Foreword

Autoimmune diseases, after heart and circulatory disorders and cancer, are the most common diseases in industrialized nations. Their prevalence, which is in addition increasing, is around 5 %. This means that in Germany alone more than four million people suffer from an autoimmune disease. Therefore, due to the high costs of treatment, hospital stays and early retirement, autoimmune diseases have both medical and economic importance.

Autoimmune processes can affect practically all organs and systems, meaning they concern almost all medical disciplines. In the last years it has been clearly demonstrated in some autoimmune diseases that early and targeted treatment improves disease management and avoids late effects. As a result, long-term healthcare costs can be reduced and, not least, patient's quality and length of life can be increased.

With the reference book 'Autoantibodies in Systemic Autoimmune Disease — a Diagnostic Guide'; the authors had initially faced the field of autoantibody diagnostics in systemic autoimmune disease. Due to the positive response to this book, within a few years the third German, second English and the first Spanish edition were published. Thanks to the suggestions of many readers and colleagues, it is now the time to present the sequel covering autoantibodies in organ specific autoimmune disease.

As the boundaries between systemic and organ specific autoimmune diseases are not always clearly defined, the guide to systemic autoimmune disease is referenced in respective places.

The following work, as the previous diagnostic guide, is addressed to all physicians and scientists who are confronted with autoimmune disease in their daily work. It is dedicated to both the autoantibodies occurring in autoimmune disease, as well as the diseases themselves and their symptoms. In addition to autoantibodies currently more or less well known as diagnostic markers, some which are no longer diagnostically relevant are included on historical grounds. Numerous newly described autoantibodies with proven or potential diagnostic and/or pathogenic relevance, which are so far not available for routine diagnostic use, are also

included so that this work is also of interest to scientists working in the field of autoimmunity.

For the critical proofreading of this document and the many useful tips and additions, the authors sincerely thank: Samantha Goddard, Reinhild Klein (Eduard-Karls University Tübingen, Germany), Michael Kirschfink (Ruprecht-Karls University Heidelberg, Germany), Matthias Schott (University Düsseldorf, Germany), Klaus-Peter Wandinger (Euroimmun AG Medical Laboratory Diagnostics, Lübeck, Germany), Silke Zwjatkow (GFID e.V., Dresden, Germany) and Klaus Zöphel (Technical University Dresden, Germany).

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Notes on the use of this book

This guide to the serological diagnosis of organ specific autoimmune diseases consists of two alphabetically organized sections. The first deals with autoantibodies, the second with autoimmune or potential autoimmune diseases as well as symptoms related to particular organ specific autoimmune diseases. Appropriate cross-references (marked with ➢) allow quick and easy navigation (from symptom to disease, from disease to relevant autoantibodies). The dark arrow (➤) refers you to the relevant chapter in the book 'Autoantibodies in Systemic Autoimmune Disease — a Diagnostic Guide'.

Due to the diversity of the subject matter, the authors decided against including comprehensive literature references. Only in some cases the first author of important historical or current publications is mentioned. Original and review articles, as well as results and experiences used as sources for this guide, can be requested from the authors.

To allow for better orientation, the 'anti-' prefix in the alphabetical listing of autoantibodies is omitted. When relevant, alternative names and synonyms are also given. In cases where these are still in use, the description favored by the authors is given in the alphabetical listing and cross-reference.

Introduction

Autoantibodies – Definitions and Characteristics

Autoantibodies, in terms of their specificity, induction, effectiveness and clinical relevance, are a very heterogenic group of immunoglobulins:

- Autoantibodies are directed against self antigens (autoantigens). Autoantibody targets can be proteins (e. g. intracellular enzymes, receptors, structural proteins), glycoproteins (e. g. β2 glycoprotein I), nucleic acids (e. g. dsDNA, tRNA), nucleic acid protein complexes (e. g. nucleosomes), phospholipids (e. g. cardiolipin) or glycolipids (glycosphingolipids, e. g. gangliosides).
- Autoantibodies are detectable in serum, and in some cases in other body fluids (e. g. synovial or cerebrospinal fluid) too. Depending on the recognized target structure, they can also be bound in tissue (e. g. autoantibodies in autoimmune blistering diseases).
- Autoantibodies can be induced through specific antigen contact (non-natural or pathogenic autoantibodies), or be present in the natural repertoire without such induction (natural autoantibodies). Whereas the natural antibodies rather have a physiological role (e. g. a first defense against infection, immune regulation), non-natural autoantibodies can have a pathological effect (e. g. blocking or stimulating of receptors).
- Regardless of whether a pathogenic effect occurs or not, a large number of non-natural autoantibodies have great diagnostic relevance. They may display significantly higher prevalence and titers in disease groups when compared to local age and sex matched control groups. The titer of pathogenic autoantibodies often correlates with disease activity.

