Encyclopedia Autoimmune Diagnostics

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Antibodies against gliadin

W. STÖCKER

Synonym(s). Anti-gliadin antibodies; coeliac disease-associated anti-gliadin fragment antibodies (C-AGFA);

Definition. Antibodies against (tissue transglutaminase-modified) gliadin are closely associated with gluten-sensitive enteropathy (infants: coeliac disease, adults: endemic sprue) and dermatitis herpetiformis (DH, Duhring disease). They are generally investigated in parallel to the antibodies against tissue transglutaminase.
Gliadin is part of the gluten protein of several grain varieties (wheat, rye, barley). Gliadin refers to 50 different proteins of which the alpha gliadin is crucial for triggering gluten-sensitive enteropathy.

Function and pathophysiology. In patients with gluten-sensitive enteropathy, the consumption of gluten-containing wheat products damages the mucous membrane of the small intestine. This leads to villous atrophy and functional impairments. The clinical condition is characterised by diarrhoea and the consequences of malabsorption, e.g. weight loss, avitaminosis and, in children, growth retardation. DH also exists in some patients with gluten-sensitive enteropathy: a chronic skin disease associated with blistering.
The (non-invasive!) investigation of the antibodies against coeliac disease-associated gliadin fragments and endomysium (autoantibodies against tissue transglutaminase) makes an important contribution to diagnosing gluten-sensitive enteropathy and dermatitis herpetiformis (DH, Duhring disease). A functional relationship exists between both target antigens: Gliadin peptides released during digestion are the substrate of the tissue transglutaminase, which is responsible for the deamination (glutamine to glutamic acid).

Analytics. Antibodies against gliadin can be analysed by indirect immunofluorescence (immunofluorescence, indirect) (initial dilution 1:10, substrate: on slide spotted with antigen (7 Fig. 1) or smears of antigen-coated erythrocytes, according to Stern and Grüttner) or by ELISA (enzyme-linked immunosorbent assay and ChLIA (chemiluminescence immunoassay. However, the detection of antibodies against native whole gliadin with conventional tests is useless for diagnosing coeliac disease, as a quarter of the normal population displays a positive reaction (especially for IgG).
As a result, “designer antigens” are used to detect antibodies against gliadin, such as a recombinant “gliadin-like fusion peptide” (GAF-3X), which shows a positive reaction almost exclusively in patients with coeliac disease and DH, but not in healthy patients or patients with other gastrointestinal diseases.
The fusion peptide consists of 2 components: An artificial gliadin fragment-like nonapeptide, which has been selected from thousands of artificial variants with regard to its reactivity with coeliac sera, and a nonapeptide section of the gliadin digestion deaminated by transglutaminase, which is likely to have a pathophysiological relevance for coeliac disease and which accounts for no more than 2% of the total size of the gliadin. The remaining 98% of the gliadin molecule is not used in the ELISA – immunological ballast, which is predominantly just a target for non-specific reactions. This results in a huge increase in specificity. The construct is also expressed in trimeric form to increase sensitivity.

Indication. According to the guideline of the “European Society of Paediatric Gastroenterology and Nutrition” (ESPGHAN, 2012), the diagnosis of coeliac disease is based on the detection of the antibodies against deaminated gliadin fragments and endomysium/tissue transglutaminase as well as on a molecular genetic HLA diagnosis (HLA-DQ2/DQ8), the histological detection of enteropathy in a biopsy of the small intestine and the symptoms.
While class IgA antibodies against deaminated gliadin and tissue transglutaminase practically do not occur in healthy people and patients with other bowel diseases, their prevalence in untreated gluten-sensitive enteropathy as well as in DH amounts to almost 100%. In general, both antibodies occur simultaneously, but they are not completely correlated with one another.
Apart from the role of the C-AGFA in the primary diagnosis of a gluten-sensitive enteropathy, its detection is suitable for monitoring the progress of the disease, compliance with a gluten-free diet, or a gluten tolerance test.
The detection of antibodies against gliadin and against tissue transglutaminase backs-up the clinical diagnosis, but is also performed for relatives of patients with coeliac disease in order to identify any predisposition to the disease. If no IgA antibodies against gliadin or endomysium/tissue transglutaminase are detected in the serum in case of suspected gluten-sensitive enteropathy, this may indicate the possibility of an IgA deficiency and the total IgA should be determined. Selective IgA deficit occurs more frequently with gluten-sensitive enteropathy. In this case, the focus is on class IgG antibodies. These kinds of patients must be warned against transfusions.

Interpretation. During treatment with a gluten-free diet, the C-AGFA falls to low values within a few months. Persistently high antibody levels indicate that the gluten-free diet is not being complied with. When exposed to gluten, a relapse will result in a rise in the C-AGFA within a few days.

Diagnostic value. In a coeliac disease study by Prause (2009), an ELISA based on recombinant designer gliadin to detect the C-AGFA showed a sensitivity of 83% (IgA) and 95% (IgG), with a 95% specificity (conventional anti-gliadin ELISA: 54% for IgA and 31% for IgG). In a group of patients with DH, the test achieved a sensitivity of 83% (IgA) and 78% (IgG) and was therefore 28% more sensitive than a conventional anti-gliadin ELISA (sensitivity IgA: 55%, IgG: 50%).
Most class IgA and IgG antibodies can be detected during the acute phase of gluten-sensitive enteropathy. Of these antibodies, the class IgA antibodies have a significantly higher disease specificity. Class IgM antibodies do not play a diagnostic role.

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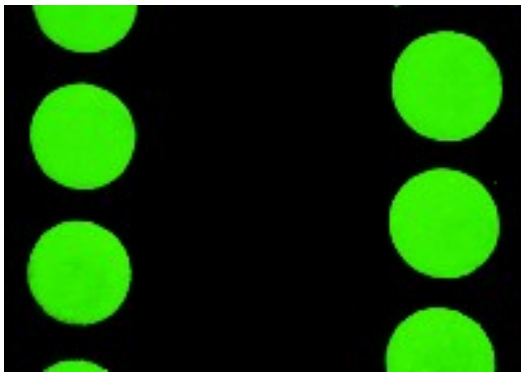

Antibodies against gliadin. Fig. 1. Substrate: deamidated gliadin antigen (GAF-3X)

Antibodies against Heparin/PF4

W. Stöcker, Chr. Krüger

Synonym(s). Anti-heparin/PF4; HIT antibodies; platelet factor 4; PF-4

Definition. Autoantibodies against heparin/platelet factor 4 (PF-4) are directed against multimolecular heparin PF4 complexes at the surface of thrombocytes.

Function and pathophysiology. Heparin-induced thrombocytopenia (HIT) is an undesirable side-effect of heparin therapy mediated by autoantibodies. The infusion of heparin leads to a rise in the concentration of PF4 in the blood with the formation of heparin PF4 complexes and the binding of these complexes to the surface of the thrombocytes. Neoepitopes are created, against which the autoantibodies are induced. These are able to activate thrombocytes via their Fc receptors, which leads to their aggregation, thrombosis in the venous and arterial system and to thrombocytopenia. As part of a disseminated intravascular coagulation, this can then also lead to the inhibition of coagulation with general bleeding.
The involvement of IgG-type heparin/PF4 antibodies in the disease incidence has been proven, while this is questionable for IgM and IgA.

Sample material/sampling conditions. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Autoantibodies against heparin/PF-4 are detected using an ELISA (enzyme-linked immunosorbent assay) or particle agglutination test (microparticle array).
In an ELISA, the target structure is a complex consisting of PF4 and polyvinyl sulfonate (PVS), which cross-react with the target autoantibodies.
Functional tests, in which the thrombocytes of healthy donors are exposed to the serum of patients, analyse the thrombocyte activation caused by the autoantibodies that may possibly be present in the serum. The most common tests are the platelet aggregation test (thrombocyte aggregation and activation), the serotonin release assay and the heparin-induced platelet activation assay (HIPA).
The functional tests are more specific than the ELISA, but are much less sensitive. As there is no “Gold Standard” for these autoantibodies, no specific information on the sensitivity and specificity of the test methods used for the diagnostics exists.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Heparin-induced thrombocytopenia (HIT)

Diagnostic value. The heparin exposure only leads to the formation of heparin-PF-4 antibodies in a subpopulation of 0.3-3% of treated patients. Thrombocytopenia or a relative decrease in the thrombocytes of more than 50% after 5 days of heparin therapy points to HIT and should be clarified using functional tests or heparin/PF-4 antibody detection. After an initial exposure as well as re-exposure to heparin, it takes 5-20 days until the antibodies are measurable.
The positive detection of antibodies does not prove the existence of clinically relevant HIT. Thrombocytopenia must also exist 5 days after a heparin dose and/or one of the other clinical manifestations.
However, a negative antibody test virtually rules out HIT, which means that this test has a high negative predictive value.
Thrombophilia exists as long as the heparin/PF4 antibodies can be detected in the patient plasma. After discontinuing treatment with heparin, the autoantibodies continue to circulate in the patient’s blood for about 2-3 months.

Literature.

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Antibodies against interferon β

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Synonym(s). Anti-IFN β antibodies; interferon β antibodies; autoantibodies against interferon β

Definition. IFN β is a glycoprotein consisting of 166 amino acids that form cells to defend against infections. Antibody formation can be induced by endogenous IFN β as well as IFN β administered as part of a treatment.

Analytics. Antibodies against interferon β are detected with an ELISA (enzyme-linked immunosorbent assay) and immunoblot techniques.

Indication. In some cases, anti-IFN β antibodies can be detected with SLE. Other autoimmune diseases with spontaneously occurring autoantibodies against IFN β are not known.
Antibodies against interferon may develop during continued treatment with interferon in patients with melanoma, autoimmune hepatitis, multiple sclerosis (MS) and other diseases, which reduce the success of the treatment. According to a Swedish study, neutralising antibodies against IFN β can be found in 5% of MS patients that are treated with IFN β 1a. By comparison, treatment with IFN β 1b leads to corresponding antibodies in 44% of all patients.

Literature.

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Antibodies against Saccharomyces cerevisiae

W. Stöcker

Synonym(s). Anti-*Saccharomyces cerevisiae* antibodies; anti-mannan antibodies; ASCA

Definition. Saccharomyces cerevisiae is also referred to as baker's or brewer's yeast and can also be used to produce wine, vinegar and ethanol. ASCA particularly react with a polysaccharide (mannan) in the cell wall of the yeast cells.

Function and pathophysiology. Antibodies against microorganisms in the intestinal flora are found more often in patients with Crohn's disease than in healthy persons. In 1988, Main et al. observed that antibodies against Saccharomyces cerevisiae often occur in patients with Crohn's disease. They are suitable for distinguishing between Chron's disease and colitis, but have no significance in relation to the development of the disease:
It is assumed that the pathogenetically relevant autoimmunity against a secretion component in the pancreas (autoantibodies against pancreatic secretion) in Crohn's disease is responsible for the triggering and continuation of the bowel inflammation and results in an adjuvant effect, strengthening patients' immunity against bacteria in the intestinal flora. There is also a higher number of antibodies against pectin, agar-agar and other polysaccharides, while mycobacteria and other pathogens have also been associated with the pathogenesis of Crohn's disease due to the associated higher antibody prevalences.

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. ASCA can be diagnosed by indirect immunofluorescence (immunofluorescence, indirect) on *Saccharomyces cerevisiae* smears (baker's or brewer's yeast), as well as in an ELISA (enzyme-linked immunosorbent assay) (on the solid phase: mannan isolated from *Saccharomyces cerevisiae*) (Fig. 1).
The initial dilution for immunofluorescence is 1:100 for IgA and 1:1,000 for IgG. The fluorescence of the yeast cells is assessed and compared with positive and negative controls.
In the case of positive sera, 31% of the ASCA consist of just IgA, 14% of just IgG and 55% of both immunoglobulin classes. IgM antibodies do not have any diagnostic value in autoimmune diseases in gastroenterology.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Discriminatory tests of chronic inflammatory bowel diseases (Crohn's disease, ulcerative colitis).

Interpretation. Antibodies against *Saccharomyces cerevisiae* occur almost exclusively with Crohn's disease, with a prevalence of 67%, if immunoglobulin classes IgA and IgG are combined. If autoantibodies against pancreatic secretion (prevalence of 39%) are also detected, 80% of patients with Crohn's diseases can be identified purely serologically, as both antibodies are not directly correlated with each other. Antibodies against *Saccharomyces cerevisiae* are also present in 25% of cases with coeliac disease.

Diagnostic value. Antibodies against *Saccharomyces cerevisiae* enhance the serological diagnosis of chronic inflammatory bowel diseases with an additional specific parameter, besides autoantibodies against the exocrine pancreas (autoantibodies against pancreatic secretion; specific to Crohn's disease), autoantibodies against intestinal goblet cells (pathognomonic for ulcerative colitis) as well as autoantibodies against granulocyte cytoplasm (pANCA).

Literature.

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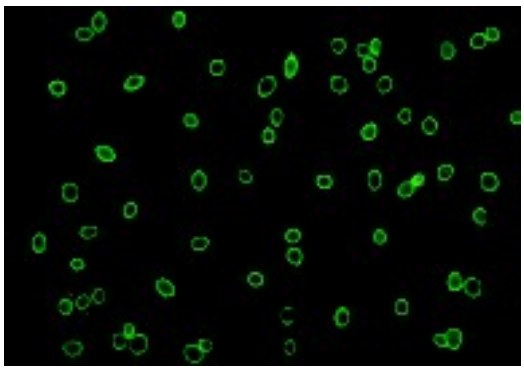


Antibodies against Saccharomyces cerevisiae. Fig. 1. Substrate: fungal smear

Antibodies against spermatozoa

W. Stöcker

Synonym(s). Antibodies against spermatozoa (in men); alloantibodies against spermatozoa (in women)

Definition. Sperm-inhibiting autoantibodies (in men) or alloantibodies (in women).

Function and pathophysiology. Autoantibodies against spermatozoa are occasionally detected in the case of infertility with an immunological genesis.
Autoantibodies against spermatozoa occur in about 10% of infertile men. These antibodies can initially appear in adolescents during puberty with, for example, urogenital diseases. The highest prevalence is recorded for people of reproductive age, while the detection rate of these autoantibodies increasingly falls with age. They also often occur after a vasectomy.

Sample material. Serum or plasma (in men or women), sperm, cervical mucus

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

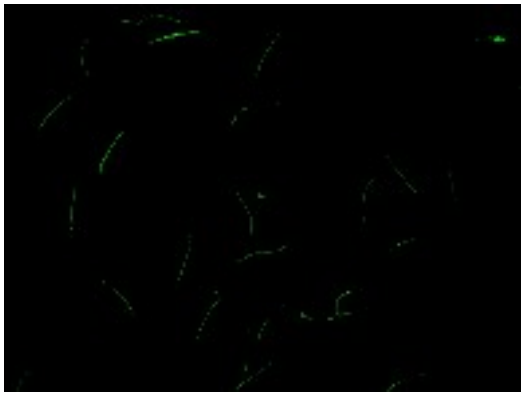
Analytics. Enzyme-linked immunosorbent assay, indirect immunofluorescence assay (immunofluorescence, indirect), mixed-antiglobulin-reaction test (MAR test; in which the sperm loading is detected with IgG or IgA via agglutination with IgA- or IgG-coated indicator particles) (Fig. 1).
Serum antibodies primarily belong to class IgG (predominantly IgG1 and IgG3), while locally produced surface antibodies in class IgA (IgA2) predominate in the sperm.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Suspected immunologically caused infertility.
An unfulfilled desire to have children is the most frequent indication for detecting antibodies against spermatozoa in men and women.

Interpretation. Immunofluorescence (positive reaction): Antibodies against spermatozoa antigens can bind to various structures of the spermatozoa. The fluorescence of the flagellum is generally analysed. Reactions on the head or middle section are also observed.
Many investigators link the fluorescence of the different spermatozoa structures to different clinical conditions.
Different ELISA methods are use spermatozoa immobilised on the solid phase. Analysis usually occurs in relation to reference sera.
The diagnostic significance of the finding “antibodies against spermatozoa positive” is extremely limited and should not be overrated.



Antibodies against spermatozoa. Fig. 1. Human spermatozoa substrate

Autoantibodies in autoimmune blistering diseases

W. Stöcker

Synonym(s). Bullous autoimmune dermatoses-associated autoantibodies, prickle cell desmosomal antibodies (see also Autoantibodies against epidermal basement membrane and autoantibodies against desmosomes)

Function and pathophysiology. Bullous autoimmune dermatoses are divided into 3 main groups, which focus on the levels of the cutis in which blisters manifest – according to the histological distribution of the target antigens against which the associated autoantibodies are directed. In addition, the fourth group of paraneoplastic pemphigus is defined for discriminatory tests:

1. Pemphigus diseases – blistering due to acantholysis, intraepidermal
   In this case, the target of autoimmunity is primarily the calcium-dependent adhesion molecules (cadherins) and desmoglein 1 and 3 (Dsg1 and Dsg3) of the prickle cell desmosomes – they bind the keratinocytes to one another. Dsg1 is expressed more strongly at the surface of both the epidermis as well as the mucosa than in the area of the stratum basale. The reverse is true for Dsg3 and Dsg3 also dominates across the entire width of the mucosa epithelium, while Dsg3 can only be found near the basal cells in the epidermis.
   Pemphigus foliaceus is associated with autoantibodies against Dsg1; consequently only superficial epidermis layers are afflicted, while the mucous membranes remain intact, as the mucosa contains sufficient Dsg3 (not affected by the autoimmune reaction). The gap forms in the stratum granulosum, where thin, flaccid blisters form. The incidence is estimated at 0.1 cases per 100,000 persons per year. In pemphigus vulgaris, the immunity against Dsg3 is dominant. The diseases manifests in two versions: If only autoantibodies against Dsg3 exist, this primarily affects the mucous membranes, while, in the epidermis, cells still have adequate stability due to the unaffected Dsg1. However, if the patient also develops autoantibodies against Dsg1, both the mucosa and the epidermis will be affected. Compared to pemphigus foliaceus, acantholysis occurs in the deeper epidermal layers and the blisters are slightly more solid. The incidence of pemphigus vulgaris amounts to 0.7-1.6 cases per 100,000 people per year. IgA pemphigus displays autoantibodies in immunoglobulin class IgA (autoantibodies against IgA) – the target antigens are Dsg1 or Dsg3 and desmocollin 1.
2. Pemphigoid diseases – sub-epidermal blistering at the level of the basement membrane
   The autoantibodies are directed against the hemidesmosomes, a complex network of structural proteins, which connect the stratum basale with the basement membrane. As a result of autoimmune reactions, the basal keratinocytes lose their contact with the basement membrane and the entire epidermis is raised – the blisters are therefore (in contrast to pemphigus vulgaris or foliaceus) absolutely full and taut. The most important representatives of this group are:
   - [5] Bullous pemphigoid – target antigens: BP180 (primarily the epitope NC16A, in some cases also the soluble ectodomain LAD-1) and BP230. The incidence amounts to 1.3-4.3 diseases per 100,000 people per year. The disease is more common than generally suspected, so antibodies against BP180 and NC16A should be analysed in all older patients with itchy skin changes that have existed for an extended period of time.
   - [5] Pemphigoid gestationis (previously: herpes gestationis) – target antigens: also BP180 (epitope NC16A) and BP230. The information on the incidence fluctuates: 1 case in every 3,000-10,000 pregnancies.
   - [5] Scarring mucous membrane pemphigoid – target antigens: 70% BP180 (predominantly soluble ectodomain LAD-1), 30% laminin 332 (a quarter of patients display a solid malignant tumour solely from this): lung, colon, mamma, cervix; detection: immunoblot based on an extract of the extracellular matrix of cultivated keratinocytes or a recombinant antigen). This parameter also has great potential in ophthalmology! The same symptoms in patients with genetically modified laminin 332.
   - [5] Anti-p200 pemphigoid – target antigen: Laminin-γ-1 (antibody detection via immunoblot).
   - [5] Linear IgA dermatosis – target antigens: LAD-1 and BP230.
   - [5] Lichen planus pemphigoides – target antigens: BP180 and BP230.
   - [5] Epidermolysis bullosa acquisita – target antigen: Collagen VII (anchoring fibrils, epitope NC1).
3. Dermatitis herpetiformis (DH, Duhring disease) – dermal blistering
   Antibodies against gliadin (specifically: coeliac disease-associated antibodies against deamidated gliadin fragments) and autoantibodies against epidermal transglutaminase or autoantibodies against tissue transglutaminase (revised designation: endomysium) are leading the way in this respect.
   DH has a special position amongst bullous autoimmune dermatoses, as the blistering occurs in deeper layers of the skin. DH is the cutaneous manifestation of coeliac disease (gluten intolerance); coeliac disease can be detected in every patient with DH (but DH is not detected in every case of coeliac disease). A lifelong gluten-free diet is the cornerstone of treating this disease.
4. In the case of paraneoplastic pemphigus, the severe skin condition occurs together with an occult or manifest tumour, generally haematologic neoplasia (non-Hodgkin's lymphoma, lymphatic leukaemia, Castleman tumour), it may be associated with autoantibodies against various desmosomal and hemidesmosomal proteins, Dsg1, Dsg3, desmoplakin 1 and 2, DBP230, envoplakin, periplakin, plectin and the protease inhibitor alpha-2-macroglobulin-like 1 (p170).

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Detection of in-vivo-bound autoantibodies by direct immunofluorescence on affected skin or mucosa. Determination of serum antibodies by indirect immunofluorescence (immunofluorescence, indirect) using epidermis, oral mucosa, tongue or oesophagus, for paraneoplastic antibodies also the urinary bladder (the urothelium also expresses desmoplakins in addition to Dsg1 and Dsg3). Primate tissue is essentially better suited than rodent tissue (higher sensitivity and specificity) Fig. 1).
Autoantibodies against the epidermal basement membrane can be partially distinguished in indirect immunofluorescence by using salt-split skin. Antibodies against BP180 and BP230 react with the top of the blister, while antibodies against LAD-1 (predominantly), laminin 332, laminin γ1 (p200) and collagen VII react with the base of the blister (Fig. 2).
Recombinant substrates with transfected human cells, which express authentic autoantigens, are also becoming increasingly popular in immunofluorescence: The immunofluorescence reactions are easier to interpret and disruptive influences by interfering antibodies are minimised, while the different autoantibodies can be directly identified.
Three dilutions are used in parallel, 1:10, 1:100 and 1:1,000, in order to detect low-titer antibodies as well as those that can only be identified from a higher dilution. This particularly plays a role for autoantibodies against epidermal basement membrane.
Normally, autoantibodies against desmosomes and against epidermal basement membrane are investigated in parallel, with the same substrates (it is often the case that only one of the two parameters are used for the analysis, but that there is a positive result for the other parameter that has not been requested).
Besides indirect immunofluorescence, corresponding enzyme-linked immunosorbent assays and immunoblot techniques using native or recombinant antigens for the solid phase are now also available.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Classification of bullous dermatosis, especially differentiating between autoimmune and hereditary forms. Determination of the disease activity based on the antibody concentration.

Literature.

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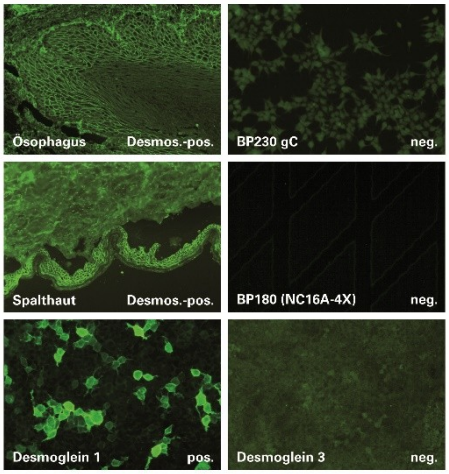
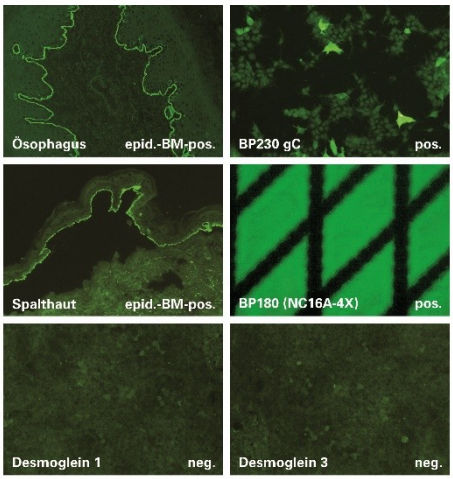


Autoimmune blistering disease -associated autoantibodies. Fig. 1. Pemphigus foliaceus: Evidence of autoantibodies against desmoglein 1 through indirect immunofluorescence. Substrates: a oesophagus, monkey, b BP230-transfected HEK-293 cells, c salt-split skin, human, d recombinant partial antigen of BP180 (NC16A-4X), e desmoglein-1-transfected HEK-293-cells, f desmoglein-3-transfected HEK-293-cells



Autoimmune blistering disease -associated autoantibodies. Fig. 2. Bullous pemphigoid: Evidence of autoantibodies against epidermal basement membrane through indirect immunofluorescence. Substrates: a oesophagus, monkey, b BP230-transfected HEK-293 cells, c salt-split skin, human, d recombinant partial antigen of BP180 (NC16A-4X), e desmoglein-1-transfected HEK-293-cells, f desmoglein-3-transfected HEK-293-cells

Autoantibodies against acetylcholine receptors

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Synonym(s). Acetylcholine receptor antibodies; anti-AChR antibodies; ACHRAB

Definition. Autoantibodies against acetylcholine receptors

Synthesis/distribution/decomposition/elimination. The corresponding autoantigen is localised on the motor end plate of the skeletal muscle fibres. The acetylcholine receptors of the skeletal muscle are comprised of two α as well as one β-, δ, γ and ε unit. Autoantibodies can be

formed against all subunits, but most are directed against a region of the extracellular part of the α chains. The α chains also contain binding sites for the neurotransmitters acetylcholine and its agonists, such as nicotine, or toxins, such as α-bungarotoxin.
A distinction must be made between acetylcholine receptors in the motor end plate and muscarinic acetylcholine receptors in the parasympathetic nervous system. These are also activated by acetylcholine as well as by muscarine; see also Autoantibodies against ganglionic acetylcholine receptors.

Function and pathophysiology. The corresponding autoantibodies bind to the acetylcholine receptors of the motor end plate and prevent the neuromuscular transmission of stimuli. The antibody-laden receptors are also absorbed into the cells and broken down, which reduces their number. Insufficient acetylcholine receptors are available for neurogenic muscle activation and muscle contraction. Recurring nerve impulses exacerbate the situation as this desensitises the remaining acetylcholine receptors. The result is muscle weakness and extreme atrophy of the skeletal muscles. The extreme weakness of vital muscles can lead to death. The corresponding pathology is referred to as myasthenia gravis (MG).

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Anti-acetylcholine receptor antibodies can be detected using radioreceptor assays: After incubating the patient serum with 125I-bungarotoxin-stained, purified acetylcholine receptors, a secondary antibody is used for precipitation and the radioactivity in the precipitate is measured. Enzyme-linked immunosorbent assay systems with recombinant antigen are also available, while human cell lines transfected with specific antigen are available for use as substrates for indirect immunofluorescence (immunofluorescence, indirect).

Reference range — Adults. Negative: < 0.25 nmol/L; marginal: 0.25–0.40 nmol/L; positive: ≥ 0.40 nmol/L

Reference range — Children. See adults

Indication. Myasthenia gravis

Interpretation. Autoantibodies against acetylcholine receptors are pathognomonic for MG. Autoantibodies can be detected in 75-90% of patients with active and generalised MG and in 45-70% of persons with ocular myasthenia. No autoantibodies are found in patients with severe forms of MG (about 5-10% of all cases). The diagnostic specificity of ACHRAB for MG is almost 100%, including in relation to other muscular diseases. The detection of ACHRAB is well-suited to monitoring the individual course of a disease, as its serum concentration correlates with the intensity of the muscle weakness.

Literature.
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Autoantibodies against α-fodrin

W. STÖCKER

Synonym(s). Anti-α-fodrin antibodies

Definition. Antibodies against α-fodrin react with the 120 kDa fragment of a molecule, which is created in connection with apoptotic processes when breaking down cytoskeletal structures. An association with Sjögren's syndrome has been described.

Function and pathophysiology. Fodrin is one of the main components of the cytoskeleton with a heterodimeric structure. The α subunit also binds with actin, calmodulin and CD45. It is presumed that an infiltration of lymphocytes into the gland tissue leads to reduced secretion and to apoptotic processes. While the native protein is not (auto)immunogenic, the 120 KDa fragment is created during cell breakdown, which may induce the autoantibodies.

Sample material. Serum

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Both IgG as well as IgA antibodies are generally found in the immune response against α-fodrin. They can be detected by enzyme immunoassays, immunoblot or immunoprecipitation. The corresponding antigen is obtained by chromatographic purification (chromatography) or, for some time, also by recombinant expression in suitable vectors.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. In 1997, Haneji et al. described an association between autoantibodies against α-fodrin and Sjögren's syndrome. In the following studies, autoantibodies against α-fodrin were found in 25 to over 90% of patients.
Autoantibodies against α-fodrin also occur in the rare cerebrovascular disorder moyamoya disease.

Diagnostic value.
The average diagnostic sensitivity of anti-α-fodrin antibodies for Sjögren's syndrome achieved in studies amounts to 39.3% with a diagnostic specificity of 83%.

Literature.
Haneji N, Nakamura T, Takio K et al (1997) Identification of alpha-fodrin as a candidate autoantigen in primary Sjogren's syndrome. Science 276:604–607

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Antibodies against aminoacyl-transfer RNA synthetase

W. Stöcker, W. Schlumberger

Synonym(s). Aminoacyl-tRNA-synthetase antibodies; anti-synthetase antibodies

Definition. Antibodies against aminoacyl-transfer RNA synthetases are directed against cytoplasmic, ribosome-associated enzymes, which catalyse the binding of the individual amino acids to the relevant t-RNA (Tab. 1). There is some evidence that aminoacyl-t RNA synthetases also occur in low concentrations in cell nuclei.

Antibodies against aminoacyl-transfer RNA synthetase. Tab. 1. Designation and function of various aminoacyl-t-RNA synthetases

Designation	Function	Abbreviation	Molecular mass
Jo-1	Histidyl-t-RNA synthetase	HisRS	55
PL-7	Threonyl-t-RNA synthetase	ThrRS	83
PL-12	Alanyl-t-RNA synthetase	AlaRS	110
OJ	Isoleucyl-t-RNA synthetase	IleRS	145
EJ	Glycyl-t-RNA synthetase	GlyRS	85
KS	Asparaginyl-t-RNA synthetase	AsnRS	63
Zo	Phenylalanyl-tRNA synthetase	PheRS	66
Ha / YRS	Tyrosyl-tRNA synthetase	TyrRS	59

Sample material. Serum and plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Antibodies against aminoacyl-t-RNA synthetases display a fine granular to homogeneous cytoplasmic fluorescence in the indirect immunofluorescence test (IIFT, immunofluorescence, indirect) with HEp-2 cells (Fig. 1). The cell nuclei also display a distinct, clear dotting in many cases: Aminoacyl-t-RNA synthetases are not exclusively localised in the cytoplasm; in some species they can also be found in the cell nuclei (Azad et al.). In the IIFT, the usual starting dilution is 1:100, where the focus is primarily on immunoglobulin class IgG.
In some cases, the different antibodies against components of the cytoplasm are difficult to distinguish using the fluorescence pattern. As a result, in the case of a positive cytoplasm reaction in the IIFT, monospecific enzyme immunoassays (enzyme-linked immunosorbent assay, chemiluminescence immunoassays) or immunoblots with purified native or recombinant target antigens, such as Jo-1, PL-7, PL-12, etc., should be used to specifically identify the antibodies.

Reference range — Adults. Negative

Reference range — Children. Negative

Interpretation. Autoantibodies against aminoacyl-t-RNA synthetases are associated with a characteristic clinical syndrome, the “anti-synthetase syndrome”. The main symptoms in 90% of antibody-positive patients are (poly)myositis, especially together with fibrotic alveolitis. Other symptoms of systematic autoimmune diseases (e.g. arthritis, Raynaud's syndrome) may arise. Autoantibodies against t-RNA synthetases are directed against Jo-1 in approx. 90% of cases.

Literature.

Nishikai M, Reichlin M (1980) Heterogeneity of precipitating antibodies in polymyositis and dermatomyositis.Characterization of the Jo-1 antibody system. Arthritis Rheum 23:881–888

Bernstein RM, Morgan SH, Chapman J, Bunn CC, Mathews MB, Turner-Warwick M, Hughes GR (1984) Anti-Jo-1 antibody: a marker for myositis with interstitial lung disease. Br Med J 289:151–152

Azad AK, Stanford DR, Sarkar S, Hopper AK (2001) Role of nuclear pools of aminoacyl-t-RNA synthetases in tRNA nuclear export. Mol Biol Cell 12(5):1381–1392

Betteridge Z, McHugh N (2016) Myositis-specific autoantibodies: an important tool to support diagnosis of myositis. J Intern Med 280(1):8-23

Lundberg IE, Miller FW, Tjärnlund A, Bottai M (2016) Diagnosis and classification of idiopathic inflammatory myopathies. J Intern Med 280(1):39-51

Legend

Antibodies against aminoacyl-transfer RNA synthetase. Fig. 1. Substrate: HEp-2 cells

Autoantibodies against amphiphysin

W. STÖCKER

Synonym(s). Amphiphysin antibodies; ti-amphiphysin antibodies

Definition. Autoantibodies initially identified in patients with stiff-person syndrome, which are directed against the amphiphysin present in the synaptic vesicles of the neurons.

Synthesis/distribution/decomposition/elimination. Two forms of amphiphysin I are described, which occur through alternative splicing. The isoform 1 migrates into the SDS-PAGE at about 128 kDa and is located in the synaptic vesicles of the nerve cells in high concentrations. The isoform 2 (108 kDa) is formed outside the nerve tissue, normally only in small quantities (mammary gland, endocrine cells, spermatozoa).

Function and pathophysiology. The involvement of amphiphysin I in the endocytosis of synaptic vesicles is being discussed. The extraneuronal expression of amphiphysin I by tumours (breast cancer, small cell lung cancer) presumably induces autoimmune reactions. Stiff-man syndrome develops in parallel to the occurrence of antibodies directed against amphiphysin I.

Sample material. Serum, plasma, cerebrospinal fluid

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Indirect immunofluorescence (immunofluorescence, indirect) with cerebellum as the substrate: Dense cytoplasmic staining of the stratum moleculare, and patchy staining of the stratum granulosum. Differentiation between autoantibodies against glutamic acid decarboxylase using Western blot (immunoblot) from brain homogenate: Antigen band with 128 kDa.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. The detection of autoantibodies against amphiphysin is used to identify neuromuscular symptoms, especially for discriminatory tests in relation to stiff-person syndrome.
Amphiphysin antibodies point to a paraneoplastic cause and necessitate a tumour screening, while GAD antibodies (autoantibodies against glutamic acid decarboxylase) indicate an idiopathic stiff-person syndrome.

Literature.

De Camilli P, Thomas A, Cofiell Rm Folli F, Lichte B, Piccolo G, Meinck HM, Austoni M, Fassetta G, Bottazzo G, Bates D, Cartlidge N, Solimena M, Kilimann MW et al. (1993) The synaptic vesicle-associated protein amphiphysin is the 128-kD autoantigen of Stiff-Man syndrome with breast cancer. J Exp Med 178(6): 2219-2223

Pittcock SJ, Luchinetti CF, Parisi JE, Benarroch EE, Mokri B, Stephan CL, Kim KK, Kilimann MW, Lennon VA (2005) Amphyphysin autoimmunity: paraneoplastic accompaniments. Ann Neurol 58(1): 96-107.

Saiz A, Dalmau J, Butler MH et al (1999) Anti-amphiphysin I antibodies in patients with paraneoplastic neurological disorders associated with small cell lung carcinoma. J Neurol Neurosurg Psychiatry 66:214–217

Autoantibodies against annexin A5

W. STÖCKER

Synonym(s). Annexin A5 antibodies; anti-annexinA5 antibodies

Function and pathophysiology. Annexin A5 (originally referred to as annexin V or “placental anticoagulant protein I”) is a strong coagulation inhibitor, which is expressed by the trophoblasts in the placenta, by thrombocytes and by endothelial cells. It prevents their coagulation-triggering effect in that it forms complexes with anionic phospholipids: Normally, no negatively charged phospholipids can be found on the outside of the plasma membrane. However, under certain conditions, such as thrombocyte activation (thrombocyte aggregation and activation) or apoptosis, the anionic phospholipid phosphatidylserine reaches the outside of the plasma membrane. Due to its high calcium-dependent affinity to phosphatidylserine, annexin A5 binds with the phosphatidylserine-containing areas of the membrane and forms a two-dimensional crystalline proteolipid complex, which inhibits phospholipid-dependent coagulation reactions, such as prothrombin activation.
Antibodies against annexin A5 disrupt the development of the crystalline structure and lead to the destabilisation of the coagulation system. The cause of the thrombosis-inducing effect may possibly be related to certain autoantibodies against phospholipids with the displacement of annexin A5.

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Enzyme-linked immunosorbent assay with recombinant annexin A5. Tomer et al. (2007) describe a flow-cytometric “annexin A5 competition test”

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Antiphospholipid syndrome. The increased arterial as well as venous risk of thrombosis in connection with pregnancies, possible intra-uterine damage in the unborn child.

Literature.

Rand JH, Wu XX, Lapinski R, van Heerde WL, Reutelingsperger CP, Chen PP, Ortel TL (2004) Detection of antibody-mediated reduction of annexin A5 anticoagulant activity in plasmas of patients with the antiphospholipid syndrome. Blood 104:2783–2790

Tomer A, Bar-Lev S, Fleisher S, Shenkman B, Friger M, Abu-Shakra M (2007) Antiphospholipid antibody syndrome: the flow cytometric annexin A5 competition assay as a diagnostic tool. Brit J Haematol 139:113–120

Autoantibodies against aquaporin 4

W. Stöcker

Synonym(s). Aquaporin 4 autoantibodies; anti-AQP4 antibodies; neuromyelitis optica IgG; NMO-IgG

Definition. The antibodies referred to as neuromyelitis optica (NMO) IgG by those who first described it, cause a characteristic colouring of the Virchow-Robin's space along the small arterioles in the grey and white matter on CNS tissue in indirect immunofluorescence. The protein aquaporin 4 (AQP4) was later identified as the target antigen.

Function and pathophysiology. Aquaporin 4 is a water channel, which is involved in regulating the water and electrolyte balance in the central nervous system (CNS) and which is expressed on astrocytes, predominantly in the area of the glial endfeet. The water channels occur particularly frequently in the sections of the CNS that are traditionally affected in the case of NMO: Optic nerves and spinal cord. Autoantibodies against aquaporin 4 are formed by peripheral plasma cells and, after binding to its target antigen in the CNS, lead to the activation of complement with locally inflammatory demyelination and necrosis. The disorder corresponds to optic neuritis and local myelitis across three or more spinal segments, primarily localised in or near the blood-brain barrier.
NMO was previously considered a localised form of multiple sclerosis (MS). According to the current state of knowledge, it is a fundamentally separate disorder with regard to the pathogenesis. In contrast to MS, which remains a disease predominantly mediated by T-cells, humoral mechanisms seem to be responsible for the occurrence of NMO, which is also reflected in the selective association with autoantibodies against aquaporin 4.

Analytics. The detection of the anti-aquaporin 4 antibodies is possible using radioimmunoprecipitation assays (radioimmunoassays), but the sensitivity only amounts to 56%. Alternatively, a fluorescence immunoprecipitation test (FIPA) has been described, and a test based on cell sorting (FACS) also exists, in which transient HEK-293 cells transfected with aquaporin are used as the antigen target.
These days, the method of choice is the indirect immunofluorescence test (IIFT, immunofluorescence, indirect) with aquaporin 4-transferred HEK cells as the substrate (“human embryonic kidney cells”). They create an easily identifiable two-dimensional, smooth to fine granular fluorescence in the cytoplasm. The sensitivity of the IIFT amounts to 70-90% with a specificity of 100%.
A BIOCHIP mosaic, which also contains frozen sections of the cerebellum, cerebrum, hippocampus and optic nerve as well as other cell substrates with recombinant expressed neuronal antigens, enables the reactions to be checked using the same approach, while autoantibodies of relevance for other discriminatory tests are detected at the same time, which, in many cases, results in a rapid and reliable (potentially unexpected) vital diagnosis.

Sample material. Serum, plasma or cerebrospinal fluid

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Diagnostic value. From a laboratory diagnostics perspective, the investigation of the autoantibodies against aquaporin 4 ensures the diagnosis of neuromyelitis optica (opticospinal encephalomyelitis, Devic’s disease). The inflammatory autoimmune disease is a rare form (~1%) of the group of acquired demyelinating diseases of the CNS with degradation of the insulating sheath of at least one optical nerve (optic neuritis) and at the same time or a few months later, the spinal cord (myelitis). The symptoms of NMO include acute visual impairment up to blindness (amaurosis) in one or both eyes within hours to days as well as symptoms of a paraplegic syndrome, in some cases with worsening symptoms, sensory disturbances, muscle weakness and paralysis of the extremities as well as loss of control of the urinary bladder, which can also develop acutely or within 1-14 days. Histologically, demyelination centres are formed (similar to those in MS), which are easy to reduce in size. However, lasting damage is also commonly caused due to tissue destruction (necrosis).
It has also been shown that anti-aquaporin 4 antibodies can be detected in patients with isolated longitudinally extensive (three or more segments) transverse myelitis (LETM) as well as in patients with isolated recurring opticus neuritis (ON). As a result of the repeatedly identified strong association between anti-aquaporin 4 antibodies and NMO, it is assumed that seropositive LETM and ON cases relate to incomplete forms of NMO. NMO and related syndromes are now grouped under the term neuromyelitis optica spectrum disorders (NMOSD). The detection of autoantibodies against AQP4 with corresponding clinical symptoms is used as an exclusion criterion for multiple sclerosis.

Literature.

Jarius S, Probst C, Borowski K, Franciotta D, Wildemann B, Stöcker W, Wandinger KP (2010) Standardized method for the detection of antibodies to aquaporin-4 based on a highly sensitive immunofluorescence assay employing recombinant target antigen. J Neurol Sci 291:52–56

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Autoantibodies against asialoglycoprotein receptors

W. Stöcker

Synonym(s). Asialoglycoprotein receptor antibodies; anti-ASGPR antibodies; autoantibodies against ASGPR

Definition. Antibodies against asialoglycoprotein receptor, a liver-specific, membrane-bound receptor (ASGPR), which is involved in the endocytosis of glycoproteins with terminal galactose. The asialoglycoprotein receptor (Ashwell receptor) is a key component of the antigen preparation LSP (liver-specific proteins) complexes.

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Antibodies against asialoglycoprotein receptors can be detected using radioimmunoassay, enzyme immunoassay and immunoblot.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Autoantibodies against asialoglycoprotein receptors do not have an adequately high disease specificity for autoimmune hepatitis and are therefore only investigated sporadically (the focus here is on detecting autoantibodies against nuclei, autoantibodies against double-stranded DNA, autoantibodies against smooth muscles (actin), autoantibodies against LC-1, autoantibodies against LKM and, primarily, autoantibodies against SLA). Prevalences: active autoimmune hepatitis 83-87%, viral hepatitis 2-57%, primary biliary cirrhosis of the liver 14%, alcoholic liver diseases 8%, non-hepatic autoimmune diseases 0-11%, liver tumours 11%.

Literature.

