Review Article

Clot composition and treatment approach to acute ischemic stroke: The road so far

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Abstract

Recent histological studies of thrombi retrieved from patients with an acute ischemic stroke using the endovascular thrombectomy devices and correlation with early vessel computed tomography (CT) and magnetic resonance imaging (MRI) characteristics have given relevant insights into the pathophysiology of thrombotic lesions and may facilitate the development of improved reperfusion treatment approaches. We present a review of recent studies on the histopathologic analysis of thrombi, studies of MRI, and CT imaging correlation with thrombus histology, and detailed structural analysis of thromboemboli retrieved by thrombectomy devices during an acute ischemic stroke.

Key Words

Clot composition, histopathology, stroke, thrombus

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Introduction

First histopathological evaluation of cerebral thrombi was carried out by Torvik and Jorgensen[1] more than 50 years ago in post-mortem cases with one and half month old obstruction. Recently, mechanical thrombectomy devices have provided an opportunity to directly investigate fresh thrombi specimens by retrieving them from the target arteries in patients with an acute ischemic stroke. On the whole, it is not known whether the choice of therapies ought to take into consideration composition of thrombus. A crucial issue is whether the structure of a thrombus ascertains its appearance on magnetic resonance imaging (MRI) and computed tomography (CT) and whether the thrombus structure also determines its susceptibility to thrombolytic agents like Tissue plasminogen activator (tPA).[2]

Examination of freshly retrieved thrombi from patients with an acute ischemic stroke could help to improve our knowledge of stroke pathophysiology, may forecast response to treatment and soon might be expected to play a role in shaping success of treatment approaches after patient selection. We did review of human and animal studies carried out on the histopathologic evaluation of thrombi, studies of MRI and CT imaging correlation with thrombus histology, and detailed structural analysis of thromboemboli by scanning electron microscopy (SEM).

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Thrombus Composition Using Light Microscopy

Marder *et al*.[3] did the systematic histological analysis of thromboemboli retrieved by Merci retriever from the middle cerebral artery and intracranial carotid artery of 25 patients with acute ischemic stroke within or beyond 8 h of symptom onset. Despite the presence of common components of the fibrin-platelets, nucleated cells (neutrophil/monocyte) and red blood cells (RBC's) in all cases, there was a large diversity of histological pattern as well as in quantitative proportion of different components. The authors accredited this to haphazard and disorganized forces of sheer and turbulence imposed on site of thrombus formation contradicting long established system of belief that a cardiac source with slow flow causes the formation of "red" (erythrocyte) clots, whereas high flow in arteries formed "white" (fibrin) clots. Since there was heterogeneity of clot composition, traditional definition of red versus white clots was not found to be truly applicable.

There was an association between thrombus size and location in intracranial circulation. There was no correlation of thrombus

histology with presumed stroke etiology, site of occlusion and reperfusion status. Marder *et al.* also stated that the interval between symptom onset and procedure initiation did not impact the recanalization rates. Calcific deposits and cholesterol crystals were not seen in the retrieved specimens.

Inference of thrombus structure from early vessel signs on computed tomography (CT) and magnetic resonance imaging (MRI) might assist to categorize patients who are not expected to respond to intravenous thrombolysis and therefore, need more aggressive treatment approaches such as intra-arterial thrombolysis or mechanical endovascular therapy. Liebeskind *et al*.[4] correlated the occurrence of Hyperdense middle cerebral artery sign (HMCAS) and blooming artefact (BA) with clot morphology and clinical results in 50 patients with acute ischemic stroke who underwent endovascular mechanical thrombectomy. Thrombi were comprehensively evaluated by histopathology regarding the fraction of red blood cells, white blood cells, and fibrin. Both HMCAS and BA were notably linked with the occurrence of red blood cell-dominant clots while lack of HMCAS and BA was suggestive for higher fibrin contents in the clots. On the other hand, the occurrence of HMCAS and BA or thrombus histopathology had no correlation with stroke severity or functional outcome. As with the study carried out by Marder *et al*., Liebeskind *et al.* too were unsuccessful to reveal an obvious association between thrombus composition and stroke etiology or mechanisms, i.e., cardioembolism or vascular atherosclerotic disease.

