# Part 1

# Autoantibodies in organ specific auto-immune diseases

## 17α-hydroxylase antibodies

17α-hydroxylase is the main target antigen of antibodies directed against steroid-producing cells (➢steroid producing cell antibodies).

## 21-hydroxylase antibodies

21-hydroxylase is the target antigen of ➢adrenal cortex antibodies.

## AADC antibodies

See ➢aromatic L-amino acid decarboxylase antibodies.

## Acetylcholine receptor antibodies, classical type

**Typical designation:** AChR antibodies.

### Autoantigen

The nicotinic acetylcholine receptor (nAChR) of the neuromuscular junction is a glycoprotein with a molecular weight of 300 kDa and is comprised of five homologous subunits with stoichiometry α2βχδ in the embryos and α2βεδ in the adults. Autoantibodies are predominantly directed against a specific region located at the α-subunits called main immunogenic region (MIR) (Tzartos et al., 1998; Lindstrom et al., 2008). In addition to the MIR region, AChR autoantibodies against the rest of the α-subunit as well as to the γ or ε AChR-subunits have also been identified (Tzartos et al., 2005; Ragheb et al., 2005; Shi et al., 2012). Furthermore, autoantibodies bind to conformational epitopes of clustered AChR (Leite et al., 2008).

**Pathologic relevance**

AChR antibodies influence nicotinic acetylcholine receptors in three ways (Gomez et al., 2010): (1) Influencing the neuromuscular function of these receptors through binding and cross-linking, accelerating their internalization and degradation. (2) To a small extent blocking acetylcholine binding sites. (3) Activating complement locally, leading to destruction of the postsynaptic membrane, with the consequence that neuromuscular stimulus transmission is wholly or partially inhibited. Transplacental transfer of IgG (including AChR autoantibodies) from MG mothers to infants may induce transient MG observed in the newborns [Tzartos et al., 1990]. Furthermore, animals immunized with AChR or injected with anti-AChR monoclonal antibodies, or with crude human MG immunoglobulin (Ig) fractions exhibit MG symptoms. Isolated α- and β-subunit antibodies were at least as efficient as the corresponding whole sera or whole Ig in causing experimental MG (Kordas et al., 2014).

**Detection methods**

- The radioimmunoprecipitation assay (RIPA) with $^{125}$I-α-bungarotoxin labeled native acetylcholine (Vincent and Newsom-Davis, 1985) is commonly used in routine diagnostics.
- Enzyme immunoassay using stable immobilized AChRTE671 cells (Nguyen et al., 1999) or EIA based on the ability of AChRAb to compete with 3 different AChR monoclonal antibodies for binding sites on affinity purified fetal and adult-type AChR preparations (Hewer et al., 2006) showed similar results compared to RIPA.
- Cell based immunofluorescence assays using HEK293 cells that co-express all AChR subunits of the adult form along with rapsyn mimicking the tightly clustered AChR that is embedded in the plasma membrane at the neuromuscular junction enables the detection of autoantibodies against clustered AChR in seronegative MG (Leite et al., 2008).

**Clinical relevance**

- Acetylcholine receptor antibodies are pathognomonic for ➢myasthenia gravis (MG) and are detectable in 80–90% of patients with generalized MG. AChR antibodies are found in 50-71% of ocular MG cases (Kupersmith et al., 1996; Peeler et al., 2015). Older age, male sex, and progression to generalized MG were significantly associated with AChR antibody positivity (Peeler et al., 2015). A positive AChR antibody test is seen as proof of MG due to the high specificity (almost 100%), however a negative result does not exclude MG ('seronegative' MG). Using cell-based assays (see methods), 16-66% of seronegative MG have been found positive for autoantibodies against clustered AChR (Leite et al., 2008; Devic et al., 2014; Zhao et al., 2015).
- AChR antibody titers do not correlate with the severity of myasthenic symptoms. However, the follow-up of AChR antibody levels can allow conclusions to be made on the prognosis of individual patients. A 50% reduction in antibody titer is often (but not always!) associated with a marked improvement in condition.

- AChR antibody positive MG had been observed in patients with ➢primary biliary cholangitis (PBC) and ➢rheumatoid arthritis (RA) treated with D-penicillamine (Morel et al., 1981; Marcus et al., 1984; Sundewall et al., 1985).
- Low titers of AChR antibodies (predominantly of the IgM isotype) had been also observed in nonmyasthenic patients with ➢AMA positive ➢PBC (Sundewall et al., 1985 & 1990). It was suggested that structural, if not functional, relationships between membrane components of liver mitochondria and muscle endplates are responsible for this phenomenon (Kyriatsoulis et al., 1988).
- In healthy individuals and patients with an inherited form of MG, as well as Lambert-Eaton myasthenic syndrome (LEMS), which has a very similar clinical picture, AChR antibodies are not detectable.

**Indications**

1. Suspicion of generalized or ocular myasthenia gravis.
2. Differential diagnostics of myasthenic syndromes.
3. Monitoring of myasthenia gravis patients.

