

# α-1-antitrypsin

## Background

α-1-antitrypsin (AAT), a 51 kD glycoprotein, is mainly synthesized in the liver, where a pair of at least 24 possible codominant alleles, which belong to the protease inhibitor (Pi) locus on chromosome 2, determine production. It functions as an inhibitor of proteases, especially elastase, collagenase, and chymotrypsin. Individuals homozygous for Pi M produce normal quantities of functionally normal AAT, whereas individuals with abnormal Pi genes, such as those designated ZZ, SZ, and PS, have serum concentrations of AAT that are 40% of normal. Such individuals are at risk for hepatic cirrhosis in childhood or pulmonary emphysema as young adults.

## Applications

AAT, AACT, and lysozyme can still provide useful diagnostic information, but they have to be used in the context of a panel of antibodies directed to the diagnostic entities considered in differential diagnosis.

1. In pleomorphic tumors of the skin, these markers are useful for the separation of atypical fibroxanthoma from its mimics, although other markers can provide more relevant information to separate such entities, and in identifying tumors rich in phagolysosomes, such as granular cell tumors.
2. Immunolabelling for AAT remains an important way of demonstrating the presence of accumulated enzyme

within hepatocytes in AAT deficiency. Hepatocyte globules characteristically associated with AAT deficiency may be mimicked by AACT deficiency, and the distinction can only be made by immunolabelling.

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## NOTES

# α-Fetoprotein (AFP)

## Background

α-Fetoprotein (AFP) is a glycoprotein composed of 590 amino acid residues. Cells of the embryonic yolk sac, fetal liver, and intestinal tract synthesize this glycoprotein. By immunostaining, the antigen is detectable in hepatocellular carcinoma and in gonadal and extragonadal germ cell tumors, including yolk sac tumors. It is otherwise not present in adult tissues.

## Applications

Staining for AFP is largely used for the identification of the glycoprotein in germ cell tumors and in the separation of hepatocellular carcinoma (HCC) from its mimics such as cholangiocarcinoma and metastatic carcinoma. AFP is of low sensitivity and estimated to be present in no more than 44% of HCCs.

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## NOTES

# Alpha-1-antichymotrypsin

## Background

$\alpha$ -1-antichymotrypsin (AACT), a 68 kD glycoprotein, is a serum protease inhibitor that is synthesized mainly by cells of the mononuclear phagocytic system. AACT was initially employed as a marker of histiocytes (monocytes/macrophages), but the demonstration of this enzyme in a large variety of normal and neoplastic tissues of both

epithelial and mesenchymal derivation has resulted in only restricted use in diagnostic immunohistology. It most likely identifies cells that are rich in phagolysosomes and has no tissue specificity. Within restricted settings, AACT can be of value in diagnostic immunohistology, although, to a large extent, more specific markers of histiocytes/macrophages have replaced this marker.

(See discussion on  $\alpha$ -1-antitrypsin.)

## NOTES

# Alpha methyl acyl CoA racemase (AMACR)

## Background

AMACR, also called P504S, is a mitochondrial and peroxisomal enzyme involved in the metabolism of branched chain fatty acid and bile acid intermediates. It catalyzes the racemization of alpha methyl branched carboxylic coenzyme A thioesters. Deficiency of AMACR is associated with certain adult onset sensory motor neuropathies. Variable levels of AMACR protein expression are seen in a wide range of tissues and cancers, including colorectal, prostate, ovarian, breast, bladder, lung, and renal cell carcinomas. Additionally, AMACR is also expressed in lymphoma and melanoma. AMACR is thus considered a common abnormality and thought to participate in the early stages of cancer development. Prostate and colorectal carcinomas show the highest expression at 92% and 83%, respectively.

AMACR has also emerged as a putative therapeutic target in cancer treatment. While overexpression of AMACR is seen in a high percentage of the cancers named in the previous paragraph, it is either negative or minimally expressed in the adjacent normal tissue. This property makes it a potential candidate for targeted therapy either as an antibody or as an enzyme inhibitor.

## Applications

### Prostate carcinoma

AMACR is a sensitive (82–100%) and relatively specific (70–100%) marker for prostate cancer. It is most specific if circumferential luminal to subluminal and diffuse cytoplasmic staining is noted. It is a commonly used tool in the diagnosis of morphologically difficult prostatic adenocarcinomas in combination with basal cell markers including p63 and 34BE12 (CK903). AMACR staining is not uniform in prostate carcinoma. Approximately 25% of carcinomas are weakly positive, 40% moderately positive, and 35% strongly positive. Histologically benign prostate tissue can sometimes be positive (5–8% of cases). Additionally, premalignant and

benign entities that are known to lose basal cell markers may also be positive for AMACR, such as atypical adenomatous hyperplasia (15% of the cases), atrophy, partial atrophy, and crowded benign glands.

It is also important to note that unusual morphologic variants, including foamy gland, pseudo-hyperplastic and atrophic prostate cancers, are less frequently positive for AMACR expression, with only 69–80% of cases staining positive. The diagnostic implications of these findings are that while AMACR is a great addition to the armamentarium of stains for the work up of atypical small acinar proliferation (ASAP), it has its limitations. About 20% of cases considered to be carcinoma on hematoxylin and eosin (H&E) may be negative for AMACR and basal cell markers. So, while the initial studies indicated that AMACR was uniformly and strongly positive in 97% to 100% of prostate cancers, only about 80–85% of cancers are positive on needle biopsies.