In organ-specific autoimmune diseases, diagnostically relevant autoantibodies are predominantly directed against autoantigens of the organ addressed (e. g. TSH-receptor autoantibodies in Graves' disease). Clinically significant autoantibodies in

organ-specific autoimmune diseases are almost always of IgG type, and rarely IgA (e. g. in autoimmune intestinal disease) or IgM (e. g. in autoimmune hemolytic anemia).

Autoantibodies in the Diagnosis of Autoimmune Diseases

About 5 % of the population of industrialized nations suffer from autoimmune diseases. The most commonly occurring organ specific autoimmune diseases are the group of autoimmune thyroid diseases, followed by gluten-sensitive enteropathy (celiac disease) and autoimmune liver diseases. The prevalence of most other organ specific autoimmune diseases is comparatively lower. As autoimmune processes can involve practically all organs, the presence of organ specific autoimmune diseases should be considered in all cases of idiopathic inflammation or dysfunction, of any type and location. The measurement of autoantibodies often points the way ahead in the diagnosis of such diseases.

In the majority of the up to now described autoimmune entities, autoantibodies with high disease-specificity are detectable. This is true even for those diseases that are predominantly cellular (autoreactive T-lymphocytes) mediated (e. g. diabetes mellitus type 1). The number of newly described autoantibodies with proven or potential diagnostic and/or pathogenic relevance in organ specific autoimmune disease is ever increasing. Considerable progress has also been made in the development and optimization of autoantibody detection methods. Therefore, autoantibody measurement is increasingly in demand in the routine diagnostic laboratory. In particular, the detection and differential diagnosis of neurological diseases has profited significantly from the insights and developments in this area of recent years. For example, neuromyelitis optica can be distinguished as a separate entity from multiple sclerosis due to the discovery of aquaporin 4 antibodies. Also, more and more diseases previously considered to be idiopathic can now be classified as having autoimmune etiology, following the discovery of new disease specific autoantibodies. In turn, it is not uncommon that particular diseases are found to have a much wider clinical spectrum (e. g. celiac disease, ganglioside and GAD associated diseases) or to occur much more frequently (e. g. autoimmune encephalopathies) than previously thought.

Part 1

Autoantibodies in organ specific autoimmune 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, 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 2009), and also in ➢ Rasmussen's encephalitis (Watson 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 antibodies**) are detectable in patients with ➢ Sjögren's syndrome, ➢ systemic sclerosis and gastrointestinal dysmotility (Singh 2009).

- Autoantibodies against the muscarinic type 2 AChR (**M2mAChR antibodies**) have been described in ➢ Chagas disease, in idiopathic ➢ dilated cardiomyopathy (in 40 % of cases) and in idiopathic atrial fibrillation (23 %) (Baba 2004; Fu 2002).

Acetylcholine receptor antibodies, classical type

Synonym: 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 subunits: α1, β, δ, γ/ε. Autoantibodies are predominantly directed against the α1 subunit.

Pathologic relevance

AChR antibodies influence nicotinic acetylcholine receptors in three ways: (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.

Detection methods

Radioimmunoprecipitation with ^{125}I-α-bungarotoxin labeled native acetylcholine receptor.

Note: Other methods, such as enzyme immunoassays or indirect immunofluorescence, are not currently popular due to their low sensitivity and specificity. An immunofluorescence test utilizing transfected AChR cells is under development.

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 only ~50 % of ocular MG cases. 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).
- 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.
- Low titers of AChR antibodies can be found, for example in ➤ rheumatoid arthritis patients taking penicillamine therapy, ➢ primary biliary cirrhosis and thymoma. These patients have an elevated risk of suffering from MG.
- 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.

Indications

1. Suspicion of myasthenia gravis.
2. Monitoring of myasthenia gravis patients.

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 antigen in the diagnosis of autoimmune hepatitis.

Detection methods

- Indirect immunofluorescence on cryostat sections of rat 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 multi-organ rodent sections (kidney, stomach, liver) is the method of choice for the detection of AIH relevant autoantibodies (Vergani 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 (ELISA, line/dot immunoassays) for the detection of actin antibodies have mostly displayed specificities too low to serve as a diagnostic of AIH.

