

### Perfusional Studies in Acute Stroke

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The perfusional evaluation of the cerebral parenchyma in acute stroke patients has its main rationale in the distinguishing the portion of brain tissue irretrievably damaged and therefore non salvageable from the one ischemic but not irreversibly infarcted and then salvageable by a rapid treatment. Such an approach is feasible and useful both in situations where no clear time of onset is disposable and in situation when the classical time window for thrombolysis is passed. Besides its usefulness is also well documented in acute stroke patients within the classical time window. There are several tools that can measure the perfusional status, both radiological and neurosonological, namely MRI with diffusion/perfusion software, CT perfusion and TCCS (Transcranial Colour Coded Sonography) with the administration of second generation contrast media and the use of non linear properties of interaction contrast-tissue of harmonic imaging generation.

No direct comparison between these methods are disposable at our knowledge and the best method is not yet well defined. For practical reasons, MRI, although well studied and documented in the literature with the diffusion-perfusion mismatch definition, is not usable in many hospitals in an acute setting and the execution time or the not applicability in a significant portion of patients (pacemakers or metal prosthesis, heart disease with the poor tolerance of the supine position for a long time, significant decrease of blood oxygen saturation in 20% of patients) limit the application of this technique. CT perfusion is a reliable and most practicable tool, with some technical limitations, as the limited brain volume examinable with a single contrast bolus, the use of iodinated contrast media (adverse reactions, impaired renal function) and the different performance of deconvolution algorithm by operator expertise. Neurosonological techniques use the same mathematical rules than radiological methods and can measure perfusional parameters in a limited portion of brain tissue, namely a single oblique scanning plane in the axial access from temporal bone window. This latter technique is markedly influenced by the suitability of temporal bone window, that is insufficient in 15% of patients, mainly in older patients and females and this feature is not overcome by contrast media use for the microvasculature examination. Therefore is not yet clear which are the threshold for hypoperfusion in gray matter versus white matter and in the single restricted time window more and more away from symptom onset.

This approach can give a more attractive and physiological tool of evaluation in acute stroke but its practical use is actually in progress.

### Neurological Functional Scales: National Institute of Health Stroke Scale (NIHSS), Modified Rankin Scale, Glasgow Scale

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Functional neurological scales, including NIHSS, Rankin scale and Glasgow scale, are routinely used in clinical practice and in randomised controlled trials in patients with cerebrovascular diseases.

Functional scales are often utilized to standardize stroke severity in the acute phase, to describe functional variations, and to quantify improvement.

NIHSS specifically assesses stroke severity while modified Rankin scale and Glasgow scale described handicap and disability, respectively. Furthermore the Barthel Index evaluates the ability to perform activity of daily living in order to draw and supervision the rehabilitative programs in stroke patients. The modified Rankin scale is worldwide used to assessed functional outcome in patients with stroke.

However a single scale describes only some features of the patient and stroke physician should take together all the information derived from different scales in order to describe the present and the future neurological status of every patient.

Only the routinely use of these scales in stroke care on clinical and research programs can ameliorate the whole clinical evaluation of stroke patient in order to select them to the most appropriate treatment in the hyperacute phase and after.

### Aspects Score

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In 1995 the National Institute of Neurological Disorders and Stroke rTPA (NINDS) study demonstrated the efficacy of systemic thrombolysis for the treatment of acute ischemic stroke when administered within 3 hours of symptoms onset. In the NINDS trial CT scan was used to exclude the presence of intracranial hemorrhage before randomization. There is now accumulating evidence that the presence of early ischemic changes (EIC) on CT can predict both functional outcome and the risk of intracerebral hemorrhage.

EIC identified on CT during the first few hours after stroke onset represent early cytotoxic edema and possibly the development of irreversible changes (the ischemic core). CT signs of early ischemia consist of sulcal effacement with loss of gray-white matter differentiation in superficial cortical infarctions and subtle hypodensity of the basal ganglia in deep cerebral infarction. The role of extensive EIC on clinical prognosis has been observed in the ECASS trial, where the presence of parenchymal hypoattenuation exceeding one third of the middle cerebral artery territory was associated with increased mortality and higher rate of hemorrhagic transformation. Given the low

reliability of the “one third” rule as an estimate of the extent of EIC, a standardised semiquantitative CT grading system was validated in 2001, the Alberta Stroke Program Early CT Score (ASPECTS).

For ASPECTS, which is calculated from two standard axial CT cuts (one at the level of the basal ganglia and one just rostral to the ganglionic structures), the territory of the middle cerebral artery is divided in 10 regions, with 1 point subtracted for an area of EIC within each of the defined regions. A normal CT scan has an ASPECTS of 10. In clinical series of patients treated with systemic rtPA, the probability of dependence, death and symptomatic hemorrhage increases as the ASPECTS value decreases. An ASPECTS > 7 predicts a good functional outcome and a lower hemorrhagic risk.

In the NINDS study, patients with ASPECTS > 7 showed no treatment-modifying effect on good outcome, with a trend towards lower mortality at 90 days with rtPA and a number needed to treat of 5. Hence, there is currently no evidence for excluding patients for thrombolytic treatment within 3 hours based on the presence of EIC.

The use of a systematic approach becomes more relevant for patients treated beyond 3 hours, with the presence of extensive EIC (ASPECTS ≤ 7) as a predictor of unfavourable outcome and high hemorrhagic risk. In the PROACT-II trial, which explored the efficacy of intra-arterial thrombolysis within 6 hours from onset, patients with ASPECTS > 7 were 3 times more likely to have an independent functional outcome with thrombolysis compared with controls. In candidates to intra-arterial thrombolysis between 3 and 6 hours, the ASPECTS score, together with a rapid acquisition of evidence of vessel occlusion (with carotid ultrasound, CT angiography), can be a useful tool for selecting patients who are most likely to benefit from treatment.

A novel CT score, called posterior circulation-Acute Stroke Prognosis Early CT Score (pc-ASPECTS) has recently been tested in patients with vertebrobasilar ischemia. In the posterior fossa, the sensitivity for ischemic changes is improved with CT angiography source images rather than with non contrast brain CT scan. The presence of extensive EIC, quantified by a pc-ASPECTS ≤ 7, may identify patients with basilar occlusion who are unlikely to have a favourable outcome.

**Conclusion:** ASPECTS is a topographic CT scoring system, that divides the middle cerebral artery territory into 10 regions. The baseline ASPECTS value predicts functional outcome and symptomatic intracerebral hemorrhage. The scoring is simple and reliable and, beyond 3 hours, identifies stroke patients unlikely to make an independent recovery, despite thrombolytic treatment.

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### **MRI: Brain Lesions in Ischemic Stroke**

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MRI offers a better (compared with CT) soft tissue contrast and multiplanar visualization of the ischemic area, but conventional imaging (T1, T2 and FLAIR) usually do not identify the ischemic changes within the therapeutic window.

In recent years, diffusion weighted imaging (DWI) has been developed, allowing the recognition of cytotoxic oedema, which

represents the first step towards the ischemic necrosis and appears in the very early few minutes from arterial occlusion as an area of markedly increased signal intensity.

This sequence, based on echoplanar fast imaging, has the advantage to be very sensitive and rapid (acquisition time about 30 seconds) and can be coupled with perfusion MR studies, thus identifying the irreversibly damaged ischemic area and the hypoperfused one. The mismatch between these two regions can recognize the so called “ischemic penumbra”, i.e. the area that can be saved with a precocious revascularization of the occluded artery.

MRI can also well recognize cerebral infarctions in the watershed areas due to extracranial severe steno-occlusive disease, differentiating between hemodynamic-hypotensive and thrombotic strokes.

Moreover, specific MRI patterns may identify non occlusive infarcts due to hypoxemia, hypoglycemia and metabolic enzymatic deficiencies, such as mitochondrial encephalopathies.

