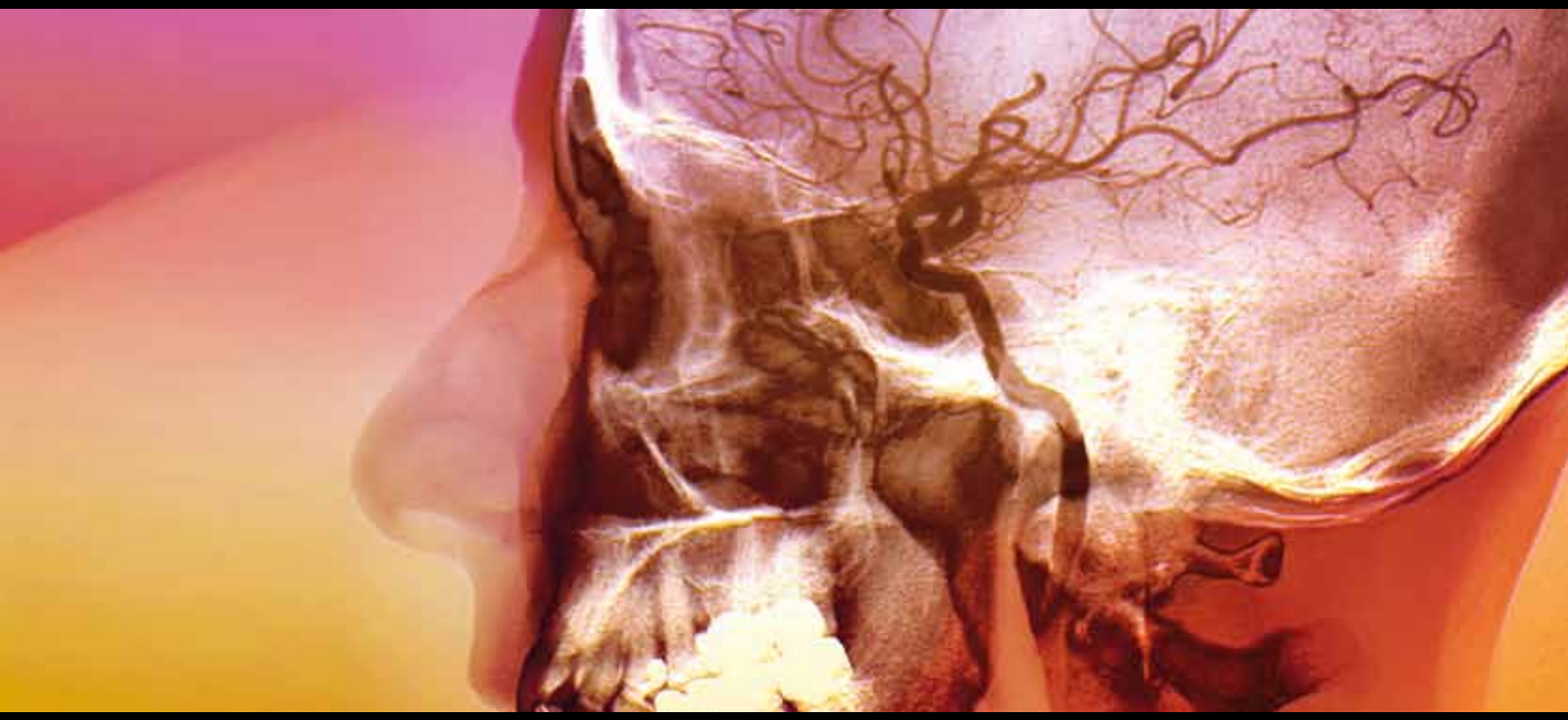
Stroke Research and Treatment

Carotid Disease and Stroke

Guest Editors: Arijana Lovrencic-Huzjan, Tatjana Rundek, Carlos A. Molina, and Michael J. Katsnelson



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Editorial Board

Contents

Carotid Disease and Stroke, Arijana Lovrencic-Huzjan, Tatjana Rundek, and Michael J. Katsnelson
Volume 2012, Article ID 264752, 2 pages

Recommendations for Management of Patients with Carotid Stenosis, Arijana Lovrencic-Huzjan, Tatjana Rundek, and Michael Katsnelson
Volume 2012, Article ID 175869, 12 pages

Genetic Susceptibility to Atherosclerosis, Sanja Kovacic and Mirjana Bakran
Volume 2012, Article ID 362941, 5 pages

Advanced Asymptomatic Carotid Disease and Cognitive Impairment: An Understated Link?, Irena Martinić-Popović, Arijana Lovrenčić-Huzjan, and Vida Demarin
Volume 2012, Article ID 981416, 4 pages

Do Clopidogrel Nonresponders Have an Increased Risk of Adverse Events during Supra-Aortal Angioplasty and Stenting?, Heiko Koerner, Christian Derveaux, Maria Alexandrou, Stefan Graeber, Christian Roth, Panagiotis Papanagiotou, Hermann Eichler, and Wolfgang Reith
Volume 2012, Article ID 904534, 4 pages

Asymptomatic and Symptomatic Carotid Stenosis: An Obsolete Classification?, Anastasios Chatzikonstantinou, Marc E. Wolf, Anke Schaefer, and Michael G. Hennerici
Volume 2012, Article ID 340798, 8 pages

Microembolus Detection by Transcranial Doppler Sonography: Review of the Literature, Vlasta Vuković-Cvetković
Volume 2012, Article ID 382361, 7 pages

Hemispheric Asymmetry of Visual Cortical Response by Means of Functional Transcranial Doppler, Marina Roje-Bedeković, Arijana Lovrenčić-Huzjan, Marijana Bosnar-Puretić, Vesna Šerić, and Vida Demarin
Volume 2012, Article ID 615406, 5 pages

Is Near-Infrared Spectroscopy a Reliable Method to Evaluate Clamping Ischemia during Carotid Surgery?, Luciano Pedrini, Filippo Magnoni, Luigi Sensi, Emilio Pisano, Maria Sandra Ballestrazzi, Maria Rosaria Cirelli, and Alessandro Pilato
Volume 2012, Article ID 156975, 7 pages

Effects of FK506 on Hippocampal CA1 Cells Following Transient Global Ischemia/Reperfusion in Wistar Rat, Zahra-Nadia Sharifi, Farid Abolhassani, Mohammad Reza Zarrindast, Shabnam Movassaghi, Nasrin Rahimian, and Gholamreza Hassanzadeh
Volume 2012, Article ID 809417, 8 pages

Safety of Early Carotid Artery Stenting after Systemic Thrombolysis: A Single Center Experience, Fabrizio Sallustio, Giacomo Koch, Alessandro Rocco, Costanza Rossi, Enrico Pampana, Roberto Gandini, Alessandro Meschini, Marina Diomedi, Paolo Stanzione, and Silvia Di Legge
Volume 2012, Article ID 904575, 5 pages

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Editorial

Carotid Disease and Stroke

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Carotid Disease and Stroke. Stroke is a major cause of disability, mortality, dementia, and depression in the world. Carotid atherosclerosis is recognized as an important cause of stroke and a modifiable factor for the risk reduction of subsequent stroke. Randomized clinical trials have shown efficacy of carotid endarterectomy (CEA) in secondary stroke prevention. Carotid stenting has emerged as a new and less invasive alternative to CEA. Continuous advances in medical therapy have made remarkable success in stroke risk reduction. These new treatment modalities were not widely applied in most interventional trials, making extrapolation of an optimal intervention strategy evermore complex in patients with symptomatic and asymptomatic carotid disease. The determination between "symptomatic" and "asymptomatic" carotid stenosis is a pivotal determinant of management of carotid disease. This special issue provides an updated synopsis of risk factors, imaging modalities, and different treatment options of atherosclerotic carotid disease.

Some of the highlights are included in the following:

(i) In the basic science rat model, the neurotrophic effect of an immunosuppressant agent after transient global cerebral ischemia is presented by Z. N. Sharifi et al.

(ii) A paper of S. Kovacic and M. Bakran provides a discussion of the main genetic investigations associated with human atherosclerotic vascular diseases.

(iii) The paper by A. Chatzikonstantinou et al. summarizes the literature of the natural history of the carotid disease and provides evidence that carotid stenosis is a sensitive marker of systemic atherosclerosis.

(iv) The causal relationship between stroke-free patients with advanced carotid stenosis and cognitive decline is presented in the paper by I. Martinić-Popović et al.

(v) The paper by V. Vuković-Cvetković presents a comprehensive review of (microembolic signal) MES detection and stroke risk by transcranial doppler (TCD)—an important ultrasound technique shown to successfully stratify asymptomatic patients at high risk for future cerebrovascular events.

(vi) The paper by M. Roje-Bedekovic et al. explores the visual evoked response in patients with severe carotid stenosis, a marker which serves as an important preoperative functional assessment of intracranial circulation.

(vii) The paper by L. Pedrini et al. discusses near infrared spectroscopy (NIRS) during CEA as a reliable method in detection of clamping ischemia and in the prevention of clamping-related neurologic deficits during CEA.

(viii) In the paper by H. Koerner et al., periprocedural ischemic lesions on diffusion-weighted imaging (DWI) during stenting procedures of supra-aortal arteries are discussed. The level of platelet inhibition is presented as an intriguing approach to determining the relationship between nonresponders to clopidogrel and clinically silent microembolization.

(ix) The paper by F. Sallustio et al. presents a case series data suggesting that early carotid artery stenting may be considered as a safe alternative to CEA after IV

thrombolysis in selected patients who are at high risk of stroke recurrence.

The primary and secondary stroke prevention approaches are exciting clinical topics in evolution with many opportunities and options for both current treatment and future discoveries. Innovations in genetics, pharmacogenomics, and vascular imaging will likely provide specific pathophysiological targets, tailored treatments, and personalized medical management for individuals with atherosclerotic carotid disease. We hope that this special issue will serve as an overview of the accomplishments, current practices, and controversies in the field as well as a useful reference for management of patients with carotid disease and future research opportunities.

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Review Article

Recommendations for Management of Patients with Carotid Stenosis

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Stroke is a one of the leading causes of morbidity and mortality in the world. Carotid atherosclerosis is recognized as an important factor in stroke pathophysiology and represents a key target in stroke prevention; multiple treatment modalities have been developed to battle this disease. Multiple randomized trials have shown the efficacy of carotid endarterectomy in secondary stroke prevention. Carotid stenting, a newer treatment option, presents a less invasive alternative to the surgical intervention on carotid arteries. Advances in medical therapy have also enabled further risk reduction in the overall incidence of stroke. Despite numerous trials and decades of clinical research, the optimal management of symptomatic and asymptomatic carotid disease remains controversial. We will attempt to highlight some of the pivotal trials already completed, discuss the current controversies and complexities in the treatment decision-making, and postulate on what likely lies ahead. This paper will highlight the complexities of decision-making optimal treatment recommendations for patients with symptomatic and asymptomatic carotid stenosis.

1. Introduction

Stroke is a major, well-recognized cause of morbidity and mortality around the world. Extracranial carotid atherosclerosis with the resulting atherothromboembolism may account for up to 20% of ischemic strokes [1]. Carotid stenosis may manifest itself in many different clinical stroke syndromes, from asymptomatic carotid disease to a TIA affecting the eye (amarousis fugax) or the brain to an ischemic stroke in the cerebral territory supplied by the vessel. Recently, cognitive impairment as a result of carotid stenosis has also been proposed [2].

Multiple treatments have been shown efficacious in treating carotid disease. Carotid endarterectomy (CEA) has been shown to be effective in significantly reducing the risk of recurrent stroke emanating from that pathological nidus [3, 4]. Angioplasty and stenting of the carotid origin have developed and evolved as a less invasive alternative to surgery, initially employed in patients with high surgical comorbidities. Multiple trials have been conducted comparing the two techniques in various subpopulations with often conflicting results. The importance of the best medical treatment cannot be overstated, as more advanced pharmacological agents and more stringent management of various risk factors of atherosclerosis have led to an overall decline in the incidence of stroke. This has altered the risk-benefit analysis of any invasive procedures which carry a nontrivial complication rate of their own. Despite numerous trials and decades of clinical research, the optimal management of symptomatic and asymptomatic carotid disease remains controversial. We will attempt to highlight some of the pivotal trials already completed, discuss the current controversies and complexities in the treatment decision-making, and postulate on what likely lies ahead.

2. Risk Factors

Risk factors for the risk of stroke in the presence of carotid stenosis are age, hypertension, coronary heart disease, irregular and ulcerated plaque morphology, absence of collateral flow, impaired cerebral reactivity, previous stroke or TIA, and microembolic signals observed on Transcranial Doppler (TCD) [5, 6].

Meta-analysis including 23,706 participants [7] of four population-based studies (Malmö Diet and Cancer Study, Tromsø, Carotid Atherosclerosis Progression Study, and Cardiovascular Health Study) showed the prevalence of severe asymptomatic carotid stenosis in the general population to be up to 3.1%. It has been shown that the risk of stroke increases with the degree of stenosis (from less than 1% per year for a <80% stenosis to 4.8% per year for a >90% stenosis).

The risk of stroke in the target vascular territory also rises with higher degree of symptomatic carotid stenosis (Hazard ratio (HR) 1.18 per 10% increase in stenosis; 95% confidence interval (CI) 1.10–1.25) [3, 4, 8]. Paradoxically, patients with ICAs severely narrowed or nearly collapsed due to markedly reduced poststenotic blood flow (pseudo-occlusion, near occlusion) have a relatively lower risk of stroke on best medical treatment alone (HR = 0.49; CI 0.19–1.24) compared to vessels with moderate degree of stenosis [9, 10]. It has been shown that the risk of stroke ipsilateral to ICA stenosis is greater in patients with recent neurological symptoms of ischemia in that vascular target artery [11, 12]. These preceding neurological symptoms have been stratified in the likelihood of subsequent ipsilateral stroke: major stroke (HR = 2.54; 95% CI 1.48–4.35), multiple TIAs (HR = 2.05; 95% CI 0.16–3.60), minor stroke (HR = 1.82; 95% CI 0.99–3.34), single TIA (HR = 1.41; 95% CI 0.75–2.66), and ocular events (HR = 1.0) [8].

Plaque instability, another important risk factor, is characterized by a thin fibrous cap, large lipid core, reduced smooth muscle content, and a high macrophage density. Studies have shown that the irregular morphology or ulceration of the plaque carries an increased risk of a clinical event (HR = 2.03; CI 1.31–3.14) [8]. A thrombotic cascade occurs primarily when the thrombogenic center of the plaque is exposed to the bloodstream carrying clotting factors. The spike in the risk of stroke recurrence in the days and weeks after an ischemic event is likely the consequence of an unstable atherosclerotic plaque, and the rapid decline in risk over the subsequent months likely reflects the healing and stabilization of the said lesion and improved collateral blood flow to the ipsilateral cerebral hemisphere [10].

3. Transient Ischemic Attacks

Transient ischemic attack (TIA) is a brief episode of neurological dysfunction resulting from focal cerebral ischemia not associated with permanent cerebral infarction [13]. Among patients who present with stroke, the prevalence of prior TIA has been reported to range from 7% to 40%, depending on factors such as how a TIA is defined, which stroke subtypes are evaluated, and whether the study is hospital or population based [14, 15]. In the population-based Northern Manhattan Stroke Study, the prevalence of preceding TIAs among those with first ischemic stroke was 8.7% [16], with the majority of TIA occurring within 30 days of the patient's first ischemic stroke.

It has long been recognized that TIA can portend stroke [17], with short-term stroke risk being particularly high, exceeding 10% in 90 days [14, 18–22] and studies confirm the elevation of that risk into the long term [23–25]. The timing of a TIA before stroke is highly time dependent, with studies showing 17% occurring on the day of the stroke, 9% within the previous day, and another 43% within the previous week of the index event [18, 26–28].

Several score systems based on clinical characteristics, like California score and the ABCD score, help to stratify patients into differing risk tiers [29]. The newer $ABCD_2$ score was derived to provide a more robust prediction standard and incorporates elements from both prior scores [29]. In addition, patients with severe extra- or intracranial stenosis carry a particularly high risk of disease recurrence [30].

Observational studies showed that urgent evaluation at a TIA clinic and immediate initiation of treatment reduces stroke risk after TIA [31, 32]. It has been shown that early management of TIA patients in a stroke unit leads to specific treatments in a significant proportion of cases [33].

4. Diagnostic Evaluation

Imaging of the brain and its supplying vessels is crucial in the treatment of patients with stroke or TIA. During the initial assessment, radiological studies distinguish ischemic stroke from intracranial hemorrhage and stroke mimics and are used to identify the penumbra and vessel occlusion, thus guiding emergent stroke care. In the acute setting, radiological studies often point to the subtype and etiology of stroke and can be utilized to predict outcome. Presence of a diffusion-weighted imaging (DWI) lesion and a vessel occlusion on a magnetic resonance image (MRI) among patients presenting acutely with a transient clinical symptoms or a minor stroke is predictive of an increased risk for future stroke and functional dependence [34]. For example, in the North Dublin TIA Study [35] of 445 confirmed TIA cases, carotid stenosis predicted 90-day stroke (HR = 2.56; CI 1.27–5.15, $P = 0.003$). Risk of stroke rose with increasing grade of carotid stenosis, ranging from 5.4% (CI 3.3%–8.7%) with <50% stenosis to 17.2% (CI 9.7%–29.7%) with severe stenosis/occlusion (HR = 3.3; CI 1.5–7.4, $P = 0.002$). Thus, prompt advanced vascular imaging is important for effective treatment in secondary stroke prevention. It has been shown that vascular evaluation assessment does identify the site and cause of arterial obstruction, and the patients at high risk of stroke or stroke recurrence [36–40].

Carotid ultrasound provides reliable assessment of the carotid bifurcation with high sensitivity and specificity [41, 42]. It is fast, inexpensive, and widely available. In TIA patients, carotid duplex and TCD performed within 24 hours of symptoms revealed a threefold greater risk for stroke in the next 90 days in those with moderate to severe extra- or

intracranial carotid stenosis compared to patients with no such findings [43].

TCD provides noninvasive monitoring of intracranial stenosis [37], with a positive predictive value (PPV) of 36% and, negative predictive value (NPV) of 86% [44]. The high NPV and the lower PPV reflect the low prevalence of intracranial stenosis in Caucasians [6], with higher rates in other ethnic groups.

TCD can also detect microembolic signals (MESs) seen with extracranial or cardiac sources of emboli. A large number of MESs on TCD is a marker of risk in patients with emboli from the carotid origin, prompting research into optimal strategies for medical treatment and the timing of endarterectomy in those with an extracranial carotid disease [6]. In a cohort of patients unselected for stroke mechanism, MESs were more common in patients with large-artery occlusive disease and were more prevalent in patients treated with anticoagulation rather than antiplatelet agents [5].

The advancement and refinement of computed tomography over the past quarter century has made it powerful tool for the visualization of the vascular system. It can provide highly detailed images of the carotid artery, with higher sensitivity and specificity than ultrasound, but does require patients to undergo radiation and contrast exposure, fares poorly with heavily calcified lesions, and involves some post acquisition image processing. Magnetic resonance imaging (MRA) has also seen an evolution in image resolution and specialized sequencing, and the modality can distinguish not only the anatomy of the vessel but also the composition of the atherosclerotic plaque with remarkable detail. MRI scanners are less widespread, and the study can overestimate the degree and morphology of high-grade stenosis. MRA with contrast provides a more accurate assessment of the vasculature image, but does involve gadolinium, which carries additional risks. Moreover, certain patients have metal implants or pacemakers, making them ineligible for scanning by this technique. Cerebral angiography is still considered "the gold standard" for evaluating the cerebrovascular system and its collaterals. However, it is expensive and has a significant radiation exposure and a discrete chance of retroperitoneal hematoma, vessel perforation, or distal emboli.

A sensible and stepwise nonemergent diagnostic work-up would usually entail an initial carotid duplex for screening purposes. If the stenosis is less than 50%, no further imaging is likely needed. If the carotid duplex comes back as >50% (and certainly >70%), CTA or MRA should be considered for more detailed plaque characterization. At that point and based on patient's presenting symptomatology, cerebrovascular and overall health and available resources, diagnostic/therapeutic cerebral angiogram, surgical intervention, or continued medical management can be undertaken.

5. Carotid Endarterectomy

Carotid endarterectomy is a surgical procedure of removing the plaque from the carotid artery, thus reducing the risk of stroke by enlarging the lumen and by removing a possible nidus of emboli. The anticipated benefit of treatment in asymptomatic patients with carotid stenosis is derived from several clinical trials.

6. Asymptomatic Carotid Stenosis

In Asymptomatic Carotid Atherosclerosis Study (ACAS) [45], patients with asymptomatic carotid artery stenosis of 60% or greater, defined by angiography or Doppler evaluation using local laboratory diagnostic criteria, were randomized to CEA or best medical management. After a median followup of 2.7 years, the aggregate risk over 5 years for ipsilateral stroke and any perioperative stroke or death was estimated to be 5.1% for surgical patients and 11.0% for patients treated medically (aggregate risk reduction of 53%; absolute risk reduction of approximately 1% per year). This net benefit was dependent upon carotid endarterectomy being performed with less than 3% perioperative morbidity and mortality.

The Asymptomatic Carotid Surgery Trial (ACST) [46] randomized asymptomatic patients with significant carotid stenosis according to Doppler criteria, to immediate CEA or indefinite deferral of surgical intervention. The mean followup was 3.4 years. The cumulative 5-year risks of surgical versus medical treatment were 6% versus 12% for all strokes, 4% versus 6% for fatal or disabling strokes, and 2% versus 4% for only fatal strokes, respectively. Subgroup-specific analyses found no significant heterogeneity in the perioperative risk or in the long-term postoperative benefits. A meta-analysis of three trials [47] reported that despite about a 3% perioperative stroke or death rate, carotid endarterectomy for asymptomatic carotid stenosis reduces the risk of ipsilateral stroke, and any stroke, by approximately 30% over 3 years. For the outcome of any stroke or death, there was a nonsignificant trend toward fewer events in the CEA group. In subgroup analysis, CEA appeared more beneficial in men than in women and more in younger patients than in older patients, although the data for age effect was less convincing. There was no statistically significant difference between the treatment effect estimates in patients with different grades of significant stenosis, but the analysis may not have been sufficiently powered.

In Asymptomatic Carotid Emboli Study (ACES), a prospective observational study in patients with asymptomatic carotid stenosis of at least 70%, followed up for 2 years, and monitored for 1 hour at 6, 12, and 18 months, HR for the risk of ipsilateral stroke, or TIA in patients with embolic signals compared with those without was 2.54 (CI 1.20–5.36; $P = 0.015$) [48]. For ipsilateral stroke, alone, HR was 5.57 (CI 1.61–19.32; $P = 0.007$). Therefore, detection of embolization on TCD may be used to help stratify patients with asymptomatic carotid stenosis in a higher and lower vascular event risk groups.

Trials of carotid surgery for asymptomatic carotid stenosis have concluded that although surgery reduces the incidence of ipsilateral stroke (RR 0.47–0.54) and any stroke, the absolute benefit is small (approximately 1% per annum) [45, 46, 49], whereas the perioperative stroke or death

rate is 3%. Medical management is the most appropriate option for most asymptomatic subjects; only centers with a perioperative complication rate of 3% or less should contemplate surgery. Patients with a high risk of stroke (men with stenosis of more than 80% and a life expectancy of more than 5 years) may derive some benefit from surgery in appropriate centers [46, 47].

7. Symptomatic Carotid Stenosis

For symptomatic carotids, the ECSCT and NASCET [9, 28] results established CEA as the treatment of choice for moderate and severe carotid artery stenosis as a secondary stroke prevention measure. The most important periprocedural risks of CEA are death (about 1%) and stroke (about 5%) [9, 28]. From a pooled analysis of data from the three largest RCTs of surgery for symptomatic carotid stenosis [50], CEA reduced the 5-year absolute risk of any stroke or death in patients with 50–69% stenosis, according to angiographic NASCET criteria (which consist of measuring the lumen at the point of the greatest stenosis divided by the diameter of the carotid beyond the carotid bulb) (absolute risk reduction (ARR) 7.8%, CI 3.1–12.5), and was highly beneficial in patients with 70–99% stenosis (15.3%, CI 9.8–20.7), but showed no benefit in patients with a near occlusion. Quantitatively similar results were seen for disabling stroke [50]. CEA, therefore, proved to be beneficial in stenosis more than 50% according to NASCET criteria, which are equivalent to 65% stenosis by ECST criteria. In ECST trial, CEA reduced the risk of recurrent TIAs in patients with a near occlusion (ARR 15%, $P = 0.007$).

While the degree of stenosis is a major determinant of benefit from CEA, there are other clinical characteristics that influence the risks and benefits of surgery. Subgroup analyses of pooled data from the large RCTs [51] showed the greatest benefit from CEA in men, patients aged ≥75 years, and patients randomized within 2 weeks after their last ischemic event. Both ECST and NASCET showed that for patients with ≥50% ICA stenosis, the number needed to treat (NNT) by CEA to prevent one ipsilateral stroke in 5 years was 9 for men versus 36 for women, 5 for age ≥75 years versus 18 for age <65 years, and 5 for patients randomized within 2 weeks after the last ischemic event versus 125 for patients randomized >12 weeks. Women had a lower risk of ipsilateral ischemic stroke on medical treatment and a higher operative risk in comparison to men [52]. CEA was more beneficial in women with ≥70% stenosis, but not in women with 50–69% stenosis. At the same time, CEA reduced the 5-year ARR by 8.0% CI 3.4–12.5 in men with 50–69% stenosis. This sex difference was statistically significant even when the analysis of the interaction was confined to the group of 50–69% stenosis [52].

8. CEA Surgical Considerations

CEA has been established as "the gold standard" for carotid stenosis treatments for many years, yet the surgical techniques of performing the procedure continue to evolve. In traditional endarterectomy, the plaque is removed via a longitudinal arteriotomy. Another technical variant is eversion endarterectomy, which employs a transverse arteriotomy and reimplantation of the carotid artery. There was no significant difference in the rates of perioperative stroke, stroke, death, or local complication rates in a review of five RCTs comparing eversion and conventional endarterectomy performed either with primary closure or patch angioplasty [53]. To reduce the risk of restenosis, many surgeons use a patch of autologous vein or synthetic material to close the artery and to enlarge the lumen. Although the patch increases the surgery time and complication rate, it was associated with a 60% reduction in the perioperative risk of stroke or death during the postoperative period and long-term followup, 85% reduction in the risk of perioperative arterial occlusion, and 80% reduction in the risk of vessel restenosis during long-term followup. Although some surgeons routinely insert a temporary intralumenal shunt [54], the number of patients who need shunting with different shunting policies has been too small, and the results of clinical studies inconclusive [55].

