NEUROSONOLOGY IN ACUTE ISCHEMIC STROKE: DIAGNOSIS AND THERAPY

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[La neurosonologia nell'ictus ischemico acuto: diagnosi e terapia]

ABSTRACT

The purpose of this review is to describe recent developments in field of neurosonology applied in acute ischemic stroke, both in diagnosis and therapy. Ultrasound allows a better etiopathogenetic definition of acute stroke, essential to impress effective thrombolytic therapy. They also have a therapeutic role enhancing thrombolysis by insonation of intracranial occlusion areas. Moreover, this technique is followed by innovative use of microbubbles, able to further accelerate fibrinolysis.

Key words: *Stroke, ischemic stroke, thrombolysis, neurosonology, sonothrombolysis*

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Neurosonology is an instrumental technique increasingly used to diagnosis of ischemic cerebrovascular disease, as this is a useful and non-invasive way to implement a morphological and functional evaluation of lesions of extra and intracranial vascular axes. It also allows a constant monitoring of evolution of arterial damage.

The use of sonology in acute stroke has taken advantage of significant development in study of intracranial circulation and then for diagnostic environment of acute stroke:

- introduction of angio-power technique
- use of contrast agent to improve intracranial vascular image and to study tissue perfusion
- study of intracranial microcirculation by multiple pulse techniques and low mechanical index.

Diagnostic objectives provide, above all, differentiation of hemorrhage from ischemia, then, detection of parenchymal and cerebral vessels abnormalities, and finally, classification of the event on pathophysiology.

As we know, in acute ischemic stroke count the maxim "time is brain", as the area around infarct is an area of "ischemic penumbra" still salvageable but over a few hours (the so-called "window period"), without adequate recovery of flow goes to meet death.

As already demonstrated in the Jansen et al.[1] study (1999), through RMN, an early and maintained recanalization (without re-thrombosis), manages to save the tissue at risk. That explains the strict interdependence between time of recanalization and improved clinical outcome, assessed by the Modified Rankin Scale (mRS) and the National Institutes of Health Stroke Scale (NIHSS)[2]. For these reasons, early recanalization is an indicator of good prognosis.

Recanalization can be monitored by ultrasound and staged using Thrombolysis in Brain Ischemia (TIBI) grading system[3], according to which are distinguished 5 levels of recanalization:

- 0: absent flow signal, complete proximal occlusion
- 1: minimal flow signal, absent end-diastolic flow
- 2: blunted flow signal, delayed systolic flow acceleration with reduced mean flow
- 3: dampened flow signal, pulsatile signal

with normal acceleration and decrease of mean flow

- 4: stenotic flow signal, focal increase of flow velocity
- 5: normal flow signal, without relevant difference to the controlateral side (<30% difference)

The information that neurosonology can provide about etiopathogenetic factors and different activation of neurovascular compensatory systems could revolutionize the concept of "therapeutic window", changing motto "time is brain" to "physiology is brain": clinical outcome after thrombolysis is not only dependent on time factor, but by multiple pathophysiological factors.

Transcranial ultrasound have important applications in acute ischemic stroke. First, to identify the location and severity of occlusion, evaluating the condition of collateral circulation, but, also, allowing recanalization monitoring over time in order to identify an early reocclusion (essential parameters for prognosis). Awareness of the site of arterial occlusion have a predictive value on response to intravenous thrombolysis[4]. So neurosonology applied at an early stage of ischemic stroke can give us useful information to determine prognosis. For example, the total absence of flow in the middle cerebral artery (MCA) is an independent predictor element of death or disability[5], representing a crucial parameter to know the clinical evolution in short and long term.

Multicenter DIAS study (Duplex-Sonography assessment of the Cerebrovascular Status in Acute Stroke)[6] evaluated intracranial circulation at six hours after acute ischemic stroke using neurosonology. On a population of patients with intracranial arterial occlusion, in the group of patients not treated with fibrinolytic therapy, only 1 patient out of 12 presented a spontaneous intracranial arterial recanalization.