### Minimal prostatic adenocarcinoma and atypical proliferations

A cocktail stain containing racemase along with p63 and CK903 is becoming increasingly common in the work up of atypical small acinar proliferations and to support the diagnosis of small foci of prostatic adenocarcinomas. Approximately 10% of cases thought to be atypical can be diagnosed as carcinoma after addition of AMACR to the basal cell marker cocktail. Approximately 45% of atypical diagnoses were converted to a definitive diagnosis of carcinoma on the basis of a positive AMACR.

### Prostatic “pseudo-neoplasms”

Mimics of prostatic adenocarcinoma include atypical adenomatous hyperplasia (AAH), adenosis, atrophy, post-atrophic hyperplasia, basal cell metaplasia, and seminal vesicles or ejaculatory duct epithelium. Only a small number of AAH cases reveal focal staining. Atrophic glands and

## A

Alpha methyl acyl CoA  
racemase (AMACR)

post-atrophic hyperplasia have also been shown to be positive for AMACR in a very small number of cases.

### Post-treatment prostate carcinoma

Radiation therapy has no effect on the AMACR staining of prostatic adenocarcinoma in needle biopsies, TURP (transurethral resection of prostate) chips, or salvage radical prostatectomies. Benign glands with radiation atypia are negative. A significant decrease in the intensity of AMACR staining is noted in hormone refractory prostatic adenocarcinoma when compared with clinically localized cancer. To assess residual, post-treatment prostatic adenocarcinoma, a panel of stains containing AMACR is useful.

### Renal tumors

AMACR has been found to be strongly expressed in papillary renal cell carcinomas (RCC), mucinous tubular and spindle cell carcinoma, Xp11 translocation RCC, and acquired cystic disease associated RCC.

Clear cell (CC) RCC and CC papillary RCC are negative for AMACR.

In a poorly differentiated RCC, or in cases with overlapping histological appearances, AMACR forms a useful adjunct to diagnosis.

### Other sites

A. In the gastrointestinal (GI) tract:

- i) Colon adenomas with high-grade dysplasia stain positive for AMACR.
- ii) Barrett-related dysplasia is found to be positive; 95% of cases with high-grade dysplasia and 45% of cases with low-grade dysplasia have been reported positive.

iii) Stomach adenocarcinoma may also show positivity for AMACR in approximately 25% of cases.

B. Extramammary Paget disease: 70% of cases are reportedly positive.

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# Amyloid

## Background

The amyloidoses are characterized by local, organ-limited, or generalized proteinaceous deposits of autologous origin. The pattern of distribution, progress of disease, and complications are dependent on the fibril protein. Amyloid is characterized by the following: (i) a typical green birefringence with polarized light after Congo red staining, (ii) non-branching linear fibrils with a diameter of 10–12 nm, and (iii) an X-ray diffraction pattern that is consistent with Pauling's model of a cross- $\beta$  fibril. Apart from the rare familial syndromes, localized forms of amyloid affect certain organs or lesions ( $A\beta$  in brain; calcitonin in medullary carcinoma; islet amyloid polypeptide in insulinomas or islets of Langerhans). The five major different fibril proteins are usually associated with the most common generalized amyloid syndromes: amyloid A (AA), amyloid of  $\lambda$ - ( $A\lambda$ ) and  $\kappa$ - ( $A\kappa$ ) light chains, and of transthyretin (ATTR) and  $\beta$ 2-microglobulin origin. These fibril proteins may be deposited in a wide variety of tissues and organs.

## Applications

1. AA-amyloidosis is commonly associated with chronic inflammatory disorders. AL-amyloidosis (either  $\lambda$ - or  $\kappa$ - light chain origin) is linked mainly to the plasma cell dyscrasias or interpreted as being idiopathic.
2. ATTR-amyloidosis is found in cases with familial amyloidosis.
3.  $A\beta$ 2microglobulin-amyloidosis is associated with long-term hemodialysis.

## Interpretation

Occasionally, more than one antibody may show immunostaining of amyloid deposits. Immunohistochemistry detects any associated contaminating component in the amyloid deposit (amyloid P component, apolipoprotein E, and glycosaminoglycans) and not merely the currently

known obligate fibril proteins. Further, the five syndromic fibril proteins originate from plasma proteins, which may themselves “contaminate” amyloid deposits. The most critical of these are the immunoglobulin light chains.

Another problem area is the false negative detection of amyloid. This can be avoided by increasing the sensitivity of detection by using both immuno- and Congo red staining methods. Long-term hemodialysis-associated  $\beta$ 2-microglobulin amyloid may also involve the gastrointestinal and reproductive systems, in addition to osteoarticular involvement.

The distinction and classification of amyloidosis has major therapeutic implications, as studies have recommended that AL-amyloidosis be treated with cytotoxic drugs (melphalan and prednisolone), whilst AA-amyloidosis responds better to colchicine and dimethylsulphoxide.

The role of antibodies against amyloid  $\beta$  precursor protein has assisted in the diagnosis of Alzheimer's disease and early detection of axonal injury in the brain. Antibodies to transthyretin amyloid protein are useful in the diagnosis of cardiac amyloidosis and familial amyloidotic polyneuropathy.

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## A

## Amyloid

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