CEA was traditionally performed under general anesthesia (GA), but surgery under local anesthesia (LA) is becoming more widespread. While a systematic review of seven small randomized trials showed the use of LA to be associated with a borderline statistically significant trend towards a reduced risk of operative death, no evidence of a reduction in risk of perioperative stroke was found [56]. A large multicenter randomized trial has shown no major difference in operative risk of stroke or death combined (risk ratio for LA versus GA RR = 0.94; CI 0.70–1.27) [57]. The anesthesiologist and surgeon, in consultation with the patient, should determine the method of appropriate anesthesia [58]. For patients with a contralateral carotid occlusion, LA may offer some benefit.

9. Carotid Stenting

Carotid angioplasty and stenting (CAS) was developed to be a less invasive and involved procedure compared to carotid endarterectomy. It has emerged as an alternative for patients who are considered to have high surgical risks due to medical comorbidities or anatomical high-risk features. Since its development over twenty years ago, the technique of endovascular carotid revascularization has been undergoing a continuous maturation process due to the shift from the initial use of balloon expandable stents to self-expanding stents, the introduction of and continuously expanding array of embolic protection devices (EPDs), and increasing operator experience.

The procedure is usually done under local anesthetic, with the subsequent expectation of less nerve injuries, venous thromboembolisms, and myocardial infarctions—all well-known clinical costs of going to the operating room. CAS also carries some potential disadvantages such as arterial dissection, dislocation of atherothrombotic debris and embolization to the brain or eye, late embolization due to thrombus formation on the damaged plaque, and

bradycardia and hypotension as a result of carotid sinus stimulation. Local complications at the site of arterial cannulation such as hematoma and aneurysm formation may also occur. Rarely, the stent may erode through the arterial wall or fracture upon deployment. In the longer term, restenosis appears to be more common after stenting than after endarterectomy.

Several trials have compared CAS and CEA in secondary stroke prevention, mostly in patients lacking high surgical-risk [49, 59–65]. Most studies were designed to assess the noninferiority of stenting compared to endarterectomy with regard to the early risks of the procedures. None of these studies were adequately powered to show the noninferiority (or superiority) of stenting looking at both the early risks and late benefits of these techniques. Initially, locating studies with the desired target populations has also proved a challenge. For example, The Stenting and Angioplasty with Protection in Patients at High Risk for Endarterectomy (SAPPHIRE) trial included more than 70% of asymptomatic patients, and therefore should not be used for decisions about secondary prevention [49]. In Carotid and Vertebral Artery Transluminal Angioplasty Study (CAVATAS), on the other hand, the majority of the patients in the endovascular group underwent angioplasty, and only 26% were treated with a stent [65].

The comparison of CEA and CAS has produced many different (and often contradictory) results. Stent-protected angioplasty versus carotid endarterectomy in symptomatic patients (SPACE) marginally failed to prove the noninferiority of CAS compared to CEA with the endpoint being ipsilateral stroke or death up to post-op day 30. The event rates for 1,200 enrolled patients were 6.8% for CAS and 6.3% for CEA patients (absolute difference 0.5%; CI −1.9 +2.9%; $P = 0.09$) [66]. The Endarterectomy versus Stenting in Patients with Symptomatic Severe Carotid Stenosis (EVA3S) trial was stopped prematurely after the inclusion of 527 patients because of safety concerns and a lack of efficacy. The RR of any stroke or death after CAS, compared with CEA, was 2.5 CI 1.2–5.1 [59].

The Stenting and Angioplasty with Protection in Patients at High Risk for Endarterectomy trial (SAPPHIRE) [61] was one of the first trials comparing carotid stenting (CAS) (with the use of an emboliprotection device) to CEA in patients considered at high surgical risk for CEA. Patients were eligible if they either had a symptomatic stenosis of 50% or greater or an asymptomatic stenosis of 80% or greater. The primary end point of the trial was the cumulative incidence of death, stroke, or myocardial infarction with 30 days after the procedure or death or ipsilateral stroke between 31 days and 1 year. The primary end point occurred in 20 patients (12%) in the CAS group and in 32 patients (20%) in the CEA cohort. For patients with asymptomatic lesions, the cumulative incidence of the primary end point at 1 year was lower among those who were treated with CAS (10%) than who underwent a CEA (22%). In the periprocedural period, the cumulative incidence of death, myocardial infarction, or stroke among patients with asymptomatic carotid artery stenosis was 5% among those who received a stent, as compared to 10% among those who underwent a CEA. The SAPPHIRE trial was one of the first trials to select high-risk patients with medical comorbidities (these criteria were the basis of *exclusion criteria* for the NASCET/ACAS trials). The major adverse events (death, stroke, and MI) at 1 year were 12.2% in the CAS group compared to 20.1% for CEA ($P = 0.053$). The trial did not include a best medical treatment arm and therefore failed to answer a question of what will happen to the surgical high-risk CEA patient if they were to receive maximal medical treatment.

The International Carotid Stenting Study (ICSS) trial [67] was a randomized, double-blinded study comparing CAS and CEA in patients with symptomatic carotid stenosis of greater than 50% within 6 months prior to randomization. Between randomisation and 120 days, there were 34 (Kaplan-Meier estimate 4.0%) events of disabling stroke or death in the stenting group compared with 27 (3.2%) events in the endarterectomy group (HR = 1.28, CI 0.77–2.11). The incidence of stroke, death, or periprocedural myocardial infarction was 8.5% in the stenting group compared with 5.2% in the endarterectomy group (72 versus 44 events; HR = 1.69, CI 1.16–2.45, $P = 0.006$). Risks of any stroke (65 versus 35 events; HR = 1.92, CI 1.27–2.89) and all-cause death (19 versus seven events; HR = 2.76, CI 1.16–6.56) were higher in the stenting group than in the endarterectomy group. Three procedural myocardial infarctions were recorded in the stenting group, all of which were fatal, compared with four, all nonfatal, in the endarterectomy group. There was one event of cranial nerve palsy in the stenting group compared with 45 in the endarterectomy group. There were also fewer hematomas of any severity in the stenting group than in the endarterectomy group (31 versus 50 events; $P = 0.02$). A magnetic-resonance-imaging (MRI) substudy was carried out at 5 ICSS centers, with scans analysis being performed blinded to the choice of treatment [68]. New ischemia was found in about half of CAS patients versus about 15% of CEA patients. On followup imaging 4 to 6 weeks later, FLAIR was abnormal at the site of early ischemia in 30% of patients after CAS versus 8% of patients after CEA, a result that was also highly significant.

Subgroups analyses from RCTs suggest some heterogeneity of risk between stenting and endarterectomy. In particular, the excess risk associated with stenting was greater in patients aged 70 years or older [62, 63]. However, owing to the drawbacks of post hoc analysis such as low statistical power and the risk of chance findings, these subgroup analyses should be interpreted with caution. The best evidence of subgroup treatment effect interaction will be obtained from a planned combined analysis of individual patient data from current larger trials that compare stenting versus endarterectomy.

In various RCTs, the risk of ipsilateral stroke beyond the perioperative period was low (<1% per year) and similar in both the stenting and endarterectomy groups, which strongly suggests that stenting is as effective as surgery for the medium-term prevention of ipsilateral stroke—at least up to 4 years after the procedures [49, 62, 65, 69]. As the incidence of recurrent carotid stenosis may be significantly higher after

CAS compared to CEA [70], there is a need to assess the long-term effects of carotid stenting, and particularly the long-term incidence of restenosis.

After analyzing the various comparison studies, CAS has not been shown to be as safe as CEA in patients with symptomatic carotid artery stenosis in RCTs. The recent meta-analyses [66, 71, 72] of RCTs that compared CAS and CEA treatment of patients with mainly symptomatic carotid artery stenosis indicated that patients who received CAS had a significantly increased risk of 30-day mortality or stroke compared with patients who received CEA (odds ratio (OR) 1.60; CI 1.26–2.01) and concluded that CEA should remain the first-line intervention in "standard risk," symptomatic patients.

What about the patients who are not "standard risk" and who cannot tolerate surgery? The registry of high-surgical risk patients undergoing CAS (recruited to postmarketing surveillance in the EXACT and CAPTURE trials) has shown different outcomes [70]. In a cohort of 6320 patients, 12% who had suffered stroke or TIA 6 months prior to CAS, a subgroup analysis was performed, stratified for age. The 30-day rate of death/stroke in 589 patients aged <80 years was 5.3% (CI 3.6–7.4), compared to 10% in 172 patients aged >80 years (CI 3.3–16). The authors concluded that CAS had demonstrated real-world outcomes consistent with established American Heart Association (AHA) guidelines in symptomatic patients and should be a viable alternative to CEA in this "high-risk" cohort. There are some questions that need to be answered before relying on these results in recommending CAS to patients who are at high risk for CEA [73]. The low procedural risk observed in nonoctogenarian patients in the amalgamated registry must be maintained and regularly audited; if it exceeds 8%, the therapeutic benefit will likely shift away from intervention. The etiology of the carotid stenosis is also important in interpreting the studies' restenosis results: primary atherosclerotic disease or nonatherosclerotic disease (e.g., radiation arteritis, restenosis after CEA, etc.) are distinct disease processes and likely behave differently after stenting. The post hoc analysis from the Acculink for Revascularization of Carotids in High-Risk Patients (ARCHeR) CAS Registry showed that the 30-day risk following CAS in patients with nonatherosclerotic disease was 14 times lower than in their atherosclerotic counterparts [74]. Clearly, what treatment is best for which particular patient is not all that clear.

Furthermore, Carotid Revascularization Endarterectomy versus Stenting Trial (CREST) [75] was the only RCT comparing CAS and CEA in patients with symptomatic and asymptomatic carotid stenosis that showed equal risk of the composite primary outcome of stroke, myocardial infarction, or death. During the periprocedural period, there was a higher risk of stroke with stenting and a higher risk of myocardial infarction with endarterectomy. Recent subgroup analysis showed sex differences of primary endpoints did exist: women fared worse with CAS compared to CEA, while men did equally well with either procedure [76]. Another study compared subgroups of patients who had suffered an MI (clinical or biochemical) and found MIs to be more common in CEA and to be independently associated to increased future mortality [77]. Given the main finding of therapeutic equivalence between CAS and CEA, the obvious questions arose about the discrepancies between CREST and the preceding trials.

There are certain key differences in trial methodology and design. The first important methodological difference was operator experience across the studies [78]. Lifetime endovascular requirements were as follows: in CAVATAS (year 2001), 504 patients, operators had training in neuroradiology and angioplasty (but not necessarily in the carotid artery), and tutor-assisted procedures were allowed; in SAPPHIRE (year 2004), 334 patients, procedures were submitted to an executive review committee, CAS periprocedural death or stroke rate had to be less than 6%, and no tutor-assisted procedures were allowed; in SPACE (2006) with 1200 patients, at least 25 successful CASs or assistance of a tutor for interventionalists who have done at least 10 CAS was required; in EVA 3S (2006), with 527 patients, operators had to have performed at least 12 CAS cases or at least 5 carotid stent procedures and more than 30 cases of endovascular treatment of supra-aortic trunks, or tutor-assisted CAS was allowed for centers not fulfilling minimum requirements; in ICSS (2010) with 1710 patients, a minimum of 50 total stenting procedures of which at least ten should be in the carotid artery or tutor-assisted procedures were allowed for interventionalists with insufficient experience. The trend across all of these studies was that many operators may have had some experience with peripheral stent placement, but that experience was not necessarily equivalent to stenting in the carotid vasculature. As aortic arch tortuosity is emerging as one of the critical factors determining procedural risk with CAS. Lack of proof of experience with carotid catheterization as a prerequisite for participation as an interventionalist across all the Europeans trials is probably the factor responsible for high rates of stroke reached in these studies. In contrast, prospective CAS registries (mentioned earlier) in North America preceding CREST required a higher level of experience with brachiocephalic catheterization and carotid interventions and have reported rates of stroke that are significantly less than those reported in the European studies. CREST study (2010), with 2502 patients, was even more rigorous and required a minimum experience of 10–30 carotid stent procedure with 0.14' wire systems, experience with EPD, and a documented 30-day stroke and death rate of 6–8% [79]. In addition, after admittance into the study, there was a required lead-in phase of up to 20 patients designed to ensure operators had adequate experience and acceptable complication rates prior to randomizing patients. The standards of rigorous vetting for proceduralists performing carotid revascularization were set by NASCET (perioperative risk of stroke or death <6% at 30 days) and ACAS [9, 45], in which only experienced surgeons chosen according to strict criteria were allowed to participate. As opposed to the stenting arm operators, carotid surgeons in the European randomized trials of CAS versus CEA were more experienced compared to their interventionalist counterparts, and there were no inexperienced surgeons allowed to perform the procedure whether or not a tutor was present.

The second major protocol difference was the use or periprocedural dual antiplatelet medications. In the ICSS and EV-3S studies, the use of dual antiplatelet medication was recommended but not required—in EVA-3S [59], 17% of patients were not on dual antiplatelet medications prior to the procedure, and nearly 15% did not have these medications after procedure. In the CREST study, the use of dual antiplatelet therapy was required and part of the protocol.

The third issue was the lack of exclusion criteria for stenting, a stark contrast to high surgical-risk criteria precluding randomization present for the CEA arms in the EVA-3S, ICSS, and SPACE trials. Absence of angiographic exclusion criteria for stenting in combination with inexperienced interventionalists may have resulted in a significant rate of perioperative stroke and death seen in the CAS arm in EVA-3S. In the CREST trial, rigorous angiographic exclusion criteria such as severe tortuosity and calcification, intraluminal, thrombi, and large, bulky plaques may explain discrepant results. Also, ICSS, EVA-3S, and SPACE all allowed the use of many different types of stents and EPD, further tipping the scales towards unfavorable outcomes, when deployed in the hands of inexperienced operators. Contrary, in the CREST study, the same stent and EPD system (Acculink stent and Accunet EPD) was used across the board, allowing the operator to become very familiar with the idiosyncrasies of one single device. Moreover, the lack of protocol in the European studies resulted in the variable use of EPDs, while in the CREST study, the protocol required the use of an embolic protection device in all enrolled patients.

10. Stenting Consideration

Certain vascular and local anatomical features are considered relative contraindications depending on experience of an interventional radiologist/neurologist/neurosurgeon and the type of anatomical substrate for CAS. These include complex bifurcation disease with long, multifocal lesions or extensive aortic or brachiocephalic trunk plaque, severe tortuosity or calcification of the aortic arch vessel, or ring-like, heavy calcifications of the carotid bifurcation. Based on experts' opinion and not on RCTs, CAS is indicated in patients with contralateral laryngeal nerve palsy and previous radical neck dissection or cervical irradiation and with prior CEA (restenosis), because the rate of cranial nerve injuries following surgery is higher in this subset. Also, CAS can be offered to patients with a high bifurcation or intracranial extension of a carotid lesion, where surgical access could be difficult or to patients with a high risk of cerebral ischemia during carotid clamping (occlusion of the contralateral ICA and anomalies of the circle of Willis).

Carotid stenting in symptomatic patients with a standard risk should only be considered in high-volume CAS centers with a 30-day risk of death/stroke as independently audited and maintained <6% [58] and where patients are treated without delay, preferably within 14 days. If these two caveats cannot be achieved, the patient should be referred for CEA.

11. Extracranial-Intracranial Anastomosis (EC-IC Bypass)

About 5–10% of patients with carotid TIA or minor stroke have occlusion of the origin of the ICA, or occasionally the distal ICA or proximal middle cerebral artery. These lesions can be bypassed by anastomosing a branch of the external carotid artery, usually the superficial temporal artery, via a skull burr hole to a cortical branch of the middle cerebral artery. Such collateral was developed to improve the blood supply in the distal middle cerebral artery bed and to reduce the risk of stroke or the severity of stroke. However, in an RCT, these anastomoses between the superficial temporal and middle cerebral arteries were not beneficial in preventing stroke in patients with middle cerebral artery or internal carotid artery stenosis or occlusion [80]. A recent Carotid Occlusion Surgery Study did not show additional benefits of bypass surgery when added to medical management in patients with symptomatic atherosclerotic internal carotid artery occlusion [81].

12. Medical Treatment of Patients with Carotid Stenosis

In patients with carotid stenosis undergoing either primary or secondary prevention, the treatment of risk factors such as hypertension, diabetes mellitus, lipid, or homocysteine metabolic disorders, as well as modification of lifestyle, particularly smoking cessation, are of utmost importance to reduce both early and long-term risks of vascular events, dementia, and death [82, 83].

Aspirin and the combination of aspirin and extended released dipyridamole, clopidogrel, ticlopidine, and triflusal have been shown to be effective as antiplatelet agents in long-term secondary prevention of ischemic stroke [84, 85]. Currently, aspirin, aspirin/extended dipyridamole, or clopidogrel is used in clinical practice.

To date, only aspirin has been shown to be safe and effective in the acute postischemic phase (first 48 hours) and should be started *immediately* in patients with TIA/ischemic stroke after the exclusion of brain hemorrhage and if iv-tPA has not been given (in that case, antiplatelets are held for the first 24 hours). Aspirin is effective in the range of doses (30–1,300 mg/day), but doses >150 mg/day are associated with more side effects [86]. In the Antithrombotic Trialists' Collaboration, a meta-analysis of >60 aspirin trials, the best risk reduction was found in trials using a 75-to-150 mg dose of aspirin [87–89]. In patients with a history of aspirin-induced ulcer bleeding, aspirin in combination with a proton-pump inhibitor was superior to clopidogrel alone in the prevention of recurrent ulcer bleeding [90].

Clopidogrel (75 mg/day) was slightly more effective than aspirin monotherapy (325 mg/day) in preventing vascular events (ischemic stroke, myocardial infarction, or vascular

death) in the CAPRIE trial, resulting in a relative risk reduction (RRR) of 8.7% (CI 0.3–16.5) [91]. The highest benefit of clopidogrel was seen in patients with peripheral artery disease.

The combination of aspirin (30–300 mg/day) and extended release dipyridamole (200 mg twice a day) was shown to be more effective compared with aspirin alone in two studies [92, 93]. Combination therapy reduced vascular events (ischemic stroke, myocardial infarction, or vascular death) by 18% (CI 9–26). The incidence of headache, a common side effect with combination therapy, can be greatly reduced by a slow titration of the drug.

The PRoFESS trial [94] was a head-to-head comparison of clopidogrel and the combination of aspirin/extended release dipyridamole. There was no difference in efficacy across all endpoints and all subgroups of patients. The combination of aspirin/extended release dipyridamole resulted in more intracranial bleeds and a higher dropout rate due to headaches compared with clopidogrel (5.9 versus 0.9%).

In the MATCH trial (secondary prevention in high-risk patients with TIA or ischemic stroke) [95] and CHARISMA (Combined Primary and Secondary Prevention Study) trial [96], comparison of clopidogrel or aspirin monotherapy with its combination failed to show superiority of the combination therapy, which had an increased bleeding rate. The Clopidogrel and Aspirin for Reduction of Emboli in Symptomatic Carotid Stenosis (CARESS) trial showed that in patients with recently symptomatic carotid stenosis combination therapy with clopidogrel and aspirin is more effective than aspirin alone in reducing asymptomatic embolization in a short-term followup [6]. The combination of clopidogrel and aspirin cohort had fewer patients with MESs, fewer MESs per hour, and fewer strokes compared to patients treated with aspirin alone in the first week after the initial clinical presentation.

A systematic review identified four randomized trials directly comparing oral anticoagulants (OAC) with high international normalized radio (INR) (3.0–4.5) versus antiplatelet therapy in patients with previous TIA or minor stroke of presumed arterial origin [97]. Therapy with OAC was associated with a significantly higher rate of recurrent serious vascular events (1.70, CI 1.12–2.59), with a highly significant increase in major bleeding complication (9.02, CI 3.91–20.84), and a significant increase of recurrent serious vascular events or major hemorrhage (2.30, CI 1.58–3.53) compared with antiplatelet therapy. Therapy with OAC was associated with a significant increase of death from any cause compared with antiplatelet therapy (RR 2.38, CI 1.31–4.32).

Therefore, the best medical treatment of patients with carotid stenosis includes treatment of hypertension, diabetes mellitus, dyslipidemia and homocysteine, metabolic disorders, modification of lifestyle, and statin and antithrombotic therapy. High-dose statins' use may have pleiotropic effects in acute and subacute settings. An LDL goal of <70 mg/dL has been recommended. A blood pressure regimen needs to be carefully selected based on patient's comorbidities and treatment goals. It is recommended that anticoagulation should not be used after noncardioembolic ischemic strokes since high-intensity anticoagulation (INR 3.0–4.5) is more hazardous than effective compared to antiplatelet therapy.

13. Consensus Challenges and Future Directions

Despite numerous RCT studies and significant resources devoted to studying carotid disease, a unified approach to treatment is still far on the horizon. Many contributing factors make consensus elusive. Professional society guidelines in the US (AHA/ASA) [98], New Zealand/Australia [99], and Europe [58] all offer differing and occasionally contradictory recommendations based on regional studies and policies. The Unites States government offers two more diverging opinions in its official statements in regulating medical devices (Food and Drug Administration) and payments for medical services (Centers for Medicare & Medicaid Services). Multiple medical specialties (primary care physicians, interventionals, cardiologists, neurologists, and vascular surgeons) are involved in treating patients with carotid stenosis, each with its own understanding and approach to the subject. The debate between specialties is alive and well [100, 101]. Best medical therapy, interventional stenting techniques, and the surgical knowhow for CEA are rapidly evolving, constantly tipping the risk-benefit ratio in a different direction. The prevalence of the underlying risk factors, the carotid disease itself, and the health care delivery are also different than they were in the past when some of the earlier trials were conducted. Current best medical therapy including statins and antithrombotics in combination with blood pressure and glucose-lowering medications and lifestyle changes has become a powerful tool for reduction of stroke risk in patients with carotid stenosis. New trials comparing CAS, CEA, and best medical therapy are once again needed, with careful selection and followup of the patients using Transcranial Doppler, carotid plaque morphology imaging, and vascular disease burden stratification [74].

14. Conclusion

Carotid stenosis accounts for up to twenty percent of ischemic strokes and TIAs. It is a potentially preventable cause of stroke, and therefore, its detection and management is of an utmost importance. Many treatment modalities exist. Best medical therapy including risk factor management and antithrombotic treatment should be administered effectively. In appropriately selected patients, interventions on carotid arteries should be considered in high-volume CEA and CAS centers with low periprocedural complication rates.

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Review Article

Genetic Susceptibility to Atherosclerosis

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Atherosclerosis is a complex multifocal arterial disease involving interactions of multiple genetic and environmental factors. Advances in techniques of molecular genetics have revealed that genetic ground significantly influences susceptibility to atherosclerotic vascular diseases. Besides further investigations of monogenetic diseases, candidate genes, genetic polymorphisms, and susceptibility loci associated with atherosclerotic diseases have been identified in recent years, and their number is rapidly increasing. This paper discusses main genetic investigations fields associated with human atherosclerotic vascular diseases. The paper concludes with a discussion of the directions and implications of future genetic research in arteriosclerosis with an emphasis on prospective prediction from an early age of individuals who are predisposed to develop premature atherosclerosis as well as to facilitate the discovery of novel drug targets.

1. Introduction

Atherosclerosis is a complex multifocal arterial disease of medium- and large-size arteries involving interactions of multiple genetic and environmental factors. It is characterized by endothelial dysfunction, vascular inflammation, and the buildup of lipids, cholesterol, calcium, and cellular debris within the intima of the vessel wall [1]. This construction results in plaque formation accumulating on the inner walls of arteries, and as the artery walls thicken, the pathway for blood narrows, and this can decrease or block blood flow diminishing oxygen supply to target organs. Atherosclerosis represents a leading global cause of death and disability [2].

Although environmental factors such as diet or smoking play an important role in atherosclerosis development, genetic factors represent consequential determinant of atherosclerotic cardiovascular disease risk. Advances in techniques of molecular genetics have revealed that genetic disorders significantly influence susceptibility to atherosclerotic vascular diseases. A large number of candidate genes, genetic polymorphisms, and susceptibility loci associated with atherosclerotic diseases have been identified in recent years and, their number is rapidly increasing. Genetically controlled arterial wall properties of carotid arteries influence atherosclerosis susceptibility. The genetic risk of atherosclerosis is conferred in part through known metabolic risk factors such as hypertension, dyslipidaemia, and diabetes mellitus, but together, the known risk features appear to be insufficient to explain the hereditary propensity to atherosclerosis. However, these risk factors alone do not account for the entire contribution to risk of atherosclerotic disease. In recent years, substantial interest in the identification of additional genetic risk factors to atherosclerosis inevitably grows.

Several types of genetic investigations and approaches have been conducted in the last years in order to prove genetic impact of atherosclerotic process.

1.1. Monogenetic Heredity. Single-gene (mendelian) disorders represent the most remarkable examples of the genetic implication to atherosclerosis [3]. Several monogenic diseases elevate plasma levels of LDL by impairing the activity of hepatic LDL receptors, which normally clear LDL from the plasma. Familial hypercholesterolemia was the first monogenic disorder shown to cause elevated plasma cholesterol levels. The primary defect in familial hypercholesterolemia is a deficit of LDL receptors, and more than 600 mutations in the *LDLR* gene have been identified in patients with this disorder [4]. The frequency of this genetic defect is 1 in

1,000,000. Patients with heterozygous familial hypercholesterolemia have only 50% of the normal number of LDL receptors in the liver. Heterozygous persons produce half the normal number of LDL receptors, leading to an increase in plasma LDL levels by a factor of 2 or 3, whereas LDL levels in those who are homozygous are 6 to 10 times normal levels. Homozygous persons have severe coronary atherosclerosis and usually die in childhood from myocardial infarction [5]. Furthermore, deficiency of lipoprotein transport abolishes transporter activity, resulting in elevated cholesterol absorption and LDL synthesis. For example, mutations in the *APOB-100* gene, which encodes apolipoprotein B-100, reduce the binding of apolipoprotein B-100 to LDL receptors and slow the clearance of plasma LDL, causing a disorder known as familial ligand-defective apolipoprotein B-100 [6]. Five different mutations located in this region of the *APOB* gene are reported to cause a high-cholesterol phenotype. One in 1000 people is heterozygous for one of these mutations. These patients are diagnosed as familial defective *APOB* (FDB), which is clinically indistinguishable from familial hypercholesterolemia [4, 7]. Mutations in *PCSK9* have recently been shown to result in Mendelian forms of increased LDL-C levels. *PCSK9* encodes NARC-1 (neural apoptosis regulated convertase), a newly identified human subtilase that is highly expressed in the liver and contributes to cholesterol homeostasis [8].

