenesis following Stroke Affecting the Adult Brain. Cold Spring Harb Perspect Biol 2015;7.
152. Quinones-Hinojosa A, Sanai N, Soriano-Navarro M, et al. Cellular composition and cytoarchitecture of the adult human subventricular zone: a niche of neural stem cells. J Comp Neurol 2006;494:415-434.
153. Hou SW, Wang YQ, Xu M, et al. Functional integration of newly generated neurons into striatum after cerebral ischemia in the adult rat brain. Stroke 2008;39:2837-2844.
154. Arvidsson A, Collin T, Kirik D, Kokaia Z, Lindvall O. Neuronal replacement from endogenous precursors in the adult brain after stroke. Nat Med 2002;8:963-970.
155. Thored P, Arvidsson A, Cacci E, et al. Persistent production of neurons from adult brain stem cells during recovery after stroke. Stem Cells 2006;24:739-747.
156. Jin K, Sun Y, Xie L, et al. Directed migration of neuronal precursors into the ischemic cerebral cortex and striatum. Mol Cell Neurosci 2003;24:171-189.
157. Parent JM, Vexler ZS, Gong C, Derugin N, Ferriero DM. Rat forebrain neurogenesis and striatal neuron replacement after focal stroke. Ann Neurol 2002;52:802-813.
158. Magnusson JP, Goritz C, Tatarishvili J, et al. A latent neurogenic program in astrocytes regulated by Notch signaling in the mouse. Science 2014;346:237-241.
159. Jin K, Wang X, Xie L, Mao XO, Greenberg DA. Transgenic ablation of doublecortin-expressing cells suppresses adult neurogenesis and worsens stroke outcome in mice. Proc Natl Acad Sci U S A 2010;107:7993-7998.
160. Wang X, Mao X, Xie L, Sun F, Greenberg DA, Jin K. Conditional depletion of neurogenesis inhibits long-term recovery after experimental stroke in mice. PLoS One 2012;7:e38932.
161. Sun F, Wang X, Mao X, Xie L, Jin K. Ablation of neurogenesis attenuates recovery of motor function after focal cerebral ischemia in middle-aged mice. PLoS One 2012;7:e46326.
162. Macas J, Nern C, Plate KH, Momma S. Increased generation of neuronal progenitors after ischemic injury in the aged adult human forebrain. J Neurosci 2006;26:13114-13119.
163. Marti-Fabregas J, Romaguera-Ros M, Gomez-Pinedo U, et al. Proliferation in the human ipsilateral subventricular zone after ischemic stroke. Neurology 2010;74:357-365.
164. Jin K, Wang X, Xie L, et al. Evidence for stroke-induced neurogenesis in the human brain. Proc Natl Acad Sci U S A 2006;103:13198-13202.
165. Ernst A, Alkass K, Bernard S, et al. Neurogenesis in the striatum of the adult human brain. Cell 2014;156:1072-1083.

166. Tornero D, Tsupykov O, Granmo M, et al. Synaptic inputs from stroke-injured brain to grafted human stem cell-derived neurons activated by sensory stimuli. Brain 2017.
167. Zhang ZG, Chopp M. Neurorestorative therapies for stroke: underlying mechanisms and translation to the clinic. Lancet Neurol 2009;8:491-500.
168. Dihne M, Hartung HP, Seitz RJ. Restoring neuronal function after stroke by cell replacement: anatomic and functional considerations. Stroke 2011;42:2342-2350.
169. Kallur T, Darsalia V, Lindvall O, Kokaia Z. Human fetal cortical and striatal neural stem cells generate region-specific neurons in vitro and differentiate extensively to neurons after intrastriatal transplantation in neonatal rats. J Neurosci Res 2006;84:1630-1644.
170. Aubry L, Bugi A, Lefort N, Rousseau F, Peschanski M, Perrier AL. Striatal progenitors derived from human ES cells mature into DARPP32 neurons in vitro and in quinolinic acid-lesioned rats. Proc Natl Acad Sci U S A 2008;105:16707-16712.
171. Hallstrom B, Jonsson AC, Nerbrand C, Petersen B, Norrving B, Lindgren A. Lund Stroke Register: hospitalization pattern and yield of different screening methods for first-ever stroke. Acta Neurol Scand 2007;115:49-54.
172. Jonsson AC, Lindgren I, Hallstrom B, Norrving B, Lindgren A. Determinants of quality of life in stroke survivors and their informal caregivers. Stroke 2005;36:803-808.
173. Jonsson AC, Lindgren I, Hallstrom B, Norrving B, Lindgren A. Prevalence and intensity of pain after stroke: a population based study focusing on patients' perspectives. J Neurol Neurosurg Psychiatry 2006;77:590-595.
174. Lindgren I, Jonsson AC, Norrving B, Lindgren A. Shoulder pain after stroke: a prospective population-based study. Stroke 2007;38:343-348.
175. Jonsson AC, Lindgren I, Norrving B, Lindgren A. Weight loss after stroke: a population-based study from the Lund Stroke Register. Stroke 2008;39:918-923.
176. Lagergren M, Fratiglioni L, Hallberg IR, et al. A longitudinal study integrating population, care and social services data. The Swedish National study on Aging and Care (SNAC). Aging Clin Exp Res 2004;16:158-168.
177. Ekstrom H, Elmstahl S. Pain and fractures are independently related to lower walking speed and grip strength: results from the population study "Good Ageing in Skane". Acta Orthop 2006;77:902-911.
178. Yamada K, Kizu O, Kubota T, et al. The pyramidal tract has a predictable course through the centrum semiovale: a diffusion-tensor based tractography study. J Magn Reson Imaging 2007;26:519-524.
179. Hoonhorst MH, Nijland RH, van den Berg JS, Emmelot CH, Kollen BJ, Kwakkel G. How Do Fugl-Meyer Arm Motor Scores Relate to Dexterity According to the Action Research Arm Test at 6 Months Poststroke? Arch Phys Med Rehabil 2015;96:1845-1849.
180. Woodbury ML, Velozo CA, Richards LG, Duncan PW. Rasch analysis staging methodology to classify upper extremity movement impairment after stroke. Arch Phys Med Rehabil 2013;94:1527-1533.