Clinical relevance

- High concentrations (titers) of actin antibodies are largely specific for ➢ autoimmune hepatitis (AIH) type 1. The sensitivity ranges from 52–85 %, although (usually low titers of) actin antibodies have also been observed in healthy individuals (3–18 %), primary biliary cirrhosis (PBC) (22 %), hepatitis C (7 %), connective tissue diseases and celiac disease (IgA antibodies).
- In about 19 % of ➢ SMA positive patients, no actin antibodies are detectable.
- 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. These antibodies can inhibit the protease function of ADAMTS13 (Furlan 1998; Tsai 1998), or induce the elimination of ADAMTS13 from the circulation (Scheiflinger 2003; Rieger 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.

Adrenal cortex antibodies

Synonyms: ACA, 21-hydroxylase antibodies.

Autoantigen

21-hydroxylase (21-OH), an enzyme with a molecular weight of 55 kDa involved in the synthesis of steroid hormones (cortisol), has been identified as the main target antigen. The conformation-dependent epitope is localized in the C terminal region of hemoproteins.

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.

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

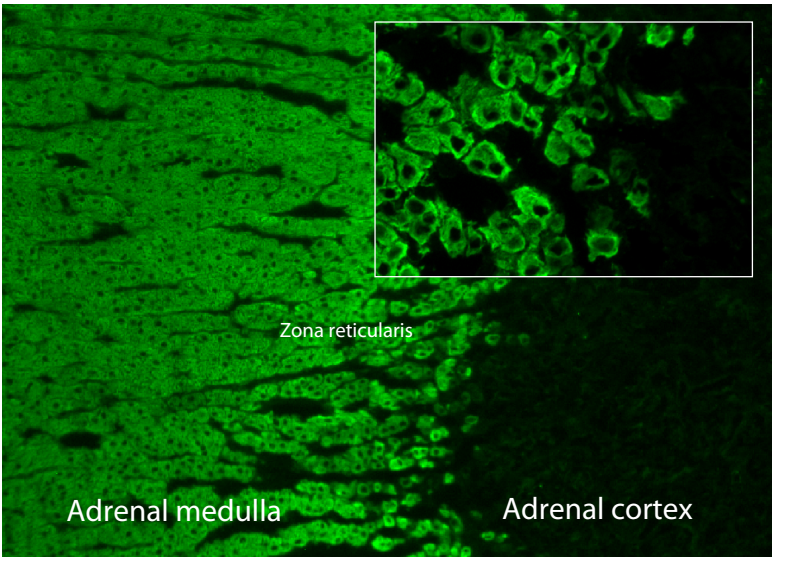

Figure 1. Indirect immunofluorescence using cryostat sections of human adrenal cortex. Adrenal cortex antibodies show a strong cytoplasmic staining pattern in the hormone producing cells of the adrenal cortex. The cells of the adrenal medulla are negative.

➢ autoimmune polyglandular syndrome. In solitary diseases, ACA are detectable in 65–81 % of cases with a specificity of 98–100 %. 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. 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.

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:

- Stimulating ➢ beta-1 adrenergic receptor antibodies are a pathologically significant marker of idiopathic ➢ dilative cardiomyopathy, as well as the cardiopathy of Chagas disease.
- Autoantibodies against beta-2 adrenergic receptors were found in patients with asthma, myasthenia gravis and Chagas disease (Eng 1992; Wallukat 1991).
- Beta-3 adrenoceptor antibodies were found in patients with heart failure (Li 2005).
- Agonistic alpha-1 adrenoceptor antibodies have been described as potential pathogenic factors in refractory hypertension (Wenzel 2008).

Alpha enolase antibodies

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 infection, 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.

Indications

Currently none.

Alveolar basement membrane antibodies

Synonym: Antibodies against lung basement membranes.

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)

Autoantigen

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 can be 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 cirrhosis (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 rat organs AMA are detectable which can be associated with various diseases due to their underlying

Table 1. Anti-mitochondrial antibody subtypes.

AMA subtype	Autoantigen	Localization in the mitochondrial membrane	Clinical association
M1	Cardiolipin	Inner	Syphilis, APS
M2	α-keto acid dehydrogenase complex	Inner	PBC
M3	?	Outer	DIL
M4	Associated with sulfite oxidase; E1 subunit of pyruvate dehydrogenase	Outer	PBC
M5	?	Inner and outer	Connective tissue diseases, APS, AIHA
M6	Monoaminoxidase B	Outer	Hepatitis (iproniazid induced)
M7	Sarcosine dehydrogenase	Inner	Myocarditis, cardiomyopathies
M8	?	Outer	PBC
M9	Glycogen phosphorylase	Outer	PBC

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

specificity (see Table 1). Most often however, ➢ 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 cirrhosis.
2. Suspicion of an overlap syndrome between PBC and autoimmune hepatitis.

