R is for Reticulin

A-Z of Staining - a series of articles where we share a little extra information about stains, staining techniques and some of the interesting chemicals associated.

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Welcome to our A-Z of staining where today we will be looking at the letter R for Reticulin. Widespread in connective tissues, muscles, and many other areas of the body, reticular fibres and are composed of type III collagen which is secreted by reticular cells. Unlike collagen fibres (which unite together to form coarse, stromal bundles), reticular fibres form a thin network which acts as a supporting mesh. They do not stain with the dyes commonly used for collagen fibres (such as the Van Gieson and Mallory trichrome methods) but as they are rich in glycoproteins, reticulin stains positive with the periodic acid Schiff (PAS) method. In addition, reticular fibres have a special affinity for binding silver but since they are argyrophilic, the silver needs to be subsequently reduced by an extraneous agent. This must

not be confused with argentaffin substances which have both the ability of binding and reducing metallic silver without the use of an extraneous reducing agent.

There are many silver impregnation methods available (such as those of Gordon & Sweet and Gomori) and they are among the most popular procedures for demonstrating reticulin fibres. Primarily, tissues are oxidized in potassium permanganate prior to destaining in a solution of oxalic acid (Mallory bleach). Subsequent sensitization of tissue in ammonium iron sulphate (iron alum) and immersion in ammoniacal silver nitrate solution allows deposition of silver onto the reticulin fibres in the form of a brown residue. Since the fibres are argyrophilic, formalin is used as an extraneous agent to accelerate reduction of the silver and render the fibres black (Figure 1). Toning in gold chloride effectively improves resolution by substituting gold for the silver and any unbound metal is removed with sodium thiosulphate. Nuclei can be visualized by counterstaining in nuclear red or similar. Van Gieson may also be used to demonstrate any collagen fibres that are present.

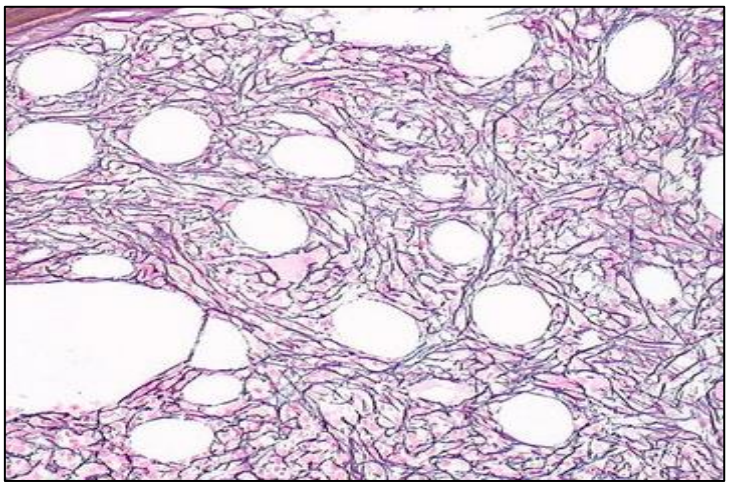

Figure 1. Silver impregnation showing the reticular network in a bone marrow

So why do we need to demonstrate reticulin?

The reticular fibre network supports the tissues of the body and is common in organs such as the liver, spleen and kidneys. The reticulin stain is an inexpensive tool and is used extensively in histology laboratories since characteristic reticulin patterns can help diagnose diseases such as fibrosis, cirrhosis and tumours of epithelial and non-epithelial tissues. In the liver for example, staining the reticular network is useful in establishing normal liver parenchyma from

that of hepatic carcinoma (Figure 2). Similarly, in the skin, the reticulin pattern is helpful in differentiating benign naevi (where reticulin fibres surround individual cells) from malignant melanomas, which usually have the fibres surrounding groups of tumour cells.

