Diagnosis of the type of amyloid in paraffin wax embedded tissue sections using antisera against human and animal amyloid proteins

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Summary. Different histochemical techniques were compared on paraffin wax embedded tissue sections for routine classification of amyloid; the following methods were used: potassium permanganate, the indirect immunoperoxidase method using polyclonal anti-human amyloid antisera (anti-AA, anti-Aβ, anti-Aκ and anti-AF) and the peroxidase-antiperoxidase (PAP) method using antisera against human, bovine, hamster and canine AA amyloid. Anti-human AA antiserum appeared to be a useful tool in this respect. Polyclonal anti-AL antisera may be helpful in diagnosing AL amyloid, but were of less value than anti-AA serum.

Strong cross reactivity between anti-bovine AA antiserum and human AA amyloid deposits was found. This indicates that animal amyloid AA antisera can also be used for the diagnosis of AA amyloid in human tissues.

Key words: Amyloid – Potassium permanganate – Immunoperoxidase

Introduction

Amyloidosis is a disease complex characterized by extracellular deposits of amyloid fibrils. These fibrils show chemical diversity depending on the underlying disease. One common feature, the β-pleated sheet conformation (Glenner and Page 1976), accounts for the characteristics of amyloid: congoophilia and green birefringence, resistance to proteolysis and insolubility in physiological solutions. Different clinicopathological entities exist and a variety of investigations has led to classifications based upon the clinical pat-
tern of organ dysfunction (Van Rijswijk and Van Heusden 1979), on the chemical nature of the protein fibrils and their precursors (Glenner et al. 1980), and on the clinicopathological conditions related to chemical types (Glenner 1980). Table 1 proposes a summary of these classifications.

The clinically important forms of systemic amyloid consist of AA proteins, AL proteins or AF proteins. AL-amyloid is derived from immunoglobulin light chains, including either lambda types (Aβ) or kappa types (Aκ). It is associated with immunocyte dyscrasia, classically with myeloma (Kahler’s disease). It also occurs in an idiopathic form, although in many such cases an underlying plasma cell dyscrasia can also be demonstrated (Osserman 1961 a, b). Since AL-amyloid consists mainly of the N-terminal variable region of a homologous light chain, which differs in each patient, there is little immunological cross reactivity between patients for immunohistology and thus a battery of antisera is needed (Glenner et al. 1970; Cornwell et al. 1977; Linke et al. 1981; Linke and Nathrath 1982; Linke et al. 1984a). If, in a number of cases, cross reactivity is found, this might be accounted for by remnants of constant regions attached to the variable light chain regions (Westermark et al. 1981).

AA-amyloid, occurring in reactive (secondary) systemic amyloidosis, is derived from a plasma precursor protein, called SAA. In contrast to AL-amyloid these AA protein fibrils are found to be almost identical between patients (Benditt and Eriksen 1977). Therefore, using an anti-AA antiserum, immunohistochemical demonstration of AA-amyloid in tissue sections is possible (Gruys and Timmermans 1979; Fujihara et al. 1980; Linke et al. 1981; Shirahama et al. 1981; Shirahama et al. 1981; Hind et al. 1983; Orfila et al. 1983; Linke 1984). Furthermore, the AA-amyloids found in animals resemble that found in man. Extensive chemical homologies of protein AA among different species have been described (Eriksen et al. 1976; Gorevic et al. 1977; Waalen et al. 1980; Gorevic et al. 1982), as well as immunohistochemical cross reactivity, although in the latter a variability in reaction intensity was found (Doepel et al. 1981; Linke et al. 1984 b; Linke et al. 1985).

It is suggested that not all amyloid precursor proteins are equally amyloidogenic, specific subclasses being more likely to be deposited as amyloid than others. In AL-amyloidosis a predominance of λ light chains in both the amyloid deposits and in the sera and urine of these patients exists in contrast to k chain predominance in myeloma proteins of non-amyloidotic myeloma cases as well as in normal immunoglobulins (Husby et al. 1974; Isobe and Osserman 1974; Franklin 1980; Franklin and Gorevic 1980; Isobe et al. 1983; Orfila et al. 1983). There is one rare subclass of λ chains (Vλ VI) which appears almost exclusively in association with amyloidosis (Solomon et al. 1982).

In prealbumin-related amyloid investigations to date have shown that only a single amino acid was altered in the amyloidogenic prealbumin of the patients with familial neuropathic amyloidosis (Saraiva et al. 1983; Tawara et al. 1983; Dwulet and Benson 1984). Recently microheterogeneity in both SAA and AA proteins has been described. Variations in size, charge