**Acetylcholine receptor antibodies, general**

Acetylcholine receptors (AChR) are transmembrane receptors, which bind the neurotransmitter acetylcholine (Ach). Two forms exist, the nicotinergic or nicotinic (nAChR, nicotine receptors) and the muscarinergic or muscarinic AChR (mAChR, muscarine receptors). Clinically relevant, or potentially relevant, antibodies can be directed against various nAChR or mAChR:

- The **"classic" ➢AChR antibodies (α1-nAChR antibodies)** in ➢myasthenia gravis are directed against α1 and other nAChR subunits in the neuromuscular junction.
- The **➢ganglionic AChR antibodies (α3-nAChR antibodies)** in autoimmune autonomic gangliopathy bind the α3 nAChR subunit in the sympathetic and parasympathetic postganglionic fibers.
- In one form of autoimmune encephalopathy (Baker et al., 2009), and also in ➢Rasmussen's encephalitis (Watson et al., 2005), autoantibodies directed against the α4 and/or α7 nAChR subunit in the central nervous system (cortex and hippocampus) have been described (**α4-nAChR antibodies, α7-nAChR antibodies**).
- Autoantibodies against the muscarinic type 3 AChR (**M3mAChR/M3R antibodies**) are detectable in patients with ➢Sjögren's syndrome, ➢systemic sclerosis and gastrointestinal dysmotility (Singh et al., 2009). Several studies showed the pathogenic role of M3R antibodies: (1) Induction of internalization of M3mAChR partly through a clathrin-mediated pathway. The results suggest M3R internalization as a potential mechanism to explain the exocrinopathy seen in pSS patients (Jin et al., 2012). (2) Inhibition of muscarinic

receptor function, thereby inhibiting the Ca2+ mobilization necessary for the activation of K+ currents and a-fodrin reorganization in human submandibular gland cells (Jin et al., 2012). (3) Suppression of AQP5 trafficking to the membrane that contributes to impaired fluid secretion in SjS (Lee et al., 2012). (4) Induction of the production of pro-inflammatory mediators (MMP-3/PGE2) that may play a role in the development of glandular inflammation (Reina et al., 2011). (5) Results that indicate the potential to mediate multiple dysfunctions of the gastrointestinal tract in primary Sjögren's syndrome, ranging from reduced esophageal motor activity to altered colonic motility (Park et al., 2011).

- Autoantibodies against the muscarinic type 2 AChR (**M2mAChR/M2R antibodies**) have been described in ➢Chagas disease and in idiopathic ➢dilated cardiomyopathy (DCM) (Fu et al., 2002; Baba et al., 2004; Wallukat et al., 2010). In DCM, M2R antibodies were detected in 40%. Purified IgG from DCM patients exhibited negative chronotropic effects and induced supraventricular arrhythmias (Baba et al., 2004). Furthermore, M2R antibodies have been found in 100% of patients with chronic Chagas' disease manifested as cardiomyopathy combined with megacolon and patients with chronic Chagas' disease manifested as megacolon only, in 98% of patients with chronic Chagas' disease manifested as cardiomyopathy, and in 42% patients with Chagas' disease in the indeterminate (asymptomatic) state compared to 0% of healthy control subjects (Wallukat et al., 2010).
- Autoantibodies against the second extracellular loop of M2R have been found in in several cardiac arrhythmias, including sinus node dysfunction, ventricular arrhythmias (Chiale et al., 2001) and, in 23%, in idiopathic atrial fibrillation (AF) (Baba et al., 2004). They may play a role in the development of AF by inducing left atrial (LA) fibrosis. Anti-M2-R levels may be associated with the severity of LA and may be implicated in the pathophysiology of AF recurrence following cryoablation (Gurses et al., 2015).

## Actin antibodies

### Autoantigen

Actin is a component of smooth muscle microfilaments, and one of the target structures of autoantibodies against smooth muscle cells (➢SMA). Polymeric F-actin, with a molecular weight of 41 kDa, has been identified as the relevant main antigen in the diagnosis of autoimmune hepatitis.

### Detection methods

- Indirect immunofluorescence on cryostat sections of rodent stomach, liver and kidney. The typical immunofluorescence picture shows staining of the smooth muscle layer of the stomach and blood vessels, as well as the septa of stomach interparietal cells. Depending on the recognized autoantigen target structures, numerous other structures may also be stained.
- Indirect immunofluorescence with HEp-2 cells or fibroblasts. The typical immunofluorescence picture shows fibrous cytoskeletal staining.

  **Note:** The detection of actin antibodies on rodent tissue sections is more sensitive than detection with HEp-2 cells.

- Enzyme immunoassay with F-actin.
- Line/dot immunoassay with F-actin.

**Note:** According to the consensus report of the International Autoimmune Hepatitis Group, indirect immunofluorescence on multiorgan rodent sections (kidney, stomach, liver) is the method of choice for the detection of AIH relevant autoantibodies (Vergani et al., 2004). Larger studies are needed to evaluate the diagnostic value of F-actin antibodies as the basis of enzyme or line/dot immunoassays. So far, commercially available immunoassays (enzyme immunoassay, line/dot immunoassays) for the detection of actin antibodies have mostly displayed specificities too low in comparison with indirect immunofluorescence to serve as a diagnostic of AIH (Himoto et al., 2016).