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### **Ultrasound Vascular Evaluation in the Acute Stroke: How to Perform an Intra and Extracranial Examination in Few Minutes**

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In patients with acute cerebral ischemia the clinician must select the optimal therapeutic strategy after careful consideration of different treatment options according to the clinical presentation, the time of symptoms onset and all the different possible underlying obstructive mechanisms. Although according to SITS MOST the assessment of the vascular status is not mandatory to state the patient eligibility to systemic or intraarterial fibrinolysis, in clinical practice a timely vascular diagnosis has proven to be a very valuable tool to make the best choice. The ultrasound vascular examination can effectively depict both the anatomical aspects and the functional characteristics of the cerebral and extracranial vascular compartments and can significantly affect both the therapeutic decisions and the prognostic characterization. Some typical examples are represented by the finding of an hemodynamic stenosis of the internal carotid stenosis in the presence of a TIA, the demonstration of a carotid dissection at the onset of sudden focal neurological symptoms, or an ultrasound demonstration of an extensive T occlusion of the internal carotid artery in a subject with stroke. All these different causes of obstruction imply different clinical outcomes and require peculiar therapeutic options that must be well considered in addition to evaluating patient eligibility to fibrinolytic treatment. In experienced hands the neurosonologic evaluation (carotid Duplex, TCCD and TCD) has proved very useful to achieve a bedside diagnosis in this delicate clinical context, and is more suited than other precise and very sophisticated neuroradiologic techniques (angio MR, angio CT) for bedside examination in the emergency room. In fact US tests can be repeated whenever needed according to the clinical fluctuations thus providing a real time continuous monitoring of the hemodynamic changes.

The questions to be answered in evaluating an acute stroke patient are almost the same as during an examination routinely planned to rule out an atherosclerotic disease of intra/extracranial vessels, with only two main differences: should always be kept in mind: the first is that the test must be clinically oriented. For example in the presence of a left hemiparesis the first site to scan will be the right side internal carotid and middle cerebral arteries. The second main peculiar characteristic is that the examination should be performed in not more than 15–20 minutes to avoid unacceptable delays in patient access to a fibrinolytic treatment (i.e. within the 3 hours therapeutic window).

The ability of TCCD and TCD to detect a vessel viability allows the monitoring of cerebral hemodynamics during the infusion of rTPA and can instantly demonstrate the persistence of an occlusion or the reopening of the artery even long time before a clinical improvement. Therefore TCD monitoring helps to decide whether all fibrinolytic attempts should be interrupted or whether it may be necessary to shift to a more aggressive intra-arterial procedural revascularization by adjunct intra-arterial thrombolysis with low-dose rTPA infusion (the so called bridging protocol).

The carotid Duplex combined with TCD allows to establish whether the extracranial obstruction extends to the origin of the middle cerebral artery and/or anterior artery or if the intracranial perfusion is preserved by intracranial activation of collateral compensatory pathways. The diagnosis of extracranial stenosis relies on both direct morphological visualization of the lumen reduction (2D echo in association with color Doppler and power-imaging) while the grading of the stenosis can be accurately determined by Doppler velocity criteria. The focal acceleration (peak systolic velocity) should be measured after 60° angle correction at the level of maximum vessel lumen reduction where the velocity jet can be observed. The velocity in the internal carotid artery should than be compared to that found in a non-stenotic segment of the common carotid artery. The cut-off point for a significant stenosis of the internal carotid artery is the finding of a greater than 230 cm/sec peak systolic velocity and a greater than 3.5 ICA/CCA peak velocity ratio. These criteria have been validated by parallel angiographic examinations and they have been published in *Radiology* in 2004. The proximal total occlusion of one of the major neck vessels can be directly diagnosed when the flow velocity signal is totally absent at color/power angio and conventional Doppler examination. The same criteria apply to total main stem MCA1 or ACA1 occlusions. When the blockage of the arterial lumen involves the more distal intracranial branches, the occlusion may not be directly visualized, but its presence can be indirectly inferred in the presence of an ipsilateral velocity reduction in one proximal segment (>21% Zante asymmetry index). Focal accelerations can also be found in intracranial arteries; they are consistent with lumen reductions whose severity can be accurately graded according to velocity indices as well. In conclusion an accurate neurosonologic characterization is feasible in the patient with acute stroke and it is a tool of great utility both for the therapeutic decisions and for the near and long term follow up of the patients.

Transcranial Doppler Sonography (TCD) is able to explore and monitor the cerebral hemodynamics regarding the study of movements of blood and of the forces concerned in brain circulation. The evaluation of flow velocities (FV) as an index for cerebral blood flow (CBF) is based on the assumption that arterial diameter and several other hemodynamic parameters are stable during the period of measurement. The normal condition for blood flow throughout most of the circulatory system occurs in long, straight blood vessels, under steady flow conditions. It is characterized by concentric layers of blood moving in parallel down the length of the vessel.

The highest velocity ( $V_{max}$ ) is found in the centre of the vessel while the lowest velocity ( $V = 0$ ) is found along the vessel wall. This gives rise to a parabolic flow profile. The disruption of laminar flow in the vessels leads to turbulence and increased energy loss. It occurs when flow velocities exceed a certain threshold value called Reynolds number (Re) which is defined by the following equation:

$$Re = 2rv\rho/\eta$$

where  $r$  = vessel radius

$v$  = flow velocity

$\rho$  = blood density

$\eta$  = blood viscosity

Therefore turbulent flow depends on flow velocity, on blood viscosity and is consequent to a sudden change of the vessel lumen that can occur in the zones distal to stenotic arterial vessels, in arteries with tortuous course and at vessel branch points. The physical laws that determine steady laminar flow are also helpful in understanding *in vivo* cerebral hemodynamics, even though blood vessels are not rigid tubes and blood is not a perfect fluid but a 2-phase system of liquid and cells.

The application of Ohm's law to cerebrovascular hemodynamics is reflected by the formula:

$$CBF = CPP/CVR$$

where CBF = cerebral blood flow

CPP = cerebral perfusion pressure

CVR = cerebrovascular resistance

This means that any change in CPP must be matched by a proportional change in CVR, and vice versa, in order for CBF to remain constant: this mechanism is called "Cerebral Autoregulation".

In cerebrovascular circulation most of the resistance is provided by the arterioles and their precapillary sphincters, while the basal cerebral arteries that form the circle of Willis play only a minor role. This implies that the vessels evaluated by TCD have a relatively constant diameter and that the role of the arterioles in regulating CVR and therefore CBF is fundamental.

Consequently the velocities measured by TCD directly reflect the hemodynamic changes occurring in the vessel segment being studied. The diameter of the basal cerebral vessels are only insignificantly responsive to  $pCO_2$  while the resistance vessels are very sensitive to changes in arterial  $pCO_2$ . Therefore the flow velocity can be assumed

to be proportional to CBF during CO<sub>2</sub> testing. Variations in PaCO<sub>2</sub> are significant vascular modulator of CBF and the flow velocity relationship with PaCO<sub>2</sub> is linear, with an approximately 4% change for every unit change in PaCO<sub>2</sub>. In conclusion TCD is a useful tool in exploring the cerebral autoregulatory mechanisms consequent to activation of collaterals (intracranial macrocirculation) and is also able to show the functional vascular reactivity of cerebral resistance arteries (intracranial microcirculation). Therefore TCD can be considered a qualified and reliable non invasive approach able to detect the variations of cerebral hemodynamics in a large variety of clinical and research settings.

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### TCD for Guiding Therapeutic Decision

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In the last few years, a tremendous progress and widespread implementation of noninvasive neurovascular techniques including transcranial Doppler (TCD), computed tomography angiography (CTA), and magnetic resonance angiography (MRA) have been achieved. These imaging modalities are being increasingly performed in the acute stroke setting without substantial delay in a large number of centers worldwide. Far beyond the simple demonstration of an intracranial artery occlusion responsible of patient's neurological deficit, detection of arterial occlusion provides valuable prognostic information and may predict response to different reperfusion strategies. Several studies have demonstrated that the absence of an intracranial artery occlusion represents an independent predictor of early clinical recover and good long-term outcome. Conversely, persistent arterial occlusion predicts clinical fluctuations and early neurological worsening. The location of arterial occlusion may represent a marker of clot burden and response to thrombolysis. So, the more proximal the occlusion the larger the clot. In a multicenter study including 253 acute strokes due to MCA occlusion, the probability of complete recanalization at 2 hours of tPA bolus varied depending on the location of MCA clot. Complete recanalization was achieved in 45% of patients with M2-M3, 29% of those with M1 and only 11% of patients with terminal ICA occlusion achieved a complete recanalization. Therefore, a rapid detection of clot location may be useful for selecting patients for more aggressive reperfusion strategies. On the other hand, in patients with basilar artery occlusion, the site of arterial obstruction may indicate the patho-physiologic underlying mechanism. While distal basilar artery occlusion suggests an embolic source, proximal occlusion basilar is in most cases the result of a complicated atherothrombotic plaque.