The various functions of ATP binding cassette transporter 1 (ABCA1) became apparent after the discovery in 1999 that mutations in the *ABCA1* gene caused Tangier disease (TD), an autosomal recessive hereditary disorder characterized by severe HDL deficiency, sterol deposition in macrophages and, premature atherosclerosis [9–11]. ABCA1 promotes cholesterol and phospholipid efflux from cells to lipidpoor apolipoprotein (apoA1), the precursor of HDL, and plays a major role in cholesterol homeostasis and reverse cholesterol transport [12]. Sitosterolemia, a rare autosomal disorder, results from loss-of-function mutations in genes encoding two ABC transporters, ABCG5 and ABCG8, which act in concert to export cholesterol into the intestinal lumen, thereby diminishing cholesterol absorption [13, 14].

Very rare hereditary hypercholesterolemia with the prevalence <1 case per 10 million persons is autosomal recessive hypercholesterolemia. The molecular cause is the presence of defects in a putative hepatic adaptor protein, which then fails to clear plasma LDL with LDL receptors [15]. Mutations in the gene encoding that protein elevate plasma LDL to levels similar to those seen in homozygous familial hypercholesterolemia [16].

1.2. Polygenetic Heredity. However, in the majority of cases it is not possible to identify single-genetic determinants, and it is likely that several major genes may contribute to the manifestation of the disease. Most of our success in understanding the genetic basis of common forms of atherosclerosis has come from studies of candidate genes, genes tested for their role in atherosclerosis *in vitro*, *in vivo,* and in association studies [17]. Within the general population, polymorphisms within genes in lipid metabolism, inflammation, and thrombogenesis are probably responsible for the wide range of atherosclerotic diseases. A good example of a candidate gene is *apolipoprotein E* (*apoE*). Yet in the 1970s, Utermann et al. identified the common genetic differences (polymorphisms) of *apoE*, and subsequent studies suggested associations with plasma cholesterol levels and type III hyperlipidemia [18]. The *apoE* gene is located at chromosome 19q13.2. Among the variants of this gene, alleles *E2*, *E3*, and *E4* constitute the common polymorphism found in most populations. Of these variants, *apoE3* is the most frequent (>60%) in all populations studied [19]. The role of *apoE* in plasma lipid metabolism has been studied intensively [20, 21]. The polymorphism has functional effects on lipoprotein metabolism mediated through the hepatic binding, uptake, and catabolism of chylomicrons, chylomicron remnants, very low-density lipoprotein (VLDL), and high-density lipoprotein subspecies [22]. Type III hyperlipidemia is an interesting example of a genetic interaction. Almost all individuals with this uncommon hyperlipidemia are homozygous for the *E2* allele, but most individuals who are homozygous for the *E2* allele do not have the disorder [23]. We can summarize from these observations that other genetic or environmental interactions are required to produce hyperlipidemia in addition to homozygosity for the *E2* allele. Another interesting example is *CYBA* gene polymorphisms, including the *C242T* (rs4673) and the $-930^{A/G}$ (rs9932581) which are implicated in the process of atherosclerosis from a very early stage to the clinical phase of cardiovascular diseases [24, 25]. The *CYBA 242T* allele consistently shows a protective association against the chronic inflammatory process presenting as impairment of endothelial function and the development of coronary artery disease [26]. In addition, the *CYBA* $-930^{A/G}$ gene polymorphisms might modify the effect of smoking and hypertension on early structural alterations on the arterial wall [27, 28].

An alternative genetic approach is conducting genomewide linkage studies to find atherogenesis-regulating quantitative trait loci (QTL). In addition, recently, the availability of whole genome sequences in humans and mice, especially the abundant SNP and haplotype information, has made it possible to perform genomewide association studies in order to identify responsible genes. Genomewide association is thought to be more powerful than genomewide linkage analysis to detect common alleles at a locus, but it is less powerful if the extreme phenotypes of interest are due to the segregation of many relatively rare alleles at that locus [29]. Recent experimental studies have revealed certain genetic background of LDL oxidation in the vessel wall, initiating the formation of an early fatty streak lesion consisting mainly of macrophages. Subsequently, a series of lipid-modifying enzymes have been identified in the vessel wall, capable of generating inflammatory mediators, which can further stimulate plaque growth by enhancing cellular influx [30]. One of the lipid-modifying enzymes that have generated a lot of interest recently is 5-lipoxygenase (5LO) which is produced by macrophages and is an important enzyme in the conversion of the lipid molecule arachidonic acid into leukotrienes. Hence, *5LO* may serve as a gene driving chronic inflammation and thereby the progression of atherosclerotic plaques.

In line with this hypothesis, *5LO* has recently been shown in genetic and knockout studies in mouse to play a major role in promoting plaque growth [31]. Based on these findings in mice, polymorphisms in the promoter ofso *5LO* have been studied in patients and were correlated with the progression of lesion formation based on measurements of the thickness of the vessel wall [32]. These seminal observations place genes interacting with *5LO* in a prime position as candidate genes for coronary artery disease (CAD). Among those genes is *ALOX5AP*, encoding 5LO-activating protein (FLAP). A recent study by Helgadottir et al. from deCODE genetics elaborated successfully on this clinical question, by performing a linkage study in a large set of Icelandic families [33]. Sum of 296 families were studied, including 713 patients with myocardial infarct, generating a highly susggestive linkage peak to a locus on chromosome 13q.

Subsequently, this chromosomal region was further investigated by association analysis with 120 microsatellite markers in a study including around 800 cases and controls. This screen resulted in the detection of a haplotype spanning two genes including *ALOX5AP*. The study by Dwyer et al. has also investigated the role of gene-environment interactions by studying the effect of diet on *5LO* promoter polymorphisms [32]. First, a subgroup of carriers of a particular promoter variant was identified, which showed increased carotid artery intima-media thickness. Second, it was shown in these allele carriers that dietary arachidonic acid, the substrate for *5LO*, increased the production of inflammatory mediators, as compared to subjects that were fed a "marine" diet with N-3 fatty acids. Another gene identified in human linkage studies of myocardial infarct, named, *myocyte-enhancing factor 2A* (*MEF2A*), is expressed in endothelial cells of coronary arteries. A 21-base pair deletion was identified in exon 11 in all ten living members within the family, and not in family members and an additional 119 individuals with normal angiograms, strongly suggesting that the deletion is responsible for myocardial infarct in this large family [34]. *MEF2A* mutations may be a rare cause of myocardial infarct because they are present in less than 2% of a US population of 207 patients [35]. Furthermore, adiponectin has thought to have variety of metabolic effects on obesity, insulin sensitivity, and atherosclerosis. To identify genes influencing variation in plasma adiponectin levels, Ling et al. performed genomewide linkage and association scans of adiponectin in two cohorts of subjects [36]. The genomewide linkage scan was conducted in 789 family members of Turkish and southern European and 2,280 northern and western European. A whole genome association (WGA) analysis was carried out on approximately 1,000 subjects with dyslipidemia and 1,000 overweight subjects with normal lipids. In conclusion, these results support an effect of DNA variation at the *ADIPOQ* locus (the adiponectin structural gene) influencing plasma adiponectin levels. However, the degree to which DNA sequence variants at this locus influence health and disease remains to be seen. Furthermore, these analyses indicated that SNPs at the *ADIPOQ* locus were the most strongly associated with adiponectin variation throughout the entire genome.

2. Genetic Models for Atherosclerosis

The use of genetic models has greatly assisted investigations of the natural history, mechanisms, and potential therapy for atherosclerosis. In the past several years, the advent of molecular techniques has enabled investigators to produce additional novel genetic models of disease that have further enhanced the study of vascular biology and medicine. Particularly valuable models in vascular diseases investigations are inbred genetic strains, transgenic animals, gene targeting by homologous recombination, and *in vivo* gene transfer [37]. Distinct types of experimental design that has used inbred mouse strains which differ in their predisposition to atherosclerosis has revealed more precise whether differences in lipid profiles were restricted to particularly dietary terms or reflect an underlying genetic factors that contribute to atherosclerotic susceptibility. The most often used mouse models of atherosclerosis were a high-fat model in which inbred mice are fed a high-fat and cholesterol diet, *ApoE*-deficient mice fed either chow, a Western diet, and *Ldlr*-deficient mice fed either a Western diet or a high-fat and cholesterol diet [38]. In order to exempt dyslipidemia as a crucial factor for the development of atherosclerosis, one of the first investigators in this particular area, has used *apoE*-deficient mice strains, extensively studied mouse model of atherosclerosis that develops atherosclerosis on a chow diet [39–41]. The authors bred *apoE*-deficient mice that had C57BL/6J or strain susceptible to atherosclerosis and C3H/HeJ, a prototypical atherosclerosis mouse resistant strain. The study demonstrated that in mice with an *apoE*-deficient background that were fed a chow diet, there were not major differences in plasma lipids. Nevertheless, The same study has revealed that mice with the C57BL/6J background developed markedly more atherosclerosis than mice with C3H/HeJ background, indicating that differences in atherosclerosis susceptibility do not reside in differences in plasma lipid level. There are variety of cell types involved in atherosclerotic process, including endothelial cells, smooth muscle cells, monocytes, macrophages, and T lymphocytes. Genetic variation that affects some of these cell types could influence susceptibility to atherosclerosis. In a second series of experiments, Shi and collaborators have analyzed the cellular compartments that have a role in genetic susceptibility to atherosclerosis in mice [39]. They have generated chimeric mice by performing bone marrow transplantation of C57BL/6J bone marrow into C3H/SW mice and C3H/SW marrow into C57BL/6J mice. They have proved that C56BL/6J mice that received C3H/SW bone marrow were not protected from atherosclerosis, and C3H/SW mice that received C57BL/6J bone marrow did not develop increased atherosclerosis. General conclusion of this study is that atherosclerosis development was determined by the genotype of the host rather than the genotype of bone marrow donor, suggesting that genetic differences in atherosclerotic susceptibility are not conditioned by the hematopoietic cells. Some novel studies have yielded similar results [42]. Sequentially *ex vivo* studies of endothelial cells isolated from C57BL/6J mice showed substantial induction of a limited panel of proinflammatory genes (*MCP-1*, *M-CSF*, *VCAM-1*, and *heme*

oxygenase-1) in response to minimally modified LDL (MM-LDL), where endothelial cells from C3H/HeJ mice did not. These results suggest the plausibility that the differences in atherosclerotic susceptibility between these two mice strains relate to genetic difference in the proinflammatory response of endothelial cells to a specific inflammatory stimulus, MM-LDL.

3. Conclusions

Beyond the traditional risk factors, less well-established risk factors for atherosclerosis development are being firmly evaluated in recent years. The outcome of these efforts will not only unveil the molecular basis of atherosclerosis but also allow prospective prediction from an early age of individuals who are predisposed to develop premature atherosclerosis as well as to facilitate the discovery of drug targets and individualized medications against the disease. Our understanding of the pathogenesis of this process has evolved in the last few decades, guiding our efforts to find treatments. The relatively recent appreciation that inflammatory response plays a key role in atherogenesis implies that inhibiting inflammation may provide new anti-atherosclerosis therapy.

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Review Article

Advanced Asymptomatic Carotid Disease and Cognitive Impairment: An Understated Link?

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Advanced carotid disease is known to be associated with symptomatic cerebrovascular diseases, such as stroke or transient ischemic attack (TIA), as well as with poststroke cognitive impairment. However, cognitive decline often occurs in patients with advanced carotid stenosis without clinically evident stroke or TIA, so it is also suspected to be an independent risk factor for dementia. Neurosonological methods enable simple and noninvasive assessment of carotid stenosis in patients at risk of advanced atherosclerosis. Cognitive status in patients diagnosed with advanced carotid stenosis is routinely not taken into consideration, although if cognitive impairment is present, such patients should probably be called symptomatic. In this paper, we discuss results of some most important studies that investigated cognitive status of patients with asymptomatic advanced carotid disease and possible mechanisms involved in the causal relationship between asymptomatic advanced carotid disease and cognitive decline.

1. Introduction

While detrimental effects of stroke on cognitive functions have been well documented in the literature, the mechanisms linking advanced carotid disease and impaired cognitive status in patients without symptomatic cerebrovascular incidents are less clear.

Advanced carotid disease is associated with the presence of multiple vascular risk factors, which most often include arterial hypertension, dyslipidemia, cigarette smoking, diabetes, and older age [1–3]. The same risk factors were shown to be associated not only with vascular dementia, but also with neurodegenerative dementia, importantly with Alzheimer's disease [4]. The vascular hypothesis of Alzheimer's disease provides substantial evidence that vascular risk factors play a critical role in the development of cognitive impairment and clinically evident dementia during aging [4]. Vascular dementia and Alzheimer's disease in their pure forms are two ends of a pathologic continuum [5]. However, many studies during the last decade implicated the overlap of their pathologies. It was shown that a substantial proportion of brains who meet neuropathological criteria for Alzheimer's disease also demonstrate lesions typical for vascular pathology, such as cerebral amyloid angiopathy, microvascular degeneration, and periventricular white matter lesions [6, 7]. Results of the seminal "Nun Study" that followed 102 elderly nuns until postmortem showed higher prevalence of clinically expressed dementia in those who met neuropathological criteria for Alzheimer's disease and simultaneously had brain infarcts [8]. Furthermore, in a 3-year follow-up study of stroke patients who were not demented before the stroke, one-third of the patients who developed poststroke dementia were diagnosed as suffering from Alzheimer's disease [9]. There is now substantial and growing evidence from studies of epidemiology, pharmacology, neuroimaging, clinical medicine, microscopic anatomy, and cellular-molecular biology to hypothesize that sporadic Alzheimer's disease is induced or accelerated by impaired cerebral perfusion [10–14].

There are few proposed mechanisms aiming to explain the link between carotid stenosis and cognitive impairment. Carotid stenosis itself could directly cause decreased cognitive functioning or it may act as a marker of cerebral atherosclerosis due to the presence of conventional vascular risk factors in such patients [15]. Preexistent silent cerebral ischemia, lacunar infarcts, or preclinical Alzheimer's disease

in patients with carotid stenosis may also influence their neuropsychological performance.

According to population studies, carotid disease of various degree can be found in more than 75% of men and in more than 62% of women older than 65 years of age. Total prevalence of carotid disease over 50% in the same age group is 7% in men and 5% in women [16]. Repercussions of carotid stenosis/occlusion on intracranial hemodynamics have been recognised by Spencer and Reid [17]. According to their study and the known "Spencer curve," in mild and moderate carotid stenosis brain blood perfusion remains stable, until high-grade carotid stenosis occurs [18]. Besides cerebral microembolisation, proposed mechanisms of cognitive impairment in individuals with advanced carotid disease thus include chronic hypoperfusion.

During normal aging process, cerebral blood flow modestly diminishes and can decrease up to 20% by the age of 65 [19, 20]. Aging is often accompanied by increase in minor memory problems which are followed by more pronounced cognitive impairment [21, 22]. Since cognitive decline during aging is commonly prodromal to Alzheimer's disease or to vascular dementia (VaD), treating disease harbingers, such as cerebrovascular and cardiovascular risk factors, may be one of the most important prevention strategies aimed at either dementia [14]. Mild, diffuse brain damage related to chronic hypoperfusion may affect the brain's ability to process information quickly and efficiently even in the absence of discrete white matter lesions [23]. Not surprisingly, carotid stenosis in asymptomatic individuals can produce a reduction of subtle cognitive abilities involving the function of the hemisphere ipsilateral to the carotid stenosis [4].

Despite the possible poor cognitive outcome, in the absence of stroke or TIA, patients diagnosed with advanced carotid disease are considered asymptomatic and their cognitive status is not routinely assessed. Decreased cognitive functioning in those patients so far has not been recognised as possible factor that could influence therapeutic decision related to the carotid disease.

2. Assessment of Cognitive Functioning in Patients with Carotid Stenosis

A number of previous studies suggested that in stroke/TIA-free patients severe carotid disease might be associated with subtle cognitive changes, but results of studies have not been consistent [3, 16, 24–26]. The principal reasons for somewhat conflicting results are differences in study designs, mostly small sample size, and nonconsistent selection of cognitive tests performed. Only few studies of cognitive status, mostly on a small number of patients, were conducted in patients with advanced carotid disease [3, 16, 25]. Patients with carotid disease were shown to have significantly worse scores on cognitive tests than matched controls. Cognitive problems experienced by patients with advanced carotid disease are mostly subtle and not severe enough to interfere with their daily activities, therefore remaining unrecognized by their closest family or friends [15]. Such changes are insufficient to meet criteria for dementia and therefore correspond to mild cognitive impairment (MCI).

Although modern cognitive neuropsychology has developed sophisticated tests in order to assess the different cognitive functions, most studies of cognitive impairment in patients with carotid disease used simple Mini-Mental-State Examination (MMSE) as a routine cognitive measure [27]. Others used more extensive neuropsychological instruments in order to analyze cognitive domains [16, 24].

The Mini-Mental State Examination (MMSE) remains the most commonly applied screening test in clinical practice. It includes five cognitive domains (with a maximum score of 30 points); however it does not contain much capacity to test frontal/executive or visuospatial functions. Another problem with MMSE is its low sensitivity in detecting subtle, early-phase cognitive changes so it is not recommended for screening for MCI [28, 29]. Patients with discrete decline of cognitive status (such as patients with carotid disease) often find tasks involved in MMSE insultingly simple.

Neuropsychological testing with standardized cognitive tests is time consuming and difficult to implement in busy clinical settings; therefore some more recent studies of cognitive functioning in patients with carotid disease successfully used Montreal Cognitive Assessment (MoCA) as a simple and brief screening tool with high sensitivity for subtle cognitive impairment [29–31]. The MoCA is a 30-point test involving eight cognitive domains in which results below cut-off score indicate cognitive impairment. A workshop group of NIH and Canadian Stroke Consortium proposed the selected MoCA subtests, including a 5-word immediate and delayed memory test, a 6-item orientation task, and a 1-letter phonemic fluency test (the letter F), for a routine use in a five-minute protocol aiming to assess individuals with possible vascular cognitive impairment [32].

Due to the lack of inclusion of frontal lobe tests in commonly applied MMSE, it appears that MoCA could be recommended as more appropriate tool for the assessment of cognitive changes in patients with carotid stenosis. Although the aim of routinely used screening tests is to detect the presence of cognitive impairment and not to distinguish between its causes, patients with predominantly vascular etiology of subtle cognitive impairment and probable significant frontal lobe pathology could be tested more accurately using MoCA as it contains both subtests with executive function and with memory.

Despite the growing number of studies that have investigated cognitive impairment in patients with carotid disease, correct pathophysiological interpretation of the underlying mechanisms remains unclear. The relationship of decreased cognitive status and asymptomatic carotid stenosis was published from the observations from the Tromsø study, including results from a heterogeneous group of patients with bilateral carotid stenosis of ≥35% [3]. Their performances in a battery of extensive neuropsychological tests were compared with results from subjects without carotid stenoses. In this study, patients with carotid disease achieved significantly lower levels of performance in several subsets of cognitive tests. In a large number of 4006 asymptomatic patients participating in the Cardiovascular Health Study

and diagnosed with various degrees of carotid stenosis, Johnston et al. used the modified MMSE in order to evaluate the cognitive status [16]. This study found that advanced stenosis (≥75%) of the left internal carotid artery was associated with a cognitive impairment and cognitive decline during the followup, but no significant correlation was detected for right-sided stenoses. As the correlation of a left carotid stenosis and reduced cognitive functioning was observed even in participants without evidence of cerebral infarction on MRI, it is likely that an asymptomatic carotid stenosis may be an independent risk factor for a cognitive impairment. In our pilot study, as well as in a study performed on a larger number of 70 patients diagnosed with advanced carotid stenosis or occlusion, we have shown cognitive impairment detectable by MoCA, but not by MMSE [30, 31]. MoCA subtests analysis in patients with carotid disease demonstrated significantly lower subscores in multiple domains, including visuospatial and executive functions, attention, and delayed recall [31]. Similar results were achieved in the Tromsø study where patients with asymptomatic carotid stenosis scored worse at attention, memory, psychomotor speed, and motor functioning [3]. However, comparing of studies results presents a major problem due to differences in cognitive testing methods. Silvestrini et al. reported on reduced cognitive scores at verbal fluency in patients with severe left-sided ICA stenosis while those with right-sided stenosis performed worse at abstract reasoning and executive functions [33]. This is similar to the results from Bossema et al. [34]. According to Komulainen et al. the degree of carotid artery stenosis correlates with the extent of cognitive loss [35]. In the Framingham Offspring study, in a followup of subgroup of 1971 participants with carotid disease, carotid disease was found to be associated with significant poorer performance on cognitive tests in asymptomatic subjects without signs of silent cerebral infarcts or white matter hyperintensities on brain MRI [36]. Study that included Canadian First Nations population who have a larger proportions of vascular risk factors showed contradictory results as individuals with left-sided carotid disease were less likely to have cognitive impairment. However, cognitive measurements were performed using only the "Trial Making Test" and only the small proportion of patients included were diagnosed with advanced carotid disease [37].

3. Conclusions

The effect of carotid stenosis on cognitive functioning remains far from being unambiguously explained. Systematic and carefully designed prospective studies with defined and consecutive inclusion of patients screened for concomitant disorders that might influence cognitive functioning, the use of uniformed neuropsychological testing, and appropriate neuroimaging (MRI) assessment in patients with carotid disease are still lacking. However, despite differences in studies results, we believe that asymptomatic advanced carotid stenosis should be considered an independent risk factor for the decline in cognitive functioning. If asymptomatic advanced carotid stenosis causes cognitive impairment, the threshold for operative treatment may change and reduction in risk for cognitive impairment may be an important benefit of interventions to treat carotid disease [15]. For the time being, effective treatment of vascular risk factors remains the most important clinical recommendation for stroke/TIA-free patients with carotid stenosis. As cognitive problems in stroke/TIA-free patients with carotid disease are so far often underestimated in routine clinical practice, the use of MoCA could aid in better recognition of this problem.

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Clinical Study

Do Clopidogrel Nonresponders Have an Increased Risk of Adverse Events during Supra-Aortal Angioplasty and Stenting?

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Objective. The aim of the present study was to correlate new periprocedural diffusion-weighted imaging (DWI) lesions during stenting of supra-aortal arteries with the level of platelet inhibition using point-of-care analysis. *Background*. Cardiological studies have shown that patients undergoing coronary PTA have a significantly elevated risk of severe thrombotic complications if patients show insufficient inhibition of platelet function. *Methods*. From August 2008 to June 2009, 44 patients with an indication of supra-aortal angioplasty and/or stenting were prospectively enrolled. Platelet reactivity was tested using a Multiplate device (Dynabyte). These patients underwent MRI before and after the intervention to determine the prevalence of new DWI lesions. The primary endpoint was the prevalence of DWI lesions; the secondary endpoint was clinical status until discharge from hospital. *Results*. There was no significant relationship between the primary endpoint and the degree of platelet function. Patients with high platelet reactivity showed the same amount of periprocedural complications as patients with sufficient inhibition of platelets. *Conclusions*. Clopidogrel did not have a protective effect on periprocedural complications, nor did it decrease the number of silent DWI lesions after the procedure. The predescribed strong relationship between high platelet reactivity and early post-procedural adverse events was not observed in our cohort.

1. Introduction

Several studies have shown that patients with a low response rate to clopidogrel, measured by in vitro function of platelets, have a significantly increased risk of early stent thrombosis after percutaneous transluminal coronary angioplasty (PTCA) and stenting compared to patients with sufficient inhibition of platelets [1–7]. The aim of this prospective trial was to examine if there is a relationship between the response to clopidogrel and clinical adverse events after supra-aortal percutaneous transluminal angioplasty (PTA) and stenting as well as the amount of newly occurred silent diffusion-weighted imaging (DWI) lesions.

The DWI lesion load is a known indicator of microembolic events after neurovascular interventions [8, 9], so it was the primary target variable. Platelet reactivity was tested using a Multiplate analyzer from Dynabyte GmbH (Munich, Germany). This device is based on multiple electrode aggregometry. One study showed a correlation between a low response to clopidogrel measured by Multiplate and clinically adverse events after supraaortal stenting [2]. In the present study, we additionally used MRI to correlate the DWI lesion load with the platelet reactivity.

2. Patients and Methods

From August 2008 to June 2009, 44 patients with stenoses of supraaortal vessels and indication for treatment were prospectively enrolled (Table 1). Inclusion criteria were

Table 1: Vessels that underwent intervention.

Total	44
Symptomatic	33 (=75%)
Stroke	26 (=59%)
TIA	7 (=16%)
Asymptomatic	11 (=25%)
Location	
Carotid artery	
Extracranial stent	35
Intracranial stent	1
Extracranial PTA	1
Vertebral artery	
Extracranial stent	4
Intracranial stent	1
Basilar artery stent	1
Middle cerebral artery PTA	1

symptomatic stenoses of at least 50% or asymptomatic high-grade (>80%) carotid artery stenoses. Patients with contraindications for MRI were excluded. 42 patients underwent PTA and stenting, and 2 patients received PTA only. 43 patients were treated with ASS 100 mg and clopidogrel 75 mg daily at least three days before the intervention, one received a loading dose of 300 mg clopidogrel and 500 mg ASS just before the intervention because an urgent treatment was necessary. Prior to the procedure, blood samples were taken directly from the arterial sheath with sample tubes equipped for the Multiplate system, which contain hirudine as anticoagulant. The test results were available after the procedure. Afterwards patients were monitored at the Stroke Unit of our institution. They underwent clinical examination to assess if new neurological deficits had occurred.

2.1. MRI. MRI was undertaken on a 1.5-T Siemens Sonata scanner (Siemens AG, Erlangen, Germany). DWI were acquired using our standard protocol. This comprised isotropic echoplanar sequences with a repetition time of 4,100 ms; echo time of 104 ms; field of view of 210 mm; a matrix of 128×128; number of excitations $= 2$; slice thickness of 6 mm; with b values of 0, 500, and 1000 s/mm^2. Images were compared with preprocedural images by two experienced neuroradiologists unaware of the clinical outcome.

2.2. Blood Analyses. Blood samples were immediately transferred to the Department of Hemostaseology of our institution. Testing was undertaken in <3 h on the Multiplate analyzer [2, 5]. This device is based on impedance aggregometry; in particular, adenosine diphosphates- (ADP-) induced aggregometry is suitable for detecting the response to clopidogrel.

Three hundred microliters of the patients' whole blood samples, anticoagulated by hirudine, were pipetted into a test cell containing 2×2 electrodes, as well as 6.4 μmol/L ADP. Aggregation of platelets continuously increased the electrical resistance between the electrodes over the test period of 6 minutes per sample. This value was shown as aggregation units (AU) over time in a graph. The area under the curve, given in arbitrary units (U) represented the platelet aggregation and provided the test results.