### Clinical relevance

- High concentrations (titers) of actin antibodies are largely specific for ➢autoimmune hepatitis (AIH) type 1. The sensitivity ranges from 52–86%, although (usually low titers of) actin antibodies have also been observed in healthy individuals (3–18%), primary biliary cholangitis (PBC) (22%), hepatitis C (10%), connective tissue diseases and celiac disease (IgA antibodies).
- The use of only anti-actin antibodies for AIH diagnosis could lead to a decline of approximately 20% of diagnosed patients because F-actin is a main but not exclusive target autoantigen of SMA (Liaskos et al., 2007).
- Actin antibodies are associated with higher γ-globulin and high titers correlate with histological evidence.
- Patients with actin antibodies have an earlier disease onset and a more severe prognosis than actin antibody negative patients with ➢anti-nuclear antibodies (ANA).

### Indications

1. Suspicion of autoimmune hepatitis type 1.
2. Differential diagnosis of autoimmune hepatitis.

## ADAMTS13 antibodies

ADAMTS13 ("**a** **d**isintegrin **a**nd **m**etalloprotease with **t**hrombo**s**pondin-1 like domains **13**") is a von Willebrand factor (vWF) cleaving protease, which strongly regulates the size of the high molecular weight vWF, and thereby its biological activity. In patients with acquired ➢**thrombotic thrombocytopenic purpura** (TTP), autoantibodies against ADAMTS13 are detectable in most cases and are considered to be strongly involved in the pathogenesis of the disease (Hovinga et al., 2012). These antibodies can inhibit the protease function of ADAMTS13 (Furlan et al., 1998; Tsai et al., 1998), or induce the elimination of ADAMTS13 from the circulation (Scheiflinger et al., 2003; Rieger et al., 2005). Therefore, the high molecular weight vWF complexes remain uncleaved, leading to formation of microvascular thrombi. Patients with ADAMTS13 antibodies have a more severe disease progression and higher mortality rates than TTP patients without these antibodies.

ADAMTS13 antibodies were further found in 13% of obese patients (Zanato et al., 2017), subjects that may have a higher risk of TTP development. Because ADAMTS13 is a metalloprotease with 8 thrombospondin repeats displaying a structural homology with thrombospondin-1 (TSP-1), increased circulating levels of TSP-1 in obese patients may stimulate the production of ADAMTS13 antibodies (Lombardi et al., 2012). This suggestion is supported by a study showing higher TSP-1 levels at baseline that significantly decreased after weight loss, in parallel with a reduction in anti-ADAMTS13 autoantibodies (Zanato et al., 2017).

## Adenylate kinase 5 antibodies

Adenylate kinases are phosphotransferase enzymes that catalyze the interconversion of adenine nucleotides (ATP, ADP, and AMP). Of the nine human isoforms, adenylate kinase 5 (AK5) is highly specific expressed in brain (Van Rompay et al., 1999), and has critical neuronal-specific functions, including energy transfer and synthesis of RNA and DNA (Ren et al., 2005). Autoantibodies against AK5, determined by immunohistochemistry, Western blotting, immunoprecipitation, mass spectrometry, and cell-based assay, have been described up to now in 12 patients with ➢**limbic encephalitis** (Tüzüm et al., 2007; Do et al., 2017). However, the clinical presentation is different from classical limbic encephalitis, characterized by subacute anterograde amnesia without seizure and sometimes preceded by a prodromal phase of asthenia or mood disturbances. Patients may have confusion and behavioral disturbances, but no epileptic symptoms were observed. Because no neoplasm could be found in these patients up to now, the anti-AK5 antibody positive limbic encephalitis seems to be not of paraneoplastic origin. According to Do et al., clinical and biological

characterizations of this syndrome are important for two reasons (Do et al., 2017): First, isolated subacute anterograde amnesia in aged patients often leads to the diagnosis of early onset dementia, and the observed data demonstrate the importance of a brain MRI and CSF examination to obtain the correct diagnosis in such patients. Second, the refractory course and the unfavorable clinical response to immunotherapy observed in most of the described patients outline the importance of early diagnosis in order to provide aggressive immunologic treatment to prevent neuronal loss. Therefore, physicians should be aware of this syndrome, but commercial assays for determination of anti-AK5 antibodies are not available yet.

## Adrenal cortex antibodies

**Synonyms:** 21-hydroxylase antibodies, 21-OH antibodies, ACA (not to confuse with anti-centromere antibodies or anti-cardiolipin antibodies).