Transcranial Doppler ultrasound is a non invasive technique that uses ultrasound to obtain information of the flow velocity in the intracranial arteries of the circle of Willis and vertebrobasilar system as a surrogate of the regional cerebral blood flow. In acute ischemic stroke, and particularly in the setting of stroke thrombolysis TCD provides rapid, reliable and real-time information on the presence and location of arterial occlusion and on recanalization at different times after stroke. Even continuous monitoring of the recanalization process can be done with TCD. The patterns of intracranial artery

occlusion on TCD have been validated against conventional angiography showing sensitivity and specificity values higher than 90%. The Thrombolysis in Brain Ischemia (TIBI) grading system has been developed to be applied rapidly in the acute stroke setting. These TCD grading system clearly reflects the dynamic nature of recanalization process during stroke thrombolysis and are based on the relative relationship between the insonation depth where the sample volume is placed and the clot location, so the closer the ultrasound beam to the thrombus the lower the TIBI score. Under good insonation conditions, an experienced operator can accurately identify the location of the offending clot in few minutes (typically 2–4 minutes), and in patients with suboptimal windows it takes <15 minutes. Ultrasound testing can be performed at bedside simultaneously with neurological examination, vital signs monitoring, and drawing blood, causing no delay in tPA administration.

In the last five years, several TCD studies have improved our knowledge and understanding of the dynamic nature of the recanalization process during stroke thrombolysis. TCD provides a unique opportunity to assess several aspects of clot dissolution by means of continuous monitoring of recanalization during and after tPA administration. This approach allows us to evaluate at the patients bedside and in real time the beginning, timing, speed and degree of artery reopening as well as to document re-occlusion after successful recanalization. Moreover, the simultaneous clinical assessment during TCD monitoring permits to correlate the hemodynamic changes with the clinical course and outcome.

Several angiographic and TCD studies have shown that spontaneous recanalization is a frequent, but unfortunately, delayed phenomenon after acute ischemic stroke. While spontaneous recanalization occurs in only 13% of patients <6 hours of cardioembolic stroke, up 66% of patients who receive tPA recanalized in the same time frame. tPA administration increases in 3- and 8-fold the rate of partial and complete recanalization, respectively. The time-to-artery reopening has been shown to be inversely correlated with clinical recovery and a time window of complete recanalization of <300 minutes from symptoms onset has been identify in human stroke to achieve full clinical recovery.

Recanalization is a continuous process that usually begins early after tPA administration. In most cases, recanalization occurs during the hour following tPA bolus, clot lysis starts at a mean time of 17 minutes and ends at 42 minutes after tPA bolus. However, the time until complete clot dissolution and restoration of blood flow may vary widely, depending on location of occlusion, clot composition, area of clot surface exposed to blood flow, and pressure-driven permeation of tPA into the clot structures. Alexandrov et al described the patterns of the speed of clot dissolution during continuous TCD monitoring. Based on the time required to achieve the maximum completeness of recanalization, the speed of clot lysis is categorized in: Sudden (recanalization is defined as an abrupt normalization of flow velocities lasting seconds shortly after tPA administration), stepwise recanalization (progressive improvement in flow velocities lasting less than 30 minutes) and slow (progressive improvement in flow velocities lasting more than 30 minutes). Sudden recanalization reflects rapid and complete restoration of flow; stepwise and slow recanalization indicate proximal clot fragmentation, downstream embolization, and continued clot migration. Unlike in acute myocardial infarction, the underlying pathophysiological mechanism of vascular arterial occlusion in acute stroke is heterogeneous. Composition of cerebral embolic material may vary, depending on specific endothelial and flow conditions of the embolic source. Old, platelet-rich,

and well-organized thrombi formed under flow conditions have been shown to be more resistant to thrombolysis than fresh, fibrin- and red cell-rich clots formed under conditions of stasis. Moreover, clot structure may differ depending on whether the embolic source is a thrombus engrafted in a proximal atherosclerotic lesion or a clot formed in cardiac cavities. In this context, stroke subtypes may represent a surrogate of the composition of offending clot. Our group demonstrated that in patients with proximal MCA occlusion treated with intravenous tPA, early recanalization is more frequent, faster, and more complete in patients with CE stroke. A cardiac source of emboli was identified in most patients who experienced sudden clot breakup during tPA administration. Sudden recanalization was associated with a higher degree of neurological improvement and better long-term outcome than stepwise and slow recanalization. On the other hand, the presence of a concomitant ipsilateral severe carotid artery disease was associated with low MCA recanalization rate and poor clinical outcome. Cardioembolic stroke probably represents the stroke subtype with more uniform fibrin-rich clots. Given the high binding affinity of tPA for fibrin, in fibrin-rich clots, tPA penetrates and distributes homogeneously, leading to an entire and rapid clot dissolution (sudden recanalization). In contrast, in well-organized and platelet-rich clots, penetration and distribution of tPA are limited, which may result in non-uniform clot softening and degradation from the outside of the clot. As a result, the clot shrinks and moves distally, lodging in smaller arteries (stepwise or slow recanalization), which would prolong ischemia. This may explain the fact that the stepwise and slow patterns of recanalization were associated with a lower degree of neurological improvement and worse long-term outcome than sudden recanalization shortly after tPA administration. The heterogeneity of clot composition is well illustrated in some patients with a documented MCA occlusion at arrival to the emergency room who experience a partial spontaneous recanalization before tPA bolus, but after treatment they remain with a persistent distal occlusion. This phenomenon may indicate that external fibrin-rich layer of the thrombus would be smooth enough to respond to endogenous fibrinolysis while the hard well-organized clot core would remain intact even in the presence of exogenous tPA (peach-like clot).

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### **Usefulness of the Ultrasound Contrast Media in Great Vessels and Microvasculature Examination of Brain**

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Ultrasound contrast media are microbubble –base moisture of different composition. First generation contrast agents are air filled bubble with a stabilized shell, but their size, durability and pressure resistance are not so favorable for the prolonged examination of cerebral vessels as echoamplificators for large vessels of the base of skull. Second generation contrast agents are gas filled microbubbles covered by lipidic and saccaridic shell, that guarantees a longer persistence in

the bloodstream and a stronger resistance to pressure. Therefore second generation contrast agents for ultrasound examination can offer some facilities in respect to first generation ones, both in large vessel examination and in microvasculature examination. Then they can provide some advantages like as the harmonic generation and the non linear behavior of this feature can improve the visualization of small vessels and perfusional status in several situations, e.g. acute stroke, intracranial stenosis, vasculitides, moya-moya disease, brain tumors, brain hemorrhages, etc.

This approach has enlarged the spectrum of application of contrast media for ultrasound from its origin, i.e. the overcoming of insufficient temporal bone window.