Animal model studies demonstrated a lesser effectiveness of tPA thrombolysis in thrombi with high fibrin content in contrast with erythrocyte-rich emboli.[5,6] Some other studies in human patients with stroke showed that strokes caused by paradoxical erythrocyte-rich embolism from a thrombus in the deep venous system were more responsive to treatment with recombinant tissue plasminogen activator in contrast with strokes of other etiologies.[7]

Almekhlafi *et al.* demonstrated chronic histopathological characteristics in thrombi mechanically retrieved by Merci device from five acute ischemic stroke patients. Early endothelialization was observed over and within the thrombus, and calcifications were also noticed, besides the usual laminar fibrin, intervening red blood cells, and neutrophils. They concluded that endothelialization, which is a subacute sign of early organization, might make tPA less successful if present in larger amounts. There was no differentiation in thrombi retrieved from the anterior or the posterior circulation, signifying a similarity of thrombotic mechanism once clotting has started.[8]

Ultrastructural Analysis of Thrombi Using Electron Microscopy

Nogueira *et al*. reported the SEM examination of thrombi retrieved from 18 ischemic stroke patients. All the thrombi samples were dissimilar in size, morphology, and fragmentation on macroscopic examination. Light microscopic analysis of the gross specimens showed that the red regions were situated where there was less flow or stasis or where the thrombus was alongside the vessel wall while white regions were likely exposed to high shear blood flow.

Two different structural organizational patterns were identified on SEM: (1) clots showing advanced maturity with all the thrombotic elements densely incorporated so that individual units were not clearly noticeable signifying a well matured and established location that had continuous exposure to shear flow; (2) clots showing dissimilar fibrin strands and trapped red cells indicative of comparatively loose cross-linking attributive of an active region where the thrombus is still in the process of maturing and probability of development in regions of stasis and recirculation. Similar to earlier light microscopic studies, the organization of the thrombotic elements, i.e., fibrin, platelets, and red cells, varied within each thrombus as well as across the various thrombi.[9,10]

Recent Advances in Imaging Assessment of Clots

Recent advances in molecular imaging have led to the detection of thrombus-specific magnetic resonance (MR) contrast agents that have an affinity to either fibrin[11] or activated platelets[12] and allow direct and selective high-signal visualization of the thrombus itself while the surrounding blood pool and soft-tissue is signal-suppressed. EP-2104R is a fibrin-targeted gadolinium-based MR contrast agent with usefulness in enhancing thrombi and has the additional benefit of allowing imaging of acute, subacute, and chronic thrombi.[13-16]

Plaque rupture with subsequent thrombosis is accepted as one of the relevant underlying pathophysiologic mechanisms in patients with acute stroke. Studies have shown that MRI can differentiate thick, thin, and ruptured caps of carotid plaque with excellent sensitivity and specificity *in vivo*.[17,18] Yuan *et al*. showed that patients with thin and ruptured fibrous caps are more prone to have had a recent transient ischemic attack (TIA) or stroke in contrast with plaques with thick caps identified by carotid plaque MRI.[19] Multidetector CT angiography can also identify high-risk morphological features (HRM) of plaques (remodeling, lipid pooling, and luminal irregularities) that are associated with an increased risk of ischemic cerebro-vascular events.[20-24]

^{18}Flurodeoxyglucose positron emission tomography (FDG-PET) has been shown to be helpful in assessing plaque inflammation. Figueroa *et al*.[25] demonstrated that inflammation as assessed by both FDG uptake and histology is increased in plaques containing HRM and increases with an increasing number of HRM. They concluded that inflammation accumulates relative to the burden of morphological abnormalities in plaques. Other prospective studies have shown that increased FDG uptake is associated with lipid-rich plaques assessed by MRI and with echo-lucency on ultrasound.[26,27]

Many studies are underway involving human and animal subjects with fibrin-specific MRI contrast agents or PET-based as well as ultrasound-based tools and perhaps will shortly permit a further precise categorization of thrombus composition.[28,29]

Summary and Conclusions

Endovascular mechanical thrombectomy devices have provided the opportunity to directly investigate freshly retrieved thrombi which until recently were inaccessible. The recent clot composition studies make available vital insights concerning the histopathology, MR and CT imaging aspects, and detailed structural analysis of thromboemboli, which commonly cause acute ischemic strokes. The histopathologic assessment of fresh thrombi with light microscopy permits new understanding of the pathogenesis of and treatment for large-vessel ischemic stroke. There is a relationship between HMCAS on CT or BA on GRE and thrombi composed mostly of RBCs while lack of these imaging findings might mean a fibrin-dominant thrombus that is likely to be more refractory to thrombolytic drugs. Presence of chronic histopathologic features in clots such as endothelization and calcification might render tPA less effective due to decreased penetration. Similar to light microscopic studies, scanning electron microscopic examination has demonstrated diverse ultrastructural patterns being present within and among thrombi causing acute ischemic stroke. The selective imaging of thrombi with fibrin specific MRI contrast agents and PET-based tools may permit further precise identification of their composition. Though, the above discussed studies are very promising and show stimulating results, still more research is required to show the way in the selection of the most suitable treatment on the basis of clot composition.

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