### Autoantigen

The steroid-21-hydroxylase (21-OH), a key intracellular steroidogenic enzyme (cytochrome P450 enzyme P450 21A2 or CYP21A2) exclusively expressed in the adrenal cortex, has been identified as the main target autoantigen of ACA (Winqvist et al., 1992). The main epitopes of 21-OH antibodies in patients with different forms of autoimmune adrenal disease are located in the C-terminal region and in a central region of 21-OH (Volpato et al., 1998)

### Detection methods

- Indirect immunofluorescence using cryostat sections of human adrenal cortex (preferably from patients with Cushing syndrome). The immunofluorescence picture shows cytoplasmic staining of the hormone producing cells of the adrenal cortex (Fig. 1).
- Radioimmunoassay with recombinant $^{125}$I labeled 21-hydroxylase.
- Radioimmunoprecipitation with $^{35}$S labeled 21-hydroxylase.
- Western blot with native or recombinant 21-hydroxylase.

**Note:** The concurrence between immunofluorescence and immunoassays is relatively good, although discrepant results can be obtained in some cases. Immunoassays have a somewhat higher sensitivity.

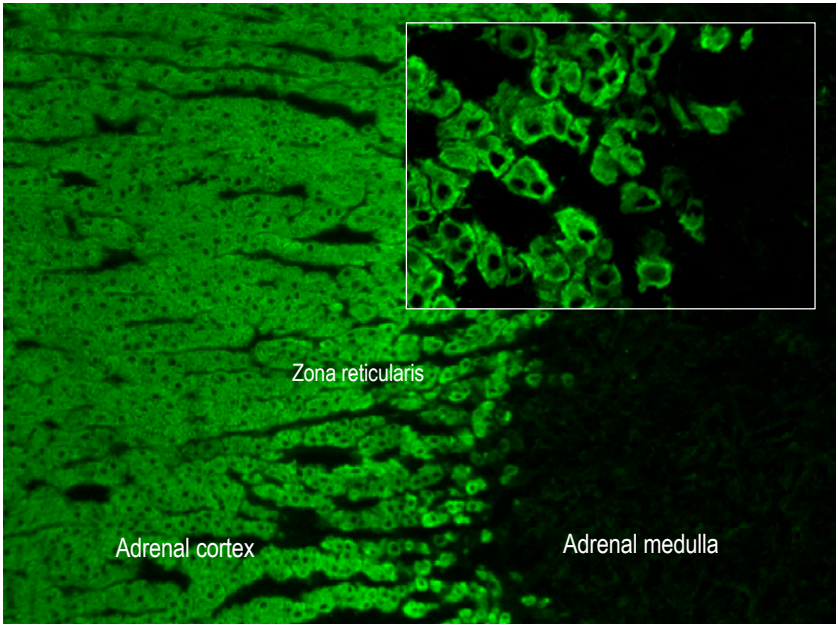



**Figure 1.** Indirect immunofluorescence using cryostat sections of human adrenal cortex. Adrenal cortex antibodies show a strong cytoplasmic staining pattern in the hormone producing cells of the adrenal cortex. The cells of the adrenal medulla are negative.

## Clinical relevance

- ACA are markers of autoimmune ➢adrenalitis (idiopathic Addison's disease). Autoimmune adrenalitis can manifest as a solitary disease or as part of an ➢autoimmune polyglandular syndrome. In solitary idiopathic Addison, ACA are detectable in 65–81% of unselected cases and in about 90% of those with newly diagnosed disease with a specificity of 98–100% (Betterle et al., 2011). With autoimmune polyglandular syndrome these autoantibodies are found in 86–92% (type 1) and 89–100% (type 2), respectively.
- The prevalence of these antibodies may decline as disease progresses.
- ACA/21-hydroxylase antibodies have a predictive role, as they can precede disturbed adrenocortical function and disease manifestation (Betterle et al., 1997). Children in particular with autoantibodies against the adrenal cortex have an increased risk of being diagnosed with Addison's disease (on average after 2.7 years). It is recommended that children with an organ specific autoimmune disease (especially idiopathic hypoparathyroidism and ➢diabetes mellitus type 1) are tested annually for adrenal cortex antibodies. Progression to autoimmune disease in ACA/21-OH antibody positive subjects depends on additional risk factors and can take months to years. Early detection of metabolic decompensation may prevent morbidity and mortality (Baker et al., 2012).

**Indications**

1. Suspicion of Addison's disease.
2. Differentiation between Addison's disease from tuberculous adrenal insufficiency or necrosis of the adrenal glands (Waterhouse-Friderichsen syndrome).
3. Suspicion of autoimmune polyglandular syndrome (APS type 1 or type 2).
4. Evaluation of the risk of developing Addison's disease, particularly in children with organ specific autoimmune diseases.

