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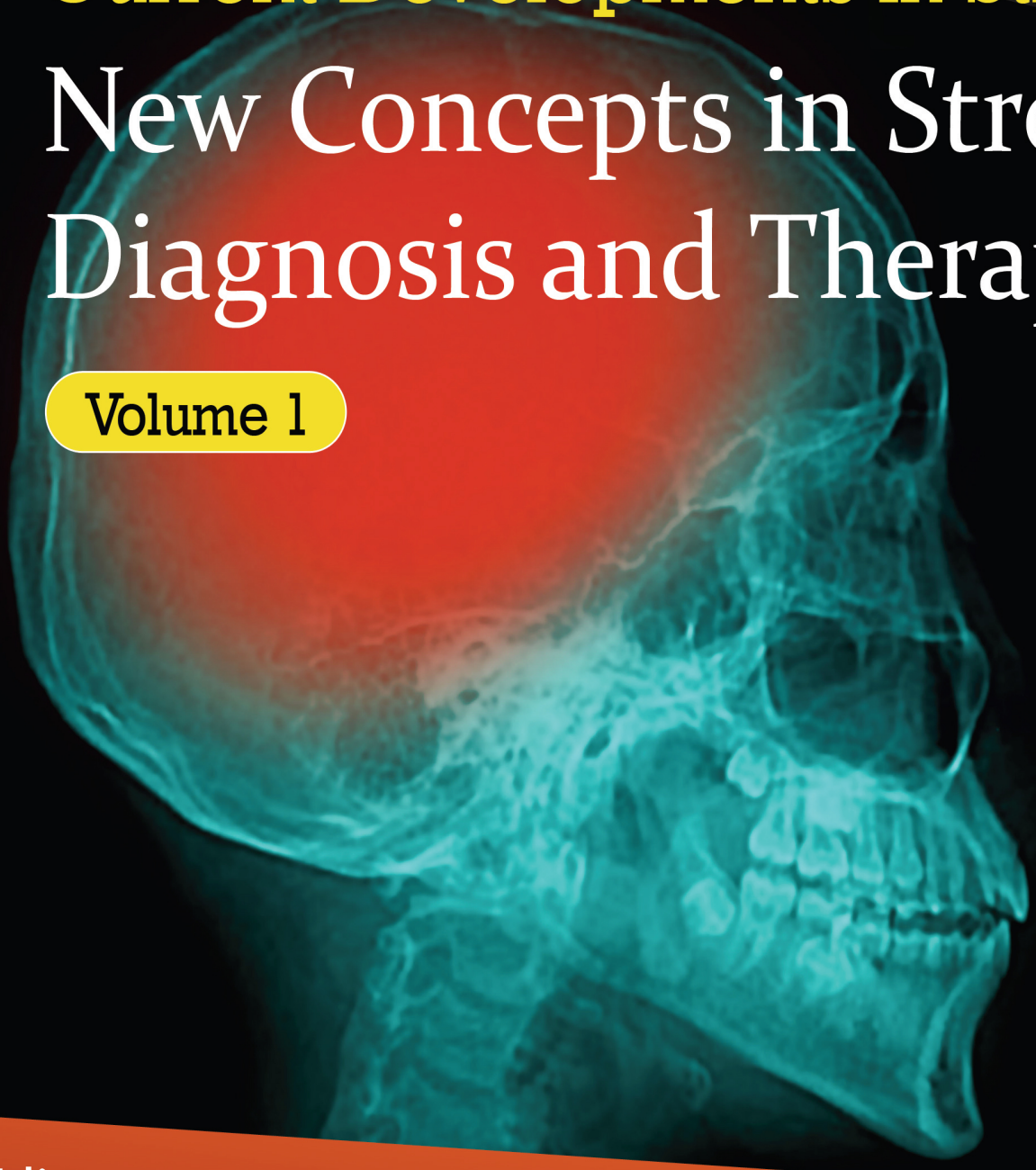
Current Developments in Stroke

New Concepts in Stroke Diagnosis and Therapy

Volume 1



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Current Developments in Stroke

(Volume 1)

(New Concepts in Stroke Diagnosis and Therapy)

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Foreword

Stroke is likely the neurological disease with the most relevant developments in all the research fields, from knowledge of the mechanisms leading to brain tissue death to codification of rehabilitation mainstays, through better definition of risk factors and consequent improvement of primary and secondary prevention, diagnosis with advanced imaging or by identification of genetic mechanisms in rare cases, treatments of the acute phase of ischemic events, with the well-established role of i.v. thrombolysis now backed up by combined endovascular treatments and with the potentialities of neuroprotection and neuroregeneration, or of iatrogenic hemorrhagic stroke, with the advent of antidotes for the new generation of direct oral anticoagulants.

As a consequence, a terribly huge literature on stroke has been produced: by typing the word "stroke" one may find approximately 140000 papers indexed in PubMed in the last 20 years. Hence, a book summarizing all the "new concepts in stroke diagnosis and therapy" is highly appreciated. The Authors report the present knowledge on all the above mentioned issue, with updated and well selected reference literature. The book can be a good "traveling companion" for neurologists working daily on stroke patient management and having little time to look for scientific literature, but also a guide for stroke basic and clinical researchers to pick up the most recent information and to get suggestions for further research.

My compliments to the Authors and "enjoy the reading" to all the readers.

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Preface 1

Stroke prevention, diagnosis and therapy are all evolving fields that give us the idea of how in progress is the clinical job and the scientific work. Stroke in particular deserves a special mention as it is perceived both by patients and by physicians and it is one of the most feared and invalidating condition.

In this regard, every single step able to improve prevention, accelerate diagnosis and therapy is welcome and need to be rapidly shared with all the scientific community.

On the one hand, despite the stabilization of stroke events in civilized countries other emerging countries contribute to new events so keeping a high prevalence of stroke worldwide. In this regard, stroke prevention still represents one of the missing opportunities and the recognition and treatment of old and new risk factors are mandatory. On the other hand, imaging and therefore diagnosis and therapeutic opportunities are now available to treat faster than ever ischemic events in order to reduce overall cerebral damage and therefore disability. These new possibilities nevertheless should not remain restricted to few golden clinical and scientific realities but be rapidly shared and diffused to the emerging countries where the prevalence of stroke is growing.

This book aims to be an aid to the diffusion and discussion of what is new in the field and what are some of the new directions of prevention, diagnosis and therapy. At least, eighty five per cent of strokes are of ischemic origin and are therefore the results of a missed prevention in vascular atherosclerosis and thrombosis. This has always been a “cardiology” field. It is evident that a tighter cooperation between cardiologists and neurologists is needed in order to share expertise and to create more powerful tools to improve prevention, diagnosis and therapy of vascular events that involve in a similar dramatic way both the heart and the brain.

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Preface 2

Only few decades ago stroke was considered a devastating condition with high mortality, high disability, without adequate prevention strategies, any tool to perform accurate diagnosis and consequently without any effective treatment available.

Scientific and technological advances in the last few years have dramatically changed the scenario: epidemiologic, genetic, imaging, biological and therapeutic advances have made it possible to effectively prevent strokes, to perform accurate differential diagnosis of stroke type and of location of vessel occlusion and new treatments for acute phase have recently demonstrated dramatic results.

In the context of this new scenario, new concepts for stroke diagnosis and therapy emerged, and this book specifically addresses this point.

Major experts and opinion leaders in respective fields extensively review and discuss new advances in the knowledge of the role of stroke risk factor for their prevention; new technological tools to perform *in vivo* imaging of cerebral collateral circulation and ischemic penumbra are widely described and their relevance for more accurate diagnosis and prognosis is discussed.

Diagnostic challenges in rare aethiologies of stroke are described in detail and new studies on recanalization and neuroprotection strategies are reported, with analysis of their impact on stroke health organization.

Finally, the hemorrhagic risk associated to older and new anticoagulants is discussed and new studies on stroke recovery are presented.

The reader of this new book may obtain a state-of-the-art knowledge of stroke diagnosis and treatment options to address the challenges of this severe, but now treatable disease.

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CHAPTER 1

Stroke and Hypertension

Cristina Sierra and **Antonio Coca***

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Abstract: Stroke, the third most-common cause of mortality after cancer and heart disease in developed countries, is one of the most common causes of cognitive impairment and vascular dementia. Stroke pathogenesis and its consequences are not completely elucidated, with various factors and biological mechanisms probably having a role. After age, hypertension is the leading modifiable cardiovascular risk factor for ischaemic/haemorrhagic stroke, small vessel disease predisposing to lacunar infarction, cerebral white matter lesions (cWML), and cerebral microbleeds. Primary stroke prevention, involving hypertension therapy and blood pressure (BP) control is now standard. At the same time, elevated post-stroke BP levels increase the risk of recurrent stroke, with recent trials suggesting that BP reduction with combinations of hypertension therapy reduces stroke recurrence. This chapter reviews the evidence on hypertension as a stroke risk factor and the part played by hypertension therapy in first/recurrent stroke prevention.

Keywords: Cerebral microbleeds, Cerebral small vessel disease, Cognitive impairment, Hemorrhagic stroke, Hypertension, Hypertension therapy, Ischemic stroke, Lacunar infarction, Recurrent stroke, Vascular dementia, White matter lesions.

INTRODUCTION

Stroke, the third most-frequent cause of death after cancer and heart disease in developed countries, is one of the most common causes of cognitive impairment and vascular dementia [1]. Stroke entails high economic and public health

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impacts. Age is the first all-stroke risk factor [1]. The stroke rate doubles each 10 years in both males and females > 55 years of age, with > 80% of strokes occurring in persons aged ≥ 65 years. Due to the aging population, the burden of stroke will rise substantially in forthcoming years. Elderly people's increased vulnerability to stroke is related to changes in the aging brain and with a higher prevalence of established stroke risk factors, including hypertension (HT), atrial fibrillation, carotid stenosis and cardiovascular (CV) disease.