McFarlane BM, McSorley CG, Vergani D et al (1986) Serum autoantibodies reacting with the hepatic asialoglycoprotein receptor protein (hepatic lectin) in acute and chronic liver disorders. J Hepatol 3:196–205

Synonym(s). ATP1A3 autoantibodies, anti-ATP1A3 antibodies

Definition. Autoantibodies against the catalytically active alpha 3 subunit of the neuronal and cardinal sodium-potassium ion pump Na+/K+-ATPase (ATP1A3).

Pathophysiology. Under the hydrolysis of ATP, the Na+/K+-ATPase catalyses the transport of 3 sodium ions from the cells and 2 potassium ions into the cells against the electrochemical potential gradient. The ATPase is therefore involved in maintaining the resting membrane potential of the nerve and muscle cells. In addition, the ion pump is believed to play a possible role in the development of memory and in the control of motor skills by the cerebellum.

Autoimmunity against the alpha 3 subunit of the neuronal Na+/K+-ATPase was described in a patient who displayed the symptoms of a combined brain stem and cerebellar syndrome. Colon cancer was diagnosed at the same time.

Sample material. Serum, plasma, cerebrospinal fluid

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Autoantibodies against AETP1A3 can be detected using indirect immunofluorescence (immunofluorescence, indirect) (Fig. 1-3): In the case of a positive reaction on cerebellum substrate, a homogeneous to fine granular fluorescence of the granular and molecular layer can be identified. The nuclei of the granular and Purkinje cells do not react. HEK cells, which express recombinant ATP1A3, are suitable for the monospecific detection of the antibodies.

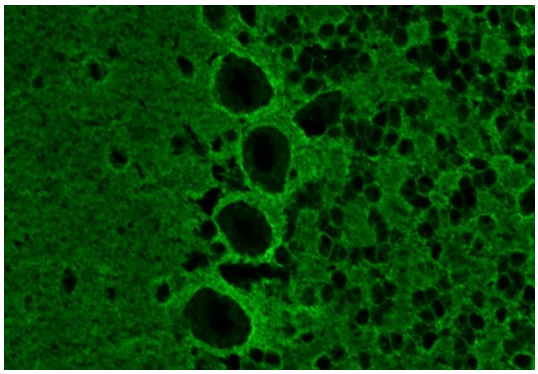
Diagnostic value. To date, there has only been one case description of a patient with IgG against ATP1A3. The woman suffered from vertical gaze palsy, progressive ataxia and spastic tetraparesis, limited vision as well as dysarthria and dysphasia. Colon cancer was also diagnosed, which indicates a paraneoplastic syndrome. Overexpressed ATP1A3 could be detected in the tumour tissue, but not in the healthy colon.

Similar neurological symptoms, including infantile hemiplegia, dystonia and CAPOS (cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss) syndrome were also described in patients with mutations in the ATP1A3 gene.

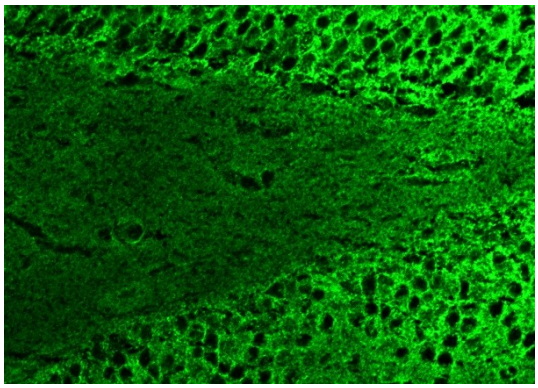
We advise investigating the most important autoantibodies against neuronal antigens in parallel in order to quickly obtain a reliable diagnosis.

Literature.

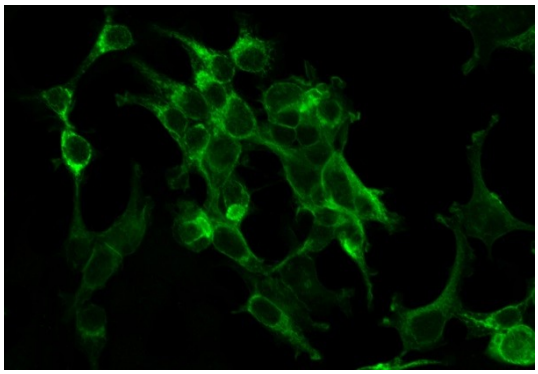
Scharf M, Miske R, Heidenreich F, Giess R, Landwehr P, Blöcker IM, Begemann N, Denno Y, Tiede S, Dähnrich C, Schlumberger W, Unger M, Teegen B, Stöcker W, Probst C, Komorowski L (2015) Neuronal Na+/K+ ATPase is an autoantibody target in paraneoplastic neurologic syndrome. Neurology 84: 1-7.



Autoantibodies against ATP1A3. Fig. 1. Substrate: cerebellum (rat)



Autoantibodies against ATP1A3. Fig. 2. Substrate: hippocampus (rat)



Autoantibodies against ATP1A3. Fig. 3. Substrate: transfected cells

Autoantibodies against eye muscle proteins

W. STÖCKER

Synonym(s). Eye muscle autoantibodies; extra ocular muscle autoantibodies

Definition. Autoantibodies that are directed against proteins in the eye muscle. They are suspected of being involved in autoimmune processes in connection with endocrine orbitopathy. Currently, insufficient evidence exists in this respect; autoimmune reactions directed against TSH receptors in the retrobulbar connective tissue appear to play a larger role.

Structure. The eye muscles contain several proteins that could represent target structures for autoimmune processes. These proteins include the G2s protein, which was identified as a subunit of the transcription factor FOXP1, the D1 membrane protein (leiomodulin), the Fp protein, the flavoprotein subunit of the enzyme succinate dehydrogenase as well as calsequestrin and sarcolumenin, two calcium-binding proteins of the sarcoplasmic reticulum in muscle cells.

Function and pathophysiology. Endocrine orbitopathy (ophthalmopathy, exophthalmos) often occurs in connection with Graves' disease (autoimmune thyroid disease). Cases of ophthalmopathy without a thyroid disorder also exist. Autoantibodies against TSH receptors in the thyroid are a pathogenically relevant indicator of Graves' disease and are directed against the thyroid tissue as well as against fibroblasts in the connective and fatty tissue of the eye sockets, the pretibial skin and several organs. This is associated with extrathyroidal manifestations, such as exophthalmos and myxoedema, with inflammation and swelling of the connective and fatty tissue in the relevant areas. In the early stages of the disease, T-lymphocytes detect antigens in the retrobulbar connective tissue. It has been shown that the fibroblasts of the eye socket tissue also contain TSH receptors on their surface. They therefore become the target for the T-cells and antibodies. The fibroblasts in the eye socket increasingly form liquid-binding molecules, so-called glycosaminoglycans. Over time, the connective tissue hypertrophies, prises the muscle fibres apart and impairs their function. The eye muscles swell (oedema). Mononuclear cells increasingly migrate into the connective, fatty and muscle tissue in the eye sockets. The triggered reaction can sustain itself and strengthen by forming cytokines, interleukins, growth factors, prostaglandins and other factors. The swelling of the tissue and the mechanical impairment lead to restricted space in the eye socket.

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Antibodies against the D1 protein are detected by a Western blot, while antibodies against G2s and Fp proteins are investigated using radioimmunoprecipitation (radioimmunoassay). Immunofluorescence (immunofluorescence, indirect) does not deliver any useful results.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Endocrine ophthalmopathy

Interpretation. Some authors consider antibodies against the G2s and Fp proteins to be sensitive markers for damage to the eye muscles. However, the detection of antibodies against eye muscle tissue does not play a role for the diagnosis of Graves' diseases, which is often associated with endocrine orbitopathy.

Literature.

Mizokami T, Salvi M, Wall JR (2004) Eye muscle antibodies in Graves' ophthalmopathy: pathogenic or secondary epiphenomenon? J Endocrinol Invest 27:221–229

Antibodies against β2 glycoprotein I

W. Stöcker

Synonym(s). Anti-β2 glycoprotein I antibodies

Definition. β2 glycoprotein I (β2GP1) is a phospholipid-binding plasma protein. In connection with autoimmune reactions, it acts as a cofactor in the antibody-binding to the phospholipid cardiolipin.

Function and pathophysiology. Autoantibodies against phospholipids

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for deep-freeze preservation of IgM.

Analytics. Antibodies against β2GP1 can only be reliably detected in enzyme-linked immunosorbent assay systems in which β2GP1 is used as the sole antigen. The antigen β2GP1 is also contained as a cofactor in anti-cardiolipin ELISA, but these are not suitable for use as screening methods for the parallel detection of autoantibodies against cardiolipin (ACA) and against β2GP1. The structural modification of the β2GP1 by binding to cardiolipin presumably leads to the loss of epitopes, which is detected by a subpopulation of the antibodies against β2GP1.
For the serological diagnosis of antiphospholipid syndrome (APS), the recommendation is to first detect the antibodies against cardiolipin (IgG and IgM; IgA is less significant) as well as lupus anticoagulant (LA). The detection of these antibodies must be repeated after 6-12 weeks, as only a double positive finding meets the serological APS criteria. In the case of a negative cardiolipin-antibody finding, class IgA, IgG and IgM antibodies against β2GP1 should be analysed. These occur in APS with a high prevalence (60-90%) as well as independent of ACA and LA. The parallel investigation of ACA and anti-β2GP1 antibodies enables the serological detection rate to be increased to almost 100%; see also autoantibodies against phospholipids.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Antiphospholipid syndrome

Diagnostic value. The clinical complications associated with the presence of antibodies against phospholipids and against β2GP1 have been summarised under the term anti-phospholipid syndrome (APS). Antibodies against β2GP1 occur with a high prevalence (60-90%) in APS patients; their presence (persisting over several weeks) is, with corresponding clinical symptoms, considered evidence of the existence of APS pursuant to the list of criteria for APS diagnosis according to Miyakis et al. (2006). Antibodies against β2GP1 are also found in anti-cardiolipin-negative APS patients and vice versa. Parallel detection of both parameters increases the serological detection rate for this disease. 15-30% of patients with SLE display antibodies against β2GP1, especially if typical APS symptoms already exist. Antibodies against β2GP1 are considered more specific for the detection of the APS than autoantibodies against cardiolipin, which can also be detected in certain infections (e.g. syphilis, Lyme disease, AIDS, hepatitis, tuberculosis) (see also autoantibodies against phospholipids).

Literature.

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Autoantibodies against BPI

W. Stöcker

Synonym(s). Anti-bactericidal permeability-increasing protein autoantibodies; anti-BPI antibodies; anti-CAP 57

Definition. BPI is a cationic membrane-associated protein toxic for gram-negative bacteria.

Molar mass. 55 kDa

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Autoantibodies against granulocyte cytoplasm
Antibodies against BPI display a cANCA pattern in immunofluorescence, which sometimes transitions to a pANCA. In case of positive or questionable immunofluorescence results (immunofluorescence, indirect), enzyme-linked immunosorbent assays with defined target antigens, isolated from human granulocytes, are required to confirm and differentiate the finding.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. None, as autoantibodies against BPI can occur in various diseases and discriminatory tests are of no use.

Diagnostic value. Autoantibodies against BPI generally appear to be formed in inflammatory reactions and have no disease specificity. They have been described in cystic fibrosis, ANCA-associated vasculitides, ulcerative colitis, Crohn's disease, autoimmune hepatitis, primary sclerosing cholangitis, HIV infections and others.

Literature.
Schultz H, Weiss J, Carroll SF, Gross WL (2001) The endotoxin-binding bactericidal/permeability-increasing protein (BPI): a target antigen of autoantibodies. J leukoc Biol 69:505–512

Autoantibodies against C1q

W. STÖCKER

Synonym(s). Autoantibodies against complement component C1q; anti-C1q antibodies

Function and pathophysiology. The glycoprotein C1q is located at the start of the complement cascade. Autoantibodies against C1q can be directed against epitopes in the globular part of the molecule as well as against the “collagen-like region” (CLR).
In SLE, Fc fragments of the immunocomplexes bind with the globular domains of the C1q and activate the classic complement path. In hypocomplementemic urticarial vasculitis syndrome (HUVS), the target structures are located in the CLR of the C1q molecule.

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. The standard method for detecting autoantibodies against C1q is an enzyme-linked immunosorbent assay. Autoantibodies against C1q primarily consist of IgG, while IgA also arises in isolated cases. The wall of the reaction vessel is coated in chromatographically purified C1q.
To prevent immunocomplexes contained in the patient serum from binding to C1q, the reaction is performed in the presence of a 1 M NaCl solution. The high salt concentration prevents circulating immunocomplexes from being acquired, but permits the bonding of the autoantibodies.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Autoantibodies against C1q were first detected in the sera of patients with systemic lupus erythematosus (SLE) in 1984. Later, these antibodies were also found in other autoimmune diseases. These include Sjögren's syndrome and microscopic polyangiitis. Of particular importance is the association with the immunocomplex diseases hypocomplementemic urticarial vasculitis syndrome (HUVS, combined with SLE in approx. 50% of cases: autoantibodies against C1q are the main criterion for the disease) and lupus nephritis.
Clinical symptoms of life-threatening HUVS are chronic autoreactive urticaria, venous oedema, polyarthritis, conjunctivitis and, in some cases, lethal glomerulonephritis and obstructive lung disease.
In SLE, the prevalence of antibodies against C1q is 30% or almost 100% for HUVS.
Autoantibodies against C1q are also sporadically detected with rheumatoid arthritis, especially in the special form of Felty syndrome (arthritis, leucopenia and splenomegalia).

Interpretation. Autoantibodies against C1q are not specific for a certain autoimmune disease, but provide important information for assessing the disease activity, especially with SLE. They are detected in an average of 45% of SLE cases and in over 90% of patients with lupus nephritis. In this case, the autoantibody against C1q concentration is, on average, about 5 times higher than for SLE patients without kidney involvement. A negative anti-C1q autoantibody test result with SLE rules out any kidney involvement with a high probability and indicates a prognosis of lupus nephritis of < 5%.
The detection of autoantibodies against C1q is also important for assessing the course of a disease and its treatment in SLE and lupus nephritis. A significant fall in the autoantibody concentration is recorded in the case of the successful immunosuppressive treatment of active lupus nephritis. The antibody titer in the serum correlates with the autoantibody concentration against dsDNA (autoantibodies against double-stranded DNA) in almost 80% of SLE cases.

Literature.

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Walport MJ (2002) Complement and systemic lupus erythematosus. Arthritis Res 4 (Suppl 3):S279–293

Autoantibodies against C3-convertase

W. STÖCKER

Synonym(s). C3-convertase antibodies; anti-C3bBb antibodies; autoantibodies against the C3 nephritic factor

Definition. These are antibodies against the C3 convertase enzyme (C3bBb), which is formed on the alternative complement activation path.

Function and pathophysiology. Antibodies against C3-convertase stabilise the C3-convertase enzyme and prevent its persistent physiological inactivation by the control protein factor H, which is reflected in the increased consumption of the complement factor C3.

Sample material. Serum, EDTA plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Several test principles are suitable for analysing the C3 nephritis factor:

- A functional test, which is based on the complement-mediated lysis of indicator cells. The test is set up so that C3bBb contained in a test system is only adequately active and causes a lysis, if it is stabilised by the antibodies against C3-convertase contained in the sample.
- An immunofixation electrophoresis, in which a defined quantity of C3 is subjected to splitting by C3-convertase. If patient serum with autoantibodies against C3-convertase is first added, the stabilising effect of the autoantibodies increases the quantity of the C3b formed. If a significant difference arises in a comparison with two approaches, once with and once without additive, the presence of the autoantibody is detected.
- An enzyme-linked immunosorbent assay with solid-phase bound C3bBb as the antigen.

Reference range — Adults. Not detectable

Reference range — Children. Not detectable

Indication. Clinical manifestations are membranoproliferative glomerulonephritis as well as partial lipodystrophy, in which the fatty tissue of the subcutis is destroyed.

Interpretation. For the diagnostic analysis of cases with membranoproliferative glomerulonephritis or lipodystrophy, the complement C3 concentration is measured at the same time as the diagnosis of the autoantibodies against C3-convertase. If functionally effective antibodies against C3-convertase exist, a low C3 concentration is detected, as the inhibition of the C3 breakdown is disrupted.

Literature.

Jelezarova E, Schlumberger M, Sadallah S et al (2001) A C3 convertase assay for nephritic factor functional activity. J Immunol Methods 251:45–52

Autoantibodies against calcium channels

W. STÖCKER

Synonym(s). Autoantibodies against voltage-gated (-dependent) calcium channels (VGCC, VDCC); calcium channel antibodies

Definition. Autoantibodies against subunits of voltage-gated calcium channels

Structure. Voltage-gated calcium channels consist of several membrane protein subunits. They can be divided into the following subtypes based on their different electrophysiological and pharmacological properties: P, Q, N, R and L. Due to their structural similarities, the P and Q subtype are jointly referred to as the P/Q type. The P/Q- and N-type calcium channels are of serological interest, as they are part of the presynaptic binding sites for vesicle-associated synaptic proteins. The P/Q-type calcium channels apparently control the release of the neurotransmitter acetylcholine from the nerve endings in the synaptic gap. The N-type calcium channels are responsible for the transmission of impulses in the vegetative nerve system. In-vitro dissolved P/Q and N-type calcium channels are differentiated based on their high-affinity bonding with labelled ω conotoxins MVIIC and GVIA (from the marine gastropod *Conus magnus*). The P/Q- and N-type calcium channels contain immunodominant epitopes on various subunits.

Function and pathophysiology. The Lambert-Eaton myasthenic syndrome (LEMS) is the most common paraneoplastic disease in neurology. It occurs in association with a tumour, without being directly caused by this. The calcium channel antibodies indicative of LEMS influence the function of various calcium channel subtypes in that they reduce the release of the neurotransmitter acetylcholine from the nerve endings in the synaptic gap of the motor end plate. In the case of a depolarisation of the presynaptic membrane, calcium flows through the channels into the nerve cells where it effects the release of acetylcholine from the vesicles. The autoantibodies in LEMS lead to a cross-linking of the channels with subsequent internalisation and degradation. This reduces the number of these channels and therefore also the release of acetylcholine. The consequence of this is an interruption of the neuromuscular signal path in the motor end plate, which ultimately leads to the development of muscle weakness.

Sample material. Serum, plasma, cerebrospinal fluid

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. P/Q-type calcium channel antibodies are detected by a radioreceptor assay in which an immunoprecipitation with 125I conotoxin-labelled P/Q-type calcium channels takes place. After incubating the patient serum with the labelled calcium channels, a secondary antibody is used for precipitation and the radioactivity in the precipitate is measured. Antibodies against N-type calcium channels are analysed in the same manner, using 125I conotoxin-labelled N-type calcium channels. Enzyme-linked immunosorbent assay systems are not yet available for these diagnostics, while the antibodies cannot be detected using indirect immunofluorescence (immunofluorescence, indirect).

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Paraneoplastic neurological syndrome, LEMS.
The LEMS-associated P/Q-type calcium channel antibodies are analysed in parallel to the autoantibodies against acetylcholine receptors (and potentially autoantibodies against MuSK) characteristic of myasthenia gravis. This confirms the often clinically difficult, but important differentiation in relation to the, in this respect, more benign myasthenia gravis due to the frequent association between LEMS and cancer. As the concentration of the calcium channel antibodies intraindividually correlates with the clinical activity of LEMS, its detection is also suitable for treatment monitoring.

Interpretation. Over 60% of patients with LEMS have small-cell lung cancer. As the diagnosis of LEMS generally precedes the clinical manifestation of the tumour by several years, the autoantigens can provide a very early and clear indication of the tumour.
The prevalence of the antibodies against P/Q-type calcium channels amounts to 90-100% in LEMS with associated small-cell lung cancer.
Autoantibodies against calcium channels also occur in other paraneoplastic syndromes, such as paraneoplastic encephalomyelopathy and cerebellar degeneration as well as in various other neurological diseases. The most commonly associated tumours are: Small-cell lung cancer, breast and ovarian cancer. Autoantibodies against calcium channels are only detected sporadically in healthy blood donors.

Literature.

Lennon VA, Kryzer TJ, Griesmann GE et al (1995) Calcium-channel antibodies in the Lambert-Eaton syndrome and other paraneoplastic syndromes. N Engl J Med 332:1467–1474

Vincent A (1999) Antibodies to ion channels in paraneoplastic disorders. Brain Pathology 9:285–291

Vincent A, Lang B, Kleopa AK (2006) Autoimmune channelopathies and related neurological disorders. Neuron 52: 123-138

Autoantibodies against cardiolipin

W. Stöcker, W. Schlumberger

Synonym(s). Anti-cardiolipin antibodies, ACLA; ACA

Definition. Antibodies against cardiolipin are directed against the complex consisting of diphosphatidylglycerol (cardiolipin) and the plasma protein β2 glycoprotein 1 (β2GP1, autoantibodies against β2 glycoprotein 1).

Function and pathophysiology. Autoantibodies against phospholipids

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Autoantibodies against phospholipids

International standard. The standards used in enzyme immunoassays are usually calibrated with a standard serum (Louisville APL Diagnostics, USA), but which is not recognised as an international reference serum by the World Health Organization (WHO). A PL-IgG unit (phospholipid IgG), for example, is defined as the cardiolipin bonding activity of 1 µg/mL of an affinity-purified IgG cardiolipin antibody in this standard serum. However, an independent standardisation of the results cannot be achieved, as numerous other influencing variables play a role in determining the results.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Antiphospholipid syndrome

Diagnostic value. Autoantibodies against phospholipids

Literature. Autoantibodies against phospholipids

Synonym(s). CARP VIII autoantibodies; anti-CARP antibodies

Definition. Autoantibodies against carbonic anhydrase-related protein (CARP) VIII, which is primarily expressed in the Purkinje cells.

Pathophysiology. CARP presumably plays an important role in the development and maturation of the Purkinje cells. It binds with the modulatory domains of the inositol 1,4,5-triphosphate receptor type 1 (ITPR1) and reduces its affinity to the substrate inositol 1,4,5-triphosphate. Moreover, an overexpression of the protein has been described in various tumours.

To date, autoantibodies against CARP have been described in 2 patients with paraneoplastic cerebellar degeneration.

Sample material. Serum, plasma, cerebrospinal fluid

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Autoantibodies against CARP VIII can be detected using indirect immunofluorescence (immunofluorescence, indirect): A fine-spotted staining of the molecular layer and the cytoplasm of the Purkinje cells can be detected on the cerebellum substrate in the case of a positive reaction (Fig. 1). Transfected HEK cells, which express recombinant CARP VIII are suitable for the monospecific detection of autoantibodies (Fig. 2).

Diagnostic value. To date, 2 patients with anti-CARP VIII antibodies and paraneoplastic cerebellar degeneration have been described. This is also associated with ataxia in the extremities and gait ataxia, dysarthria and nystagmus. At the same time, one patient was diagnosed with a melanoma, while the other had ovarian cancer.

Literature.

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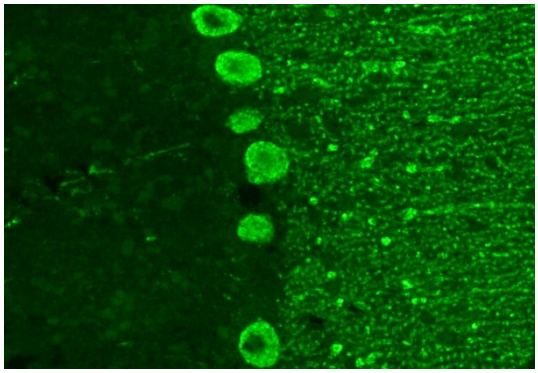
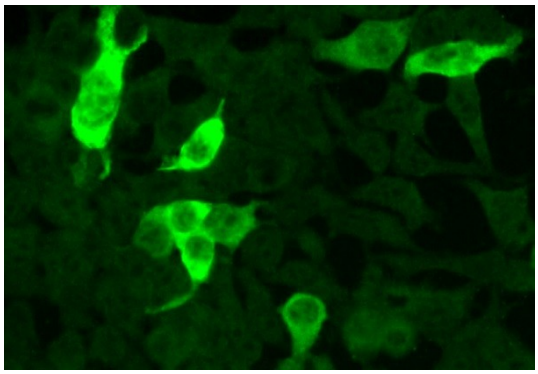
Höftberger R, Sabater L, Velasco F, Ciordia R, Dalmau J, Graus F (2014) Carbonic anhydrase-realted protein VIII antibodies and paraneoplastic cerebellar degeneration. Neuropathol Appl Neurobiol 40(5): 650-653.



Autoantibodies against CARP VIII. Fig. 1. Substrate: cerebellum (rat)



Autoantibodies against CARP VIII. Fig. 2. Substrate: transfected cells

Autoantibodies against CENP-F

W. Stöcker

Synonym(s). Autoantibodies against CENP-F kinetochore protein; anti-p330; anti-mitosin; anti-cylin-2; MSA-3; NSp-II; CENP-F antibodies

Definition. The gene that codes for CENP-F is located on chromosome 1q32-4, a region that is associated with various types of cancer. The cell cycle-dependent expression primarily takes place in the S, G2 and M phase of mitosis.

Function and pathophysiology. The target antigen is mitosis, which is expressed in the interval between the S2 phase of the cell cycle and mitosis. It triggers the mitosis and controls its progress. Although autoantibodies against CENP-F are predominantly found in cancer patients, CENP-F cannot currently be referred to as a tumour antigen.

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Autoantibodies against CENP-F/mitosin are detected by indirect immunofluorescence (immunofluorescence, indirect). Analysis using immunoblot is also possible. The starting dilution in immunofluorescence is 1:100; generally, antibodies in all immunoglobulin classes are investigated.
A typical picture with HEp-2 cells is: half of the interphase nuclei displays a distinct, fine- to coarse-granular fluorescence, while the remaining interphase nuclei react in the same manner, but ten times weaker (Fig. 1). In addition, the mitotic cells, apart from the chromosome region, display a particularly distinct, smooth to fine-granular, stain. There is a risk of confusion with autoantibodies against PCNA (cyclin-1): These also only stain part of the cell nuclei, but the mitotic cells remain dark. Very fine dots are detected in the centromere region of the mitotic cells, which are reminiscent of autoantibodies against centromeres, but are much more delicate (in this case, the antigen appears to attach to the chromosomes in points), while the centromeres of the interphase nuclei are not stained.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Autoantibodies against CENP-F are generally not specifically analysed, rather they are detected as an incidental finding.

Diagnostic value. A malignant underlying disease exists in 50% of patients with antibodies against CENP-F, which include a range of possible tumours. In particular, high-titer sera with anti-CEPNP-F antibodies should give rise to an oncological evaluation.

Literature.

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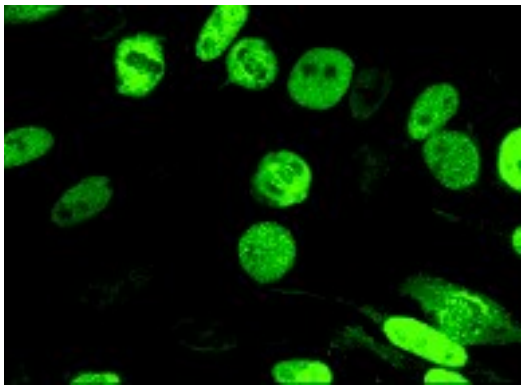

Autoantibodies against CENP-F. Fig. 1. Substrate: HEp-2 cells

Autoantibodies against citrullinated peptides

W. Stöcker, W. Schlumberger

Synonym(s). CCP antibodies; autoantibodies against cyclic citrullinated peptides (CCP)

Definition. Autoantibodies against cyclic citrullinated peptides (CCP) are directed against annular synthetic peptides that contain the amino acid citrulline. Citrulline is located in an exposed position due to the cyclisation of the peptide and so appears to be particularly accessible to the corresponding autoantibodies.

Function and pathophysiology. Autoantibodies against proteins, which contain the rare amino acid citrulline, are associated with rheumatoid arthritis (RA). Citrulline does not belong to the group of amino acids that are coded by human DNA, it occurs posttranslationally by deaminating arginine. The reaction is catalysed by peptidyl arginine deiminase (PAD enzyme). Citrullinated proteins could also be identified in the inflamed synovial mucous membrane of RA patients, but not in healthy tissue. It must be assumed that citrullinated proteins, e.g. citrullinated α-enolase (CEP-1) are targets of autoimmune reactions in RA and, in this respect, are involved in inflammatory reactions and tissue destruction (see also autoantibodies against Sa).
Antibodies against citrullinated peptides therefore presumably have a closer aetiological disease relationship than the rheumatoid factors, which have been know about for longer. These display a low diagnostic specificity and also occur with other rheumatic diseases, in infectious diseases and in healthy patients. By contrast, antibodies against CCP are found virtually exclusively with rheumatoid arthritis.

Sample material. Serum, plasma, puncture fluid

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Autoantibodies against CCP are detected by enzyme-linked immunosorbent assays (ELISA), chemiluminescence immunoassays (ChLIA) (see enzyme immunoassays) or immunoblots. Immunoglobulin class IgG is diagnostically relevant. Various citrullinated proteins can essentially act as antigens to detect RA-specific autoantibodies in an ELISA and ChLIA.
These are likely the same antibodies that are also detected in the indirect immunofluorescence test (immunofluorescence, indirect): in this case, they are analysed as autoantibodies against “RA keratin” using rat oesophagus or as a “perinuclear factor (PNF)” with epithelial cells of the human oral mucosa as the substrates. These antibodies have long been known to be directed against filaggrin, a citrulline-containing epidermal structural protein with affinity to cytokeratin.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Rheumatoid arthritis

Diagnostic value. Autoantibodies against CCP are found virtually exclusively with rheumatoid arthritis (RA). They are often also detected in rheumatoid factor-negative rheumatoid arthritis, and vice versa. Both parameters can therefore complement one another. Anti-CCP are observed in the very early stages of the disease, often even before its onset, and have a high prognostic value: Patients with anti-CCP antibodies develop significantly more radiologically detectable joint damage than anti-CCP-negative patients.
Antibodies against CCP occur independent of rheumatoid factors. The term “seronegative RA” to identify RF-negative cases is obsolete and should no longer be used. Many studies have shown that antibodies against CCP can be detected in 20-57% of all RF-negative RA patients. The parallel detection of both antibodies therefore increases the serological detection rate in RA patients. However, compared to the rheumatoid factors, antibodies against CCP have a much higher specificity for RA (anti-CCP: 97%, RF: 62%; Table 1) with the same specificity (80%). The titer generally correlates with the severity of the disease. Antibodies against CCP predominantly belong to the IgG class. They are predictive markers, as they can be detected very early in the course of the disease in 70-80% of patients, often even several years before the initial symptoms, in the serum as well as in the synovial fluid. This means that adequate treatment can take place the earlier the diagnosis is made.
Antibodies against CCP can also be used as discriminatory test markers, for example, in order to distinguish between patients with hepatitis-associated arthropathy and patients with rheumatoid arthritis (e.g. anti-CCP-negative and RF-positive in HCV infections). The generally high significance of the anti-CCP antibody detection for diagnosing rheumatoid arthritis is limited for checks of suspected juvenile idiopathic arthritis (JIA), as antibodies against CCP only occur with a prevalence of between 2 and 12% in patients with JIA. Likewise, the detection of anti-CCP has limited suitability for monitoring RA treatment, as no uniform correlation to the disease activity could be detected.
Up to 60% of anti-CCP-positive RA patients also display autoantibodies against CEP-1. CEP-1 reacts with 37-62% of the sera of RA patients, but only with 2-3% of the sera of healthy blood donors and patients in a control group. Anti-CEP-1 antibodies therefore have a similar specificity to anti-CCP antibodies, but with a lower prevalence. Anti-CEP-1 antibodies may characterise a subtype of RA, which is associated with certain genetic (HLA-DRB1 “shared epitope” alleles and a PTNP22 polymorphism) and environmental risk factors, such as smoking. Associations between anti-CEP-1 antibodies and erosive RA as well as RA with lung involvement have also been described. Autoantibodies against CEP-1 can also occur in the case of an infection with *Pophyromonas gingivalis*, the main cause of periodontitis. *P. gingivalis* expresses a separate PAD enzyme, which can citrullinate endogenous as well as human proteins and it has been shown that anti-CEP 1 antibodies from the serum of RA patients cross-react with the citrullinated enolase of *P. gingivalis*. In fact, RA and periodontitis have a similar pathophysiology and similar risk factors; they often occur together.

Autoantibodies against citrullinated peptides. Tab. 1. Prevalence of autoantibodies against citrullinated peptides and rheumatoid factors

Prevalence	Autoantibodies against citrullinated peptides	Rheumatoid factors
Rheumatoid arthritis	79	75
Other arthropathies	6	22
Systemic lupus erythematosus	8	46

Sjögren's syndrome	3	73
Scleroderma	5	25
Polymyositis/ Dermatomyositis	0	27
Autoimmune thyroiditis	0	20
Lyme disease	2	22
Viraemia	1	62
Healthy blood donor	0	5
Specificity for rheumatoid arthritis	98	63

Literature.

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Fisher BA, Plant D, Brode M, van Vollenhoven RF, Mathsson L, Symmons D, Lundberg K, Rönnelid J, Venables PJ (2011) Antibodies to citrullinated α-enolase peptide 1 and clinical and radiological outcomes in rheumatoid arthritis. Ann Rheum Dis 70:1095-1098.

Autoantibodies against desmosomes

W. STÖCKER

Synonym(s). Autoantibodies against prickle cell desmosomes; autoantibodies against desmoglein; autoantibodies against intercellular substance; desmoglein autoantibodies (see also autoimmune blistering disease-associated autoantibodies).

Definition. Autoantibodies against components of the desmosomes of epithelial keratinocytes

Function and pathophysiology. Target antigens are the glycoproteins desmoglein (Dsg) 1 and 3 – calcium-dependent adhesion molecules (cadherins) important for the cohesion of the epithelial cell bond. The materno-foetal transmissibility of the disease, among other things, indicates that these autoantibodies play a pathogenic role.
The disruption of the desmoglein-mediated cell-to-cell contacts by autoantibodies against desmoglein forms the pathophysiological basis for the blistering of the skin and mucous membrane observed with pemphigus. Numerous plasma cells, which secrete the autoantibodies against desmoglein, can be found in the subcutis of the affected skin area. These antibodies penetrate into the basement membrane and occupy the desmosomes in the epithelium, in which case they lose their “adhesive power”: intraepithelial blisters form.

Sample material. Serum or plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. In-vivo-bound antibodies are detected by direct immunofluorescence in the affected areas of the skin. Indirect immunofluorescence (immunofluorescence, indirect) and ELISA (enzyme-linked immunosorbent assay) predominate when detecting serum antibodies.
Standard substrates for the indirect immunofluorescence test are primate oesophagus, epidermis or tongue (Fig. 1, Fig. 2), dilution of 1:10, 1:100 and 1:1.000 in parallel. The “intercellular substance” is fluorescent in the case of a positive intraepithelial result: A mostly honeycomb-like, partially granular, extranuclear staining of the prickle cells.
However, it is difficult to distinguish between pemphigus foliaceus (reaction exclusively with Dsg1) and pemphigus vulgaris (Dsg3 alone or Dsg1 and Dsg3) with tissue sections and the samples are often also overlaid by non-specific reactions (e.g. by antibodies against keratin). These days, the use of recombinant cell substrates belongs to the state-of-the-art, one with each of the relevant target antigens Dsg1 and Dsg3. Both are synthesised in human cell lines in which they undergo species-faithful and authentic posttranslational modification. Together with the tissue sections, the transfected cells form a highly diagnostic mosaic, which enables a prima-vista diagnosis in a single assay.
Corresponding antigens are also used to produce modern ELISA reagents. This leads to sensitivities of 96.0% (Dsg1) and 100% (Dsg3) with specificities of 99.1% (Dsg1) and 99.6% (Dsg3) respectively.
The reagents are equally suited for primary diagnostics, to assess the course of a disease and for treatment monitoring.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Pemphigus vulgaris is a prognostically serious disease of the squamous epithelium-bearing skin and mucous membrane, which is characterised by acantholysis. It mostly affects adults between 30 and 60 years of age, but newborns can also catch the disease as a result of the diaplacental transmission of antibodies. In pemphigus foliaceus, autoantibodies against Dsg1 are present, while in pemphigus vulgaris, this either involves autoantibodies against Dsg3 alone or against both Dsg1 and Dsg3.
The pemphigus group also includes: Pemphigus vegetans, herpetiformis, erythematosus as well as paraneoplastic, drug-induced and IgA pemphigus (autoimmune blistering disease-associated autoantibodies).

Interpretation. Patients with pemphigus foliaceus display antibodies against Dsg1 and blistering is restricted to the skin.
In pemphigus vulgaris, only autoantibodies against Dsg3 can initially be detected and, in this stage, the diseases predominantly manifests in the mucosa. However, as the disease progresses, antibodies against Dsg1 also develop in over half of those affected, in which case the epidermis is then also involved in the disease. The antibody titer correlates with the disease activity. In rare cases, antibodies against Dsg1 and Dsg3 can also be detected after burns or in the case of drug exanthema.

Literature.

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Legends

Autoantibodies against desmosomes. Fig. 1. Substrate: primate oesophagus

Autoantibodies against desmosomes. Fig. 2nd Substrate: primate tongue

Synonym(s). DFS70 antibodies, anti-DFS70 antibodies, LEDGF/p75 antibodies, anti-LEDGF/p75 antibodies

Definition. Autoantibodies against the nuclear dense fine speckled 70 antigen (DFS70), also known as the lens epithelium-derived growth factor (LEDGF/p75). The protein acts as a transcriptional co-activator.

Pathophysiology. DFS70 is expressed in a large number of different human tissues. It presumably plays a role in the cellular stress reaction. Moreover, the protein has also been identified as an important co-factor for the integration of HIV-1 into the host genome. Antibodies against DFS70 occur in healthy persons as well as in connection with various diseases (atopic dermatitis, asthma, Vogt–Koyanagi–Harada disease, interstitial cystitis as well as prostate cancer and, occasionally, in collagenosis). The antibodies are not disease-specific. They were first described in the 1990s as part of a study on antinuclear antibodies in interstitial cystitis.

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Autoantibodies against DFS70 are primarily detected on HEp-2 cells using indirect immunofluorescence (immunofluorescence, indirect) (gold standard, Fig. 1). In addition, monospecific methods, such as enzyme-linked immunosorbent assays or immunoblots with recombinant antigen can be used as the confirmation test.

Diagnostic value. The dense fine-granular fluorescence pattern of the anti-DFS70 antibodies on HEp-2 cells in indirect immunofluorescence assays can be found in the nucleoplasm of the interphase cells as well as in mitotic cells. These must be distinguished from the homogeneous and granular patterns of other ANA.

The antibodies are often also found in healthy blood donors. In patients with systemic rheumatic autoimmune diseases, anti-DFS70 antibodies often also occur in parallel with other disease-related ANA; only rarely do they have an independent ANA specificity. Anti-DFS70 are therefore also considered exclusion markers for systemic rheumatic autoimmune diseases in ANA-positive persons. In any case, a careful differentiation of the autoantibodies is necessary; a positive anti-DFS70 finding can at least explain part of the ANA pattern in the indirect immunofluorescence assay, which cannot be assigned to a disease-specific antibody.

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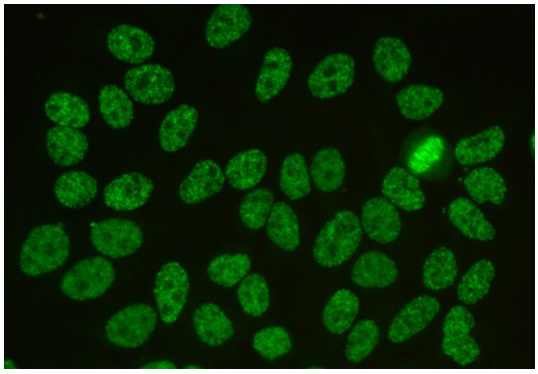

Autoantibodies against DFS70. Fig. 1. Substrate: HEp-2 cells

Autoantibodies against double-stranded DNA

W. Stöcker, W. Schlumberger

Synonym(s). Double-stranded DNA antibodies; anti-dsDNA antibodies; autoantibodies against dsDNA

Definition. A distinction is essentially made between two types of autoantibodies against DNA: antibodies against double-stranded, native DNA (double-stranded-DNA, dsDNA, nDNA) and antibodies against single-stranded denatured DNA (single-stranded-DNA, ssDNA). Antibodies against dsDNA react with epitopes in the (outer) deoxyribose phosphate backbone of the DNA. By contrast, antibodies against ssDNA primarily bond with epitopes from the purine and pyrimidine bases.

Function and pathophysiology. The involvement of antibodies against double-stranded DNA in the pathogenesis of systemic lupus erythematosus has been largely confirmed: During the course of the disease, immunocomplexes consisting of double-stranded DNA and the corresponding autoantibodies are also deposited in the capillaries of the subcutis, the kidneys and other organs. Here, they lead to tissue damage by activating the complement system.

Sample material. Serum or plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Autoantibodies against dsDNA can be analysed with indirect immunofluorescence (immunofluorescence, indirect), enzyme immunoassays (enzyme-linked immunosorbent assay [ELISA], chemiluminescence immunoassay [ChLIA]) or radioimmunoassay (RIA).
The standard substrate for immunofluorescence is the haemoflagellate *Crithidia luciliae*. This possesses a giant mitochondrion containing dsDNA (kinetoplast), which does not show any of the other antigens present in the cell nuclei. Antibodies reacting with the kinetoplast are therefore only directed against dsDNA. In *Crithidia luciliae* they create a homogeneous, in some cases, rimmed fluorescence of the kinetoplasts. A reaction in the cell nuclei is not analysed; the fluorescence of the basal body of the flagellum is insignificant. Autoantibodies against single-stranded DNA cannot stain the kinetoplasts (Fig. 1).
In HEp-2 cells, autoantibodies against dsDNA display a homogeneous fluorescence of the cell nuclei. The precipitated chromosomes of the mitotic cells are highlighted and the area around the chromosomes is dark. A peripheral fluorescence ("rim") described by many authors is due to the artefacts of the substrate (Fig. 2). A homogenous fluorescence of the cell nuclei is also displayed on primate liver (Fig. 3).
When detecting antibodies against dsDNA, the sensitivity is much higher with *Crithidia luciliae* than with HEp-2 cells or frozen sections. The higher antigen density in the substrate means that the sera can be diluted by a factor of 10 more with *Crithidia luciliae* than with HEp-2 cells when using the standard ANA solution. The immunofluorescence test with *Crithidia luciliae* is highly specific for systemic lupus erythematosus (SLE): Antibody titers ≥ 1:10 are evidence of the disease if the relevant symptoms exist. However, the sensitivity of the immunofluorescence test with Crithidia luciliae is not as high as with other test methods. Biochemically processed dsDNA is used for the enzyme immunoassays and RIA, where artificial epitopes may be exposed from inside the DNA during preparation. This may occasionally lead to non-specific positive reactions by antibodies against ssDNA. The specificity of the test systems is highly dependent on the careful preparation of the dsDNA used and eukaryotically expressed DNA tends to display fewer non-specific reactions than bacterially expressed DNA. When setting up the ELISA, one of the great challenges is to link the isolated dsDNA to the surface of the reagent wells. Poly-L-lysine and protamine sulphate are primarily used as the linking substance, but they often lead to false-positive reactions. Nucleosomes also have a distinct adhesive capacity, which can be utilised to coat surfaces with DNA without a decrease in specificity, as nucleosomes are also an exclusive target antigen of the autoantibodies with SLE. In an SLE panel by Biesen (2008), a specificity of 98.15% with a sensitivity of 66.7% was determined for a corresponding ELISA test system (anti-dsDNA RIA according to Farr: 55.6%, conventional anti-dsDNA ELISA: 41.5%).