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### **Differences and/or Analogies Between Coronary and Cerebrovascular Risk Factors**

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Atherosclerosis is a systemic, chronic inflammatory process that mainly involves medium-sized arteries and affects the arteries of different vascular beds simultaneously, but with differing degrees of progression. Atherosclerosis tends to develop in the coronary arteries that supply blood to the heart, to the brain (the carotid, vertebral, and cerebral arteries) and the lower extremities (the iliac, femoral, popliteal and infra-popliteal arteries). Clinically, it can become apparent as ischemic heart disease, cerebrovascular disease, or peripheral arterial disease. The presence of atherosclerosis in a particular vascular bed is frequently associated with disease in other vascular territories. The risk factors are the same for all affected vascular beds, regardless of location, and there is compelling evidence that established causal risk factors such as smoking, hypertension, diabetes mellitus, hyper-lipidemia play an important role in the development of vascular disease. Although the exact mechanisms have yet to be clearly explained, causal risk factors promote the development of arteriosclerosis, predispose to coronary artery disease, act independently of one another and their effects are additive. There is also accumulating evidence supporting the role of emerging risk factors such as hyper-homocysteinemia, protrombotic factors (fibrinogen, PAI-1) and inflammatory markers (C-reactive protein). Emerging risk factors are associated with an increased risk of ischemic heart disease, but for which a causal relationship has not been documented, because their atherogenic potential is lower and/or because their prevalence among the population is not high enough. Although all the risk factors favor the development of atherothrombotic disease in the different vascular beds, the predictive power of the risk factors differs from one territory to another. For example, the cholesterol level has a greater predictive power for the coronary territory, smoking for the peripheral vascular territory and hypertension for the cerebrovascular territory.

About the prognostic role of established risk factors, *smoking* is a powerful risk factor for all vascular diseases. A large number of epidemiological studies have clearly demonstrated that there is a linear relationship between cigarette smoking and the increased risk of

ischemic heart disease, stroke, peripheral arterial disease and sudden death. Cigarette smoking impairs flow-mediated endothelium-dependent arterial vasodilatation, and, additionally, nicotine itself alters the structural and functional characteristics of vascular smooth muscle and endothelial cells. In addition, smoking favors atherothrombosis through multiple mechanisms, including endothelial damage produced by circulating carbon monoxide, increased fibrinogen and factor VII, increased platelet adhesion and aggregability, increased LDL oxidation, and decreased HDL-C concentration. Low-nicotine cigarettes increase cardiovascular risk to the same extent as the regular type. Even passive smoke exposure has a considerable effect on the flow-mediated vasodilatation and the risk of coronary heart disease in passive smokers is between 10% and 30% higher. Vice versa, the smoking cessation decreases by 50% the risk of coronary heart disease during the first year and approaches that of nonsmokers in two years. Smoking is associated with a variable magnitude of cardiovascular risk, because the risk is highest for peripheral arterial disease and abdominal aortic aneurysm and lowest for cerebrovascular disease.

*Hypertension* is one of the major risk factors, independently of age, sex, or race. Arterial blood pressures, both systolic and diastolic, are correlated with the incidence of coronary heart disease and stroke. In a recent study of Tacoy et al, after evaluation of coronary segments, it was demonstrated that hypertension is associated with distal rather than proximal atherosclerosis of coronary arteries. A high blood pressure is more than just a predictor of hemorrhagic stroke and a systematic review of 54 studies, by Cordonnier et al in 2007, showed that hypertension is a robust predictor (OR 3.9) of brain microbleeds, associated with both first-ever and recurrent ischemic and hemorrhagic stroke. Little is known about the role of hypertension in the atherothrombotic process: it has been postulated that the excessively high pressure would damage the endothelium and increase its permeability. In addition, hypertension could stimulate the proliferation of smooth muscle cells or induce the rupture of the plaque. A number of clinical trials have demonstrated that a decrease in arterial blood pressure is associated with significant reductions in the rate of stroke and, to a lesser extent, in that of coronary events, circumstances that produce an overall decrease in cardiovascular mortality. Thus, reductions in diastolic arterial pressure of 5 mmHg reduce the five-year incidence of stroke by 34%, that of ischemic heart disease by 19% and that of cardiovascular mortality by 23%.

The association between *hypercholesterolemia* and the incidence of ischemic heart disease and/or peripheral arterial disease has been demonstrated in epidemiological studies. The relationship between cholesterol and ischemic heart disease is continuous, gradual and highly intense. The risk attributed to hypercholesterolemia is due to low-density lipoprotein cholesterol (LDL-C). A number of intervention studies have demonstrated that the lowering of LDL-C by means of hypolipidemic agents (eg, with statins) is accompanied by significant reductions in cardiovascular morbidity and mortality, both in primary and secondary care. Statin use in patients with peripheral arterial disease is also associated with an improvement in renal function and carotid intima-media thickness. An independent, inverse correlation between high-density lipoprotein cholesterol (HDL-C) and the risk of ischemic heart disease has been observed in several epidemiological studies. The protection provided by HDL-C is independent of the LDL-C concentration. The National Cholesterol Education Program (NCEP) considers a HDL-C level below 40 mg/dL to be a risk factor, whereas concentrations over 60 mg/dL are reported to be a negative risk factor. An increased risk of ischemic heart disease of 3%

to 4%, in the six-year, is associated with a decrease in HDL-C of 1%. The HDL-C concentration correlates negatively with smoking, body weight and triglyceride concentration, and positively with fat and alcohol intake and physical exercise. There is no clear correlation between the concentrations of cholesterol and the incidence of stroke, although treatment with statins reduces the risk of stroke in patients with ischemic heart disease or with a history of previous stroke. Opposing results have also been published: in the Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) study, aggressive LDL-C lowering treatment with atorvastatin 80 mg/d in patients with a recent (1–6 months) stroke or transient ischemic attack was associated with an increase in the risk of hemorrhagic stroke (compared with placebo), but also with an overall significant decrease in risk of stroke and cardiac-related events.

*Diabetes mellitus* is associated with an elevated risk for ischemic heart disease and/or peripheral arterial disease and/or cerebrovascular disease, regardless of whether or not the individual is insulin-dependent. Cardiovascular disease is the leading cause of death among diabetics. There is a direct relationship between the duration of diabetes in years and risk of ischemic heart disease. Type 2 diabetics present elevated cardiovascular risk which, on occasion, is similar to that of nondiabetic subjects who have experienced a coronary event. For this reason, the major guidelines consider diabetics to be subjects at high cardiovascular risk who should receive the same treatment as patients who have a history of a previous cardiovascular event. Diabetes mellitus favors atherothrombosis through a number of mechanisms: an unfavorable lipid profile (high triglyceride levels, low HDL-C levels, small dense LDL particles), presence of modified LDL, hyperinsulinism, hypercoagulability and increased inflammatory markers. However, surprisingly there is evidence of a negative association between diabetes mellitus and the development of abdominal aortic aneurysm. In a recent pilot animal study of Alnaeb et al, in 2007, were demonstrated differences in the regional distribution of eNOS activity, as well as ET-1 and 5HT receptors between the aorta, renal and femoral arteries obtained from control and diabetic rabbits: these regional receptors differences may explain why diabetes mellitus is linked with a predilection for atherosclerosis in distal arteries and not in the aorta of patients.

In conclusion, current data suggest that established risk factors are associated with specific vascular diseases with a variable magnitude. These risk factors may also influence each other. Smoking is associated with high LDL-C, triglyceride and serum cholesterol levels, as well as low HDL-C concentrations. Smoking acts synergically with other risk factors such as hypertension, hyperlipidemia and diabetes mellitus, to increase vascular morbidity and mortality. In addition, smoking adversely influences several emerging risk factors (eg, fibrinogen and C-reactive protein). Family history may have an effect on the development of a specific vascular disease: the negative association between diabetes mellitus and abdominal aneurysm may support that a certain genetic background may be prone to the development of a specific vascular disease. Future studies may clarify the association between vascular risk factors and development of certain arteriopathies and may influence both prevention and treatment strategies.