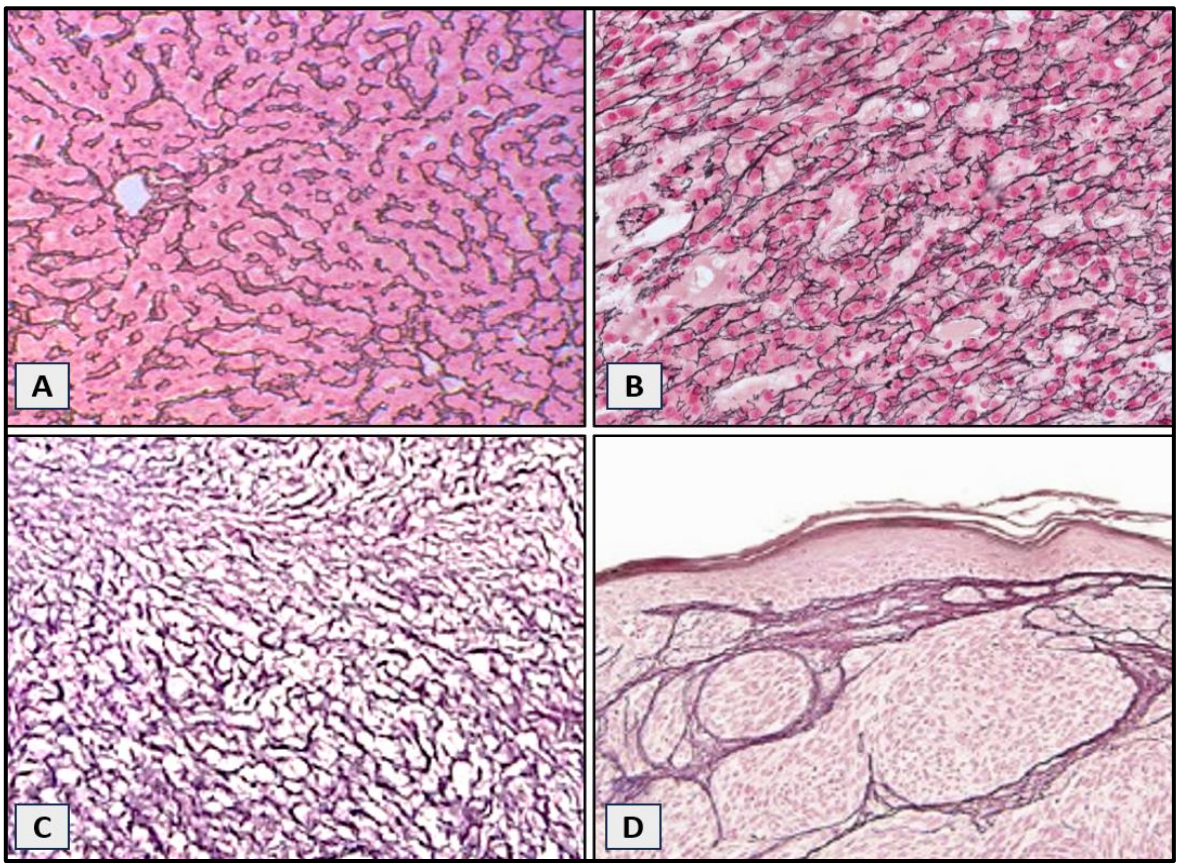

Figure 2. Reticulin stain showing normal liver architecture (A), hepatic cell carcinoma with the reticulin surrounding individual tumour cells (B), benign naevus with the network around individual melanocytes (C) and reticulin surrounding large melanoma nests (D)

Because several neoplastic and non-neoplastic pathological conditions are associated with increased reticulin fibrosis, both the distribution and thickness of fibres can be evaluated. In bone marrow samples, fibrosis or an excess of fibrous tissue is usually seen in myeloproliferative disorders such as polycythaemia, thrombocytosis and chronic myeloid leukaemia. The fibrosis is characterized by an increase in reticulin fibres (reticulin fibrosis) or a combination of reticulin and collagen fibres (collagen fibrosis). Differentiation between the transition of various myeloproliferative states has shown to be improved by viewing bone marrow sections that have been stained with both silver impregnation and Van Gieson. By grading the fibrosis using the WHO (or European) reticulin fibrosis system, it allows the precise status of both the reticulin and collagen fibre content which may help to predict prognosis.

Whether the need is to evaluate a necrotic lymph node or to differentiate an astrocytoma from a benign growth in the brain, the staining of reticulin remains a highly dependable test in the histological diagnosis of disease processes.

Further reading

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