International standard. The standards used in ELISA, ChLIA and RIA were generally calibrated with the World Health Organization's (WHO) international reference serum Wo/80, but this is no longer available. However, an independent standardisation of the results could not be achieved by this, as numerous other influencing variables play a role in determining the results. This is reflected in the fact that the limits recommended by various manufacturers differ significantly.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Serological diagnostics of systemic lupus erythematosus

Diagnostic value. Autoantibodies against dsDNA are exclusively found in systemic lupus erythematosus, in 60-90% of cases, depending on the method of analysis and disease activity.
Its high specificity means that the presence of anti-dsDNA antibodies is one of the most important criteria for diagnosing SLE. Apparently healthy persons with antibodies against dsDNA develop this disease within 5 years of investigation in 85% of cases. Given that the concentration of the antibody correlates with the disease activity, titer determinations are suitable for monitoring treatment. However, systemic lupus erythematosus cannot be ruled out if no anti-dsDNA antibodies are detected.
Besides autoantibodies against dsDNA, if SLE is suspected, antibodies against nucleosomes, autoantibodies against Sm, autoantibodies against SS-A, autoantibodies against ribosomal phosphoproteins, autoantibodies against cardiolipin and autoantibodies against β2 glycoprotein must also be analysed; one or more of these antibodies are detected in over 90% of cases with active SLE.

Literature.

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Legend

Autoantibodies against double-stranded DNA. Fig. 1. Substrate: *Crithidia luciliae*

Autoantibodies against double-stranded DNA. Fig. 2nd Substrate: HEp2 cells

Autoantibodies against double-stranded DNA. Fig. 3. Substrate: primate liver

Synonym(s). DPPX autoantibodies, anti-DPPX antibodies

Definition. Autoantibodies against dipeptidyl-peptidase-like protein-6 (DPPX), an accessory subunit of the Kv4.2 potassium channel.

Pathophysiology. The voltage-gated Kv4 potassium channels are particularly expressed in the somata and dendrites of the neurons of the central nervous system. DPPX interacts with the KV4.2 potassium channel and influences its functional surface expression and electrophysiological properties.

Patients with autoantibodies against DPPX display symptoms of progressive encephalomyelitis, in some cases in combination with severe gastrointestinal complaints.

Sample material. Serum, plasma, cerebrospinal fluid

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Autoantibodies against DPPX can be detected using indirect immunofluorescence (immunofluorescence, indirect): In the case of a positive finding, the granular and molecular layer reacts on the substrate cerebellum and the translucent layer on the substrate hippocampus (Fig. 1, 2). Transfected HEK cells, which express recombinant DPPX are suitable for the monospecific detection of autoantibodies (Fig. 3).

Diagnostic value. Anti-DPPX antibodies were first found in 2013 in 4 patients with progressive encephalomyelitis, whose clinical symptoms included agitation, hallucinations, states of confusion, myoclonus, tremors and fits. In addition, 3 of the patients had severe diarrhoea. Subsequently, 3 patients with anti-DPPX antibodies and progressive encephalomyelitis with rigidity and myoclonus (PERM) were described. In a study with 20 anti-DPPX-positive patients, various manifestations of encephalitis (amnesia, delirium, psychosis, fits, ataxia, dysphagia, dysarthria), the hyperexcitability of the central nervous system (myoclonus, excessive nervousness, diffuse rigidity and hyperreflexia) and dysautonomia, especially in the gastrointestinal system and the bladder were identified. Malignant B-cell neoplasia was diagnosed in 2 patients.

Anti-DPPX antibodies are another important parameter in differential diagnostics of neurological diseases with central hyperexcitability and/or encephalomyelitis. Parallel analysis of additional neuronal antibodies is advised in order to obtain a rapid diagnosis. In most cases, immunotherapy leads to prompt improvement of the symptoms.

Literatur

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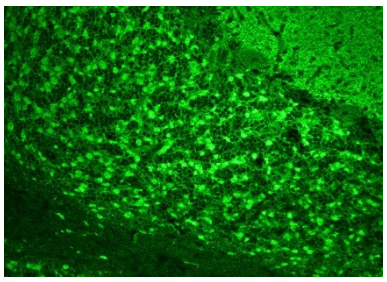
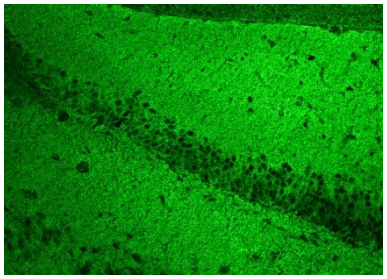
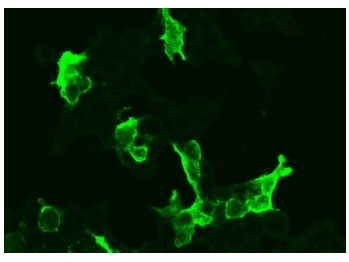


Autoantibodies against DPPX. Fig. 1. Substrate: cerebellum (rat)



Autoantibodies against DPPX. Fig. 2nd Substrate: hippocampus (rat)



Autoantibodies against DPPX. Fig. 3. Substrate: transfected cells

Autoantibodies against single-stranded DNA

W. Stöcker, W. Schlumberger

Synonym(s). Autoantibodies against ssDNA; anti-ssDNA antibodies; anti-ssDNA antibodies

Definition. Autoantibodies against DNA can be divided into two groups: antibodies against double-stranded, native DNA (double-stranded-DNA, dsDNA, nDNA; autoantibodies against double-stranded DNA) and antibodies against single-stranded denatured DNA (single-stranded-DNA, ssDNA). Antibodies against dsDNA react with epitopes that are located in the (outer) deoxyribose phosphate backbone of the DNA, while antibodies against ssDNA predominantly bind to epitopes from the area of the purine and pyrimidine bases.

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Autoantibodies against ssDNA primarily belong to immunoglobulin class IgG and are detected using enzyme immunoassays with heat-denatured DNA in the solid phase. The species source for purifying the DNA is insignificant, as DNA is a highly conservative structure and should be free of associated proteins.
Antibodies against ssDNA are not detected with HEp-2 cells or with *Crithidia luciliae* with indirect immunofluorescence (immunofluorescence, indirect), because the DNA is present in its native form and the corresponding epitopes are largely concealed.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. None, as antibodies against ssDNA can occur in various diseases and discriminatory tests are of no use.

Diagnostic value. The detection of autoantibodies against ssDNA does not play a significant role in diagnostics. While autoantibodies against dsDNA are virtually exclusively found in patients with systemic lupus erythematosus (SLE), autoantibodies against ssDNA are also present in many other rheumatic diseases with a higher prevalence (Tab. 1).

Autoantibodies against elastin

W. STÖCKER

Synonym(s). Elastin-reactive autoantibodies

Definition. The collective reference for autoantibodies directed against tropoelastin, elastin fibres and their degradation products (α-elastin).

Function and pathophysiology. The degeneration of elastin fibres is discussed as a possible cause of vascular damage. The involvement of autoantibodies against elastin in the pathogenesis of various forms of vasculitis has not yet been confirmed.

Sample material. Serum or plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Enzyme-linked immunosorbent assay, indirect immunofluorescence assay (immunofluorescence, indirect)

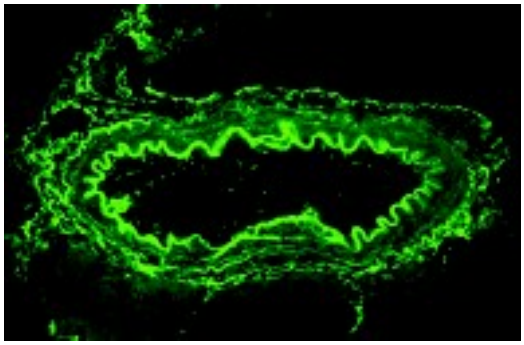
Reference range — Adults. Not known

Reference range — Children. Not known

Indication. Vasculitis, 5% in multiple sclerosis.

Interpretation. Autoantibodies against elastin display a typical wave-like staining of the laminae elastica interna and externa on arterial sections in immunofluorescence (Fig. 1).
To date, autoantibodies against elastin are only investigated for scientific research purposes.

Literature.
Colburn KK, Langga-Shariffi E, Kelly GT, Malto MC, Sandberg LB, Baydanoff S, Green LM (2003) Abnormalities of serum antielastin antibodies in connective tissue diseases. J Investig Med Mar 51:104–109



Autoantibodies against elastin. Fig. 1. Substrate: rat kidney.

Autoantibodies against enterocytes

W. STÖCKER

Synonym(s). Colon epithelium antibodies

Definition. Autoantibodies against antigens of the intestinal epithelium. In contrast to the much more diagnostically important autoantibodies against intestinal goblet cells, these antibodies are of no diagnostic relevance.

Function and pathophysiology. The intestine's immune system seems to produce antibodies against all possible antigens present in the intestinal wall, especially in Crohn's diseases, as a result of disease-specific autoimmune reactions, due to the adjuvant effect of the immune system's specific conflict with the relevant autoantigen. Similar to antibodies against the colonic epithelium, in Crohn's disease, antibodies against *Saccharomyces cerevisiae* (ASCA) and antibodies against various pathogens can also be found in the patients' serum.

Sample material. Serum or plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Indirect immunofluorescence (immunofluorescence, indirect) is used to analyse autoantibodies against enterocytes; the starting dilution is 1:10. The cytoplasm in the intestine's epithelial cells reacts, including the goblet cells. The same affinity exists to the various sections of the intestine.

Interpretation. Under identical incubation conditions, 39% positive results are obtained for Crohn's disease (autoantibodies against pancreatic secretion occur with the same prevalence, but do not cross-react; cross-reactivity), while this value is 33% for ulcerative colitis, 10% for coeliac disease and 14% in healthy people.

Diagnostic value. The low disease specificity of these autoantibodies means that they do not have to be considered any further.

Literature.

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Autoantibodies against epidermal basement membrane

W. STÖCKER

Synonym(s). Epidermal basement membrane antibodies; autoantibodies against hemidesmosomes (see also autoantibodies against desmosomes and autoimmune blistering disease-associated antibodies)

Definition. The epidermal basement membrane ("dermoepidermal junction zone"; DEJ) contains several potential targets for autoimmune reactions, which can manifest in the form of different bullous autoimmune dermatoses. The most important target antigens identified to date are:

- Transmembrane protein BP180 (collagen type XVII)
- Intracellular protein BP230

Both are components of the hemidesmosomal plaque. Other target antigens are laminin 5 (laminin 332), laminin γ1 (p200), β4 integrin and collagen type VII.

BP180 is a transmembrane glycoprotein with an intracellular localised C-terminus and an extracellular N-terminus. The ectodomain consists of 15 collagenous and 16 non-collagenous (NC) domains, of which NC16A (extracellular) is directly adjacent to the keratinocyte membrane and is the most important immunogenic epitope for the autoantibodies in bullous pemphigoid. However, BP230 was the first target antigen to be identified for bullous pemphigoid ("BP-AG-1"). Its C-terminus domain contributes to the anchoring of the keratin filament system. The N-terminus end of BP230 is important for its integration into the hemidesmosomes, it interacts with BP180 and the β4 subunit of the α6β4 integrin.

Function and pathophysiology. The autoantibodies are formed by the plasma cells in the subcutis of the affected skin area and diffuse in the direction of the epidermal basement membrane. This is where the binding of the autoantibodies leads to the activation of complement and subsequently to inflammatory reactions with subepidermal blistering; by contrast, pemphigus vulgaris (target: desmosomes) leads to the formation of intraepithelial blisters.

Sample material. Serum or plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. In immunofluorescence, frozen sections of the oesophagus and tongue display a linear colouring between the stratum basale and the connective tissue (Fig. 1, Fig. 2). The starting dilution is 1:10. Monospecific enzyme-linked immunosorbent assays are an alternative to indirect immunofluorescence (immunofluorescence, indirect). The serum concentration of the autoantibodies against BP180 correlates with the disease activity of bullous pemphigoid.

Recombinant designer antigens have also been developed to detect autoantibodies against BP180, which only display the diagnostically relevant target structure of BP180, the NC16A domain, and do so many times in succession. These antigens are used in the ELISA or in the indirect immunofluorescence test and provide a sensitivity of 90% with an unprecedented specificity of 98% [Sitaru (2007)]. Similar diagnostics based on recombinant epitopes that only represent the relevant target antigens are also available for detecting antibodies against BP230

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Pemphigoid diseases are associated with various autoantibodies against epidermal basement membrane (subepidermal blistering; Tab. 1)

Autoantibodies against epidermal basement membrane. Tab. 1. Autoantibodies against epidermal basement membrane associated with pemphigoid diseases

Disease	Target antigen
Bullous pemphigoid	BP180, BP230
Pemphigoid gestationis	BP180 (BP230 only in exceptional cases)
Anti-p200-pemphigoid	p200
Mucosal pemphigoid	BP180, laminin 5, beta-4-integrin and BP230
Scarring pemphigoid	Laminin 5 (laminin 332) and C-terminus end of the BP180
Linear IgA dermatosis	Predominantly proteolytic fragments of the entire ectodomain of BP180
Lichen planuspemphigoides	BP180, BP230
Epidermolysis bullosa acquisita	Collagen VII (anchoring fibrils)
Bullous systemic lupus erythematosus	see autoantibodies against cell nuclei

From a differential diagnostic perspective, pemphigoid diseases must primarily be distinguished from pemphigus vulgaris, which is caused by autoantibodies against desmosomes. Dermatitis herpetiformis (Duhring disease) (autoantibodies against epidermal transglutaminase, autoantibodies against tissue transglutaminase as well as Z-AGFA: antibodies against gliadin; see also autoimmune blistering disease-associated autoantibodies) must also be ruled out.

Literature.

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Legends

Autoantibodies against epidermal basement membrane. Fig. 1. Substrate: primate oesophagus

Autoantibodies against epidermal basement membrane. Fig. 2. Substrate: primate tongue

Autoantibodies against erythrocytic antigens

W. Stöcker

Synonym(s). Erythrocyte antibodies; EA; red blood cell antibodies

Definition. Antibodies that are directed against antigens on erythrocytes

Synthesis/distribution/decomposition/elimination. Natural EA normally occur in individuals who lack the corresponding antigen without a clear reason for the immunisation (most important representatives: anti-A/B/AB).
Irregular EA (representatives: anti-rhesus-D, -kell) are formed as an immune reaction to clear antigen contact (blood transfusion, pregnancy).

Function and pathophysiology. EA with specificity against blood type characteristics (blood typing), which the individual does *not* have themselves (Alloantibodies), can react with transfused erythrocytes and cause haemolytic transfusion reactions. If antibodies formed by pregnant mothers are transferred via the placenta and react with the foetus' blood type characteristics, this may lead to foetal haemolysis haemolytic disease of the newborn).
EA with specificity for blood type characteristics, which the individual has themselves (autoantibodies), can lead to autoimmune haemolytic anaemia. These kinds of antibodies often occur idiopathically or in connection with other diseases (e.g. systemic lupus erythematosus). Various drugs can also induce erythrocytic autoantibodies.
In many cases, this can result in the accelerated breakdown of EA-loaded erythrocytes, either intravascular or in the reticuloendothelial system of the liver and spleen (intravascular or extravascular haemolysis). In particular, the intravascular haemolysis may become highly acute (within just a few minutes). This is associated with life-threatening systemic reactions (example: acute transfusion reaction after the inadvertent administration of an AB0-incompatible blood reserve).

Sample material. Indirect Coombs test: Serum or plasma. Haemolysis test: Serum. Direct Coombs test: Red cell sediment from plasma.

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Indirect Coombs test: The incubation of antigen-positive test erythrocytes with patient serum; binding of the EA to the erythrocytes; agglutination of the EA-loaded erythrocytes with anti-human immunoglobulin to detect alloantibodies. This is generally carried out using test erythrocytes in the O blood type so that the irregular antibodies to be detected are not naturally concealed by natural anti-A and anti-B antibodies.
Direct Coombs test: Detection of EA bound to patient erythrocytes in-vivo via agglutination by anti-human immunoglobulin, potentially with subsequent elution and specificity determination to detect autoantibodies.
The use of enzymes to increase the sensitivity is no longer considered appropriate. The same applies for tests in the "cooking salt phase" without anti-human globulin.
Occasionally, haemolysis tests are used to detect the anti-A/B EA instead of the agglutination tests.

Reference range — Adults. Indirect Coombs test (irregular antibodies): negative. Direct Coombs test: negative. Anti-A/B haemolysis test: Reference values must be determined for the specific laboratory based on a control group.

Indication. Irregular (allo)antibodies against EA are detected before any blood transfusion and as part of prenatal care (indirect Coombs test). A direct Coombs test is carried out to detect immunohaemolytic anaemia. Both analyses take place if a haemolytic transfusion reaction is suspected.

Literature.
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Autoantibodies against extractable nuclear antigens

W. Stöcker, W. Schlumberger

Synonym(s). Anti-ENA

Definition. Autoantibodies against extractable nuclear antigens (ENA) are a subgroup of the autoantibodies against cell nuclei. They react with certain nuclear proteins, which can be extracted from the thymus, spleen and cultivated cells with physiological buffer solutions. These target antigens were previously classified under the (now redundant) term “extractable nuclear antigens” (ENA). In the narrower sense, these include ribonucleoproteins U1-nRNP (autoantibodies against U1-RNP), Sm (autoantibodies against Sm) and Ro/SS-A (autoantibodies against SS-A), the phosphoprotein La/SS-B (autoantibodies against SS-B) and Scl-70 (autoantibodies against Scl-0). In general, Jo-1 (autoantibodies against aminoacyl-tRNA-synthetase) are also included in the ENA, even though this relates to a cytoplasmic antigen.

Literature.

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Autoantibodies against F-actin

W. Stöcker

Synonym(s). Autoantibodies against filamentous actin (F-actin); anti-F-actin antibodies

Definition. Autoantibodies against filamentous actin (F-actin) of the cytoskeletal microfilament and the muscle fibres are a subgroup of autoantibodies against smooth muscles ("anti-smooth muscle antibody", ASMA). In contrast to other ASMA, autoantibodies against F-actin are extremely specific markers of type I autoimmune hepatitis (AIH).

Function and pathophysiology. High concentrations of the autoantibodies against smooth muscles indicate autoimmune hepatitis (AIH). Part of the antibodies is directed against conformational epitopes of the F-actin. Actin as well as actin-binding proteins, such as filamin, actinin or tropomyosin, has been described as molecular targets of autoantibodies against F-actin.

Analytics. Autoantibodies against F-actin can be detected by indirect immunofluorescence (immunofluorescence, indirect) with vascular smooth muscle cells (VSM47) as the substrate and can be identified by the typical microfilamentous fluorescence pattern. They typically stain the vessel walls (smooth musculature) as well as the glomeruli and tubules of the kidneys.

Sample material. Serum

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Diagnostic value. The detection of autoantibodies against F-actin is of particular importance for diagnosing autoimmune hepatitis (AIH, prevalence of about 50%), excluding a combined liver disease (overlap syndrome) and distinguishing between AIH and alcohol- or drug-induced cirrhosis and other chronic inflammations of the liver, such as virus-induced hepatitis, primary biliary cirrhosis of the liver (PBC) and primary sclerosing cholangitis (PSC; see also autoantibodies against smooth muscles).

Literature.

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Synonym(s). Flotillin autoantibodies; anti-flotillin antibodies

Definition. Autoantibodies against flotillin-1/2 complexes. Flotillin-1 and -2 are peripheral membrane proteins, which have a higher rate of expression in the muscle, fat and lung tissue as well as in the brain.

Pathophysiology. Flotillin-1 and -2 play a role in the growth and regeneration of damaged axons in retinal ganglion cells. In addition, functions in endocytosis, signal transduction and processing of the amyloid precursor protein in neurons have been described. Little information is available on the function of the flotillin-1/2 homo- or heterocomplexes.

Antibodies against flotillin-1/2 complexes have been reported in patients with multiple sclerosis and optical neuritis. A possible pathogenetic role is being discussed.

Sample material. Serum, plasma, cerebrospinal fluid

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Anti-flotillin antibodies are detected using indirect immunofluorescence (immunofluorescence, indirect). A fine granular staining of the molecular layer can be detected on frozen sections of the hippocampus or cerebellum (rat, primate) in the presence of specific IgG (Fig. 1, Fig. 2). Transfected HEK cells, which express recombinant flotillin-1 and -2, are suitable for the monospecific detection of autoantibodies (Fig. 3).

Diagnostic value. Anti-flotillin antibodies were first found in 14 patients with multiple sclerosis (MS), often in connection with encephalomyelitis or an inflammation of the optic nerve. The patients did not display any antibodies against aquaporin-4 or myelin-oligodendrocyte glycoprotein (autoantibodies against myelin-oligodendrocyte glycoprotein). Over 500 tested control samples were negative for anti-flotillin. The prevalence of the autoantibodies in MS patients is estimated at 1-2%.

Literature.

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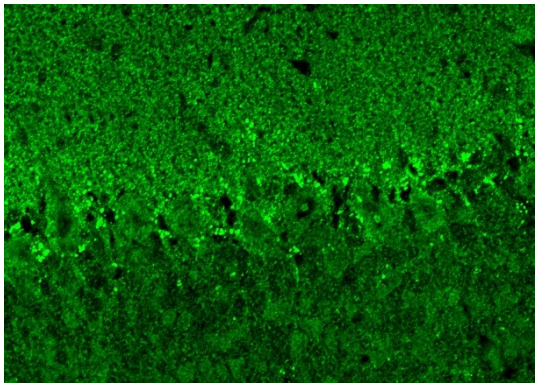
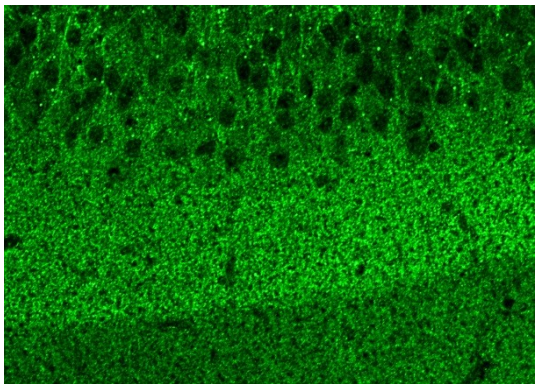
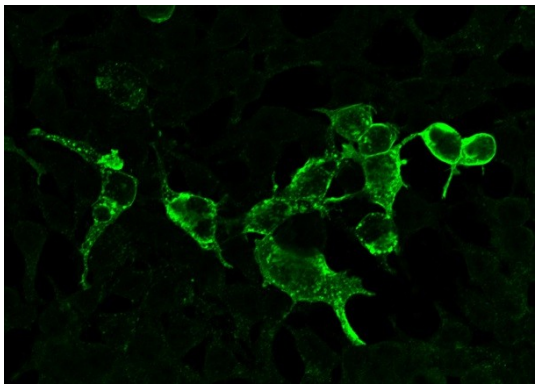


Autoantibodies against flotillin. Fig. 1. Substrate: cerebellum (rat)



Autoantibodies against flotillin. Fig. 2. Substrate: hippocampus (rat)



Autoantibodies against flotillin. Fig. 3. Substrate: transfected cells

Autoantibodies against $GABA_B$ receptors

W. Stöcker

Synonym(s). Antibodies against γ-aminobutyric acid-B receptors; anti-GABAB-receptor antibodies; GABAB-receptor antibodies; GABABR antibodies

Definition. Autoantibodies against transmembrane receptors, which are present in pre- and post-synaptic membranes of the entire central nervous system (CNS; especially the hippocampus, thalamus, cerebellum). GABAB-receptors are heterotetramers, each consisting of two GABAB1 and GABAB2 subunits. They are associated with KCTD proteins ("potassium channel tetramerization domain-containing proteins"), which determine the kinetic and pharmacological receptor properties. The immunorelevant epitopes are primarily localised in the GABAB1 subunit.

Function and pathophysiology. GABAB receptors are metabotropic G-protein-linked receptors. The binding of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) to the GABAB1 subunit leads, via a pre- and post-synaptic G-protein-mediated signal cascade, to the activation of potassium channels, to the closure of calcium channels and, via the reduction of the calcium concentration, to a reduced transmitter release from the presynapsis. The binding of specific antibodies inhibits the receptor function; the autoimmune reactions lead to limbic encephalitis (epileptic seizures, disorientation, memory deficits, etc.). This results in an increased risk of temporal lobe epilepsy. The frequent association between anti-GABAB-receptor encephalitis and small cell lung cancer (SCLC) and its ability to express synaptic proteins indicates the possibility of a tumour-induced pathological immune response against GABAB receptors.

Analytics. Autoantibodies against GABAB receptors are displayed as coarse granular fluorescence, predominantly of the stratum moleculare, in the indirect immunofluorescence test (immunofluorescence, indirect) with frozen sections of the hippocampus and the cerebellum. Monospecific detection occurs using transfected HEK-293 cells, which recombinantly express the GABAB1/GABAB2-subunits.

Sample material. Serum, plasma or cerebrospinal fluid

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Diagnostic value. Autoantibodies against GABAB-receptors are found in the serum or cerebrospinal fluid of patients with a special type of autoimmune limbic encephalitis, which is associated with the occurrence of tumours, generally SCLC in 50-80% of cases (facultative paraneoplastic). Some of the patients display additional autoantibodies (e.g. autoantibodies against thyroid peroxidase, autoantibodies against glutamate decarboxylase, autoantibodies against glial cell nuclei, autoantibodies against calcium channels). After autoantibodies against Hu, anti-GABAB-receptor antibodies are the second most common immunoreactivity in limbic encephalitis with SCLC. Positive antibody findings should therefore be followed-up with an intensive tumour screening.
The investigation of the other key autoantibodies against neuronal antigens in parallel, which, in many cases, results in a rapid and reliable (potentially unsuspected) vital diagnosis, is also advisable.
Acute therapy: Methylprednisolone i.v. and immunoglobulin concentrate or plasmapheresis.
Escalation: Cyclophosphamide and rituximab. Long-term treatment: potentially azathioprine.

Literature.

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Lancaster E, Lai M, Peng X, Hughes E, Constantinescu R, Raizer J, Friedman D, Skeen MB, Grisold W, Kimura A, Ohta K, Iizuka T, Guzman M, Graus F, Moss SJ, Balice-Gordon R, Dalmau J (2010) Antibodies to the GABAB receptor in limbic encephalitis with seizures: case series and characterisation of the antigen. Lancet Neurol 9:67–76

Wandinger KP, Klingbeil C, Gneiss C, Waters P, Dalmau J, Saschenbrecker S, Borowski K, Deisenhammer F, Vincent A, Probst C, Stöcker W (2011) Neue serologische Marker zur Differentialdiagnose der Autoimmun-Enzephalitis. J Lab Med 35:329–342

Autoantibodies against bile duct epithelium

W. STÖCKER

Synonym(s). Bile duct antibodies

Definition. Autoantibodies against the epithelium of the bile ducts

i In the immunofluorescence test (immunofluorescence, indirect) with rat or primate liver as the substrate, a characteristically smooth fluorescence of the bile duct epithelium is observed in the area of the Glisson's triad (Fig. 1). At the same time, similar fluorescence of the ductus pancreaticus and the peritoneal epithelium are always found in positive sera. These reactions were previously considered non-specific [Jeffrey (1990)], but, surprisingly, they are quite regularly found in patients with paraneoplastic autoimmune dermatoses; this is presumably due to cross-reactions (cross-reactivity) or antigen communities with plakins or plectins exist, which are also expressed in the urinary bladder's transitional epithelium (as well as in the epithelium of the gall bladder). A strong reaction of the bile ducts in the liver substrate in indirect immunofluorescence should therefore always be checked with the urinary bladder or gall bladder substrates, or with monospecific enzyme-linked immunosorbent assays (detection of antibodies against desmoplakin 1 and 2, envoplakin, periplakin and plectin). A positive result should be followed by a dermatological and internal examination (see also autoimmune blistering disease-associated autoantibodies).

Literature.

Jeffrey GP, Swanson NR, Yarred LJ et al (1990) Bile duct antibodies crossreacting with blood group antigens in primary sclerosing cholangitis. Gut 31:698–701

Legend

Autoantibodies against the bile duct. Fig. 1. Substrate: primate liver

Autoantibodies against ganglionic acetylcholine receptors

W. Stöcker, Chr. Krüger

Synonym(s). Autoantibodies against ganglionic nicotinic acetylcholine receptors; GN-AChR antibodies; antibodies against neuronal acetylcholine receptors in autonomic ganglia

Definition. Antibodies against nicotinic acetylcholine receptors in autonomic ganglia

Function and pathophysiology. Nicotinic acetylcholine receptors are membrane receptors in various areas of the nervous system and the motor end plate, which are activated by the neurotransmitter acetylcholine and belong to the group of ligand-gated ion channels. They occur in the muscle, the autonomic ganglia and the brain, but have different structures. Ganglionic nicotinic acetylcholine receptors mediate the rapid synaptic transmission in the parasympathetic and enteric autonomic ganglia, the autoantibodies are directed against the α3-subtype of the receptor and can disrupt the cholinergic synaptic transmission processes. The syndrome associated with the antibodies is referred to as “autoimmune autonomic ganglionopathy” (AAG).

Analytics. Anti-GN-AChR antibodies are detected using radioreceptor assays: After incubating the patient serum with 125I-epibatidine-labelled, ganglionic nicotinic acetylcholine receptors, a secondary antibody is used for precipitation and the radioactivity in the precipitate is measured.

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Diagnostic value. Autoantibodies against ganglionic nicotinic acetylcholine receptors cause autoimmune autonomic ganglionopathy (AAG) and are found in the serum of up to 50% of all patients with AAG. The most important clinical features of AAG are orthostatic hypotony, gastrointestinal motility disturbances, anhidrosis, bladder function disorder as well as Sjögren’s syndrome. The detection of the autoantibodies is used to distinguish it from other types of dysautonomia (see also autoantibodies against acetylcholine receptors).

Literature.

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Autoantibodies against gangliosides

W. Stöcker, W. Schlumberger

Synonym(s). Ganglioside antibodies

Definition. Autoantibodies against gangliosides (anti-GM1, -GM2, -GM3, -GD1a, -GD1b, -GQ1b) are found in patients with peripheral neuropathies. For example, these diseases include Guillain-Barré syndrome (GBS), chronic-inflammatory demyelinating polyneuropathy (CIDP), multifocal motor neuropathy (MMN) and the Miller-Fisher syndrome (MFS; a subtype of GBS).
(GM1,2,3: monosialogangliosides; GD1a,b; disialogangliosides; GT1b: trisialoganglioside; GQ1b: tetrasialoganglioside).

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Monospecific test methods (enzyme-linked immunosorbent assay or immunoblot) with purified gangliosides are used to detect antibodies against gangliosides.

Reference range. Negative

Interpretation. In the case of inflammatory peripheral neuropathies (GBS, CIDP, MMN or MFS), class IgG and IgM autoantibodies against gangliosides can be detected with prevalences of 20-80% [Tab. 1 and Tab. 2 (according to Meyer (2000)]. Moreover, IgG autoantibodies against GQ1b are diagnostically indicative for autoimmune-mediated inflammations in the area of the brain stem (Bickerstaff encephalitis).

Literature.

Heidenreich F (1998) Autoantibodies associated with peripheral neuropathies. In: Conrad K, Humbel RL, Meurer M, Shoenfeld Y, Tan EM (eds) Pathogenic and diagnostic relevance of autoantibodies. Pabst Science Publishers 316–327

Meyer W, Schneider B, Klotz M, Schlumberger W, Stöcker W (2000) EUROLINE ganglioside profile: A new membrane test for the detection of autoantibodies against gangliosides. In: Conrad K et al (eds) Autoantigens and Autoantibodies: Diagnostic Tools and Clues to Understanding Autoimmunity. Pabst Science Publishers 619–620

Autoantibodies against gangliosides. Tab. 1. Prevalences of IgG autoantibodies against gangliosides (%)

Patient group (number of patients)	GM1	GM2	GM3	GD1a	GD1b	GT1b	GQ1b
GBS (71)	6	1	0	0	1	0	1
CIDP (13)	0	0	8	0	0	0	0
MMN (18)	0	6	6	0	0	0	0
MFS (5)	0	0	0	0	0	0	80
Blood donors (60)	0	0	0	0	0	0	0

Autoantibodies against gangliosides. Tab. 2nd Prevalences of IgM autoantibodies against gangliosides (%)

Patient group (number of patients)	GM1	GM2	GM3	GD1a	GD1b	GT1b	GQ1b
GBS (71)	13	10	1	1	3	4	1
CIDP (13)	0	8	15	23	8	0	0
MMN (18)	28	22	17	11	11	6	0
MFS (5)	0	0	0	0	0	0	0

Blood donors (60)	3	15	0	0	0	0	0

Autoantibodies against tissue transglutaminase

W. Stöcker

Synonym(s). Tissue transglutaminase antibodies; anti-tTG antibodies; anti-endomysium antibodies

Definition. Antibodies against tissue transglutaminase (tTG) are found (generally together with antibodies against gliadin) in gluten-sensitive enteropathy (GSE, coeliac disease). They are also associated with dermatitis herpetiformis (Duhring disease), which is often associated with gluten-sensitive enteropathy.

Function and pathophysiology. In predisposed persons, gluten-sensitive enteropathy is caused by consuming gluten-containing cereal products (gluten). The disease is characterised by atrophy of the intestinal villi, chronic diarrhoea and the consequences of malabsorption. Some patients with GSE also suffer from dermatitis herpetiformis (Duhring disease), a recurrent skin disease characterised by subepidermal blisters, which can also occur independently.
The phenomena of gluten-sensitive enteropathy are only partially based on allergic reactions as a result of gluten intolerance. An additional autoimmunity is expressed by antibodies against (deaminated) gliadin fragments associated with coeliac disease (Z-AGFA; antibodies against gliadin) and, often, also autoantibodies against tissue transglutaminase. Both antibodies are practically non-existent in healthy people and patients with other bowel diseases. Anti-tTG are often also detected in the inactive stage and indicate a predisposition for the disease. Both antibodies can also be distinguishing features of dermatitis herpetiformis (Duhring disease).
Antibodies against endomysium are apparently identical to the antibodies against reticulin discovered by Seah in 1971. In 1983, Chorzelski and coworkers identified that immunoglobulin class IgA was decisive for diagnosing coeliac disease with these antibodies that react with the connective tissue. They proposed the name “anti-endomysium” and recommended primate oesophagus (lower third) as the substrate for immunofluorescence. The name was too narrowly worded, as many other tissue structures also react and oesophagus is possibly unsuitable due to the risk of confusion with autoantibodies against smooth muscles. In 1997, Dieterich et al. were able to identify tissue transglutaminase as the target antigen.

Sample material. Serum or plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. The gold standard for detecting anti-tTG is indirect immunofluorescence (immunofluorescence, indirect) with frozen sections of primate organs as the antigen substrates. The following are better suited than oesophagus: intestine, liver (Fig. 1), placenta and umbilical cord. When using the ideal substrate, bowel tissue, a positive result displays a typically membranous fluorescence of the smooth muscles as well as a honeycomb-like staining of the lamina mucosae propria; the endothelium of the blood vessels reacts simultaneously (Fig. 2). A starting dilution of the patient serum of 1:10 is recommended, while antibody titers of up to 1:1000 are not uncommon.
For the ELISA (enzyme-linked immunosorbent assay), native tissue transglutaminase from human placenta or recombinant human antigen is highly purified and used as a substrate to coat the ELISA slides. If the coating antigen is isolated from guinea pig liver, there is a risk that many coeliac disease patients are not detected due to the insufficient antigen relationship, while too many non-specific reactions also occur.
Anti-tTG-associated GSE predominantly consists of IgA, immunoglobulin class IgG, in a lower concentration, only occurs in about 50% of IgA-positive sera, while IgM does not play a role. Despite this, IgA and IgG should be analysed in parallel, as GSE is often associated with a selective deficiency of IgA. In this case (in the case of negative IgA), class IgG anti-tTG is detected in high titers. Patients with this constellation must be warned against whole-blood transfusions.
The concordance between immunofluorescence and ELISA is almost 100% for titers from 1:32, if human (native or recombinant) antigens are used in the ELISA. In addition, line blots (immunoblot) and chemiluminescence immunoassays (see also enzyme immunoassay) are now also available for diagnosing coeliac disease.

Indication. Chronic diarrhoea, failure to thrive, retarded development. Chronic dermatitis.
An anti-tTG test, together with the detection of antibodies against deaminated gliadin, is able to secure the clinical diagnosis of GSE or dermatitis herpetiformis (Duhring disease). This type of analysis is also used for relatives of patients with coeliac disease in order to detect an associated predisposition.
In Germany, manifest GSE has a prevalence of 90 cases per 100,000 inhabitants and can be clinically detected without any further measures. Diagnosing latent coeliac disease is already much more difficult, e.g. in children with a failure to thrive and retarded development. As a colonoscopy cannot always be immediately arranged and a gluten-free diet cannot always be prescribed for all of these patients, many suspected cases are not clarified and some patients suffering from coeliac disease are consequently not consistently treated. The frequency of latent gluten-sensitive enteropathy is significantly underestimated; 330-900 cases per 100,000 persons is assumed, for every diagnosed patient (90 per 100,000) there are up to 10 whose disease is not detected. Fortunately, these days, a simple laboratory test can provide clarity.

Interpretation. Anti-tTG practically do not occur in healthy people and are extremely rare in patients with other bowel diseases, while its prevalence is almost 100% in untreated GSE. Most patients with GSE also display antibodies against deaminated gliadin fragments (Z-AGFA) (prevalence of 95%). These are used to monitor the progress and a gluten-free diet or for a gluten tolerance test. While antibodies against native gliadin are often also found in healthy people, such as infants, whose diet has recently been enriched with cereals, Z-AGFA are highly specific.

Literature.
Chorzelski TP, Sulej J, Tchorzewska H et al (1983) IgA class endomysium antibodies in dermatitis herpetiformis and coeliac disease. Ann NY Acad Sci 420:325–334

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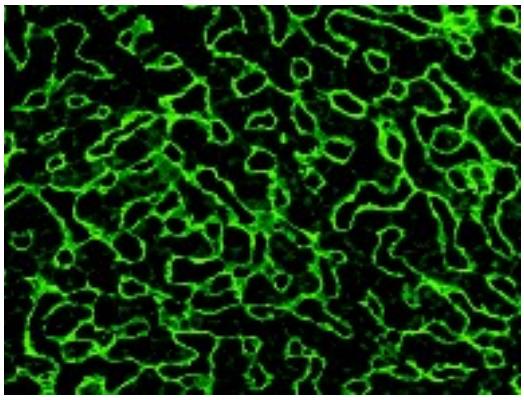
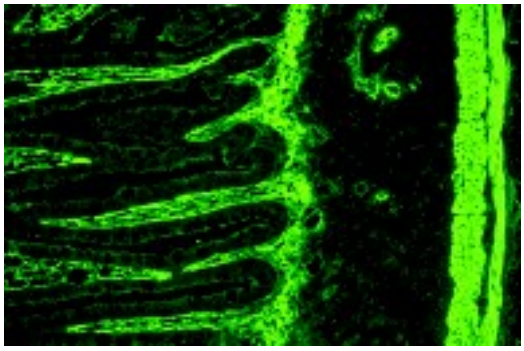


Autoantibodies against tissue transglutaminase. Fig. 1. Substrate: primate liver.



Autoantibodies against tissue transglutaminase. Fig. 2nd Substrate: primate intestine.

Autoantibodies against smooth muscles

W. STÖCKER

Synonym(s). Actin autoantibodies: SMA; ASMA

Definition. Antibodies against various smooth muscle antigens. The most important target antigens of ASMA is the protein actin; autoantibodies against F-actin.

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Frozen sections of various organs can be used to detect autoantibodies against smooth muscle using indirect immunofluorescence (immunofluorescence, indirect; starting dilution 1:100). The stomach and intestine, etc., of many species, with a muscle layer or an adequate number of arteries can be used. The portal venous sections of the Glisson's triad in rat liver also react. The standard substrate is rat stomach. In this case, ASMA display a clear cytoplasmic fluorescence of the tunica muscularis propria, the lamina muscularis mucosae, including the interglandular contractile fibrils, which stretch through the mucous membrane between the fundus glands, as well as the muscle sections of the arteries. In the case of negative samples, the contractile elements do not stain. The fluorescence of other structures is not assessed.
Part of the antibodies against smooth muscles is directed against the protein actin (autoantibodies against F-actin) and is analysed in the indirect immunofluorescence test with combinations of cell (HEp-2 cell, VSM47 cell) and tissue substrates (primate liver, rat stomach, rat kidney) (starting dilution: 1:100; see Fig. 1–5). The fluorescence of the cytoskeleton of the HEp-2 cells (individual to multiple bound fibre structures, which primarily occur in the cytoplasm) and the microfilamentous pattern on VSM47 cells (vascular smooth muscle) as well as the staining of the biliary canaliculi of the primate liver are typically displayed. To date, it has not been possible to detect antibodies againstactin, either by enzyme-linked immunosorbent assay or by Western blot, as they are directed against conformation epitopes.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Suspected autoimmune hepatitis (autoimmune (lupoid) chronic active hepatitis)

Interpretation. High concentrations of the antibodies against smooth muscle are associated with autoimmune hepatitis (AIH) with a prevalence of 70%. IgG and IgM antibody titers can correlate with the activity of the disease. Low ASMA titers are also found in patients with primary biliary cholangitis (previously: primary biliary cirrhosis, approx. 20%), alcohol-caused cirrhosis of the liver, occlusion of the bile duct and in 5% of healthy persons.
Autoantibodies against smooth muscle are also detected in infectious mononucleosis and other viral infections as well as in systemic lupus erythematosus, breast and ovarian cancers and malignant melanomas, but they do not play a diagnostic role in these cases. After viral hepatitis, the titer generally quickly decreases again.

Literature.
Johnson GD, Holborow EJ, Glynn LE (1965) Antibody to smooth muscle in patients with liver disease.Lancet 2:878–879

Legends

Autoantibodies against smooth muscle. Fig. 1. Substrate: HEp-2 cells

Autoantibodies against smooth muscle. Fig. 2. Substrate: primate liver

Autoantibodies against smooth muscle. Fig. 3. Substrate: rat stomach

Autoantibodies against smooth muscle. Fig. 4. Substrate: rat kidney

Autoantibodies against smooth muscle. Fig. 5. Substrate: rat liver

Autoantibodies against glial cell nuclei

W. STÖCKER

Synonym(s). AGNA; anti-glial-nuclear antibodies;

Definition. Autoantibodies against nuclear antigens of the Bergmann glial cells. New research findings indicate that AGNA are not identical to antibodies against SOX1, as previously assumed.

Analytics. Autoantibodies against glial cell nuclei are identified using indirect immunofluorescence (immunofluorescence, indirect) with frozen sections of primate cerebellum. They react with cell nuclei of the Bergmann glia in the Purkinje cell layer.
Due to their discriminatory testing relationship between associated diseases and other paraneoplastic neurological syndromes, AGNA are investigated in parallel with the other autoantibodies against neuronal antigens.

Sample material. Serum, plasma or cerebrospinal fluid

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Diagnostic value. Autoantibodies against glial cell nuclei are associated with the following neurological diseases: Lambert-Eaton myasthenic syndrome (LEMS), cerebellar degeneration and sensitive neuropathy. Anti-SOX1 and AGNA often occur together in paraneoplastic neuropathies, but can also occur independent of one another. Anti-SOX1 antibodies are now considered general tumour markers, especially for the presence of small cell lung cancer.

Literature.

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Autoantibodies against the glomerular basement membrane

W. Stöcker

Synonym(s). Autoantibodies against renal glomeruli; GBM (auto-)antibodies; Goodpasture antibodies

Definition. The target antigen of the Goodpasture antibodies are type IV collagen α-3 chains in the basement membrane of the renal glomeruli. These contain the relevant epitopes of the GBM antigen (NC-1 domain).