The test cell contained two pairs of electrodes, so the results were already the mean value of two simultaneous measurements. As suggested in [10], the patients' platelet reactivity has been defined as "high on treatment" if the results exceeded 468 AU*min in response to ADP.

2.3. Statistical Analysis. Acquired data were analyzed using SPSS software (SPSS, Chicago, IL, USA).

The patients' platelet aggregation values, which were given in arbitrary units (U), were correlated with the amount of new DWI lesions by using a nonparametric test for independent values (Mann-Whitney test).

Additionally, a 2 by 2 table consisting of platelet activity status (according to the consensus [10]) and DWI lesions yes/no has been created and analyzed with a chi-square test.

3. Results

The values from the ADP-induced aggregometry ranged from 3 units to 88 AU (mean, 34.6 AU; standard deviation (SD), 20.24) (Figure 1). The distribution of ADP test values was similar in patients with and without new DWI lesions (Figure 3).

In these cases in which aggregation exceeded 468 AU*min, platelet reactivity was defined as "high." This resulted in 11 patients with high platelet reactivity out of 44 enrolled patients (25%).

After the procedure 16 out of 44 patients (36%) showed new DWI lesions. These were silent DWI lesions in 13 cases. Two patients suffered a periprocedural stroke, and one patient suffered a reperfusion trauma. The ADP-test values of these cases were 18, 24, and 64 units. That is, one of the two patients that suffered a periprocedural stroke showed high platelet reactivity whereas the other case as well as the patient with reperfusion trauma had sufficient suppression of platelets.

Of the 11 patients with insufficient platelet suppression who underwent MRI, 4 (36%) showed new DWI lesions (Figure 2). Of 33 patients with sufficient platelet inhibition, 11 (36%) had DWI lesions after the procedure (Figure 2). A statistically significant relationship between ADP-test values respectively platelet reactivity and MRI evidence of new DWI lesions ($P = 0.893$ from the Mann-Whitney test, $P = 0.635$ from the chi square test) or between outcome was not observed. However, three clinically affected patients is not sufficient for a statistical conclusion.

4. Discussion

Clopidogrel resistance is dependent upon the genetic background of the patient. Cytochrome CYP2C19 is an important enzyme that metabolizes clopidogrel. Individuals with an impaired allele (which seems to represent about one-third of the population) show ≤30% less active metabolism of

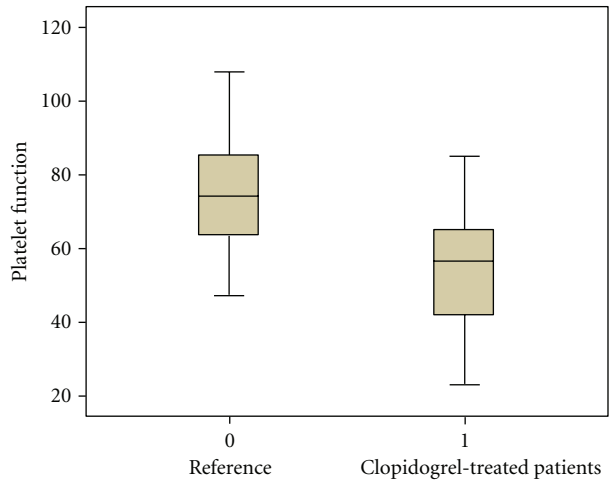

Figure 1: Distribution of ADP values from the study population, including three patients that received a loading dose, and those from the reference group ("Reference" in this figure means a combined reference range of healthy male and female blood donors).

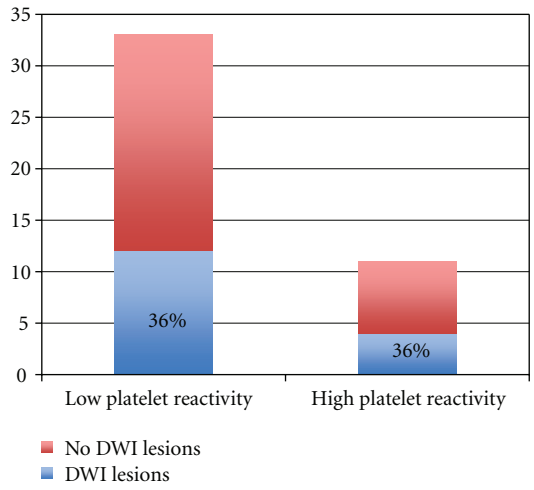

Figure 2: Shows the number of patients with (blue) and without (red) DWI lesions among responders and non-responders.

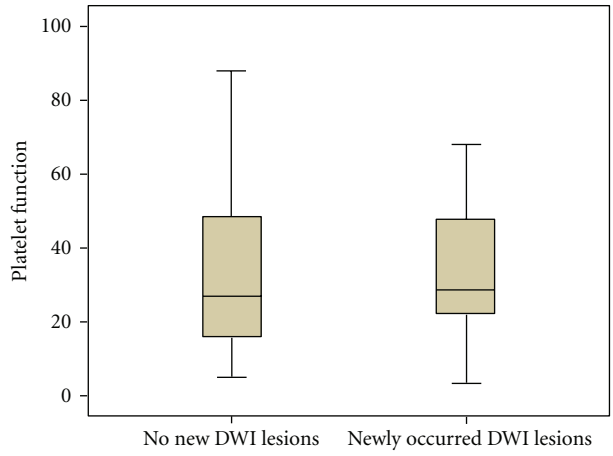

Figure 3: Boxplots showing the distribution of ADP values for patients with (right) and without DWI lesions (left) after the procedure.

clopidogrel in plasma. Furthermore, carriers of such an allele showed an elevated risk of death by cardiovascular causes, infarction, or stroke of 53% in the TRITON-TIMI 38 trial (Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel-Thrombolysis in Myocardial Infarction) [11].

Studies focusing on the relationship between an inadequate response to clopidogrel and adverse events in cardiovascular or neurovascular interventions have shown a significant (or at least a considerable) connection [1–7, 11]. Most of these studies concerned cardiovascular interventions. Because of the differences in supra-aortal interventions with respect to stent types, vessel sizes, and flow characteristics, the results of these studies should not be readily transferred to other vessel territories. Furthermore, a common complication such as in-stent thrombosis shortly after PTCA is extremely rare in supra-aortal stenting, therefore the target variables in both issues must be different. Müller-Schunk et al. [2] were the first to analyse the correlation between clopidogrel resistance and procedural complications in supra-aortal stenting. They found a significant relationship between non-response to clopidogrel and the clinical outcome.

In supra-aortal stenting, attention must be focused on periprocedural strokes or the more frequently appearing cerebral DWI lesions (which are usually clinically unremarkable). These DWI lesions could help in the highly sensitive detection of microembolic events.

If we compare a subgroup of our study population who received unprotected carotid artery stenting (CAS, $n = 35$), 11 patients (31%) showed post-procedural DWI lesions. In the literature, the percentage of DWI lesions after unprotected CAS varies from 22% to 67% [8, 9, 12].

In contrast to previous studies, a relationship between low response to clopidogrel and thromboembolic events was not observed. These thromboembolic events are represented by DWI lesions or peri- and post-procedural ischemic strokes.

Of the 16 patients who showed MRI evidence of new DWI lesions, three had clinically noticeable symptoms (as mentioned above). Studies that rely only on the patients' clinical outcomes will only detect small part of the adverse events.

If patients undergo carotid stenting or vertebral stenting with high platelet reactivity without negative consequences, the need for screening of clopidogrel non-responders before supra-aortal stenting must be questioned. Further studies should show if low response to clopidogrel leads to a higher rate of stent restenosis.

The non-response to clopidogrel has been investigated in various studies. The definition of "non-response" in the literature is inconsistent, making interstudy comparison difficult. The effect of clopidogrel responsiveness in a patient population forms a Gaussian distribution, so De Miguel [13] suggested measurement of the degree of platelet inhibition as

a nominal variable instead of dividing patients into groups of responders or non-responders. In [10], Bonello et al. suggest to focus on the absolute level of platelet reactivity instead of clopidogrel responsiveness. They present cut-off points for several platelet aggregation analyzers which help to stratify thromboembolic risks.

There are several possible alternatives for clopidogrel low-responders undergoing coronary interventions, such as increasing the loading dose from 300 mg to 600 mg, which leads to a higher and stronger ADP-induced platelet inhibition [14]. Another possibility is to try different agents such as prasugrel and ticagrelor. The latter recently showed the characteristic of significantly affecting platelet inhibition in clopidogrel non-responders in the RESPOND study [15].

5. Study Limitations

This study tries to correlate patients' platelet aggregation with the amount of new dwi lesions which is a common indicator for the safety of neurovascular interventions. The results differ from preceding studies regarding the relation to adverse events. However, 44 patients may not be enough to evaluate a statistical relation. Especially the amount of three clinically affected patients is not enough for a conclusion. Further studies with more participants are needed for verification.

6. Conclusion

In contrast to the preceding studies, we found no relationship between the patients' response to clopidogrel and adverse events represented by microembolism, detected by mri imaging. So the need for point-of-care testing of platelet function prior to angioplasty and stenting of supraaortal arteries must be questioned.

Acknowledgments

The authors state that there is no conflict of interest. The study had been approved by the ethics committee. Patients gave their informed consent prior to inclusion in the study.

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Review Article

Asymptomatic and Symptomatic Carotid Stenosis: An Obsolete Classification?

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Since many years, clinical decisions about the management of patients with carotid stenosis have been based on the distinction between "asymptomatic" and "symptomatic" presentations. This was also reflected by the design of previous studies on the surgical versus conservative treatment and of current studies on interventional treatment versus surgery. Both terms, however, only address different phases of activity of the one and the same condition and blur the significant message that carotid stenosis is a most important marker of systemic atherosclerosis, which is accompanied by a much higher risk of cardiovascular events rather than stroke. As a consequence, early diagnosis and followup during best medical treatment, life-style management, regular cardiovascular assessment, and good control of all vascular risk factors should be recommended in all patients with carotid stenosis—whether identified in the long-lasting "silent" or short-lasting "vulnerable" period lasting only a few weeks after cerebral ischemia. Patients in this short time window benefit from additional carotid intervention, under the condition of an individually favorable benefit-risk ratio ("individual vulnerability").

1. Introduction

Carotid stenosis is common, especially in patients with vascular risk factors or with coexistent pathology of coronary or peripheral arteries [1, 2]. Since many decades, the classification between "symptomatic" and "asymptomatic" carotid stenosis has dominated the management of affected patients. This distinction corresponds to the design and results of previous clinical trials on surgical versus medical treatment of carotid stenosis for stroke prevention as well as of current studies comparing surgical with interventional procedures (angioplasty with or without stent) [3–8].

However, careful review of these studies, as well as long-term natural history observations for many decades, shows that carotid stenosis is much more sensitive as marker of systemic atherosclerosis than a cause of stroke [9, 10]. Only within a small time window, when carotid stenosis shows progression and high plaque vulnerability, either in the presence of or without clinical signs or symptoms, there is a higher incidence of stroke.

First descriptions of carotid stenosis related to cerebrovascular events date back to T. Willis (1621–1675) and J. J. Wepfer (1620–1695). However, it was not before the 1950s when surgical interventions in the acute phase of stroke or for secondary stroke prevention were reported by DeBakey and Eastcott [11, 12].

In the 1980s, with the introduction of vascular ultrasound, the true dimensions of carotid disease became apparent. Quite unsurprisingly, a high number of patients were diagnosed with a so-called "asymptomatic" carotid stenosis [2], but the prognosis and management of these patients were totally uncertain. Studies performed since then showed already very early an eminent contrast between a very low incidence of stroke (1-2%/year) and a rather high cardiovascular morbidity and mortality (5–10%/year), in particular in patients with clinically silent presentation (so-called "asymptomatic carotid disease") [9, 10]. The same is true for the "symptomatic" carotid stenosis too, but with one difference: there is a higher risk of stroke (10–20%) within the first 14–28 days following a cerebrovascular event (TIA

or stroke) [13]. After this vulnerable period, the stroke risk declines to that of the "asymptomatic" carotid stenosis.

2. Pathophysiology of Carotid Stenosis

In most cases, carotid stenosis is the result of atherosclerotic changes of the vessel wall. Other causes (e.g., dissection, often observed in patients under 55 years of age) only account for a small fraction of carotid lesions.

Atherosclerosis is a progressive disease with periods of stability and sometimes reparation, which begins with endothelial damage of the vessel wall already in infancy and adolescence. There are many factors promoting this damage: arterial hypertension, elevated blood lipid levels and problems of cholesterol or glucose metabolism, reduced release of nitrogen oxide, but also genetic components such as expression of vascular cell adhesion molecules (VCAM-1) [14–19]. Functional disturbance of laminar blood flow, particularly in arterial junctions, promotes the accumulation of LDL and consecutively lead to remodeling of the vessel wall. Monocyte migration and adhesion in the region of endothelial damage then follows, induced by proinflammatory cytokines [20, 21]. Identification of these mechanisms as biomarkers for the activity of atherosclerosis has confirmed the association of general risk factors with vascular degeneration but unfortunately did not contribute much to the sensitivity/specificity of a biomarker to predict the individual stroke risk in a "vulnerable patient."

3. Diagnostic Methods

Significant improvements in diagnostic methods used for detection of carotid stenosis have influenced our knowledge about causes, spontaneous course, and risks of this disease. Initially, auscultation of carotid bruits was the gold standard in clinical practice. Systematic evaluation of this method, however, revealed a very low specificity and sensitivity [2]. Digital subtraction angiography (DSA) was for a long time the only method able to reliably identify a carotid stenosis, but it was only used in preselected patients because of its invasive nature and the risks associated with the procedure. It was the introduction of vascular ultrasound, with its noninvasive nature and good visualization, that made better carotid screening possible in a large number of patients, including many in clinically silent periods. Nowadays, highly developed ultrasound systems allow visualization of vessel wall changes in early stages, various grades of vessel stenosis and of intraluminal flow phenomena, as well as analysis of plaque texture and structure in real time and 3D or even 4D [22]. An experienced user can perform a highly sophisticated and reliable examination, which can be repeated during long-term monitoring without any risks or complications for the patient. Such technologies have recently been introduced in large clinical trials (e.g., PERFORM study) [23]. Ultrasound imaging can be used for screening and control of intima-media thickness (IMT), plaque morphology, grade and progression in clinical practice, as well as in clinical and epidemiological studies [24, 25]. Today, DSA is only rarely necessary—as opposed to the time when NASCET [3], ECST [4] and ACAS [5] were carried out. In addition to ultrasound, examinations such as CT and MR angiography allow collection of supplementary diagnostic insights, for example, perfusion studies, detection of vessel anomalies (aneurysms, angiomas), and plaque imaging, and can also be useful in cases where vessels are not well accessible via ultrasound or as an alternative investigation [26, 27].

Despite these technical approaches and changes in diagnostic practice, the current classification of the degree of stenosis still corresponds to the modalities of angiographic measurements used in the aforementioned clinical trials, because they formed the basis for evidence-based regimens. However, the information provided by ultrasound, CTA, and MRA today is by far better for an individual decision than the restricted use of only three stages of obstruction: high grade (>75%), moderate (60–75%), and low grade (<50% of local lumen reduction). If combined with other parameters such as individual clinical presentation ("the vulnerable patient"), modern brain imaging, and plaque structure analysis ("the vulnerable plaque"), a best estimation of the individual stroke risk associated with carotid disease can be made regarding the therapeutic consequences. These parameters allow a differentiation between "active" (vulnerable plaques) and "stable" disease. Echomorphologic features (plaque ulcerations, hemorrhage, lipid accumulation), which can be identified through high-definition ultrasound imaging and/or application of contrast agent, correlate with histopathologic characteristics and signalize a high risk for arterioarterial embolization [28]. HITS (high-intensity transient signals) registered over the middle cerebral artery indicate microembolization and can facilitate the decision of individual treatment strategies with suitable medication (CARESS [29]), as can detection of progressive carotid stenosis or silent infarcts in cerebral CT/MRT be interpreted as a sign of activity or acuity of a carotid stenosis [30–32].

4. Studies

Most of our knowledge on the topic of carotid disease, especially regarding treatment and course of the disease, was derived from studies done in the last 25 years. Some studied the natural history and reported clinical and vascular followup, as did Chambers and Norris (1986) [9] and Hennerici et al. (1987) [10].

As far as the so-called "symptomatic" carotid stenosis is concerned, two major, multicenter, randomized controlled studies are widely known: the North American Symptomatic Carotid Endarterectomy Trial (NASCET) with 2885 patients and the European Carotid Surgery Trial (ECST) with 3024 patients [3, 4].

The two randomized controlled studies mostly referenced on the subject of "asymptomatic" carotid stenosis are the Asymptomatic Carotid Atherosclerosis Study (ACAS) with 1662 and the Asymptomatic Carotid Surgery Trial (ACST) with 3120 patients [5, 6].

Table 1 summarizes the most important results of the aforementioned studies [3–6, 33–38].

Table 1: Outline of the most important characteristics and results of large carotid surgery trials. DSA: digital substraction angiography.

	NASCET		ECST		ACAS	ACST
n (total)	2885		3024		1662	3120
Observation period prior to inclusion (max.)	120 days		180 days		120 days	180 days
Ratio TIA/stroke as qualifying event prior to inclusion	61%/39%		50%/50%		—	—
Diagnostic method for determination of stenosis grade (SG)	DSA (distal SG)		DSA (local SG)		Doppler sonography/DSA	Duplex sonography
Significant correlation of stroke risk with SG	Yes		Yes		No	No
Controlled medical treatment	No		No		No	No
Indication for carotid surgery	Limited at 50–69% SG	>70–99% SG	Limited at 50–69% SG	>70–99% SG	No	Limited at >60–99%
Stroke risk (surgical treatment) (%)	15.7 (5 yrs)	8.9 (3 yrs)	15.0 (5 yrs)	10.5 (5 yrs)	5.1 (5 yrs)	6.4 (5 yrs)
Stroke risk (medical treatment) (%)	22.2 (5 yrs)	28.3 (3 yrs)	12.1 (5 yrs)	19.0 (5 yrs)	11.0 (5 yrs)	11.7 (5 yrs)
Absolute risk reduction (%)	6.5	19.4	−2.9	8.5	5.9	5.4
Relative risk reduction (%)	29	69	—	45	53	46
Numbers needed to treat	15	5	—	12	17	19

There is also a plethora of meta-analysis data available in the literature [34, 36–38].

5. "Symptomatic" Carotid Stenosis

In NASCET, the annual stroke rate under uncontrolled medical treatment (within 2 years of followup) was 13% for high-grade stenosis (>70% distal degree of stenosis) and about 7% for moderate stenosis. Carotid occlusions were found to be associated with a low risk of stroke (2.1%/year for ipsilateral and 5.5%/year for all strokes) [3]. Therefore, carotid endarterectomy was not recommended for low degree of stenosis, as well as for patients of subtotal carotid stenosis ("pseudoocclusion"). The annual stroke rate in ECST was up to 7% for high-grade stenosis (>90% local degree of stenosis) in the—also uncontrolled—medical treatment branch within a 3-year followup and as low as 1.5% for 70–89% local degree of stenosis [36, 39]. Both studies recruited patients with a qualifying event (stroke, TIA or retinal TIA, the latter being the case in about 1/3 of NASCET patients) within a time window of 4 to 6 months prior to recruitment. Many of these cerebrovascular events were not evaluated with modern brain imaging (CT or MRI), and the association with the carotid stenosis was made only based on patients' histories. Other coincident potential sources of stroke or TIA, such as lacunar infarcts due to small vessel disease or cardioembolic infarcts from atrial fibrillation, were not taken into consideration [40], which is a major drawback compared to modern clinical workup.

The results of both studies were interpreted as a significant advantage of carotid surgery over medical treatment, which mainly consisted of acetylsalicylic acid, but lacked systematic, prospective study design of other risk factor management and monitoring of compliance during the study.

Data in the literature indicates that relevant carotid stenosis (>75%) is the underlying cause in only 5–12% of all cerebral ischemic events [3, 4]: about 20% of strokes in the territory of a "symptomatic" carotid stenosis cannot be without any doubt attributed to carotid stenosis, for example, in case of coexisting lacunar or cardioembolic causes [41].

The new ASCO stroke subtype classification [42] is suited to display such coexisting stroke causes. We prospectively studied 158 consecutive patients (89 men and 69 women, mean age 75 ± 11 years) with carotid stenosis ≥50% (as diagnosed on Doppler/Duplex vascular imaging) admitted to our stroke unit in 2010 with the diagnosis of ischemic stroke (142; 89.9%) or TIA (16; 10.1%), based on clinical features and brain imaging (CT/MRI). Patients' characteristics, risk factors, and the distribution of the grades of the 232 carotid stenosis detected are displayed in Table 2. The ASCO score (A: atherothrombosis; S: small vessel disease; C: cardioembolism; O: other causes with corresponding grades: (1) definitely a potential cause, (2) causality uncertain, (3) disease present, but unlikely a cause, (0) no disease present, and (9) no suitable tests performed) was used to classify stroke etiology after a thorough stroke workup was completed.

As determined by ASCO (Figure 1), carotid stenosis was the probable cause of the ischemic event only in less than half of the patients recruited (grade A1; 65; 41.1%). However, in 17 patients (10.8%) this was combined with other equally probable stroke causes (2; 3.1% S1 and 15; 9.5% C1). Interestingly, only if degrees of stenosis ≥70% (78; 49.3%) were considered, the majority of patients were identified correctly. However, there were still 15 patients with A1 and

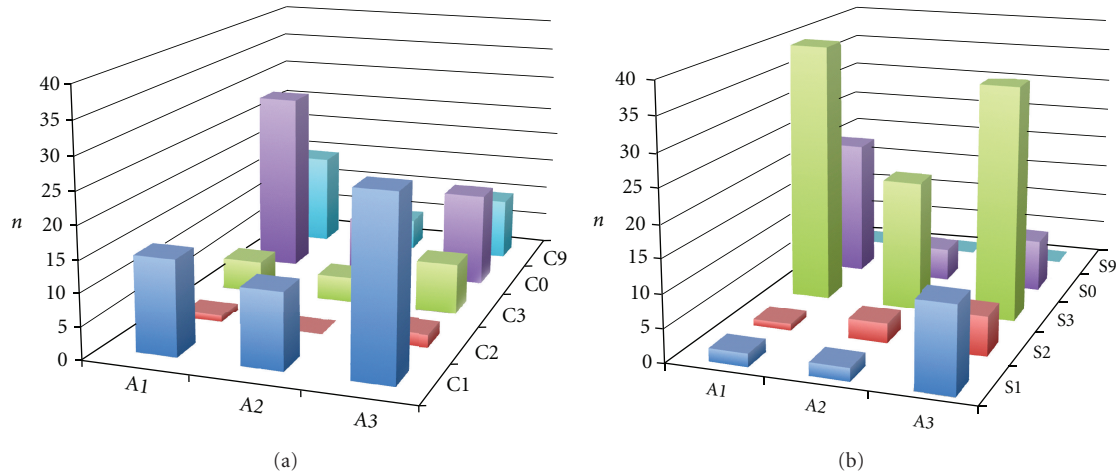

Figure 1: ASCO classification of stroke etiologies. The ASCO classification for "A" (atherothrombosis), "S" (small vessel disease), and "C" (cardioembolic). The "O" (other causes) group was omitted because only 4 patients received a grade other than "0." As all patients had Duplex/Doppler imaging and carotid stenosis, no patient had "A0" or "A9," so these groups were omitted. The figure visualizes competing/coexisting etiologies.

Table 2: Patient characteristics.

Parameter	Value	%
Total of patients	158	100
Men	89	56.3
Women	69	43.7
Mean age (±SD)	75 ±11	—
Cerebral ischemia	142	89.9
TIA	16	10.1
Unilateral stenosis	84	53.2
Bilateral stenosis	74	46.8
Carotid stenosis grades		
Carotid stenosis in total	232	
≥50–59%	74	(31.9)
≥60–69%	68	(29.3)
≥70–79%	24	(10.3)
≥80–89%	28	(12.1)
≥90–99%	29	(12.5)
100%	9	(3.9)
Concomitant vascular diseases		
Coronary artery disease	38	24.1*
Peripheral artery disease	18	11.4
Vascular risk factors		
Arterial hypertension	140	88.6
Hyperlipidemia	77	48.7
Diabetes mellitus	54	34.2

* Correlation with stenosis grade: $P < 0.05$.

C1 (9.5%). Cardioembolism was the most probable stroke cause in 55 (34.8%) and small vessel disease in 17 (10.8%) patients. Hence, 93 patients (58.9%) had an "asymptomatic" carotid stenosis despite suggested acute stroke from carotid stenosis.

6. "Asymptomatic" Carotid Stenosis

Both natural history studies, as well as several large, multicenter, randomized controlled studies, have shown that "asymptomatic" carotid stenosis is quite a benign disease, with an annual stroke rate of only 1-2% and a stroke-related annual mortality rate of merely <0.05% [5, 10].

Carotid stenosis, however, is an established risk factor for myocardial infarction. The annual mortality, mainly because of cardiovascular events, is about 6–8%. About 40% of patients with "asymptomatic" carotid stenosis have coincident coronary artery disease (CAD) [10, 43, 44]. In many patients, silent atherosclerotic alterations of coronary vessels lead to a significantly increased mortality. ACAS showed a mortality rate of 6% with one year of followup [5]. The highest prevalence (28%) of carotid stenosis was found in patients with peripheral artery disease (PAD); these patients also have the highest mortality [2].

In our patient group described above, about one-fourth of the patients (38; 24.1%) had CAD and 18 (11.4%) PAD. There was even a statistically significant correlation between grade of stenosis and presence of CAD ($P = 0.018$). Classic vascular risk factors were very common: arterial hypertension (140; 88.6%), hyperlipidemia (77; 48.7%), and diabetes mellitus (54; 34.2%).

ACAS and ACST could show a minor preventive effect of carotid surgery in carotid stenosis between 60% and 99%. A refined assessment of risk according to stenosis grade could not be made because of various methodological problems. In ACAS, absolute risk reduction of stroke and/or death was 5.9% and in ACST 5.4%, in a time frame of 5 years. However, it should be taken into consideration

Table 3: Factors that increase stroke risk in patients with carotid stenosis.