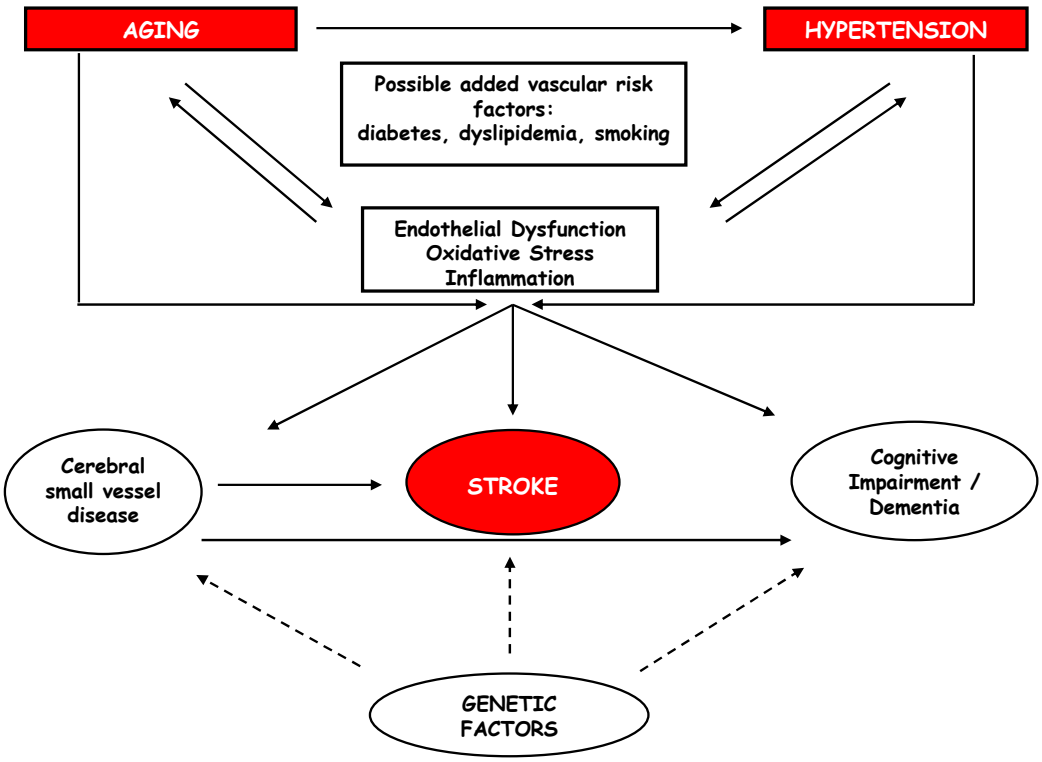

Fig. (1). Multiple connected biological mechanisms that participate in the pathogenesis of stroke (Adapted from Sierra C *et al.* [2]).

Stroke pathogenesis and its consequences are not completely elucidated, with various factors and biological mechanisms possibly playing a role (Fig. **1**) [2]. Elevated blood pressure (BP) is a major stroke risk factor, with an established, continuous relationship between stroke and BP [1, 3]. However, trials of

hypertension therapy demonstrate that relatively-small BP reductions (5-6 mmHg in diastolic BP (DBP), 10-12 mmHg in systolic BP (SBP) for 3-5 years) cut the stroke risk by > 33% [3]. Primary stroke prevention through BP control and hypertension treatment is now standard [1, 3]. In the same way, elevated post-stroke BP increases the recurrent stroke risk [3, 4], with some trials demonstrating that BP lowering plus combination hypertension therapy has benefits in lowering stroke recurrence [3, 4].

HT, known to be the leading factor for macrovascular cerebral complications, such as stroke and, therefore, vascular dementia [1, 3, 5], may also predispose to more-subtle cerebral changes due to narrowing of the arterioles or pathological microvascular changes. Cerebral microvascular disease has been suggested as a factor in vascular cognitive impairment [6, 7]. The complex underlying mechanisms of HT-related cognitive changes are not completely elucidated. Associations between cerebral white matter lesions (cWML) and BP elevation indirect suggest that long-term structural/functional brain changes may result in worse cognitive functioning when BP control is poor or absent. At the same time, some evidence suggests hypertension therapy may aid the prevention of cognitive impairment/vascular dementia by controlling BP [5].

Older age and HT are consistently reported as the leading risk factors for cWML, which, in turn is a leading factor in the prognosis of stroke and cognitive impairment/dementia [1, 3, 5, 6, 8]. Hypertensives present more and a greater area of cWML than normotensives [6, 8]. At the same time, treated and controlled hypertensives have been shown to have a lower prevalence of cWML than untreated/treated uncontrolled hypertensives [9]. A randomized BP-lowering trial of perindopril *vs.* placebo in normotensives and hypertensives with cerebrovascular disease (CeVD) found average total new WML volume was significantly lower in actively treated patients than in the placebo group [10].

The idea that, in hypertensives, cWML may be an early, silent marker of brain damage is strongly supported by recent evidence.

STROKE EPIDEMIOLOGY

HT increases the stroke risk six-fold [11], with stroke being most common

CHAPTER 2

Autonomic Nervous System Dysfunction and Risk of Stroke

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Abstract: Cerebrovascular disease is predicted to remain the second leading cause of mortality reaching almost eight million annual deaths by 2030. Cerebral arteries are innervated and are therefore potential targets for autonomic nervous system dysfunction. In particular, a dynamic baroreflex mediated sympathetic modulation of cerebral blood flow has been demonstrated, confirming the role that the autonomic nervous system exerts on cerebral flow regulation. Moreover, it has been shown that the vagus nerve may influence neuro-inflammation therefore producing an inflammatory mediated vascular damage in case of dysfunction. Dynamic interactions between cerebral blood flow and the autonomic nervous system activity are therefore important and can be analyzed by studying the rhythms that characterize both cerebral blood flow, blood pressure and heart rate. With this regard, variability analysis performed together with techniques that investigate cerebral blood flow distribution and together with functional evaluation of the brain could provide new insight on the role played by the autonomic nervous system in the progression of cerebral vascular disease.

Keywords: Autonomic nervous system activity, Baroreflex function, Blood pressure variability, Heart rate variability, Neuro inflammation, Parasympathetic activity, Stroke, Sympathetic activity.

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INTRODUCTION

Since the last three decades, stroke remains the second most common cause of mortality [1] and recently, it has become the third leading cause of global disease burden if we consider disability-adjusted life years [2]. Cerebrovascular disease is predicted to remain the second leading cause of mortality reaching almost eight million annual deaths by 2030 [3]. Hypertension and aging are the most important risk factors for stroke. Both of them share the presence of important alterations of the activity of the autonomic nervous system. In particular, there is a clear evidence now that an increase in the activity of the sympathetic nervous system plays an important role not only in the development of hypertension but also of the related organ damage [4]. In addition to this, it is becoming more and more evident that autonomic nervous system dysfunction does not play an ancillary role in cardiovascular disease but is able to promote atherosclerosis and vascular remodeling [5 - 7]. So far emphasis has been placed on the effect of stroke on autonomic nervous system and not on the role that an alteration of autonomic nervous system activity could play in the genesis of strokes. The issue is important as autonomic nervous system dysfunction is a common condition shared by many diseases. Moreover, evidence is accumulating indicating that the autonomic nervous system plays an important role in the dynamic regulation of cerebral blood flow that has to adapt continuously to changes in blood pressure. The aim of this review is therefore to discuss the actual evidence on the influence that the autonomic nervous system activity has on cerebral blood flow and on the possible effect of an alteration of the autonomic nervous system activity on the cerebral circulation.

Stroke Subtypes

Strokes can be subdivided into three subtypes [8]: 1) ischemic strokes, caused by an occluded vessel, (80% of strokes), 2) intracerebral hemorrhages (15% of all strokes), and 3) subarachnoid hemorrhages (5%). In humans ischemic strokes occur most often in the brain region supplied by the middle cerebral arteries which are relatively large vessels arising from the circle of Willis. Occlusion of one of these arteries produces a large area of ischemic injury and neuronal death. Ischemic strokes can be further subdivided into: large artery atherothrombotic

strokes, (40% of strokes), lacunar strokes (20% of stroke) that occur when small intracranial arteries are occluded (Fig. **1**). In 20% of the patients, strokes are caused by a cardiac emboli lodging in a cerebral artery and originating from the heart and much more rarely from the aorta, other large arteries or the venous side of the circulation [9]. Additionally, hemodynamic strokes are the result of cerebral hypoperfusion in the absence of a clot nor emboli.

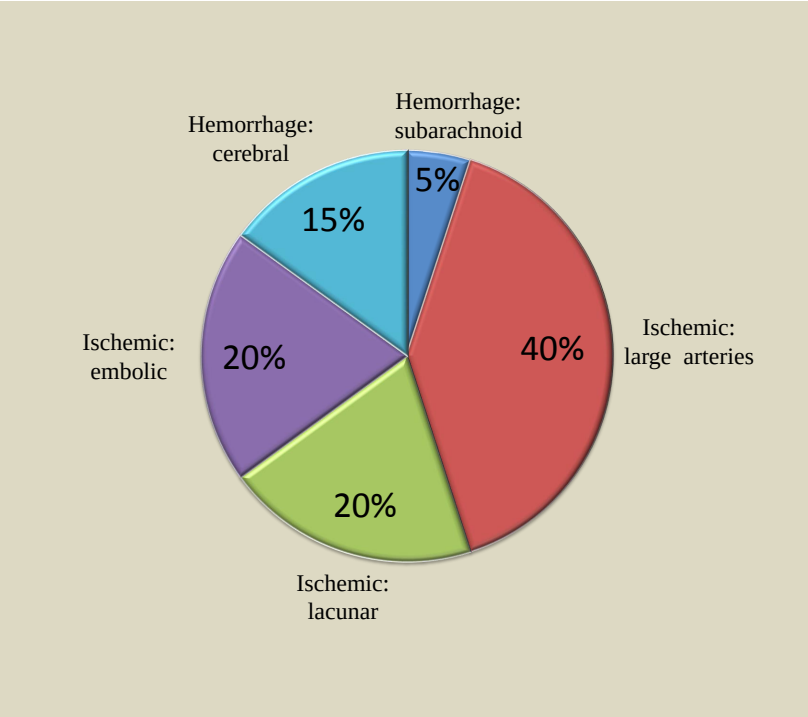

Fig. (1). Percent distribution of different types of stroke.