AMA-M2

Anti**m**itochondrial **a**ntibodies of subtype M2.

Autoantigen

AMA-M2 are directed against proteins of the α-keto acid dehydrogenase complexes. The central target antigens of these complexes are:

- Pyruvate dehydrogenase (PDH),
- Branched chain keto acid dehydrogenase (BCKD), sometimes known as branched chain oxoacid dehydrogenase complex (BCOADC),
- α-keto glutarate dehydrogenase (KGD), also known as 2-oxoacidglutarate dehydrogenase complex (OADC, OGDC).

Each of these antigens is composed of three subunits (E1, E2, E3), with the immunodominant epitope of each being E2. The main antigen of PBC is PDH-E2. The autoantigens of AMA-M2 are summarized in Table 2.

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 (Figs. 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.
- Enzyme immunoassay with recombinant proteins PDH-E2, BCOADC-E2 and OGDC-E2. This Elisa has a 12 % higher sensitivity than previously used Elisas utilizing PDH (Norman 2006).

Table 2. Autoantigens of AMA-M2 and their respective classification on the M2 complex (according to Gershwin 1991 and Strassburg 2004).

	MW (kDa)	Prevalence	M Classification
PDH			
• PDH-E2 (dihydrolipoamide S-acetyl transferase)	74	95 %	M2a
• PDH-E1α (pyruvate dehydrogenase E1α)	41	41–66 %	M2d
• PDH-E1β (pyruvate dehydrogenase E1β)	36	2–7 %	M2e
• E3-binding protein (protein X, dihydrolipoamide dehydrogenase binding protein)	55	95 %	M2b
BCKD			
• BCKD-E2 (acyltransferase)	55	53–55 %	M2c
• BCKD-E1α (2-oxoisovalerate dehydrogenase E1α)	46	?	
• BCKD-E2β (2-oxoisovalerate dehydrogenase E2β)	38	?	
KGD			
• KGD-E2 (succinyl transferase)	48	39–88 %	M2c
• KGD-E1 (ketoglutarate decarboxylase)	113	Low	M2c
• KGD-E3 (lipoamide dehydrogenase)	55	38 %	

- Particle based immunoassay with the recombinant proteins PDH-E2, BCOADC-E2 and OGDC-E2.
- Line immunoassay with recombinant or native PDH.
- Western blot with purified antigen fractions (rarely used and not recommended for routine diagnostics).
- Complement binding reaction with purified antigen fractions (rarely used).

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!