### Effects of Aging and Hypertension on Cerebrovascular Reactivity, Endothelial Function and Cognitive Performance (Fair Culture Test)

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Elevated arterial blood pressure is strongly related with stroke, dementia, and silent cerebrovascular disease. Arterial hypertension, especially in the elderly, can lead to a shift to the left of the lower threshold of cerebral pressure autoregulation curve and it can reduce the CO<sub>2</sub> dependent cerebral vasodilatation. On the other hand blood pressure normalization leads to a significant reduction in both ischemic and hemorrhagic strokes and in the prevalence of cognitive deterioration often found in hypertensives. Despite these well known associations, the intimate pathophysiological mechanism underlying the neuronal damage in elderly hypertensives is not fully understood. To investigate the hypothesis that hypertension can alter the autoregulation threshold not only at the lower end but also at its upper limit, and to evaluate the relation between cerebral vasomotion, endothelial function and acute cognitive performance we have evaluated the cerebrovascular reaction to different adrenergic stimulations (i.e. pressor - handgrip, cognitive-fair culture test; ventilatory - 1 minute breath holding) in 5 groups (10 subjects/group): 2 groups of young and elderly healthy subjects; 2 groups of age matched (respectively young and elderly) systo-diastolic hypertensives, one group of elderly patients with isolated systolic hypertension. In all subjects, blood pressure increased significantly during handgrip and in the elderly hypertensive subjects, the pressure increase persisted during the recovery period. The pressure elevation caused a significant increase in mean flow velocity in the middle cerebral arteries (MCA) in the elderly and in young hypertensives ( $p < 0.05$ ), a slight though not significant MCA velocity reduction in young normotensives (consistent with an autoregulatory vasoconstriction), a slight though not significant increase in MCA velocity in elderly normotensives. Cognitive stimulation significantly increased mean MCA blood flow velocity ( $p < 0.001$ ) and blood pressure ( $p < 0.001$ ) in all subjects. The velocity increase and the cognitive performance (FCT scoring) was significantly higher in the young than in the elderly ( $p < 0.001$ ); the absolute velocity increase and FCT scoring were greater in young normotensives than in hypertensives. Cerebral vasodilatation and cognitive performance was similar in both elderly groups. Mean MCA flow velocity rose less during FCT in young hypertensives than in age matched normotensives, and the FCT scoring was lower thus suggesting a possible link between the sluggish cerebral hemodynamic response and the relative reduction in acute neuronal cognitive function. In elderly hypertensives, on the contrary, there was no evidence of a close neuronal-flow velocity coupling since the vasomotor reaction to the cognitive stimulation was maintained but the cognitive performance (FCT scoring) was low. In all elderly hypertensives, the blood pressure elevation and the mean flow velocity increase persisted well into the recovery period, consistent with a defect of systemic

blood pressure and cerebral pressure autoregulation. This defect of feed-back mechanisms can contribute to magnify the pressure burden on cerebral hemodynamics and explain the greater predisposition of the elderly hypertensives to cerebral damage, such as vascular cognitive impairment or stroke. Breath holding increased mean MCA flow velocity by the same extent in all subjects thus indicating that oxygen dependent cerebral vasomotion is not affected by age or hypertension. Endothelial function was studied in all subjects by ultrasound evaluation of the changes in brachial artery internal dimensions after forearm transient ischemia (Flow Mediated Dilatation-FMD). FMD was significantly higher in young normotensives than in the other groups. In addition elderly ISH patients showed a reduced FMD consistent with an impaired endothelial function compared with elderly normotensives ( $p = 0.04$ ). Nonetheless all elderly subjects showed similar FCT scoring. In conclusion the ability of cerebral and systemic arteries to vasodilate in response to a cognitive or hypoxic stimulation are not related with acute cognitive performance. This results confirm the lack of correlation between maximum vasodilatation and cognition in elderly. Compared to the other groups significant differences were found in the elderly after adrenergic HG stimulation suggesting a reduced adaptability of cerebral perfusion to blood pressure oscillations. These findings suggest that an altered cerebrovascular blood pressure autoregulation may be the mechanism underlying the greater prevalence of neural damage such as stroke and/or of vascular dementia in aging subjects, especially in those affected by hypertension.

### Stroke in Patients with Coronary Artery Disease: Natural History and In-Hospital Events (Iatrogenic Morbidity in the Interventional and Cardio-Surgical Era)

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Stroke in the course of percutaneous coronary interventions (PCI) is fortunately a rare complication (0.27–0.5%), but nevertheless a devastating event for patients and caregivers. The incidence of stroke is greater in patients undergoing surgical coronary artery bypass graft (CABG). Stroke is considered to represent a periprocedural complication when occurring <24 hours following PCI, and is most commonly ischemic in etiology (cerebral hemorrhage being considerably less common). Its low incidence hinders the identification of predictive factors. Nevertheless, based on the available evidence, periprocedural stroke is more common in the elderly, in women, in patients with diabetes, chronic renal failure, diffuse atherosclerotic disease, and in those with other periprocedural complications (coronary perforation, no reflow), intra-aortic balloon pump, or requiring high doses of contrast medium. In patients with periprocedural stroke, in-hospital mortality is high as 27% at six months, as shown by the OASIS study. In this, as in prior studies, most cerebrovascular episodes occur in patients undergoing CABG, compared to PCI.

## State of the Art in Carotid Surgery

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The aim of current surgical approach (CEA and PTA and stenting) to cerebrovascular disease is essentially prophylactic against vascular ischemic stroke.

The high number of studies in literature and the analysis of international trials about the guidelines on carotid endoarterectomy make it right of the huge spread of such intervention: currently in vascular surgery it is the more widely performed intervention in the world. Recently the conceptual settings and methods of these trials were challenged, especially about the treatment of asymptomatic patients. It is argued that in these patients, the majority of stroke occurs on a cardioembolic basis or lacunar stroke. Therefore it is essential to identify patients with carotid plaque “at risk”, or those patients who have a high risk of stroke when treated in a conservative way.

If anatomic-pathological features of carotid lesions are known, still lacks the ability to identify in vivo and in a non-invasive way these lesions.

Some contributions in literature and two recent European multicenter studies have identified the echocolor Doppler as the tool that could identify the carotid plaque “at risk” on the basis of the assessment not only quantitative but primarily of the quality of the morphology of the lesion.

In literature have been proposed many types of classification of plaque that have generated some confusion. A recent consensus, however, suggested that the evaluation of echogenicity should be indicated by the brightness of the plaque, dividing the lesions in hypoechoic, isoechoic and hyperechoic, identifying the structures of reference: echogenicity of blood for hypoechoic plaques, echogenicity of sternocleidomastoid muscle for isoechoic plaques and echogenicity of bones of the cervical vertebrae for hyperechoic plaques.

A further development was obtained through the “normalization” of the B-mode imaging that allows an objective and quantitative study by reference to minimum gray scale blood and adventitial sheet. In this way the characterization is not expressed by an adjective but a number (GSM - Median Gray Scale), eliminating the variability caused by subjective interpretations and numerous classifications.

The relation between ultrasound morphological study of carotid plaque and immediate and late results of the procedure, surgical or endovascular, could allow to detect lesions that are expected of either treatment.

About this, the recent study ICAROS has shown that the periprocedural risk undergoing carotid stenting increases significantly with the decrease of the echogenicity of the lesion. So the ideal lesions to be treated with endovascular procedure are those iso-hyperechoic, that is full of fibrous tissue and calcifications and those with low lipidic and hematic structure.

Therefore it is clear that the possibility to characterize with non-invasive approach these components can potentially allow not only to identify the lesion at risk but also to choose which type of procedure is most appropriate in order to minimize the risk of intra and post-procedural thrombo-embolic events.

Previous studies published by our group have enabled us to demonstrate statistically significant correlation between hypogenic plaque and appearance of omolateral neurological symptoms; similarly hypoechogenicity of the lesion appeared related to the presence of silent brain lesions evaluated at CT scan. Moreover, in the plaque, the presence of large cholesterinic and haemorrhagic areas, assessed in an histological examination, were correlated with the appearance of ultrasonographic hypoechogenicity of the lesion.

In the last years we have also evaluated the preliminary results obtained with a new technique of ultrasonology called RULES (Local Radiofrequency Ultrasonic Estimator), based on elaboration of ultrasonographic signals at different radiofrequencies, which allows to have spectral parameters related to space and mechanical properties of the tissue examined and developed in a Laboratory of the Faculty of Engineering of University of Florence, lead by Prof. Leonardo Masotti, obtaining a good correlation between the histological data and those reported with ultrasonographic technique of radiofrequency in vitro.

In particular, was shown a correlation in 100% of the cases in identification of lipidic and calcific areas in the carotid plaque.

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### Statins and Plaque Passivation

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The concept of plaque stabilization was proposed in the 1990s in an attempt to explain the discrepancy between a small amount of angiographically demonstrated plaque regression, and the large reduction in clinical events in trials of lipid-lowering drugs. “Plaque passivation” is a therapeutic concept by which either the structure or the content of the vulnerable and active atherosclerotic plaque is modified to reduce the risk of subsequent rupture and thrombosis.