## Adrenoceptor antibodies

Adrenoceptors are adrenergic receptors, that are responsible for adrenalin and noradrenalin mediated effects. Autoantibodies can exert an agonistic or antagonistic influence on the corresponding adrenoceptor, and therefore have a pathogenic effect. Pathologically relevant or potentially relevant autoantibodies can be directed against various adrenoceptors (reviewed in Wallukat & Schimke, 2014):

- Stimulating ➢**beta-1 adrenergic receptor antibodies** are a pathologically significant marker of idiopathic ➢dilative cardiomyopathy, as well as the cardiopathy of Chagas disease.
- Inhibitory autoantibodies against **beta-2 adrenergic receptors** were found in patients with asthma, agonistic autoantibodies in myasthenia gravis, Chagas cardiomyopathy, open angle glaucoma, and Alzheimer's disease.
- **Beta-3 adrenoceptor antibodies** were found in patients with heart failure (Li et al., 2005).
- **Agonistic alpha-1 adrenoceptor antibodies** were first described in patients with malignant hypertension [Fu et al., 1994], but they are also present in patients with refractory hypertension, essential and pulmonary hypertension, as well as ➢postural tachycardia syndrome (POTS) and idiopathic orthostatic hypotension. They may be involved in the pathogenesis of hypertension due to their ability to influence the contractile state of the blood vessels (Luther et al., 1997).
- Autoantibodies against beta-2 and alpha-1a adrenergic receptors have been found in a subset of patients with ➢complex regional pain syndrome (CRPS) (Dubois et al., 2014; Hendrickson et al., 2016).

## Alpha enolase antibodies

**Typical designation:** Anti-alpha-enolase antibody (AENO).

### Autoantigen

The ubiquitously occurring glycolytic enzyme α-enolase. It has 82% homology with the two other isoforms β- and γ-enolase, as well as homology with the soluble lens protein tau (a crystalline).

### Detection methods

Enzyme immunoassay or immunoblot with purified α-enolase.

### Clinical relevance

Autoantibodies against α-enolase are not disease specific. They are found in infections, as well as a number of inflammatory and autoimmune diseases, such as systematic vasculitis, connective tissue diseases, inflammatory kidney diseases (primarily ➢membranous nephropathy), ➢endometriosis, ➢autoimmune hypophysitis, autoimmune liver disease, ➢chronic inflammatory bowel disease, ➢Hashimoto's encephalitis as well as paraneoplastic retinal degeneration (➢CAR syndrome, see also ➢retina antibodies). The induction of autoantibodies against the ubiquitously occurring α-enolase can follow microbial infections or hyperproliferative processes in specific organs under particular pathophysiological processes, which explains their presence in varying diseases. A pathological effect on endothelial cells through complement activation, inhibition of the binding of plasminogen with α-enolase and apoptosis induction is suspected.

AENO was detected in about 70% of patients with both primary and secondary ➢membranous nephropathy (MN), while ➢phospholipase A2 receptor antibody (PLA2R) was restricted to primary MN. The absence of glomerular deposition of alpha-enolase in subepithelial area suggests that AENO binding to glomerular cell surface might increase access to the podocyte of other antibodies such as anti-PLA2R, and thus be an enhancing event in primary and secondary MN. (Kimura et al., 2017)

### Indications

Currently none.

## Alveolar basement membrane antibodies

**Synonym:** Lung basement membrane antibodies

### Autoantigen

The target antigen has been identified as the C-terminal globular domain 1 (NC1) (previously known as the M2 region) of the α3 chain of collagen type IV, with a molecular weight of ~43 kDa. The α3 chain of collagen type IV is predominantly localized in the basement membranes of the kidneys and lungs, which explains the selective diseases of the kidneys and lungs.

### Detection methods

Indirect immunofluorescence using cryostat sections of primate lung.

### Clinical relevance

Alveolar basement membrane antibodies are detectable in 10–30% of patients with Goodpasture syndrome (see ➢glomerular basement membrane antibodies).

### Indications

Due to the significantly higher sensitivity of glomerular basement membrane antibodies, the measurement of alveolar basement membrane antibodies has no diagnostic value.

## AMA (anti-mitochondrial antibodies)

### Autoantigens

Varying, but mostly biochemically defined, proteins of the inner and outer mitochondrial membrane. On the basis of fluorescence intensity and distribution in rat kidney sections, 10 AMA subtypes have been differentiated (Storch 1982). In a second classification (by Berg and Klein, 1995), 9 subtypes were differentiated (see table 1), wherein AMA-1 to AMA-6 were consistent with the classifications described by Storch. The most practically relevant are those seen in ➢primary biliary cholangitis (PBC) associated AMA (see ➢AMA-M2, ➢AMA-M4, ➢AMA-M8, ➢AMA-M9).

**Detection methods**

- Indirect immunofluorescence on cryostat sections of rodent kidney, stomach and liver. The immunofluorescence picture is characterized by granular cytoplasmic staining of hepatocytes, distal and proximal tubules of the kidney, as well as parietal cells of the stomach (Fig. 2a-c).
- Specific immunoassays with recombinant or native proteins for the measurement of PBC-typical AMA (see➢AMA-M2, ➢AMA-M4, ➢AMA-M8, ➢AMA-M9).

**Clinical relevance**

- Using indirect immunofluorescence on cryostat sections of rodent organs, AMA are detectable which can be associated with various diseases due to their underlying specificity (see table 1). Most often however, ➢primary biliary cholangitis (primary biliary cirrhosis) specific ➢AMA-M2 are found. Therefore, indirect immunofluorescence on rat organ cryostat sections is still useful in the screening of suspected PBC patients, especially since this method can also detect autoantibodies relevant to the diagnosis of other autoimmune liver diseases (➢LKM, ➢LC-1 antibodies, ➢SMA).
- With the exception of AMA-M7, the non-PBC associated AMA have not yet acquired any clinical relevance.