181. Grut M, Fratiglioni L, Viitanen M, Winblad B. Accuracy of the Mini-Mental Status Examination as a screening test for dementia in a Swedish elderly population. Acta Neurol Scand 1993;87:312-317.
182. Pendlebury ST, Cuthbertson FC, Welch SJ, Mehta Z, Rothwell PM. Underestimation of cognitive impairment by Mini-Mental State Examination versus the Montreal Cognitive Assessment in patients with transient ischemic attack and stroke: a population-based study. Stroke 2010;41:1290-1293.
183. Pendlebury ST, Mariz J, Bull L, Mehta Z, Rothwell PM. Impact of different operational definitions on mild cognitive impairment rate and MMSE and MoCA performance in transient ischaemic attack and stroke. Cerebrovasc Dis 2013;36:355-362.
184. Webb AJ, Pendlebury ST, Li L, et al. Validation of the Montreal cognitive assessment versus mini-mental state examination against hypertension and hypertensive arteriopathy after transient ischemic attack or minor stroke. Stroke 2014;45:3337-3342.
185. Mai LM, Sposato LA, Rothwell PM, Hachinski V, Pendlebury ST. A comparison between the MoCA and the MMSE visuoexecutive sub-tests in detecting abnormalities in TIA/stroke patients. Int J Stroke 2016;11:420-424.
186. Muir KW, Lees KR, Ford I, Davis S, Intravenous Magnesium Efficacy in Stroke Study I. Magnesium for acute stroke (Intravenous Magnesium Efficacy in Stroke trial): randomised controlled trial. Lancet 2004;363:439-445.
187. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis 1987;40:373-383.
188. Feigin VL, Carter K. Editorial comment--Stroke incidence studies one step closer to the elusive gold standard? Stroke 2004;35:2045-2047.
189. Pendlebury ST, Chen PJ, Welch SJ, et al. Methodological Factors in Determining Risk of Dementia After Transient Ischemic Attack and Stroke: (II) Effect of Attrition on Follow-Up. Stroke 2015;46:1494-1500.
190. Pendlebury ST, Chen PJ, Bull L, et al. Methodological factors in determining rates of dementia in transient ischemic attack and stroke: (I) impact of baseline selection bias. Stroke 2015;46:641-646.
191. Pendlebury ST, Klaus SP, Thomson RJ, et al. Methodological Factors in Determining Risk of Dementia After Transient Ischemic Attack and Stroke: (III) Applicability of Cognitive Tests. Stroke 2015;46:3067-3073.
192. Chatfield MD, Brayne CE, Matthews FE. A systematic literature review of attrition between waves in longitudinal studies in the elderly shows a consistent pattern of dropout between differing studies. J Clin Epidemiol 2005;58:13-19.
193. Hasegawa Y, Fisher M, Latour LL, Dardzinski BJ, Sotak CH. MRI diffusion mapping of reversible and irreversible ischemic injury in focal brain ischemia. Neurology 1994;44:1484-1490.
194. Kidwell CS, Saver JL, Starkman S, et al. Late secondary ischemic injury in patients receiving intraarterial thrombolysis. Ann Neurol 2002;52:698-703.