The process of plaque destabilization begins with endothelial dysfunction against a background of inflammation. Postmortem pathologic studies have revealed that the vulnerable plaque has three hallmark histologic features: (i) a large lipid core occupying more than 40% of the plaque volume; (ii) an abundance of inflammatory cells; and (iii) a thin fibrous cap that lacks proper collagen and smooth muscle cell support. In an unstable plaque, almost every cell type is activated. These cells are mostly monocytes–macrophages, but can also be activated T cells and mast cells. These inflammatory cells secrete certain enzymes (proteases) that degrade collagen. In addition, apoptosis of smooth muscle cells, which are the chief source of collagen, further weakens the plaque. The lipid core is composed of free cholesterol, cholesterol esters, and crystals. In addition, it contains oxidized lipids, and is impregnated with tissue factor derived from macrophages, making it highly thrombogenic. The family of enzymes—matrix metalloproteinases (MMPs)—expressed by macrophages erodes the thin fibrous cap resulting in exposure of thrombogenic subendothelial material to the circulating blood. The acute clinical event is precipitated by the formation of an intimal, platelet-rich thrombus followed in some cases by a fibrin–red cell intraluminal thrombus.

The biology of plaque instability and rupture suggests that therapeutic strategies must revolve around three potential target sites: (i) platelets and the coagulation cascade leading to dissolution of the thrombus and restoration of luminal patency; (ii) passivation of dysfunctional endothelial cells for the reduction of pro-inflammatory and procoagulant activity; and (iii) modification of plaque contents by an aggressive reduction of serum low-density lipoprotein cholesterol (LDL-c) levels and inhibition of LDL oxidation. These target sites are not mutually exclusive. Therefore, an integrated approach to plaque passivation in ACS is required to reduce future adverse clinical events.

Based on evidence from human and animal studies, it can be assumed that lipid-lowering drugs stabilize plaque by several mechanisms. Statin-mediated lowering of lipids may stabilize vulnerable plaque by changes in the lipid core itself. There is a reduction in the levels of oxidized LDL in the plaque's core accompanied by reduction in plaque macrophage content, and increase in the volume of collagen and smooth muscle cells with statins. In a small, nonrandomized study of patients undergoing carotid endarterectomy, statin therapy given for 3 months resulted in a decrease in the lipid pool and increase in fibrosis in carotid plaque. There was 75% less lipid core, 40% less oxidized LDL and MMP, and twice the amount of collagen. In experimental studies, these changes require at least a few months to occur and, therefore, may not fully explain the early benefits observed with statin therapy in patients with ACS.

Statins have "pleiotropic actions" that go beyond the lowering of LDL-c levels, and are relevant to the pathophysiology of ACS. In comparison to lipid-lowering actions, these pleiotropic effects on vascular and cardiac cells may be effective after early initiation of therapy.

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### **Carotid Artery Disease: Identification of Novel Pathophysiological Mechanisms by Gene Expression Profiling of Peripheral Blood Perturbation**

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Carotid artery disease (CAS) is the most frequently identified cause of ischemic stroke and is mostly due to atherosclerotic disease. Inflammation plays an important role in the pathogenesis of atherosclerosis.

Aims of this study were to investigate the systemic gene expression profile of patients affected by CAS versus control subjects, and validate and extend microarray data in two further independent populations of patients and controls.

Total RNAs were extracted from whole peripheral blood of 46 patients affected by CAS and 46 controls comparable for age and sex. We determined the expression of 14,000 genes by two colors microarray technology in n = 10 pooled RNA from patients and n = 10 pooled RNA from controls and validated data by real time PCR in n = 36 CAS patients and n = 36 controls. 82 genes showed altered expression levels between CAS patients and controls: 61 genes resulted up-regulated and 21 down-regulated. Gene ontology analysis indicated an alteration of the following biological processes: immune response, oxygen transport, cytoskeleton organization and lipidic metabolism. Some of the biological process found in CAS and the relative associated genes resulted similarly altered in patients affected by atherosclerotic lesions in an other district (abdominal aortic aneurysm patients). In particular we focused our attention in validating genes associated with the biological process peculiarly observed in CAS patients. These genes encode for the major histocompatibility complexes, expressed by the human leukocyte antigens (HLA) or are involved in the immune response (HLA-DPA1, HLA-DPB1, HLA-DQB1, HLA-DRB3, IFIT1, IGKV1D, TRBJ2-1, DBNL, HLA-B).

This study provides new insights into the regulatory mechanisms controlling the development and the progression of plaque emphasizing the central role of inflammatory and immune cells.

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### **Detrimental and Beneficial Effects of Ethanol by Stimulation of TRPV1 in Sensory Neurons**

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The risk of coronary artery disease (CAD) is reduced by red wine and more in general by consumption of alcoholic beverages (Mukamal et al., *N Engl J Med.* 2003; 348: 109–18). However, the mechanism by which ethanol (EtOH) exerts protection is not fully understood. Ethanol stimulates neuropeptide-containing primary sensory neurons via the activation of transient receptor potential vanilloid 1 (TRPV1), also known as the capsaicin receptor, thus causing a burning sensation and the release of the neuropeptides, substance P (SP) and calcitonin gene related peptide (CGRP). TRPV1 belongs to a large family of ion channels and is gated by noxious heat (42–53°C). Ethanol potentiated the response of TRPV1 to capsaicin, protons and heat and lowered the threshold for heat activation of TRPV1 from approximately 42°C to approximately 34°C. Thus, it is possible that ethanol-induced sensory responses that occur at normal body temperature (Trevisani et al., *Nat Neurosci* 2002; 5: 546–51). Recently we addressed the question as to whether ethanol may cause coronary vasodilatation by activation of TRPV1 on perivascular sensory fibers and the release of the vasodilating peptide CGRP. Indeed we observed that ethanol relaxes porcine isolated coronary and human isolated gastro-epiploic arteries by TRPV1 stimulation and CGRP release. Ethanol by the same mechanism caused vasodilatation in the guinea pig coronary circulation. Ethanol-induced release of vasodilatory CGRP may contribute

to the reduced risk of CAD (Gazzieri et al., *Cardiovasc Res.* 2006; 70: 589–99).

In addition to protective actions ethanol also contribute to various diseases. In susceptible individuals ethanol is known to trigger attacks of asthma. We reported that ethanol contracts isolated guinea pig bronchi, via activation of TRPV1 on terminals of airway sensory neurons and releasing the bronchomotor peptide SP. This excitatory effect of EtOH, distinct from that of Acetylcholine, results also in neurogenic inflammatory responses that may contribute to the mechanism of EtOH-induced asthma (Trevisani et al., *J Pharmacol Exp Ther.* 2004; 309: 1167–73).

Alcoholic beverages, are not only cardioprotective, but they also trigger migraine attacks and activation of trigeminal neurons plays a role in migraine. We found that ethanol evokes release of neuropeptides from nerve terminals of the rodent meninges by TRPV1 stimulation, thus provoking a vasodilatation that is abolished by the CGRP receptor antagonist, olgeceptant, and is considered to be mediated by CGRP released from perivascular sensory nerve endings. Olgeceptant has been reported effective in the treatment of the migraine attack. Thus, arterial vasodilatation of meningeal vessels by TRPV1 activation and CGRP release may be relevant to the mechanism by which alcohol ingestion triggers migraine attacks (Nicoletti et al., *Cephalalgia* 2008; 28: 9–17).

Finally, it is known that ethanol induces hemorrhagic gastric lesions in rodents, but the mechanism is only partly understood. A series of *in vivo* experiments in rats and mice (including neurokinin-1 receptor deleted mice) showed that via stimulation of TRPV1 in gastric nerve terminals, ethanol releases SP that activates epithelial neurokinin-1 receptors to generate damaging reactive oxygen species (ROS) and the highly reactive aldehyde 4-hydroxynonenal. We conclude that gastric lesions are caused by an initial detrimental effect of ethanol, which is damaging only if associated with TRPV1 activation, SP release from sensory nerves, stimulation of neurokinin-1 receptors on epithelial cells, and ROS generation (Gazzieri et al., *Free Radic Biol Med.* 2007; 43: 581–9). The present data indicate that the ability of ethanol to target TRPV1 may be associated to a series of detrimental effects, that range from production of a burning painful sensation to contributing to airway inflammation and migraine. However, it is possible that the neurogenic vasodilatation caused by ethanol in the coronary circulation contributes to the cardioprotective effect of moderate alcohol consumption.