Cerebral Blood Vessels Innervation

Cerebral arteries are innervated and therefore they are potential targets for autonomic nervous system (ANS) dysfunction. Two types of cerebral vessels innervation are distinguished [10]. Extrinsic innervation: the pial arteries on the surface of the brain receive innervation from the peripheral nervous system. The majority of these nerves arise from the superior cervical ganglion, although a small percentage of nerves also arise from the sphenopalatine, otic and trigeminal ganglia [11]. Intrinsic innervation: the parenchymal arterioles supplying the cerebral cortex receive innervation from within the cerebral parenchyma [12] *i.e.*

CHAPTER 3

Stroke and the Immune System: Therapeutic Targeting of Toll-Like Receptors

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Abstract: Local and systemic inflammatory responses have been shown to play an important role in post-stroke damage. Recent studies suggest that the innate immune cells contribute to stroke-induced brain injury by activating an inflammatory response that further increases local ischemic damage. Innate immune signaling, *via* Toll-like receptors (TLRs), has been shown to be involved in several neuropathological processes. This chapter summarizes the current knowledge concerning the involvement of TLRs in acute ischemic brain injury. In particular, the therapeutic role of TLR2 and TLR4 antagonists will be discussed. Moreover, since TLR3 stimulation could play a beneficial role through the production of anti-inflammatory molecules, including I type interferons (IFNs), the potential benefits of TLR3 agonist administration to counteract stroke-related inflammation will be also focused.

Keywords: DAMPs, IFNα/β, Immunomodulators TLR-targeting, Inflammatory Responses, Innate Immunity, Ischemia/Reperfusion, Pro-inflammatory Cytokines, Stroke, TLR Agonists/Antagonists, TLRs.

1. INTRODUCTION

Brain injury from stroke represents the most common cause of death following

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ischemic heart disease [1, 2], the leading cause of permanent adult physical disability, and the second cause of dementia in adults worldwide [3, 4], despite the considerable variability between countries and regions, partly explained by differences in environmental risk exposure, lifestyle, genetic predisposition, stroke management factors, and different statistical methods used [5]. Strokes is a heterogeneous group of vascular disorders that can be caused both by sudden and focal interruption of cerebral blood, that leads to ischemic brain damage, or by a cerebral vessel rupture, which causes hemorrhagic injury. Ischemic stroke can be thrombotic, when a blood clot, the so-called thrombus, is generated in an artery that supplies blood to the brain, or embolic, when a blood clot travels through the bloodstream to an artery in the brain. Hemorrhagic stroke refers to the two main types of intracerebral and subarachnoid hemorrhage. Despite the progresses made in improving the care of stroke patients, the therapeutic options for acute and post-acute stroke remain limited. Stroke-related focal and systemic inflammation has recently been shown to play an important role in cerebral ischemic and hemorrhagic injury, due to the significant interaction existing between the immune system and nervous system [6, 7]. In addition, systemic inflammatory status at the onset of ischemic or hemorrhagic strokes, has been shown to be a key determinant of mortality and long-term prognosis [7 - 9]. This inflammatory reaction is mediated by various molecules, such as chemokines and pro-inflammatory cytokines [10] produced by several immune cell types, glial cells and neurons [11]. The immune system has been classically divided into innate and adaptive immunity, with distinct roles and functions. In contrast to previous hypotheses considering the CNS as fully isolated from the immune system, most recent studies have shown that the CNS is in dynamic bidirectional communication with the peripheral immune system across the blood–brain barrier (BBB) [12]. As a result of the stroke, the well-balanced interaction between CNS and immune system can be affected, and a few minutes after ischemic events, innate immune responses become active, inducing both beneficial and adverse effects on the disease evolution. In fact they are capable of promoting both necrotic cell clearance (tissue repair), and the initiation and amplification of post-stroke inflammation, that further increases the extent of brain injury [13, 14]. According to Yin and Yang effects of innate immunity, unspecific immune suppression or activation can be harmful, whereas the most promising therapeutic

approach could be represented by the specific inhibition of the detrimental effects of the immune response, without affecting the beneficial immune-mediated processes, mainly consisting in tissue regeneration [10]. Therefore, stroke-induced immune responses should be carefully modulated. Taking into consideration the complex events occurring before and post stroke, innate immune response could be manipulated by using immunotherapeutic compounds able to limit the stroke-induced damage and to stimulate repair processes caused by the injury.

2. INNATE IMMUNE CELLS AND THEIR REGULATION IN THE CNS

The innate immune system is considered as the first line of host defenses against invading microorganisms, malignant cells, and viruses. Innate defense mechanism is mediated by phagocytes, including basophils, dendritic cells, eosinophils, Langerhans cells, mast cells, monocytes, macrophages, and cytotoxic cells (neutrophils, and NK cells). In addition, most innate immune cells, such as dendritic cells, macrophages, Langerhans cells and B cells, mediate cellular immune responses by acting as antigen presenting cells and also producing cytokines involved in the activation, proliferation and growth of other immune cells. Despite the CNS is considered an immune-privileged site, it is continuously monitored by resident and blood-borne immune cells. Current data show that CNS and immune cells interact with each other in both physiological conditions and pathological events [15, 16]. Immune responses in healthy brain are mediated by resident immune cells, including parenchymal microglia, astrocytes and endothelial cells that contribute to maintain homeostasis and to provide neuronal protection [17 - 23]. Microglia are considered hematopoietic-derived brain-resident macrophages capable of self-renewal without requirement of replenishment, unlike other tissue-specific macrophages, which are initially released from the bone marrow as immature monocytes, also called bone marrow-derived cells (BMDCs), and recruited from the circulation after stroke [25]. Microglia are ramified brain macrophages, capable of responding appropriately to injury signals released by damaged cells through the production and release of cytokines that promote the phagocytic clearance of apoptotic cells and cellular debris and that attract other immune cells to the injury site in a stimulus-dependent way [24]. Pro- and anti-inflammatory cytokines can polarize microglia into two distinct activation states. In the pro-inflammatory status, they produce

CHAPTER 4

Intracerebral Bleeding and Oral Anticoagulant Therapies: Clinical Relevance and Management

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Abstract: Vitamin K antagonists such as warfarin and acenocumarol are the most widely used oral anticoagulants. Their clinical indications include both stroke prevention and prophylaxis and treatment of venous thromboembolism. Intracerebral hemorrhage (ICH) is the most important side effect of anticoagulant therapy accounting for almost 20% of all ICH. Non vitamin K anticoagulants or direct oral anticoagulants (DOACs) have been recently introduced in clinical practice due to their practical advantages over VKA. They are at least as effective as warfarin in the management of thromboembolic diseases and in the thromboprophylaxis of non-valvular atrial fibrillation, moreover, they have a more favorable safety profile. The present chapter will focus on vitamin K antagonists and DOACs mechanisms of action, on their pharmacokinetics and pharmacodynamics, and on the relative risk of bleeding during treatment.

Keywords: Anticoagulation reversal, Anticoagulants, Bleeding risk, Intracerebral hemorrhage.

Anticoagulant drugs are the cornerstone of prevention and treatment of thromboembolic diseases. Vitamin K anticoagulants (VKA) have been used for over six decades [1 - 3] in patients with atrial fibrillation, in patients with mechanical valve prosthesis and in patients with venous thrombosis.

Novel target-specific oral anticoagulants, known as “direct oral anticoagulants” (DOACs) or “non - vitamin K oral anticoagulants” (NOACs) have become

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available for various indications in the last few years, and their use is rapidly spreading. The number of patients receiving anticoagulant drugs is expected to double by 2050, because of aging and because of the increasing indications for their use.

Prospective controlled trials have demonstrated that in atrial fibrillation DOAC are associated with a similar or even increased reduction of thromboembolic events compared with VKA [4 - 8]. Furthermore, major bleeding complications, such as intracranial hemorrhage and fatal bleeding, occur less frequently with DOAC than with VKA. Nonetheless, concerns regarding the absence of specific reversal agents in case of life-threatening bleeding exist and guidelines for the management of bleeding complications are actually based on pathophysiological rationales and pre-clinical studies.

This chapter will focus on the following sections:

1. VKA-associated cerebral hemorrhages
2. DOACs for stroke prevention: update on intracranial bleeding risk
3. Reversal of DOACs activity and management of bleeding complications

1. VKA-ASSOCIATED CEREBRAL HEMORRHAGES

Anticoagulant-associated intracerebral hemorrhages (ICH) have been reported accounting for almost 20% of all ICHs, most of them being VKA-ICHs [9 - 11]. Nowadays, it remains unclear whether VKA should be considered as a cause or as a risk factor for ICH. Both experimental and clinical observations have demonstrated that VKA-ICHs are more severe than other ICHs, although the mechanism by which VKA worsen the severity of ICH is not completely understood [12, 13].

1.1. Warfarin: Mechanism of Action and Main Characteristics

Despite the availability of newer drugs, warfarin is still the most widely used oral anticoagulant. From a chemical point of view, warfarin is a racemic mixture of enantiomers, with the S enantiomer being recognized as the most effective of the racemic mixture. Warfarin acts as a powerful inhibitor of the vitamin K epoxide reductase (VKORC1), a liver enzyme required for the synthesis of many

coagulation factors such as FVII, FII, FIX, FX and the natural anticoagulant protein C and protein S (Fig. **1**) [14].

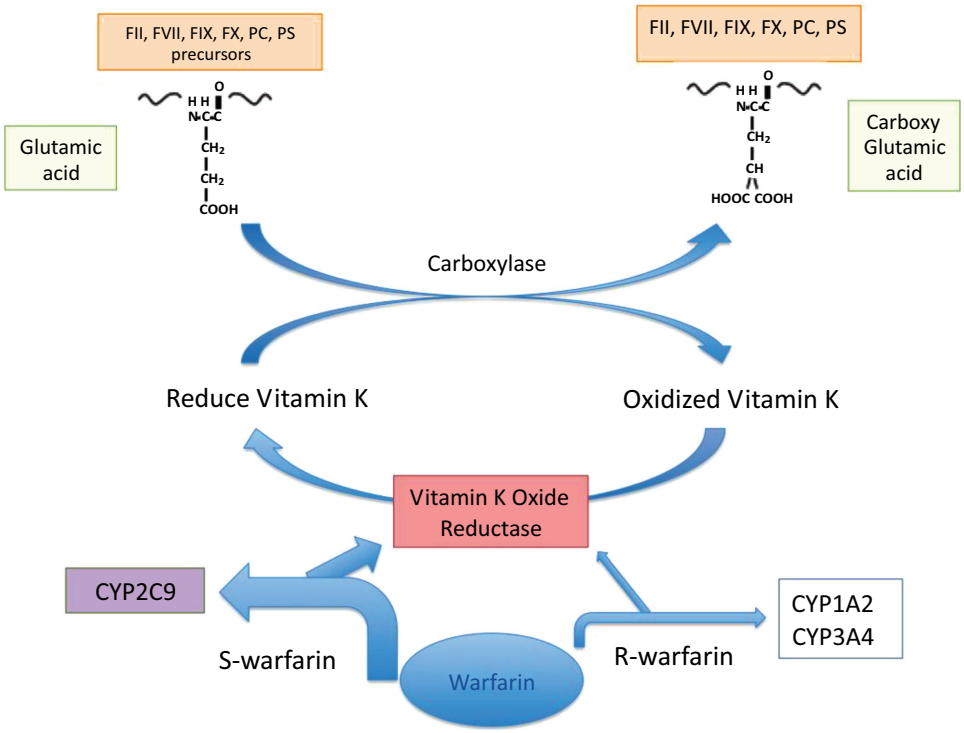

Fig. (1). Mechanism of action of warfarin: it inhibits the synthesis of vitamin K dependent coagulation factors acting on VKORC1, and it is metabolized by liver cytochromes.