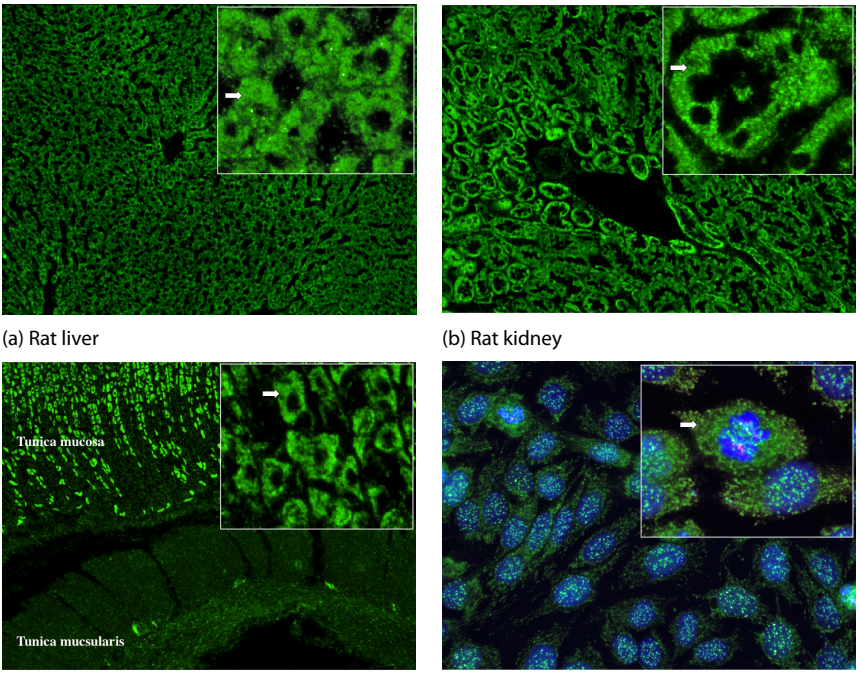

Figure 2. Immunofluorescence patterns of anti-mitochondrial antibodies (AMA) from patients with primary biliary cirrhosis (PBC): granular fluorescence of hepatocytes (a), kidney tubules (b) and stomach parietal cells (c) on rat organ cryostat sections, as well as granular finely-filamentous staining 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 and are detectable in 90–95 % 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 1988; Zurgil 1992). In patients with SLE, the presence of AMA-M2 is significantly associated with increased ➢ amino transferase (Li 2006).

- 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.
- 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 1996).
- AMA-M2 persist following liver transplantation.

Indications

1. Suspicion of primary biliary cirrhosis.
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 2009).

AMA-M4

Anti-**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 E1-subunit of the pyruvate hydrogenase (PDH) was described as autoantigen (Klein 1991; Brunn 1995; Berg 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 cirrhosis (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 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 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

Anti-**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 cirrhosis (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.

AMA-M9

Anti-**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 cirrhosis (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 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. Other studies are required to evaluate the clinical relevance of these autoantibodies.

AMPA receptor antibodies

AMPAR antibodies. Autoantibodies against **a**lpha-amino-3-hydroxy-5-**m**ethyl-4-isoxazole**p**ropionic **a**cid (AMPA) receptors, belonging to the group of glutamate receptor antibodies.

The ionotropic (i) AMPA receptors belong to the family of ligand-gated cation channels, and generally form heteromere-tetrameric receptor structures, from combinations of the subunits iGluR1, iGluR2, iGluR3 and iGluR4. Presently, the most important target structures for autoantibodies are believed to be the iGluR1 and iGluR2 subunits. The measurement of AMPA receptor antibodies is performed through indirect immunofluorescence on transfected HEK cells, or radioimmunoprecipitation using *in vitro* transcribed and translated ^{35}S-methionine labeled human receptor protein (iGluR1, iGluR2, iGluR3). AMPA receptor antibodies are mainly found in ➢ limbic encephalitis (GluR1/2 antibodies). Their relevance in the diagnostic of ➢ Rasmussen's encephalitis (GluR3 antibodies) is controversial.

Amphiphysin antibodies

Amphiphysin (128 kDa), a dimeric protein, occurs in high concentrations in the synaptic vesicles of nerve cells. It has two isoforms, and is involved in clathrin-mediated endocytosis. In addition to nerve tissue, they are expressed in small amounts in endocrine cells, mammary glands, spermatozoa and retina.

Detection methods

- Indirect immunofluorescence (IIF) on cryostat sections of primate cerebellum. Amphiphysin antibodies display fluorescence of the presynaptic nerve endings of the cerebellum. The axons of the stratum moleculare are more intensely stained than the stratum granulosum (Fig. 3).
- Western blot (WB) with cerebellar extracts.

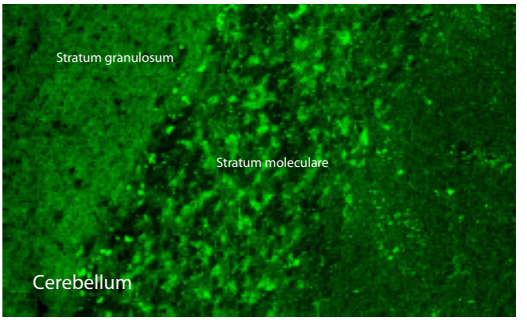

Figure 3. Indirect immunofluorescence on cryostat sections of primate cerebellum. Amphiphysin antibodies show fluorescence of the presynaptic nerve endings of the cerebellum. The axons of the stratum moleculare are more intensively stained than the stratum granulosum. Immunofluorescence courtesy of Klaus-Peter Wandinger, Euroimmun AG Medical Laboratory Diagnostics.

- Line immunoassay (LIA) with native or recombinant amphiphysin.

Note: A positive IIF result is indicative, but not conclusive, evidence for the presence of amphiphysin antibodies, and should therefore **always** be repeated with a specific detection method (i. e. LIA; see ONA antibodies). Amphiphysin antibodies are usually present in high titers, and are almost always of the IgG type.