Acute hemispheric ischemia associated with carotid stenosis in the last 4 weeks
Ipsilateral silent infarcts in CT/MRI
Intracranial artery stenosis
Contralateral carotid occlusion
Insufficient medical treatment (risk factors)
Insufficient collateralisation over the circulus Willisii
Coexistent coronary/peripheral artery disease
Leukoaraiosis
HITS detection
Rapid progression of carotid stenosis
Plaque ulcerations
Highly echolucent plaques in carotid duplexsonography
High Lp-PLA_2 Concentration

that the perioperative complication risk in both studies was extremely low (2.3% and 2.9%), because of a very strict selection of surgeons [5, 6]. Nevertheless, many patients were treated unnecessarily because of the large number needed to treat (17–19).

Control of risk factors was reported in ACST on a retrospective analysis suggesting that the already small benefit of surgery was minimized or lost if adequate monitoring of risk factor management had been achieved [6, 45].

Results from studies with very long followups from our department support these findings of low stroke mortality rate in these patients [10, 46].

7. New Assessment of Prognosis and Management

Based on the available data [47–51], a number of factors associated with a high risk of stroke on the basis of a carotid stenosis can be identified (Table 3).

Associated with low risk of stroke are Retinal TIA, no (or few) vascular risk factors, effective intracranial collateralisation over the circulus Willisii, as well as subtotal stenosis [52–54].

Indications of an unstable ("vulnerable") plaque are plaque ulcerations, high concentration of lipoprotein-associated phospholipase A_2 (Lp-PLA_2), and highly echolucent plaques [55–59].

Beside the simple clinical risk score $ABCD^2$ (age, blood pressure, clinical features, duration, diabetes) [60], the risk model proposed by Rothwell can help to assess the individual risk for ipsilateral cerebrovascular events in patients with carotid stenosis [54, 61]. It is based on meta-analysis data of the major carotid studies (particularly the ECST) and may support in making an individual treatment decision (Table 4).

8. Conclusions

Uncritical usage of the results from the aforementioned studies often misguides and leads to false conclusions, generalizations, and uncertainty about best available modern treatment. This problem rises at least partly from the different methods used to evaluate the stenosis grade (angiography versus ultrasound) and from the nonvalidated evaluation criteria. Therefore, ACAS and ACST could not show any connection between stenosis grade and stroke risk, whereas this connection was clearly shown not only in natural history studies but also in NASCET and ECST. Because patients nowadays do not usually undergo conventional angiography before carotid surgery, the direct justification for surgery by these studies' results is actually missing.

Other problems and insecurities in the management of carotid stenosis result from the fact that a significantly positive effect of carotid surgery (in NASCET and ECST) was shown only in patients who underwent surgery within 2–4 weeks, but not in those operated upon later, up to 4–6 months. Furthermore, there are significant asymmetries in the recruitment of men/women in all 4 major studies. These asymmetries were not corrected in the analysis of the primary endpoints, thus undermining the results of ACAS and ACST in particular [62]. Possible overlapping of different stroke pathogenesis was not taken into consideration, which limits the direct transfer of study results to the individual patient in everyday clinical practice. In our patient collective, for example, carotid stenosis was the probable cause of stroke in less than half of patients and even in part of these patients there was an overlapping with other (mostly cardiac) causes. The ASCO score is especially suitable to demonstrate overlapping stroke etiologies and to quickly create a rudimental risk profile of the patient [42]. Often lacking a sufficient brain imaging (especially MRI) and thorough stroke workup, as it is performed today before every carotid surgery, the studies failed to take some of these factors into account. Finally, adequate medical management with monitoring and controlled treatment of vascular risk factors (e.g., statin and/or antihypertensive treatment) was missed.

A strict classification in "symptomatic" and "asymptomatic" carotid stenosis and decisions on the degree of carotid stenosis alone could therefore be misleading. Carotid disease should be rather regarded as an entity with active and stable phases. When deciding about treatment, one should differentiate between those two phases utilizing clinical findings, followups (e.g., rapid progression, repeated TIAs), or other parameters (silent infarcts in brain imaging, HITS, plaque configuration). During an active phase, carotid surgery is advisable under the condition that it is performed early and that the perioperative complication risk is low (<5%) [47, 63]. Carotid angioplasty with or without stent is currently not a routine option but can be performed by highly experienced interventionalists in selected patients who cannot undergo a surgery. None of the major studies

Table 4: Risk model by Rothwell for ipsilateral territorial infarcts in all patients in the medical treatment branch of ECST [61]. CI: confidence interval.

	P	Hazard ratio	95% CI
Cerebral events versus ocular events	0.008	2.45	1.27–4.75
Residual neurological signs after 7 days	0.006	1.30	1.08–1.57
Diabetes	0.007	1.82	1.18–2.80
Any ischemic event within the last 2 months	0.003	1.71	1.20–2.44
Number of events within the last 3 months (per event)	0.01	1.02	1.01–1.03
Previous myocardial infarction	0.02	1.31	1.04–1.65
Degree of carotid stenosis	0.0000	1.34	1.30–1.38
Plaque surface irregularity	0.01	1.80	1.14–2.83
Poststenotic collapse of the internal carotid artery	0.03	0.40	0.17–0.94
Age (per year)	0.62	1.01	0.98–1.03
Male sex	0.31	1.23	0.83–1.82
Systolic blood pressure (per 10 mmHg)	0.82	1.05	0.90–1.15
Diastolic blood pressure (per 10 mmHg)	0.61	1.10	0.80–1.30
Peripheral vascular disease	0.90	1.03	0.65–1.63
Angina without previous myocardial infarction	0.77	0.96	0.71–1.29
ECG signs of left ventricular hypertrophy	0.90	1.07	0.40–2.10
Cerebral infarct on symptomatic side on CT	0.18	1.32	0.88–1.96
Occlusion of the contralateral internal carotid artery	0.96	1.00	0.72–1.63

until today (SPACE, EVA3S, CAVATAS, ICSS, CREST) could show a noninferiority of angioplasty compared with carotid surgery [64, 65].

During a stable phase, the patient with carotid stenosis is a patient in high risk for cardiovascular events. In accordance with results from previous studies [10, 43, 44], our patients had a high percentage of concomitant vascular diseases and risk factors. Almost all patients had an arterial hypertension, and hyperlipidemia and diabetes were also very common. Furthermore, PAD and CAD were quite common, and the presence of CAD even correlated with the grade of carotid stenosis. The risks derived from these diseases are not diminished by a surgical or interventional treatment of the carotid stenosis [52]. Because of the high cardiovascular risk, however, these patients do profit from controlled medical treatment. Every risk factor increases vascular risk by its own, so there is a summation effect of their combined treatment on the prognosis [66]. Particularly under statin treatment, it could be shown that not only there is a reduction of TIA/stroke and of cardiovascular morbidity and mortality associated with coincidental coronary heart disease but also a decrease in the necessity of vascular interventions (56% of relative risk reduction) [67]. SPACE2 will possibly clarify if there is need of an interventional carotid treatment in these cases. Other cerebrovascular risk factors, such as atrial fibrillation, should certainly be also taken into consideration. Conservative treatment strategies and duplex sonographic followups (in most cases every 3–6 months) are essential in order to detect a phase of activity in time.

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Review Article

Microembolus Detection by Transcranial Doppler Sonography: Review of the Literature

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Transcranial Doppler can detect microembolic signals which are characterized by unidirectional high intensity increase, short duration, random occurrence, and a "whistling" sound. Microembolic signals have been detected in a number of clinical settings: carotid artery stenosis, aortic arch plaques, atrial fibrillation, myocardial infarction, prosthetic heart valves, patent foramen ovale, valvular stenosis, during invasive procedures (angiography, percutaneous transluminal angioplasty), surgery (carotid, cardiopulmonary bypass, orthopedic), and in certain systemic diseases. Microembolic signals are frequent in large artery disease, less commonly detected in cardioembolic stroke, and infrequent in lacunar stroke. This article provides an overview about the current state of technical and clinical aspects of microembolus detection.

1. Introduction

1.1. Clinical Aspects. Acute stroke is one of the leading cause of morbidity and mortality worldwide. In developed countries, stroke ranks as either second or third most common cause of death and embolization is the cause of ischemic stroke in 40%–80% of cases [1]. TCD is a sensitive technique for real-time detection of microembolic signals (MESs). In the last 20 years, a substantial number of studies dealing with emboli detection have been carried out, showing that MES are proven to represent emboli passing within cerebral circulation. MES have been detected in a number of clinical conditions: carotid artery stenosis, aortic arch plaques, atrial fibrillation, myocardial infarction, prosthetic heart valves, patent foramen ovale, valvular stenosis, during carotid surgery, surgery on open heart, stent implantation, percutaneous transluminal angioplasty and angiography, and in patients with migraine and patent foramen ovale (PFO). Patients who have detectable MES, especially in larger number, should be considered as high-risk patients for stroke. MES detection may help in localization of embolic source, identification of patients with high stroke risk, monitoring during invasive procedures and surgery, and monitoring of the effectiveness of therapy.

Consensus on MES detection by TCD has been established [2]; MES can be identified as short lasting (<0.01–0.03 s), unidirectional intensity increase, and intensity increase (>3 dB) within the Doppler frequency spectrum; intensity increase is focused around 1 frequency. MESs appear randomly within the cardiac cycle and produce a "whistle," "chirping," or "clicking" sound when passing through the sample volume.

1.2. Technical Aspects. TCD is a very convenient tool to monitor intracranial circulation, however, the thick temporal bone window is an obstacle in certain patients. A new transcranial modality, power M-mode Doppler (PMD), has been developed to overcome the difficulties in location and insonation through transcranial ultrasound windows. PMD has 33 sample gates placed with 2 mm spacing for display of Doppler signal power, colored red and blue for directionality, in an M-mode format. The spectrogram from a user-selected depth is displayed simultaneously. PMD facilities window location and alignment of the ultrasound beam to view blood flow from multiple vessels simultaneously, without sound or spectral clues. MES appear as characteristic sloping high-power tracks in the PMD image [3, 4].

The optimal time of monitoring depends on the clinical entity. In patients with implanted artificial heart valves in whom MES can be detected in large proportion, monitoring during 30 minutes will be sufficient. In patients with carotid artery stenosis, atrial fibrillation, or other cardiac disease, frequency of MES is usually low, 1-2 MES over 60 minutes. Extended monitoring up to 8 hours, or repetitive monitoring over a couple of days in succession, is in relation with the percentage of MES positive patients [5, 6]. The embolic activity is highest in the first couple of hours after stroke; however, MES may be detectable days and weeks after cerebrovascular incidents which means that those patients are under higher risk for stroke [7–11].

Although technological improvement in the area of MES detection has recently developed, it is still impossible to reliably distinguish the composition of emboli (particles of fat, platelet aggregates, or particles of atheroma). Differentiation between solid and gaseous microemboli is based on the principle that solid emboli reflect more ultrasound at higher frequency, whereas the opposite is the case for gaseous emboli. This principle is used in multifrequency TCD instrumentation where the vessels are insonated simultaneously with 2.5 and 2.0 MHz and can be used for the differentiation between gaseous and solid emboli [12]. A recent study has shown that there is a significant relationship between low- and high-intensity MES, indicating that many MESs routinely rejected because of their low intensity are real and may predict future occurrence of high-intensity MES [13].

2. Clinical Condition in Which MES May Be Detected

2.1. Atherosclerotic Disease

2.1.1. Carotid Stenosis. Carotid artery stenosis is a well-known source of cerebral MES [7, 8, 14–19]. Systematic review of the literature showed that MES can be detected in 43% of patients with symptomatic and in 10% with asymptomatic carotid stenosis; presence of one MES indicated an increased risk of future events (OR 7.5, 95% confidence interval (CI): 3.6–15.4, $P < 0.0001$ for symptomatic and OR 13.4, 95% CI: 6.5–27.4, $P < 0.0001$ for asymptomatic disease) [20]. A meta-analysis of the literature revealed that MESs are most frequent in large artery disease, less frequent in cardioembolic stroke, and infrequent in lacunar stroke. For symptomatic carotid stenosis, ES predicted stroke alone (OR, 9.57; $P = 0.02$) and stroke/TIA (OR, 6.36; $P < 0.00001$). For asymptomatic carotid stenosis, ES predicted stroke alone (OR, 7.46; $P = 0.001$) and stroke/TIA (OR, 12.00; $P = 0.002$) but with heterogeneity ($P = 0.004$). In acute stroke, ES predicted stroke alone (OR, 2.44; $P = 0.02$) and stroke/TIA (OR, 3.71; $P = 0.002$). A high frequency of ES immediately after carotid endarterectomy predicted stroke alone (OR, 24.54; $P < 0.00001$) and stroke/TIA (OR, 32.04; $P < 0.00001$). The meta-analysis suggests that MES predict stroke risk in acute stroke, symptomatic carotid stenosis, and postoperatively after carotid endarterectomy; however, in asymptomatic carotid stenosis, the predictive value of MES is less clear [21].

In the asymptomatic carotid emboli study (ACES), a prospective observational study, patients with asymptomatic carotid stenosis of at least 70% were monitored by TCD; the results showed that the hazard ratio for the risk of ipsilateral stroke and transient ischaemic attack from baseline to 2 years in patients with MES compared with those without was 2.54 (95% CI 1.20–5.36; $P = 0.015$). For ipsilateral stroke alone, the hazard ratio was 5.57 (1.61–19.32; $P = 0.007$). The absolute annual risk of ipsilateral stroke or transient ischaemic attack between baseline and 2 years was 7.13% in patients with MES and 3.04% in those without, and for ipsilateral stroke was 3.62% in patients with MES and 0.70% in those without [22].

Studies have shown that intraluminal thrombosis, irregular plaque surface, and ulceration are in relation with emboli frequency [23–25]. Carotid plaque inflammation is associated with cerebral microembolism in patients with recent transient ischemic attack or stroke [26].

Despite optimum standard antiplatelet therapy, cerebral microembolisation occurs in 30% of patients with symptomatic carotid artery disease [27]. Patients with symptomatic high-grade ICA stenosis and who were on antiplatelet treatment underwent bilateral MES monitoring for 30 minutes. The study has shown that the presence of MES is independent of intrastenotic blood flow disturbances and gray-scale ultrasound plaque characteristics. The authors concluded that the presence of MES is an indicator of an unstable plaque [27].

Carotid plaques in patients with severe unilateral carotid restenosis at least one year after surgery are similar to patients with primary severe stenosis in their embolic potential and ultrasonic characteristics [28].

Dissection of carotid arteries is an embolic source and MES in those patients can be detected in a high proportion [29]. Among patients with cervical artery dissection presenting with TIA or stroke, 50% had MES compared with 13% of patients with local symptoms ($P = 0.006$) [20].

2.1.2. Aortic Arch Atheroma. Aortic arch atheroma has long been underestimated as an embolic source. Studies have shown that severe atheroma of the aortic arch has now been established as an important and independent risk factor for stroke. The prevalence of severe arch atheroma among patients presenting with acute ischaemic stroke is approximately 20%.

The odds ratio for stroke or peripheral embolism in patients with severe arch atheroma (>4 mm) is greater than four, and for mobile atheroma it is greater than twelve, particularly in patients with other stroke risk factors. Patients found to have severe atheroma are at high risk of recurrent events (14.2% per year) and may, therefore, need an aggressive secondary prevention strategy [19, 20].

2.1.3. Intracranial Stenosis. Intracranial stenosis is a significant source of cerebral emboli, although in most studies not appreciated as important as carotid stenosis. A recent study has shown that MESs were reported in 25% of 220 patients with symptomatic versus 0% of 86 patients with asymptomatic intracranial stenosis ($P < 0.0001$) [20].

2.1.4. Thrombolysis and MES Occurrence. Thrombolysis is a well-established therapy in acute stroke. TCD has proven to serve as an enhancement tool in clot dissolution. MES detected by TCD at the site of arterial obstruction can indicate clot dissolution and recanalization of intracranial arteries, and therefore serve as a predictor for outcome [30].

2.1.5. Carotid Endarterectomy (CEA), Carotid Artery Stenting (CAS), and Percutaneous Transluminal Angioplasty (PTA). After carotid endarterectomy, MESs disappear or the frequency is significantly lower [31, 32]. However, patients with clinically significant postoperative microembolism have an approximately 15 times higher risk of ipsilateral stroke or TIA [33]. Efficacy of CEA largely depends on postoperative results, that is, perisurgical complications. Intraoperative monitoring of hemodynamic changes and detection of MES may significantly influence postsurgical outcome [34]. Different antiplatelet regimens (combination of dipyridamole, aspirin and clopidogrel) following CEA does not show a significant influence on postoperative TCD embolization [35]. However, administration of dual antiplatelet therapy (clopidogrel 75 mg plus aspirin 75 mg) prior to CEA reduces postoperative embolisation and thromboembolic events [36].

Meta-analysis of trials to date shows that symptomatic and asymptomatic CAS patients had significantly higher 30-day postprocedure incidence of death/stroke/MI when compared with CEA patients [37, 38].

Experimental studies have shown that during PTA, the majority of MESs are gaseous in origin, while MESs detected during balloon inflation are probably attributable to solid particles [39].

The use of cerebral protection devices appears to reduce thromboembolic complications during PTA and stenting. The combined stroke and death rate within 30 days in patients treated with cerebral protection devices was 1.8% compared with 5,5% in patients without [40].

2.1.6. Angiography. MESs detected during angiography are attributable to gas bubbles, contrast injection, clot formation in catheter, and disrupted atheromatous material [41, 42]. Magnetic resonance studies have shown increased number of brain parenchymal lesions after angiography, although the majority of patients had nor neurological deficit [43]. However, angiography of the aortic arch is associated with 1% risk for stroke with permanent deficit, and in 3% mild-to-moderate stroke or TIA [41, 42]. In a study with 24 patients undergoing angiography, all had detectable MES, approximately 51 per patient. The majority of MES had the characteristics of gas bubbles. The number of detected MESs correlated with the volume of injected contrast and all patients except one who had stroke were asymptomatic [42].

2.2. Cardiac Sources. Approximately 15–30% of strokes are caused by cardiac diseases [44]. Prospective studies have shown that recurrent stroke or systemic embolization in that group of patients is high, approximately 20% [7, 45].

2.2.1. Atrial Fibrillation. Atrial fibrillation (AF) is present in 1,7% of population aged 60–64 years, and in 6% of population older than 75 years [46]. Incidence of stroke in patients with AF is 4,5% per year [47]. In cases when AF is associated with mitral valve stenosis, risk of stroke is 17 times higher [48]. Majority of strokes in patients with AF are embolic in origin; MES can be detected in 15–30% [49, 50].

2.2.2. Patent Foramen Ovale. The prevalence of patent foramen ovale (PFO) in common population is 22–34.4% [51, 52]. In patients with cryptogenic stroke, especially younger patients, a high prevalence of PFO has been found [53, 54]. The rate of recurrent stroke or TIA is 9.9% [55]. A multicentric study has shown that stroke risk over 4 years is 5,6% in patients with isolated PFO and 19,2% in patients with PFO and atrial septal aneurysm (ASA) [56]. However, a prospective population-based study has established that PFO is not an independent risk factor for stroke when age and comorbiditiy are considered [57].

A connection between migraine (especially migraine with aura), stroke, and PFO has been established [58–63]. Studies have shown that the prevalence of PFO in patients with migraine is about 2.5 times greater in comparison with patients who do not suffer from migraine [64, 65]. Patients with migraine with aura have twice as high risk for stroke. Patients with probable migraine are 1, 5 times higher risk. If female patients with migraine smoke and take oral contraceptives, the risk increases up to seven times compared to those who do not have migraine [66–68].

According to one of the proposed hypothesis, paradoxal embolization causes segmental hypoperfusion and vasodilatation, that is, PFO enables vasoactive substances (hypotheticaly serotonin) and microemboli from venous circulation to pass the pulmonal filter (normally they would be stopped in lungs), and entering the brain they can induce cortical spreading depression which characterizes migrainous attack [69]. This hypothesis could explain the decrease of migraine prevalence and intensity after PFO closure (in most studies). In favor of microembolization as a provoking factor speaks the MRI study in which a 13.7 times higher incidence of white matter lesions has been found in patients with migraine with aura compared to brains of controls [70–72]. Furthermore, most TCD studies have shown that most paradoxical MES can be detected in posterior brain circulation [70], which is interesting because in these patients PFO and migraine are more frequently present. However, up to now, there is no definite evidence for proposed theories regarding migraine onset caused by circulating microemboli.

2.2.3. Prosthetic Heart Valves. Thromboembolism is a significant complication in patients with prosthetic heart valves (PHV). MES can be detectable in cerebral circulation ranging from 50 to 90% [73–75]. The majority of HITS in patients with PHV correspond to gaseous, for example, due to cavitations at mechanical heart valves [76].

2.2.4. Myocardial Infarction. Approximately 2.5% of patients with acute myocardial infarction (MI) will have stroke within 2–4 months [77]. In a prospective study in patients with anterior wall MI, MES could be detected in 21% [78].

2.2.5. Infective Myocarditis. The prevalence of stroke in patients with infective myocarditis is 15–20% [79]. The size of vegetations has a predictive value: risk for embolization is higher in patients with vegetations larger than 10 mm, in patients with endocarditis of the mitral valve and mobile vegetations [79].

2.2.6. Dilatative Congestive Myocardiopathy. Incidence of embolic complications in patients with cardiomyopathy is 4% per year [78]. MESs are detectable in one third of patients with dilatative congestive myocardiopathy [44].

2.2.7. Mitral Valve Prolapse. Prevalence of mitral valve prolapse (MVP) in common population is 1–15% [80]. Isolated MVP is not considered as a significant risk factor for stroke in older patients, but in younger patients myxomatous change of mitral valves is considered to be in relationship with cerebrovascular incidents [80].

2.2.8. Cardiac Tumors. Although rare, atrial myxoma is the most frequent primary cardiac tumor, usually present in the left atrium. Due to its fragile nature, myxoma has a high potential for embolization to the brain as well as to other organs [81].

2.2.9. Systemic Diseases. MESs in cerebral circulation are detectable in primary antiphospholipid syndrome, Sneddon's syndrome, systemic lupus, Behcet's disease, and Takayasu's arteritis and are in temporal relation with cerebrovascular incidents in those patients [82–85].

2.2.10. Coagulation. Clinical studies have shown that hyperfibrinogenemia is in a positive correlation with cardiovascular and cerebrovascular diseases [86–88]. Patients with progressive atherosclerosis have higher levels of fibrinogen compared with those with nonprogressive disease [89].

3. Monitoring of Therapeutic Efficacy

Monitoring of therapeutic effect has shown that after CEA, MESs disappear or decrease in frequency, this finding is consistent through studies [31, 32]. Studies that investigated the efficacy of anticoagulant and antiaggregation therapy and frequency of MES have shown various results. Although some studies have shown decrease in MES frequency after aspirin or heparin administration, consistent correlation has not been found [90].

The first multicenter, randomized, double-blind trial that used MES detection as an endpoint to evaluate antiplatelet therapy, the Clopidogrel (CARESS and Aspirin for Reduction of Emboli in Symptomatic Carotid Stenosis) trial, has shown that clopidogrel plus aspirin is superior to aspirin alone in reducing the frequency of MES in patients with recent symptomatic carotid stenosis [91]. Glycoprotein IIb/IIIa receptor antagonist tirofiban is a highly selective platelet aggregation inhibitor. Administration of tirofiban resulted in microembolic rate drop down to zero in patients with severe carotid artery stenosis in two studies. However, the inhibitory effect of tirofiban is reversible [92, 93].

4. Clinical Significance of Emboli Detection

It is important to emphasize that the vast majority of MESs do not produce immediate symptoms. MESs in cerebral circulation indicate asymptomatic patients with increased stroke risk. Nevertheless, correlation of cerebral microemboli with clinical symptoms (stroke or TIA ipsilateral to carotid stenosis, higher incidence of stroke in patients with cardiac diseases, neurological complications after angiography, postoperative neurological complications after CEA, in patients after CABG) in a number of studies has been shown. In symptomatic patients, the etiology of neurological deficit may be elucidated and adequate therapy may be introduced.

Patients with cerebral microembolism have higher cognitive deficits; cumulative effect of embolism is thought to be the cause. Even minor neuropsychological impairment should not be underestimated, the presence of an embolic source should be regarded as the possible cause for cognitive decline. Imaging techniques (CT scan, MRI, DWI-MRI) can show "silent" areas of cerebral ischemia. These small and multiple areas of acute or subacute brain infarction may occasionally present with clinical features atypical for brain embolism or will not produce any apparent symptoms [94].

Despite disputable clinical significance in certain conditions, the potential benefit of TCD detection of MES remains substantial.

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Clinical Study

Hemispheric Asymmetry of Visual Cortical Response by Means of Functional Transcranial Doppler

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We assessed the visual evoked response and investigated side-to-side differences in mean blood flow velocities (MBFVs) by means of functional transcranial Doppler (fTCD) in 49 right-handed patients with severe internal carotid artery (ICA) stenosis and 30 healthy volunteers, simultaneously in both posterior cerebral arteries (PCAs) using 2 MHz probes, successively in the dark and during the white light stimulation. Statistically significant correlation ($P = 0.001$) was shown in healthy and in patients ($P < 0.05$) between MBFV in right PCA in physiological conditions and MBFV in right PCA during the white light stimulation and in the dark. The correlation between MBVF in right PCA and contralateral left PCA was not statistically significant ($P > 0.05$). The correlation between ipsilateral left PCA was significantly higher than the one with contralateral right PCA ($P < 0.05$). There is a clear trend towards the lateralisation of the visual evoked response in the right PCA.

1. Introduction

Functional transcranial Doppler (fTCD) studies indicated that simultaneous bilateral stimulation during a different visual tasks caused greater blood flow velocities in right hemisphere in healthy individuals, indicating that it may identify visual hemispheric dominance [1, 2]. Results of the studies testing the posterior cerebral circulation using visual stimuli in patients with severe carotid stenosis and consecutively compromised anterior cerebral circulation suggest the existence of an independent cerebral vascular reserve capacity of the posterior part of Willis circle [3–7]. Undoubtedly, collateral variations in circle of Willis have to be taken into consideration since they are highly variable [8]. In patients with carotid occlusive disease collateral flow from posterior to anterior circulation could be through the posterior communicating artery or via the leptomeningeal collaterals. In the first case, the blood flow in the vertebrobasilar artery system is shunted into the anterior cerebral circulation before the P2 segment branches leading to a decrease in the P2 flow velocities, and in the latter it is shunted through the P2 branch into the leptomeningeal collaterals leading to an increase resting blood flow velocity in the P2 [9, 10].