VKAs produce their anticoagulant effect by interfering with the cyclic interconversion of vitamin K and its 2,3 epoxide (vitamin K epoxide), thereby modulating the beta-carboxylation of glutamate residues (Gla) on the N-terminal regions of vitamin K-dependent proteins. The vitamin K-dependent coagulation factors II, VII, IX, and X require beta-carboxylation for their procoagulant activity, and treatment with VKAs results in the hepatic production of partially carboxylated and decarboxylated proteins with reduced coagulant activity. Carboxylation is required for a calcium-dependent conformational change in coagulation proteins that promotes binding to cofactors on phospholipid surfaces. In addition, the VKAs inhibit carboxylation of the regulatory anticoagulant proteins C and S.

CHAPTER 5

Cerebral Collateral Circulation in Acute Ischemic Stroke: Translational Evidence for Outcome Prediction and Modulation Strategies

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Abstract: Cerebral collateral circulation is a subsidiary vascular network, which is dynamically recruited after arterial occlusion, and represents a powerful determinant of ischemic stroke outcome. Although several methods may be used for assessing cerebral collaterals in the acute phase of ischemic stroke in humans and rodents, they are generally underutilized. The assessment of collateral status in acute stroke patients may improve patient selection and maximize benefit-to-risk ratio of acute recanalization therapies. The systematic assessment of collaterals in experimental stroke models may be used as a "stratification factor" in multiple regression analysis of neuroprotection studies, in order to control the within-group variability. Exploring the modulatory mechanisms of cerebral collaterals during acute ischemic stroke may promote the translational development of therapeutic strategies for increasing collateral flow and directly compare them in terms of efficacy, safety and feasibility. Collateral therapeutics may have a role in the hyperacute (even pre-hospital) phase of ischemic stroke, prior to recanalization therapies.

Keywords: Acute ischemic stroke, Cerebral collaterals, Collateral therapeutics, CT angiography, Experimental stroke models, Infarct size variability, Ischemic penumbra, MRI angiography, patient selection, Recanalization therapies.

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INTRODUCTION

Cerebral collateral circulation is an auxiliary vascular network that is dynamically recruited after arterial occlusion and has the potential to provide residual blood flow to ischemic tissue. During acute ischemic stroke, cerebral collateral flow is highly variable among different individuals and is emerging as a strong prognostic factor both in unselected stroke patients and in patients treated with intravenous rtPA or endovascular recanalization therapy [1]. Experimental stroke models may be fundamental for a better understanding of the adaptive and modulatory mechanisms that regulate cerebral collateral circulation. Researching this further may lead to the translational development of new stroke therapies centred on collateral flow modulation in the hyperacute stage of ischemic stroke, prior to recanalization therapies [2].

Cerebral Collateral Circulation in Humans and Rodents

Many similarities can be found between humans and rodents cerebral collateral circulation. In both species the circle of Willis represents a compensatory system to rapidly redistribute blood flow in case of occlusion of cervical arteries. Moreover in both humans and rodents, each cerebral artery ramifies along the cortical surface to form a pial arteriolar network, creating anastomotic connections among different vascular territories, known as leptomeningeal anastomoses (LMAs). LMAs are mostly developed between cortical branches of middle cerebral artery (MCA) and ACA or posterior cerebral artery. When proximal occlusion of a cerebral artery occurs, dynamic blood flow diversion through these anastomoses might provide residual (retrograde) blood flow to the cortical surface of the occluded artery territory, distally from the occlusion (Fig. **1**). However there are also some notable differences in the cerebral collateral circulation anatomy in the two species. The circle of Willis in rodents does not include the anterior communicating artery and the proximal segments of anterior cerebral arteries (ACA) converge to form one single median artery called Azigos ACA. Another difference between the two species is that in rodents, the pterygopalatine artery originates from the proximal internal carotid artery (ICA) providing additional extracranial collateral connections between external carotid

artery and ICA *via* many arterial branches to facial, orbital and meningeal districts.

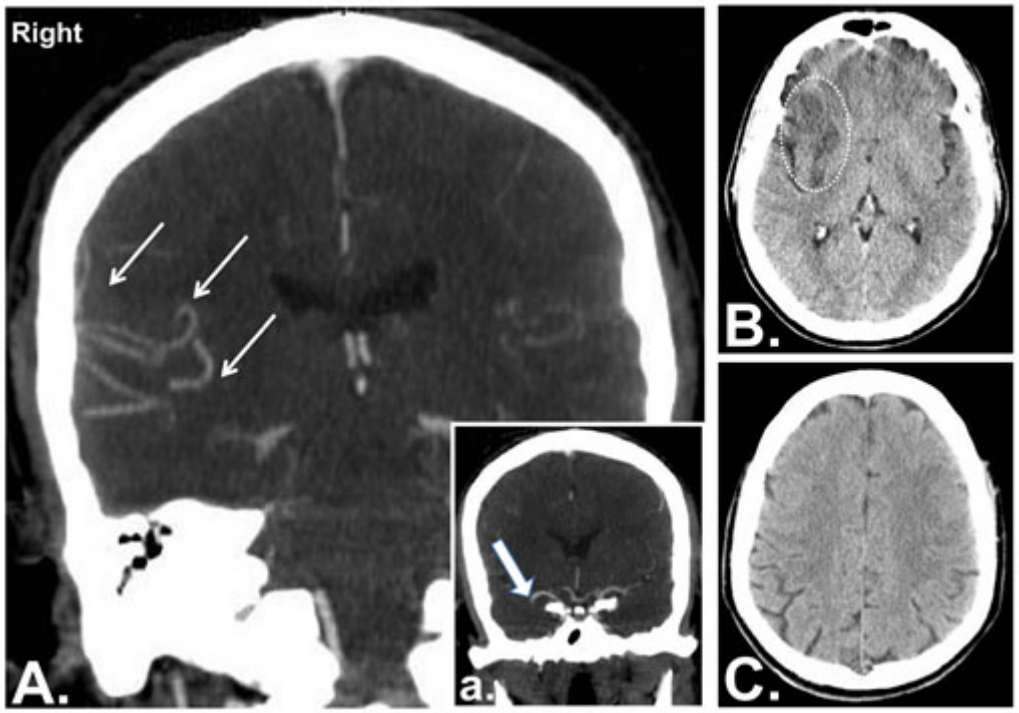

Fig. (1). Clinical imaging of cerebral collaterals during acute ischemic stroke using CT-angiography. Collateral vessels (**A**. small arrows) are visible in the right hemisphere. These vessels have been recruited after acute right MCA occlusion (**a**. large arrow). This patient was treated with intravenous thrombolysis and developed a small subcortical lesion (**B**), while the entire cortical territory was intact (**C**).

Assessment of Cerebral Collateral Flow in Acute Stroke Patients

The anatomy of cerebral collaterals in acute stroke patients can be assessed using different diagnostic modalities (Table **1**) such as conventional digital subtraction angiography (DSA), CT angiography (CTA) or MR angiography (MRA). Functional performance can be studied through tissue perfusion evaluation *via* CT and MR perfusion techniques (PCT and PWI). At present, there is no agreement in clinical practice on which imaging should be performed, when after stroke and which patients would benefit most from cerebral collateral imaging. Recent studies have investigated the role of the circle of Willis in the development of collateral flow in ICA obstruction. [3, 4]. These studies were based on

CHAPTER 6

Life in the Penumbra with the BRODERICK PROBE®

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Abstract: The penumbra is an area of living brain tissue immediately surrounding the necrotic core of an ischemic or thrombolytic stroke. The penumbra may remain viable several hours post-stroke due to blood flow from collateral arteries. Thus, this area of peri-infarct tissue is a therapeutic target for post- stroke and neuroprotective treatment modalities. Due to the fact that the ratio of viable to non-viable tissue decreases with time, Factor Xa and Factor II inhibitors such as enoxaparin and thrombolytics such as recombinant tissue plasminogen activator (r-tPA) must be administered immediately for optimal, synergistic treatment outcomes. The Broderick Lab is the first to study penumbral brain neurochemistry after causal acute ischemic stroke (AIS) by middle cerebral artery occlusion (MCAO) *in vivo*, as well as to comprehend the effects of enoxaparin (Lovenox®) and reperfusion *via in vivo* biochip nanotheranostics actually imaging the penumbra and its surrounding tissue intravascularly. Indeed, using Neuromolecular Imaging (NMI) with BRODERICK PROBE® nanobiosensors, animals were studied as their own control and each side of the brain was imaged *in vivo*, online, and in real time. NMI is a technology that uniquely images the baseline state of subjects before a disease state occurs, thereby establishing an intra-subject control

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model. In the same subject, with no gliosis, both the infarcted and peri-infarcted regions were imaged before, during, and after enoxaparin administration. Such imaging is available only with NMI nanobiosensor technology. In fact, with this new NMI nanobiosensor technology, the specificity of comparing baselines during drug and disease states is high because of the ability of NMI to compare baselines in thousands of previous studies of prescient and non-prescient mammals *in vivo*. Concurrently, Dual Laser Doppler Flowmetry (DLDF) was used to monitor cerebral blood perfusion. The results of this study demonstrate that, using intra-subject studies online (a) NMI profiles for dorsal striatum in basal ganglia are baseline values, ipsilaterally and contralaterally. (b) Diminished cerebral blood perfusion from AIS produces a significant increase of DA and 5-HT neurotransmitter concentrations, as well as associated metabolites and precursors, in motor neurons. (c) Enoxaparin alleviates oxygen deficiency by enhancing blood perfusion and reduces DA-induced brain trauma, enabling brain repair and regeneration. (d) Enoxaparin increases 5-HT release from motor neurons within the ipsilateral, lesioned hemisphere, as well as in the contralateral, non-lesioned hemisphere, particularly during the reperfusion stage. This serotonergic effect demonstrates the potential use of enoxaparin as an antidepressant, which would be clinically relevant for treating the depression that oftentimes is co-morbid with AIS. (e) The area of post-stroke infarcts is significantly reduced upon reperfusion; and (f) Cerebral blood perfusion is augmented in a compensatory manner by both enoxaparin therapy and reperfusion within both hemispheres, particularly the contralateral hemisphere. Thus, this research demonstrates the efficacy of enoxaparin in preserving the viability of the penumbra in stroke victims and supports consideration of the combined use of enoxaparin with r-TPA in standard stroke treatment protocols in order to harness the brain's intrinsic repair system. Moreover, these studies demonstrate the power of NMI nanotechnology in conjunction with BRODERICK PROBE® theranostic nanobiosensors to reliably study the intricacies of stroke in order to develop further neuroprotective treatments and allow personalized medicine to be realized.