195. Wu O, Koroshetz WJ, Ostergaard L, et al. Predicting tissue outcome in acute human cerebral ischemia using combined diffusion- and perfusion-weighted MR imaging. Stroke 2001;32:933-942.
196. Rohl L, Ostergaard L, Simonsen CZ, et al. Viability thresholds of ischemic penumbra of hyperacute stroke defined by perfusion-weighted MRI and apparent diffusion coefficient. Stroke 2001;32:1140-1146.
197. Li F, Liu KF, Silva MD, et al. Transient and permanent resolution of ischemic lesions on diffusion-weighted imaging after brief periods of focal ischemia in rats : correlation with histopathology. Stroke 2000;31:946-954.
198. Campbell BC, Purushotham A, Christensen S, et al. The infarct core is well represented by the acute diffusion lesion: sustained reversal is infrequent. J Cereb Blood Flow Metab 2012;32:50-56.
199. Chemmanam T, Campbell BC, Christensen S, et al. Ischemic diffusion lesion reversal is uncommon and rarely alters perfusion-diffusion mismatch. Neurology 2010;75:1040-1047.
200. Cumming TB, Churilov L, Linden T, Bernhardt J. Montreal Cognitive Assessment and Mini-Mental State Examination are both valid cognitive tools in stroke. Acta Neurol Scand 2013;128:122-129.
201. Huttner HB, Bergmann O, Salehpour M, et al. The age and genomic integrity of neurons after cortical stroke in humans. Nat Neurosci 2014;17:801-803.
202. Hess D. Cell Therapy for Brain Injury. Springer International Publishing Switzerland, 2015: 351-364.
203. Persson HC, Danielsson A, Sunnerhagen KS. A cross sectional study of upper extremity strength ten days after a stroke; relationship between patient-reported and objective measures. BMC Neurol 2015;15:178.
204. Kokmen E, Whisnant JP, O'Fallon WM, Chu CP, Beard CM. Dementia after ischemic stroke: a population-based study in Rochester, Minnesota (1960-1984). Neurology 1996;46:154-159.
205. Ivan CS, Seshadri S, Beiser A, et al. Dementia after stroke: the Framingham Study. Stroke 2004;35:1264-1268.
206. Boy S, Sauerbruch S, Kraemer M, et al. Mobilisation of hematopoietic CD34+ precursor cells in patients with acute stroke is safe--results of an open-labeled non randomized phase I/II trial. PLoS One 2011;6:e23099.
207. Sun C, Sun H, Wu S, et al. Conditional ablation of neuroprogenitor cells in adult mice impedes recovery of poststroke cognitive function and reduces synaptic connectivity in the perforant pathway. J Neurosci 2013;33:17314-17325.
208. Misra V, Hicks WJ, Vahidy F, Alderman S, Savitz SI. Recruiting Patients With Stroke Into Cell Therapy Trials: A Review. JAMA Neurol 2016;73:1141-1144.
209. Steyerberg EW, Moons KG, van der Windt DA, et al. Prognosis Research Strategy (PROGRESS) 3: prognostic model research. PLoS Med 2013;10:e1001381.
210. Stinear CM, Barber PA, Petoe M, Anwar S, Byblow WD. The PREP algorithm predicts potential for upper limb recovery after stroke. Brain 2012;135:2527-2535.

211. Weimar C, Konig IR, Kraywinkel K, Ziegler A, Diener HC, German Stroke Study C. Age and National Institutes of Health Stroke Scale Score within 6 hours after onset are accurate predictors of outcome after cerebral ischemia: development and external validation of prognostic models. Stroke 2004;35:158-162.
212. Schwartz CE, Andresen EM, Nosek MA, Krahn GL, Measurement REPoHS. Response shift theory: important implications for measuring quality of life in people with disability. Arch Phys Med Rehabil 2007;88:529-536.
213. Kim YS, Chung DI, Choi H, et al. Fantasies about stem cell therapy in chronic ischemic stroke patients. Stem Cells Dev 2013;22:31-36.
214. Gargano JW, Reeves MJ, Paul Coverdell National Acute Stroke Registry Michigan Prototype I. Sex differences in stroke recovery and stroke-specific quality of life: results from a statewide stroke registry. Stroke 2007;38:2541-2548.
215. Ullberg T, Zia E, Petersson J, Norrving B. Perceived Unmet Rehabilitation Needs 1 Year After Stroke: An Observational Study From the Swedish Stroke Register. Stroke 2016;47:539-541.
216. Hingorani AD, Windt DA, Riley RD, et al. Prognosis research strategy (PROGRESS) 4: stratified medicine research. BMJ 2013;346:e5793.

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