**Indications**

1. Suspicion of primary biliary cholangitis.
2. Suspicion of an overlap syndrome between PBC and autoimmune hepatitis.

**Table 1.** Anti-mitochondrial antibody subtypes.

| AMA subtype | Autoantigen | Localization in the mito-chondrial membrane | Clinical association |
|---|---|---|---|
| M1 | Cardiolipin | Inner | Syphilis, APS |
| M2 | E2 components of the 2-oxoacid dehydrogenase family of enzyme complexes (2-OACD) including pyruvate dehydrogenase complex (PDC-E2), branched chain 2-oxoacid dehydrogenase complex (BCOADC-E2) and 2-oxo-glutarate dehydrogenase complex (OGDC-E2) | Inner | PBC |
| M3 | unknown | Outer | DIL |
| M4 | Associated with sulfite oxidase; E1 subunit of pyruvate dehydrogenase | Outer | PBC |
| M5 | unknown | Inner and outer | Connective tissue diseases, APS, AIHA |
| M6 | Monoaminoxidase B | Outer | Hepatitis (iproniazid induced) |
| M7 | Sarcosine dehydrogenase | Inner | Myocarditis, cardiomyopathies |
| M8 | unknown | Outer | PBC |
| M9 | Glycogen phosphorylase | Outer | PBC |

Abbreviations: APS: anti-phospholipid syndrome, AIHA: autoimmune hemolytic anemia, PBC: primary biliary cholangitis, DIL: drug-induced lupus erythematosus

## AMA-M2

**A**nti**m**itochondrial **a**ntibodies of subtype **M2**.

### Autoantigens

AMA-M2 are directed against proteins of the E2 components of the 2-oxoacid dehydrogenase family of enzyme complexes (2-OACD). The central target antigens of these complexes are:

- Pyruvate dehydrogenase complex (PDC-E2, PDH-E2)
- Branched chain 2-oxoacid dehydrogenase complex (BCOADC-E2), sometimes known as branched chain keto acid dehydrogenase (BCKD)

- 2-oxoglutarate dehydrogenase complex (OGDC-E2, OADC-E2), also known as α-keto glutarate dehydrogenase (KGD
- Dihydrolipoamide dehydrogenase (E3)-binding protein (E3BP)
- E1α subunit of pyruvate dehydrogenase complex (PDC-E1α)

Each of these antigens is composed of three subunits (E1, E2, E3), with the immunodominant epitope of each being E2. Although the E3BP subunit is distinct from E2 subunits, it has common structure with the E2 antigens. The lipoic acid residue attached to the epitopes is essential for AMA binding. AMA can be directed against one or more antigens, but in PBC the main antigen is PDH-E2.

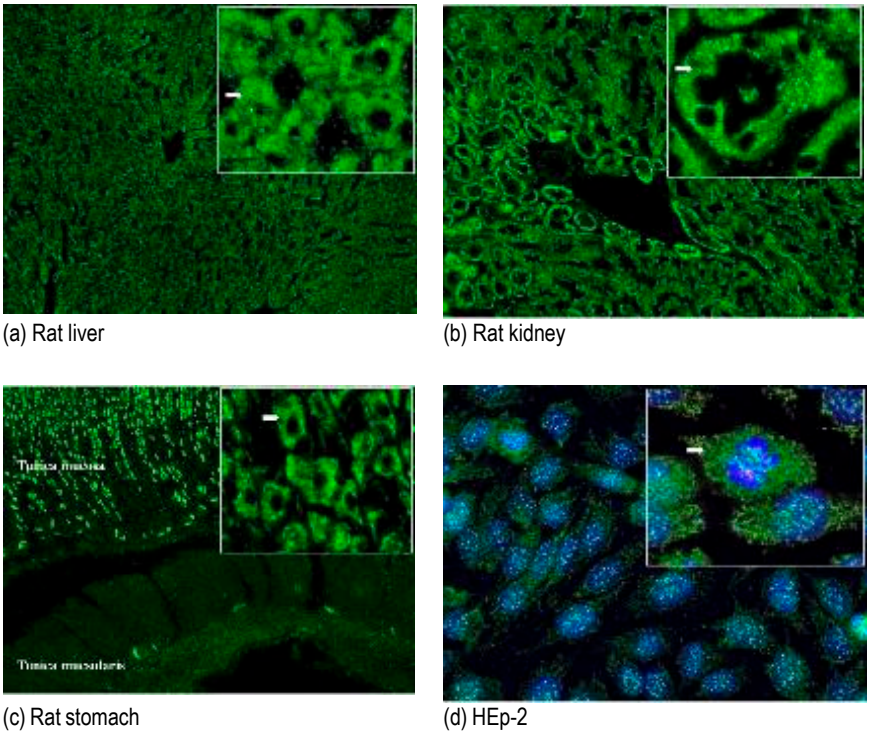
**Detection methods**

- Screening of AMA-M2 is usually performed by indirect immunofluorescence on cryostat sections of rodent kidney, stomach and liver. Typically, a granular cytoplasmic staining of the tubular epithelial cells (kidney), parietal cells (stomach) and hepatocytes (liver) is seen. The distal tubules, loop of Henle and the parietal cells show significantly stronger staining than the proximal tubule cells and the hepatocytes (Fig. 2a–c).
- Indirect immunofluorescence on HEp-2 cells. The immunofluorescence picture displays a coarse granular, finely dotted network of cytoplasmic staining (Fig. 2d).

