

A simple trichromic staining method detects microcalcifications in atherosclerotic plaques: ex vivo study of human carotid endarterectomies embedded in epoxy resin

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The term vulnerable plaque is used to identify thrombosis-prone plaques and plaques with a high probability of undergoing rapid progression¹, thus becoming responsible of acute coronary syndrome or stroke. The role of microcalcifications about 10 µm in diameter in atherosclerotic plaque vulnerability is still debated²⁻⁴. From a morphological point of view, microcalcifications are not so easy to detect just by observing paraffin-embedded histopathological sections (3-5 µm thick). Diffuse calcification can be observed using Von Kossa staining⁵ but such small calcifications cannot be accurately measured. A good characterization of microcalcifications may be achieved observing at light microscopy semithin sections obtained from epoxy resin-embedded tissue samples thinner than 1 µm. Therefore, on the basis of the method reported by D'Amico⁶ we have developed a simple, rapid and trichromic method, which considers the use of methylene blue-azur B, basic fuchsin and alizarin red. Our aim was to analyze microcalcifications on semithin sections from epoxy resin embedded samples of carotid endarterectomies. Comparisons were performed between the present method and methylene blue-azur B (with or without basic fuchsin) followed or not by Von Kossa staining. Our method is simple and fast (ca. 2 min), gives a sharp general contrast for all structures, and allows to easily identify collagen and elastin. In addition, gray-green colour associated to intracellular lipid droplets evidences foam cells, which are particularly abundant in endarterectomies samples. Mast cells and their metachromatic granules are also well recognized. Calcifications over 0,5 µm are clearly recognizable. Microcalcifications are clearly distinguished from the extracellular matrix in spite of their reduced dimensions. Methylene blue-azur B-basic fuchsin-alizarin red method is easy to use, reproducible, and is particularly suitable for the identification of microcalcifications in the morphological analysis of atherosclerotic plaques.

References

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Key words

Atherosclerosis, carotid endarterectomy, calcification, polychromatic stain, epoxy resin, transmission electron microscopy