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### **Intrarterial Thrombolysis for Acute Ischemic Stroke**

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Thrombolysis, in its broad sense of recanalization of a vessel occluded by thrombus, is probably the most challenging and promising therapy of acute stroke so far; it has the merit of having played a key role in changing the general perception of acute stroke, which switched from the mere contemplation of a bedridden patient to the consideration of stroke as a medical emergency.

The favourable results of the *National Institute of Neurological Disorders and Stroke* (NINDS) study on recombinant tissue plasminogen activator (rt-PA), a thrombolytic agent already used for myocardial infarction and pulmonary embolism, were followed by the approval of intravenous (IV) rt-PA for ischaemic stroke within 3 hours from symptoms onset in 1996 in United States and three years later in Canada.

Albeit IV r-TPA is today the standard treatment for acute ischemic stroke, just 10–12% of the patients can be treated within 3 hours, as IV rt-PA has many contraindications, and about 50% of those treated die or remain severely disabled. Hence, there is a need for other recanalizing treatments that are more effective and have less contraindications. IA thrombolysis might provide many advantages compared to the intra-venous one, such as to titrate the dosage of the thrombolytic agent, having high drug concentration locally and low in systemic circulation, facilitate clot disruption with mechanical thrombolysis, extend the therapeutic time window and increase angiographic recanalization rates. For these characteristics IA in place of IV thrombolysis for acute ischemic stroke has been proposed for patients with severe neurological deficits, vertebrobasilar stroke, occlusion of major supra-aortic and cerebral arteries, presentation beyond 3 hours from symptoms onset, acute stroke caused by cervical artery dissection, recent surgical and invasive diagnostic procedures, pregnancy and anticoagulant therapy. However no data are available on the effectiveness of IA thrombolysis compared to the IV one that is known to be quicker to initiate, cheaper and easier to use.

Intra-arterial (IA) thrombolysis in acute ischemic stroke has a different story from the intravenous (IV) one. The first report was in 1958 about a case of acute carotid thrombosis treated with IA plasmin but this approach was developed later with the diffusion of cerebral angiography and endovascular treatment. IA thrombolysis, indeed, was at first used to dissolve thrombi forming during cerebral angiography or endovascular procedures. The current procedure was introduced by Zeumer in 1982. It starts with an angiogram to identify the site of occlusion and collateral supply to the affected region. The guiding catheter is introduced percutaneously into the femoral, radial or brachial artery and advanced in the supra-aortic vessel of interest. A microcatheter is then positioned close to, or within the thrombus, allowing local thrombolytic agent delivery.

Thrombolytic agent may be aided by mechanical thrombus disruption through the manipulations of the microcatheter tip and multiple advancements of the microguide. In some cases an alternative strategy for thrombus removal is the use of mechanical recanalization techniques such as percutaneous transluminal angioplasty with balloon catheters or clot extraction using retrieval devices. Also, specialised intravascular catheters that emit ultrasound, pressurised saline solution with resorption, or photoacoustic energy are being studied. Some of these devices have been created for other purposes and readapted to perform IA thrombolysis. Their risk to benefit ratio is still uncertain. The description of patients with complete or partial early arterial recanalization during IA thrombolysis and early improvement of their neurological symptoms suggests the likely efficacy of these techniques, but no substantive data exist.

For these purposes the Synthesis Expansion Trial has been designed, to compare IV thrombolysis with rtPA with IA thrombolysis (farmacological and/or mechanical).

## Intrarterial Thrombolysis. New Devices

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Most of ischemic strokes are due to thrombo-embolic events; angiographic studies on patients with acute ischemic stroke within 8 hours from symptoms onset showed complete occlusion of a main cerebral artery in 80% of cases, mostly in the carotid territory. When angiography is performed within 12 hours from the event, in 80–90% of patients a stenosis or an occlusion matching with the neurologic deficit territory can be found. Therefore, the aim of the acute treatment of a patient with severe brain ischemia is to early recanalize the occluded vessel and to restore brain perfusion in order to limit the extension of the ischemic core.

The studies about intravenous systemic thrombolysis with rtPA in acute ischemic stroke showed that this therapy can improve the outcome if the infusion is performed within 3 hours from symptoms onset. However, systemic thrombolysis has strict inclusion criteria and its results are promising but not very satisfying. Locoregional intrarterial thrombolysis (LIT) has many advantages over systemic therapy:

1) intrarterial injection allows higher drug concentration inside the clot, therefore the pharmacological efficacy is enhanced and the systemic activation of the drug is decreased.

2) thrombolytic injection can be joined with mechanical manoeuvres to fragment and aspire the clot.

3) dose of the thrombolytic drug can be set on the basis of the anatomical response (recanalization). The local infusion can be stopped at any time when the clot has resolved, limiting the delivered dose and the systemic concentration; otherwise, the administered dose with the systemic infusion is predetermined on the basis of the patient's weight.

4) LIT allows a wider therapeutic window compared with systemic therapy, because the treatment can be performed within 6 hours from symptoms onset in anterior territory stroke and 24 hours in case of vertebrobasilar stroke.

5) anatomical efficacy of the treatment can be evaluated during the procedure and the recanalization rate can be calculated to assess and compare different techniques.

The main limit to the diffusion of LIT is the low number of published studies on this topic, often with few patients enrolled, while most of observations have been reported outside randomized and controlled trials.

In only two randomized and controlled trials (PROACT I and II), LIT performed with rt-PA within 6 hours from symptoms onset has been compared with controls. A meta-analysis of these trials showed that treated patients compared with control cases, had a reduction of the combined endpoint of death and 90 days dependence of 15% (OR: 0.55, 95% CI 0.31–1.00); the hemorrhagic transformation rate was 10% in the PROACT II (OR: 2.39; 95% CI 0.88–6.47). However, both studies only included patients with middle cerebral artery

occlusion and did not allow adjunctive mechanical manoeuvres. Another meta-analysis that included different series and patients with- out selected site of vascular occlusion, reported a good outcome in 41.5% of treated patients vs. 23% of control patients ( $p = 0.002$ ) and a mortality rate of 40% vs. 23% ( $p = 0.004$ ); the symptomatic hemorrhage rate was higher in the group of the LIT patients (9.5% vs 3% ;  $p = 0.046$ ). The outcome was influenced by the site of the occlusion, being better in the anterior than in the vertebrobasilar circulation. Overall, the results of clinical series report that the efficacy of LIT in the anterior circle varies from 30% to 70% and that the clinical outcome depends on the degree of recanalization; similar results are reported for the vertebrobasilar circulation.

Although these results are encouraging, LIT has some limitations:

Difficult recruitment of patients within the limits of the therapeutic window, which appears still too short.

Increased incidence of hemorrhagic complications compared with systemic thrombolysis (10% versus 6.4%).

The clinical results depend on the arterial recanalization rate (a complete recanalization is obtained in only 30–40% of cases) and the reperfusion time.

When the flow has been only partially restored, reocclusion can occur.

For these reasons, industry and clinical research have been oriented towards the project on new treatment modalities in order to enhance the effects of LIT. The main fields of interest are mechanical recanalization (by means of aspiration or fragmentation of the clot) and the association of LIT and systemic thrombolysis to improve the recanalization time and rate.