  **Note:** The typical cytoplasmic fluorescence can be masked by other cytoplasmic antibodies.

- Enzyme immunoassays employing native PDH. This is the most widely used test, however it does not detect all PBC-specific AMA-M2.
- Enzyme immunoassay with recombinant proteins PDC-E2, BCOADC-E2 and OGDC-E2. This assay has a 12% higher sensitivity than previously used EIAs utilizing PDH (Norman et al., 2006).
- Particle based immunoassay with the recombinant proteins PDC-E2, BCOADC-E2 and OGDC-E2.
- Line immunoassay with recombinant PDC-E2 or native PDH.
- Western blot with purified antigen fractions and complement binding reaction with purified antigen fractions are not used anymore.

**Note:** Incidental findings of mitochondria typical fluorescence during ANA screening on HEp-2 cells (see Fig. 2d) generally indicate the presence of AMA-M2. Since rheumatic symptoms may precede the PBC manifestation, in these cases the measurement of AMA-M2 can enable earlier diagnosis and treatment of PBC!




(a) Rat liver (b) Rat kidney

(c) Rat stomach (d) HEp-2

**Figure 2.** Immunofluorescence patterns of anti-mitochondrial antibodies (AMA) from patients with primary biliary cholangitis (PBC): granular fluorescence of hepatocytes (a), kidney tubules (b) and stomach parietal cells (c) on rat organ cryostat sections, as well as granular finely-filamentous staining of cytoplasm on HEp-2 cells (d); HEp-2 cells centromeres are also stained by this serum because of additional expression of anti-centromere antibodies (chromatin is counterstained with DAPI).

### Clinical relevance

- AMA-M2 are marker antibodies of ➢**primary biliary cholangitis** (PBC) and are detectable in nearly 95% of cases. They count towards the three diagnostic criteria for PBC.
- Although they are highly specific for PBC, AMA-M2 can also be detected in patients with chronic inflammatory rheumatic diseases. It is believed that these patients are at an increased risk of developing PBC in addition to the underlying disease. Particularly in AMA-M2 positive CREST variant of ➢systemic sclerosis there is an increased risk of PBC

development (Fregeau et al., 1988; Zurgil et al., 1992). In patients with ➢SLE, the presence of AMA-M2 is significantly associated with increased ➢aminotransferase (Li et al., 2 006).

- AMA-M2 are detectable in 3–6% of autoimmune hepatitis (AIH) type 1 patients. These are most often cases of an ➢AIH/PBC overlap syndrome. AIH/PBC overlap should be considered when the ALP to aminotransferase ratio is less than 1.5, IgG is elevated and the ➢SMA are present with a titer greater than 1:80 (Bowlus & Gershwin, 2014).
- AMA can be predictive. They can appear years before manifestations of PBC. Individuals with persistently high AMA-M2 antibody levels have a higher risk of developing PBC. Prospective studies have shown that 76% of asymptomatic AMA-M2 positive patients over a period of observation from 11–24 years are diagnosed with PBC (Metcalf et al., 1996). The prevalence of AMAs in the first-degree relatives of PBC patients is high (13.1%) (Nakamura et al., 2014).
- AMA titers do not change over time and are not associated with disease severity or progression (Benson et al., 2004). On the other hand some groups have been shown that the AMA titer decrease with the treatment with UDCA (see ➢PBC) (Nakamura et al., 2014).
- AMA-M2 persist following liver transplantation.

**Indications**

1. Suspicion of primary biliary cholangitis (PBC).
2. Suspicion of an overlap syndrome between PBC and autoimmune hepatitis.
3. Patients with ➢systemic sclerosis (scleroderma), as they have an increased risk of developing PBC (Norman et al., 2009).

## AMA-M4

**A**nti-**m**itochondrial **a**ntibodies of subtype **M4**.

### Autoantigen

The autoantigen of AMA-M4 has been not clearly identified up to now. Both, the association with sulfite oxidase (however not identical to this enzyme) as well as the E1- and E2-subunit of the pyruvate hydrogenase (PDH) was described as autoantigen (Klein et al., 1991; Brunn et al., 1995; Berg et al., 2006).

### Detection methods

- Enzyme immunoassay with native antigens.
- Complement binding reaction with purified antigen fractions (rarely used method).

**Note:** AMA-M4 is not detectable by Western blot or indirect immunofluorescence. Methods utilizing a recombinant sulfite oxidase basis are not specific for AMA-M4.