Function and pathophysiology. Goodpasture syndrome is a rare kidney disease. Clinically, it consists of a combination of rapid-progressive glomerulonephritis and haemoptysis with recurrent parenchymal haemorrhage (lung haemosiderosis). Lung haemorrhages often occur as the first sign. Both fulminant as well as abortive forms have been observed. About 70% of those affected are men. Early treatment (immunosuppression and plasmapheresis until remission) can ensure that the liver function is maintained in 60% of patients. Relapses are possible.

Sample material. Serum or plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Tissue sections of primate kidneys are used as the standard substrate to detect autoantibodies against glomerular basement membrane using indirect immunofluorescence (IIFT, immunofluorescence, indirect) (Fig. 1). At the same time, lung tissue can be used as the substrate to also examine antibodies against the basement membrane of the lung alveoli. A combination of the tissue substrates with highly purified, recombinant GBM antigen as a substrate in the IIFT allows positive reactions on the tissues to be monospecifically confirmed in the same assay. The starting dilution is 1:10. During titration, for this antibody, pure PBS is not used for dilution, as this inevitably leads to non-specific positive reactions. A dilution of 1:10 in PBS/Tween prediluted normal human serum is used.
Monospecific 7enzyme immunoassays (enzyme-linked immunosorbent assay (ELISA), chemiluminescence immunoassay) and line blots (immunoblot) often use the highly purified autoantigen from collagen IV.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Autoantibodies against the basement membrane of the renal glomeruli are associated with Goodpasture syndrome (pulmorenal syndrome).

Diagnostic value. A special form of autoimmune glomerulonephritis is the Goodpasture syndrome, named after the US pathologist Ernest William Goodpasture (1886–1960), who described the combination of glomerulonephritis and lung haemorrhages in 1919. This rare syndrome affects men six times as frequently as women, primarily as young adults. Clinically, the combination of rapid-progressive anti-basement membrane glomerulonephritis and lung haemosiderosis is indicative, while lung haemorrhages often occur as the first sign.
Due to the great importance of this diagnosis, the parallel use of the IIFT and ELISA and the establishment of the result on the day of receipt of the sample is advisable.
With the relevant symptoms, antibodies against GBM antigens are pathognomonic of Goodpasture syndrome, which account for 0.5-2% of all glomerulonephritis. In cases without lung involvement, anti-GBM antibodies with Goodpasture syndrome (verified by a positive IgG reaction on the basement membrane in the direct immunofluorescence test on the patient's renal biopsy) can be detected in the serum in 60% of cases or, with additional lung involvement, in 80-90% of cases. A reaction of the Goodpasture serum with the basement membrane of the lung alveoli only occurs in exceptional cases.
In many cases of active Goodpasture syndrome with the positive detection of antibodies against the basement membrane in the biopsy, no antibodies can be found in the serum; a negative serological finding must therefore not be used to rule out Goodpasture syndrome. In these cases, it is assumed that the autoantibodies formed were absorbed by the affected tissue.
Caution is advised when interpreting a borderline test result, so that no unnecessary follow-up examinations are initiated as a result of a diagnostic uncertainty. The clinical progression of Goodpasture syndrome correlates with the concentration of the autoantibodies against the glomerular basement membrane. High antibody titers indicate an unfavourable development. In the case of a negative finding and continued suspicion of anti-GBM glomerulonephritis, renal biopsy is indicated.

Literature.
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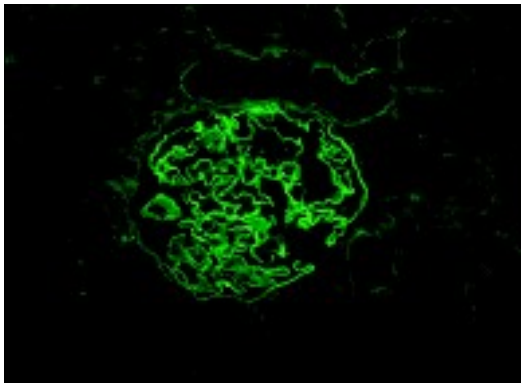

Autoantibodies against the glomerular basement membrane. Fig. 1. Substrate: primate kidney

Autoantibodies against glutamic acid decarboxylase

W. Stöcker

Synonym(s). Autoantibodies against GAD; GAD65 antibodies; GADA; glutamic acid decarboxylase antibodies

Definition. The enzyme glutamic acid decarboxylase (GAD) catalyses the synthesis of the neurotransmitter γ-aminobutyric acid (GABA) and occurs in the 2 isoenzymes GAD65 and GAD67. The detection of antibodies against the 65 kDA protein of glutamic acid decarboxylase is part of the diagnosis of insulin-dependent diabetes mellitus. Another strong association exists with neurological diseases, incl. stiff person (previously: stiff man) syndrome as well as cerebellar ataxia and limbic encephalitis, ocassionally also in combination with a tumour (paraneoplastic syndrome).

Function. The reaction catalysed by GAD is the only natural way to biosynthesise GABA, the most important neurotransmitter of the inhibitory synapsis in the CNS. Accordingly, the isoforms GAD65 and GAD 67 are exclusively expressed in GABA neurons in the CNS, while GAD65 is also formed in the pancreas.

Analytics. Within the scope of diabetes diagnostics, autoantibodies against GAD can be detected by indirect immunofluorescence (IIFT, immunofluorescence, indirect), radioimmunoassays and enzyme immunoassays (incl. enzyme-linked immunosorbent assay (ELISA), chemiluminescence assay). A specially configured ELISA, in which the autoantibodies of solid phase-immobilised GAD and labelled GAD from the liquid phase are surrounded, shows a similar sensitivity (sensitivity, diagnostic) and specificity (specificity, diagnostic) to the radioactive methods currently in use. The ELISA is easy to reproduce and implement and therefore suited to investigating large and small sample series in routine analysis. In this case, the IIFT is less sensitive than the radioimmune assay or the ELISA. Primate pancreas and primate cerebellum tissue sections are used as the substrates (Fig. 1, Fig. 2). The pancreas islets display an extremely fine granulated cytoplasmic staining with positive sera. The fact that antibodies against pancreatic islets can also be directed against the brain's grey matter (against glutamic acid decarboxylase, GAD) in type I diabetes mellitus, was first described by Baekkeskov et al. (1990) and Stöcker et al. (1990). The fluorescence of both the islet cells as well as the grey matter could be neutralised by incubating the diabetic sera with homogenised human precentral gyrus. Some pancreatic islet-antibody-positive sera of patients with diabetes mellitus do not react with grey matter. In these cases, preliminary incubation with the homogenate does not reduce the antibody titer.

Pancreatic tissue and primate cerebellum are also used as the IIFT substrate in neurological diagnostics. The granular layer of the cerebellum shows a (stronger) "leopard skin-like" fluorescence, while the molecular layer displays a (weaker) even, granular fluorescence, apart from the cell nuclei. The grey matter of all other regions of the central nervous system also reacts. GAD-transfected cells and immunoblots are also available for detecting anti-GAD65 antibodies in neurological samples.

Sample material. Serum or cerebrospinal fluid.

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Diagnostic value. During an immune reaction, in the case of insulin-dependent diabetes mellitus (IDDM), this quickly results in the formation of autoantibodies against various islet cell antigens, whose detection is highly significant for diagnosing type I diabetes and its prediction in first-degree relatives of diabetics: Autoantibodies against glutamic acid decarboxylase (GAD65), autoantibodies against tyrosine phosphatase ("insulinoma associated antigen", IA2; autoantibodies against insulinoma associated antigen 2), autoantibodies against zinc transporter ZnT8, autoantibodies against other cytoplasmic islet cell components and insulin (autoantibodies against insulin). One or more of these autoantibodies can be detected in almost all patients at the time of diagnosis of type I diabetes. Autoantibodies against GAD65 are found in 70-90% of newly diagnosed type I diabetics, but cross-reactions (cross-reactivity) with GAD67 exist. Before the onset of the disease, anti-GAD antibodies indicate a high individual risk of diabetes and are considered a marker of the prediabetic phase. A combination of all of the aforementioned diabetes mellitus-related autoantibodies should be tested in order to accurately assess a risk of diabetes in the individual case. If one of the parameters is positive, appropriate measures can be taken to prevent the onset of diabetes mellitus: immunosuppression or a diabetic diet over several years (unoccupied pancreatic islets expose fewer autoantigens; see also autoantibodies against pancreatic islets). If autoantibodies against pancreatic islets or their constituents are present, diabetes mellitus must generally be treated with insulin and not with insulin-stimulating drugs, which would only instigate the autoimmune process with increased antigen expression.
With regard to a specific form of type I diabetes (LADA, latent autoimmune diabetes in adults), the detection of autoantibodies against pancreatic islets and their constituents helps to distinguish between type 2 diabetes as well as acting as a predictor of the secondary insulin dependence of patients.

Antibodies against GAD in serum and cerebrospinal fluid are also found in 60-100% of patients with stiff-person syndrome (SPS), a rare neurological disease with progressive muscle rigidity, spasms and resulting skeletal deformations, or its clinical variants (stiff-limb syndrome, progressive encephalomyelitis with rigidity and myoclonus). The possible accompanying manifestations of stiff-person syndrome include type 1 diabetes mellitus, autoimmune thyroiditis, breast cancer, small cell lung cancer and colon cancer. GAD-specific antibodies can also occur in cases of non-paraneoplastic limbic encephalitis or cerebellar ataxia. The risk of a paraneoplastic neurological syndrome rises in anti-GAD positive patients over 50 years of age.

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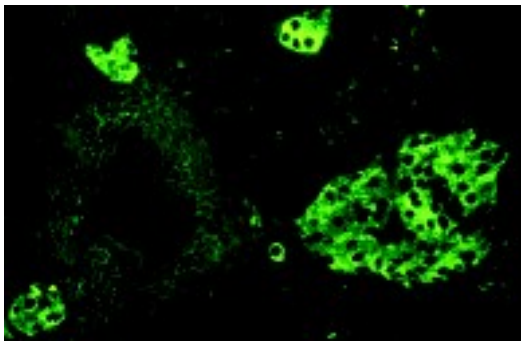
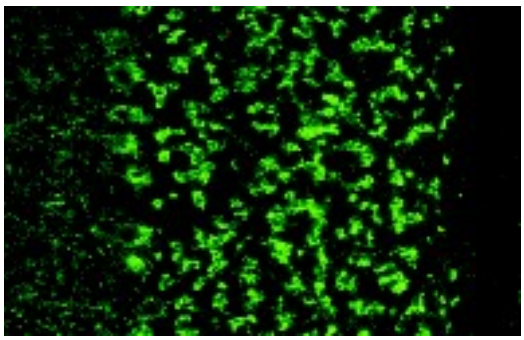


Autoantibodies against glutamic acid decarboxylase. Fig. 1. Substrate: primate pancreas



Autoantibodies against glutamic acid decarboxylase. Fig. 2. Substrate: primate cerebellum

Autoantibodies against AMPA-type glutamate receptors

W. Stöcker

Synonym(s). AMPA receptor autoantibodies; anti-AMPA receptor antibodies

Definition. Autoantibodies against α-amino-3-hydroxy-5-methyl-4-isoxazol propionic acid A(MPA) receptors; see also autoantibodies against neuronal antigens

Function and pathophysiology. AMPA receptors are a subgroup of the glutamate receptors, the most common neurotransmitter receptors in the central nervous system. They consist of 4 subunits, each with a mass of about 100 kDa, which are referred to as GluR1 to GluR4 (“alternatively gria1-gria4”). AMPA receptors are important for the synaptic plasticity. In many synapses, such as in the hippocampus or cerebellum, the density of the AMPA receptors in the post-synaptic membrane is regulated based on the activity of the synapses.

Analytics. Autoantibodies against AMPA receptors can be detected using indirect immunofluorescence (IIFT, immunofluorescence, indirect): In a positive reaction there is a characteristic staining of the molecular layer of the hippocampus, the granular and molecular layer of the cerebellum as well as the Purkinje cells. For monospecific detection, transfected HEK (“human embryonic kidney”) cells, which express AMPA receptors, are used as the substrate.

Sample material. Serum or cerebrospinal fluid.

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Diagnostic value. Autoantibodies against subunits GluR1 and GluR2 of the AMPA receptors can be found in a subgroup of patients with autoimmune-mediated limbic encephalitis, inflammation of the medial temporal lobe, the corpora amygdala and the orbitofrontal cortex. Characteristic symptoms are short-term memory disorders, behavioural problems and epileptic seizures. Lung cancer, breast cancer or a malignant thymoma (paraneoplastic syndrome) also exits in 50-70% of patients with antibodies against AMPA receptors.
Rasmussen's encephalitis is associated with antibodies against the subunit GluR3, encephalitis in childhood, which manifests as chronic-progressive epilepsy. It is only restricted to one cerebrum hemisphere and can lead to the atrophy of the entire region of the brain. Patients increasingly lose motor and language skills over time. This is also associated with progressive dementia. The antibody titer correlates with the frequency of the seizures and a plasma exchange leads to improvement. Surgical excision of the affected region alone prevents the disease from progressing [Theodore Rasmussen (1910–2002) was a famous neurosurgeon in Montreal].

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Autoantibodies against NMDA-type glutamate receptors

W. STÖCKER

Synonym(s). NMDA receptor autoantibodies; anti-NMDA receptor antibodies

Definition. Autoantibodies against N-methyl-D-aspartate (NMDA) receptors. N-methyl-D-aspartate is a synthetic amino acid, which does not normally occur in nature, but is used for neurophysiological experiments. Structurally, NMDA receptors are heterodimers, consisting of the subunits NR1 and NR2; see also autoantibodies against neuronal antigens.

Function and pathophysiology. NMDA receptors belong to the ionotropic glutamate receptors. These are ion channels in the cell membrane, which are activated by the binding of the glutamate ligands. Localised in the post-synaptic membrane, they control the flow of ions to the downstream nerve cells of the synapsis selectively by the type of ions. The channel has different binding sites for different ligands, which control the receptor function. Besides the binding sites for the actual messenger substance glutamate (agonist) and a binding site for the coagonist glycine, the NMDA receptor also has binding sites for other substances, which influence activity increase (agonists such as NMDA) or decrease (antagonists such as amantadine, dextromethorphan or kynurenic acid). The function of the NMDA receptor is presumably a key element for the induction of synaptic plasticity and is therefore a molecular mechanism for learning and memory.

Analytics. Autoantibodies against NMDA receptors can be detected using immunohistochemical detection methods. A positive reaction in the indirect immunofluorescence test (IIFT, immunofluorescence, indirect) results in a characteristic staining of the inner molecular layer on the hippocampus substrate (Fig. 1, Fig. 2) or the staining of the granular layer on the cerebellum substrate. Transfected HEK cells (human embryonic kidney cells) as the IIFT substrate are suitable for the monospecific detection of the autoantibodies.

Sample material. Serum or cerebrospinal fluid.

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for deep-freeze preservation of IgM.

Diagnostic value. Anti-NMDA receptor encephalitis (limbic encephalitis) is an autoimmune disease that was first described in 2007. In a third of cases it is associated with a teratoma of the ovaries (occasionally: the testes; the tumours also contain neuronal structures). The disease often starts with a flu-like preliminary stage, followed by psychiatric symptoms such as anxiety, excitement, strange behaviour, delusions and hallucinations. Many patients initially receive psychiatric treatment (and remain there if they are not rescued by simple antibody test!). Initial signs are often epileptic seizures and catatonia-like impairments of consciousness. Autoantibodies against NMDA receptors (NR1 subunit) are characteristically located in the serum and cerebrospinal fluid. In the case of a positive finding, the treating physicians must be informed that in some affected women, the ovaries are afflicted by tumours! Resection of the tumour and immunosuppressive treatment over several months – acute: methylprednisolone i.v.; escalation: plasmapheresis, steroids, long-term: azathioprine, cyclophosphamide, rituximab. The effectiveness of the proteasome inhibitor bortezomib was displayed in a retrospective study with 5 patients with severe and/or refractory anti-NMDAR encephalitis. Cognitive deficits (sclerosis of the hippocampus) and the recurrence rate are reduced with early and aggressive treatment. Positive reactions in IgA and IgM have previously been observed in dementia, deliria symptoms (organic psychosyndromes) and peripheral neuropathies. They are of no relevance for diagnosing limbic encephalitis.
The investigation of the other key autoantibodies against neuronal antigens in parallel, which, in many cases, results in a rapid and reliable (potentially unsuspected) vital diagnosis is also advisable.

Literature.

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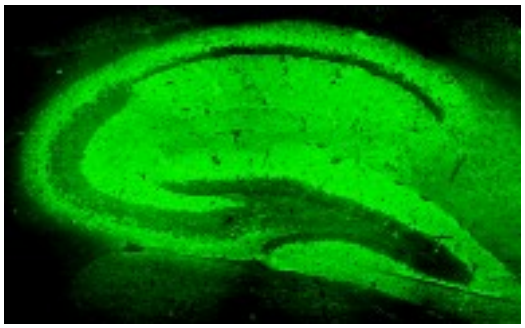
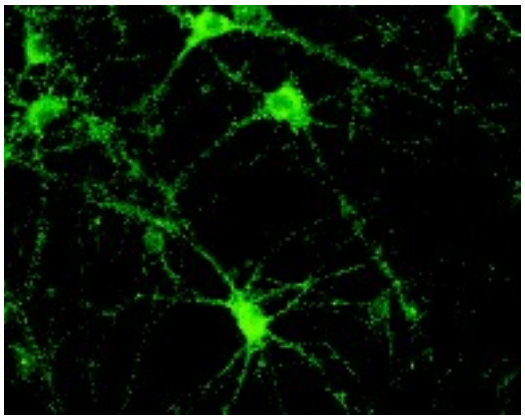


Autoantibodies against NMDA-type glutamate receptors. Fig. 1. Substrate: hippocampus (mouse)



Autoantibodies against NMDA-type glutamate receptors. Fig. 2. Substrate: ex vivo cultivated mouse neurons of the hippocampus

Autoantibodies against glycine receptors

W. Stöcker

Synonym(s). Anti-GlyR antibodies; anti-GLR antibodies; anti-glycine receptor antibodies

Definition. Autoantibodies against a transmembrane, postsynaptic protein complex. In the CNS, glycine receptors are concentrated in the brain stem and spinal cord. The native, functional receptor consists of 5 subunits (3α2β), which are arranged in a circular shape around the central ion channel. Four isoforms of the α-subunits are known, which are interchangeable and represent the neurotransmitter-binding unit.

Function and pathophysiology. Glycine receptors belong to the class of inhibitory ligand-controlled ion channels. The binding of the neurotransmitter glycine to the receptor results in the inflow of chloride ions into the cell and leads to a reduction in the cellular excitability. The inhibitory glycerin mechanism suppresses the excessive activity of neurons and is disrupted by the autoantibodies against glycine receptors. The resulting symptoms include hyperekplexia, which also has a hereditary cause due to mutations in the GLRA1 gene, which codes the α1 subunit of the glycine receptor.

Analytics. Autoantibodies against glycine receptors can be detected using GlyR-transfected human cells in an indirect immunofluorescence test (immunofluorescence, indirect).

Sample material. Serum, plasma or cerebrospinal fluid

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Diagnostic value. Autoantibodies against glycine receptors have been detected in some patients with stiff-person plus syndrome (SPS-plus), so-called progressive encephalomyelitis with rigidity and myoclonus (PERM). However, individual cases show that the clinical picture associated with autoantibodies against glycine receptors can go beyond that of the traditional PERM disease. As this relates to an extremely rare disease, only a few cases have previously been described. The diagnostic significance of the indirect immunofluorescence test is extremely high with this parameter.

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Antibodies against glycoprotein 210

W. Stöcker

Synonym(s). Anti-GP210 antibodies; autoantibodies against GP 210; autoantibodies against the nuclear pore glycoprotein 210; glycoprotein 210 autoantibodies

Definition. The GP210 autoantigen is a glycoprotein in the nuclear membrane and an integral part of the nuclear pore complex. The protein structure is comprised of three domains. At least two of these contain epitopes which react with autoantibodies.

Function and pathophysiology. In a third of patients with primary biliary cholangitis (PBC, chronic non-suppurative destructive cholangitis, previously: primary biliary cirrhosis), indirect immunofluorescence can be used to detect autoantibodies against several specific cell nucleus antigens, including antibodies against Sp100, proteins from cancer cells (promyelocytic leukaemia: PML), antigens in the nuclear membrane (lamins, lamin-B receptors) as well as components of the nuclear pore complex (GP 210); PBC-associated antinuclear autoantibodies.

Sample material. Serum, plasma.

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. In immunofluorescence (immunofluorescence, indirect), autoantibodies against GP210 react with the membrane of the cell nuclei and are displayed as a linear fluorescence. HEp-2 cells and tissue sections of primate liver can be used as the substrate, where these antibodies are more clearly displayed in the hepatocytes and easier to distinguish from occasionally additionally present cell nuclei antibodies (autoantibodies against cell nuclei) with a homogenous pattern on the liver. The starting serum dilution is 1:100.
In enzyme immunoassays (enzyme-linked immunosorbent assay, chemiluminescence immunoassays) or immunoblot systems, potentially recombinant GP 210, isolated from cell cultures, is used to detect these antibodies.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Primary biliary cholangitis (PBC) and overlap syndrome (autoimmune hepatitis and PBC).

Diagnostic value. Autoantibodies against GP 210 are found in about 20-30% of patients with primary biliary cholangitis and indicate a particularly severe course of the disease. These antibodies are occasionally also observed in autoimmune hepatitis or hepatitis B and C.
The joint detection of autoantibodies against PML, SP100, GP 210, AMA-M2 and M2-3E (autoantibodies against mitochondria) increases the diagnostic sensitivity for PBC to 94% with a specificity of 99% and serves to differentiate it from other autoimmune liver diseases, see also PBC-associated antinuclear autoantibodies.

Literature.

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Autoantibodies against Golgi apparatus antigens

W. STÖCKER

Synonym(s). Autoantibodies against the Golgi apparatus; anti-Golgi apparatus antibodies

Definition. Autoantibodies directed against antigens in the Golgi apparatus in the cytoplasm. The Golgi complex includes the antigenic determinants indicated in Tab. 1.

Autoantibodies against Golgi apparatus antigens. Tab. 1. Antigenic determinants of the Golgi apparatus

Autoantigen	Molar mass
Giantin/macrogolgin	376–364
Golgin-245	245
Golgin-160	160
Golgin-97	97
Golgin 95/gm130	130
Golgin-67	67

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. In indirect immunofluorescence (immunofluorescence, indirect), autoantibodies against the Golgi apparatus are displayed as a net-like granular structure on HEp-2 cells, located on one side of the cell nucleus (Fig. 1). The Golgi apparatus is mostly dissolved in HEp-2 cells in mitosis. In this case, the antibodies do not react. The cytoplasm of the hepatocytes is also stained on frozen sections of primate liver.
The starting dilution for the serum is 1:100.

Reference range — Adults. Negative

Reference range — Children. Negative

Diagnostic value. Autoantibodies against antigens in the Golgi apparatus occur in various autoimmune diseases, especially in systemic lupus erythematosus and Sjögren's syndrome. The low disease specificity means that their detection has no significant diagnostic importance.

Literature.
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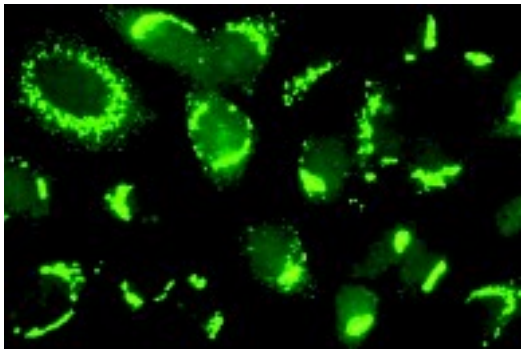

Autoantibodies against Golgi apparatus antigens. Fig. 1. Substrate: HEp-2 cells

Autoantibodies against granulocyte membrane

W. STÖCKER

Synonym(s). Anti-granulocyte membrane antigen; GMA; anti-GMA

Definition. Antibodies against proteins in the membrane of granulocytes. Not to be confused with ANCA (autoantibodies against granulocyte cytoplasm)

Pathophysiology. GMA are part of the blood group antigens and are codominantly inherited. A distinction is essentially made between 5 antigen systems (HNA1-5) of which 1-3 different forms are known in each case. One part of the antigens is localised on the Fc receptor of the granulocytes.
Clinically, GMA incompatibilities are primarily of importance for neonatal immune granulocytopenia; in this case, the antibodies are transferred diaplacentally from the mother. They are also responsible for severe pulmonary transfusion reactions (TRALI syndrome, transfusion associated lung injury). In this case, a blood donor's GMA activate the patient's alveolar granulocytes and cause pulmonary oedema.

Sample material. Serum

Analytics. GIFT (granulocyte immunofluorescence test) with granulocyte smears or in a flow cytometer: screening test.
MAIGA ("monoclonal antibody immobilisation of granulocyte antigens") test: antigen-specific test in case of positive GIFT results.

Reference range. Negative

Diagnostic value. Positive test results in connection with the corresponding symptoms play an essential role in the diagnosis. Negative results particularly do not rule out the presence of a TRALI syndrome!

Literature.
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Autoantibodies against granulocyte cytoplasm

W. Stöcker

Synonym(s). ANCA; ANCA (atypical); cANCA; pANCA; antineutrophil cytoplasmic antibodies; autoantibodies against cytoplasm of neutrophil granulocytes, cytoplasmic and perinuclear type

Definition. Autoantibodies against cytoplasm components of neutrophil granulocytes. According to the microscopic image, which arises in the indirect immunofluorescence test, a distinction is made between two types: cANCA (cytoplasmic type) and pANCA (perinuclear type). The primary target antigen of cANCA is proteinase 3, while antibodies against BPI and, sometimes, antibodies against myeloperoxidase (MPO) also react with this image. The pANCA pattern is displayed by autoantibodies against myeloperoxidase, autoantibodies against elastase, cathepsin G, lactoferrin, lysozyme, β-glucuronidase, azurocidin, LAMP2, α-enolase and defensin.

Pathophysiology. The pathogenetic role of ANCA has not been conclusively established to date.

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. The diagnostics of autoantibodies against granulocyte cytoplasm is primarily based on the indirect immunofluorescence test (IIFT, with unfixed or specifically fixed granulocytes or immortalised leukaemia cells as well as with antigen dots as substrates; immunofluorescence, indirect); it is logically complemented by monospecific 7enzyme immunoassays (enzyme-linked immunosorbent assays, chemiluminescence immunoassays) and immunoblots. The standard substrates for immunofluorescence are ethanol- and formalin-fixed human granulocytes. A distinction can be made between at least two fluorescence patterns: a granular fluorescence, which is largely evenly distributed over the entire cytoplasm of the granulocytes and leaves the cell nuclei free (cANCA: cytoplasmic pattern, granulomatosis with polyangiitis) (Fig. 1), and a predominantly smooth, in some cases also fine granular fluorescence, which winds around the cell nuclei of the granulocytes as a ribbon shape (pANCA; perinuclear pattern) (Fig. 2).
The cANCA pattern is primarily caused by antibodies against proteinase 3. Ethanol and formalin-fixed granulocytes react with antibodies against proteinase 3 in the same manner. The perinuclear fluorescence pattern of the pANCA arises due to the fact that the antigens diffuse from the granules on the nuclear membrane during incubation with the patient serum, to which they have a high affinity. With the exception of anti-myeloperoxidase, these generally only react on ethanol-fixed granulocytes with the described pANCA pattern. However, for antibodies against MPO, the primary target antigen of pANCA, a clear granular fluorescence can be detected in the cytoplasm of the formalin-fixed granulocytes, as the antigen is bound to the granules by the formalin and cannot diffuse to the nuclear membrane. By contrast, the pANCA associated with ulcerative colitis and primary sclerosing cholangitis (PSC) tend not to react with formalin-fixed granulocytes, but rather only with ethanol-fixed granulocytes; the corresponding target antigen is generally lactoferrin bound to DNA (DNA-ANCA). Granulocyte substrates specially depleted with high salt concentrations and selectively supplied with lactoferrin specifically react with the sera of patients with ulcerative colitis and with primary sclerosing cholangitis, which is useful from a diagnostic perspective.
Indirect immunofluorescence is a global test, which essentially detects all autoantibodies against granulocytes, if they are present in an adequate concentration: For confirmation purposes, a number of (more sensitive) ELISA are used in parallel to detect the anti-PR3 and anti-MPO antibodies. While cANCA can be directly identified by indirect immunofluorescence, pANCA cannot be precisely differentiated under the microscope. Test substrates with defined individual antigens are used to find out against which of these individual antigens the pANCA are directed. Sometimes immunofluorescence detects pANCA that do not react with any of the antigens mentioned: obviously not all relevant antigen-antibody systems have yet been discovered.
In addition, the HEp-2 cells and primate liver substrates are used to differentiate from ANA (autoantibodies against cell nuclei). On the primate liver, the cell nuclei of the hepatocytes and the granulocytes contained in the sinusoids are located in the same field of view and it is often even possible to detect whether ANA and pANCA are simultaneously present in the same serum: in this case, the granulocytes fluoresce much more brightly than the hepatocyte nuclei. HEp-2 cell substrates overlaid with granulocytes also exist, which provide the same effect: microscopic assessment of the autoantibodies against cell nuclei and against granulocytes in the same field of view.

Reference range. Negative

Diagnostic value. cANCA have a high sensitivity and specificity for granulomatosis with polyangiitis (GPA, previously referred to as Wegener's granulomatosis) (prevalence of approx. 90%), the titer correlates with the disease activity. The sensitivity of indirect immunofluorescence is now exceeded by modern anti-PR3 ELISA systems (95%), which use combinations of native and recombinant antigen substrates. However, occasionally cANCA can also be detected in microscopic arteritis and polyarteritis nodosa. The primary antigen of cANCA is proteinase 3, while the presence of other target antigens (e.g. “bactericidal permeability increasing protein”, BPI) is being discussed.
The pANCA, autoantibodies against perinuclear granulocyte cytoplasm, which are induced by antibodies against myeloperoxidase, are primarily associated with microscopic polyangiitis (prevalence of approx. 60%) and pauci-immune necrotising glomerulonephritis (prevalence of 65-90%). In addition, autoantibodies against myeloperoxidase also occur in classic polyarteritis nodosa and eosinophilic granulomatosis with polyangiitis (EGPA, previously referred to as Churg-Strauss syndrome). In very rare cases, MPO-ANCA occur in systemic lupus erythematosus and rheumatoid arthritis.
The detection of pANCA (IgA and IgG, formalin-sensitive antigen, DNA-ANCA) also plays an important role in the serological discriminatory tests of the chronic inflammatory intestinal disorders ulcerative colitis (CU, prevalence of approx. 67%) and Crohn’s disease (CD; prevalence of approx. 7%). Granulocytes enriched with lactoferrin react in 72% of cases in the case of UC (CD 3%, healthy blood donors 0% and PSC 42%). Sensitivities of 87% (UC) and 54% (PSC) are achieved for the combination of the two different substrates.

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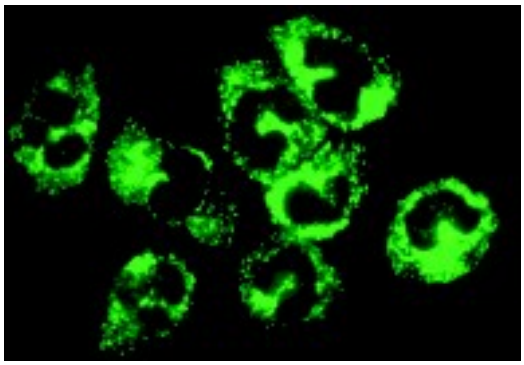
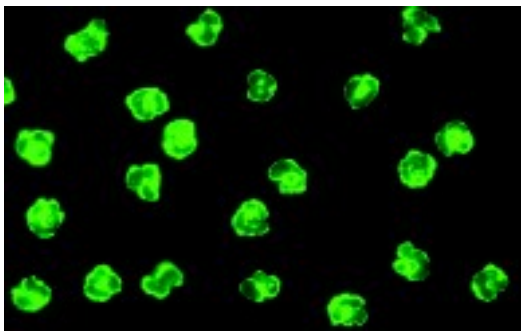


Autoantibodies against granulocyte cytoplasm. Fig. 1. cANCA, substrate human granulocytes (ethanol-fixed)



Autoantibodies against granulocyte cytoplasm. Fig. 2. pANCA, substrate human granulocytes (ethanol-fixed)

Autoantibodies against cardiac antigens

W. STÖCKER

Synonym(s). Anti-heart muscle antibodies; HMA; anti-beta receptor antibodies

Definition. Autoantibodies that specifically react with antigens in the heart musculature

Function and pathophysiology. In the case of dilative cardiomyopathy, myocarditis, severe angina pectoris or a condition after a myocardial infarction, cardiotomy and traumatic events affecting the heart, antigens in the destroyed tissue lead to a physiological immunisation of the organism. There are clinical indications that autoimmune reactions can then cause an inflammation of the heart muscle: For example, in 1956, Dressler observed severe inflammatory reactions in the late stage after a heart attack (Dressler's syndrome), or some patients develop similar symptoms after heart operations, which is known as post-cardiotomy syndrome.
It must be assumed that the sera of these kinds of patients also display autoantibodies directed against parts of the heart muscle and other heart-specific structures (pericardium, endocardium, conducting tissue, heart valves). The targets of the heart muscle antibodies primarily include antigens that are not expressed in other organs (the healthy organs apparently limit autoimmune reactions), but exclusively or predominantly in the heart, such as cardiac troponin I (troponin I, cardiac) and troponin T (troponin T, cardiac), α-hydroxybutyric acid hydrogenase, variant CK-MB of creatine kinase (creatine kinase isoforms), atrial alpha-myosin, ventricular beta-myosin (antigen community with skeletal muscle). Other candidates would be antigens of the intercalated disc and cardiomyolemmal proteins.
Antibodies against conducting tissue are also investigated on a sporadic basis. They may be associated with disruptions to the saltatory conduction. According to the latest findings, autoantibodies against Ro/SS-A from the blood of pregnant women with systemic lupus erythematosus have a similar impact, which cause bradycardia up to a congenital heart block in foetuses and newborns (in this case, this does not relate to “cardiac antibodies”): It has been shown that these antibodies react with proteins in the calcium channels of the conducting tissue and retard the saltatory conduction. An extension of the QT interval in the ECG (as an expression of a delay in the conduction system) is also found in adults with autoantibodies against SS-A.
In particular, beta-adrenergic and muscarinic receptors must be considered in the search for a relevant autoimmune mechanism, which could be associated with the pathogenesis of forms of myocarditis and dilatative cardiomyopathy. However, for the most part, only animal experiments provided evidence of this for a long period of time [Jahns (1994)]. Whether autoantibodies against these receptors contribute to the pathogenesis, similar to autoantibodies against TSH receptors in Graves' disease or autoantibodies against acetyl choline receptors, and their occurrence in the blood can be used for diagnostic purposes, is not generally accepted.
However, autoimmune reactions against the heart can now be objectified by direct investigation of biopsied tissue; in most cases, serological analyses that are often performed an expression of an unjustified expectation.

Analytics. In the indirect immunofluorescence test (immunofluorescence, indirect), frozen sections of primate heart are used as substrate. The starting dilution is 1:100, which is used to examine class IgA, IgG and IgM antibodies with a trivalent antiserum. The serum of a patient with myasthenia gravis is used as the positive control, which displays a typical horizontal striping on the heart tissue, to check the function of the test system.
An enzyme-linked immunosorbent assay, which is based on synthetic peptide analogues of partial sequences of the β1- and β2-receptors, has been described to detect antibodies against cardiac beta-adrenergic receptors. At the same time, insect cells transfected with gene sequences of β-receptors have also been used as the substrate for indirect immunofluorescence.

Indication. To date, a broad-based diagnostic application of antibodies against heart muscle has no authority due to the insufficient relevance.

Diagnostic value. Antibodies against the horizontal striping are primarily only found in connection with myasthenia gravis. While the heart specificity of antibodies against intercalated discs, as can be detected by indirect immunofluorescence, cannot be denied, they lack disease specificity and are also found in healthy blood donors.
Antibodies against stimulating adrenergic β1 receptors have been detected in 26% of patients with dilatative cardiomyopathy (ischaemic cardiomyopathy: 13%). It is doubtful that the detection of this parameter at such a low specificity will be generally accepted.

Literature.

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Autoantibodies against histones

W. Stöcker, W. Schlumberger

Synonym(s). Histone antibodies; anti-histone antibodies

Definition. Histones are alkaline, DNA-associated proteins with a molar mass of between 11.2 and 21.5 kDa. A distinction is made between 5 different histone fractions: H1, H2A, H2B, H3 and H4. Autoantibodies can be directed against each of the 5 fractions.

Function and pathophysiology. Histones are alkaline nuclear proteins with a high affinity to DNA.

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. In indirect immunofluorescence (immunofluorescence, indirect), HEp-2 cells display a homogenous fluorescence of the cell nuclei in the presence of antibodies against histones. The condensed chromosomes of the mitotic cells are emphasised. On primate liver, a homogenous, in parts also a coarse to fine clumpy fluorescence of the cell nuclei can be observed. The kinetoplasts of the flagellate *Crithidia luciliae* are not stained by antibodies against histones.
In the case of a positive result in indirect immunofluorescence, monospecific test systems (enzyme-linked immunosorbent assay, chemiluminescence immunoassay, immunoblot), which contain highly purified histones as test antigens, can be used for more precise identification.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Antibodies against one or more histone fractions or against the H2A-H2B complex indicate drug-induced (procainamide, hydralazine and others) lupus erythematosus.
Antibodies against histones also occur in around 50% of patients with non-drug-induced lupus erythematosus and in 5% - 50% of patients with rheumatoid arthritis.

Interpretation. About 50-75% of patients treated with procainamide and about 25-30% of those treated with hydralazine develop antinuclear antibodies in long-term therapy, initially without symptoms of lupus erythematosus – in a third of these patients, the antibodies are also directed against histones. After different lengths of therapy, patients then display the clinical signs of drug-induced lupus erythematosus: polyarthralgia, pleuritis, pericarditis. The anti-nuclear antibodies persist for years after the drugs have been discontinued and the clinical symptoms have disappeared.

Diagnostic value. Antibodies against one or several histone fractions or against the H2A-H2B complex are a consistent finding in drug-induced (procainamide, hydralazine and others) lupus erythematosus.

Literature.
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Autoantibodies against heat shock proteins

W. Stöcker

Synonym(s). Anti-HSP (auto)antibodies

Definition. Autoantibodies against various cell proteins that are increasingly expressed in stress (e.g. raised body temperature).

Pathophysiology. Autoantibodies against heat shock proteins (anti-HSP 60) may play a causal role in the pathogenesis of atherosclerosis. Increased anti-HSP titers have also been described in autoimmune diseases (rheumatoid arthritis, chronic inflammatory bowel diseases). The cause could either be cross-reactions with bacterial HSP or increased HSP expression of the chronically inflamed or damaged tissue with subsequent autoimmunisation.

Sample material. Serum, plasma

Analytics. Enzyme-linked immunosorbent assay or Western blot

Reference range. Autoantibodies against heat shock proteins also occur in healthy people. Laboratory-specific limits must therefore be defined.

Evaluation. No reliable indication for the detection of autoantibodies against heat shock proteins currently exists.

Literature.

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Autoantibodies against Hu

W. STÖCKER

Synonym(s). Anti-Hu autoantibodies; ANNA-1; anti-neuronal nuclear antibodies 1

Definition. Autoantibodies against Hu proteins of neuronal cell nuclei in paraneoplastic encephalitis. The name is derived from the index patient with the name Hull; Hu antibodies.

Function and pathophysiology. Hu proteins are expressed in peripheral and central neurons as well as, in antibody-positive patients, in tumour tissue.

Sample material. Serum, plasma or cerebrospinal fluid

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. The indirect immunofluorescence test (IIFT, immunofluorescence, indirect) with frozen sections of primate cerebellum (Fig. 1) is suitable for detecting autoantibodies against neuronal cell nuclei (ANNA 1–3: autoantibodies against Hu, autoantibodies against Ri and autoantibodies against neuronal cell nuclei type 3). Autoantibodies against Hu often have a high antibody titer, sometimes up to 1:100,000. In addition, frozen sections of primate intestine are used to distinguish between autoantibodies against Hu and autoantibodies against Ri (Fig. 2): Anti-Hu react with the cell nuclei of the plexus myentericus, while anti-Ri do not. In the case of a positive anti-Hu result in the IIFT, a Western blot with cerebellum antigens or a line blot (immunoblot) with purified defined (recombinant) antigens can be used to confirm the finding.

Reference range — Adults. Negative

Reference range — Children. Negative

Diagnostic value. Anti-Hu antibodies may provide an initial indication of an underlying tumour (paraneoplastic neurological syndrome). Antibodies against Hu should be analysed in all patients with unexplained neuropathies, especially in the case of sensitive neuropathies and encephalitides focussed in the brain stem, cerebellum and limbic system (for differential diagnostics, see also: autoantibodies against neuronal antigens). The tumours most commonly associated with anti-Hu antibodies are small cell lung cancer (SCLC), neuroblastoma, prostate cancer, breast cancer.

Literature.

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Legends

Autoantibodies against Hu. Fig. 1. Substrate: primate cerebellum

Autoantibodies against Hu. Fig. 2. Substrate: primate intestine

Autoantibodies against IgA

W. STÖCKER

Synonym(s). Anti-IgA

Definition. Autoantibodies against IgA are directed against class IgA immunoglobulins; they can essentially belong to all immunoglobulin classes, including the IgA class itself. However, in most cases, this does not relate to autoantibodies in the actual sense, but rather to alloantibodies, as, at least in absolute IgA deficiency, this immunoglobulin does not belong to the autoantigen repertoire.

Function and pathophysiology. Actual autoantibodies against IgA occur very rarely and have the same effect as antibodies against IgA induced by the immunisation of IgA-deficient persons, and they are often found in persons with an absolute or relative selective IgA deficiency following the parenteral administration of blood or blood components. The reaction to IgA donor blood following a repeat administration can cause severe non-haemolytic transfusion reactions in these persons.

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Most anti-IgA autoantibodies belong to the immunoglobulin class IgG. Detection often occurs by passive haemagglutination (haemagglutination test).
Sensitive enzyme-linked immunosorbent assays are able to detect the relevant autoantibodies, even in low concentrations in normal persons without an immunoglobulin deficiency. In this case, highly purified IgA extracted from several myeloma sera is bound to the surface via streptococcal protein B to coat the solid phase.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Clarification of a transfusion reaction, preparation of a transfusion.

Diagnostic value. Autoantibodies against IgA are found in 20% of patients with a relative IgA deficiency (values below 5 mg/dL), while the prevalence is almost 100% if systemic lupus erythematosus is present at the same time.
In Caucasians, a prevalence of 1:500–1:100 is stated for selective IgA deficiency. The autoantibodies against IgA, which are often associated with this, do not have any negative consequences for the affected persons, apart from the possibility of severe transfusion reactions.
If a transfusion is necessary for patients with anti-IgA antibodies, washed erythrocyte concentrate must be administered.

Literature.
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Autoantibodies against IgE receptors

W. Stöcker

Synonym(s). Fc-epsilon receptor antibodies

Definition. IgE receptors are expressed on the surface of the basophilic granulocytes and mast cells

Function and pathophysiology. If the autoantibodies against the IgE receptor react with their target antigens, these are linked to one another, imitating a physiological reaction triggered by specific IgE, and histamine is released.

Sample material. Serum

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Autoantibodies against IgE receptors primarily consist of IgG. A functional test based on the release of histamine (basophilic degranulation) generally enables these autoantibodies to be detected more reliably than with the usual immunoassays. The degranulation test achieves a sensitivity of about 3 mg/mL.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Autoantibodies against Fcε receptors are associated with chronic idiopathic urticaria with a prevalence of between 10 and 40%.