NAIS study[7] (Neurosonology in Acute Ischemic Stroke) monitored the profiles of extra/intracranial arterial occlusion in a group of 452 patients with acute cerebral ischemia, within six hours of the event.

Through neurosonology, Molina et al.[8] have defined the different ways of arterial recanalization in response to fibrinolytic therapy and in relation to different types of ischemic stroke. From this study it has been pointed-out that cardioembolic stroke have more rapid intracranial arterial recanalization and a better clinical outcome, both short and long term.

ELIGIBLE study[9] of Malferrari et al. has demonstrated how neurosonology, through the study of intracranial circulation, allows to program fibrinolytic therapy in acute stroke, in order to optimize the efficacy and reduce side effects.

Other possible applications is the detection of microembolic signals (MES), using systems (still being improved) that allow to automatically distinguish artifact from embolic signal and solid microemboli from those gaseous.

Sonothrombolysis

A particularly promising application of neurosonology is the sonothrombolysis, or the use of ultrasound (U.S.) - at frequencies lower than those normally used to diagnosis (2 MHz) - alone or concomitantly with thrombolytic drugs (rtPA), with the aim of increase lytic effect. This effect appears to be greater especially at relatively low frequencies, from a few MHz to KHz. The only limits of transcranial doppler are operator-dependence and possible lack of adequate temporal bone window, from which it is possible to identify middle cerebral artery, anterior cerebral artery, posterior cerebral artery and the two connecting front and rear. The sonothrombolysis is proving to be particularly important in acute ischemic stroke by occlusion of major intracranial arterial trunks. Lithic effect increase, is only partially understood. It would seem that ultrasound are able to accelerate fibrinolysis through non-thermal mechanisms of cavitation, which would allow a reversible increase of flow through the fibrin's mesh. Ultrasound's effect on fibrin has been studied using a scanning electron microscope. Studies carried out on non-crosslinked fibrin gels have shown that ultrasounds disintegrate fibrin's meshes, by increasing both input of thrombolytic drugs and the sites of attachment of thrombus, with acceleration of its dissolution[10].

CLOTBUST study[11,12] (Combined Lysis of thrombus in brain ischemia using transcranial ultrasound systems and t-PA) has used ultrasonic insonation of intracranial arteries continued for 2 hours and associated with administration of thrombolytic drugs in acute phase of stroke by middle cerebral artery occlusion. Two hours following tPA's bolus administration, 49% of group receiving this treatment has reached a complete recanalization, while in the control group (treated only with tPA) only 30%. At 24 hours, 44% of target group and 40% of control group had a clear clinical recovery.

At three months, 42% of target group and 29% of control group showed a good clinical outcome (as assessed by mRS).

In 2008 Eggers et al.[13] have used transcranial color-coded sonography (TCCS) at 1.8 MHz for an hour: in a group of 37 patients with complete occlusion of middle cerebral artery, 19 patients were treated with thrombolytic drugs and ultrasounds and 18 with thrombolytic therapy alone. Compared to the latter, the group treated with U.S. + rtPA showed a best TIBI score at 1 hour and a best NIHSS increase at distance of 1 and 4 days.

In 2005 TRUMBI study[14] (Transcranial low-frequency US-Mediated Thrombolysis in Brain Ischemia) used, for sonothrombolysis, a low frequency transducer (approximately 300 kHz) with an intensity of 700 mW/cm2, applied simultaneously with administration of tPA (in 60 minutes) and for the next 30 minutes (for a total of 90 minutes). However, it was necessary to stop study because of high number of symptomatic intracranial hemorrhage (SICH), demonstrating the inapplicability of low frequencies for sonothrombolysis.

Since 2006 numerous clinical and experimental data[15,16,17,18] have shown that thrombolytic's effectiveness can be further enhanced using gas microbubbles or gas-filled microspheres associated at insonation of intracranial circulation with probe at double frequency. Clinical studies have confirmed these effects in patients with acute stroke[19,20,21].