The aim of this study was to assess visual evoked response in PCA in patients with severe carotid stenosis and compromised anterior cerebral circulation, as the most powerful and fully noninvasive test of cerebral autoregulation, in vascular territory mostly supplied by posterior circulation, in order to investigate if flow through the circle of Willis might result in changes in the blood flow distribution in the PCA territory. Also, the aim was to investigate side-to-side differences of simultaneously measured PCAs blood flow velocities during white light stimulation in patients with severe carotid disease, in order to establish a possible functional hemispheric asymmetry of the visual cortex in patients.

2. Methodology

The study cohort consisted of 49 right-handed patients (mean age ± SD = 67 ± 8; 37 men), with no eye abnor-

Table 1: Mean blood flow velocities in posterior cerebral artery at baseline.

Mean blood flow velocities in posterior cerebral artery (cm/s ± 2SD) at baseline (eyes opened)				
		Patients ($n = 49$)	Healthy ($n = 30$)	P
PCA at baseline	right	29.08 ± 10.46	25.8 ± 7.32	0.14
	left	28.65 ± 9.58	25.93 ± 8.67	0.21

cm/s: centimeters in second.
n: number of patients/healthy subjects.
PCA: posterior cerebral artery.
SD: standard deviation.

Table 2: Mean blood flow velocities in posterior cerebral artery during the white light stimulation and in the dark in the group of healthy subjects.

Mean blood flow velocities in posterior cerebral artery (cm/s ± 2SD)			
		Light	Dark
Healthy ($n = 30$)	Right PCA	29.99 ± 9.5	20.12 ± 8.33
	Left PCA	30.56 ± 8.34	21.40 ± 7.32

cm/s: centimeters in second.
n: number of healthy subjects.
PCA: posterior cerebral artery.
SD: standard deviation.

malities, with high-grade (70–99%) symptomatic or asymptomatic ICA stenosis (20 right and 19 left ICAs) as measured by Doppler ultrasonography and 30 healthy volunteers (mean age ± SD = 67 ± 7; 22 men) with normal ICAs. The two groups were matched in age ($P = 0.91$) and sex ($P = 0.52$). For the comparison, in the group of healthy individuals, the proportion of the left and right ICA was matched to the proportion of left and right ICA in the group of patients with severe carotid stenosis.

Exclusion criteria were limited ultrasound temporal bone window, detectable stenosis or occlusion of any of the arteries of the Willis circle or vertebrobasilar circle by means of TCD, uncooperative patients (dementia, coma, etc.), heart disease (atrial fibrillation, myocardial infarction, patent foramen ovale, atrial septum aneurysm, and mitral valve prolapse), uncontrolled hypertension, diabetes mellitus, and migraine.

In all patients, the risk factors were under control for at least one year before the study was performed. None of the patients used vasoactive medications. Patients had abstained from alcohol, caffeinated beverages, and smoking, as well as certain drugs that may alter blood pressure or cause vasodilatation (nitrates, β-blocking agents, calcium channel blockers, anticoagulants, and vasodilatatory agents) for at least 24 hours prior to the study.

Carotid artery disease was assessed and defined using the carotid color Doppler flow imaging (CDFI) and power Doppler imaging (PDI) according to validated criteria [11]. The intracranial arteries were evaluated by TCD according to validated criteria [12]. Visual evoked response was obtained by means of TCD (MultiDop X4 DWL, Elektronische Systeme GmbH, Sipplingen) using a special application for evoked flow. It included transtemporal simultaneous insonation of P1 or proximal part of P2 segment if the P1 was not able to insonate, of both PCAs while obtaining the signal away from the probe at the depth of 60–70 mm using two 2 MHz probes mounted on an individually fitted head band. The test was performed while patient in a supine position, in a dark, quiet room, after an accommodation period of resting and closed eyes for 10 minutes. For the visual stimuli, a 100 W electric bulb was used, located 50 cm in front of the head of the examinee. After the accommodation period, mean blood flow velocities (MBFVs) in each PCA were measured, in a dark (closed eyes) and during a white light stimulation (opened eyes, looking at an electric bulb). The measurements were performed successively in the dark and during the white light stimulation, during three consecutive repetitive periods of 1 minute each. Mean values of MBFV during a one-minute period with and without visual stimuli were analyzed. Before the testing, all the subjects were introduced to the testing method and the testing measurements were performed before the study measurements in order to establish which subjects were suitable for the study.

Institutional Ethical Committee approved the study. All patients signed informed consent.

For statistical analyses, we used statistical program package Statistica for Windows, Kernel release (5.5) A (StatSoft, Inc. Tulsa, OK) (StatSoft, Inc. (2000); statistics for Windows (Computer program manual). Tulsa, OK: StatSoft, Inc.

We used paired Student t-test to compare quantitative variables between the two groups, t-test for dependent variables to compare values of repetitive measurements within the same group and linear regression analyses to analyze the correlation of quantitative variables.

From nonparametric statistics model, we used Pearson chi-square test to compare a distribution of qualitative characteristics of the group. Results with P-values of <0.05 were considered statistically significant.

3. Results

Regarding the symptomatic status, 9 of 49 patients were symptomatic, at least one year or more before the study was performed. Concerning the presence and functionality of the collateral cerebral circulation by TCD before testing for vasomotor reactivity, the patient's statuses were as follows: 5 patients had anterior collateral pathway, 17 patients had developed collateral flow through the ophthalmic arteries (2 of them were symptomatic), and 4 patients had developed both anterior collateral pathway and collateral pathway through the ophthalmic artery (2 of them were symptomatic). With regard to vascular risk factors, 30 patients had no vascular risk factors, 18 patients had arterial hypertension, 10 had cardiomyopathy, 4 had diabetes mellitus, 6 hypercholesterolaemia, 2 patients were cigarette smokers, and 1 patient was an alcohol abuser.

There was no difference at baseline between right PCAs ($P = 0.14$; Student t-test) and left PCAs ($P = 0.21$; Student t-test) between healthy and patients (Table 1).

Table 2 displays the difference in MBFV between dark and white light stimulation in the group of healthy subjects.

Table 3: Mean blood flow velocities in posterior cerebral artery during the white light stimulation and in the dark in the group of patients with severe carotid stenosis.

		Mean blood flow velocities in posterior cerebral artery (cm/s ± 2SD)	
		Light	Dark
Patients with severe carotid stenosis ($n = 49$)	Right PCA	29.88 ± 8.6	21.32 ± 7.08
	Left PCA	32.6 ± 11.49	21.40 ± 7.32

cm/s: centimeters in second.
n: number of patients.
PCA: posterior cerebral artery.
SD: standard deviation.

Table 4: Correlation between internal carotid artery stenosis and mean blood flow velocities in posterior cerebral artery.

		Mean blood flow velocities in posterior cerebral artery (cm/s)			
		Light		Dark	
Patients ($n = 49$)		Right PCA	Left PCA	Right PCA	Left PCA
Stenosis	Right ICA	0.1891, $P = 0.193$	0.0546, $P = 0.710$	0.2009, $P = 0.166$	0.1349, $P = 0.355$
	Reft ICA	−0.1092, $P = 0.455$	−0.008, $P = 0.955$	−0.072, $P = 0.622$	−0.0390, $P = 0.790$

cm/s: centimeters in second.
n: number of patients.
ICA: internal carotid artery.
PCA: posterior cerebral artery.

MBFV during the white light stimulation and in the dark in the group of patients with severe carotid stenosis are displayed in Table 3.

Linear regression analysis showed no statistically significant correlation between the degree of right ICA stenosis and MBFV either in any PCA in the dark and during a light stimulation (Table 4), but there was a trend of negative correlation between the degree of left ICA stenosis and MBFV both in contralateral PCA as well as in ipsilateral PCA, but with no statistical significance (Table 4).

In the same time, significant correlation ($P = 0.001$) of the measured parameters both during the white light stimulation and in the dark, regardless of the side of the measurement, was found in the group of healthy individuals (Table 5).

In the group of patients with severe carotid stenosis, correlation between MBFV in left and right PCA at baseline and MBFV in left and right PCA during the white light stimulation and in the dark showed statistically significant correlation (linear regression analyses; $P < 0.05$) between MBFV in right PCA at baseline and MBFV in right PCA during the white light stimulation and in the dark (Table 6). On the contrary, correlation between MBFV in right PCA at baseline and contralateral PCA either during the white light stimulation or in the dark was not statistically significant (linear regression analyses; $P > 0.05$) (Table 6). The results showed the higher flow response under various conditions in the right PCA compared to the left one.

Analyzing the correlation between MBFV in left PCA at baseline conditions, statistically significant correlation between MBFV in both ipsilateral and contralateral PCA, both during the white light stimulation and in the dark, was found, but the correlation with ipsilateral side was significantly higher than with contralateral side (linear regression analyses; $P < 0.05$) (Table 6).

Considering the small number of symptomatic patients, which is not suitable for statistical analyses, and the fact that they had symptoms at least one year or more before the study was performed, we did not find it suitable to separate the results by symptomatic status. Additionally, the number of symptomatic patients was too small to be suitable for separate analyses regarding the presence and functionality of the collateral cerebral circulation.

4. Discussion

We found no statistical difference in MBFV at baseline conditions between right and left PCAs between healthy subjects and patients. According to our results, a degree of ICA stenosis does not influence the MBFV in ipsilateral nor in contralateral PCA, showing that visual evoked response of the PCA remains similar both on the stenosed and the unstenosed side of ICAs in the case of more pronounced metabolic demands of the region and that the degree of ICA stenosis has no impact, or only exceptionally, on the collateralizing capacity of the PCAs. Those results that are in concordance with the results of the previous studies demonstrate an independent cerebral posterior circulation mechanism that compensates very successfully the anterior circulation insufficiency in severe carotid disease [3–7]. Concerning the correlation of the ICA stenosis, one of the limitations of our study was limited number of patients. Another limitation is that the ICA stenosis might serve as an indicator of vascular risk since it was shown that patients with increased risk factors have decreased hemodynamic response due to functional activation [13]. Our results show that our group of patients with severe carotid stenosis had the same response as healthy volunteers suggesting that carotid stenosis have no or only little impact on PCA flow velocities during various

Table 5: Correlation between mean blood flow velocities in physiological conditions and mean blood flow velocities during the white light stimulation and in the dark in healthy subjects.

Healthy ($n = 30$)					
		Light		Dark	
Mean blood flow velocities in posterior cerebral artery (cm/s)		Right	Left	Right	Left
Baseline(eyes opened)	Right	0.8812, $P = 0.001^*$	0.7035, $P = 0.001^*$	0.8459, $P = 0.001^*$	0.6690, $P = 0.001^*$
	Left	0.7556, $P = 0.001^*$	0.9235, $P = 0.001^*$	0.7553, $P = 0.001^*$	0.9450, $P = 0.001^*$

n: number of healthy subjects.
cm/s: centimeters in second.
*statistically significant.

Table 6: Correlation between mean blood flow velocities in physiological conditions and mean blood flow velocities during the white light stimulation and in the dark in severe carotid disease patients.

Pateints with severe carotid disease ($n = 49$)					
		Light		Dark	
Mean blood flow velocities in posterior cerebral artery (cm/s)		Right	Left	Right	Left
Baseline(eyes opened)	Right	0.7953, $P = 0.001^*$	0.2421, $P = 0.094$	0.7589, $P = 0.001^*$	0.2125, $P = 0.143$
	Left	0.4463, $P = 0.001^*$	0.6851, $P = 0.001^*$	0.3384, $p = 0.017^*$	0.6944, $P = 0.001^*$

n: number of patients.
cm/s: centimeters in second.
*statistically significant.

stimulation conditions suggesting an independent cerebral vascular reserve capacity of the posterior part of Willis circle.

Since the primary and associative visual areas located in the occipital lobes receive blood supply almost exclusively from the PCAs [14], every change in arterial blood flow due to differences in the metabolism of the visual cortex neurons is expected to have reflection on arterial blood flow in PCAs. Therefore, changes in blood flow in PCAs could indirectly reflect changes in metabolism of the visual cortex [15]. Although different authors used different methods and different kind of visual stimulation, making direct comparison quite difficult, previous fTCD studies have provided converging support for the view that visual stimulation might cause a greater activation of the visual cortex in right occipital lobe [16–22]. These studies on hemispheric asymmetry of the visual cortex were done on healthy subjects, raising questions about the potential impact of pathology on the research findings. In the present study, we, therefore, used fTCD to investigate hemispheric asymmetries of the visual cortex in patients with severe carotid stenosis and compromised anterior cerebral circulation, considering the importance of posterior collateral pathway via PCA. In the group of healthy subjects, we recorded statistically significant difference between MBFV in left and right PCA at baseline and MBFV in both PCAs during the white light stimulation and in the dark, regardless of the side of the measurement ($P = 0.001$). In the group of severe carotid disease patients, statistically significant correlation between MBFV in right PCA at baseline and MBFV in right PCA during the white light stimulation and in the dark was also found. On the contrary, correlation between MBVF in right PCA at baseline conditions and contralateral left PCA either during the white light stimulation or in the dark was not statistically significant. Moreover, analyzing the correlation between MBFV in left PCA at baseline, statistically significant correlation between MBFV in ipsilateral left PCA, as well as contralateral right PCA was found, both during the white light stimulation and in the dark, but the correlation between ipsilateral left side was significantly higher than the one with contralateral right side. Following our results, the correlation of MBFV with functional reserve capacity is more evident in the healthy subjects while the right-sided lateralization of the evoked responses more pronounced in the patients. Furthermore, the lateralization is less evident in the healthy subjects. The lower correlation coefficients in the group of patients with severe carotid stenosis could imply that carotid stenosis affects functional reserve capacity in the patients.

5. Conclusions

Considering the presented results obtained in our study, we can conclude that there is a clear trend towards the lateralization of the visual evoked response in the right PCA, being highly consistent with results of the previous studies and showing a general dominance of the right occipital lobe in the visual process. The right occipital lobe is more responsive to visual stimuli in terms of functional activation than the left one, indicated by a consistently higher blood

flow velocity response on the right. fTCD was able to assess hemispheric visual dominance not only in healthy individuals, but also in patients with severe carotid disease and thus compromised anterior cerebral circulation. Furthermore, velocity changes between sides as a measure of hemispheric perfusion lateralization could be an indicator of posterior collateral pathway. The demonstrated right-sided asymmetry in posterior cerebral circulation in patients with severe carotid disease could possibly be taken into consideration in further trials according to stroke risk or outcome.

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Clinical Study

Is Near-Infrared Spectroscopy a Reliable Method to Evaluate Clamping Ischemia during Carotid Surgery?

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Guidelines do not include cerebral oximetry among monitoring for carotid endarterectomy (CEA). The purpose of this study was to evaluate the reliability of near-infrared spectroscopy (NIRS) in the detection of clamping ischemia and in the prevention of clamping-related neurologic deficits using, as a cutoff for shunting, a 20% regional cerebral oxygen saturation (rSO_2) decrease if persistent more than 4 minutes, otherwise a 25% rSO_2 decrease. Bilateral rSO_2 was monitored continuously in patients undergoing CEA under general anesthesia (GA). Data was recorded after clamping, declamping, during shunting and lowest values achieved. Preoperative neurologic, CT-scan, and vascular lesions were recorded. We reviewed 473 cases: 305 males (64.5%) mean age 73.3 ± 7.3. Three patients presented transient ischemic deficits at awakening, no perioperative stroke or death; 41 (8.7%) required shunting: 30 based on the initial rSO_2 value and 11 due to a decrease during surgery. Using the ROC curve analysis we found, for a $>25\%$ reduction from baseline value, a sensitivity of 100% and a specificity of 90.6%. Reliability, PPV, and NPV were 95.38%, 9%, and 100%, respectively. In conclusion, this study indicates the potential reliability of NIRS monitoring during CEA under GA, using a cutoff of 25% or a cutoff of 20% for prolonged hypoperfusion.

1. Introduction

The European Society for Vascular and Endovascular Surgery (ESVS) guidelines [1] state that there is no evidence for the routine use of shunts during carotid endarterectomy (CEA) (grade A) and that both local and general anesthesia are safe (grade A). A critical issue confirms "there is insufficient evidence from RCTs to support or refute the use of routine or selective shunting during CEA."

Italian SPREAD guidelines [2] regarding monitoring during CEA also state that there is little evidence to support specific monitoring in cases of selective shunting and that electroencephalography (EEG) combined with stump pressure (SP) measurement can reduce the number of shunts required without increased risk of perioperative stroke [3].

The European and Italian Society for Vascular Surgery guidelines do not include cerebral oximetry (CO) using near-infrared spectroscopy (NIRS) among methods for the evaluation of cerebral ischemia during CEA under general anesthesia (GA). Many authors do not recommend this monitoring, assuming many limits of this method, such as the wide range of baseline regional cerebral oxygen saturation (rSO_2) values and lack of an absolute lower cut-off value. However, NIRS is widely used in cardiac, pediatric, neurosurgery and ICU for monitoring during and after operations [4]; moreover, it is used by anesthesiologists for the manipulation of blood pressure to optimize cerebral perfusion [5].

NIRS and its possible use in the evaluation of clamping ischemia has been reported in many papers [6]. Parameters reported by different available NIRS instruments are rSO_2, changes in intracerebral saturation (CsO_2), and tissue oxygen index (TOI) [7]. Parameters obtained using different tools are not comparable [8].

Firstly, we compared EEG and NIRS in a small sample of patients submitted to CEA under GA, showing a lowering of EEG records for decrease of rSO_2 values between 13% and 25% over baseline and a flattening for decreases between 19% and 23% [9]. Then, in a sample of 248 patients, using a cutoff

of 15% baseline decrease, we recorded 99.59% reliability, sensitivity of 66.6%, and specificity of 100% [10].

In patients operated under local anesthesia (LA), Roberts observed the appearance of neurologic symptoms for decreases of rSO_2 greater than 27% [11]. Using a 20% cut-off in patients operated under LA, Samra et al. [12] showed a sensitivity of 80% and specificity of 82%.

Since January 2000, we have routinely used NIRS with a 20% rSO_2 decrease cut-off value for the decision to shunt patients submitted to CEA under GA.

The purpose of this study was to evaluate NIRS reliability in the prevention of cerebral clamping ischemia during CEA under GA, with persistent neurologic deficit at awakening, using a 20% rSO_2 decrease as cutoff, if persistent more than 4 minutes, otherwise a 25% rSO_2 decrease.

As there is no valuable gold standard, and as many recent papers compare their results to those obtained using the stump pressure measurement, we estimated diagnostic accuracy across the group, also comparing accuracy between NIRS and SP measurement.

2. Materials and Methods

In this study, we evaluated prospective data of all patients consecutively submitted to elective CEA between 2006 and 2008 for symptomatic and asymptomatic carotid stenosis. Patients who underwent routine shunting (due to large contralateral infarction at preoperative CT-scan and patients treated by junior surgeons), plus those treated for urgent CEA and under remifentanil anaesthesia, were excluded from analysis.

Preoperative evaluation included CT-angiography of supraortic and intracranial vessels and brain-CT.

All patients were operated under GA, using standardized protocol with a combination of fentanyl and sevoflurane and including the i.v. administration of 2,500 IU of heparin during vessel preparation, before clamping. Vessel preparation was performed without distal clamping. Infiltration of carotid sinus with lidocaine was used in those few patients who showed heart rate modifications during vessel dissection. Antiplatelet treatment was begun at least one week before surgery or maintained. At the end of the procedure, heparin was not reversed; a completion duplex ultrasonography was performed before skin closure.

Bilateral regional cerebral oxygen saturation (rSO_2) was measured in the frontoparietal area using an INVOS 4100 cerebral oximeter (Somanetics, Troy, Mich, USA), considering oxygen saturation percentage as an absolute number and measuring rSO_2 changes using the formula: delta = (basal value − actual value)/basal value. Vital software, implemented in recent years, treats rSO_2 values as absolute numbers and calculates percent changes between basal value and actual value using the same formula, also showing a 20% desaturation threshold line. The rSO_2 was monitored continuously throughout the procedure; data was recorded at 20 second intervals. Probes were placed before positioning the head of the patient; rSO_2 value modifications during head positioning lead to modification of surgical position suspecting reduced efficacy of the vertebral collateral pathway.

All NIRS evaluations recorded for each patient throughout the procedure were collected and stored for detailed analysis.

For this study we chose the value recorded after induction of GA as rSO_2 basal value (T0), when oxygenation and systemic blood pressure were considered normal for the patient. We recorded rSO_2 values one minute after clamping (C1), one minute after insertion (S1) and after removal (S2) of the shunt in shunted patients, then in the first three minutes after declamping (D1-D2-D3). Finally, we recorded the lowest rSO_2 value (L-rSO_2), the maximum percentage decrease of rSO_2 over the basal value (delta rSO_2), and the time extent of delta greater than 20% (T3). In 59 cases (12.5%), when rSO_2 dropped more than 25% at carotid clamping, we declamped within one minute, to improve systemic blood pressure and to prepare for quick shunting; this value was recorded as trial clamping (T1).

Stump pressure (SP) was measured, but indication for shunting was based on a delta rSO_2 greater than 20%, if not improved within 3 minutes by increasing the systemic blood pressure, or greater than 25%. Shunt insertion was not performed in patients at risk of dissection and during the arteriotomy suture, when we presumed a further ischemic time of less than 4-5 minutes.

Other parameters, including neurological classification, presence or absence of ischemic lesions at CT-scan, and preoperative and completion ultrasound evaluations, are routinely recorded in a dedicated database. For preoperative neurological classification, we considered as asymptomatic all patients without ipsilateral hemispheric focal symptoms in the previous six months. Cerebral CT-scan was considered positive in presence of ischemic lesions including lacunar infarcts.

Anesthesiologists and surgeons assessed clinical results at patient awakening, confirmed by an independent neurologist within 24–36 hours after the operation, before discharge. All patients with neurologic deficits or headshake underwent urgent CT-scan, repeated after 24 hours if symptoms persisted. In addition, patients with an intraoperative rSO_2 decrease >20% for more than four minutes underwent CT scan before discharge.

The Local Research Ethics Committee granted approval for an observational study in consecutive patients treated for CEA.

3. Statistical Analysis

Data was analyzed using SPSS statistics version 17.0 for Windows (SPSS Inc. Chicago, Ill, USA). Categorical data was analyzed using Chi square or Fisher's exact test where appropriate. Differences in mean values of age, SP, and CO in both patient groups (those who required a shunt and those who were not shunted) were evaluated by 2-tailed *t*-Student test for unpaired samples. Mean rSO_2 trend during surgery was evaluated using a 2-tailed *t*-Student test for paired samples. Relationships between rSO_2 and SP were analyzed using

Pearson's correlation test (two tails). Data were tested for normality using the Kolmogorov-Smirnov test.

For assessment of the accuracy of cerebral oximetry in discriminating ischemic from nonischemic patients, we performed receiver operating characteristic (ROC) analysis.

Reliability was also calculated determining true and false positives and negatives, positive predictive value (PPV), and negative predictive value (NPV) in subgroup of nonshunted patients, using specificity and sensitivity tests.

Data is presented as mean (or median) ± SD. Probability values < 0.05 were considered statistically significant.

4. Results

A total of 473 cases were reviewed: 305 males (64.5%) mean age 73.3 ± 7.3 (range 50–86); 333 were asymptomatic for the ipsilateral hemisphere, but 70 of these had ischemic lesions in the ipsilateral hemisphere at cerebral CT-scan. Overall brain CT scan presented ipsilateral preoperative ischemic lesions in 162 (34.2%) patients. Detailed patient characteristic data is reported in Table 1.

No strokes or deaths were reported in the postoperative period.

Three patients (all women), operated for mono- and bilateral stenosis, presented transient neurologic symptoms (TIA) at awakening; patients no. 1 and no. 3 required extended clamping time. In case one we inserted a shunt between the common and external carotid artery, as it was impossible to perform a carotid reconstruction with an intraluminal shunt in the normal position. RSO_2 increased, but remained lower than 20% overall for 17 minutes subdivided into three periods of three, eight, and six minutes, respectively. The patient awoke slowly with uncertain neurologic deficits but recovered after a few minutes. In case three the shunt did not work due to distal kinking. On awakening she demonstrated mild paresis of the hand but recovered after 30 minutes. Patient no. 2 had very low SP and basal rSO_2, quickly recovered to 47% after shunt insertion; low perfusion lasted three minutes before shunting and for a further three minutes after shunt removal. At awakening the patient showed paresis of the upper limb which recovered within one hour. All patients had a decrease >25% which lasted for two—five minutes, none had changes at brain CT-scan, and none presented new neurologic symptoms (see Table 2).

5. Monitoring Evaluation

In general rSO_2 decreased after carotid clamping (C1), but in some patients it spontaneously increased after clamping due to carotid bulb baroreflex. Clamping induced a significant decrease of mean rSO_2 value ($P < 0.0001$) in both patient groups. Mean rSO_2 increased slightly over base soon after declamping (D1-D2-D3). No difference was noted in rSO_2 after trial clamping (when performed) and definitive clamping.

After declamping we observed an immediate increase of rSO_2; one minute after declamping, mean values were similar to base, but significantly slightly higher ($P < 0.002$) (Tables 3 and 4). In 12 cases, prevalently nonshunted patients, with delta rSO_2 < 20% (5 cases) and >20% (7 cases), we observed a temporary rSO_2 increase considered as an instrumental sign of hyperperfusion.

The shunt significantly increased mean rSO_2 one minute after insertion ($P < 0.0001$), even though mean data remained significantly lower than baseline ($P < 0.0001$) (see Table 4).

One hundred and four patients reached a delta rSO_2 greater than 20% and only 46 greater than 25%. SP was <50 mmHg in 172 cases. An intraluminal shunt was inserted in 41 cases as some rSO_2 decreases lasted for less than two minutes or arose during carotid suture.

Relationships between SP and C1 values were not significant; in fact there was a weak but significant inverse relationship between SP and delta-rSO_2 (-0.273; $P < 0.0001$). Evaluating the differences between two categories of SP (<50 mmHg and ≥50 mmHg) and delta rSO_2 using two cut-off values, a significant difference emerged between the two parameters using both a delta rSO_2 cut-off of 20% ($P < 0.002$) and 25% ($P = 0.016$, Fisher's exact test).

Twenty-four patients presented rSO_2 lower than 40% during clamping with a further five lower than 30%. Two symptomatic patients, at awakening, reached 28% and 27% during clamping, respectively, while the lowest value of other symptomatic patients was 49%. (See Table 2.)

6. Reliability

Using the ROC curve analysis, we calculated the sensitivity and specificity of many different threshold reductions of rSO_2 in detecting neurologic deficits after carotid endarterectomy. Plotting these thresholds on a graph of sensitivity versus, 1−specificity in order to find that with the best performance we found, for a >25% reduction from baseline value, a sensitivity of 100% and a specificity of 90.6% (CI (95%) 0.934–0.997). The shape of the curve is very square indicating excellent performance of cerebral oximetry in preventing neurologic deficits (Figure 1). The lower rSO_2 value did not result predictive (AUC 0.075, SE 0.059, CI (95%) 0-1).