Literature.

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Autoantibodies against IgLON5

Synonym(s). IgLON5 autoantibodies; anti-IgLON5 antibodies

Definition. Autoantibodies against IgLON family member 5 (IgLON5), a cell adhesion protein.

Pathophysiology. IgLON5 belongs to the Ig superfamily and is a neuronal cell adhesion protein. The function of the protein is currently unknown.

Autoantibodies against IgLON5 are associated with a new form of parasomnia with respiratory dysfunctions. Neuropathologically, the disease displays a loss of neurons and accumulations of hyperphosphorylated Tau protein, especially in the area of the tegmentum (brain stem) and hypothalmus (tauopathy).

Cell culture experiments with hippocampal neurons have shown that the antibodies cause an irreversible reduction in the surface density of IgLON5.

Sample material. Serum and cerebrospinal fluid

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Autoantibodies against IgLON5 can be detected using indirect immunofluorescence (immunofluorescence, indirect): In positive findings, the molecular layer of the substrates cerebellum (Fig. 1) and hippocampus reacts. The granular layer of the cerebellum displays a speckled fluorescence. Transfected HEK cells, which express recombinant IgLON5 are suitable for the monospecific detection of autoantibodies (Fig. 2).

Diagnostic value. Antibodies against IgLON5 are markers for an independent neurological syndrome, which was first described in 2014. The anti-IgLON5 syndrome is characterised by abnormal sleeping behaviour and obstructive sleep apnoea. Other symptoms may include gait disorders, ataxia, dysphasia and dysarthria. The disease is often fatal.

The course of the syndrome is usually protracted and delayed and was generally only diagnosed at a late stage in most known cases. Immunotherapy did not result in any material improvement at this time. It must be assumed that a rapid diagnosis and immediate treatment could stop the loss of IgLON5. It is unclear whether this would also lead to an improvement of tauopathy and a higher chance of survival. Indirect immunofluorescence is extremely important as a screening method for the parallel investigation of important neuronal autoantigens.

Literature.

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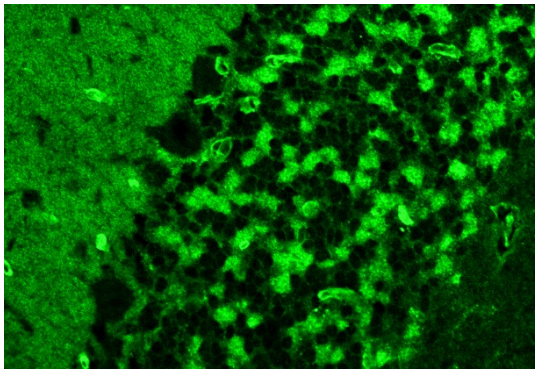
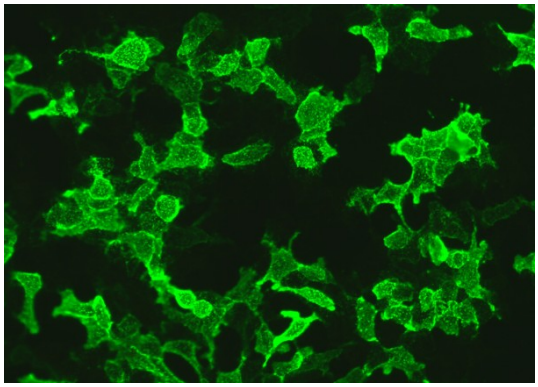


Autoantibodies against IgLON5. Fig. 1. Substrate: cerebellum (rat)



Autoantibodies against IgLON5. Fig. 2. Substrate: transfected cells

Autoantibodies against insulin

W. Stöcker, Chr. Krüger

Synonym(s). Insulin autoantibodies; IAA

Definition. The proteohormone insulin is formed from proinsulin in the beta cells of the pancreatic islets and released into the blood if this is physiologically required. It has a molecular weight of about 5.8 kDa and consists of two peptide chains linked by two disulphide bridges. Insulin is the most important hormone for blood sugar regulation. It reduces the blood glucose concentration and also has a direct or indirect influence on other metabolic reactions, such as the fat metabolism. Disorders of the carbohydrate metabolism due to an insulin deficiency or the reduced insulin effectiveness lead to the clinical pictures of diabetes mellitus type I and II. One of many causes of insulin deficiency is the autoimmunity directed against pancreatic islets and their components. If antibodies against insulin are present, an increased need for insulin arises. A distinction must be made between these antibodies and the rare autoantibodies against insulin receptors, which can be associated with hyperglycaemia as well as hypoglycaemia.

Function and pathophysiology. During an immune reaction, in the case of insulin-dependent diabetes mellitus, autoantibodies against various islet cell antigens form, whose detection is highly significant for diagnosing type I diabetes and its prediction in first-degree relatives of diabetics: Autoantibodies against glutamic acid decarboxylase (GAD), tyrosine phosphatase ("insulinoma associated antigen", IA2; autoantibodies against insulinoma associated antigen 2), autoantibodies against zinc transporter ZnT8 and other cytoplasmic islet cell components and insulin.
Although the immune system is already in contact with insulin as an essential proteohormone before birth, the destruction of the insulin-producing islet cells leads to the secondary formation of autoantibodies against insulin, which is presumably triggered by the insulin precursors, preproinsulin and proinsulin. One or more of these autoantibodies can be detected in almost all patients at the time of diagnosis of type I diabetes. Moreover, during treatment with insulin, some patients develop antibodies against the active agent, which is the reason why, particularly in the era before human insulin, the insulin species often had to be changed with an increased need for insulin (however, this does not relate to autoantibodies).
The prevalence of the autoantibodies against insulin is strongly correlated to the age of the patient. They are found in half of cases in children with a new onset of type 1 diabetes before the age of five, while they are only rarely detected in newly diagnosed adults. They do not only react with human insulin, but also display a cross-reactivity with the insulin of other species.

Analytics. Autoantibodies against insulin can be detected via radioimmunoassay and enzyme-linked immunosorbent assay, while ELISA methods have not established themselves to date. The IAA detected via a radioimmunoassay (liquid phase 125I insulin binding assay) have a higher diabetes relevance than those measured in the ELISA. In the liquid phase radioimmunoassay, all epitopes of the insulin molecule are accessible for the IAA, moreover, the insulin is used in a lower concentration than in the ELISA. In the ELISA, the insulin is bound to the solid phase, whereby some epitopes can be concealed.
Autoantibodies against insulin receptors are investigated using a radioimmunoassay.

Sample material. Serum

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Diagnostic value. Children below 5 years of age with diabetes mellitus display autoantibodies against insulin in over 90% of all investigated cases, while the prevalence in diabetics > 12 years of age is only about 40% and even lower in adults. A high concentration of the antibodies is associated with a higher risk of the disease. A combination of all of the diabetes mellitus-related autoantibodies should be tested in order to accurately assess a possible risk of diabetes in the individual case. If one or several of the parameters are positive, appropriate measures can be taken to prevent the imminent onset of diabetes mellitus: immunosuppression or a diabetic diet over several years (unoccupied pancreatic islets expose fewer autoantigens).
The rare, but important, autoantibodies against insulin receptors must be distinguished from the autoantibodies against insulin. They may be associated with hyperglycaemia (type-B insulin resistance) as well as with hypoglycaemia (in this case the antibodies may have an agonistic effect; in these kinds of patients the following are often found in addition to diabetes mellitus: systemic lupus erythematosus (SLE), obesity, hyperfunction of the ovaries or acanthosis nigricans). Both disorders can be cured in the long-term by treatment with cortisone. In particular, the investigation of autoantibodies against insulin receptors must be considered in SLE and recurrent idiopathic hypoglycaemia as well as in diabetics with an extremely high insulin requirement: see also autoantibodies against pancreatic islets.

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Autoantibodies against insulinoma-associated antigen 2

W. Stöcker, Chr. Krüger

Synonym(s). Insulinoma-associated antigen 2; IA2 antibodies; IA2A; tyrosine phosphatase autoantibodies; (ICA512) autoantibodies

Definition. Insulinoma-associated antigen 2 is an enzymatically inactive protein-tyrosine phosphatase, which is expressed in the β cells of the Langerhans islets and neuroendocrine tissues and which is involved in regulating insulin secretion. The detection of autoantibodies against IA2 is used to diagnose insulin-dependent diabetes mellitus (IDDM).

Function and pathophysiology. In the course of the autoimmune reaction in insulin-dependent diabetes mellitus, autoantibodies to various islet cell antigens are formed at a very early stage, the determination of which has gained great significance for the diagnosis of type 1 diabetes and for the prediction of the disease in first-degree relatives of diabetics: Autoantibodies against glutamic acid decarboxylase (GAD), tyrosine phosphatase ("insulinoma associated antigen", IA2; autoantibodies against zinc transporter ZnT8, other cytoplasmic islet cell components and insulin (autoantibodies against insulin). One or more of these autoantibodies against GAD, IA2, ZnT8, cytoplasmic islet cell antigens (ICA) and insulin can be detected in almost all patients at the time of diagnosis of type I diabetes.
Autoantibodies against IA2 are directed against epitopes of the cytoplasmic C-terminal domain of IA2. The islet cell antigens ICA512 as well as the 40 kDa islet cell antigen obtained from immunoprecipitates after typical treatment are fragments of IA2. The islet cell antigens IA2β (mouse) or phogrin (rat, human) related to IA2, 74% of whose intracytoplasmic domains are identical to IA2, contain epitopes that cross-react with IA2 (cross-reactivity) as well as independent epitopes.
The occurrence of autoantibodies against IA2 is associated with a relatively rapid manifestation of insulin dependence.

Analytics. Autoantibodies against IA2 can be detected via radioimmunoassay and enzyme immunoassay (enzyme-linked immunosorbent assay, chemiluminescence immunoassay). A specially designed ELISA, in which the IA2 antibodies are surrounded by solid phase-immobilised and labelled IA2 of the liquid phase, shows a similar sensitivity and specificity to the radioactive methods currently in use. The ELISA is easy to reproduce and implement and is therefore suited to investigating large and small sample series in routine analysis.

Sample material. Serum or cerebrospinal fluid.

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Diagnostic value. Autoantibodies against IA2 are found in 50-70% of children and adolescents and 30-50% of adults with newly diagnosed type I diabetes mellitus. In non-diabetics, they have a high predictive value with regard to the individual risk of developping from type I diabetes. In most cases, the antibodies are already positive before the manifestation of the disease and are therefore considered a marker in the prediabetic phase. A combination of all of the diabetes mellitus-related autoantibodies (against GAD, IA2, ZnT8, insulin and pancreatic islets) should be tested in order to accurately assess a possible risk of diabetes in the individual case. If one of the parameters is positive, appropriate measures can be taken to prevent the onset of diabetes mellitus: immunosuppression or a diabetic diet over several years (unoccupied pancreatic islets expose fewer autoantigens; see also autoantibodies against pancreatic islets.

Literature.

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Autoantibodies against intestinal goblet cells

W. STÖCKER

Synonym(s). Goblet cell antibodies (GAB)

Definition. Autoantibodies to intestinal goblet cells Goblet cells show the same reactivity from the duodenum to the rectum; there is no antigen commonality with other goblet cells of the organism, such as the stomach mucosa.

Function and pathophysiology. The exclusive presence of autoantibodies against intestinal goblet cells in ulcerative colitis (UC) may be an expression of a pathogenetically significant autoimmunity. In comparison, disease-specific autoantibodies are also found in the second chronic-inflammatory bowel disease, Crohn's disease. These are directed against a secretion product of the pancreas (autoantibodies against pancreatic secretion) and are presumably also highly relevant for the pathogenesis.
The distribution of the goblet cells reflects the disease localisation at both the macroscopic and microscopic level: Only a few goblet cells are present in the duodenum, while their number continuously increases towards the rectum. Accordingly, the duodenum is never affected in ulcerative colitis, the disease starts in the rectum and expands upwards as the disease activity increases. A high goblet cell density can be found in the crypts of the colon; while cryptitis is also always considered a sign of ulcerative colitis when examining biopsies, cryptitis tends to be an exception in Crohn's disease.
The key target antigen for ulcerative colitis has not yet been precisely identified.

Sample material. Serum or cerebrospinal fluid.

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. All four antibodies of relevance in chronic inflammatory bowel diseases are generally analysed by indirect immunofluorescence (immunofluorescence, indirect): Antibodies against intestinal goblet cells, autoantibodies against granulocyte cytoplasm (pANCA), autoantibodies against the exocrine pancreas and antibodies against *Saccharomyces cerevisiae*. To date, primate intestine has been used as the substrate for diagnosing goblet cell antibodies (Fig. 1) (human-foetal intestinal tissue would be optimal: First of all, it originates from the right species and, secondly, it has not yet been contaminated with bacteria or exogenous antigens). In contrast to the recommendations made by some authors, rodent tissue is absolutely unsuitable. These days, a colon cell line (HT29-18N2) is also available for immunofluorescence, which is also a good source of antigens for the development of 7enzyme-linked immunosorbent assay systems and for antigen characterisation (Fig. 2).
The starting dilution for goblet cell antibodies is 1:10. A cloudy fluorescence over the goblet cells with a fuzzy border is obtained in the case of a positive result. Unfortunately, the prevalence of positive results in UC only amounts to 28% (Crohn's disease 0%, healthy patients 0%). The immunoglobulin classes are distributed as follows: IgA 8%, IgG 23% and IgA and IgG 69%.
The prevalence of goblet cell antibodies in case of ulcerative colitis predominates in male patients (m:f = 3.3:1), but not the prevalence of pANCA (m:f = 0.9:1).

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Goblet cell antibodies, as well as pANCA, antibodies against the exocrine pancreas and antibodies against *Saccharomyces cerevisiae* could significantly enrich the differential diagnosis of chronic inflammatory bowel diseases (Crohn's disease, ulcerative colitis). Many patients would be spared from an unpleasant painful intervention if clinicians made greater use of this diagnostically conclusive serology. Autoantibody diagnostics seems to be in undue competition with endoscopy in view of its unrivalled accuracy, especially in gastroenterology (in the case of positive findings).

Interpretation. Autoantibodies against intestinal goblet cells are pathognomonic for ulcerative colitis. They have a diagnostic sensitivity of 28% and a diagnostic specificity of 100% for this disease. An additional investigation of ANCA of the perinuclear type (pANCA) allows the identification of 83% of patients with ulcerative colitiis.

Diagnostic value. Additional antibodies against granulocytes may occur (pANCA, autoantibodies against granulocyte cytoplasm) in ulcerative colitis as well as (occasionally) in Crohn's disease. They are detected by indirect immunofluorescence with smears of human ethanol-fixed granulocytes and display a smooth, in some cases also fine granular, perinuclear fluorescence of the cytoplasm (pANCA) and do not react with formalin-fixed granulocytes. The same pANCA are also found in primary sclerosing cholangitis, which is often associated with ulcerative colitis. In 2009, DNA-bound lactoferrin was identified as the target antigen. The prevalence of pANCA for UC amounts to 67% (Crohn's disease 7%, healthy patients 0-1%); distribution of the immunoglobulin classes: IgA 3%, IgG 39%, IgA plus IgG 58%.

Literature.

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Legends

Autoantibodies against intestinal goblet cells. Fig. 1. Substrate: primate intestine

Autoantibodies against intestinal goblet cells. Fig. 2. Substrate: HT29-18N2 cells

Autoantibodies against intrinsic factor

W. STÖCKER

Synonym(s). Anti-IF antibodies; anti-IFA, intrinsic factor antibodies

Definition. The intrinsic factor is a secretion product of parietal cells of the stomach and is required for the resorption of vitamin B12 in the ileum. Antibodies against intrinsic factor are associated with pernicious anaemia.

Function and pathophysiology. Intrinsic factor is a glycoprotein with a molar mass of 70 kDa. It is used as a transport and protective protein: in the stomach and duodenum, orally ingested vitamin B12 combines with intrinsic factor to form a complex and is therefore protected against decomposition or consumption by the intestinal flora until the complex is resorbed in the distal ileum.
A distinction is made between 2 types of autoantibodies against intrinsic factor:
- Type 1 antibodies react with the vitamin B12 binding sites, thus blocking the formation of the complex.
- Type 2 antibodies, by contrast, bond outside the vitamin B12 binding sites.

Autoantibodies against intrinsic factor (IFA) (as well as autoantibodies against parietal cells) are associated with pernicious anaemia (PA), but cannot be detected in the serum of every PA patient. Type-1 IFA occur in the serum of 70% of PA patients, while type-2 IFA only occur in 35% and only if type-1 IFA are also present.

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Autoantibodies against intrinsic factor are usually detected with an enzyme-linked immunosorbent assay or radioimmunoassay, but can also be analysed using indirect immunofluorescence (7immunofluorescence, indirect) (Fig. 1) on surfaces coated with intrinsic factor.
In the fluorescence test, the starting dilution of the sera amounts to 1:10.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Autoantibodies against intrinsic factor may already exist in some patients with chronic atrophic gastritis (fundal type) without clinical indications of the simultaneous presence of pernicious anaemia. It is highly likely that these patients will develop pernicious anaemia at a later stage.
Before the era of parenteral therapy with vitamin B12 (cyanocobalamin), patients with pernicious anaemia were administered preparations of porcine stomach mucosa. In these cases, class IgA heterologous antibodies against intrinsic factor often formed; at the same time the patients became refractory to the treatment.

Literature.
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Legend

Autoantibodies against intrinsic factor. Fig. 1. Substrate: intrinsic factor antigen.

Synonym(s). ITPR1 autoantibodies; anti-ITPR1 antibodies

Definition. Autoantibodies against inositol-1 4 5-trisphosphate receptor type 1, a ligand-controlled calcium channel.

Pathophysiology. ITPR1 is expressed particularly strongly in the Purkinje cells of the cerebellum and the hippocampal neurons as well as in the neurons and glial cells of the peripheral nervous system. ITPR1 is bound to the metabotropic glutamate receptor 1 (mGluR1) via Homer3. Activated mGluR1 leads to the synthesis of the ligand inositol-1 4 5-trisphosphate, which effects the release of calcium from the endoplasmic reticulum via ITPR1.

Autoantibodies against ITPR1 have previously been described in four patients with cerebellar ataxia and three patients with symptoms of peripheral neuropathy. Malignant neoplasia was additionally diagnosed in two patients (adenocarcinoma of the lung, multiple myeloma).

Sample material. Serum, plasma, cerebrospinal fluid

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Autoantibodies against ITPR1 can be detected using indirect immunofluorescence (immunofluorescence, indirect): A finely speckled staining of the molecular layer and the cytoplasm of the Purkinje cells can be detected on the cerebellum substrate in case of a positive finding (Fig. 1). Transfected HEK cells expressing recombinant ITPR1 are suitable for the monospecific detection of autoantibodies (Fig. 2).

Diagnostic value. Anti-ITPR1 antibodies may be associated with cerebellar ataxia or also with peripheral neuropathy with possible paraneoplastic aetiology.

The monospecific detection using recombinant cells plays an important role in diagnostics, as the fluorescence pattern of anti-ITPR1 antibodies is barely distinguishable from that of the other Purkinje cell antibodies.

Literature.

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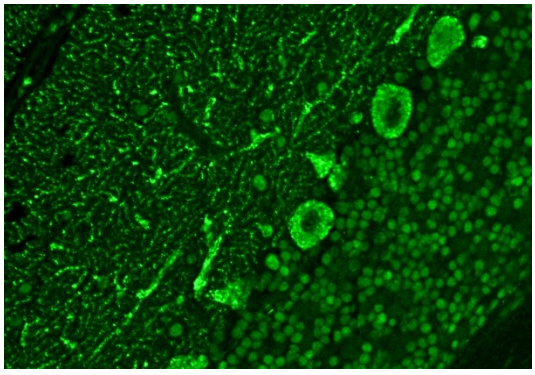
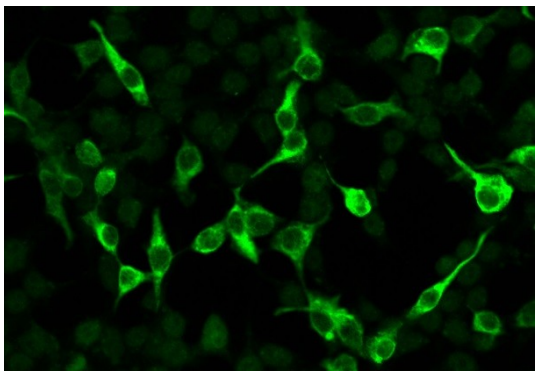


Autoantibodies against ITPR1. Fig. 1. Substrate: cerebellum (rat)



Autoantibodies against ITPR1. Fig. 2. Substrate: transfected cells

Autoantibodies against potassium channels

W. Stöcker

Synonym(s). Potassium channel complex autoantibodies; anti-VGKC complex autoantibodies

Definition. Autoantibodies against voltage-gated potassium channels (VGKC) were first defined by their reactivity in a radioimmunoassay. Of these antibodies, 3% are directed against the Kv1.1-, Kv1.2- and Kv1.6-subunits of the potassium channels, 80% are directed against the VGKC-associated proteins LGI1 ("leucine-rich glioma-inactivated protein 1"), CASPR2 ("contactin-associated protein 2") and (rarely) TAG1 ("transient axonal glycoprotein 1/contactin 2"). About 20% of the RIA-positive autoantibodies bind to epitopes other than LGI1 or CASPR2, including cytosolic epitopes of the Kv1 subunits.

Function and pathophysiology. Voltage-gated potassium channels are also responsible for the repolarisation of the neuronal cell membrane following activation potentials. LGI1 is present in synaptic VGKC complexes, regulates the VGKC inactivation and is involved in glutamate receptor-mediated signal transduction (type AMPA). CASPR2 belongs to the neurexin superfamily and also mediates interactions between the nerve cells. Autoantibodies against LGI1 impair its function and cause increased excitability. According to the high density of the antigen in the hippocampus, autoimmunity results in the symptoms of limbic encephalitis. Anti-CASPR2 autoantibodies appear to effect a reduction in the VGKC density on the axons of peripheral nerves which leads to neuromuscular hyperexcitability characteristic of acquired neuromyotonia.
Autoantibodies against components of the VGKC complexes may possibly arise as a result of an irregular ectopic expression of the antigens in neoplastic tissue. An indication of the involvement of autoimmune reactions in the pathogenesis of the associated neurological symptoms is that, in most cases, an immunosuppressive intervention leads to clinical improvement.

Analytics. For the radioimmunoassay, VGKC are isolated from brain homogenate and labelled with the snake venom 125I-α dendrotoxin. After incubation with patient serum (immunoprecipitation), the complexes are centrifuged and washed. The radioactivity measured in the precipitate is proportional to the concentration of the anti-VGKC autoantibodies.
Anti-LGI and anti-CASPR2 antibodies are shown as a smooth to finely granular fluorescence, predominantly of the stratum moleculare, in the indirect immunofluorescence test (7immunofluorescence, indirect) with frozen sections of hippocampus and cerebellum. Monospecific detection is performed using transfected HEK-293 cells, which recombinantly expressed LGI1, CASPR2 or TAG1.
It is recommended in addition to investigate in parallel the most important other 7autoantibodies against neuronal antigens to onconeuronal antigens, which in many cases will result in a fast and secure (perhaps also unexpected) vital diagnosis.

Sample material. Serum, plasma, cerebrospinal fluid

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Diagnostic value. In ≥ 90% of cases, autoantibodies against LGI1 are associated with a special form of autoimmune limbic encephalitis. Anti-CASPR2 autoantibodies are predominantly (53%) found in patients with neuromyotonia or Morvan's disease, but also occur in connection with limbic encephalitis (37%) or isolated epilepsy (10%). They belong to the group of facultative paraneoplastic antibodies: In 10–30% of cases, the neurological syndromes are based on paraneoplastic aetiology, i.e. a positive antibody finding may indicate the presence of a tumour (thymoma or the like). Rapid treatment can prevent cognitive deficits and reduce the recurrence rate. A subsequent sclerosis of the affected area of the brain (hippocampus) must be countered and requires early and aggressive treatment. Acute: Methylprednisolone plus immunoglobulin concentrate, then, if necessary, azathioprine and oral steroids or rituximab.
Anti-VGKC antibodies against antigens other than LG1 and CASPR2 often occur in low titers; they are presumably of no clinical relevance and should be interpreted with care.

Literature.

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Autoantibodies against collagen

W. STÖCKER

Synonym(s). Collagen antibodies

Definition. Autoantibodies against collagen include a group of antibodies that may be directed against various types of collagen. These are associated with different autoimmune diseases.

Function and pathophysiology. Collagens are a heterogenous protein class, which (to date) includes 25 different types of collagen. Their primary function is to form the structure of the extracellular matrix.

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Autoantibodies against citrullinated collagen II are analysed in the enzyme-linked immunosorbent assay, in some cases, using recombinant antigens. Antibodies against glomerular basement membrane, collagen VII and collagen XVII are detected using the ELISA, immunoblot and indirect immunofluorescence (immunofluorescence, indirect). Autoantibodies against collagen VII display a reaction of the basement membrane on the base of the blister with frozen section of human salt-split skin (“1M NaCL split human skin”); for autoantibodies against collagen XVII, the basement membrane reacts in the area of the top of the blister.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Several collagen types are suspected of being target antigens in various autoimmune diseases. This has currently only been established for the following structures:

- Collagen II: Belongs to the fibrillar collagens. Autoantibodies against intact native collagen II can be found in rheumatoid arthritis with a low prevalence (10-20%). The detection rate increases to 70% if the arginine modules of the collagen II used for analysis is replaced by citrulline (in the pathogenesis of rheumatoid arthritis, this conversion to inflamed tissue takes place with the involvement of the enzyme peptidyl arginine deiminase). Citrullination takes place at the carboxy terminal telopeptides. To date, corresponding tests have not established themselves with regard to the detection of autoantibodies against citrullinated peptides (q.v.).
- Collagen IV in Goodpasture syndrome. The target structure is the globular NC1 domain of collagen IV, which is usually referred to as the GBM (glomerular basement membrane) antigen. Autoantibodies against glomerular basement membrane provide evidence of Goodpasture syndrome.
- Collagen VII in epidermolysis bullosa acquisita (EBA). It is the main component of the anchoring fibrils by which the epidermis is connected to the dermis in the area of the basement membrane. The autoantibodies have a direct influence on the pathogenesis: after bonding to the target structures, they activate the alternative complement path and cause blisters to form. The detection of these antibodies is used to differentiate EBA from genetic dystrophic epidermolysis bullosa, which is caused by abnormal or a lack of collagen VII.
- Collagen XVII = BP180, one of the target antigens in bullous pemphigoid (Autoantibodies in autoimmune blistering diseases) and pemphigoid getationis (autoantibodies against epidermal basement membrane).

Diagnostic value. It is no longer appropriate to generally request the determination of "antibodies to collagen" in the laboratory, especially as the indication can relate to different areas of medicine: rheumatology, nephrology and dermatology.

Autoantibodies against Ku

W. Stöcker

Synonym(s). Ku antibodies; anti-Ku (p70/p86) antibodies

Function and pathophysiology. Antibodies against Ku are directed against a DNA-binding, nuclear heterodimer, which is involved in repairing dsDNA breaks, preventing the recombination of telomere ends as well as their length regulation (see also autoantibodies against cell nuclei).

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Antibodies against Ku display a fine granular fluorescence of the nuclei in the indirect immunofluorescence test (IIFT, immunofluorescence, indirect) with HEp-2 cells, while the nucleoli are positive in some cases (Fig. 1). There is virtually no difference between 7autoantibodies against SS-A, autoantibodies against SS-B, autoantibodies against Sm and autoantibodies against U1-RNP, in contrast, primate liver incubated in parallel in the same field, where possible, displays a typical clumpy-speckled staining of the cell nuclei, which almost umistakeably points to autoantibodies against Ku (Fig. 2). The starting dilution of the serum amounts to 1:100.
If uncertainty exists, in the case of a positive result in the IIFT, an appropriate monospecific immunoblot can be used to precisely identify the target antigen.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Autoantibodies against Ku occur with the following prevalences:
- [5] Poly/dermatomyositis progressive systemic sclerosis overlap syndrome 25-50% (often accompanied by primary pulmonary hypertension),
- [5] Various forms of myositis 5-10%
- [5] Systemic lupus erythematosus 10%
- [5] Progressive systemic sclerosis ≤ 5%.

Literature.

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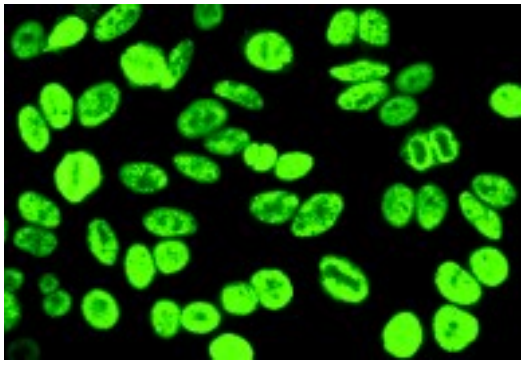
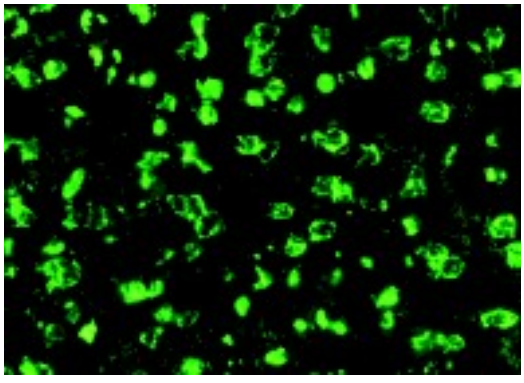


Autoantibodies against Ku. Fig. 1. Substrate: HEp-2 cells



Autoantibodies against Ku. Fig. 2. Substrate: primate liver

Autoantibodies against lactoferrin

W. STÖCKER

Synonym(s). Autoantibodies against DNA-bound lactoferrin

Function and pathophysiology. Lactoferrin (lactotransferrin) is an iron-bound protein, which consists of a peptide chain with two asparagine-bound oligosaccharides and belongs to the transferrin family. It is formed in neutrophilic granulocytes as well as in glandular epithelial cells. Lactoferrin can be found in serum, bile, sperm, pancreatic secretion, urine, stool, bronchial secretion and, especially in breast milk (~5.5 g/L). Every lactoferrin molecule can bind two iron-III ions and inhibit the growth of bacteria and fungi in the mucous membrane, which need iron to grow.

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. The target antigen of autoantibodies against lactoferrin is lactoferrin localised in the cytoplasmic granules of the neutrophilic granulocytes. Detection via indirect immunofluorescence (immunofluorescence, indirect). Granulocyte substrates specially depleted with high salt concentrations and selectively supplied with lactoferrin specifically react with the sera from patients with ulcerative colitis and primary sclerosing cholangitis.
To detect anti-lactoferrin antibodies when diagnosing ulcerative colitis and primary sclerosing cholangitis, the lactoferrin must be present in DNA-bound form. This was not considered in the past, so the importance of these antibodies in gastroenterology was judged controversially and underestimated.

Reference range — Adults. Negative

Indication. The serological detection of autoantibodies against DNA-bound lactoferrin can contribute to the diagnosis of chronic-inflammatory bowel and liver diseases. Granulocytes enriched with lactoferrin react in 72% of cases of ulcerative colitis (Crohn's disease 3%, primary sclerosing cholangitis 42%, healthy blood donors 0%).

Literature.

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Autoantibodies against lamin B receptors

W. STÖCKER

Synonym(s). Lamin B receptor antibodies

Function and pathophysiology. Lamin B receptors are proteins of the inner nuclear membrane, between 58 kDa (avian erythrocytes) to 61 kDa (rat liver). The autoantibodies are directed against an epitope consisting of 60 amino acids; see also PBC-associated antinuclear autoantibodies.

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Antibodies against lamin B receptors display a linear fluorescence of the nuclear membrane in the indirect immunofluorescence test (IIFT, immunofluorescence, indirect) with HEp-2 cells and primate liver (Fig. 1, Fig. 2). However, the same fluorescence pattern arises with autoantibodies against glycoprotein 210, which are present in 20-30% of patients with primary biliary cholangitis (PBC), and with antibodies against lamins (autoantibodies against cell nuclei), which are often found in anti-cardiolipin-positive systemic lupus erythematosus. Therefore, findings with positive nuclear membrane should always be differentiated using monospecific test systems (immunoblot).

Reference range — Adults. Negative

Reference range — Children. Negative

Diagnostic value. Antibodies against lamin-B receptors appear to be highly-specific for PBC, but only have a prevalence of 1-3%. The diagnostic relevance is therefore quite low.

Literature.

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Legends

Autoantibodies against lamin B receptors. 1. Nuclear membrane positive. Substrate: HEp-2 cells

Autoantibodies against lamin B receptors. Fig. 2. Nuclear membrane positive. Substrate: primate liver

Autoantibodies against LAMP-2 (granulocytes)

W. Stöcker

Synonym(s). Anti-hLAMP-2 antibodies; autoantibodies against the lysosomal-associated membrane protein 2

Definition. The autoantibodies are directed against the extracellular domain of the human lysosomal-associated membrane protein 2 of the granulocytes (LAMP-2)

Function and pathophysiology. The lysosomal-associated membrane protein LAMP-2 plays a role in cell adhesion, antigen presentation and autophagy. It is heavily glycosylated and is expressed on the cell surface and in the membrane of the myeloperoxidase- and proteinase 3-containing vesicles of neutrophilic granulocytes, while LAMP-2 is also present on endothelial cells and is therefore directly accessible to circulating autoantibodies. Their pathogenetic importance is highlighted by experimental findings on rats: After injecting these antibodies, the animals developed pauci immune focal necrotising glomerulonephritis.
The antibodies detect an epitope in human LAMP-2 (P41–49), which displays a 100% homology with the bacterial fimbrial protein FimH. In some types of bacteria, fimbria serve as adhesive organelles, with which they attach via adhesins of a host cell's membrane. A FimH-induced autoimmunity could explain the pathogenesis of pauci immune focal necrotising glomerulonephritis. For instance, it is also known that an infection with fimbria-containing bacteria often occurs before the start of focal necrotising glomerulonephritis.

Sample material. Serum, plasma, cerebrospinal fluid

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Autoantibodies against human LAMP-2 display a cANCA pattern in indirect immunofluorescence (immunofluorescence, indirect) with ethanol-fixed granulocytes. The antigen is also expressed by human epithelial cells; the antibodies are therefore observed purely by chance when detecting autoantibodies against cell nuclei in the immunofluorescence test. The HEp-2 cells display a typical fine to coarse droplet-shaped ("lysosomal") fluorescence of the cytoplasm.

Reference range — Adults. Negative

Indication. The membrane protein hLAMP-2 was described as a new ANCA antigen in 1995. The autoantibodies occasionally occur in active granulomatosis with polyangiitis (GPA; obsolete: Wegener's granulomatosis) and are treated as a possible new, additional biomarker for ANCA-associated vasculitis. Autoantibodies against hLAMP-2 can be detected in 93% of patients with pauci immune focal necrotising glomerulonephritis. This acute inflammatory disease leads to rapid irreversible renal failure, typically with ANCA-associated vasculitis of small vessels, including in GPA or microscopic polyangiitis.

Literature.

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Autoantibodies against LC-1

W. Stöcker

Synonym(s). Autoantibodies against cytosolic liver antigen type 1; autoantibodies against formiminotransferase cyclodeaminase; autoantibodies against liver cytosolic antigen 1; LC-1 antibodies

Definition. In 1999, the enzyme formiminotransferase cyclodeaminase, a liver-specific enzyme with a molecular weight of 62 kDa, was identified as the specific target antigen of antibodies against LC-1.

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. In indirect immunofluorescence (immunofluorescence, indirect), LC-1 antibodies display patchy pericentral fluorescence on rat and primate liver, while the area directly around the Glisson's triad shows no reaction (contrary to other information) (Fig. 1). In addition, anti-LC-1 often displays a fine crystalline fluorescence above the focussing plane, which probably relates to immunocomplexes from partially released antigen and the autoantibodies. All other tissue (stomach, kidney, HEp-2 cells, etc.) does not display any reaction.
Autoantibodies against LC-1 can be reliably detected with enzyme immune tests (enzyme-linked immunosorbent assay, immunoblot) based on a recombinant antigen as well as with Western blot using a whole liver extract.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Unclear increase in the transaminases, suspected autoimmune hepatitis (AIH).

Diagnostic value. The 7prevalence of the autoantibodies against LC-1 in autoimmune hepatitis (AIH) amounts to 5%. They are a clear indication of AIH. No association with viral hepatitis is described for LC-1 antibodies, as is the case for autoantibodies against LKM, which are even rarer than anti-LC-1, but which are traditionally more popular in diagnostics.
The serological detection of autoantibodies against LC-1 enables a precise differentiation from viral hepatitis in some patients with AIH, which has significant consequences for the hepatological symptoms: The incorrect treatment of AIH with interferon can also have fatal consequences, similar to an immunosuppressive treatment of the viral infection.
The parallel detection of the other AIH-associated autoantibodies is recommended to differentiate from viral hepatitis, such as autoantibodies against cell nuclei, autoantibodies against granulocyte cytoplasm, autoantibodies against smooth muscles, autoantibodies against LKM, autoantibodies against SLA.

Literature.
Lapierre P, Hajoui O, Homberg JC et al (1999) Formiminotransferasecyclodeaminase is an organ-specific autoantigen recognized by sera of patients with autoimmune hepatitis. Gastroenterology 116:643–649

Legend

Autoantibodies against LC-1. Fig. 1. Substrate: rat liver

Autoantibodies against LKM

W. STÖCKER

Synonym(s). Autoantibodies against liver kidney microsomes

Definition. LKM is localised in the microsomes of the liver and kidneys. Sequencing and cloning allowed the antigen to be identified as cytochrome P450 IID6.

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. To detect autoantibodies against LKM, frozen sections of rat kidney, rat liver and HEp-2 cells are used as a substrate combination in the indirect immunofluorescence test (immunofluorescence, indirect). This small mosaic also enables a differentiation from autoantibodies against mitochondria (AMA). Rat kidney: A smooth to fine granular cytoplasmic fluorescence of the proximal tubules is visible in the area of the cortex. The distal tubules and the glomeruli are negative (Fig. 1). Rat liver: Antibodies against LKM react well with rat liver and create a smooth staining of the cytoplasm of the hepatocytes. The intensity of the fluorescence of the liver cells is generally just as bright as that of the proximal renal tubules (Fig. 2). HEp-2 cells: Negative, in contrast to autoantibodies against mitochondria. Autoantibodies against LKM can be reliably detected with enzyme-linked immunosorbent assay or immunoblot (line blot), among others, based on a recombinant antigen as well as with Western blot using a whole liver extract.

Reference range — Adults. Negative

Reference range — Children. Negative

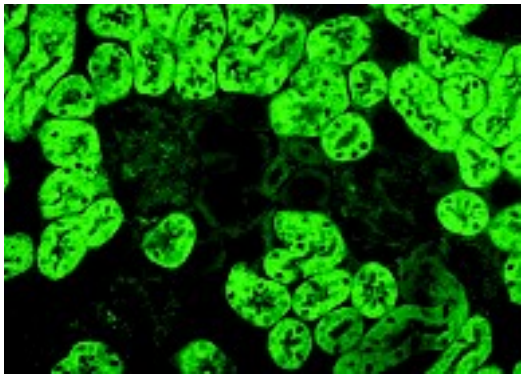
Indication. Unclear increase in the transaminases, suspected autoimmune hepatitis (AIH)

Diagnostic value. Autoantibodies against liver/kidney microsomes only occur in 1% of adult AIH patients, and more frequently in children. However, they are also found in about 5% of patients with positive hepatitis C serology. As they are not associated with antibodies against SLA/LP and do not occur together with the other AIH-relevant antibodies, their detection enables an increase in the serological detection rate in AIH diagnosis, especially in children. The parallel detection of the other AIH-associated autoantibodies, such as 7autoantibodies against cell nuclei, autoantibodies against granulocyte cytoplasm, autoantibodies against smooth muscles, autoantibodies against LC-1 and autoantibodies against SLA, is recommended to differentiate from viral hepatitis; see also autoimmune liver disease-associated autoantibodies.

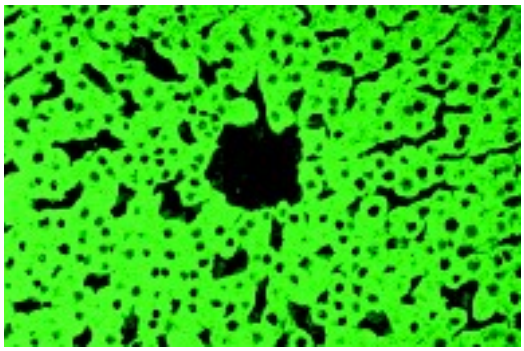
Literature.

Homberg JC, Abuaf N, Bernard O, Islam S, Alvarez F, Khalil SH, Poupon R, Darnis F, Levy VG, Grippon P (1987) Chronic active hepatitis associated with antiliver/kidney microsome antibody type 1: a second type of against “autoimmune” hepatitis. Hepatology 7:1333–1339

Manns MP, Johnson EF, Griffin KJ, Tan EM, Sullivan KF (1989) Major antigen of liver kidney microsomal autoantibodies in idiopathic autoimmune hepatitis is cytochrome P450db1. J Clin Invest 83:1066–1072



Autoantibodies against LKM. Fig. 1. Substrate: rat kidney



Autoantibodies against LKM. Fig. 2. Substrate: rat liver

Autoantibodies against Ma

W. Stöcker

Synonym(s). Ma (ma1, Ma2/Ta) autoantibodies; autoantibodies against paraneoplastic antigen 1/2; autoantibodies against PNMA

Definition. Autoantibodies against proteins (PNMA1, Ma1, 37 kDa; PNMA2, Ma2/Ta, 40 kDa) in the nucleoli of neuron nuclei; see also autoantibodies against neuronal antigens.

Function and pathophysiology. Ma proteins are expressed in peripheral and central neurons as well as, in antibody-positive patients, in tumour tissue.

Analytics. Autoantibodies against Ma can be detected by the indirect immunofluorescence test (IIFT, immunofluorescence, indirect) using primate frozen sections of cerebellum and cerebrum. They are characterised by a reaction of the nerve cell nucleoli.
In a line blot (immunoblot), anti-Ma1 and -Ma2/Ta autoantibodies cause a reaction with the recombinant Ma2/Ta (PNMA2) antigen.

Sample material. Serum, plasma, cerebrospinal fluid

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Diagnostic value. Autoantibodies against Ma are associated with brainstem encephalitis and limbic encephalitis. The antibodies as well as the clinical symptoms may provide an initial indication of underlying lung, testicular or breast cancer.

Literature.

Graus F, Delattre JY, Antoine JC, Dalmau J, Giometto B, Grisold W, Honnorat J, Smitt PS, Vedeler CH, Verschuuren JJ, Vincent A, Voltz R (2004) Recommended diagnostic criteria for paraneoplastic neurological syndromes. J Neurol Neurosurg Psychiatry 75:1135–1140

Rosenfeld MR, Eichen JG, Wade DF, Posner JB, Dalmau J (2001) Molecular and clinical diversity in paraneoplastic immunity to Ma proteins. Ann Neurol 50:339–348

Autoantibodies against MAP-2

W. STÖCKER

Synonym(s). Microtubule-associated neuronal protein 2

Function and pathophysiology. MAP-2 is a component of the cytoskeleton of neuronal cells. It is exclusively present in the nerve cells and can therefore be specifically labelled in order to immunohistochemically identify ganglion cells in the CNS or in the autonomic nervous system and, for example, to differentiate from (GFAP-positive) glial cells (glial fibrillary acidic protein).