Mechanical thrombolysis is performed to reduce the thrombus burden and to obtain a faster recanalization, sometimes even immediate. This approach has also the potential advantage to reduce the need of thrombolytic drugs and so to decrease the symptomatic hemorrhage rate. Therefore, a mechanical treatment can be performed even after 6 hours (depending on the series, within 8 hours). The most evaluated mechanical device is the Merci system, which allows to catch and extract the clot inside a guide catheter. This device has been evaluated in two randomized trials alone or in conjunction with pharmacologic LIT. In the last, the multimerci trial, 164 patients were enrolled and the recanalization rate was 54% with merci alone and 69% with merci and rt-PA; the favourable outcome (m-RS 0–2) was 39%. Interestingly, in patients treated with the new generation Merci device clinical results were improved; that underlines the continuous improvement of these devices. Other mechanical devices are available, but most of them have not been evaluated in clinical randomized trials. A recent trial concerns the new device Penumbra, composed by an aspiration system and a thrombectomy device. Preliminary results are good, since recanalization rate (TIMI 2–3) was 85 %.

Another mechanical approach is intracranial angioplasty, that can now be more safely performed than in the past thanks to high compliance balloons. In a series of PTA of the middle cerebral artery, a recanalization rate of 91% and m-RS of 73% were reported.

Recently, new attention has been oriented towards primary clot stenting, with or without simultaneous anti-GP IIb-IIIa injection. Actually this must be considered a bailout procedure but results are very promising.

## Indications, Techniques, Catheters for Mechanics Disobstruction. The Stents

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Indications to mechanical thrombolysis are mainly represented by an acute arterial occlusion with a high thrombus load (T siphon, Basilar artery) in a patient presenting out of the necessary time window (3–6 hrs) or with a wake-up stroke or after unsuccessful iv thrombolysis (bridging therapy). The most common techniques are constituted by clot suction with large bore microcatheter (21–35), clot retrieval devices (Merci, IN TIME), clot fragmentation and aspiration (Penumbra) and soft angioplasty and stenting.

Our experience is mainly derived from the use of angioplasty and stenting in combination with asystemic glycoprotein (gp) IIb/IIIa inhibitor Tirofiban in patients with acute basilar occlusion.

Outcome in acute ischemic stroke due to basilar artery (BA) occlusion remains poor with high mortality or severe disability, despite the proliferation of numerous endovascular techniques. In patients with stroke due to BA occlusion a wider time window acute intervention is allowed, because of the poor prognosis and the progression of symptoms. We assessed the hypothesis that an intra-arterial approach with primary stenting combined with systemic glycoprotein (gp) IIb/IIIa inhibitors is feasible and might lead to high recanalization rates. We retrospectively reviewed 23 consecutive patients with acute BA occlusion, treated with intra-arterial approach at the Careggi Hospital (Florence, Italy) from February 2004 to May 2008. We evaluated the recanalization rate using the Thrombolysis in Myocardial Infarction (TIMI) score and 3 month outcome using the modified Rankin scale (mRS) in patients treated with primary basilar stenting and gp IIb/IIIa inhibitor tirofiban, compared with intra-arterial thrombolysis with urokinase and/or mechanical disruption.

Nine patients (45% male, mean age  $62 \pm 16$  years, median NIHSS 27) were treated with intracranial stenting and gp IIb/IIIa inhibitors and 14 patients (78% male, mean age  $70 \pm 15$  years, median NIHSS 26) were treated with intra-arterial urokinase and/or mechanical disruption. Eight out of 9 (89%) patients treated with stenting had recanalization of BA (TIMI 2–3), versus 10/14 (71%) of patients treated with urokinase. Complete recanalization (TIMI 3) was achieved in 67% of patients treated with stent versus 14% of subjects treated with urokinase. Good outcome (mRS 0–3) rate in the stenting group was similar to the urokinase group (33% versus 28%, respectively). Mortality rate was higher in the stenting group (44% versus 36%). One symptomatic hemorrhage was observed in the urokinase group.

**Conclusions** In our series, the approach with systemic administration of gp IIb/IIIa antagonists and placement of intracranial stent appears to be feasible and safe, reducing the use of thrombolytic agents. The high recanalization rate obtained with this procedure makes this approach promising. More data are needed to confirm the efficacy of this treatment.

## National Organization of Stroke Assistance

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Regarding all the interventions directed to the reduction of death and disability following a stroke, it was demonstrated that a early recovery in a dedicated unit, a Stroke Unit, determines an absolute reduction of the risk of dead/dependency of 5.6%; practically for every 1000 patients admitted in Stroke Unit, 56 avoid died/dependency.

In order to make a correct evaluation of the “stroke question”, it is important to know the data of prevalence and incidence in western countries.

Prevalence increases with age. In international population-based studies, prevalence reaches values between 4,61 and 7,33 for 100 inhabitants in the subjects older than 65 years.

In Italy available data are from the *Italian Longitudinal Study on Aging* (ILSA) (1), that refers to a population between 65 and 84 years. Among this population the prevalence rate is a 6,5% (IC<sub>95</sub>: 5,8–7,2), with an higher prevalence rate in male (7,4%; IC<sub>95</sub>: 6,3–8,5) than the female (5,9%; IC<sub>95</sub>: 4,9–6,9).

In other European population-based studies with a similar methodological approach, the incidence rate

was 8, 72 per 1·000 (IC<sub>95</sub>: 7, 47–10, 06) in subjects between 65 and 84 years.

These data reveal that we are in front of a real “stroke epidemia” and that it needs structural and coordinated interventions both at regional and national level. Considering even the severity of strokes (110,000 strokes per year and 230,000 those with disability from previous stroke) it has become indispensable to make an operative reorganization and to promote the idea that stroke is a curable medical emergency. It is also necessary to stimulate the prompt beginning of a correct secondary prevention.

On the epidemiological data shown, that characterize stroke like one of the main causes of dead, the following interventions should be adopted:

### *Prevention in the general population*

- Health education programs
- Programs to aware of the stroke question the General Medicine Doctors
- Emanation of specific regional guidelines. Useful for technical, methodological, and organizational support for the Local Sanitary Companies, based on the national guidelines (SPREAD).

### *Prevention in high risk individuals*

- To improve the identification of individuals at greater cardiovascular risk
- To guarantee the therapeutic continuity to the high risk patients both with pharmacological and non-pharmacological strategies, according to the international guidelines.
- To improve the accessibility to the early intensive units and then to the follow-up and post event programs.

### *Improvement of the organizational efficiency and effectiveness*

- To help the early admission, in specialized units possibly, of the stroke patients in order to facilitate the prompt institution of specific

therapies such as pharmacological or surgical re-vascularization in ischemic stroke or neurosurgical evaluation in the hemorrhagic.

All this is possible by the institution of the Stroke Unit that synthesizes how much was previously debated.

A Stroke Unit is:

1. a ward that admit patients with acute stroke (within 48 h)
2. presence of beds dedicated exclusively to the cure of patients with acute stroke ( at least 80% )
3. staff (doctors and nurses) dedicated exclusively to the cure of the patients with stroke (at least a doctor or nurses occupied for 80% of their working hours).

From the experiences matured up to now in international and national contest, characterizing points for a SU are:

- multi professional integration
- early rehabilitation
- staff education
- particular attention to the communication with the patients, their relatives and with the doctors of general medicine.
- engagement to guarantee a continuity in the cure and the attendance.

The chance to have a continuous monitoring of vitals sign (Blood pressure, heart rate, temperature) leads to a better surveillance of the patient in the acute phase and characterizes the intensive-subintensive type of stroke unit.

The stroke unit must be integrated with all the other units that are involved with the management of the patient during his acute phase.

It must also be opened to the territory of competence, through the collaboration with the general medicine doctors and with Local Sanitary Company and the Districts to the aim to guarantee the *dimissioni protette* and the programs of rehabilitation and reinsertion of the patient.

**The implementation of the subintensive dedicated units (Stroke Unit) represent** a part of a more wide process that tends to guarantee the best management for the patients and the therapeutic continuity.

This must be carry out even by the integration with the territory, the rehabilitation structures, the ambulatorial activity of follow-up and secondary prevention.

The objective is to realize the standardization and the adaptation of the regional welfare levels as well as the correct distribution of the human and technological sources in order to answer in the right way to the Stroke Emergency.

(1) The Italian Longitudinal Study on Aging Working Group. Prevalence of chronic diseases in older Italians: comparing self-reported and clinical diagnoses. *Int J Epidemiol* 1997; **26**: 995–1002.

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