Mechanisms implicated are only partially understood. The application of high acoustic pressure U.S. could induce non-linear oscillations to microspheres, which absorb energy until it explodes. The explosion would have a mechanical damage on thrombus, allowing greater penetration of thrombolytic in fibrin's mesh with consequent acceleration of lysis[22,23,24,25].

Fragmentation of microbubbles after insonation can determine the release of any possibly associated substance, which penetrate more easily within cells thanks to increased permeability of cell's membranes determined by ultrasound[26].

There are different substances used as microbubbles. In the study by Molina et al. of 2006 was used Levovist (first generation molecule) to evaluate effects on recanalization in 345 patients with ischemic stroke at a distance of 3 hours. 32% was treated with only tPA, 33% with tPA + U.S. and 34% with tPA + U.S. + microbubbles of Levovist at a dose of 400 mg / ml administered at 2, 20 and 400 minutes. Two hours later, subjects with complete recanalization was 24% in first group, 41% in the second and 54% in the third. In addition, 55% of third group patients had an increase of 4 points to NIHSS at 24 hours.

In 2008, the study by Perren et al.[27] has reported an increased recanalization and an improvement of clinical outcomes by administration of SonoVue microbubbles (gases sulfur hexafluoride, a second generation molecule) at a dose of 5 ml, coupled with Transcranial color-coded duplex (TCCD) at 2MHz and monitored for 60 minutes. While subjects treated with thrombolytic drugs and TCCD showed complete recanalization rates (assessed with TIBI) at around 45%, addition of microbubbles allowed achieving of 70%.

This fact was reflected in clinical improvement at 24 hours as measured by NIHSS. All this without increasing risk of hemorrhagic transformation. This study therefore confirms results of Molina with a different echocontrast agent, a different ultrasonographic technique (TCCD versus TCD) and a different range of insonation (1 versus 2 h).

Similar results have also been obtained through the use of microspheres (μS) of perflutren (octafluoropropane encapsulated in an outer lipid shell) at 2.8 ml[28] associated with insonation of Transcranial Doppler (TCD). Target group (tPA+TCD+2.8 mL μS) showed recanalization rates higher than control group (tPA+TCD, protocol used in CLOTBUST study), without thereby an increased risk of bleeding. After treatment, in fact, have been found four asymptomatic bleeding: three in target group (25%) and one in controls (33%); in none of two groups occurred SICH. Best rate of recanalization was confirmed by an increase in NIHSS score.

Considering advantages conferred by microbubbles, subsequent studies have attempted to examine the safety of this method. The randomized phase 2 TUCSON study[29] (2009), compares a first group of patients treated with microbubbles of perflutren at 1.4 ml, a second group with microbubbles at 2.8 ml and a third control group treated only with the thrombolytic. 27% of group 2 (at 2.8 ml) had a SICH. In group 1, however, there was no bleeding, a result comparable to group 3. The study therefore concluded that at a dose of 1.4 ml of perflutren, the use of microbubbles does not increase risk of intracranial hemorrhage compared to treatment with only tPA, unlike what happens at a dose of 2.8 ml.

A recent metanalysis[30] analyzes benefits and risks of various methods. It confirms the excellent results obtained with protocol U.S.+ tPA (with or without microbubbles), capable of improving both recanalization that functional independence compared to treatment with tPA alone, without however significantly increase risk of SICH. The latter is instead result significantly increased in treatment with tPA and LFUS, as already seen for the TRUMBI study.

The use of microbubbles can be a valid strategy for intervention in case of exceeding therapeutic window (3 hours of symptom onset) or intracranial arterial reocclusion after thrombolysis, a condition that occurs in 20% of cases. Moreover it can represent a real alternative for patients not candidates to intravenous thrombolysis.

Recent developments

Use of ultrasound to arterial level

In 2003 it was tried for the first time direct insonation of thrombus through a microcatheter (EKOS MicroLysUS infusion catheter)[31], simultaneously with arterial infusion of thrombolytic. The findings, which need further future confirmation, were positive, indicating a real possibility of use in treatment of acute ischemic stroke.