Reliability was also calculated in the group of 433 patients nonshunted or with nonfunctioning shunts, including the patient with the shunt in the external carotid artery. Using a delta cutoff of 25%, reliability, sensitivity, specificity, PPV and NPV were 95.38%, 100%, 95.36%, 9% and 100% respectively. When using a 20% cutoff they were 82.67%, 100%, 82.83%, 2.26% and 100% respectively.

Using an SP of 50 mmHg as cutoff in the same group, reliability, sensitivity, specificity, PPV, and NPV were 36.6%, 66%, 47%, 0.6%, and 99.4%, respectively.

7. Discussion

Endarterectomy under GA needs cerebral monitoring to avoid ischemic deficits and/or the routine use of shunts. EEG is the most studied neurophysiologic technique which shows functional deficit when blood flow is <20 mL/100 g/min; however, we know that cerebral damage occur at a flow of <6–10 mL/100 g/min. EEG also presents some disadvantages

Table 1: Patient characteristics and analysis of the difference between the groups in relation to shunting and symptoms at awakening.

Characteristics		Num./mean	%/SD	Not shunted	Shunted	*P*	Asympt.	Sympt.	*P*
Patients		473		432	41		470	3	
Sex	M	305		279	26	NS	305	0	0.0044
	F	168		153	15		165	3	
Age		73.3	7.4	73.3 ± 7.4	73.3 ± 7	NS	73.3 ± 7.3	74.6 ± 9	NS
Symptoms	none	333	70.4	305	28	NS	332	1	NS
	TIA	46	9.7	42	4		45	1	
	ocular	11	2.3	9	2		11	0	
	VB/ borderline	30	6.4	27	3		30	0	
	m-stroke	32	6.8	27	5		31	1	
	stroke	21	4.4	20	1		21	0	
Contralat. stenosis	None	311	65.7	291	20	<0.0001	310	1	NS
	>70%	134	28.3	123	11		132	2	
	Occlusion	28	5.9	18	10		28	0	
Positive CT scan	Ipsilateral only	60	12.7	52	8	NS	59	1	NS
	Bilateral	102	21.6	94	8		101	1	
	Contralat. only	42	0.9	37	5		42	0	
Monitoring	T0	64.8	±9.8	64.9 ± 9.7	64.4 ± 10.3	NS	64.9 ± 9.7	50.6 ± 14.4	0.012
	Delta-RSO_2	13.5	±9.8	12.3 ± 8.7	26.1 ± 11.1	<0.0001	13.4 ± 9.7	32.4 ± 5.4	<0.001
	Stump pressure	59.8	±23.13	61.9 ± 22.6	38.6 ± 16.1	<0.0001	59.6 ± 23.1	52.3 ± 28.5	NS

Table 2: Characteristics of patients with TIAs at awakening.

N	Age	carotid lesion	Bain CT		Preop. symptoms	Stump press.	RSO_2				Shunt	20	Time > 25
No.			Omo	Ctr		mmHg	T0	C1	Min	Delta (%)		min	Min
1	84	BS	+	–	TIA	50	67	62	49	26.87	ECA	3 + 8 + 6	5
2	66	MS	–	–	Asympt.	25	40	27	27	32.50	Yes	3 + 3	3 + 2
3	74	BS	+	+	mStroke	82	45	35	28	37.78	NF	2 + 1	2 + 1

MS: monolateral stenosis, BS: bilateral stenosis, omo: omolateral brain, ctc: contralateral brain CT, ECA: shunt inserted in external carotid artery for technical reason, NF: not functioning, time: time length delta-RSO_2 >20%/25% recorded consecutively in two or more steps of the operation.

as its use requires the presence of a neurologist and has other limits due to anesthetic drugs and its inability to highlight ischemic complications of the subcortical and internal capsule area [13]. One review shows an incidence of false positives of between 8% and 13% while false negatives range between 5% and 50% [14]. False negatives are often associated to lacunar infarct [15], under LA reaching up to 40.6% [16] and are particularly dangerous.

The incidence of ischemic complications due to carotid clamping is fortunately low; therefore, it is difficult to find significant data for each monitoring tool.

Cerebral oximetry by NIRS is the latest monitoring proposed; it has been criticized for the wide range of values in normal conditions and for the lack of a sure cut-off value. Moreover, there are many confounding factors that may change rSO_2, such as modification of systemic oxygen saturation and blood pressure, bronchodilatation, and so forth, but these same factors could also modify brain perfusion during LA with an increase of neurologic symptoms. As these factors can be quickly modified by the anesthesiologist, with consequent return to normality, we did not take them into account. Otherwise, all medications used for GA influence all neurophysiologic monitoring. Notwithstanding these criticisms, many studies confirm NIRS reliability. When compared with SSEP, NIRS showed a greater rSO_2 decrease in patients with flattening of potentials [17]. Studies obtained using INVOS showed good relationships between NIRS, jugular bulb venous oxygen saturation (SjO2), and trans-cranial Doppler (TCD) [18]. To date, the proposed cut-off for NIRS ranges between a 15% and 25% reduction over baseline.

Samra et al. [12] using a cutoff of 20%, in patients operated under LA, recorded a sensitivity of 80% and a specificity of 82% concluding that using a cutoff greater than 20%, NIRS

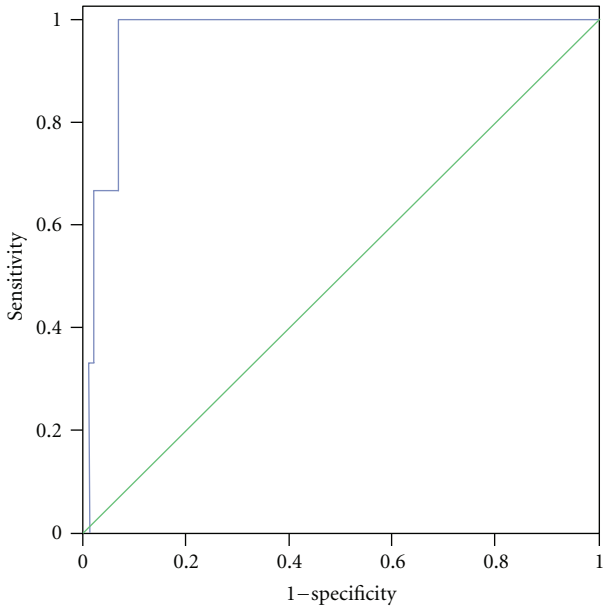

Figure 1: ROC curve of delta rSO_2. Area under the curve 0.966, SE 0.016, CI (95%) 0.934–0.997.

Table 3: Mean values of rSO_2 recorded in the different steps of operation compared to mean T0 values. P = P value (two-sided) t-Student test for paired samples.

RSO_2 values	Mean	SD	P
T0	64.85	9.8	
T1	59.44	10.3	<0.0001
C1	59.34	10.4	<0.0001
L- RSO_2	56.29	10.3	<0.0001
D1	65.42	10.3	0.064
D2	66.38	34.3	N.S.
D3	64.61	9.7	N.S.

Table 4: Mean values of rSO_2 recorded in the two groups of shunted and not shunted patients.

Time	Shunted		Not shunted	
	Mean	SD	Mean	SD
T0	64,41	10,31	64.89	9.76
T1	53,88	10,842	59.99	10.12
C1	52,37	11,467	64.53	10.27
S1	59,27	12,994		
S2	51,46	11,567		
D1	63,88	11,122	65.73	9.87
D2	62,80	15,302	65.26	9.84
D3	64,15	11,421	64.69	9.55

has a sensibility and specificity similar to EEG and SSEP, with a low incidence of false negatives but a high incidence of false positives.

Another study [19], also performed under LA, comparing NIRS, TCD, SP, SSEP, with neurologic surveillance, found as cutoff: a 20% decrease of basal rSO_2, a 48% decrease of TCD, 40 mmHg of SP, 50% decrease of SSEP and a lower rSO_2 value of 59%.

This data contrasts with the results of another study which showed that cerebral saturation lower than 54–56.1% and a decrease greater than 15.6–18.2% predicts neurological impairment [20].

A recent study reported the relationship between prolonged rSO_2 desaturation and cognitive changes, showing impairment below a rSO_2 threshold of 50% [21].

In our sample, mean lowest rSO_2 value was 56.3 ± 10.2 (95% CI 55.4–57.2, range 20–91), and the median value was 57%. However, 298 patients had an L-rSO_2 < 59, 194 patients <54 and five <30; only two of these were symptomatic at awakening. We also performed studies on cognitive changes; however, these modifications were not included as part of this particular study.

In a study including 594 patients operated under GA without shunting, the best cutoff was 11.7%, with a sensitivity of 75% (95% CI 71–78) and a specificity of 77% (95% CI 74–80). A cutoff of 20% showed a lower sensitivity (30%) but a higher specificity (98%) in identifying complications, with a PPV of 37% and a NPV of 98% [22].

In another recent study under LA, the cut-off drop indicating the need for shunt was 19%, with a sensitivity of 100%, a specificity of 98%, a PPV of 82%, and a NPV of 100% [23].

Brain perfusion is modified by head position, blood pressure, and heart and respiratory rate, plus autoregulatory response (also in GA) or by administration of vasoactive drugs. For this reason, it is difficult to compare results obtained under LA with those obtained under GA. Moreover, we agree with Gough [24] and McClearly et al. [25] about the impossibility of applying criteria for shunt insertion under GA derived by CEA under LA.

As we know that neurological deficit follows a persistent flow decrease, we introduced a variable "time" in the shunt decision. On this basis, we decided to evaluate the diagnostic accuracy of this monitoring for an rSO_2 decrease greater than 20% or 25%, in the prevention of cerebral clamping ischemia during CEA. A single center study avoided the bias linked to multiple surgical and anesthesiological staff.

In our sample, using NIRS to identify the need for shunt, we did not observe any strokes. The three TIAs occurred in patients with a delta-rSO_2 greater than 25%; patients no. 1 and no. 3 presented a very low rSO_2 value after clamping; SP indicated the need for shunt only in patient no. 2.

NIRS allowed us to monitor modifications of cerebral oximetry during all surgery phases, from anesthesia to head positioning and awakening. Utilizing this tool, we used a shunt in 41 (8.7%) of patients; based on the initial rSO_2 value, the number patients with indication to shunting would have been 30 but in the other 11 patients CO decreased during surgery without correction by anesthetic management. Here NIRS was useful in showing delayed rSO_2 reduction leading to shunt insertion also in these patients. Furthermore, it was useful to alert promptly of shunt malfunction. Using a delta rSO_2 cutoff ≥ 15%, 20%, 25%, and 30%, we would have used a shunt in 185, 107, 47, and 22 patients, respectively. Using SP at 50 mmHg cutoff (derived

by our previous experiences), we would have used a shunt in 172 cases or in 102 cases using a cutoff of 40 mmHg.

The reliability of NIRS in our sample was high (95.38%), with a sensitivity of 100% and a specificity of 95.36%. The number of false positives was 4.6% using NIRS with rSO_2 cutoff of 25% against 44.6% using SP. The lack of false negatives with CO in our study, even using a 25% decrease as cutoff, offers an important safety benefit.

Lee et al. [26] showed a relationship between rSO_2 decrease and SP ($r = -0.57$, $P = 0.002$); however in our sample this relationship was very weak ($r = -0.26$ $P < 0.0001$).

Evaluation of our postdeclamping data is very useful. Generally we observed a prompt rSO_2 increase (within 5 seconds), slightly higher than baseline. In patients with a preserved baroreflex, systemic blood pressure decreased with the secondary lowering of CO. The absence of an increase may be related to carotid thrombosis or hemodynamic failure. Significantly high values can be predictive of hyperperfusion [27]. In our sample we observed only 12 (2.5%) cases of temporary hyperperfusion that induced a strict control of systemic blood pressure.

8. Conclusions

It is not possible to show functional changes under GA without using EEG and/or SSEP (with the reported limits) as can done under LA or GA with remifentanyl conscious sedation, being only possible to show transient or permanent neurologic deficits at awakening.

For this reason, the purpose of our monitoring was to prevent TIAs and strokes related to carotid clamping, without routine shunting and to demonstrate the suitability of the cutoff. This protocol did not include a routine postoperative neuroimaging; however, we performed a CT-scan in all patients showing any symptoms or at risk, without evidence of new ischemic areas.

The results of this study suggest that during CEA, under GA, monitoring of cerebral oxygenation by NIRS is a reliable method, using a cutoff of 25% or a cutoff of 20% for prolonged hypoperfusion. The absence of false negatives confirms its safety. Based on our study sample, we are unable to propose a lower value as cutoff for shunting; however, it is certainly lower than those proposed under LA. In consideration of data reported by Slater et al. [21], we agree that there is need for further study of this complication in CEA.

In conclusion, NIRS cannot exclude ischemic damages due to embolism during carotid preparation or after declamping, which can only be showed by TCD, a useful tool particularly in teaching hospitals. Our study also does not provide information about clamping ischemia not followed by neurologic deficits at awakening, which are not important if they are not responsible for cognitive deficits, as suggested by some studies.

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Research Article

Effects of FK506 on Hippocampal CA1 Cells Following Transient Global Ischemia/Reperfusion in Wistar Rat

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Transient global cerebral ischemia causes loss of pyramidal cells in CA1 region of hippocampus. In this study, we investigated the neurotrophic effect of the immunosuppressant agent FK506 in rat after global cerebral ischemia. Both common carotid arteries were occluded for 20 minutes followed by reperfusion. In experimental group 1, FK506 (6 mg/kg) was given as a single dose exactly at the time of reperfusion. In the second group, FK506 was administered at the beginning of reperfusion, followed by its administration intraperitoneally (IP) 6, 24, 48, and 72 hours after reperfusion. FK506 failed to show neurotrophic effects on CA1 region when applied as a single dose of 6 mg/kg. The cell number and size of the CA1 pyramidal cells were increased, also the number of cell death decreased in this region when FK506 was administrated 48 h after reperfusion. This work supports the possible use of FK506 in treatment of ischemic brain damage.

1. Introduction

Reperfusion injury plays an important role in the brain ischemia cascade, which is involved in stroke and brain trauma. It is due to the inflammatory response of injured tissues [1]. Returned blood flow can reintroduce oxygen which can damage proteins, DNA, and plasma membranes of the cells. Plasma membrane damage may in turn induce the release of more free radicals. These processes may play an important role in redox signaling indirectly and cause cell apoptosis [2].

Certain areas of the brain and certain types of neurons which are more sensitive to cerebral ischemia are pyramidal neurons of the CA1 region of the hippocampus [3, 4].

Apoptosis is the most important process in CA1 neurons exposed to transient global ischemia. Apoptotic cell death of neurons can be limited by inhibition of macromolecular synthesis and of caspase activity [5]. Therefore, the attempts have predominantly been concentrated on prevention of acute cell death to help stroke patients. More than one hundred agents have proven to be neuroprotective in experimental models [6].

Unlike the promising results from animal models in preventing these types of cell death, unfortunately, no effective pharmacologic strategy has been found to deal with the problem of ischemia. This may be due to lack of efficacy and presence of unwanted side effects [7, 8].

Recently, using immunophilin ligands has been considered as a potential and appropriate strategy for neuroprotection. Since it was observed that tacrolimus (FK506), a useful immunosuppressant used in organ transplantation, provides

neuroprotection against glutamate-induced neurotoxicity in vitro and prevents hippocampal neuronal damage in a model of transient global ischemia, the importance of immunophilins in the development of neuroprotectors has emerged [9, 10].

Neuroimmunophilin ligands are a class of compounds that can be used for the treatment of nerve injuries and neurological diseases. These ligands can readily penetrate the blood-brain barrier and are effective in a variety of animal models of ischemia, traumatic nerve injury, and neurodegenerative disorders in human being [11].

Cyclosporin A and FK-506 have already been used in humans (as immunosuppressant drugs). Both of them exhibit neuroprotective actions, but only FK506 and its derivatives have significant neuroregenerative properties. Tacrolimus is a macrolid antibiotic compound which exhibits immunosuppressant effects and interact with the immunophilin FKBP12 (FK506-binding protein). The resulting complex, in turn, can inhibit calcineurin, a Ca^{2+} calcium/calmodulin-dependent serin/threonine phosphatase [12, 13].

Neuroprotective and neuroregenerative properties of tacrolimus seem to act via different mechanisms, because FK506-related compound that bind to FKBP-12 do not block calcineurin (as an immunosuppressant). Thus, FK506 has the ability to enhance nerve regeneration and acts via a calcineurin-independent mechanism [11, 14, 15].

Many studies assessed neuroprotective effects of FK506, but little is known about the neurotrophic effects of this drug [11, 15, 16].

The time of injection may also play an important role in the process of neurogenesis, so comprehensive research regarding the effects of different times of injection seems necessary [17].

In the present study, neurotrophic properties of FK506 in CA1 area of Wistar rat hippocampus were assessed by histological outcome following ischemia/reperfusion in different periods of the drug injection.

2. Methods

In this study, we evaluate the effects of repeated doses of FK506 after ischemia at various times (IV injection exactly at the time of reperfusion and repeated IP at 6-24-48-72 hours after reperfusion) on hippocampus CA1 pyramidal cell numbers, diameters and number of apoptotic bodies by using Nissl stain and TUNEL-labeled sections Figure 3.

All of the data was collected from the CA1 sector of the hippocampus. The number of surviving pyramidal cells, their diameters and number of dead cells were counted in control, sham and experimental groups which received single and repeated doses of FK506 in various times of injection.

2.1. Animals. Adult male Wistar rats 12 to 13 weeks old and weighing 250–300 g from the Pharmacology Department of Tehran University of Medical Sciences were used in all experiments. The rats were housed under a 12-hour light/dark cycle. They were allowed free access to food and water.

All of them were housed in animal house for at least 5 days prior to experiments. All experiments were performed at the Department of Anatomy, School of Medicine of Tehran University of Medical Sciences and Institute of Cognitive Science Studies in 2010.

All procedures used in the study were approved by the ethics committee for the use of experimental animals at Institute for Cognitive Science Studies, Tehran, Iran.

2.2. Experimental Groups and Drugs. Animals were divided randomly into 7 groups ($n = 35$) as described below.

(1) Control group: rats only anesthetized by pentobarbital sodium (40 mg/kg).

(2) Sham group: After anesthesia by pentobarbital sodium, common carotid arteries on both sides were occluded for 20 minutes followed by reperfusion.

(3) Experimental Group 1: After anesthesia and ischemia for 20 min followed by reperfusion, 6 mg/kg Tacrolimus was injected intravenously (IV) at the beginning of reperfusion phase.

(4) Experimental Group 2: After anesthesia and ischemia for 20 minutes followed by reperfusion, 6 mg/kg Tacrolimus was injected (IV) at the beginning of reperfusion phase. After 6 hours Tacrolimus was injected intraperitoneally (IP) again with the same dose.

(5) Experimental Group 3: After anesthesia and ischemia for 20 min followed by reperfusion, 6 mg/kg Tacrolimus was injected (IV) at the beginning of reperfusion phase. After 24 hours, the injection was repeated with the same dose intraperitoneally.

(6) Experimental Group 4: After anesthesia and ischemia for 20 min followed by reperfusion, 6 mg/kg Tacrolimus was injected (IV) at the beginning of reperfusion phase. After 48 hour injection was repeated with the same dose (IP).

(7) Experimental Group 5: After anesthesia and ischemia in the same period, injecting a dose of 6 mg/kg Tacrolimus (IV) at the beginning of the reperfusion phase was done. After 72 hours injection was repeated with the same dose (IP).

Rats in groups ((1) to (7)) were sacrificed after 4 days, and all brains were removed for histological assessment (Nissl and TUNEL methods).

FK506 (solution, 5 mg/mL ampule) was kindly gifted by the Astellas Pharmaceutical Co (Osaka-Japan).

2.3. Surgical Procedures. To induce transient cerebral ischemia, rats were anesthetised with sodium pentobarbital anesthesia (40 mg/kg, IP). A rectal temperature probe was inserted, and body temperature was monitored and maintained at 37°C using heating lamps. Both common carotid arteries were exposed and freed from its carotid sheet, then

the vagus nerves were carefully separated. Both common carotid arteries were occluded for 20 min using Yashargil Aneurism microclips.

During ischemia, the animals were monitored for body temperature, loss of righting reflex, and unresponsiveness to gentle touch.

Subsequently, the carotid arteries were released and inspected for immediate reperfusion. Recirculation of blood flow was established by releasing the clips and restoration of blood flow in the carotid arteries was confirmed by observation. Animals were returned to their home cage after the surgery and kept separately for 4 days (96 h). After this period of time, rats were anesthetized intraperitoneally with pentobarbital sodium (40 mg/kg) and transcardiac perfusion was performed with heparin (10 U/mL) in 0.9% salin, followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PH = 7.4). Their brains were removed and postfixed in the same fixative for more than 3 days. Brains were rapidly removed and put in the fixator for more than 3 days.

2.4. Histopathologies. Paraffin-embedded coronal sections at different thicknesses were cut for different staining methods, 3 and 10 μm thickness for Nissl and Tunel staining respectively, between 2.3 and 5 mm posterior to bregma fortune.

2.5. Nissl Staining. For Nissl staining, 10 μm-thick sections were mounted directly onto gelatin-coated glass slides and air-dried. The slides were stained with 1.0% cresyl violet, dehydrated, and cover-slipped with Entellan. Eight photomicrographs were prepared from each animal (between the level of 2.3 and 5 mm posterior to bregma fortune according to the paxinos atlas). Three of them were selected and counted at ×400 magnification of light microscope by a blinded investigator. Only cells with an evident nucleus and nucleolus were included. Images were taken at ×400 magnification with a microscope (Olympus *AX-70*) and analyzed by using image tool 2 software.

2.6. TUNEL Staining. For TUNEL staining, paraffin blocks were cut into 3 μm thickness coronal sections. To detect apoptotic cells, TUNEL staining was performed using an In Situ Cell Death Detection Kit (Roche, Mannheim, Germany) according to the manufacturer's protocol. Briefly, the sections were deparaffinized in xylol, rehydrated by successive series of alcohol, washed in phosphate-buffered saline (PBS), and deproteinized (or permeabilized) by proteinase K (20 μg/mL) for 30 min at room temperature. Then, the sections were rinsed and incubated with 3% H_2O_2 in methanol for 10 min in the dark to block endogenous peroxidase (POD) then the sections were incubated in the TUNEL reaction mixture for 60 min at 37°C in humidified atmosphere and rinsed with PBS. Then, sections were visualized by using converter-POD for 30 min in 37°C at humidified atmosphere in the dark then rinsed with PBS and 50–100 μL DAB substrate [diaminobenzidine (DAB)] was added and rinsed with PBS then all slides were mounted by cover slip and analysis by light microscope. Coronal sections of the CA1 area of hippocampus at the level of 2.3 and 5 mm posterior to bregma according to the paxinos atlas (four sections per animal) [18] were examined and TUNEL-positive cells were quantified using light microscopy at ×400 magnification. Values (per mm^2) from four sections were averaged to calculate the number of TUNEL-positive cells. All counting procedures were performed blindly.

2.7. Statistical Analysis. The data were expressed as mean ± standard error of measurement (S.E.M.). Statistical analysis of the data for multiple comparisons were performed by one-way ANOVA (SPSS 13 software program) followed by Tukey's test. Statistical significance was defined as a P value ≤ 0.05.

3. Results

After 20 minutes of bilateral Common Carotid Arteries (CCA) occlusion, marked CA1 cell loss was noticed in all subject groups (Figure 1). We found a statistically significant difference in CA1 cell loss among controls and all other subjects groups except group 6 ($P = 0.233$). Although marked decrease in CA1 hippocampal region cell diameters was observed after 20 minutes of bilateral CCA occlusion in all groups, differences were statistically significant only for group 2 versus controls ($P = 0.03$).

Figure 2 shows the relationship of repeated injections of FK506 on ischemia reperfusion-induced CA1 cell death in various times on cell diameter.

We next carried out TUNEL staining which detects DNA damage characteristic of apoptosis after bilateral common carotid occlusion for 20 minutes.

The number of TUNEL-positive cells was significantly increased compared with control group in the CA1 region of the hippocampus after ischemia. There was a significant difference between control group versus groups 2 ($P = 0.001$) and 3 ($P = 0.023$), which means that apoptotic cells were significantly decreased by repeated injection of FK506 in this region.

Figure 4 shows the relationship of FK506 treatment on number of apoptotic cell bodies in CA1 region in all groups with various times of injection.

Our results show that neurodegenerative changes were not mitigated by a single FK506 application and that repeated applications of tacrolimus ameliorate not only CA1 damage and neurodegeneration, but also have protective effects, which can reduce CA1 cell death.

4. Discussion

Our study demonstrates that rats which are subjected to 20-min brain ischemia showed loss of the pyramidal cell in number followed by Delayed Neuronal Death (DND) in CA1 region of hippocampus. Our data extends previous findings of the CCO models of animals which showed transient global cerebral ischemia/reperfusion causing neuronal cell death [19, 20].