Clinical relevance

- Amphiphysin antibodies are diagnostic markers for ➢ paraneoplastic neurological diseases. They are, however, not specific for a particular paraneoplastic disease, or an underlying malignancy.
- The neurological symptoms of amphiphysin antibody positive patients can be quite different. The clinical spectrum ranges from ➢ stiff person syndrome, to ➢ paraneoplastic encephalomyelitis, to sensory and sensorimotor neuronopathy (see ➢ subacute sensory neuronopathy). Amphiphysin antibody positive paraneoplastic syndromes are most commonly associated with mammary and small cell lung carcinoma.
- Amphiphysin antibodies are markers of paraneoplastic ➢ stiff person syndrome (SPS), whereas ➢ GAD antibodies suggest an idiopathic cause of SPS.
- Amphiphysin antibodies are rarely found in patients without a detectable tumor (5 %).
- In patients positive for amphiphysin antibodies, other antibodies can be found in addition in 31–74 % of cases (e. g. ➢ CV2/CRMP5 antibodies in 19 %, ➢ Hu antibodies in 8 %, ➢ Ri antibodies in 4 %).

- Due to the high specificity of amphiphysin antibodies for paraneoplastic disease, rapid and intensive tumor screening is essential, in particular for mammary and small cell lung carcinoma. In cases where this screening is unsuccessful, close monitoring is recommended as tumor presence can not be excluded.

Indications

1. Suspicion of a paraneoplastic syndrome.
2. Patients with a neuropathy of unclear etiology, particularly sensory and sensorimotor neuropathy.
3. Differential diagnosis of stiff person syndrome.

Angiotensin 1 receptor antibodies

Agonistic autoantibodies against the **a**ngiotensin II **t**ype **1** **r**eceptor (AT1R) are detectable in ~70 % of patients with preeclampsia. They appear to be responsible for hypertension, expression of tissue factor and intrauterine growth retardation in patients with ➢ preeclampsia (Wallukat 1999; Dechend 2000; Irani 2009). AT1R antibodies have also been found in some patients with secondary malignant hypertension (Fu 2000) and kidney transplant recipients with graft rejection (Dragun *et al.,* 2005). Recently, probably pathogenically relevant AT1R antibodies were found using a novel developed sandwich Elisa in the majority of patients with ➢ systemic sclerosis (Riemekasten 2011). Although AT1R antibodies seem to have a pathogenic relevance in these diseases, their diagnostic determination is currently not indicated due to the lack of evaluation studies and detection methods suitable for routine use.

ANNA-1

Anti-**n**euronal **n**uclear **a**ntibodies type 1. See ➢ Hu antibodies.

ANNA-2

Anti-**n**euronal **n**uclear **a**ntibodies type 2. See ➢ Ri antibodies.

ANNA-3

Anti-**n**euronal **n**uclear **a**ntibodies type 3 (paraneoplastic autoantibodies against neuronal cell nuclear antigens, type 3).

Autoantigen

A 170 kDa brain protein with unknown function has been identified as the possible autoantigen.

Detection methods

- Indirect immunofluorescence (IIF) with cryostat sections of primate cerebellum. ANNA-3 show a characteristic homogenous staining, particularly in the Purkinje cells, sparing the nucleoli, and weak fluorescence of the neurons. ANNA-3 antibodies do not react with the myenteric plexus, but demonstrate discrete staining of kidney glomeruli.
- Western blot (WB) with cerebellum antigens.

Note: High ANA titers (homogenous pattern) can resemble ANNA-3. Conversely, ANA and other autoantibodies can mask ANNA-3. ANNA-3 are usually present in high titers and are almost always of the IgG type. See ➢ ONA antibodies.

Clinical relevance

- ANNA-3 are rarely detected ➢ ONA antibodies, and are diagnostic markers of ➢ paraneoplastic neurological diseases. They are, however, not specific for particular paraneoplastic diseases.
- The neurological symptoms of ANNA-3 positive patients are varied, and are usually multifocal. The clinical spectrum ranges from ➢ sub-acute sensory neuropathy, ➢ paraneoplastic cerebellar degeneration (➢ cerebellar ataxia), myelopathy to ➢ limbic encephalitis.
- ANNA-3 are associated with small cell lung carcinoma and adenocarcinoma.
- ANNA-3 are occasionally found in patients without tumors (9 %).
- In ~30 % of ANNA-3 positive patients, additional ➢ ONA antibodies can be found (➢ CV2/CRMP5 antibodies in 20 %, Hu antibodies in 10 %).