Keywords: Anti-platelet, Brain attack, Brain repair, Cerebral blood perfusion, Enoxaparin, Imaging, Infarct, Intravascular, Ischemia, Lovenox®, Micro-circulation, Nanobiosensors, Nanodiagnostics, Nanotheranostics, Nano-therapeutics, Neuromolecular Imaging, Occlusion, Peri-infarct, Personalized medicine, Point of care, Reperfusion, Sensors, Stroke, Surgery, Theranostics, thrombolytic, Tissue imaging.

INTRODUCTION

"Umbra," "penumbra," and "antumbra" are Latin terms used to describe different eclipse relationships between the earth and the moon. "Umbra" is Latin for shadow and can either describe a total or partial planetary eclipse caused by the inner core of Earth's shadow blocking all sunlight from reaching the moon. An umbra generates a dramatic effect with a total lunar umbra creating a blood moon and a partial umbra casting a bite-like lunar shadow on the moon's surface. Similarly, an "antumbra" creates a striking appearance as the total circumference of the earth is encompassed by the luminous body of the moon, thereby creating a glistening, ring-like lunar eclipse. In contrast, a "penumbra" describes the moon falling into the outer, annular shadow of the earth, which results in a subtle darkening of the moon's exterior that is often missed by the naked eye. [1]

In medicine, penumbra refers to the living neural tissue encircling an infarcted core secondary to ischemic stroke. This viable peri-infarct region is visually analogous to the circular shadow left by a penumbral eclipse. The penumbra is literally a region of brain suffering from a dimming of its former vitality as a result of arterial occlusion. In this shadow zone, components of the ischemic lesion tentatively remain alive. However, the viability of penumbral tissue decreases with time if it remains in a prolonged ischemic state. In contrast, the necrotic core encompassed by the tenuously living penumbra is analogous to the umbra. Indeed, the necrotic core has already been muted by total cell death. Thus, the penumbra is an effective therapeutic target for the prevention of additional neuronal cell death after stroke. Due to the fact that the ratio of viable to non-viable penumbral tissue decreases with time, clotting factor inhibitors such as enoxaparin and thrombolytics such as recombinant tissue plasminogen activator (r-tPA) must be administered immediately for optimal treatment outcomes. Fig. (**1**) shows an image of a thrombus occluding an artery that can result in ischemia with viable penumbral tissue.

Indeed, enoxaparin (Lovenox®) is a first-line anti-platelet and anti-thrombotic agent that inhibits Factor Xa and Factor II in the clotting cascade. Enoxaparin is a low molecular weight heparin that impedes the formation of clots, reduces brain edema [2], reduces cerebral infarcts [3], restores both cognitive and motor

CHAPTER 7

On the Influence of Normalization Strategies for Perfusion MRI in Acute Stroke

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Abstract: Normalization of magnetic resonance images with a given reference is a common preprocessing task which is rarely discussed. We review and address this question for a specific neuro-imaging problem of practical huge interest. We investigate the influence of the location of region of interest used for normalization of perfusion maps obtained with perfusion magnetic resonance imaging in the framework of the study of acute stroke. We demonstrate that a slice by slice normalization based on the whole hemisphere strategy optimally reduces the variability of the predictive value of the different perfusion maps. Interestingly, this is obtained for all the tested perfusion maps both from numerical simulation of perfusion MRI and from perfusion maps of real patients through a Neyman-Pearson detection strategy. These are important results to ease the quantitative assessment of stroke lesion from perfusion MRI on cohorts of patients. The proposed methodology could easily be transposed to other medical imaging problems where normalization of images is necessary.

Keywords: Acute stroke, Image processing, Ischemic penumbra, Ischemic stroke, Medical Imaging, MRI, Neuroimaging, Normalization, Perfusion maps, Perfusion MRI.

INTRODUCTION

Perfusion-weighted imaging (PWI) is a magnetic resonance sequence increasingly

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used in the acute stroke setting. In its quantitative version, as pioneered in [1], maps of Cerebral Blood Flow (CBF), Cerebral Blood Volume (CBV), Mean Time Transit (MTT) and Time To Peak (TTP) can be derived noninvasively by dynamic imaging of a bolus injection of gadolinium contrast agent and subsequent data analysis from the time course of the tracer in both tissue and middle cerebral artery and deconvolution as shown in Fig. (**1**) for two human patients. Although used in clinics, there are still unsolved open questions on the determination of the optimal processing for the quantitative extraction of hemodynamic parameters. This is highlighted when PWI is compared to positron emission tomography (PET) imaging [2 - 5]. As stressed in [6], this is especially true for acute stroke because the images are of rather low quality due to emergency of the clinical context and the risk of possible movement of the patient. Because the method is inherently sensitive to vascular delays and dispersion effects, these maps have to be normalized to facilitate inter-individual quantitative evaluation [7 - 9]. Normalization can be considered in this context at two levels. Temporal normalization by deconvolution of the arterial input function [10, 11] and spatial normalization by division with hemodynamic values taken in a reference area. Please note that spatio-temporal normalization has also recently been introduced in [12]. In this chapter, we deal with spatial normalization. This normalization is usually done in the contralateral hemisphere unaffected by the stroke lesion. However, there are multiple ways of performing this normalization [13 - 20]. Spatial normalization by division of hemodynamic parameters from the contralateral hemisphere has been done for stroke with mirror ROI of the same slice [13, 14], with contralateral white matter of the whole hemisphere [15, 16], with mean white matter of a single slice [17] with contralateral voxel [18, 19] or with average in grey matter [20]. To the best of our knowledge, however, no comparison of various possible methods for this spatial normalization has been undertaken so far. In this work, we compared nine different normalization strategies in four different perfusion hemodynamic parameters (TTP, MTT, CBF, and CBV). This includes three normalization levels: to a reference slice (RS), to a volume (V) or slice by slice (SS) as shown in Fig. (**2**).

In addition, the region of interest for normalization can be chosen in the white matter (WM), can be taken as the whole contralateral hemisphere (WH), or can be

defined as the contralateral "mirror" volume of the diffusion mask lesion (DIFF) as illustrated in Fig. (**3**).

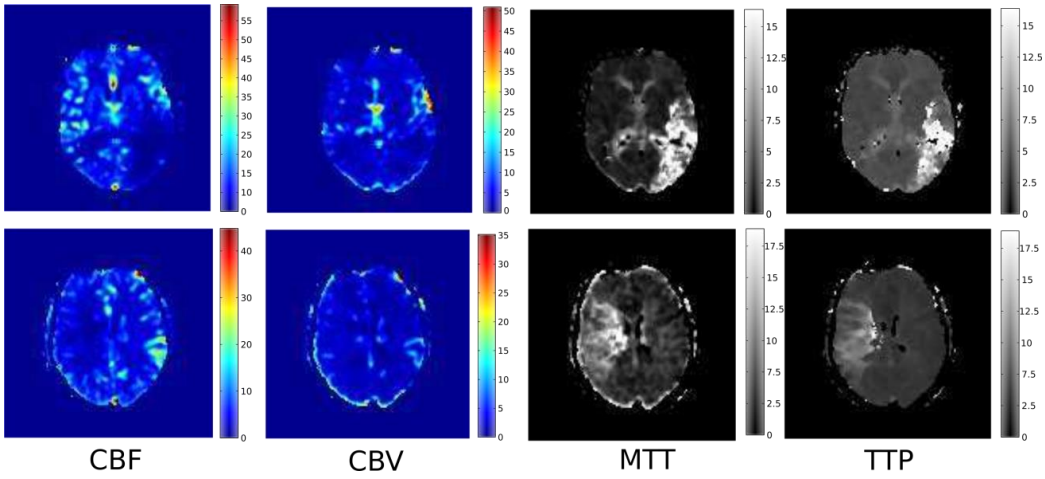

Fig. (1). Examples of perfusion parametric maps for two patients (one patient per line) with ischemic stroke. CBF and CBV are expressed in arbitrary units and, MTT and TTP in seconds. One can observe that the value ranges are not the same between the two patients.

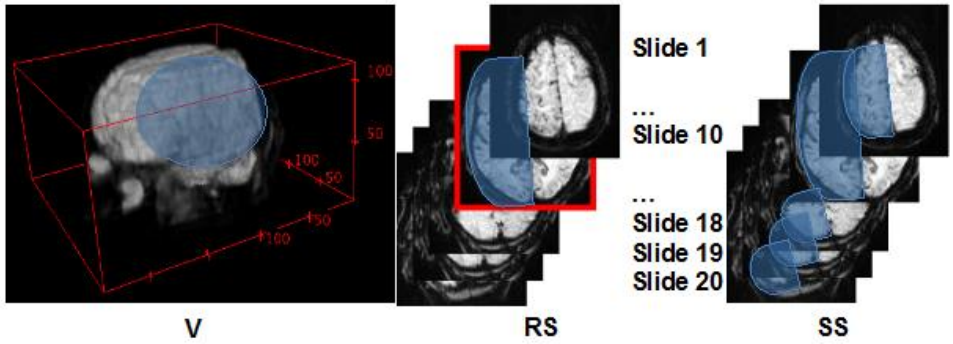

Fig. (2). Various levels tested for the normalization of perfusion parametric maps. The normalization is made using a unique reference slice just under the ventricle (RS), the entire contralateral volume (V) or slice by slice (SS).