### Clinical relevance

- AMA-M4 are found in ~50% of patients with ➢**primary biliary cholangitis** (PBC). However, they always occur together with ➢AMA-M2, so their diagnostic value is limited.
- The role of AMA-M4 as unfavorable prognostic autoantibodies is controversial in the literature. It has been shown that 97% of patients with ➢AMA-M4 and/or AMA-M8 and/or AMA-M2 have progressive PBC (Klein et al., 1991). These results could not be confirmed by other groups.
- The sulfite oxidase antibodies detectable in patients with primary sclerosing cholangitis (PSC) and other diseases do not correspond with the AMA-M4 in PBC (Preuß et al., 2007)!

### Indications

Due to the co-existence with AMA-M2, and the inconsistent results regarding prognostic relevance, there is currently no indication for AMA-M4 determination.

## AMA-M8

**A**nti-**m**itochondrial **a**ntibodies of subtype **M8**.

### Autoantigen

Antigens of the outer mitochondrial membrane. The biochemical identity is so far undetermined.

### Detection methods

- Enzyme immunoassay with native antigens.
- Complement binding reaction with purified antigen fractions (rarely used method).

**Note:** AMA-M8 is not detectable by Western blot or indirect immunofluorescence.

### Clinical relevance

- AMA-M8 are found in ~50% of patients with ➢primary biliary cholangitis (PBC). However, they always occur together with ➢AMA-M2, so their diagnostic value is limited.
- The role of AMA-M8 as unfavorable prognostic autoantibodies is controversial in the literature. It has been shown that 97% of patients with ➢AMA-M4 and/or AMA-M8 and/or AMA-M2 have progressive PBC (Klein 1991). These results could not be confirmed by other groups.

### Indications

Due to the co-existence with AMA-M2, and the contradictory results regarding prognostic relevance, there is currently no indication for AMA-M8 determination.

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## AMA-M9

**A**nti-**m**itochondrial **a**ntibodies of subtype **M9**.

### Autoantigen

Glycogen phosphorylase, with a molecular weight of 98 kDa, has been identified as the autoantigen of AMA-M9 antibodies.

### Detection methods

- Enzyme immunoassay with purified protein.
- Western blot with purified M9 fractions from rat liver mitochondria.

### Clinical relevance

- AMA-M9 are detectable in ~37% of patients with an ➢AMA-M2 positive primary ➢**biliary cholangitis** (PBC), as well as in 82% with an AMA-M2 negative PBC. However, the AMA-M9 present in most AMA-M2 negative patients, and 33% of AMA-M2 positive patients, are of the IgM type (Klein 1988).
- AMA-M9 appear to signal a good prognosis in PBC (Klein et al., 1991), although these findings could not be validated by other groups.
- Similarly, the discussion of the relevance of AMA-M9 as an early marker for PBC is controversial in the literature.

### Indications

None at present, due to the controversial results.

## AMPA receptor antibodies

**Synonyms:** Anti-AMPAR antibodies, anti-iGluR antibodies.

### Autoantigens

The AMPA (**a**lpha-amino-3-hydroxy-5-**m**ethyl-4-isoxazole**p**ropionic **a**cid) receptors belong to the family of ligand-gated cation channels, and generally form heteromere-tetrameric receptor structures, from combinations of the subunits of ionotropic glutamate receptor iGluR1, iGluR2, iGluR3 and iGluR4. The most important target structures for autoantibodies are believed to be the iGluR1 and iGluR2 subunits.

### Detection methods

The measurement of AMPA receptor antibodies is performed through indirect immunofluorescence on transfected HEK cells, or radioimmunoprecipitation using *in vitro* transcribed and translated 35S-methionine labeled human receptor protein (iGluR1, iGluR2, iGluR3).

**Note:** In rare cases anti-iGluR autoantibodies were only found in cerebrospinal fluid.

### Clinical relevance

- **AMPAR1 (iGluR1) antibodies** have been found in patients with ➢paraneoplastic cerebellar degeneration (Gahring et al., 1995) and ➢limbic encephalitis, in part in association with malignant tumors (Lai et al., 2009). Furthermore, using synthetic peptide fragments of iGluR1, autoantibodies were found in patients with epileptic syndromes and children with posttraumatic headache after contusion cerebri (Dambinova et al., 1997; Goryunova et al., 2007).
- **AMPAR2 (iGluR2) antibodies** have been observed in association with AMPAR1 receptors (Gahring et al., 1995; Lai et al., 2009; Bataller et al., 2010). Anti-iGluR1/2 antibodies may alter the synaptic localization and number of AMPAR in patients with limbic encephalitis (Lai et al., 2009). Two recent studies describing 29 anti-iGlu1/2 antibody positive patients showed that the **anti-AMPAR encephalitis** usually manifests as **limbic encephalitis**, but can present with other symptoms or psychosis, and is paraneoplastic in 48-64% of cases (Höftberger et al., 2015; Joberts et al., 2015).
- **AMPAR3 (iGluR3) antibodies** were first described in children with ➢Rasmussen's encephalitis (RE) (Rogers et al., 1994). This could not be confirmed by other studies, but