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Indirect immunofluorescence (immunofluorescence, indirect) with tissue sections of cerebrum, cerebellum, spinal cord, stomach, intestine or with recombinantly expressed MAP-2 antigen in HEK-293 cells as antigen substrates. Alternatively, enzyme-linked immunosorbent assay and immunoblot methods (Western blot: 210 kDa).

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Systemic lupus erythematosus involving the central nervous system (neuropsychiatric SLE), prevalence of around 77% (all of 100 SLE cases together: only 17%).

Interpretation. Limited disease specificity, the antibodies also occur with alcoholic liver disease, viral hepatitis and primary biliary cholangitis.

Literature.

Komatsu M, Goto M, Yamamoto A, Toyoshima I, Masamune O (1990) A new autoantibody, anti-210 kDa microtubule associated protein antibody, detected in the serum of patients with various liver diseases and SLE. Nihon Shokakibyo Gakkai Zasshi 87:2451–2456

Williams RC Jr., Sugiura K, Tan EM (2004) Antibodies to microtubule-associated protein 2 in patients with neuropsychiatric systemic lupus erythematosus. Arthritis Rheum 50:1239–1247

Autoantibodies against Mi-2

W. STÖCKER

Synonym(s). Anti-Mi-2 antibodies; Mi-2 antibodies

Definition. Autoantibodies against Mi-2 bind to a multi-component complex of the nucleus. Molecular biological analyses led to the target antigen with a molar mass of 218 kDa, which displays histone deacetylase and “nucleosome remodelling” activity (see also autoantibodies against cell nuclei).

Sample material. Serum

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Antibodies against Mi-2 display a fine granular fluorescence of the nuclei in the indirect immunofluorescence test (IIFT, immunofluorescence, indirect) with HEp-2 cells. The nucleoli are partially partly spared (Fig. 1). The starting dilution is 1:100.
With a positive result in the IIFT, an appropriate monospecific immunoblot with Mi-2 antigens, which are isolated from HeLa nuclei, are used to specifically identify the target antigen.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Autoantibodies against Mi-2 are serological markers of dermatomyositis Available data show a prevalence of between 5% and 30% exist.

Literature.

Ghirardello A, Borella E, Beggio M, Franceschini F, Fredi M, Doria A (2014) Myositis autoantibodies and clinical phenotypes. Auto Immun Highlights 5(3): 69-75.

Meurer M, Hausmann-Martinez-Pardo G, Braun-Falco O (1989) Spectrum of antinuclear and anti-cytoplasmic antibodies in dermatomyositis and polymyositis overlap syndromes. Hautarzt 40:623–629

Mierau R, Genth E (1995) Diagnostische Bedeutung Sklerodermie- und Myositis-assozierter Autoantikörper. Z Rheumatol 54:39–49

Rozman B, Bozic B, Kos-Golja M et al (2000) Immunoserological aspects of idiopathic inflammatory muscle disease. Wien KlinWochenschr 112:722–727

Legend

Autoantibodies against Mi-2. Fig. 1. Substrate: HEp-2 cells

Autoantibodies against midbody

W. STÖCKER

Synonym(s). Midbody antibodies

Function and pathophysiology. Antibodies against the overlapping area of the spindle fibers during cell division (midbody). The target antigen is a protein with ATPase activity, which is involved in rejecting the overlapping spindle fibres. This is a double polypeptide with a molecular weight of 330 kDa each.
Background: During mitosis, the spindle fibres grow radially in all directions, starting from the centrioles, primarily towards the median plane of the cell. Some of them bind with the kinetochores of the chromatids and pull these towards the relevant centriole during the anaphase. However, most spindle fibres do not establish contact with the chromosomes; rather they come together with the spindle fibres on the opposite side. The rejection and division of the cell into daughter cells occurs in the overlapping area (midbody); see also autoantibodies against mitosis-associated antigens.

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

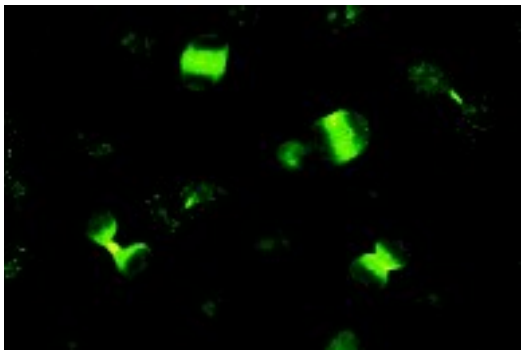
Analytics. In the indirect immunofluorescence test (starting dilution of 1:100; immunofluorescence, indirect), HEp-2 cells in the metaphase of mitosis show a fine granular fluorescence of the median plane in the presence of midbody antibodies (Fig. 1). In contrast to the pattern found with 7autoantibodies against centromeres, this fluorescing line remains in the middle until the end of mitosis. Their length corresponds to the overall width of the cell in the midbody and the line becomes shorter until only a fluorescing dot, which connects the daughter cells to one another (“parting kiss”), is visible in the telophase. Half of the interphase cells contain numerous coarse fluorescing droplets, while the other cells are dark.

Reference range — Adults. Negative

Reference range — Children. Negative

Diagnostic value. The diagnostic importance of these antibodies has not yet been conclusively established.

Literature.
Casiano CA, Landberg G, Ochs RL, Tan EM (1993) Autoantibodies to a novel cell cycle regulated protein that accumulates in the nuclear matrix during S phase and is localized in the kinetochores and spindle midzone during mitosis. J Cell Sci 106:1045–1056



Autoantibodies against midbody. Fig. 1. Substrate: HEp-2 cells

Autoantibodies against mitochondria

W. Stöcker, W. Schlumberger

Synonym(s). AMA; mitochondria antibodies; M(1-9) antibodies; anti-mitochondrial antibodies

Definition. Autoantibodies against components of the mitochondria

Function and pathophysiology. Mitochondria contain numerous different biochemically definable antigens, of which some are significant for autoimmune diseases and are referred to as mitochondria antigens M1-M9.
The most important representative, the M2 antigen (AMA-M2), is part of three biochemically related multi-enzyme complexes of the inner mitochondrial membrane. These catalyse the oxidative decarboxylation of the pyruvate, the α-ketoglutarate and the branched-chain α-keto acids. Other antigens are M4 (sulphite oxidase) and M9 (phosphorylase a, actually an extramitochondrial cytoplasmic enzyme, but it is associated with the mitochondrial membrane).
To date, 4 of 9 different AMA types (antibodies against the antigens M2, M4, M8 and M9) have been detected in the serum of patients with primary biliary cholangitis (PBC, chronic non-suppurative destructive cholangitis, formerly: primary biliary cirrhosis). Antibodies against the M2 antigen are present in up to 94% of all PBC patients. A positive serological anti-M2 antibody finding with a high titer is an important indicator in the diagnosis of PBC and an extremely useful predictor in the early detection of PBC. Antibodies against M2 occasionally also occur in other diseases that occur at the same time as PBC, such as autoimmune hepatitis (overlap syndrome of PBC and AIH) as well as with autoimmune diseases, which do not primarily affect the liver, such as progressive systemic sclerosis (6%) and Sjögren's syndrome. AMA in low titers have also been described in chronic hepatitis C and systemic lupus.
The molecular target antigens of the autoantibodies against M2 are different subunits of enzymes of the mitochondrial respiratory chain from the family of 2-oxo acid dehydrogenase complexes (2-OADH family). This includes the E2 subunit of branched-chain 2-oxo acid dehydrogenase (BCOADH-E2), the E2 subunit of pyruvate dehydrogenase (PDH-E2), the E2 subunit of 2-oxoglutarate dehydrogenase (OGDH-E2), the E1t subunits of PDH and the E3 binding protein (protein X). Under these enzyme components, the E2 component of PDH is the main autoantigen with which the majority of the serum samples (80-90%) reacts in case of PBC. In addition, 60% of PBC patients also display antibodies against BCOADH-E2. Interestingly, 4-25% of the sera from PBC patients only detect BCOADH-E2 and not PDH-E2. The E2 component of OGDH-E2 is reactive in 30-80% of the sera from PBC patients. The immunodominant epitopes of BCOADH-E2, PDH-E2 and OGDH-E2 are lipoyl-binding sites, while antibodies directed against these do not cross-react (cross-reactivity).

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Rat kidney is the standard substrate for AMA detection via indirect immunofluorescence (immunofluorescence, indirect). The starting dilution of the patient serum is 1:100, immunoglobulin classes IgA, IgG and IgM are analysed. The cytoplasm of the proximal and distal tubule cells displays a granular, basally emphasized fluorescence with a positive serum (Fig. 1). The glomeruli are only weakly stained by AMA. HEp-2 cells contain the antigens M2, M3, M5 and M9; in this case the antibodies create a coarse granular fluorescence of the cytoplasm, which does not include the nucleus (previously, the co-reacting "nuclear dots", also relevant in PBC, were wrongly suspected of being stray mitochondria) (Fig. 2).
AMA in a serum sample can create a granular fluorescence of the cytoplasm on almost all cell substrates. They must not be confused with organ-specific autoantibodies!
Various defined AMA antigens can be reliably identified using monospecific test systems (enzyme-linked immunosorbent assay, chemiluminescence immunoassay, immunoblot, Western blot).
The 3 lipoyl-binding sites of BCOADH-E2, PDH-E2 and OGDH-E2 can be merged using recombinant techniques in order to produce the artificial BPO protein, which contains all relevant epitopes. This fusion protein in combination with native M2 (purified protein of porcine pyruvate hydrogenase complex) increases the sensitivity in a monospecific test system for the detection of antibodies against M2 compared to test systems that only use native M2.

Reference range — Adults. Negative

Interpretation. Autoantibodies against mitochondria can be detected in various diseases (Tab. 1). They often occur together with other autoantibodies, such as with autoantibodies against cell nuclei.

Autoantibodies against mitochondria. Tab. 1. Presence of autoantibodies against mitochondria in selected clinical pictures

M1	Syphilis (indication of activity)	100
	Systemic lupus erythematosus	50 5–15
	Progressive systemic sclerosis, Sjögren's syndrome, Sharp's syndrome, rheumatoid arthritis	
M2	Primary biliary cholangitis (high titer)	98
	Other chronic liver diseases	30
	Progressive systemic sclerosis	7–25
M3	Pseudo-lupus syndrome	100
M4	Primary biliary cholangitis	≤ 55
M5	Undefined collagenosis	Rare

M6	Hepatitis (iproniazid-induced)	100
M7	Acute myocarditis Cardiomyopathies	60 30
M8	Primary biliary cholangitis	≤ 55
M9	Primary biliary cholangitis, Other forms of hepatitis	37–82 3–10

Antibodies against mitochondria are of particular significance for diagnosing PBC. Various types of AMA have been detected in the sera of PBC patients: antibodies against the antigens M2, M4, M8 and M9. Antibodies against the M2 antigen are the diagnostic marker with the highest sensitivity and specificity, they can be detected in over 90% of patients.

Note:
In the case of a negative M2 finding and the continued suspicion of PBC, the additional detection of antibodies against nuclear granules (autoantibodies against cell nuclei, PBC-associated antinuclear autoantibodies) and nuclear membrane, whose significance has also been recognised, is advised.

Literature.

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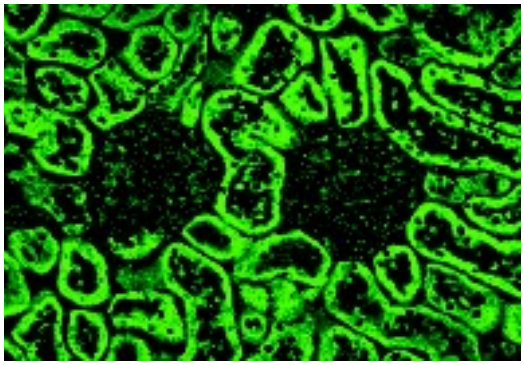
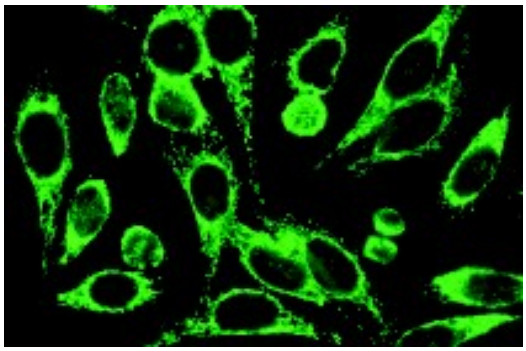


Autoantibodies against mitochondria. Fig. 1. Substrate: rat kidney



Autoantibodies against mitochondria. Fig. 2. Substrate: HEp-2 cells

Autoantibodies against mitosis-associated antigens

W. STÖCKER

Definition. Autoantibodies against structures that predominantly occur or exercise their function in the mitosis phase of the cell cycle. These antigenic structures include:

- MSA-1 (mitotic spindle apparatus, antigen 1, autoantibodies against the spindle apparatus (inappropriate name: NuMa))
- MSA-2 (mitotic spindle apparatus, antigen 2, autoantibodies against the spindle apparatus (HsEg5))
- Midbody (autoantibodies against midbody),
- CENP-F (autoantibodies against CENP-F),
- Centrioles (autoantibodies against centrioles/centrosomes),
- Centromeres (autoantibodies against centromeres).

Autoantibodies against cN-1A (Mup44)

W. Stöcker, Chr. Krüger

Synonym(s). Autoantibodies against cytosolic 5'-nucleotidase 1A (cN-1A); anti-cN-1A autoantibodies; autoantibodies against Mup44

Definition. Autoantibodies against cytosolic 5' nucleotidase 1A are specific markers for sporadic inclusion body myositis, sIBM).

Function and pathophysiology. Cytosolic 5'-nucleotidase 1A primarily exists in cells in the skeletal musculature and catalyses the hydrolysis of adenosine monophosphate. Deposits of cN-1A can be found in the abnormal inclusion bodies in the muscle fibres typically associated with sIBM. Autoantibodies against cN-1A can be found in sIBM patients with a moderate prevalence and are the first specific biomarker for sIBM.

Analytics. Autoantibodies against cN-1A can be detected with enzyme immunoassays (enzyme-linked immunosorbent assays, chemiluminescence immunoassays) and immunoblots using full-length recombinant human cN-1A protein.

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Diagnostic value. The detection of autoantibodies against cN-1A is of particular significance for diagnosing sIBM, as this is currently the only specific biomarker. The detection of anti-cN-1A especially facilitates the sometimes difficult differentiation from other myopathies and is extremely important in the case of ambiguous or a lack of muscle biopsy results. Anti-cN-1A autoantibodies occur in sIBM patients with a moderate prevalence of 30-40% and are distinguished by a high specificity for sIBM of >95% determined in control groups.

Literature.

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Autoantibodies against MuSK

W. Stöcker, Chr. Krüger

Synonym(s). MuSK antibodies; autoantibodies against muscle-specific tyrosine kinase

Definition. MuSK is a transmembrane protein of the neuromuscular junction (motor end plate) associated with the acetylcholine receptor.

Function and pathophysiology. The function of muscle-specific tyrosine kinase remains largely unclear. MuSK presumably plays a role in mediating the agrin effect on the aggregation of the acetylcholine receptor. The autoantibodies against MuSK that occur in myasthenia gravis are directed against the extracellular *N*-terminal end of the MuSK.

Sample material. Serum or plasma

Sample stability. Antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. They are detected using radioreceptor assays (RRA) or 7enzyme-linked immunosorbent assays.

Reference range — Adults. < 0.05 nmol/L

Reference range — Children. < 0.05 nmol/L

Indication. Myasthenia gravis (ocular and generalised form), especially if no autoantibodies against acetylcholine receptors (ACHRAB) are detected.

Interpretation. Myasthenia gravis, a neuromuscular autoimmune disease, is often associated with the detection of autoantibodies against acetylcholine receptors (ACHRAB) in the blood. However, these cannot be detected in 10-20% of patients with generalised myasthenia gravis (currently referred to as seronegative myasthenia). Antibodies against MuSK are found in about 40-70% of cases of myasthenia without ACHRAB. Together with ACHRAB, more than 90% of myasthenia patients can be identified by serology.

Literature.

Hoch W, McConville J, Helms S et al (2001) Auto-antibodies to the receptor tyrosine kinase MuSK in patients with myasthenia gravis without acetylcholine receptor antibodies. Nat Med 7:365–368

Vincent A, Bowen J, Newsom-Davis J et al (2003) Seronegative generalised myasthenia gravis: clinical features, antibodies, and their targets. Lancet Neurol 2:99–106

Autoantibodies against myelin

W. Stöcker

Synonym(s). Myelin antibodies

Definition. Autoantibodies against myelin of the nerve sheath of the myelinated nerves are considered to be associated with neurological diseases, especially multiple sclerosis.

Function and pathophysiology. Myelin is localised in Schwann's sheath, which forms an isolating layer around the myelinated nerves. Antibodies against myelin have been described in multiple sclerosis and other neurological diseases by some investigators, but are also found in healthy control persons with the same frequency.

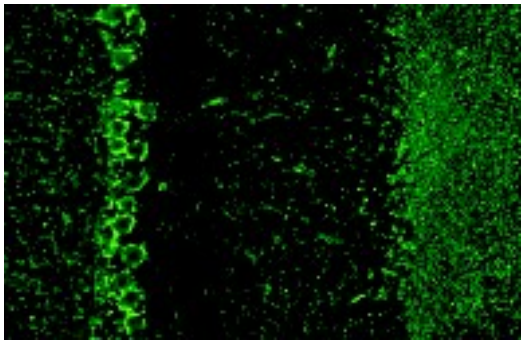
Sample material. Serum

Sample stability. Antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

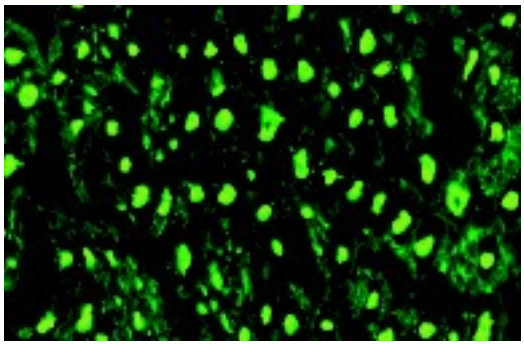
Analytics. Indirect immunofluorescence (immunofluorescence, indirect) is suitable for detecting antibodies against myelin. Frozen sections of primate tissue (N. suralis) and small animal nerves are used as the standard substrates (Fig. 1, Fig. 2). The starting dilution of the patient sera amounts to 1:10.
Autoantibodies against myelin are displayed as hyaline fluorescing cylinders in which the dark axon can sometimes be identified.

Diagnostic value. The diagnostic value of this serum antibody is disputed as high titers also exist in healthy persons. The presumption that the anti-myelin antibodies, which can be detected by indirect immunofluorescence, are associated with multiple sclerosis could not be confirmed by our own investigations with 500 patients; see also autoantibodies against myelin-associated glycoprotein.

Literature.
Genain CP, Cannella B, Hauser SL, Raine CS (1999) Identification of autoantibodies associated with myelin damage in multiple sclerosis. Nature Med 5:170–175



Autoantibodies against myelin. Fig. 1. Substrate: primate cerebellum



Autoantibodies against myelin. Fig. 2. Substrate: primate nerve

Autoantibodies against myelin-associated glycoprotein

W. Stöcker

Synonym(s). Autoantibodies against MAG; anti-MAG antibodies

Definition. Autoantibodies directed against myelin-associated glycoprotein in Schwann's sheath, whose presence is associated with diseases of the peripheral nervous system.

Function and pathophysiology. Myelin-associated glycoprotein (MAG) is a 100-kDA integral membrane protein from the family of neuraminic acid-binding lectins. The protein has a carbohydrate percentage of 30%. As an adhesion molecule, MAG mediates the interaction between the cells. In animal testing, the injection of anti-MAG antibodies caused a local demyelination.

Sample material. Serum, plasma, cerebrospinal fluid

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for deep-freeze preservation of IgM.

Analytics. Antibodies against MAG are detected by indirect immunofluorescence (immunofluorescence, indirect), with peripheral myelinated nerves and cerebellum of primates as the antigen substrates (Fig. 1, Fig. 2). When reacting with nerves, they display a striped fluorescence, which can easily be distinguished from the picture of the diagnostically less relevant autoantibodies against myelin. In the cerebellum, it is particulary the white substance that is entirely stained.

Moreover, 7enzyme-linked immunosorbent assays and Western blot techniques are also suitable for detecting anti-MAG. Antibodies against classes IgG and IgM are analysed; the IgM reaction is generally monoclonal (paraprotein) and is directed against the epitopes of the carbohydrate part.

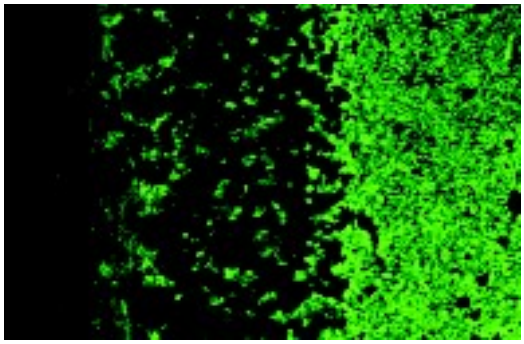
Reference range — Adults. Negative

Reference range — Children. Negative

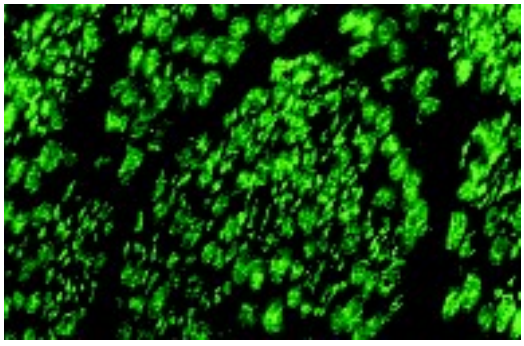
Indication. Antibodies against MAG are analysed whenever peripheral demyelinating neuropathy is suspected. Typical indications are symmetrical distal somatosensory and motor function disorders with electroneurographic abnormalities, which point to demyelination or axon degeneration.

Diagnostic value. Anti-MAG antibodies can be detected in half of all patients with IgM gammopathy-associated peripheral neuropathy. This is associated with muscular atrophy, paresis, ataxia and intention tremors.
Autoantibodies against MAG can sometimes also be detected in Guillain-Barré syndrome. The disease is characterised by multifocal inflammations with cell infiltrations in the myelin sheath of peripheral nerves and in the dorsal root ganglia. Clinical symptoms include somatosensory and motor function disorders, starting with reduced reflexes in the legs, progressing to signs of paralysis up to tetraplegia and respiratory paralysis.

Literature.
Jaskowski TD, Martins TB, Litwin CM et al (2004) Immunoglobulin (Ig)M antibody against myelin associated glycoprotein (MAG): A comparison of methods. J Clin Lab Anal 18:247–250



Autoantibodies against myelin-associated glycoprotein. Fig. 1. Substrate: primate cerebellum



Autoantibodies against myelin-associated glycoprotein. Fig. 2. Substrate: primate nerve

Autoantibodies against myelin oligodendrocyte glycoprotein

W. STÖCKER

Synonym(s). Antibodies against MOG; anti-MOG antibodies

Definition. Autoantibodies against an integral membrane protein of the central nervous system (CNS) myelin. MOG is exclusively expressed in myelinating oligodendrocytes. It is localised in the oligodendrocyte plasma membrane and on the extracellular side of the outer myelin lamella, but is largely lacking in the compact myelin. Its share in the total myelin protein amounts to 0.01-0.05%.

Function and pathophysiology. A possible immunopathogenic role is attributed to antibodies to MOG, or MOG is considered as a relevant target antigen of autoreactive T and B cells in the development of inflammatory demyelinating diseases of the CNS. The potential of anti-MOG antibodies to induce demyelination was demonstrated on brain cell cultures and in animal models of autoimmune encephalomyelitis.

Analytics. Autoantibodies against myelin oligodendrocyte glycoprotein should be detected using test systems that use MOG with authentic, membrane-bound conformation and native glycosylation as the antigen substrate. The indirect immunofluorescence test (immunofluorescence, indirect) with MOG-transfected HEK-203 cells is suitable.

Sample material. Serum, plasma or cerebrospinal fluid

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Diagnostic value. Autoantibodies against myelin oligodendrocyte glycoprotein are found in a percentage of patients with demyelinating diseases of the CNS, especially in paediatric cases. These include acute disseminated encephalomyelitis (ADEM) and “clinically isolated (episodic, demyelinating) syndrome”. In addition, the antibodies are also found in anti-AQP4-negative patients with NMO spectrum diseases (optic neuritis and/or longitudinal extensive transverse myelitis), where they are indicative of a monophasic course of the disease and a better prognosis.

Literature.

Jarius S, Ruprecht K, Kleiter I, Borisow N, Asgari N, Pitarokoili K, Pache F, Stich O, Beume LA, Hümmert MW, Trebst C, Ringelstein M, Aktas O, Winkelmann A, Buttmann M, Schwarz A, Zimmermann H, Brandt AU, Franciotta D, Capobianco M, Kuchling J, Haas J, Korporal-Kuhnke M, Lillevang ST, Fechner K, Schanda K, Paul F, Wildemann B, Reindl M (2016) MOG-IgG in NMO and related disorders: a multicenter study of 50 patients. Part 1: Frequency, syndrome specificity, influence of disease activity, long-term course, association with AQP4-IgG, and origin. J Neuroinflammation 13(1): 279.

McLaughlin KA, Chitnis T, Newcombe J et al (2009) Age-dependent B cell autoimmunity to a myelin surface antigen in pediatric multiple sclerosis. J Immunol 183:4067–4076

Pröbstel AK, Dornmair K, Bittner R, Sperl P, Jenne D, Magalhaes S, Villalobos A, Breithaupt C, Weissert R, Jacob U et al (2011) Antibodies to MOG are transient in childhood acute disseminated encephalomyelitis. Neurology 77:580–588

Autoantibodies against myeloperoxidase

W. Stöcker

Synonym(s). Myeloperoxidase antibodies; anti-MPO antibodies

Definition. Antibodies against the peroxidase of granulocytes and monocytes (myeloperoxidase) (autoantibodies against granulocyte cytoplasm)

Molar mass. 120 kDa

Function and pathophysiology. A possible pathogenetic role of the antibodies is the subject of controversial debate. The antibodies may possibly lead to a release of lysosomal granules from granulocytes and therefore initiate a vasculitic inflammatory process.

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. The diagnostics of autoantibodies against neutrophil granulocytes (antineutrophil cytoplasmic antibodies; ANCA) is primarily based on the indirect immunofluorescence test (IIFT, immunofluorescence, indirect); it is usefully complemented by monospecific 7enzyme immunoassays (enzyme-linked immunosorbent assays, chemiluminescence immunoassays) and immunoblots. The standard substrates for immunofluorescence are ethanol- and formalin-fixed human granulocytes. Ethanol-fixed granulocytes with anti-MPO display a band-shaped perinuclear pattern, occasionally, with high avidity, also a granular cytoplasm fluorescence (Fig. 1). After treating the granulocytes with formalin, a pure cytoplasmic granular pattern is displayed, as is the case for autoantibodies against proteinase 3 (Fig. 2, Fig. 3). Methanol-fixed granulocytes do not react with anti-MPO, but with most other subspecificities, which can be used for differential diagnosis. The serum starting dilution is 1:10 and immunoglobulin class IgG is analysed.
The ribbon-like perinuclear fluorescence pattern of pANCA develops during incubation with the patient serum through antigens diffusing out of the granula into the nuclear membrane to which (as well as to the cell wall of bac-teria) they have a high affinity. (Previously, it was wrongly asserted that the fixation with ethanol led to this cellular redistribution: This is contradicted by the fact that formalin-fixed granulocytes also display a cANCA pattern with anti-MPO if they were previously fixed with ethanol).
Myeloperoxidase is the main target antigen of pANCA, but not all pANCA are positively displayed in an anti-MPO ELISA. Other target antigens of pANCA can be: granulocyte elastase, lactoferrin, lysozyme, cathepsin G, beta glucuronidase, azurocidin, h-lamp-2, alpha enolase and defensin. Enzyme immunoassays are based on native myeloperoxidase, which is isolated from human granulocytes.

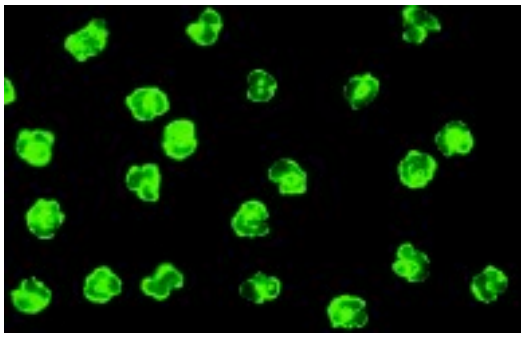
Reference range — Adults. Negative

Reference range — Children. Negative

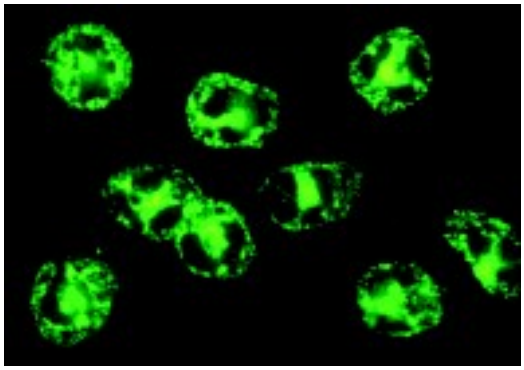
Indication. Microscopic polyangiitis, rapid-progressive glomerulonephritis, other forms of vasculitis.

Diagnostic value. The pANCA, which are induced by antibodies against myeloperoxidase, are primarily associated with microscopic polyangiitis (prevalence of approx. 60%) and pauci-immune necrotising glomerulonephritis (prevalence of 65-90%). In addition, autoantibodies against myeloperoxidase also occur in classic polyarteritis nodosa and eosinophilic granulomatosis with polyangiitis (EGPA, obsolete: Churg-Strauss syndrome). In very rare cases, MPO-ANCA occur in systemic lupus erythematosus and rheumatoid arthritis.

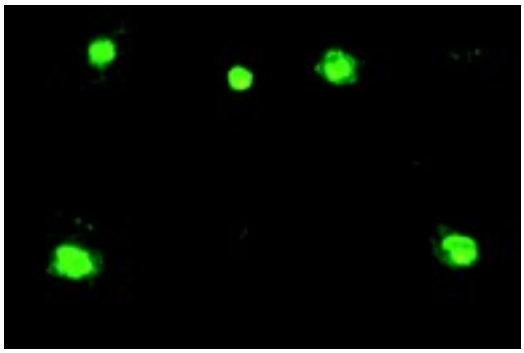
Literature.
Gross WL (1995) Antineutrophil cytoplasmic autoantibody testing in vasculitides. Rheum Dis Clin North Am 21:987–1011



Autoantibodies against myeloperoxidase. Fig. 1. Substrate: human granulocytes (ethanol-fixed)



Autoantibodies against myeloperoxidase. Fig. 2. Substrate: human granulocytes (formalin-fixed)



Autoantibodies against myeloperoxidase. Fig. 3. Substrate: primate liver

Autoantibodies against adrenal cortex

W. STÖCKER

Synonym(s). Adrenal cortex antibodies; anti-AC antibodies; autoantibodies against steroid hormone-producing cells of the adrenal cortex

Function and pathophysiology. Autoantibodies against the adrenal cortex are associated with autoimmune adrenalitis. This contributes to more than half of cases with Addison's disease (primary adrenocortical insufficiency, characterised by a lack of adrenal cortex hormones: glucocorticoids, mineralocorticoids).
The antibodies are primarily directed against the enzyme 21-hydroxylase (21-OH), which is involved in the synthesis of the steroid hormones. This converts 17-α-progesterone and progesterone into 11-deoxycortisol and deoxycorticosterone.

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Antibodies against the adrenal cortex can be detected by indirect immunofluorescence (immunofluorescence, indirect). With a starting dilution of a positive serum of 1:10, the cytoplasm in the steroid hormone-producing cells displays a granular to smooth fluorescence in the area of the cortex, while the adrenal medulla is not stained (Fig. 1). In general, all 3 zones of the adrenal cortex react more or less evenly: zonae glomerulosa, fasciculata, reticularis. The emphasis of the zonae glomerulosa and reticularis that is sometimes observed is mostly due to technical reasons, as these areas also contain antibodies from neighbouring areas.
The use of a substrate combination of adrenal glands and rat kidney enables a reliable differentiation from antibodies against mitochondria (AMA) in the same test.

Diagnostic value. The prevalence of autoantibodies against the adrenal cortex is over 80% at the onset of autoimmune adrenalitis. The antibodies disappear with the increasing atrophy of the adrenal glands, in which case sometimes only antibodies against parietal cells of the stomach (autoantibodies against intestinal goblet cells), thyroid-specific peroxidase (autoantibodies against thyroid peroxidase) or against other endocrine organs, which are often associated with autoimmune adrenalitis, indicate the autoimmune pathogenesis of Addison's disease. The detection of anti-AC antibodies can contribute to the differentiation from other possible causes of Addison's disease: tuberculosis of the adrenal glands, Waterhouse–Friderichsen syndrome (necrosis of the adrenal glands, especially as a result of meningococcal septicaemia) and others.
Anti-AC antibodies may also be an indicator of the clinical pictures from the group of autoimmune polyendocrinopathies, which are characterised by the association of at least two endocrine diseases and the presence of one or more autoantibodies against endocrine organs as well as against parietal cells of the stomach and against striated musculature. In type I (juvenile autoimmune polyendocrinpathy), adrenocortical insufficiency is joined by hypoparathyroidism and, in most cases, pernicious anaemia as well as mucocutaneous candidiasis. The more common type II is characterised by adrenocortical insufficiency in combination with autoimmune diseases of the thyroid (Schmidt syndrome) and, on a case-by-case basis, also with diabetes mellitus type I (Carpenter syndrome).

Literature.

Anderson JR, Goudie RB, Gray KG, Timbury GC (1957) Autoantibodies in Addison's disease. Lancet 272:1123–1124

Betterle C, Dal Pra C, Mantero F, Zanchetta R (2002) Autoimmune adrenal insufficiency and autoimmune polyendocrine syndromes: Autoantibodies, autoantigens, and their applicability in diagnosis and disease prediction. EndocrRev 23: 327–364

Legend

Autoantibodies against the adrenal cortex. Fig. 1. Substrate: Primate adrenal gland

Autoantibodies against the parathyroid gland

W. STÖCKER

Synonym(s). Autoantibodies against epithelial bodies; parathyroid gland antibodies

Definition. Autoantibodies against the parathyroid gland occur in idiopathic hypoparathyroidism and also in polyendocrinopathy in combination with other autoantibodies.

Function and pathophysiology. In some patients with idiopathic hypoparathyroidism, autoantibodies (predominantly in class IgG) against the parathyroid gland are found in the serum. The presence of these antibodies provides evidence of autoimmune pathogenesis in these patients, but can also indicate autoimmune polyendocrinopathy.

Sample material. Serum

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

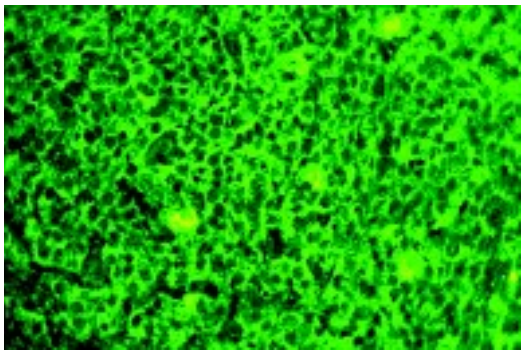
Analytics. To detect autoantibodies against cells in the parathyroid gland through indirect immunofluorescence (immunofluorescence, indirect), frozen sections of the glandula parathyroidea of primates are used as the standard substrate. In the case of a positive result, the main cells and (accentuated) the oxyphilic cells of the parathyroid gland display a smooth to fine granular fluorescence (Fig. 1). The parallel use of rat kidney enables a reliable differentiation from autoantibodies against mitochondria (AMA).
Human adenoma tissue is a suitable antigen substrate, whose function must however first be checked with positive patient sera and an adequate number of negative controls.

Reference range — Adults. Negative

Reference range — Children. Negative

Interpretation. Antibodies against the parathyroid gland indicate idiopathic hypoparathyroidism, potentially in connection with autoimmune polyendocrinopathy type I (hypoparathyroidism, adrenocortical insufficiency and pernicious anaemia as well as mucocutaneous candidiasis).

Literature.
Eisenbarth GS, Gottlieb PA (2004) Autoimmune polyendocrine syndromes. N Engl J Med 350:2068–2079



Autoantibodies against the parathyroid gland. Fig. 1. Substrate: primate parathyroid gland

Synonym(s). Neurochondrin autoantibodies; anti-neurochondrin antibodies

Definition. Autoantibodies against neurochondrin, a protein expressed in the neurons of the cerebellum, the amygdala and the hippocampus.

Pathophysiology. Neurochondrin displays an intracellular, somato-dendritic distribution in the neurons. The protein interacts with G-protein-bound receptors (metabotropic glutamate receptors), which play an important role in the synaptic plasticity of the cerebellum and the hippocampus.

Sample material. Serum, plasma, cerebrospinal fluid

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

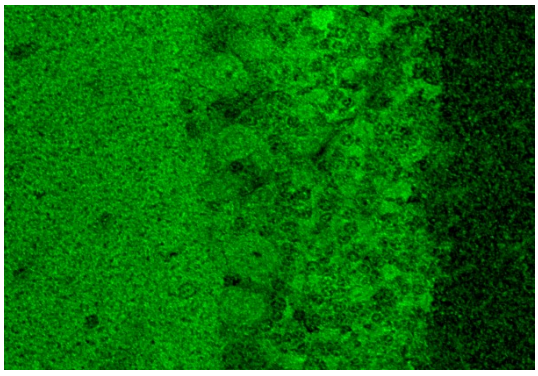
Analytics. Anti-neurochondrin antibodies are detected using indirect immunofluorescence (immunofluorescence, indirect). A fine granular staining of the granular and molecular layer can be detected on frozen sections of the hippocampus or cerebellum (rat, primate) in the presence of specific IgG (Fig. 1, 2). Transfected HEK cells, which express recombinant neurochondrin, are suitable for the monospecific detection of these autoantibodies (Fig. 3).

Diagnostic value. Anti-neurochondrin antibodies were first described in connection with 3 cases of autoimmune cerebellum degeneration. Malignant tumours were not found. Only long-term immunosuppressive therapies in patients led to a clinical stabilisation or improvement.

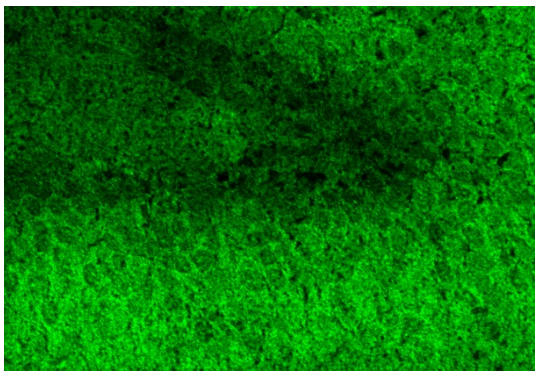
Anti-neurochondrin-associated cerebellum degeneration is another subtype of autoimmune cerebellum ataxia; a parallel analysis of all antibodies associated with cerebellum ataxia using indirect immunofluorescence is recommended.

Literature.

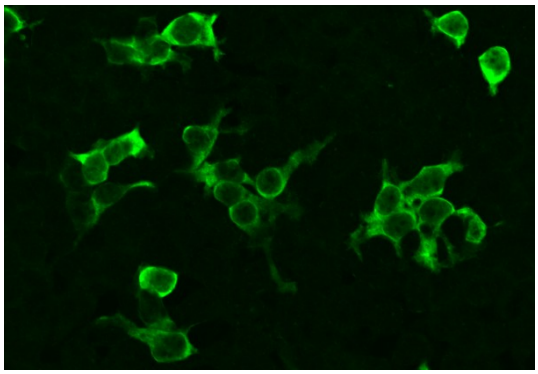
Miske R, Gross CC, Scharf M, Golombeck KS, Hartwig M, Bhatia U, Schulte-Mecklenbeck A, Bönte K, Strippel C, Schöls L, Synofzik M, Lohmann H, Dettmann IM, Deppe M, Mindorf S, Warnecke T, Denno Y, Teegen B, Probst C, Brakopp S, Wandinger KP, Wiendl H, Stöcker W, Meuth SG, Komorowski L, Melzer N (2017) Neurochondrin is a neuronal target antigen in autoimmune cerebellar degeneration. Neurol Neuroimmunol Neuroinflamm 4(1):e307.



Autoantibodies against neurochondrin. Fig. 1. Substrate: cerebellum (rat)



Autoantibodies against neurochondrin. Fig. 2. Substrate: hippocampus (rat)



Autoantibodies against neurochondrin. Fig. 3. Substrate: transfected cells

Autoantibodies against neuronal antigens

W. Stöcker

Synonym(s). Anti-neuronal antibodies; autoantibodies against onconeuronal antigens

Definition. Neuronal antigens can be found in normal cells in the nervous system, some of them are also expressed in various tumours (onconeuronal antigens). Antibodies against (onco)neuronal antigens are therefore often associated with neurological syndromes, which are obligate (>95%) or facultative paraneoplastic depending on the antigen, i.e. as the complications in tumour diseases that are not triggered by the tumour itself, its metastases or by vascular, infectious, metabolic or therapy-related causes. Moreover, antibodies against antigens of the nervous system, which occur with neurological syndromes without a tumour association also exist (Tab. 1); see also autoantibodies against acetylcholine receptors, autoantibodies against amphiphysin, autoantibodies against ATP1A3, autoantibodies against calcium channels, autoantibodies against CARP, autoantibodies against DPPX, autoantibodies against GABAB-receptors, autoantibodies against ganglionic acetylcholine receptors, autoantibodies against glutamate decarboxylase, autoantibodies against glutamate receptors type AMPA, autoantibodies against glutamate receptors type NMDA, autoantibodies against glycine receptors, autoantibodies against Hu, autoantibodies against IgLON5, autoantibodies against ITPR1, autoantibodies against potassium channels, autoantibodies against Ma, autoantibodies against neurochondrin, autoantibodies against neuronal cell nuclei type 3, autoantibodies against PCA-2, autoantibodies against Ri, autoantibodies against titin, autoantibodies against Tr/DNER, autoantibodies against Yo. See also autoantibodies against aquaporin 4, autoantibodies against glial cell nuclei (AGNA), autoantibodies against myelin oligodendrocyte glycoprotein; however, this does not relate to neuronal antigens in the narrow sense, but rather specifically to neural antigens, as they are primarily expressed by glial cells (Tab. 1).

Sample material. Serum, plasma or cerebrospinal fluid

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. The gold standard for analysing autoantibodies against neuronal antigens and the differentiation of non-neuron-specific autoantibodies is the indirect immunofluorescence test (IIFT, immunofluorescence, indirect) with frozen sections of the following primate tissue: Hippocampus, cerebellum, cerebrum, peripheral nerve, foetal intestine, pancreas, liver. Transfected HEK cells ("human embryonic kidney cells), which ensure the recombinant expression of the different neuronal antigens, are also used for the monospecific detection of these autoantibodies. Two starting dilutions are incubated in parallel, 1:10 and 1:100; immunoglobulin class IgG is primarily analysed, the clinical relevance of specific IgA or IgM is still unclear in most cases.
Positive results can be confirmed using immunoblots: This requires the use of Western blots based on cerebellum and hippocampus extracts or line blots with recombinant antigens. Western blots offer the complete spectrum of antigens, while line blots only provide a selection of recombinant antigens. However, line blots are easier to read.
It is recommended, in addition to the requested analyses, to analyse the most important other autoantibodies against neuronal antigens at the same time (see also Fig. 1). This provides a rapid and unexpected vital diagnosis in many cases (for every third positive reaction). Biochip mosaics are suitable for detecting the entire spectrum of anti-neuronal antibodies with immunofluorescence: 20 or more substrates from tissue sections and different recombinant cells are arranged in succession on a reaction field and incubated with the sample (Fig. 2).