To confront the performance of these nine combinations of normalization levels and normalization ROI, the comparison endpoint was the variability of the prediction of the final lesion after thresholding the parametric perfusion hemodynamic maps. Several studies have performed comparisons of various PWI post-processing methods using a variety of perfusion maps, normalization

CHAPTER 8

Genetic Causes of Ischemic Stroke

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Abstract: The pathogenesis of ischemic stroke remains unknown but a better knowledge of its pathogenetic mechanism may help us in identifying more effective therapies to reduce the the disease burden. Family and twin studies support the role of genetic factors in stroke pathophysiology. A number of monogenic conditions presenting with stroke have been described. They account for only a small proportion of strokes, but it is believed they are underestimated and the study of these diseases may provide insight in the pathogenic pathways of stroke, given also the existence of animal models in which examine disease mechanisms. However, in most cases, stroke is believed to be a multifactorial disorder. A number of genetic association studies using the candidate gene approach have failed in demonstrating reliable associations between stroke and genetic variants. Although several molecular variants resulted from GWAS strongly associated with ischemic stroke risk, they account for only a small part of the risk of ischemic stroke. Moreover, the pathogenic significance of many of these genetic variants has yet to be determined by functional studies so that the significance of these findings in clinical practice has been limited. Interestingly, the studies conducted so far demonstrated that the majority of the identified genetic variants found were associated with specific stroke subtypes, supporting the hypothesis that distinct genetic architecture and pathophysiological mechanisms underlie specific stroke subtypes.

INTRODUCTION

Evidence from epidemiological and twin studies support a genetic predisposition to stroke occurrence and outcome [1 - 4]. Family history data suggest that genetic risk differ by stroke subtype, with greater familial associations for large-artery and

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small-artery (lacunar) than for cardioembolic stroke [5]. A number of monogenic diseases have been described as responsible for stroke. In some of these strokes may be a part of a systemic disorder and may manifest in late disease phases. However, they are considered rare diseases accounting only for a small percentage of strokes and the genetic contribution to stroke is believed to be polygenic [1, 2]. This case is much more complex since genetic contribution results from the interaction of multiple risk alleles with small effect and genetic factors could act at various levels by predisposing to common risk factors, or, alternatively, by a direct effect on stroke risk occurrence, infarct size and response to ischemic injury [6].

Several methodological difficulties have been highlighted in studies on stroke genetics. The late onset, the difficulty in collecting genealogical trees, the phenotypic heterogeneity, and the presence of confounding risk factors make it difficult to apply linkage-based methods [1, 2].

A number of pathways including hemostatic and inflammatory systems, homocysteine metabolisms, renin-angiotensin-aldosterone system have been explored applying candidate gene approach. Recently, developed advanced high throughput platforms such as genome-wide association studies (GWAS) and Next generation sequencing as well as bioinformatic approaches are considered promising in tools for identification of novel biological mechanisms that underlie the pathogenesis of cerebrovascular diseases. This effort might lead in the future to the development of preventive strategies and individual tailored treatments.

The purpose of this chapter is to provide an update on the most well characterised monogenic disorders associated with stroke and on the most recent advances on genetic variants associated with stroke.

MONOGENIC STROKE DISORDERS

Despite monogenic diseases are considered rare and believed to account for about 1% to 5% of all strokes, they are probably underestimated. They can be misdiagnosed simply because physicians may not include them in the differential diagnoses or they are difficulty recognised given the pleomorphic phenotypic spectrum in which stroke may be only a part of a systemic disorder [4]. However,

although rare, these diseases may have important implication in the comprehension of pathogenic pathways multifactorial stroke [5], given also the existence of animal models in which to examine the disease pathophysiology. Moreover, their identification and diagnosis is an important challenge for clinicians for the implementation of a correct management of these patients, including genetic counseling, preventative and therapeutic measures [5 - 8], since they are usually life-threatening or chronically debilitating diseases. A large number of single-gene disorders is well known as cause of stroke (Table **1**). Interesting progresses have been particularly reported in discovering new heritable entities underlying small vessel diseases.

Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) (OMIM 125310-600276)

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an autosomal dominant disease caused by mutations in the NOTCH3 gene on chromosome 19q12 encoding a trans-membrane receptor with an extracellular domain containing 34 epidermal growth factor repeats (EGFRs) with six cysteine residues [9, 10]. Particularly, CADASIL results from mutations, mostly missense although small in-frame deletions or splice-site mutations have also been reported, in exons encoding EGFRs (exons 2-24) altering the number of cysteine and leaving one unpaired. Although a mutation cluster in exons 3 and 4 has been reported, a wider variation in mutational spectrum has been described [11 - 13]. CADASIL is the most common mendelian disorder associated with stroke (estimated prevalence of about 2-4/100000 inhabitants) [14 - 16]. Clinical presentation is essentially characterised by four main clinical features, which are migraine, lacunar strokes, mood disorders and cognitive impairment. Migraine, usually with aura, which has been observed in 20- 40% of patients, is the presenting clinical feature in the 60-75% (onset around the 20s or 30s). The most frequent manifestation are recurrent subcortical ischaemic events (TIA or stroke), which are reported in 60-85% of patients (mean age at onset 46+9.7 years) [17 - 19]. Ischemic strokes are lacunar and occur in most cases in the absence of common cerebrovascular risk factors, although some risk factors such as smoking and high cholesterol level have been recognised to condition an earlier stroke onset [18, 19]. Ischemic events are

CHAPTER 9

Diagnostic Challenges: Cryptogenic Ischemic Stroke

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Abstract: Cryptogenic ischemic stroke defines the situation where the cause of the thromboembolic event is not evident. Atrial fibrillation (AF) is a possible cause of cryptogenic stroke, even if asymptomatic. AF is the most common arrhythmia, and its incidence increases with age. AF is associated with an increased risk of ischemic stroke and systemic embolism, independent of the type of AF and also of the symptoms caused by the arrhythmia. The thromboembolic risk is associated to some clinical variables like age, hypertension, heart failure, diabetes, previous stroke, vascular diseases and gender. The mechanism of thrombosis in AF is complex, and comprises blood stasis in the left atrial appendage, but also atrial and endothelial damage, alteration of the extracellular matrix, and activation of humoral factors with prothrombotic significance.

AF is often asymptomatic or "silent", as demonstrated in many clinical situations. Silent AF may be a frequent cause of otherwise unexplained stroke. The association between stroke and silent AF was demonstrated in many trials, especially in patients with an implanted device, like pacemakers, implantable cardiac defibrillators (ICD) and cardiac resynchronisation therapy (CRT) devices, where a reliable report of the arrhythmia is available. The best system to discover if a patient with cryptogenic stroke has AF is the continuous monitoring with an implantable loop recorder. However, the temporal relationship between AF and stroke is not often evident, so that the decision to prevent thromboembolisms in patients with silent AF should be based on the evaluation of risk with the appropriate score index. In the secondary prevention of stroke and transient ischemic attack (TIA), however, anticoagulation is generally indicated if a silent AF is demonstrated.

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Keywords: Atrial fibrillation, Cryptogenic stroke, Implantable defibrillators, Implantable loop recorder, Left atrial appendage, Pacemaker, Silent atrial fibrillation, Thromboembolic risk score, Thromboembolism.

Cryptogenic ischemic stroke (CIS) is defined as the situation where, after the examinations of the acute phase, including an electrocardiogram (EKG), a transthoracic and transesophageal echocardiogram, a 24 hour EKG monitoring, or a telemetric EKG recording, and after assessing the regular neurologic and neurovascular exams, the origin of the ischemic event is not evident.

Epidemiological studies document that between 50 and 60% of strokes are due to cardiovascular documented problems; among them about 15% are due to documented atrial fibrillation, but in about 25-40% of ischemic strokes, no etiologic factor is evident [1 - 5]. A portion of these cerebral ischemic episodes is often attributed to subclinic or silent atrial fibrillation (AF) as the etiologic cause, that is one or more arrhythmic episodes elapsed without symptoms before the index episode [6].

ATRIAL FIBRILLATION: PREVALENCE AND THROMBOEMBOLIC RISK

Atrial fibrillation is the most common sustained arrhythmia, with a prevalence of between 1 and 2% in the general population, increasing with age, reaching and exceeding 8% in subjects over 80 years of age. AF is associated, as an independent factor, to an increased risk of stroke. Stroke risk is about 5-fold in AF patients compared with controls [7].

The cause of ischemic strokes is AF in about 15-17% of all cases. It is relevant to point out that AF related strokes show a higher mortality and higher disability level than non AF related strokes [8].

It is well known that AF can be classified as *paroxysmal* (when spontaneously ceases, usually within 48 hours from onset, and anyway within 7 days), *persistent* (when it lasts more than 7 days, or can be interrupted with drugs or electrical cardioversion) and *long lasting persistent* or *permanent* (when it lasts a long period and/or is considered not possible to be further interrupted). In spite of what

is often considered, the thromboembolic risk does not depend on the type of AF, and, with some limitation that will be discussed later on, neither on the duration of episodes of paroxysmal AF [9, 10].

Conversely, the thromboembolic risk depends on a series of clinical variables, used to construct specific scores to evaluate the risk: the first score was the "$CHADS_2$" [11] (Fig. **1**), which considers congestive heart failure, hypertension, age, diabetes and previous stroke or TIA, the last scoring two points; more recently the "CHA_2DS_2-VASc" score [12] (Fig. **2**), where age > 74, with double score, the vascular peripheral or coronary disease, and female sex were added (female gender is considered only for more advanced age). As shown in Fig. (**2**), the CHA_2DS_2-VASc score is capable to stratify the risk even for the lowest levels, and in particular to identify, for subjects with score 0, a group of patients with a very low risk, similar to patients without AF.

Stroke Risk Factor	Score
Congestive Heart Failure	1
Hypertension	1
Age (> 75 years)	1
Diabetes	1
Prior **S**troke / TIA	2
Max Score	**6**

Fig. (1). $CHADS_2$ score for calculation of thromboembolic risk in non-valvular atrial fibrillation.

MECHANISM OF THROMBOSIS AND THROMBO-EMBOLI IN NON-VALVULAR ATRIAL FIBRILLATION

Non-valvular atrial fibrillation (NVAF) is defined as AF in the absence of significant mitral valve disease (stenosis) and mechanical prosthetic valves. The definition is not negligible, inasmuch as the mechanism of atrial thrombosis may be partly different in these situations comparing to NVAF.