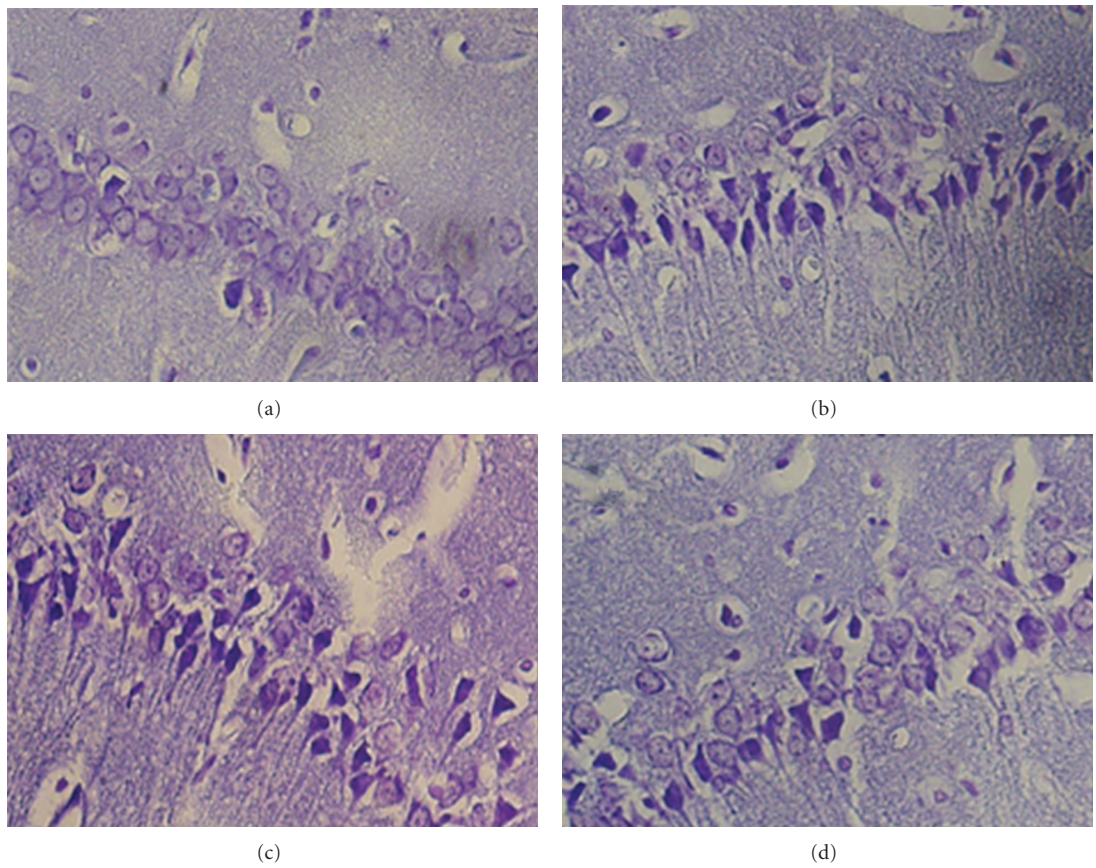

Figure 1: Photomicrographs of coronal sections (cresyl violet stain) of the hippocampus. (a) Control group. (b) Rats subjected to ischemia operation. (c) Ischemia plus single dose of FK506 (IV injection exact the time of reperfusion. (d) Ischemia plus repeated doses of FK506 (IV injection exact the time of reperfusion and IP injection at 48 h after reperfusion). Scale bar = 30 μm.

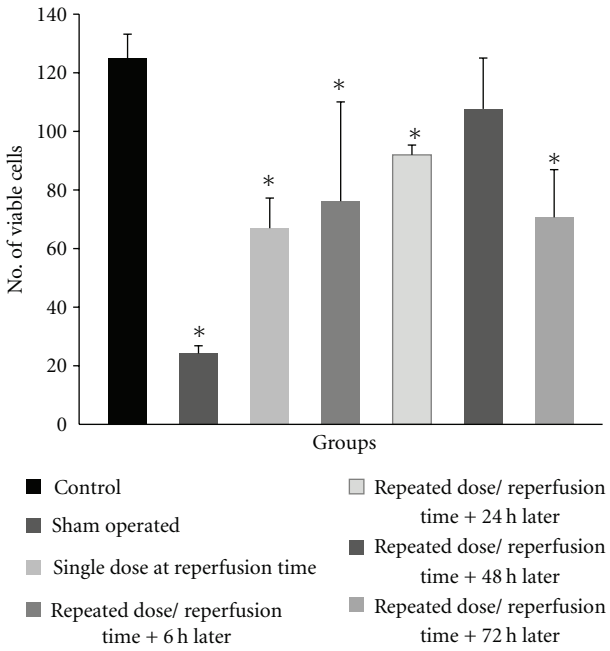

Figure 2: The relationship of repeated injections of FK506 on ischemia reperfusion in various time on pyramidal cell number of CA1. $^*P < 0.05$ versus control and repeated dose/reperfusion time, 48 h later groups.

In the first experiment, (group 3) a single intravenous injection of FK506 (6 mg/kg) exactly at the time of reperfusion, did not significantly increase the number and size of pyramidal cells of the CA1 region of the hippocampus; however, when the repeated dose of this drug was used after 48 h with the same concentration, significant increase was observed in the number and size of these cells. our data supported the previous study which demonstrated that repeated FK506, application in contrast to a single injection regime reduces ischemia induced CA1 pyramidal cell [17]. We also found out that the single dose could not decrease the number of Tunel-positive cells in the ischemic region but repeated dose of this drug can decrease the number of Tunel-positive cells in the ischemic region. The potent immunosuppressant was shown to be neuroprotective confirming previous studies [21–23].

There are studies reporting that FK506 can speed up nerve regeneration after peripheral nerve injury in rats [24]. In addition, it increases axonal regeneration following spinal cord injury in a dose-dependent fashion [25]. Applied FK506 enhances the sprouting of axonized central intrinsic neurons such as retinal ganglion cells after optic nerve crush [26]. FK506, and its derivative L-685,818 treatment of rats with crushed sciatic nerves, enhances both functional recovery and regenerated myelinized fibers [27]. FK506 can exert

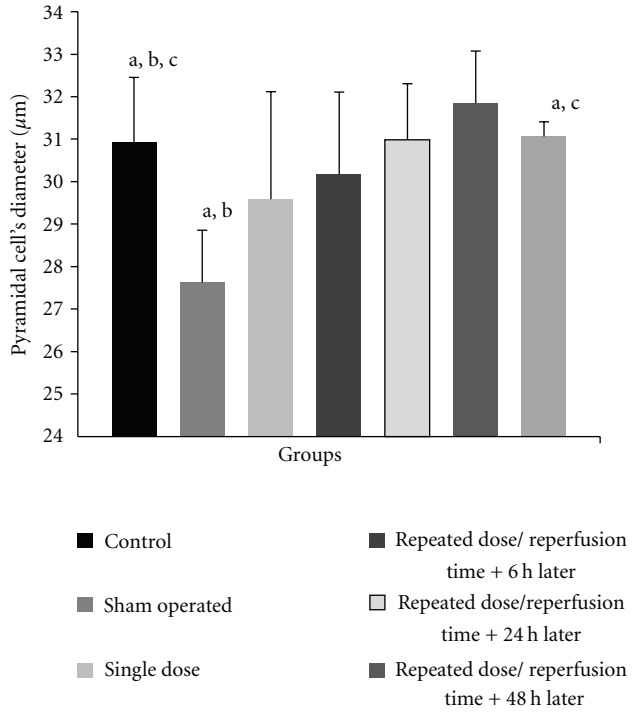

Figure 3: The effect of FK506 (6 mg/kg injection IV and 4 injections IP in various times) on pyramidal CA1 cells diameters after transient global ischemia. $^{a,b,c}P < 0.05$ control (a) versus sham-operated (b) and repeated dose/reperfusion time +72 h later (c) groups.

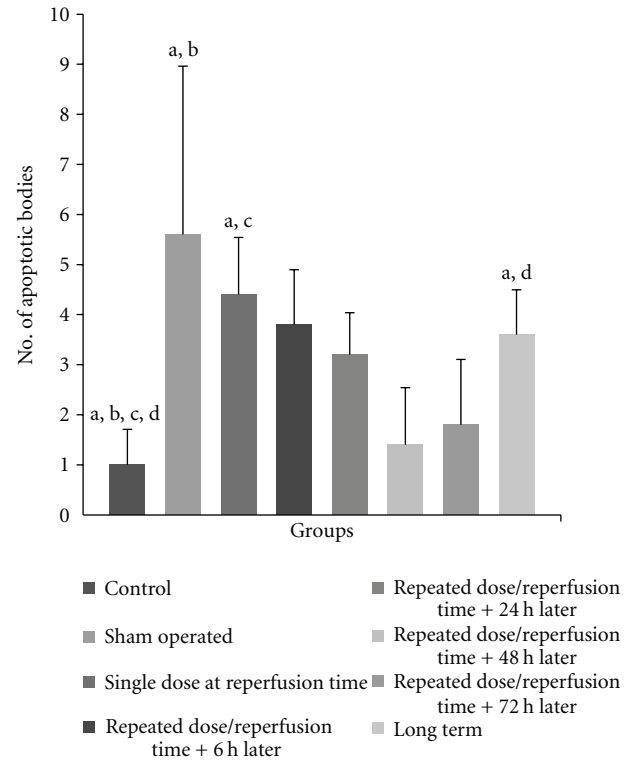

Figure 4: Bar graph shows the effect of repeated doses of FK506 on ischemia-induced, hippocampal pyramidal CA1 cell death (apoptotic body) in various times of injection. $^{a,b,c}P < 0.05$ control versus sham, single dose and long term groups.

neurotrophic effects on the penile innervations that promote erectile function recovery in rats after extensive CNS injury [28]. Our findings also show that FK506 can improve the number and size of pyramidal neurons in the hippocampal CA1 region after global transient brain ischemia in a time-dependent fashion of repeated injection.

Tissue injury due to ischemia is the result of a pathophysiological cascade which can increase glutamate concentration and stimulate NMDA and other glutamate receptors which can increase Ca^{2+} entry and cause cell death [29].

DND in the hippocampus following global ischemia was first recognized [30]. Feature of DNA ladders on gel electrophoresis of extracted nuclear DNA is a key feature in apoptotic cells which have been identified after ischemia of 12 or 20 minutes in the rat and 5 or 20 minutes in the gerbils [31, 32].

Several studies were done in order to find the mechanism of ischemic cell death, but up to now, the definite mechanism underlying DND followed by ischemia is not found. Calcineurin is one of the most important enzymes which is necessary to regulate the phosphorilation activity of many proteins in neurons [33, 34]. Brain calcineurin concentration is 3–10 times higher than other tissues [35].

FK506, which is one of the immunosuppressive drugs, binds to the immunophilin FKBP12 (FK506 binding protein) and creates a new complex. This FKBP12-FK506 complex interacts with and inhibits calcineurin [36]. Calcineurin is a common target of FKBP-FK506 complexes [37]. This drug crosses the blood-brain barrier and has neuroprotective effect [38–40]. In this study, it seems that the same mechanism causes this protective effect on the pyramidal neurons of CA1 region.

Several studies have reported that FK506 acts as a neuroprotective agent in several models of focal and global cerebral ischemia in rats, gerbils, and primates, and it can inhibit NO production which contributes to the neuroprotective effect of this drug on DND in the hippocampus [41–46].

Our results are not only in accordance with previous studies, but also shows that FK506 as a neuroprotective agent can ameliorate DND.

Our findings also confirm with the previous finding which reported that a single IV injection of FK506 provided no protection, but this result does not correlate with the study which reported that a single-dose injected (3 or 10 mg/kg) IV has neuroprotective effect on pyramidal cells of robust when injected immediately after ischemia [47, 48].

Several studies reported that neurogenesis had occurred after ischemia in the CA1 region [49]. Subgranular and subventricular zones are two sites which are neurogenic areas in intact adult mammalian brain and produce newborn neurons [50]. In normal conditions, there is no neurogenic region, but in animal models of stroke, newborn neurons from the subependymal zone might be induced [51]. The new cells which are generated in subgranular zone of hippocampus differentiate into granular neurons which efficiently develop and change to mature neuronal cells [52–54].

As mentioned before, there was no statistically significant difference in number of pyramidal cells between the rats that were given repeated doses of tacrolimus after 48 hours and the control group. According to previous studies, further investigations are needed for determining whether these cells are induced by brain endogenous factors or not.

5. Conclusion

It was concluded that 20-min brain ischemia showed loss of the pyramidal cells in number and size followed by DND in the hippocampal CA1 region. Repeated dose of FK506 via different mechanisms of action can increase the number and the size of pyramidal cells of the CA1 region of hippocampus and has a neuroprotective effect against DND.

It seems that when FK506 was given repeatedly as a combination of IV (at the beginning of reperfusion phase) plus IP injection (48 h after reperfusion), it may have stimulated the endogenous zones of the brain, and a neurotrophic effect was provided. Such an effect may depend on a repeated dose protocol.

Conflict of Interests

The authors report no conflict of interests.

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Clinical Study

Safety of Early Carotid Artery Stenting after Systemic Thrombolysis: A Single Center Experience

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Background. Patients with acute ischemic stroke due to internal carotid artery (ICA) disease are at high risk of early stroke recurrence. A combination of IV thrombolysis and early carotid artery stenting (CAS) may result in more effective secondary stroke prevention. *Objective*. We tested safety and durability of early CAS following IV thrombolysis in stroke patients with residual stenosis in the symptomatic ICA. *Methods*. Of consecutive patients treated with IV rtPA, those with residual ICA stenosis ≥70% or <70% with an ulcerated plaque underwent early CAS (>24 hours). The protocol included pre-rtPA MRI and MR angiography, and post-rtPA carotid ultrasound and CT angiography. Stroke severity was assessed by the NIH Stroke Scale (NIHSS). Three- and twelve-month stent patency was assessed by ultrasound. Twelve-month functional outcome was assessed by the modified Rankin Scale (mRS). *Results*. Of 145 consecutive IV rtPA-treated patients, 6 (4%) underwent early CAS. Median age was 76 (range 67–78) years, median NIHSS at stroke onset was 12 (range 9–16) and 7 (range 7-8) before CAS. Median onset-to-CAS time was 48 (range 30–94) hours. A single self-expandable stent was implanted to cover the entire lesion in all patients. The procedure was uneventful in all patients. After 12 months, all patients had stent patency, and the functional outcome was favourable (mRS ≤ 2) in all but 1 patient experiencing a recurrent stroke for new-onset atrial fibrillation. *Conclusion*. This small case series of a single centre suggests that early CAS may be considered a safe alternative to CEA after IV rtPA administration in selected patients at high risk of stroke recurrence.

1. Introduction

The incidence and management of early recurrent ischemic stroke after intravenous (IV) administration of recombinant tissue plasminogen activator (rtPA) have not been extensively investigated. Among stroke subtypes, patients with stroke due to large-artery atherosclerosis carry the highest risk of stroke recurrence, accounting for 37% of recurrences within 7 days [1]. A combination of IV thrombolysis and early carotid revascularization might result in more effective secondary stroke prevention strategy. However, at least in the first 24 hours after rtPA administration, the risk of intracranial haemorrhage associated with early reperfusion of a recently ischemic brain tissue might be increased [2, 3]. Safety of early carotid endarterectomy (CEA) in patients with residual severe carotid stenosis following IV rtPA administration has been reported in small single-centre case series [4–6]. Emergency carotid artery stent (CAS) placement has safety comparable to that of emergent CEA [7] and may be indicated in selected patients with small infarct volume and mild neurological deficit [8]. Further, there is a substantial risk that a decrease in cerebral blood flow during emergency CEA in the affected hemisphere may turn the brain more vulnerable to ischemia. Conversely, early CAS might offer the advantage of treating a critical stenosis with a rare reduction in cerebral blood flow in the affected hemisphere during

the intervention. However, the required double antiplatelet regimen that is administered before and after CAS procedure in order to prevent stent occlusion increases the potential risk of bleeding complications.

The aim of the present study was to analyze a cohort of patients undergoing early CAS after intravenous administration of rtPA for acute ischemic stroke. Safety of the procedure, patient outcome, and twelve-month durability of this approach were described.

2. Methods

Among consecutive patients treated with intravenous (IV) rtPA up to 4.5 hours [9], those with residual stenosis ≥70% or <70% with an ulcerated plaque in the symptomatic internal carotid artery (ICA) underwent urgent CAS. All patients had (i) pre-rtPA treatment diffusion-weighted (DW) and perfusion-weighted (PW) MRI protocol and MR angiography (MRA) showing involvement of less than 1/3 of middle cerebral artery (MCA) territory; (ii) head CT scan after rtPA administration and after CAS to rule out intracranial bleeding complications; (iii) post-rtPA treatment CT angiography (CTA) to identify the extent of residual ICA stenosis and features of plaque ulceration supporting an atherothrombotic origin of the stroke.

Age, sex, vascular risk factors, list of current medications, clinical and imaging data were collected for all patients. Stroke severity was assessed by the NIH Stroke Scale (NIHSS) at the following time points: before rtPA treatment, at the end of rtPA infusion, at 24 hours, at discharge, and 3 and 12 months after stroke onset. Severity of the stenosis was assessed by the NASCET criteria [10]. CAS was performed as soon as possible (but >24 hours) by an experienced team of interventional radiologists (EP, RG). No stent was placed within the first 24 hours after IV rtPA administration due to the absolute contraindication to start double antiplatelet treatment during this time frame. Prior to CAS procedure, ASA (100 mg/day) and clopidogrel (75 mg/day) were administered to all patients. During the procedure, intravenous heparin (5000 IU) was administered. To prevent bradycardia and hypotension during balloon inflation and stent deployment, prophylactic atropine (0.5–1 mg) was administered in all patients 1 minute before stent deployment. Following the CAS procedure, ASA (100 mg/day) and clopidogrel (75 mg/day) were administered to all patients during 6 weeks, later only ASA (100 mg/day) was prescribed. All procedures were performed in an angiographic room; patients were assisted by an anaesthesiologist for invasive arterial pressure measurement and oximetry monitoring. The patients, under light sedation, were kept conscious. The procedure adopted in our institution has been previously described [11]. A single self-expandable stent was implanted to cover the entire lesion in all patients. The stent used was the Cristallo Ideale (Carotid Self-Expanding Stent System; Medtronic) and the WALLSTENT Monorail Endoprosthesis (Boston Scientific, US). Protection devices were the EPI Filter Wire EZ Embolic Protection System (Boston Scientific Corporation, USA) or The SPIDER Embolic Protection Device (ev3, Plymouth, MN). All stent were postdilated with noncompliant balloons (range 5-6 mm). In all patients, after stent deployment, a postprocedural angiography was performed to evaluate the eventual residual stenosis and intracranial circulation.

After the procedure, patients were admitted to the stroke unit and managed according to the guidelines. Three-month and twelve-month stent patency was assessed by ultrasound. The rate of recurrent cerebrovascular events was assessed at 3, 6 and 12 months. Twelve-month followup included assessment for stroke severity by the NIHSS and functional outcome by the modified Rankin Scale (mRS).

Table 1: Demographic and clinical data of six patients who had early carotid artery stenting after thrombolysis.

	No. of patients*
Age, years	76 (67–78)
Females, n (proportion)	*2* (*0.33*)
Onset-to-CAS time, days	2.5 (2–4)
Onset-to-CAS time, hours	48 (30–94)
NIHSS onset (pre-rtPA)	12 (9–16)
NIHSS score before CAS	7 (7-8)
Right ICA involvement/n (proportion)	*4* (*0.67*)
Vascular risk factors:	
Smoking	2
Hypertension	6
Diabetes	1
Previous TIA/stroke	2
Hypercholesterolemia	3
12-month NIHSS score	2.5 (2–5)

*Numbers are medians (±IQR); CAS: carotid artery stenting; NIHSS: National Institute of Health Stroke Scale; TIA: transient ischemic attack.

3. Results

Since October 2006, 145 patients received IV rtPA within 4.5 hours after the onset of symptoms of acute ischemic stroke in our Institution. Within this group, subsequent CAS was performed in 6/145 (4%) patients with evidence of residual stenosis ≥70% or <70% with ulcerated plaque in the symptomatic ICA associated with unstable or ulcerated plaque, as detected by post-rtPA CTA. They were 4 males and 2 females, median age (IQR) was 76 (67–78) years, median admission NIHSS (IQR) score was 12 (9–16), and median (IQR) onset-to-IV rtPA administration was 175 (120–240) minutes. Median (IQR) 24-hour NIHSS change was −3 (−9;−2). The median NIHSS (IQR) score before CAS was 7 (7-8). Median (IQR) onset-to-CAS time was 48 (30–94) hours. Median (range) pretreatment DWI lesion volume was 26 cm^3 (6–52), and median (range) DWI lesion volume on follow-up examination (days 5–7 after stroke onset) was 31 cm^3 (5–57). The procedure was uneventful in all patients. Demographic, clinical, and outcome data are shown in Tables 1 and 2. On pre-rtPA treatment MRA, an intracranial occlusion was detected in 3 patients (Table 2). On CTA performed before CAS, there was a persisting MCA steno/occlusive disease in one patient only. However, angiographic study performed at the end on the CAS procedure yielded normal findings

Table 2: Clinical, imaging, and outcome data for 6 patients who had early CAS after intravenous thrombolysis.

Case no/sex/age (years)	Onset-to-rtPA time (minutes)	Site of DWI lesion	Clinical symptoms	Intracranial occlusion	Onset NIHSS score	Onset-to-CAS time (hours)	post-rtPA NIHSS score	Pre-CAS stenosis	Type of stent	12 months mRS score	12 months NIHSS score
1/M/57	175	I, BG, TP	Dysarthria, hemianopia, FP, HP, hypoesthesia	MCA (M1)	16	50	7	90%	Wallstent 7×40 mm	1	2
2/M/75	230	Prerolandic, periventricular	Dysarthria, FP, HP, hypoesthesia	MCA (M1)	10	94	7	70% ulcerated	Cristallo Ideale $6.0/9.0 \times 40$ mm	0	0
3/F/78	270	IC, BG	FP, HP	none	6	28	3	65% ulcerated	Cristallo Ideale $6.0/9.0 \times 40$ mm	3	8
4/F/78	150	BG, FP	Dysarthria, FP, HP, hypoesthesia	none	18	110	7	80%	Cristallo Ideale $6.0/9.0 \times 40$ mm	2	3
5/M/77	200	CR, prerolandic	Aphasia, FP, HP	none	9	46	8	55% ulcerated	Wallstent 7×40 mm	1	2
6/M/67	90	BG	Dysarthria, FP, hypoesthesia	MCA (M1)	14	30	12	90%	Cristallo Ideale $7.0/10 \times 40$ mm	2	5

IC: internal capsule; BG: basal ganglia; CR: corona radiata; FP: frontoparietal; TP: tempoparietal; I: insula; HP: hemiplegia or hemiparesis; FP: facial palsy; MCA M1: M1 segment of the middle cerebral artery; mRS: modified Rankin score; NIHSS: National Institutes of Health Stroke Scale.

in all patients. There were no bleeding complications on posttreatment CT scan, and all patients were discharged on daily ASA (100 mg/day) plus clopidogrel (75 mg/day). Regarding the discharge time, the two subgroups of rtPA-treated stroke patients in whom the early CAS was performed (mean ± SD 5.6 ± 1.8 days) and rtPA-treated stroke patients without the CAS procedure (mean ± SD 6.8 ± 2.6 days) did not differ in mean discharge time ($P = 0.29$).

All stents were patent at 3-month and 12-month ultrasound examination. Twelve-month functional outcome was favourable (mRS ≤ 2) in all but 1 patient experiencing a recurrent stroke after 4 months for new-onset atrial fibrillation.

4. Discussion

This small case series suggests that early treatment with CAS is both feasible and safe in selected patients previously treated with IV rtPA for an acute ischemic stroke. All stents were patent at 3- and 12-month follow-up evaluation. The outcome at 12 months was favourable in all but one patient experiencing a recurrent stroke four months following the index event associated with a new-onset paroxysmal atrial fibrillation.

A major concern about the safety of this approach is that previous systemic thrombolysis might increase the risk of hemorrhagic transformation thus making early reperfusion procedures potentially dangerous [2, 3]. For this reason, CAS was performed at least 24 hours after IV rtPA administration (with a median onset-to-CAS time of 2.5 days) when the hemocoagulative effects of thrombolytic therapy decline toward baseline [12]. Further, additional risk of hemorrhagic transformation could be determined by the need of administer double antiplatelet regimen to prevent in-stent restenosis. Feasibility and safety of urgent CAS after stroke have been previously investigated. In the study of Zaidat et al. [8], clinical and radiological data on a total of 38 patients with 39 procedures were reviewed. The median initial NIHSS score was 8. The carotid artery showed severe to high-grade stenosis in 28 patients, dissection was present in 6, and the rest ($n = 4$) had an acute occlusion treated with thrombolysis followed by CAS. The mean time from stroke onset to CAS was 55 (±34) hours. Complete recanalization was achieved in 95% procedures. Neurological deterioration occurred after three procedures (8%), with minor nondisabling stroke in two and death from intracranial hemorrhage in one. The authors concluded that early CAS seems to be safe after acute ischemic stroke if infarction volume is small and neurological deficit is mild. In a single-centre Italian registry [13], early treatment with protected carotid stenting was both feasible and safe in 43 selected patients with TIA or minor stroke (with cerebral ischemic lesion smaller than 2.5 cm). Patients who had a TIA underwent urgent CAS within 24 hours of the cerebral event, while patients who had had a minor stroke underwent deferred CAS (mean time, 6.5 days; range, 2–28 days). Similar to our findings, an urgent endovascular approach was associated with a satisfactory outcome considering the very high-risk profile of the patient population. However, the novelty of our study, compared to earlier ones, is that we have focused our attention on patients who had received IV rtPA before being submitted to a CAS procedure. Conversely, in one study [8], only a minority of patients ($n = 4$) had received IV rtPA before CAS. On the other hand, safety of early CEA following intravenous thrombolysis has been described in small single-centre case series of patients with an acute ischemic stroke. In the first study [4] five patients underwent early CEA (range 6–45 hours) after rtPA administration. The procedure was safe, and the outcome was favourable in all patients with no recurrent stroke (follow-up of 5–22 months). In the study of Bartoli et al. [5] early CEA was performed with a median of 8 days (range 1–16 days) after rtPA treatment in patients with median NIHSS of 12 (range 5–21). Also in this case series ($n = 12$) full vessel patency was achieved in all patients, and 3-month outcome was favourable in 10/12 (83%) patients. In the study of Crozier et al. [6] only 10/450 (2%) of patients treated with IV rtPA were eligible for early CEA; surgery was performed with a median of 8 (range 2–23) days after the index event. The procedure was safe, and the clinical outcome (up to six weeks) was favourable in all patients. Taken together, the results of these studies suggest that first, CEA can be safely performed shortly after IV rtPA administration; second, only a small percentage of patients treated with IV rtPA are appropriate candidates for reperfusion treatment; third, the best time to perform the procedure remains to be determined. Based on our results, similar considerations can apply to CAS after thrombolysis.

Although not as safe as elective carotid stent placement, early CAS performed with a mean time from stroke onset of 55 (±34) hours seems to be safe after an acute ischemic stroke if infarction volume is small and neurological deficit is mild [8]. Further, emergency carotid stent placement has safety comparable to that of emergent CEA, sharing similar risks of hemorrhagic transformation related to early reperfusion [7]. A major concern might be in the increased risk of hemorrhagic transformation related to the double antiplatelet treatment required after CAS. However, we did not observe any hemorrhagic complications, and none of the patients presented clinical deterioration after the procedure.

In conclusion, this is the first paper evaluating safety, durability, and patients outcome of early CAS after systemic IV rtPA administration. This small case series of a single centre suggests that CAS may be considered a safe alternative to CEA after rtPA administration in preventing early stroke recurrence in selected patients. These results apply to patients with residual significant ICA stenosis or ulcerated plaque after IV thrombolysis and pretreatment DWI lesion volume not exceeding 1/3 of the MCA territory.

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