Reference range — Adults. Negative

Interpretation. Two-thirds of patients with paraneoplastic neurological syndromes display autoantibodies against onconeuronal antigens in the serum or in the cerebrospinal fluid. This finding is often the first sign of the underlying tumour. It not only provides evidence of the paraneoplastic aetiology, but also facilitates the tumour screening due to the association with certain types of tumours.

Literature.

Blaes F, Grisold W, Grabbe S, Hübner J, Kleeberg U, Krege S, Leypoldt F, Rauer S, Roelcke U, Schreckenberger M, Singer S, Stummer W, Voltz, R, Wandinger KP, Weller M, Wörmann B (2012) Paraneoplastische neurologische Syndrome, in: Hans-Christoph Diener, Christian Weimar (Hrsg.): Leitlinien für Diagnostik und Therapie in der Neurologie, Herausgegeben von der Kommission "Leitlinien" der Deutschen Gesellschaft für Neurologie, Thieme Verlag, Stuttgart, September 2012

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Autoantibodies against neuronal antigens. Tab. 1. Antibodies as a marker of possible paraneoplastic aetiology, directed against antigens with clinical relevance as well as against other antigens whose clinical significance is still unclear.

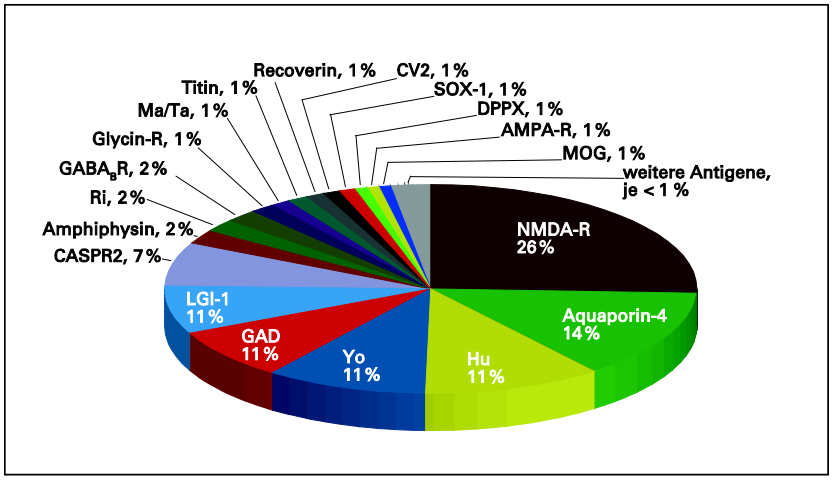
Antigen or antibody against	Alternative name (anti-)	Antigen	Antigen localisation	Function	Syndrome	Most commonly associated tumours
Paraneoplastic antibodies against neuronal antigens, tumour association > 90%						

Amphiphysin		Amphiphysin	Intracellular (cytoplasmic)	Vesicle endocytosis	Stiff-person syndrome	Breast cancer, SCLC
CV-2	Collapsin response mediator protein 5 (CRMP5)	CRMP5	Intracellular (cytoplasmic)	Neuronal development	Encephalitis	SCLC, thymoma
Hu	ANNA-1	Hu proteins	Intracellular (nuclear)	RNA bonding	Encephalomyelitis, neuropathy	SCLC, neuroblastoma
Ma1	PNMA1	Ma protein (37 kDa)	Intracellular (nuclear)	Unknown	Rhombencephalitis, PLE	Breast cancer
PCA-2		Microtubule-associated protein (MAP) 1B	Intracellular (cytoplasmic)	Cytoskeleton organisation, axon growth	Cerebellitis, Encephalitis, LEMS, neuropathy	SCLC
PP	ANNA-3	Unknown (170 kDa)	Intracellular (nuclear)	Unknown	Neuropathy, PCD, PLE	SCLC
Recoverin		Recoverin	Intracellular	Photoreceptor protein	Retinopathy	Lung cancer
Ri	ANNA-2	NOVA	Intracellular (nuclear)	RNA bonding	Opsoclonus myoclonus ataxia	Breast cancer, SCLC
Ma2/Ta	PNMA2	Ma protein (40 kDa)	Intracellular (nuclear)	Unknown	Rhombencephalitis, PLE	Testicular cancer
Sry-like high mobility group box protein 1	Sox1	Sox1	Intracellular (nuclear)	Transcription factor	LEMS, neuropathy	SCLC
Tr	PCA-Tr, Tr/DNER	Delta/Notch-like Epidermal Growth Factor–related Receptor (DNER)	Extracellular (membrane-bound)	Ligand of NOTCH1	PCD	Hodgkin's disease
Yo	PCA-1	cdr2, cdr62	Intracellular (nuclear)	DNA bonding	PCD	ovarian, breast, uterine cancer
Zic-4		Zic proteins	Intracellular (nuclear)	DNA bonding	PCD	SCLC
Facultative paraneoplastic antibodies against neuronal and neuromuscular antigens						
Acetylcholine receptors	AChR	Nicotinergic AChR	Extracellular (membrane-bound, neuromuscular end plate)	Neurotransmitter receptors	Myasthenia gravis	Thymoma
Carbonic anhydrase-related protein VIII	CARP	CARP	Intracellular (cytoplasmic)	Bonding to ITPR1	Cerebellum degeneration	Melanoma, ovarian cancer
Contactin-associated protein-like 2	CASPR2	VGKC-associated protein	Extracellular (membrane-bound)	Component of adhesion complexes	Neuromyotonia, Morvan's disease, limbic encephalitis	Thymoma
Dipeptidyl-peptidase-like protein-6	DPPX	DPPX	Extracellular (membrane-bound)	Accessory subunit of the Kv4.2 potassium channel	Progressive encephalomyelitis (with rigidity and myoclonus); autonomic dysfunction	B-cell neoplasia
Gamma-aminobutyric acid B-receptors	GABAB-R	Extracellular domain of the GABAB1 subunit of the receptor	Extracellular (membrane-bound, synapsis)	Neurotransmitter receptors	Limbic encephalitis, psychosis	SCLC, thymus carcinoid
Glutamate decarboxylase	GAD	GAD65	Intracellular (cytoplasmic)	Neurotransmitter synthesis	Stiff-person syndrome, limbic encephalitis, cerebellum degeneration	SCLC, thymoma, renal cell, pancreatic carcinoma
Ganglionic acetylcholine receptors	GN-AChR	Ganglionic (α3) AChR	Extracellular (membrane-bound, synapsis)	Neurotransmitter receptors	Autonomic neuropathy	SCLC, lymphoma, bladder, breast, prostate, rectal
Glutamate receptors, type AMPA	AMPA-R	GluR1 and GluR2 subunit of the receptor	Extracellular (membrane-bound, synapsis)	Neurotransmitter receptors	Limbic encephalitis, atypical psychosis	Lung cancer, Breast cancer, Thymoma
Glutamate receptors, type NMDA	NMDA-R	Extracellular domain of the NR1 subunit of the receptor	Extracellular (membrane-bound, synapsis)	Neurotransmitter receptors	Anti-glutamate receptors (type NMDA) encephalitis (limbic encephalitis)	Teratomas (ovaries, testes)
Glycine receptors	Gly-R	α1-subunit of the receptor	Extracellular (membrane-bound, synapsis)	Neurotransmitter receptors	Progressive encephalomyelitis with rigidity and myoclonus	Hodgkin's disease
Inositol-1,4,5-triphosphate receptor type 1	ITPR1	ITPR1	Intracellular (membrane-bound)	Ligand-controlled calcium channel	Cerebellum ataxia, peripheral neuropathy	Adenocarcinoma, myeloma
Leucin-rich glioma-inactivated 1 protein	LGI1	VGKC-associated protein	Extracellular (secreted)	Part of the transsynaptic complexes	Limbic encephalitis, dementia	Thyroid cancer, thymoma, SCLC, renal cell carcinoma, ovarian teratoma

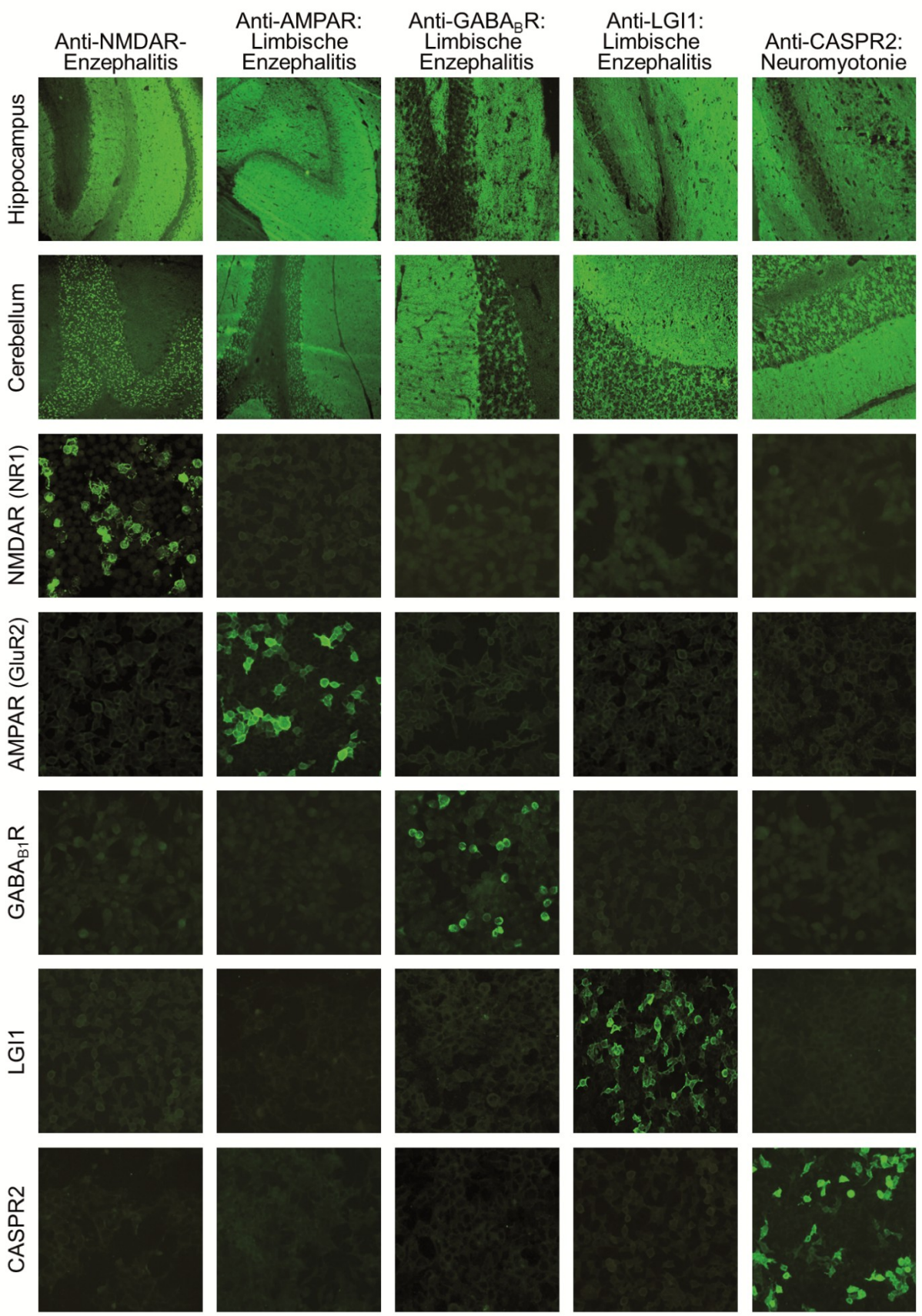
Metabotropic glutamate receptors 1	mGluR1	Extracellular domain of mGluR1	Extracellular (membrane-bound, synapsis)	Neurotransmitter receptors	Cerebellum degeneration	Hodgkin's disease, adenocarcinoma
Metabotropic glutamate receptors 5	mGluR5	Extracellular domain of mGluR5	Extracellular (membrane-bound, synapsis)	Neurotransmitter receptors	Ophelia syndrome (psychosis)	Hodgkin's disease
Muscle-specific tyrosine kinase	MuSK	MuSK	Extracellular (membrane-bound, neuromuscular end plate)	Formation of neuromuscular end plates, aggregation of acetylcholine receptors	Myasthenia gravis	Thymoma
Neuronal Na+/K+-ATPase	ATP1A3	Alpha 3-subunit	Extracellular (membrane-bound)	Neuronal and cardiac sodium-potassium ion pump	Progressive ataxia, paresis	Colon cancer
Rho GTPase activating protein 26	ARHGAP26, Ca	ARHGAP26	Intracellular (cytoplasmic)	Clathrin-independent endocytosis	Cerebellum ataxia	Ovarian cancer
Titin		Titin	Intracellular (cytoplasmic, muscle fibres)	Muscle filament	Myasthenia gravis	Thymoma
Voltage-gated calcium channels	VGCC	VGCC	Extracellular (membrane-bound)	Voltage-gated calcium channels	LEMS	SCLC
Voltage-gated potassium channel subfamily A	KCNA2, Kv1.2	KCNA2, Kv1.2	Extracellular (membrane-bound)	Voltage-gated potassium channel	Neuromyotonia	Thymoma, SCLC
Non-paraneoplastic antibodies against neuronal antigens (no tumour associations described to date)						
Contactin 1/Contactin-associated protein 1	CNTN1/CASPR1	CNTN1/CASPR1	Extracellular (membrane-associated)	Cell adhesion, formation of axon connections	Chronic-inflammatory demyelinating polyradiculoneuropathy	None known
ELKS/Rab6-interacting/CAST family member 1	ERC1	ERC1	Intracellular (cytoplasmic)	Synaptic transmission of stimuli, associated with β4-subunit of the VGCC	LEMS	None known
Flotillin	FLOT1/2	FLOT1/2	Extracellular (membrane-bound)	Axon growth and regeneration (optic nerve)	Optic neuritis, multiple sclerosis	None known
Glutamate receptor delta 2	GluRδ2	GluRδ2	Extracellular (membrane-bound, synapsis)	Synapsis development	Encephalitis, transverse myelitis	None known
Homer-3		Homer-3	Intracellular (cytoplasmic)	Modulated activity of metabotropic glutamate receptors	Cerebellum ataxia	None known
IgLON family member 5	IgLON5	IgLON5	Extracellular (membrane-associated)	Neuronal cell adhesion protein	Parasomnia with respiratory dysfunction	None known
Neurochondrin	NCDN	Neurochondrin	Intracellular (cytoplasmic)	Interaction with metabotropic glutamate receptors	Cerebellum degeneration	None known
Antibodies against neural antigens (occur with tumours in rare cases)						
AQP-4	NMO-IgG	Aquaporin-4 protein	Extracellular (membrane-bound, astrocytes)	CNS water channels	Neuromyelitis optica, longitudinal extensive transverse myelitis, recurring optic neuritis	Breast and lung cancer, thymoma
Glial cell nuclei	AGNA	Unknown	Intracellular (Bergmann glia)	Unknown	LEMS, cerebellum degeneration, neuropathy	Lung cancer
Myelin oligodendrocyte glycoprotein	MOG	MOG	Extracellular (membrane-bound, oligodendrocytes)	Part of the myelin sheath	Acute disseminated encephalomyelitis, multiple sclerosis, neuromyelitis spectrum diseases	None known
Antibodies against partially characterised neural antigens; uncertain disease associations and clinical relevance (Komorowski et al., 2017)						
Carnitine O-palmitoyl transferase 1	CPT1C	CPT1C	Intracellular (membrane-bound)	Lipid metabolism sensor in neurons	_	_
C-terminal binding protein 1	CTBP1	CTBP1	Intracellular (nuclear, cytoplasmic)	Transcription factor	_	_
Rho-associated protein class 2	ROCK2	ROCK2	Intracellular (cytoplasmic)	Serine/threonine kinase	_	_
Septin complex	Septin complex	Septin complex	Intracellular (cytoplasmic)	Regulation of the neuronal cell architecture	_	_
Syntaxin 1B	STX 1B	STX1B	Extracellular (membrane-bound)	Receptor for transport vesicles (release of	_	_

				neurotransmitters)		

LEMS Lambert Eaton myasthenia syndrome; *PCD* paraneoplastic cerebellum degeneration; *PLE* paraneoplastic limbic encephalitis; *SCLC* small cell lung cancer; *VGCC* voltage-gated calcium channels; *VGKC* voltage-gated potassium channels



Autoantibodies against neuronal antigens. Fig. 1. Percentage proportions of the autoantibodies (IgG) against neural antigens in the lab analysis request "Neurology autoantibody profile" (n = 16,741 samples, sent to Clinical Immunological Laboratory, Lübeck, 1 April 2012 - 31 March 2013).



Autoantibodies against neuronal antigens. Fig. 2. Detection of autoantibodies against neuronal surface antigens by indirect immunofluorescence. Antigen substrates: hippocampus and cerebellum (rat), variously transfected HEK-293 cells. While a differentiation of the autoantibodies is difficult on tissues, they can be identified immediately using recombinant cells. See also 7 autoantibodies against GABAB-receptors, [7] autoantibodies against glutamate receptors type AMPA, [7] autoantibodies against glutamate receptors type NMDA, [7] autoantibodies against potassium channels

Autoantibodies against neuronal cell nuclei type 3

W. Stöcker

Synonym(s). ANNA-3

Definition. Autoantibodies against a 170-kDa protein in the cell nuclei of the Purkinje cells of the cerebellum (which is also expressed in the podocytes of the renal glomeruli).

Function and pathophysiology. ANNA-3 proteins are expressed in peripheral and central neurons as well as, in antibody-positive patients, in tumour tissue.

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Autoantibodies against neuronal cell nuclei type 3 can be detected with the indirect immunofluorescence test (IIFT, immunofluorescence, indirect) based on frozen sections of primate cerebellum. Autoantibodies against neuronal cell nuclei type 3 display a fluorescence of the Purkinje cell nuclei as well as the podocytes of the renal glomeruli.
In the Western blot with separated cerebellum extract, a reaction with 170 kDa is detected.
ANNA-3 are investigated in parallel with the other 7autoantibodies against neuronal antigens owing to the differential diagnostic relationship of the associated encephalitis to other paraneoplastic neurological syndromes.

Sample material. Serum, plasma or cerebrospinal fluid

Diagnostic value. ANNA-3 are found in cerebellar ataxia, myelopathy and limbic/brain stem encephalitis; they are associated with small cell lung cancers and adenocarcinomas and can provide an initial indication of an underlying tumour.

Literature.
Chan KH, Vernino S, Lennon VA (2001) ANNA-3 anti-neuronal antibody: marker of lung cancer-related autoimmunity. Ann Neurol 50:301–311

Autoantibodies against renal tubules

W. STÖCKER

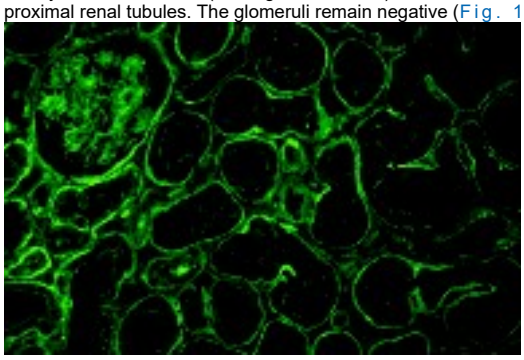
Synonym(s). Autoantibodies against the basement membrane of the renal tubules; tubular basement membrane antibodies

Definition. Antibodies against antigens of the renal tubule basement membrane

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. In the case of a positive reaction in the indirect immunofluorescence test (immunofluorescence, indirect) with primate kidney as the substrate (starting dilution 1:10), the tubular basement membrane displays a linear fluorescence predominantly in the area of the proximal renal tubules. The glomeruli remain negative (Fig. 1).



Autoantibodies against renal tubules. Fig. 1. Substrate: primate kidney

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Antibodies against the tubular basement membrane can be found in various forms of nephritis, including rejection reactions after transplants and help in the differential diagnosis of tubulointerstitial diseases. Sera of patients with Goodpasture syndrome and autoantibodies against glomerular basement membrane (GBM) in some cases also react with the basement membrane of part of the tubules, in addition to staining the GBM. Likewise, some sera from patients with progressive systemic sclerosis display a reaction with the tubular basement membrane; however, in those cases it is mainly caused by autoantibodies against Scl-0.

Literature.

Steblay RW, Rudofsky U (1971) Renal tubular disease and autoantibodies against tubular basement membrane induced in guinea pigs. J Immunol 107:589–594

Komorowski L, Scharf M, Teegen B, Rentzsch K, Schlumberger W, Stöecker W. Autoantibodies against tubular basement membrane in progressive systemic sclerosis are directed against Scl-70. In: Conrad K, Chan EK, Andrade LEC, Steiner G, Prujin GJ, Shoenfeld Y, editors. From Autoantibody research to standardized diagnostic assay in the management of human diseases. Report on the 12th Dresden Symposium on Autoantibodies. 10 ed. Dresden: Pabst Science Publishers; 2015. p. 127-128

Autoantibodies against nucleoli

W. STÖCKER

Synonym(s). Antinucleolar antibodies

Definition. Autoantibodies against nucleoli are a subgroup of the antinuclear antibodies, the former are directed against antigens of the nucleoli. Antigens of the nucleolus: U3-(n)RNP/fibrillarin, RNA-polymerase I, PM-Scl (PM-1), 7-2-RNP (To), 4-6-S-RNA, nucleolus organiser

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Autoantibodies against nucleoli display a granular (Us-nRNP/fibrillarin) (Fig. 1), a fine droplet (RNA-polymerase I) (Fig. 2), a homogeneous (PM-Scl (PM-1), 7-2-RNP (To), 4-6-S-RNA) (Fig. 3) or a dot-like (NOR-90 nucleolus organiser region) (Fig. 4) fluorescence of the nucleoli in the indirect immunofluorescence test (IIFT; immunofluorescence, indirect) with HEp-2 cells, depending on the target antigen. NOR-90 antibodies also display a particularly distinct coarse-dot fluorescence in the chromosome region of the metaphase cells.
In the case of a positive result in the IIFT, a monospecific test (enzyme-linked immunosorbent assay, line blot) with purified or recombinant antigens or a Western blot with nuclear antigens can be used to precisely identify the target antigen.

Reference range — Adults. Negative

Diagnostic value. Autoantibodies against nucleoli in the serum of patients are a characteristic finding in progressive systemic sclerosis (diffuse form); Tab. 1.

Autoantibodies against nucleoli. Tab. 1. Antibody prevalence in progressive systemic sclerosis (diffuse form)

Antigen	Prevalence
Fibrillarin	5–10
PM-Scl	50–70 (including overlap syndrome)
Scl-70 (nuclear antigen, not limited to the nucleoli)	25–75
RNA-polymerase I	4
7-2-RNP (To)	Rare
NOR-90 (nucleolus organiser region)	Rare

Literature.

Schlumberger W, Olbrich S, Müller-Kunert E et al (1994) Autoantikörper-Diagnostik mit der Substratkombination Humane Epithelzellen (HEp-2) und Primatenleber. Differenzierung der Antikörper durch Enzymimmuntests. Eigenverlag der EUROIMMUN AG, Lübeck, S 1–28

Legends

Autoantibodies against nucleoli. Fig. 1. Autoantibodies against fibrillarin. Substrate: HEp-2 cells

Autoantibodies against nucleoli. Fig. 2. Anti-RNA polymerase I. Substrate: HEp-2 cells.

Autoantibodies against nucleoli. Fig. 3. Anti-PM-Scl. Substrate: HEp-2 cells.

Autoantibodies against nucleoli. Fig. 4. Autoantibodies against nucleolus organiser; NOR. Substrate: HEp-2 cells

Autoantibodies against nucleosomes

W. Stöcker

Synonym(s). Nucleosome antibodies; anti-nucleosome antibodies; ANuA

Function and pathophysiology. Nucleosomes are functional subunits of chromosomes and contribute to the dense packing of the DNA in the cell nuclei. They consist of a nucleus, which consists of the histone proteins H2A, H2B, H3 and H4 and of 2 coils of DNA (altogether 146 base pairs), which surround the histone part. An area of free DNA (linker DNA), which is associated with histone H1, is located between two nucleosomes.

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Antibodies against nucleosomes are detected with enzyme immunoassays (enzyme-linked immunosorbent assay), whose antigen coating consists of highly purified nucleosomes. The nucleosomes must not be contaminated with DNA topoisomerase I, the autoantigen Scl-70, which has a high affinity to nucleosomes.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Systemic lupus erythematosus (SLE)

Diagnostic value. Systemic lupus erythematosus (SLE) is characterised by the presence of various antinuclear antibodies (ANA): For example, autoantibodies against double-stranded DNA (dsDNS), autoantibodies against Sm and autoantibodies against ribosomal phosphoproteins are specific and sensitive markers for SLE. In addition, autoantibodies against nucleosomes (ANuA) can be detected with a prevalence of 50-70% with SLE, especially for SLE with kidney involvement (lupus nephritis, LN). However, its relevance as a characteristic disease marker for SLE has been limited to date, given that 10–68% of the sera of scleroderma patients also reacted with conventionally prepared nucleosomes. Using a preparation of mononucleosomes, which is free of H1, Scl-70 and other non-histone proteins, as the ELISA antigen, eliminates the false-positive reactions of the scleroderma sera.
Autoantibodies against nucleosomes occur independent of anti-dsDNA antibodies: 18% of the SLE sera reacted exclusively with nucleosomes and not with dsDNA. The additional detection of the anti-nucleosome antibodies can therefore significantly increase the serological detection rate in SLE diagnosis.
Antibodies against nucleosomes also indicate a severe course of SLE with LN: prevalence in patients with transplant-associated LN: 79%, without transplant: 18%, SLE without nephritis: 9%.

Literature.

Mastroianni-Kirsztajn G, Hornig N, Schlumberger W (2015) Autoantibodies in renal diseases – clinical significance and recent developments in serological detection. Front Immunol 6:221

Stinton LM, Barr SG, Tibbles LA, Yilmaz S, Sar A, Benedikttson H, Fritzler MJ (2007) Autoantibodies in lupus nephritis patients requiring renal transplantation. Lupus 16:394–400

Suer W, Dähnrich C, Schlumberger W, Stöcker W (2004) Autoantibodies in SLE but not in scleroderma react with protein-stripped nucleosomes. J Autoimmun 22:325–334

Autoantibodies against oxidised LDL

W. Stöcker, Chr. Krüger

Synonym(s). oxLDL antibodies

Function and pathophysiology. Oxidised low-density lipoprotein (LDL) is the main part of the lipoproteins contained in the atherosclerotic lesions. Complexes of oxidised LDL (oxLDL) and the phospholipid-binding plasma protein β2 glycoprotein (β2GPI) can be detected in various autoimmune and chronic inflammatory diseases (systemic lupus erythematosus, antiphospholipid syndrome, progressive systemic sclerosis, diabetes mellitus, myocardial infarction).

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Autoantibodies against oxidised LDL as well as against the complex consisting of oxLDL/β2GPI have previously been described. They are detected using an enzyme-linked immunosorbent assay.

Indication. Possible risk assessment of arterial thrombosis in patients with systemic lupus erythematosus and antiphospholipid syndrome as well as a general marker of the progression of atherosclerosis, especially in connection with various autoimmune and chronic-inflammatory diseases.

Diagnostic value. Autoantibodies against oxLDL/β2GPI have been found in patients with systemic lupus erythematosus as well as with antiphospholipid syndrome. They are an indication of arterial thrombosis. Research is currently looking at whether these autoantibodies have the potential to be a prognostic marker.

Literature.

Salonen JT, Yla-Herttuala S, Yamamoto R et al (1992) Autoantibody against oxidised LDL and progression of carotid atherosclerosis. Lancet 339:883–887

Autoantibodies against p53

W. Stöcker, Chr. Krüger

Synonym(s). p53 antibodies; autoantibodies against the tumour suppressor factor p53

Definition. p53 is a protein which, as a transcription factor in the cell nuclei, is involved in transmitting anti-proliferative and proapoptotic signals. It can protect cells against malignant degeneration and prevent the occurrence of tumours. p53 is the product of a tumour suppressor gene. These genes code proteins which inhibit cell growth, and therefore represent a counterbalance to the proliferation-activating proto-oncogenes.
The p53 gene is the most frequently mutated tumour suppressor gene in human tumours. Mutations of the p53 gene lead to the accumulation of the protein in the cell.

Function and pathophysiology. In its mutated form, p53 can bond with various cellular proteins (e.g. to heat shock proteins). In some cases, it can then also be detected in the blood. This may potentially be the reason for the formation of autoantibodies against (mutated) p53 in patients with various malignant diseases, as shown by numerous studies.
Besides tumours, anti-p53 antibodies (occasionally) also occur in a small number of non-malignant diseases, such as with various autoimmune diseases: systemic lupus erythematosus, rheumatoid arthritis, granulomatosis with polyangiitis (obsolete: Wegener's granulomatosis) or Graves' disease. However, a possibly undetected tumour must always be considered as the cause when anti-p53 antibodies are detected.

Sample material. Serum

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Enzyme-linked immunosorbent assay with p53 as the target antigen

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Autoantibodies against p53 can be detected in numerous malignant diseases and are used as a marker for diagnosis, prognosis and the course of treatment.
The prevalence is particularly high in tumour patients with mutations in the p53 gene and amounts to 30-50%.

Diagnostic value. The detection of anti-p53 autoantibodies enables the early diagnosis of colon, ovarian and liver cancer in individual cases. As the occurrence of the autoantibodies generally indicates a malignant disease, this parameter is also suitable for monitoring patients at risk, such as heavy smokers, patients with long-term colorectal adenomas and where colon cancer is suspected as well as for persons who work with carcinogenic substances or who have a genetic risk of tumours (e.g. for lung, liver or breast cancer).

Literature.
Soussi T (2000) p53 Antibodies in the sera of patients with various types of cancer: a review. Cancer Res 60:1777–1788
Crawford LV, Pim DC, Bulbrook RD (1982) Detection of antibodies against the cellular protein p53 in sera from patients with breast cancer. Int J Cancer 30:403–408

Autoantibodies against pancreatic islets

W. Stöcker

Synonym(s). Pancreatic islet cell antibodies; antibodies against endocrine pancreatic tissue; islet cell antibodies; autoantibodies against endocrine pancreatic tissue

Definition. Autoantibodies against antigens in the pancreatic islets; 3 relevant target antigens have been identified to date: the enzymes glutamate decarboxylase (GAD), tyrosine phosphatase (insulinoma-associated antigen 2, IA2) and the zinc transporter ZnT8.

Function and pathophysiology. The serological detection of autoantibodies against pancreatic islets confirms the diagnosis of type I diabetes mellitus. Moreover, it also enables the detection of preclinical autoimmune reactions in persons at risk.
The following observations indicate that islet cell antibodies do not play a role in the pathogenesis of diabetes mellitus type I:
- [5] The disease is not transmitted from the mother to the foetus.
- [5] Plasmapheresis does not lead to the improvement of the disease.
- [5] In animal testing, the disease cannot be transmitted via ICA-containing sera.

Sample material. Serum or plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. A smooth to granular cytoplasmic fluorescence of all islet cells is observed in the indirect immunofluorescence test (immunofluorescence, indirect) with primate pancreas as the substrate (parallel starting dilutions of 1:10 and 1:100, duration of the first incubation step 18 h) (Fig. 1).
Enzyme-linked immunosorbent assays, chemiluminescence immunoassays and radioimmunoassays are available as monospecific test systems to detect autoantibodies against the most important antigens of GAD (autoantibodies against glutamate decarboxylase), IA2 (tyrosine phosphatase; autoantibodies against insulinoma-associated antigen 2) and zinc transporter 8 (autoantibodies against zinc transporter ZnT8).

International standard. The Juvenile Diabetes Foundation (JDF) provides a reference serum for the immunofluorescence test. Accordingly, the results should be indicated in JDF units (JDFU). At the same time, indirect immunofluorescence is used to determine the titer of the positive patient samples and the reference serum and the JDF units are calculated for the patients, with awareness of the JDF units of the reference serum, based on the rule of three.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. The detection of autoantibodies against antigens of the pancreatic islets is used to confirm the diagnosis of type I diabetes mellitus as well as to identify preclinical autoimmune reactions in persons at risk: In 90% of cases, one or more diabetes mellitus-associated autoantibodies can be detected in the serum before the clinical manifestation. Their detection then enables the early identification of persons with an increased risk of the disease. Appropriate interventions, e.g. regulation of the glucose concentration at a low level or immunosuppressive measures, enable the onset of the disease to be prevented in some cases.
There are over 4 million diabetics in Germany (approx. 7% of the population), of which more than 90% are persons classified as type II diabetics. However, about 10% suffer from type I (“latent autoimmune diabetes of adults”, LADA). Misdiagnosis has fatal consequences: Oral antidiabetics should essentially not be administered to these patients, rather they must be exclusively supplied with insulin so as not to unnecessarily stimulate the pancreatic islets and thereby expose them to further autoaggression. After insulitis subsides, a residual function of the endocrine pancreas remains intact in most cases. The diabetes mellitus-associated autoantibodies should therefore be analysed in all newly diagnosed diabetics in order to identify cases with type I.

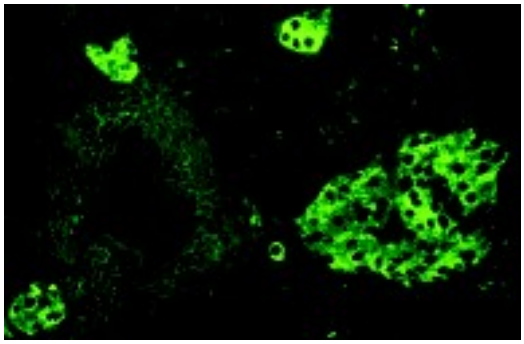
Diagnostic value. The antibody titer decreases as the disease progresses.

Literature.

American Diabetes Association (2013) Standards of Medical Care in Diabetes. Diabetes Care 36.

Bottazzo GF, Florin-Christensen A, Doniach D (1974) Islet-cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. Lancet 2:1279–1283

Landin-Olsson M (2002) Latent autoimmune diabetes in adults. Ann N Y Acad Sci 958:112-116.



Autoantibodies against pancreatic islets. Fig. 1. Substrate: primate pancreas

Autoantibodies against pancreatic secretion

W. Stöcker

Synonym(s). Autoantibodies against pancreatic acinar cells; pancreatic acinar cell antibodies; aab against the exocrine pancreas; aab against pancreatic juice

Definition. Autoantibodies in Crohn's disease, which are directed against the acinar cells and secretion of the pancreas

Function and pathophysiology. Autoantibodies against pancreatic acinar cells are a reliable identifier of Crohn's disease; they are the serological equivalent to autoantibodies against intestinal goblet cells (goblet cell antibodies) in ulcerative colitis. With regard to their organ specificity and disease association as well as their often high serum concentrations, both antibodies have a high significance in a way similar to that of other autoantibodies for diseases whose autoimmune pathogenesis is already generally accepted, such as autoantibodies against desmosomes for pemphigus vulgaris or autoantibodies against glomerular basement membrane for Goodpasture syndrome. The fact that only part of the patients exhibit autoantibodies is not a counter-argument, rather it also corresponds to the conditions found in diseases with a confirmed autoimmune pathogenesis. Pancreatic antibodies can only be detected in patients with acute or chronic pancreatitis in exceptional cases, the titers are always very low; in contrast to Crohn's disease, IgG is practically non-existent with pancreatitis.
Pancreatic acinar cell autoantibodies can be neutralised by pancreatic secretion. They are possibly an expression of a pathogenetically significant autoimmunity: It seems reasonable to assume that the inflammation of the intestinal wall in Crohn's disease is caused by the autoantigen contained in the pancreatic secretion. Only bowel segments from the ileum down are affected, in which the antigen concentration is sufficient to stimulate the sensitised immune system. The physiologically intended long period of retention of the intestinal contents in the ileum makes this bowel segment a predilection site; the autoimmune potential can extensively interact with the autoantigen at this site of the most frequent manifestation of Crohn's disease ("ileitis terminalis"). The discontinuous transition from colon areas with severe inflammation to completely normal mucous membrane, which is typical for Crohn's disease, could be explained by the fact that a connected stool column does not move for a long period of time, which enables the autoantigen it contains to initiate the autoaggression mechanisms, which lead to a distinctly localised inflammation, in the meantime.
The antibodies against brewer's yeast (antibodies against *Saccharomyces cerevisiae*) and against various infectious pathogens, which occur in parallel with Crohn's disease with an even higher prevalence, presumably originate from a secondary immunisation caused by the adjuvant effect of the specific interactions between the immune system and the pancreatic secretion.
The detection of antibodies against the exocrine pancreas was a purely incidental finding and the direct result of the use of BIOCHIP mosaics. Previously, antibodies to components of the intestinal mucosa were investigated at the most. An autoimmune mechanism directed against a pancreatic antigen was never considered, as this organ is generally not involved in the disease process.

Sample material. Serum or plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Autoantibodies against pancreatic secretion are detected by indirect immunofluorescence (immunofluorescence, indirect), while frozen sections of primate pancreas are used as the substrate (Fig. 1). The starting dilution amounts to 1:10. Both IgA and IgG are analysed, IgM does not play a role.
Two relevant patterns can be differentiated in the case of positive sera: A reticular-granular and drop-like fluorescence in the area of the acinar cells, while the islets are not stained. Only precisely these two patterns should be evaluated as positive; the large number of other fluorescence patterns that can be found with exocrine pancreas have nothing to do with Crohn's disease. The reticular-granular fluorescence is based on a reaction with what has now been identified as autoantigen CUZD1; the target antigen that corresponds to the drop-like fluorescence is glycoprotein GP-2. Instead of frozen sections of the pancreas, these days, HEK-293 cells transfected with these two autoantigens can be used as the substrate, which increases the detection sensitivity by 25%.
Autoantigens against pancreatic secretion consist exclusively of IgA in 9% of positive cases and exclusively of IgG in 36% of cases, while both immunoglobulin classes are present in 55% of cases. Titers from 1:32 confirm Crohn's disease.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Discriminatory tests of chronic inflammatory bowel diseases (Crohn's disease, ulcerative colitis)

Interpretation. Autoantibodies against pancreatic secretion (acinar cells) are pathognomonic for Chron's disease. Their prevalence amounts to 39% on average, or 50% if the disease has existed for more than 2 years.
In ulcerative colitis, pancreatic antibodies only occur in exceptional cases and practically never in healthy blood donors.

Diagnostic value. In addition to pancreatic acinar cell autoantibodies, anti-*Saccharomyces -cerevisiae* antibodies (ASCA) are also found in 67% of the sera with Crohn's disease. These two antibodies are only rarely observed with ulcerative colitis. Together with autoantibodies against pancreatic secretion, the serological detection rate for Crohn's disease amounts to 80%. With the involvement of the autoantibodies against intestinal goblet cells (BAk, 28% in ulcerative colitis) and against neutrophilic granulocytes (pANCA; 67% in ulcerative colitis, 7% in Crohn's disease), a serological diagnosis alone, without knowledge of the symptoms, enables a distinction to be made between ulcerative colitis and Crohn's disease in four of five patients with chronic inflammatory bowel diseases (as all autoantibodies are present independent of one another and detect completely different target antigens). However, autoantibodies against granulocyte cytoplasm are not adequately specific.

Literature.

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Stöcker W, Otte M, Ulrich S, Normann D, Finkbeiner H, Stöcker K, Jantschek G, Scriba PC (1987) Autoimmunity to pancreatic juice in Crohn's disease. Results of an autoantibody screening in patients with chronic inflammatory bowel disease. Scand J Gastroenterol Suppl 139:41–52

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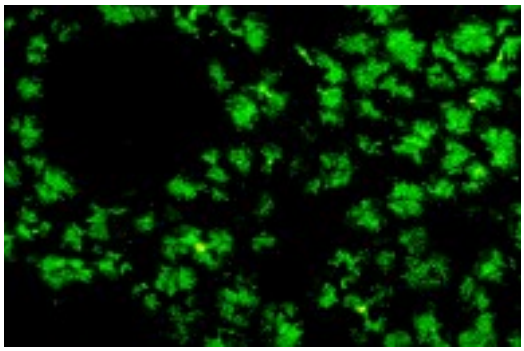
Stöcker W, Teegen B, Probst C, Aulinger-Stöcker K, Ludwig D, Glocker MO, and Komorowski L (2009) CUZD1 and GP2 are the exocrine pancreas autoantigens in Crohn's disease. In: Conrad K et al (eds) From pathogenesis to therapy of autoimmune diseases. Pabst Science Publishers:463–473



Autoantibodies against pancreatic secretion. Fig. 1. Substrate: primate pancreas

Autoantibodies against parietal cells

W. STÖCKER

Synonym(s). Autoantibodies against parietal cells of the stomach; parietal cell antibodies; PCA; autoantibodies against H+/K+-ATPase

Definition. Autoantibodies against parietal cells of the stomach. These cells produce muriatic acid and intrinsic factor, which is required to resorb vitamin B12. The enzyme H+/K+-ATPase, which is substantially involved in muriatic acid production, was identified as a target antigen of the autoantibodies against parietal cells. In addition, PCA may also be directed against gastrin receptors. Both antigens are located on the surface of the parietal cells.

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. In the indirect immunofluorescence test (immunofluorescence, indirect) with primate stomach as the substrate (starting dilution 1:10) the cytoplasm of the parietal cells in the gastric mucosa fluoresces in the case of a positive reaction; the fluorescence is fine to coarse clumpy. All other structures are darker. In a negative reaction, the parietal cells of the stomach display the same dark fluorescence as the surrounding area (Fig. 1). Parietal cell antibodies are often confused with autoantibodies against mitochondria (AMA) under a microscope. They display an even fine granular fluorescence of the cytoplasm of the parietal cells, while the environment displays a (weaker) co-reaction. Pretreating the frozen sections of the stomach with urea almost fully suppresses the typical pattern of the mitochondria antibodies. This allows PCA to be reliably detected if AMA are also present, the evaluation of the immunofluorescence is facilitated and the sensitivity and specificity are increased.
A monospecific 7enzyme-linked immunosorbent assay can be used to detect antibodies against H+/K+-ATPase, the main target antigen of PCA.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Autoantibodies against parietal cells can be detected in patients with chronic atrophic gastritis, pernicious anaemia and funicular myelosis, but also in patients with autoimmune endocrinopathies. They primarily belong to immunoglobulin classes IgA and IgG.
Chronic atrophic gastritis can be endoscopically detected in almost all patients with PCA, the prevalence amounts to almost 100%, as long as the gastric mucosa has not yet completely atrophied.
While the diagnostic sensitivity for pernicious anaemia is very high at 80-90%, the specificity for chronic atrophic gastritis, pernicious anaemia and funicular myelosis is limited due to the high number of other clinical pictures associated with PCA (e.g. Hashimoto thyroiditis, Graves' disease, diabetes mellitus type I, autoimmune adrenalitis, idiopathic primary hypoparathyroidism) and the high prevalence in healthy blood donors (5-10%, increasing with age). The prevalence of antibodies against parietal cells decreases during the course of chronic atrophic gastritis.

Literature.
Taylor KB, Roitt IM, Doniach D et al (1962) Autoimmunephenomena in perniciousanaemia: Gastricantibodies. Br Med J 2:1347–13527

Legend

Autoantibodies against parietal cells. Fig. 1. Substrate: primate stomach.