CHAPTER 10

Emerging Concepts for Neuroprotection

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Abstract: Following an ischemic insult, the early damage associated with the energy defect gives rise to molecular events, which may occur even during reperfusion to sustain a progression of the damage in the ensuing hours or days. N*europrotection* refers to interventions that are supposed to beneficially interfere with the maturation of the ischemic damage. The post-ischemic molecular events embrace a huge variety of mechanisms. Each mechanism is entangled with others, to configure a pathogenic process that has the characteristics of a near-chaotic phenomenon. To add to the complexity, the ischemic process includes both mechanisms that fuel the pathogenic process promoting cell death, and mechanisms reflecting the effort of the organism to oppose the process.

Interventions aimed to oppose the maturation process activate brain repair and epigenetic mechanisms to promote survival pathways. Examples of potential neuroprotective approaches are: pre- and post-conditioning, hypothermia, and a number of drugs. All the treatments, however, that were proven to be effective in animal models, failed in clinical trials. No unique explanation accounts for the gap between laboratory animal and clinical studies.

Keywords: Acute ischemic stroke, Clinical trials, Experimental stroke models, Ischemic cascade, Ischemic conditioning, Mynocycline, Neuroprotection, Therapeutic hypothermia.

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INTRODUTION

In a classical experimental set up, 5 minutes of bilateral occlusion of the carotid arteries in the gerbil causes no apparent, immediate brain tissue damage. The animal survives the transient loss of blood flow to the entire brain without evident behavioral abnormality or structural tissue damage. Parts of the brain, however, eventually dye hours or days following the ischemic insult. Typically, following the 5-minute occlusion the animals develop a focal lesion in the CA1 section of the hippocampus. The lesion becomes structurally evident 3 or 4 days post ischemia. Remarkably, the pathogenic process that eventually leads to cell death occurs under conditions of normal cerebral blood flow (CBF), as the perfusion returns back to normal in a few minutes after the release of the occlusion. Two phenomena, therefore, characterize the 2-vessel 5-minute occlusion in the gerbil: 1) an interval of time during which there is no structural change before the damage eventually becomes apparent, 2-4 days following recirculation (*maturation phenomenon*); 2) a focal distribution of the damage in spite of the whole brain ischemia (*selective vulnerability*).

If the vessel occlusion lasts just 2-minutes, the animal survives to the insult without any apparent lesion to be observed in the ensuing days. The non-lethal 2-minutes occlusion, however, makes the animal tolerant to the 5-minute insult. Thus, performing a 2-minute vessel occlusion in the gerbil 2 days before a subsequent 5-minute occlusion makes the typically lethal insult ineffective. This is to say that a subliminal, or non-lethal, insult acts as a *preconditioning* stimulus, which makes the animal tolerant to ischemia (*ischemic tolerance*).

The findings (*maturation phenomenon*, and *preconditioning*) indicate that the ischemic insult triggers a number of changes, which have a progress over time. The changes seem to include mechanisms that promote the ischemic damage and, at the same time, mechanisms that counteract the insult. In addition, the *selective vulnerability* phenomenon clearly suggests that intrinsic tissue factors contribute heavily to the damage, as the same blood flow reduction (in terms of intensity and duration) causes tissue damage in certain areas of the brain and not in others. Severe hypoxia or hypoglycemia as well may produce brain injuries limited to specific brain areas, consistently with the notion that variables other than blood

flow, both local and systemic, are relevant in determining the vulnerability to the energy deprivation.

The report of the maturation phenomenon has boosted research in the field, as it became evident that ischemic damage is not just a sudden event causing necrosis. It can be a process triggered, but not sustained, by the initial ischemic insult. In the last three decades a huge amount of data and high number of reports have characterized the mechanisms associated with the ischemic insult. The findings all together depict a complex picture in which several variables interact in a conflicting mode. It is worth stressing that deleterious mechanisms may coexist with protective mechanisms. As a whole the post-ischemic event configures a sort of chaotic system, which is affected by both local and systemic variables. Dissecting the different components is an impossible task. We might grossly consider two conceptual frameworks to describe the ischemic pathogenic process, as suggested by experimental observations [1].

One aspect refers to the hemodynamic abnormalities, which cause a defect in supply of energy substrates to the brain. Following an ischemic insult, the mismatch between energy demand and supply from blood circulation is the main mechanism involved in causing an early damage and in triggering the maturation process. The initial mismatch is obviously related to the low residual blood flow. In absence of energy deposits, the brain tissue uniquely relies on continuous supply of oxygen and nutrients. Within minutes after ischemia loss of ATP results in decreased function of the ion pumps and consequent break-down of the ion gradient, which is the basic mechanism for the electrochemical force that drives the neuronal signaling. Under physiological conditions, a partial decrease of the electrochemical energy is restored by a transient increase of the local blood flow (*functional hyperemia*). During ischemia, the lack of fuel causes a sustained failure of the sodium pumps and sustained depolarization. Such a depolarization is characterized by near-complete break-down of the ion gradient across the membrane, loss of electrical activity and neuronal swelling (*cytotoxic edema*). The depolarization may spread to the surrounding naive tissue (*spreading depolarization*). Neuronal depolarization causes glutamate release. Both NMDA and AMPA/kainate receptors contribute to the excitotoxic neuronal response, which amplifies the initial energy mismatch. All these phenomena occur in the

CHAPTER 11

Pharmacological and Endovascular Recanalization Therapy

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Abstract: The major aim of acute stroke treatment is to save the hypoperfused cerebral parenchyma in order to minimize residual disability. Early recanalization of occluded arteries with thrombolytic therapy is the most efficient procedure for protecting the brain parenchyma which is not yet infarcted.

Intravenous administration of Alteplase (recombinant tissue-type plasminogen activator – rtPA) has proven to be effective in reducing disability at 90 and 180 days after stroke in patients treated within 4.5 hours from symptoms onset.

Recently, striking results have been provided by the endovascular treatment, unless in a selected population. The use of the stent-retrievers or direct thrombus aspiration is now a good option for acute stroke treatment giving the opportunity of an effective multimodal therapeutic approach.

Keywords: Actilyse, Acute therapy, Alteplase, Endovascular, Pharmacological, Recanalization, Stent retrievers, Stroke, Thrombectomy, Thrombolysis.

INTRODUCTION

The major aim of acute stroke treatment is to save the hypoperfused cerebral parenchyma in order to minimize residual disability in the medium and long term after the acute event. Therefore, the main goals of treatment in the acute phase of

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ischemic stroke concern two main aspects: 1. The attempt to bring the situation of arterial occlusion back to its previous condition of vessel patency, improving the supply of oxygen and glucose correlated to artery reperfusion; 2. To block dysmetabolic processes which, in an anaerobic environment, contribute to the increase in volume of the infarction of brain parenchyma. In the acute phase of stroke, vascular reperfusion and neuroprotection treatments should be practiced respecting the concept of maximum urgency of intervention. The scenario of the therapy offered also includes more invasive procedures requiring surgery. Very briefly, these interventions (*e.g.*: carotid thromboendarterectomy) are aimed at reducing the risk of early recurrence of stroke and preventing deterioration of the anatomical and clinical situation. Another range of surgical procedures (decompressive hemicraniectomy, placement of external ventricular deviation) aim at preventing clinical deterioration in the presence of intracranial hypertension due to the "mass" effect of the lesion

Early recanalization of occluded arteries with thrombolytic therapy is the most efficient procedure for protecting the brain parenchyma which is not yet infarcted. While lysis of the thrombus occluding the vessel is the immediate result that is pursued through this procedure, improvement in terms of clinical outcome is the final objective of such treatment. An earlier meta-analysis published in 2002, which analyzed the data of 2006 patients, confirmed the positive predictive role of recanalization in achieving a positive outcome after 3 months (OR: 4.43; 95% CI: 3.32 – 5.91), as well as in reducing death (OR: 0.24; 95% CI: 0.16 – 0.35) [1]. In the same meta-analysis, 24.1% of the patients showed spontaneous recanalization. However, the highest percentages of reperfusion were observed in the group of patients treated with mechanical thrombectomy (83.6%), followed by a combination of systemic and loco regional therapies (67.5%) and the intra-arterial procedure (63.2%). Several factors are associated with favourable recanalization. First of all, size and location of the thrombus: higher volumes of thrombus, or thrombosis of the large vessels of previous atherosclerotic stenosis, seem to be factors for resistance to thrombolysis, as well as involvement of the extra-cranial internal carotid, occlusions in the carotid artery or the T basilar artery [2, 3]. The status of pial collateral circulation is also a factor that affects the success of reperfusion of the artery [4]. Thrombolytic treatment can be delivered in a well-

defined time window, beyond which its effectiveness is significantly reduced at the expenses of safety [5]. Patient management in the hyper acute phase of stroke must provide a quick pathway leading to prompt treatment. In addition to drug therapy, recent findings have meant that therapeutic potentials can be increased by giving a positive presentation of using different devices to offer mechanical recanalization techniques to selected patients [6].

Systemic Thrombolysis

Intravenous administration of Alteplase (recombinant tissue-type plasminogen activator – rtPA) has proven effective in reducing disability at 90 and 180 days after stroke [5, 7]. However, the benefit of the drug tends to decrease significantly as time goes by, and the time window currently applied is 4.5 hours [8]. At first, Alteplase was administered within three hours from onset of symptoms because of evidence in previous studies such as NINDS, in which 38% of the treated patients reached a favourable outcome compared to 21% of the placebo group, with no significant increase in the risk of mortality [9]. ECASS III assessed the efficacy of the treatment by extending the time window to 4.5 hours. The main result of the study was the effectiveness of Alteplase compared to placebo (OR: 1.34; 95% CI: 1.02 – 1.76; number needed to treat [NNT]: 14), with no significant difference between the two groups regarding mortality and symptomatic haemorrhages [10]. Further evidence emerged from the SITS-ISTR observational study that confirmed data already presented in a previous randomized trial [11]. To date no evidence has emerged from literature concerning the efficacy and safety of rtPA between 4.5 and 6 hours. IST-3 is the most important trial that has taken this therapeutic window into consideration and has enrolled more than 3,000 patients [12]. In particular, the sub analysis of 1,007 patients treated within 4.5 and 6 hours has shown a significant difference between the groups of treated and untreated patients (47% *versus* 43%; OR: 1.31; but with a confidential interval across the line of 1 (95% CI: 0.89 – 1.93). A previous meta-analysis published in 2012 involved over 7,000 patients treated within 6 hours [7]. Globally, the results showed that thrombolytic treatment was superior to placebo (OR: 1.17; 95% CI: 1.06 – 1.29), with a net benefit for those who were treated within 3 hours. In fact, the patients treated between 3 and 6 hours did not benefit significantly from the treatment (OR: 1.07; 95% CI: 0.96 – 1.20). In conclusion, a recent meta-analysis

CHAPTER 12

Neuroregeneration after Stroke

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Abstract: Promoting tissue plasticity is a very important therapeutic approach to reduce post-stroke disability. The neurological damage occurring after stroke indeed is a consequence of disrupted brain connectivity circuits due to cellular degeneration and impairment of plasticity processes. Axonal degeneration is also invariably seen in remote brain structures that have neuroanatomical links to the ischemic area. Recovery from stroke is thus very much depending on the possibility to develop treatments able to halt the neurodegenerative process and to foster adaptive tissue plasticity. Due to the intricacy of the systems involved, therapies that foster endogenous repair processes in a spatially and time targeted manner are required. We here discuss the physiology of recovery processes occurring after stroke and the main strategies to foster compensatory neuronal networks aiming to reduce stroke-related disability.