Effects on microcirculation

In recent years, possible side effects of sonothrombolysis on microcirculation have been extensively investigated. A study[32] of 2008 conducted in rats, demonstrated no adverse effects of sonothrombolysis with microbubbles (MB) of SonoVue on ischemic stroke with ACM occlusion. This result was obtained measuring, by means of intracerebral microdialysis, the levels of glutamate, pyruvate, lactate and glycerol both before and after induction of stroke, and after application of ultrasound and microbubbles for 20 hours. After 24 hours it was rated cerebral infarct volume, apoptosis degree and levels of IL-6 and TNF-alpha. Volume was significantly reduced in group treated with U.S. and microbubbles, as well as level of apoptosis, while IL6 and TNFa were the same in both groups.

Not only microcirculation would not be damaged, but it would seem even benefit from the combined action of tPA, U.S. and MB. A study[33] conducted on animals have monitored, by means microTC and nanoTC, the complete recovery, at 60 minutes, of cerebral microcirculation earlier interrupted. Recovery was greatest after administration of tPA, U.S. and MB; treatment with U.S. and MB (without tPA), tPA and U.S. (without MB) and with only tPA led to a partial recovery of microcirculation; whereas, using of only U.S. did not bring any benefit.

MRI-guided sonothrombolysis

Currently, several works are taking advantage of opportunity to focus U.S. on the area of interest through the guidance of RM. In a study[34] of 2011conducted in rats, this combined technique has been exploited for the introduction of stem cells in ischemic penumbra area. U.S., in fact, appear to increase BBB's permeability, so concentrating them in infarcted area, stem cells introduced systemically (in the current study from carotid artery) may exceed more easily BBB and distributed in the affected area. After 24 hours from rats sacrifice, immunohistochemical analysis confirmed presence of stem cells (marked with iron) and that these were alive (as expressed markers nestin and polysialic acid) and able to differentiate (as demonstrated by 'expression of doblecortina, microtubule-associated protein, expressed in neuronal precursors and immature neurons).

This approach is clearly more advantageous than surgery stereotactic introduction of stem cells: as well as being less invasive, avoids the dissemination of stem cells in different areas.

Microbubbles "loaded" with thrombolytic

It is imposing more and more to attention of researchers the opportunity to take advantage of MB as a "carrier" for thrombolytic therapy. The idea is to "load" microbubbles and, by means insonation, make them explode at the site in question to allow release of thrombolytic into place. The use of these "intelligent" particles would allow a targeted action with a significantly smaller amount of thrombolysis, thus representing a promising strategy for treatment of acute ischemic stroke. Marsh et al. in 2009[35] developed a perfluorocarbon nanoparticle specific for fibrin, "loaded" with plasminogen activator streptokinase. The nanoparticles, tested in vitro, bound to fibrin thrombus and induced it to a rapid lysis (in less than 60 minutes),

without further training or cavitation of microbubbles.

The study of Tiukinhoy-Laing et al.[36], conducted in vitro as the previous, evaluated potential of echogenic liposomes (ELIP) loaded with tPA and effect that ultrasound exerted on ELIP "loads" for lytic action. The exposure of charged molecules to ultrasound has enabled a net enhancement of thrombolysis, which was as effective as the systemic, although the doses used were infinitely smaller.

Last frontiers of sonothrombolysis pass through genetic engineering and nanotechnology. The objective, as difficult as fascinating, is to create particles able to bind in a selective manner the fibrin through the use of monoclonal antibodies. A study[37] in 2011 evaluated efficacy of fibrinolytic perfluorocarbon nanoparticles "loaded" with urokinase and monoclonal anti-fibrin. To values greater than 100-400 antibodies for nanoparticle lytic effect was considerably increased.

Finally, thrombolytic substances are also loaded in nanoparticles of silica mesoporus; a study[38] of 2012, although postponing to future work, prove how with these nanoparticles have been achieved excellent results, both in terms of lysis that reperfusion.

Latest developments in sonothrombolysis, together with attempts to extend the "window period", let us hope in a ever more efficient therapy of acute ischemic stroke, able to achieve, even with lower amounts of thrombolytic, an improvement of clinical outcome and a reduced occurrence of complications.

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