Keywords: Axonal sprouting, Critical period, Growth factors, Inflammation, Ischemic stroke, Neural stem cell, Plasticity, Transplantation.

INTRODUCTION

Stroke is a highly disabling neurological disease representing, the third leading cause of death worldwide. Whatever the cause that triggers the occlusion of the brain artery, the inadequate blood supply initiates a series of pathophysiological events that, if not reversed quickly in time, results in irreversible damage of the brain tissue. Victims of stroke experience different clinical manifestations depending on the brain area(s) affected: symptoms might include hemiparalysis,

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impairment of speech, or loss of vision and many other neurological dysfunctions. Partial functional recovery can occur and depends on the entity of damage and on the spared neuronal networks that can take over the lost brain functions. Unfortunately of all stroke patients about 50% are left with a motor impairment, 30% are not able to walk and about 26% have a severe disability requiring care [1].

Following the acute and the subacute phase of stroke, that are characterized by acute injury mechanisms such as spreading depressions, excitotoxicity, calcium release, formation of free radicals, endoplasmic reticulum dysfunction and mitochondria failure, the chronic phase, lasting for months after ischemic insult, follows [2, 3]. Even though in this phase secondary injury mechanisms contribute to the progression of tissue damage, repair mechanisms begin to emerge. Inflammation, which begins few hours after the onset of ischemia, importantly shapes also this delayed injury phase. Early after stroke microglia and astrocytes are activated as well blood borne cells (*e.g.* neutrophils, lymphocytes and monocytes) are recruited to the brain by locally expressed cytokines, adhesion molecules and chemokines. Even areas remote from the site of injury, degenerate and induce an inflammatory response resulting in secondary damage and atrophy. Consequently, loss of function during stroke is on the one hand due to brain tissue loss in the infarct site and on the other hand due to neuronal dysfunction in the non-ischemic peri-ischemic zone and in more remote areas connected to the infarct core. In the months following stroke some neurological functions spontaneously recover [1, 4]. In fact it is essential to remember that ischemic tissue, and especially the peri-infarct zone, are also sites of active recovery processes. Many evidences support the concept that mechanisms underlying recovery and damage are not separate entities but often diverge in time or space and components involved in damage in the acute phase of stroke can, early after, contribute to recovery processes.

Indeed the transition from injury to repair is guided by mediators of the extracellular matrix, components of the excitatory pathway and from signals derived from activated inflammatory cells; the same players which have a key role in the initial neurodegeneration [5]. An example can be the N- methyl-D-aspartate (NMDA) receptors: in the acute phase their over-activation seems detrimental

while, in the chronic phase, the same receptor activation is important to stimulate neuroplasticity and protection against apoptosis. Further examples are matrix metalloproteinases (MMP): although MMPs are able to degrade the neurovascular matrix and to mediate damage of the blood-brain barrier contributing to neuronal death, oedema and haemorrhagic transformation [6] the same MMPs can at a later stage also promote plasticity processes such as remodelling of the neurovascular unit in the boundary of the lesion. Accordingly while inhibitors of MMPs in acute stroke have been shown to reduce tissue infarction, the same inhibitors worsen in sub-acute or chronic phases of stroke the outcome, since they inhibit plasticity processes [7].

These examples show that at the same time of degeneration restorative processes are initiated in brain tissue in response to cerebral ischemia. Recovery after stroke comprehends also central nervous system (CNS) reorganization. The multifaceted mechanisms such as vascular remodelling, neurogenesis and gliogenesis, axonal sprouting, dendritic arborisation modification, spine remodelling and cortical function relocation are often synergistic and tightly orchestrated in time. For succeeding in favourably manipulating neuroregeneration it is essential to study and comprehend in the minimal detail the pathophysiology of stroke. All too often broad pharmacological approaches have failed in triggering effective neuroregeneration since timing, location, duration or dosages were not properly set. In this chapter we review the main biological mechanism underlying regeneration and discuss how these processes can be fostered for eliciting neuroregeneration.

Reparative Processes after Stroke

Inflammation

Inflammation is a very important actor in the pathophysiology of stroke. Recent evidence suggests that the immune system is involved in all stages of the ischemic cascade, from the very acute vascular occlusion phase, or even before, to the ensuing tissue repair [8]. The past decade has witnessed a revolution in our understanding of CNS inflammation, in particular regarding the involvement of immune cells in CNS maintenance and repair [9]. Although an inflammatory

CHAPTER 13

Post-Stroke Rehabilitation

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Abstract: Stroke is one of the main cause of chronic disability in industrialized countries and, in 2010, it was among the top eighteen diseases that most frequently lead to "years lived with disability". All the quoted conditions decreased between 1990 and 2010, with the exception of the age-standardized rates for stroke. Caring addressed to post-stroke patients involves multiple challenges, due to stroke causes a wide range of impairments, among them motor skills deficits are the most common, even if should be enumerated other areas of impairment such as sensory, visual, swallowing, cognitive and language related. Rehabilitation provides the possibility of reducing the burden of disability and, nowadays, one of the most exciting areas of stroke research is the prospect to increase rehabilitation effectiveness, influence neurological recovery and, subsequently, impact clinical outcomes. In recent years, following the spreading of studies regarding neuroplasticity, innovative rehabilitative approaches have been based on reasonable and intriguing theoretical assumptions. The restorative methods represent significant tools for stroke rehabilitation; nevertheless, they become meaningless if not integrated within the context of a rigorously personalized care plan. A comprehensive rehabilitation plan should begin as soon as the stroke occurs. Management must be directed toward preventing functional deterioration, restoring lost abilities and functions, gaining compensatory strategies, suggesting environmental changes, increasing participation and consequently achieving the highest quality of life after the brain damage. The grade to which a rehabilitation program meets the challenge of improving dignity and independence of stroke survivors is assessable through the adoption of a systematic approach that relates the diseased-state and disability to outcomes of care. The following can be considered as stroke rehabilitation strongholds: (1) to provide a consistent rehabilitation plan throughout the acute, sub-acute, and chronic phases: stroke rehabilitation is a dynamic pathway starting following the

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symptoms onset and accompanying the patient in the care pathway until his going back to community; (2) to set goals based on the prediction of the single cases evolution (short, medium and long-term goals) and design an appropriate pathway: the first step for a well-conceived rehabilitation management is to establish appropriate setting addressed to the patient; (3) to provide a comprehensive, coordinated, interdisciplinary approach: since the strokes clinical manifestations are multidimensional, rehabilitation is best realized through the coordinated and well harmonized actions among the whole members of a specialized team; (4) to build up a tailored rehabilitative treatment.

Keywords: Action observation, Comprehensive approach, Individual rehabilitation project, Multidisciplinary, Neuroplasticity, Non Invasive brain stimulation, Prognosis, Robotic device, Setting, Tailored treatment, Task-oriented treatment.

POST-STROKE REHABILITATION: ACUTE PHASE MANAGEMENT

Systematic Reviews of clinical trials have formerly demonstrated that Stroke Unit, decisively, contributes to reduce mortality, institutionalization and long-term disability compared to general wards [1]. Common characteristics that establish effectiveness of Stroke Unit appear to be:

1. medical and nursing staff deep specialization;
2. multidimensional assessment;
3. early management of risk factors for complications;
4. early mobilization out of bed;
5. caregivers involving;
6. timely design of discharge plan [2].

Most of them belong to acute stroke rehabilitation domain and contribute to the success of Stroke Units indeed (Table **1**).

Early mobilization (out of bed mobilization) is perceived as one of the strategic constituents of acute stroke care responsible for good outcomes. It reduces bedsores, orthostatic hypotyension, deep venous thrombosis and pulmonary embolism, pneumonia. In such a context, a significant issue appears to be the standardization in terms of timing, duration and intensity of treatment: mobilization procedures remain poorly defined and differ both geographically and

by the characteristics of the unit where patients are being managed.

Table 1. Model of rehabilitation care in stroke acute phase.

Objectives	Actions	Operators
Functional Assessment	Evaluation of consciousness, comorbidity, continence, mobility, depression, language, cognitive abilities, global and selective disability. Swallowing Screening Test	Physician (Neurologist, Physiatrist, Nutritionist), Nurse, Physiotherapist, Speech Therapist, Neuropsychologist, Occupational Therapist
Prognosis definition	Definition of the real level of global and selective functional recovery achievable on the basis of identified clinical elements	Physiatrist
Management of continuity of care and definition of rehabilitation plan	Choice of rehabilitation setting. Information and education for the patient and the caregiver	Physiatrist
Prevention of complications	Management of dysphagia, early mobilization, positioning, falls risk management	Nurse, Physiotherapist, Speech Therapist, Occupational Therapist
Promotion of independence in primary Activity Daily Living (ADL)	Early mobilization, occupational therapy	Nurse, Physiotherapist, Speech Therapist, Occupational Therapist

In this background the AVERT [3], published in 2015, is the first randomized controlled clinical trial, conducted on a sample of 2104 patients, that has evaluated the effect of intensive and early mobilization (a few hours after stroke) on medium-term outcome of stroke subjects. The working hypothesis was intended to prove the efficacy of precocious and intensive treatment to improve functional outcome three months after the acute event. The results showed that in the precocious and intensive treatment group, the outcome at three months was worse, in terms of mortality and disability, mainly in patients suffering from severe ischemic stroke and intraparenchymal hemorrhage [3]. The results of the AVERT are still under discussion within the scientific community: far from reducing the emphasis on the importance of early mobilization in stroke patients [4], the stated outcome show that the definition of the timing, frequency and duration of the mobilization “out of bed” in the acute phase is more complex than believed until now.

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