Autoantibodies against PCA-2

W. Stöcker

Synonym(s). Purkinje cell cytoplasmic autoantibodies 2; autoantibodies against Purkinje cells 2;

English term. Purkinje cell cytoplasmic autoantibodies 2

Definition. Autoantibodies against a 280-kDa protein of the Purkinje cells of the cerebellum. The microtubule-associated protein (MAP) 1B was identified as a target antigen in 2017.

Function and pathophysiology. The autoantigen of PCA-2 antibodies is expressed in peripheral and central neurons as well as in the tumour tissue of antibody-positive patients.

Analytics. The indirect immunofluorescence test (IIFT, immunofluorescence, indirect) with frozen sections of primate cerebellum is suitable for detecting autoantibodies against PCA-2. Autoantibodies against PCA-2 display a fluorescence of the Purkinje cell cytoplasm, which extends as far as to the dendrites.
In the Western blot with separated cerebellum extract, a reaction with 280 kDa is detected.

Sample material. Serum, plasma or cerebrospinal fluid

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Diagnostic value. PCA-2 antibodies are extremely rare and can provide an initial indication of an underlying tumour. They are associated with limbic/brain stem encephalitis, cerebellar ataxia, Lambert-Eaton myasthenic syndrome (LEMS), autonomic and motor neuropathy and often with gynaecological tumours and small cell lung cancer; see also autoantibodies against neuronal antigens.

Literature.

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Autoantibodies against PCNA

W. Stöcker

Synonym(s). PCNA antibodies; anti-PCNA; anti-cyclin I

Definition. The autoantibodies are directed against PCNA epitopes (proliferating cell nuclear antigen). This is an auxiliary protein of the DNA polymerase delta and has a molar mass of 36 kDa. Due to its function, PCNA takes on a key role in controlling the cell cycle: The S-phase begins upon its appearance. The protein is broken down again in the middle of the G2 phase.

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Antibodies against PCNA display a cell cycle-dependent fluorescence pattern in the indirect immunofluorescence test (IIFT, immunofluorescence, indirect) (Fig. 1). Half of the cell nuclei of all interphase cells display a bright, fine granular basic fluorescence except for the nucleoli. The other half display the same fluorescence pattern, but the intensity is lower by a factor of 10. In mitosis, the area of condensed chromosomes is not stained; the area around the chromosomes only displays a weak fine granular fluorescence, similar to the pattern and intensity of the darker nuclei of the interphase cells.
Antibodies against PCNA are often confused with autoantibodies against mitosin (cyclin II), which are associated with or are identical to CENP-F (autoantibodies against CENP-F). Antibodies against PCNA as well as against mitosin have the same special feature in that only half of the cell nuclei display a strong reaction, while the other cell nuclei display a reaction that is many times weaker. However, the mitotic cells outside the chromosome region show only a weak fluorescence with antibodies to PCNA, whilst they present a perichromosomal, particularly strong smooth to fine-speckled fluorescence with antibodies to mitosin.
In the case of a positive result in the IIFT, a monospecific test (enzyme-linked immunosorbent assay, line blot) with purified or recombinant PCNA can be used to precisely identify the target antigen.

Reference range — Adults. Negative

Reference range — Children. Negative

Diagnostic value. Anti-PCNA antibodies are specific for systemic lupus erythematosus. However, the prevalence is only 3%.

Literature.

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Legend

Autoantibodies against PCNA. Fig. 1. Substrate: HEp-2 cells

Autoantibodies against phospholipase A2 receptors (PLA2R)

W. Schlumberger, W. Stöcker

Synonym(s). Autoantibodies against PLA2R; anti-PLA2R autoantibodies

Definition. Autoantibodies against type M phospholipase A2 receptors are a specific marker for primary membranous nephropathy (pMN, synonym: primary membranous glomerulonephritis, pMGN).

Function and pathophysiology. Membranous nephropathy is characterised by a chronic inflammation of the renal corpuscles (glomeruli) with increasing restriction of renal function. The primary form of MN is based on autoimmune reactions, which are directed against the transmembrane proteins PLA2R and THSD7A (autoantibodies against THSDA) and potentially other, previously unidentified antigens. The proteins are located on the surface of the podocytes in human glomeruli, which are damaged by the binding of the autoantibodies. Immunocomplexes are deposited “in situ” in the area of the glomerular basement membrane and activate the complement system; this leads to the overproduction of extracellular matrix proteins, the destruction of the cytoskeleton of the podocytes, thickening of the basement membrane and proteinuria.

MN is the most frequent cause of a nephrotic syndrome (symptom complex consisting of proteinuria, hypoproteinemia with hypoalbuminemia, hyperlipoproteinemia, oedema). The more pronounced the proteinuria, the higher the long-term risk of kidney failure, while a high mortality also exists in connection with thromboembolic and cardiovascular complications.

Analytics. Autoantibodies against PLA2R are detected by enzyme-linked immunosorbent assay and indirect immunofluorescence (immunofluorescence, indirect) using PLA2R-transfected HEK293 cells as the substrate or Western blot.

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Diagnostic value. Until PLA2R was identified as the specific target antigen in primary membranous nephropathy, MN was exclusively diagnosed by histological as well as electron microscopic analysis of renal biopsies. In this case, the deposition of immunocomplexes on the outside of the glomerular basement membrane is a characteristic feature. The detection of autoantibodies against PLA2R and THSD7A now represents a non-invasive alternative for diagnosing MN. Anti-PLA2R antibodies belong to immunoglobulin class IgG, they are highly specific and can be detected in the serum of up to 75% of patients with primary MN. In patients with secondary MN (MN as a consequence of another underlying disease), Anti-PLA2R antibodies have only been described in isolated cases, but it could not be ruled out that the underlying diseases occurred in parallel with primary MN in these cases. The anti-PLA2R antibody titer correlates with the disease activity, where an increase, decrease or disappearance of the antibodies precedes the clinical picture. As a result, the determination of the antibody titer has a high predictive value for predicting a clinical remission (spontaneous or with successful treatment) or a relapse as well as for assessing the risk of the reoccurrence of MN after a kidney transplant. The analysis of anti-THSD7A antibodies (cf. 7autoantibodies against THSDA) is useful in patients with suspected MN and a negative anti-PLA2R finding.

Literature.

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Autoantibodies against phospholipids

W. Stöcker, W. Schlumberger

Synonym(s). Phospholipid antibodies; aPL antibodies

Definition. Antibodies against phospholipids are directed against complexes consisting of phospholipids and plasma proteins

Structure. The basic building block of phospholipids is phosphatidic acid, consisting of a phosphoric acid esterified with glycerin and 2 fatty acids, which, in turn, is esterified with a polar group (e.g. serine, glycerin). If, for example, the polar group is serine, the phospholipid is referred to as phosphatidylserine. In cardiolipin, 2 phosphatidic acids are linked with an additional glycerin.

Function and pathophysiology. Background: Autoantibodies against phospholipids were initially discovered as disruptive factors in infection serological examinations (Wassermann test, VRDL test). The discovery that patients with autoantibodies against phospholipids often suffered from SLE and other autoimmune diseases was only made in the 80s.
Pathogenesis: The various points of attack by autoantibodies against phospholipids mean that the pathogenesis is diverse. Besides activating the endothelium, possibly associated with direct damage, a direct activation of thrombocytes and disruptions in the humoral coagulation factors may also be involved. The combined impact of all these changes is increased coagulation with pathological thrombus formation.

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Antibodies against phospholipids can only be reliably detected with enzyme-linked immunosorbent assays or chemiluminescence immunoassays, in which the relevant phospholipid as well as the plasma protein β2 glycoprotein I is used as the antigen.
Clinically relevant autoantibodies have been described against anionic phospholipids (cardiolipin, phosphatidylserine, phosphatidylglycerol, phosphatidylinositol) as well as against neutral phospholipids (phosphatidylethanolamine, phosphatidylcholine).
The presence of autoantibodies against cardiolipin (ACA) is one of the diagnostic criteria of antiphospholipid syndrome [International Concensus Statement, Myakis (2006)]. Due to their distinct structural homologies, antibodies against cardiolipin display a cross-reactivity with other anionic phospholipids. The detection of the relevant antibodies (against phosphatidylserine, phosphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine, phosphatidylcholine) only has an additional diagnostic benefit in rare cases.
For the serological diagnosis of antiphospholipid syndrome (APS), the recommendation is to first detect the antibodies against cardiolipin (IgG and IgM; IgA is less significant) as well as lupus anticoagulant (LA). The detection of these antibodies must be repeated after 3-6 weeks, as only a double positive finding meets the serological APS criteria. In the case of a negative ACA finding, antibodies of classes IgA, IgG and IgM against β2 glycoprotein I (β2GP1; a plasma protein cofactor; autoantibodies against β2 glycoprotein I) should be analysed. These occur in APS with a high prevalence (60-90%) as well as independent of ACA and LA. The parallel investigation of ACA and anti-β2GP1 antibodies enables the serological detection rate to be increased to almost 100%.
The clinically relevant antibodies against cardiolipin are reliant on the plasma protein β2GP1 as a cofactor in antigen detection. The binding of β2GP1 to cardiolipin is likely to lead to conformational changes within the overall structure and therefore to new antigenic epitopes. An anti-cardiolipin ELISA therefore seems to detect 3 different types of antibodies:

- Autoantibodies against cardiolipin (often with infectious diseases)
- Autoantibodies against the complex of cardiolipin and β2GP1
- Autoantibodies against β2GPI (probably structurally modified)

Anti-cardiolipin ELISAs are not suitable for use as screening methods for the parallel detection of antibodies against cardiolipin and against β2GP1, even though β2GP1 is contained as an antigen. The structural modification of the β2GP1 by binding to cardiolipin presumably leads to the loss of epitopes, which is detected by a subpopulation of the antibodies against β2GP1. The reliable and sensitive detection of antibodies against β2GP1 is only possible with an ELISA that exclusively contains this protein as an antigen.

Reference range — Adults. Negative

Diagnostic value. The clinical complications associated with the presence of antibodies against phospholipids have been summarised under the term anti-phospholipid syndrome (APS). ACA prevalence in 1,000 APS patients [according to Cervera (2002)] (Tab. 1).
The antiphospholipid syndrome is divided into 3 different subtypes:

- Primary APS: Isolated occurrence, no other identifiable autoimmune disease.
- Secondary APS: Combination with other autoimmune diseases, generally in SLE patients, less frequently in patients with scleroderma or Sjögren's syndrome.
- Catastrophic APS: Extremely rare complication that occurs equally in primary and secondary APS. This manifestation is associated with a high mortality rate of over 50% and must be considered a possible cause in all patients with multiple organ failures.

Autoantibodies against phospholipids. Tab. 1. ACA prevalence in APS patients

Ig class	Prevalence with APS
IgG only	44
IgM only	12
IgG/IgM	88

Antibodies against cardiolipin occur with a high prevalence (60-90%) in patients that suffer from symptoms of antiphospholipid syndrome. Their detection (persisting over more than 12 weeks) is a serological criterion for APS diagnosis according to the International Consensus Statement [Miyakis (2006)]. Accordingly, APS is considered proven, if one of 2 clinical criteria and one of 3 serological criteria are met (Tab. 2).

Autoantibodies against phospholipids. Tab. 2. APS detection

Clinical criteria	Serological criteria
Vascular thrombosis	Presence of lupus anticoagulant
Pregnancy complications (e.g. premature or stillbirths)	Autoantibodies against cardiolipin (IgG/IgM)
	Antibodies against β2GP1 (IgG/IgM)

20-40% of patients with SLE display antibodies against cardiolipin, especially if typical APS symptoms already exist. There are indications that, in patients with SLE, IgG antibodies against cardiolipin correlate with thrombocytopenia, and IgM antibodies correlate with haemolytic anaemia.
ACA can also be detected in the serum of 5-15% of patients with other systemic autoimmune diseases (rheumatoid arthritis, scleroderma, Sjögren's syndrome, Sharp's syndrome and others). However, they also occur in infections, such as syphilis or viral hepatitis, as well as in 1-5% of seemingly healthy persons. The prevalence in persons with thrombosis in their anamnesis amounts to 20-30%. The frequency with which ACA is measured in infectious diseases and blood donors depends heavily on the test system used.
Cardiology: Persistently high antibody titers of anti-cardiolipin antibodies are considered a risk factor for thrombosis and vascular complications in heart attacks or strokes. These complications occur in ~80% of cases in the case of high antibody titers against cardiolipin.
Gynaecology: Habitual abortions, stillbirths or premature births are to be expected in 64% of women with antibodies against phospholipids, regardless of whether symptoms of an autoimmune disease exist or not. In this respect, patients with systemic lupus erythematosus are particularly affected by the aforementioned pregnancy complications (up to 77% of cases). Infarcts triggered in the placenta by venous thrombosis are being discussed as a possible cause. ACA concentrations should be checked in persons at increased risk of thrombosis, in women with a miscarriage in their anamnesis and in infarct patients.

Literature.

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Autoantibodies against PML

W. STÖCKER

Synonym(s). Anti-PML antibodies; autoantibodies against promyelocytic leukaemia proteins

Definition. The PML antigen is part of the “promyelocytic leukaemia nuclear bodies” (PML-NB, nuclear granule).

Function and pathophysiology. Autoantibodies against cell nuclei (ANA) can be detected in about a third of patients with primary biliary cirrhosis (PBC) using indirect immunofluorescence (IIF). In the past ten years, a number of nuclear structures have been able to be identified as specific ANA target antigens in PBC. These include promyelocytic leukaemia proteins (PML proteins) and Sp100 as well as two components of the nuclear pore complex (GP 210, see autoantibodies against glycoprotein 210, and p62).

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Autoantibodies against PML can be detected using indirect immunofluorescence (IIF, immunofluorescence, indirect) and display a nuclear-dot pattern. Accurate detection is also possible with line blots (immunoblot) or enzyme-linked immunosorbent assays using recombinant PML.

Reference range — Adults. Negative

Indication. Primary biliary cholangitis (PBC) and combined liver disease (overlap syndrome).

Diagnostic value. Autoantibodies against PML are found in about 13% of patients with primary biliary cholangitis (PBC), but also occur in 4% of patients with autoimmune hepatitis (AIH). These antibodies are also occasionally observed in sera from patients with virus-induced hepatitis B and C.
The joint detection of autoantibodies against PML, SP100, GP 210, AMA-M2 and M2-3E increases the diagnostic sensitivity for PBC to 94%, with a specificity of 99%, and serves to differentiate it from other autoimmune liver diseases, see also PBC-associated antinuclear autoantibodies.

Literature.

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Autoantibodies against PM-Scl

W. Stöcker

Synonym(s). Anti-PM-Scl; PM-Scl antibodies; anti-PM-1; antibodies against PM-1; anti-PM-Scl autoantibodies; autoantibodies against PM-1; PM-1 antibodies

Definition. Autoantibodies against PM-Scl bind to a protein complex consisting of 16 polypeptides with molecular masses between 20 and 110 kDa, which is predominantly localised in the nucleoli and is involved in the formation of ribosomal RNA. The main antigens of the complex have molecular masses of 75 kDa (PM-Scl-75) and 100 kDa (PM-Scl-100).

Sample material. Serum

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Autoantibodies against PM-Scl display a homogeneous fluorescence of the nucleoli with a simultaneous weaker, fine granular reaction of the nucleoplasm in the immunofluorescence test (immunofluorescence, indirect) with HEp-2 cells (Fig. 1). The condensed chromosomes of the mitotic cells do not react, while a fine, granular fluorescence is displayed outside the chromosomes. A homogeneous fluorescence of the nucleoli, as well as a very weak, fine granular to reticular staining of the cell nuclei, also occurs with frozen sections of primate liver (Fig. 2). The starting dilution is 1:100.
Positive results in indirect immunofluorescence should be confirmed with monospecific test systems, such as enzyme-linked immunosorbent assays or immunoblots.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Anti-PM-Scl antibodies are detected in about 15% of patients with myositis; 50-70% of anti-PM-Scl-positive patients are affected by polymyositis/systemic sclerosis overlap syndrome. Here, the autoantibodies are generally directed against both main antigens: PM-Scl-75 and PM-Scl-100. If only progressive systemic sclerosis is present, antibodies against PM-Scl-75 show a prevalence of almost 10%, while those against PM-Scl-100 have a prevalence of 7%. In test systems that exclusively detect anti-PM-Scl-100, a percentage of patients with progressive systemic sclerosis remains undetected; see also autoantibodies against cell nuclei and myositis-specific autoantibodies.

Literature.

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Legend

Autoantibodies against PM-Scl. Fig. 1. Substrate: HEp-2 cells

Autoantibodies against PM-Scl. Fig. 2. Substrate: primate liver

Autoantibodies against proteinase 3

W. STÖCKER

Synonym(s). Anti-PR3 antibodies; proteinase 3 antibodies

Definition. Autoantibodies against proteinase 3 (PR3), a cationic serine proteinase with a molecular weight of 27 kDa. It is localised in the granules of neutrophilic granulocytes and in the lysosomes of the monocytes; see also autoantibodies against granulocyte cytoplasm (antineutrophil cytoplasm antibodies, cytoplasmic type)

Function and pathophysiology. Some clinical observations and animal models indicate a direct pathogenetic role of the antibodies for the vasculitic inflammation process.

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. The International Consensus Statement recommends the use of indirect immunofluorescence (IIF, immunofluorescence, indirect) for ANCA screening tests and the use of both anti-PR3 and anti-MPO ELISAs to confirm positive IIF results. With this approach, the sensitivity for newly diagnosed cases of granulomatosis with polyangiitis (GPA; obsolete: Wegener's granulomatosis) amounted to 73%, while the sensitivity for microscopic polyangiitis (MPA) amounted to 67%. The exclusive use of IIF or enzyme-linked immunosorbent assays provided an insufficient diagnostic specificity. Combining IIF with the anti-PR3 and anti-MPO ELISA resulted in a specificity of 99% for the detection of vasculitis of the small vessels.
The detection of anti-proteinase 3 antibodies is based on IIF, which universally detects autoantibodies against neutrophilic granulocytes (ANCA), as well as on monospecific enzyme immunoassays (enzyme-linked immunosorbent assays, chemiluminescence immunoassays) and immunoblots and on antigen dots for IIF. The standard substrates for immunofluorescence are ethanol- and formalin-fixed human granulocytes (Fig. 1, Fig. 2). Anti-proteinase 3 antibodies present as cANCA on ethanol-fixed as well as formalin-fixed granulocytes: A granular fluorescence pattern, the granules are evenly distributed across the entire cytoplasm of the granulocytes and leave the cell nuclei free. The granular pattern caused by cANCA corresponds to the distribution of proteinase 3 (PR3). However, a cANCA fluorescence can also be caused by antibodies against the "bactericidal permeability increasing protein" (autoantibodies against BPI) or, on formalin-fixed granulocytes, also by autoantibodies against myeloperoxidase, which then appear as pANCA on ethanol-fixed granulocytes.
Proteinase 3 is the main target antigen of cANCA, but not all cANCA are positive in an anti-PR3 ELISA. The parallel analysis of the cANCA (IIFT) and the antibodies against PR3 (ELISA) enables a significant increase in the diagnostic detection rate in patients with GPA compared to the use of just one of the two methods.
Enzyme immunoassays are mostly based on native proteinase 3, which is isolated from human granulocytes. PR3 antigen is either bound directly to microplates (classic anti-PR3 ELISA) or it is fixed to microplates using a "capture antibody" (anti-PR3 capture ELISA), in which case the autoantigen epitopes of PR3 are particularly easily accessible for the relevant antibodies. The anti-PR3 capture ELISA is therefore characterised by a higher sensitivity for GPA compared to a classic ELISA, but with a slightly lower specificity.
The use of recombinant PR3 (based on human cDNS, expressed in human cells) enables the use of modern ELISA, which are characterised by outstanding sensitivity and specificity. In recombinant PR3, the proteolytic active centre of the enzyme can be switched off in the active centre, for example, by replacing the serine in position 176 with alanine, so that the proteinase activity no longer disrupts the cell metabolism and the PR3 culture cells can accumulate in a higher concentration, without this intervention they die off prematurely. The synthesised PR3 does not digest itself in an unmanageable manner during the various preparation steps and can be produced in large quantities. This enables a previously unsurpassed sensitivity of over 95% to be achieved as opposed to indirect immunofluorescence.

Reference range — Adults. Negative

Indication. ANCA-associated vasculitis, granulomatosis with polyangiitis

Diagnostic value. Autoantibodies against PR3 display a high diagnostic sensitivity and specificity for GPA (prevalence of up to 93%). However, the absence of the antibodies does not rule out the presence of the disease. A correlation between the titer and the disease activity has been described, but is the subject of controversial debate. Significantly increasing antibody titers often precede a relapse, but the positive predictive values are too low to justify the administration of medication based on the ANCA titer alone. But significant rises in the titer should cause clinicians to monitor their patients more closely.
Anti-PR3 can also be detected in eosinophilic granulomatosis with polyangiitis (EGPA; obsolete: Churg-Strauss syndrome) (10%) and, ocassionally in microscopic polyangiitis and polyarteritis nodosa.

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Legends

Autoantibodies against proteinase 3. Fig. 1. Substrate: human granulocytes (ethanol-fixed)

Autoantibodies against proteinase 3. Fig. 2. Substrate: human granulocytes (formalin-fixed)

Autoantibodies against prothrombin

W. STÖCKER

Synonym(s). Prothrombin antibodies; aPT; aPS/PT

Definition. Autoantibodies belonging to the group of antiphospholipid antibodies that are directed against endogenous prothrombin.

Function and pathophysiology. Antibodies against prothrombin (aPT) were first discussed as possible cofactors of lupus anticoagulants (LA) in 1959. Prothrombin is a vitamin K-dependent glycoprotein formed in the hepatocytes with a molar mass of 70 kDa and, besides β2 glycoprotein I (β2-GPI) and annexin A5, is one of the most important phospholipid-binding proteins. The first ten N-terminal glutamate residues are enzymatically γ-carboxylated during biosynthesis. This γ-carboxyglutamate-containing region (Gla domains) mediates the calcium-dependent binding to phosphatidylserine, which leads to a conformational change of the prothrombin. The enzymatic activation of the prothrombin to α-thrombin during a blood coagulation reaction takes place via the prothrombinase complex, consisting of factors Va and Xa (coagulation factor Xa) as well as phospholipids and calcium ions.

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Enzyme-linked immunosorbent assay

Reference range — Adults. Not detectable

Reference range — Children. Not detectable

Interpretation. The anti-prothrombin autoantibodies, which frequently occur in patients with systemic lupus erythematosus (SLE), are considered risk factors for arterial, but not venous, thrombosis, in addition to anti-β2-GPI autoantibodies. The thrombosis-inducing effect of aPT was shown in animal testing. Its involvement in habitual abortions is being discussed.
The anti-prothrombin autoantibodies are heterogeneous. They are detected in an ELISA. In this case, solid phase-bound isolated prothrombin (PT) or a complex consisting of phosphatidylserine and prothrombin (PS/PT) are used as antigens.
Comparative studies show that, in contrast to aPT, aPS/PT correlates very well with the clinical manifestation of antiphospholipid syndrome (APS) and that it has the same specificity as β2-GPI-dependent anti-cardiolipin (aCL or ACA; autoantibodies against cardiolipin) with APS. Accordingly, aPS/PT-IgG is also correlated with lupus anticoagulant. Besides β2-GPI-dependent ACA, aPS/PT is a very good marker for APS.

Literature.
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Autoantibodies against striated muscle

W. STÖCKER

Definition. Autoantibodies against striated muscle react with various proteins in the skeletal and cardiac muscles. One of the target antigens is the protein titin, whose physiological function consists of preventing the overstretching of the muscle fibres.

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. A positive reaction in the indirect immunofluorescence test (IIFT, immunofluorescence, indirect) with skeletal and/or cardiac muscles as the substrates (starting dilution 1:100) displays a characteristic horizontal striping of the tissue (Fig. 1, Fig. 2).
Line blots (immunoblot) with purified defined antigen are suitable for detecting autoantibodies against titin, as they cannot be clearly distinguished from other autoantibodies with the same fluorescence image by IIFT.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Autoantibodies against striated muscle occur in myasthenia gravis patients with a prevalence of 70% and can confirm the diagnosis. However, only high antibody titers from 1:1,000 are diagnostically relevant. Moreover, these antibodies can also be detected in the serological investigation of patients with various inflammatory myopathies (in low titers) (polymyositis and others). These autoantibodies are also found in patients with Chagas disease in the chronic stage. An asymptomatic occurrence may be detected in patients with Graves' disease or autoimmune polyendocrinopathy.

Literature.
Strauss AJ, Seegal BC, Hsu KC et al (1960) Immunofluorescence demonstration of a muscle binding, complement-fixing serum globulin fraction in myasthenia gravis. ProcSocExpBiol Med 105:184–191

Legends

Autoantibodies against striated muscle. 1. Substrate: primate skeletal muscle

Autoantibodies against striated muscle. 2. Substrate: primate cardiac muscle

Autoantibodies against RA33

W. Stöcker, W. Schlumberger

Synonym(s). Anti-A2/RA33; RA33 antibodies

Definition. Antibodies against the A2 core protein of heterogeneous nuclear ribonucleoprotein complexes (A2-hnRNP).

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Autoantibodies against RA33 can be detected with enzyme immunoassays using native or recombinant antigens.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Potentially if rheumatoid arthritis is suspected, but has limited diagnostic uses.

Diagnostic value. Autoantibodies against RA33 occur in about a third of patients with rheumatoid arthritis. Their specificity is limited, as they also occur in patients with SLE and other rheumatic diseases.

Literature.

Lee YH, Bae SC (2016) Diagnostic accuracy of anti-Sa and anti-RA33 antibodies in rheumatoid arthritis: a meta-analysis. Z Rheumatol doi: 10.1007/s00393-016-0134-y

Steiner G, Smolen J (2002) Autoantibodies in rheumatoid arthritis and their clinical significance. Arthritis Res 4(Suppl 2):1–5

Autoantibodies against Ri

W. Stöcker

Synonym(s). Anti-Ri; ANNA-2 (anti-neuronal nuclear antibodies 2); autoantibodies against the nuclei of neuronal cells type 2; Ri antibodies

Definition. Onconeuronal antibodies, which are directed against various tumours as well as against the nuclei of neuronal cells. The target antigens are the RNA-binding proteins NOVA-1 and NOVA-2. The reference was derived from the name of the index patient (Richards).

Sample material. Serum, cerebrospinal fluid

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. The indirect immunofluorescence test (IIFT, immunofluorescence, indirect) with frozen sections of primate cerebellum is suitable for detecting autoantibodies against neuron nuclei (Ri, Hu) (Fig. 1). The autoantibodies against Ri often have a high antibody titer, sometimes up to 1:100,000. An additional frozen section of primate intestine is used to distinguish between autoantibodies against Ri and autoantibodies against Hu (Fig. 2): Anti-Hu react with the cell nuclei of the plexus myentericus, while anti-Ri do not. In the case of a positive result in the IIFT, a Western blot with cerebellum antigens or a line blot with purified defined, potentially recombinant, antigens can be used to confirm the finding.

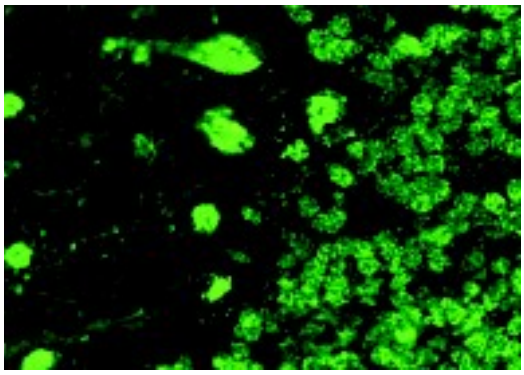
Reference range — Adults. Negative

Reference range — Children. Negative

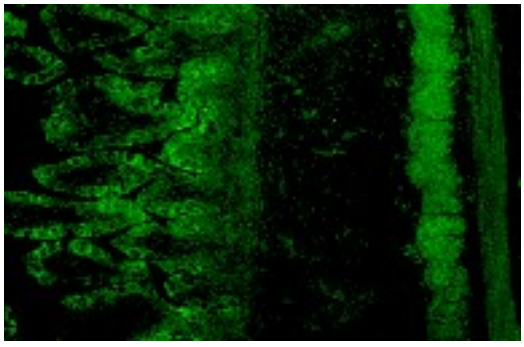
Diagnostic value. Autoantibodies against the onconeuronal Ri proteins NOVA-1 and NOVA-2 have been described with opsoclonus myoclonus syndrome in connection with a gynaecological tumour, predominantly with breast cancer. Anti-Ri antibodies can provide an initial indication of an underlying tumour (autoantibodies against neuronal antigens). The tumours most often associated with anti-Ri antibodies: Small cell lung cancer (SCLC) and breast cancer.

Literature.

Voltz R (2002) Paraneoplastische neurologische Autoimmunerkrankungen. Nervenarzt 73:909–929



Autoantibodies against Ri. Fig. 1. Substrate: primate cerebellum



Autoantibodies against Ri. Fig. 2. Substrate: primate intestine

Autoantibodies against ribosomal phosphoproteins

W. Stöcker, W. Schlumberger

Synonym(s). Autoantibodies against ribosomal P proteins; ribosomal phosphoprotein antigens; anti-RPP antibodies; ARPA

Definition. The ribosomal P protein antigen consists of three proteins from the 60S subunit of eukaryotic ribosomes. These proteins are referred to as:

- P0 (molecular weight of 38 kDa),
- P1 (molecular weight of 19 kDa),
- P2 (molecular weight of 17 kDa).

The immunoreactive main epitope is localised at the carboxyterminal end, which consists of an identical sequence of 17 amino acids in all 3 proteins. One of the first people to describe the ribosomal phosphoproteins was A.M. Gressner.

Sample material. Serum, cerebrospinal fluid

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Autoantibodies against ribosomal P proteins display a smooth to fine granular staining of the cytoplasm in the indirect immunofluorescence test (IIFT, immunofluorescence, indirect) with HEp-2 cells as the substrate (Fig. 1). Hepatocytes in primate liver display a full-surface cytoplasmic fluorescence with patchy highlights (Fig. 2). The cytoplasm of the kidney and stomach also displayed a positive reaction. These should be detected using monospecific test systems (enzyme-linked immunosorbent assay, chemiluminescence immunoassay, immunoblot) to confirm the finding.

Reference range — Adults. Negative

Indication. Autoantibodies against ribosomal P proteins are an indicator of systemic lupus erythematosus (SLE). The prevalence amounts to about 10%.

Diagnostic value. Due to their high disease specificity, it is worthwhile analysing these antibodies in addition to autoantibodies against double-stranded DNA, autoantibodies against nucleosomes, autoantibodies against Sm, autoantibodies against SS-A, autoantibodies against histones and autoantibodies against cardiolipin if SLE is suspected, as these occur independently of the other antibodies.
The disease activity with SLE does not correlate to the titer of ARPA. A previously assumed connection between CNS involvement, nephritis or hepatitis with the occurrence of ARPA can presumably be ruled out.

Literature.

Elkon KB, Bonfa E, Weissbach H, Brot N (1994) Antiribosomal antibodies in SLE, infection, and following deliberate immunization. Adv Exp Med Biol 347:81–92

Caponi L, Giordano A, Bartoloni EB, Gerli R (2003) Detection of anti-ribosome antibodies: a long story of lights and shadows. Clin Exp Rheumatol 21:771–778

Gressner AM, Wool IG (1974) The phosphorylation of liver ribosomal proteins in vivo. J Biol Chem 249:6917–6925

Legends

Autoantibodies against ribosomal phosphoproteins. Fig. 1. Substrate: HEp-2 cells

Autoantibodies against ribosomal phosphoproteins. Fig. 2. Substrate: primate liver

Autoantibodies against ribosomes

W. Stöcker

Synonym(s). Anti-ribosomal antibodies

Definition. Autoantibodies against ribosomes have nothing to do with autoantibodies against ribosomal phosphoproteins. The designation was used as an attempt to interpret a fluorescence pattern without any knowledge of the nature of the antigen.

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Autoantibodies against ribosomes are analysed by indirect immunofluorescence (immunofluoresence, indirect). The starting dilution is 1:100; analyses can generally be restricted to immunoglobulin class IgG.
Autoantibodies against ribosomes display a smooth to fine granular fluorescence of the cytoplasm on HEp-2 cells, which weakens towards the edge. In more diluted samples, the cells stain with different intensities. In mitosis, the perichromosomal region is clearly highlighted. Only part of the hepatocytes react on primate liver, with a smooth fluorescence of the cytoplasm. Positive cells are distributed across non-reactive regions, either individually or in groups. Rat liver displays a smooth fluorescence distributed across the entire organ. On the stomach, the main and parietal cells also display a smooth and even stain.
The fluorescence on all organs is finer and smoother compared to autoantibodies against ribosomal P proteins. Modern monospecific enzyme-linked immunosorbent assay systems must be used to establish a clear distinction.

Reference range — Adults. Negative

Reference range — Children. Negative

Diagnostic value. Under the microscope, autoantibodies against ribosomes are often confused with ribosomal P proteins, which have a high specificity for systemic lupus erythematosus (SLE). As a result, it is impossible to rely on statements that assign these antibodies a specificity for both SLE and autoimmune hepatitis.

Literature.
Storch W (1997) Immunfluoreszenzfibel. Blackwell Wissenschaftsverlag, Berlin Wien, S 139–141

Autoantibodies against Sa

W. Stöcker, W. Schlumberger

Synonym(s). Sa autoantibodies; anti-Sa antibodies

Definition. Autoantibodies against Sa are directed against a protein from the human placenta with a molecular weight of 50 kDa, in which this relates to the citrullinated form of the intermediate filament vimentin.

Function and pathophysiology. Autoantibodies against proteins, which contain the rare amino acid citrulline, are associated with rheumatoid arthritis (RA). Citrullinated proteins could also be identified in the inflamed synovial mucous membrane of RA patients, but not in healthy tissue. It must be assumed that citrullinated proteins are targets of autoimmune reactions in RA and, in this respect, are involved in inflammatory reactions and tissue destruction.
Antibodies against citrullinated peptides therefore presumably have a closer aetiological disease relationship than the rheumatoid factors (autoantibodies against immunoglobulins; autoantibodies against IgA), which have been know about for longer. These display very low disease specificity and also occur with other rheumatic diseases, in infectious diseases and in healthy patients. By contrast, antibodies against Sa as well as against citrullinated peptides (autoantibodies against citrullinated peptides) are found almost exclusively with rheumatoid arthritis.

Analytics. Autoantibodies against Sa can be detected using enzyme immunoassays or immunoblot. Immunoglobulin class IgG is diagnostically relevant.

Sample material. Serum

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Diagnostic value. Autoantibodies against Sa, together with autoantibodies against CCP and CEP-1 (see also autoantibodies against citrullinated peptides) belong to what are currently the most important markers of rheumatoid arthritis. They have a specificity of almost 100%. The target antigen is the citrullinated vimentin expressed in the synovial tissue. While anti-Sa antibodies have a lower sensitivity than anti-CCP (anti-Sa Western blot 40%, anti-Sa ELISA 55-60%), their prognostic value for a severe progressive form of RA is unmatched (severe joint involvement, extraarticular manifestations). The detection of anti-Sa antibodies must be considered an RA risk in healthy persons. However, it can take 10-15 years for these persons to suffer from RA: The higher the anti-SA titer, the shorter the interval.
The antibody titers vary with the disease activity and their normalisation is considered a compulsory feature of a remission. Patients with active RA display significantly higher anti-Sa antibody titers compared to patients with milder RA.
Autoantibodies against CCP and against SA can be detected very early in the course of the disease in about 75% and 60% of RA patients respectively, often even many years before the initial symptoms, in the serum as well as in the synovial fluid. This means that, these days, diagnosis as well as adequate treatment can take place at an earlier stage. With regard to the disease prognosis, radiological analyses show that more severe joint damage occurs in patients with anti-CCP antibodies or anti-Sa antibodies than in anti-CCP-negative or anti-Sa-negative patients. Anti-Sa detection also has a prognostic value in this respect.

Literature.

Després N, Boire G, Lopez-Longo FJ, Ménard HA (1994) The Sa system: a novel antigen-antibody system specific for rheumatoid arthritis. J Rheumatol 21:1027–1033

Vossenaar ER, Després N, Lapointe E, van der Heijden A, Lora M, Senshu T, van Venrooij WJ, Ménard HA (2004) Rheumatoid arthritis specific anti-Sa antibodies target citrullinated vimentin. Arthritis Res Ther 6:R142–150

Ménard HA (2007) Anti-CCP versus anti-Sa antibodies for the diagnosis of RA. Nat Clin Pract Rheumatol 3:76–77

Autoantibodies against Scl-70

W. Stöcker, W. Schlumberger

Synonym(s). Anti-DNA topoisomerase I antibodies; anti-Scl-70 antibodies

Definition. Anti-Scl-70 antibodies are directed against epitopes of DNA topoisomerase I.

Function and pathophysiology. The molecular weight of the native enzyme amounts to 100 kDa, but only a fission product of 70 kDa was originally found in the Western blot. DNA topoisomerase I is localised in the nucleoplasm and is particularly highly concentrated in the nucleolus. The enzyme is involved in the replication and transcription of the DNA double helix: It splits the DNA chain and settles on the resulting free ends. As soon as a certain section is replicated or transcribed, the strands are joined once again and the topoisomerase is released.

Sample material. Serum

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Anti-Scl-70 antibodies display a virtually homogenous nuclear fluorescence of the interphase cells in the indirect immunofluorescence test (IIFT, 7immunofluorescence, indirect) with HEp-2 cells (7Fig. 1). The nucleoli are highlighted and also display a homogenous fluorescence, while the cytoplasm remains dark. In mitotic cells, only the region of the condensed chromosomes fluoresces. The liver displays a predominantly homogenous fluorescence of the cell nuclei (7Fig. 2).
A positive result in the IIFT provides grounds for an accurate identification of the target antigen with a monospecific enzyme immunoassay (enzyme-linked immunosorbent assay, chemiluminescence immunoassay) or immunoblot (line blot) with native purified Scl-70 antigen (100 kDa protein) or a Western blot with nuclear antigens.

Reference range. Negative

Diagnostic value. Anti-Scl-70 antibodies are detected in 25-75% of patients with progressive systemic sclerosis (diffuse form) depending on the analysis method and activity of the disease.

Literature.

Tan EM, Chan EKL, Sullivan KF, Rubin RL (1988) Antinuclear antibodies (ANAs): Diagnostically specific immune markers and clues toward the understanding of systemic autoimmunity. Clin Immunol Immunopathol 47:121–141

Fritzler MJ (1993) Autoantibodies in Scleroderma. The Journal of Dermatology 20:257–268

Hanke K, Dähnrich C, Brückner C, Huscher D, Becker M, Jansen A, Meyer W, Egerer K, Hiepe F, Burmester G, Schlumberger W, Riemekasten G (2009) Diagnostic value of anti-topoisomerase I antibodies in a large monocentric cohort. Arthritis Res Ther 11:R28

Legends

Autoantibodies against Scl-70. Fig. 1. Substrate: HEp-2 cells

Autoantibodies against Scl-70. Fig. 2. Substrate: primate liver

Autoantibodies against SLA

W. Stöcker

Synonym(s). Anti-SLA/LP antibodies; autoantibodies against soluble liver antigen; anti-liver/pancreas antigen; SLA autoantibodies

Definition. An autoantibody against an antigen expressed in the liver and pancreas, among other areas, relevant for the diagnosis of autoimmune hepatitis (AIH).

Function and pathophysiology. SLA/LP was identified at the DNA level in 1998 by cloning the target antigen. SLA/LP is presumably a cytoplasmic enzyme with a molar mass of 50 kDa, which plays a role in regulating the selenoprotein biosynthesis (a UGA-suppressor tRNA-associated protein). The former descriptions of SLA as liver cytokeratin 8 and 18 or as glutathione-*S* transferase proved to be patently incorrect.

Sample material. Serum or plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Autoantibodies against SLA/LP can be sensitively and specifically detected with 7enzyme immunoassays (7enzyme-linked immunosorbent assay, chemiluminescence immunoassay) using a recombinant antigen, and in the indirect immunofluorescence test (7immunofluorescence, indirect) with SLA/LP-transfected cells as the substrate. Classic Western blot are not suitable for detecting these antibodies, as the use of denatured SLA/LP leads to a reduction in detection sensitivity, while a positive band reaction of 50 kDa can be caused by other unidentified autoantibodies.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Unclear increase in the transaminases, suspected autoimmune hepatitis.

Diagnostic value. Antibodies against SLA/LP provide the highest diagnostic accuracy for autoimmune hepatitis of all autoantibodies. Anti-SLA/LP occur with AIH alone or together with other autoantibodies. However, their prevalence only lies between 10 and 30%, but the predictive value is almost 100%. Every positive anti-SLA result essentially provides evidence of autoimmune hepatitis (if the corresponding clinical symptoms are present).
The serological detection of autoantibodies against SLA/LP enables a precise differentiation from viral hepatitis in many patients with AIH, which has significant consequences for the hepatological symptoms: The incorrect treatment of AIH with interferon can also have fatal consequences, similar to an immunosuppressive treatment of the viral infection.
The parallel detection of the other AIH-associated autoantibodies, such as autoantibodies against cell nuclei), pANCA (autoantibodies against granulocyte cytoplasm), ASMA (autoantibodies against smooth muscles) or autoantibodies against LC-1 and autoantibodies against LKM, is recommended to differentiate from viral hepatitis.

Literature.

Baeres M, Herkel J, Czaja AJ et al (2002) Establishment of standardized SLA/LP immunoassays: specificity for autoimmune hepatitis, worldwide occurrence, and clinical characteristics. Gut 51:259–264

Berg PA, Stechemesser E, Strienz J (1981) Hypergammaglobulinämische chronisch aktive Hepatitis mit Nachweis von Leber-Pankreas-spezifischen komplementbindenden Autoantikörpern. Verh Dtsch Ges Inn Med 87:921–927

Wies I, Brunner S, Henninger J et al (2000) Identification of target antigen for SLA/LP autoantibodies in autoimmune hepatitis. Lancet 355:1510–1515

Autoantibodies against Sm

W. Stöcker, W. Schlumberger

Synonym(s). Sm antibodies; anti-Sm antibodies; Anti-Sm

Definition. The name of the anti-Sm antibody is derived from the name of the indicator patient (Smith). The corresponding antigens are a group of 7 components of small nuclear ribonucleoproteins (snRNP) referred to as core proteins B/B', D1, D2, D3, E, F and G. The RNA part is referred to as U1, U2, U4 and U5, depending on the chromatographic behaviour, and the ribonucleoproteins are referred to U1-, U2-, U4- and U5-snRNP accordingly. Autoantibodies against Sm can be directed against one or more of the core proteins.

Function and pathophysiology. Autoantibodies against Sm have a high specificity for systemic lupus erythematosus.

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Autoantibodies against Sm generally display a coarse granular, sometimes also a medium to fine granular fluorescence, which is distributed across the entire cell nucleus except for the nucleoli, in the indirect immunofluorescence test (IIFT, immunofluorescence, indirect) on HEp-2 cells. In mitosis cells, the condensed chromosomes are dark, while the periphery displays an almost homogenous, smooth fluorescence. Tissue sections of primate liver also display a granular fluorescence, apart from the nucleoli. Autoantibodies against U1-nRNP and Sm react with the primate liver just as strongly as with HEp-2 cells, in contrast to antibodies against Ro/SS-A (autoantibodies against SS-A) and La/SS-B (autoantibodies against SS-B).
In the case of a positive result in the IIFT, a monospecific enzyme immunoassay (enzyme-linked immunosorbent assay, chemiluminescence immunoassay) or immunoblot (line blot) with native purified Sm antigens or a Western blot with nuclear antigens can be used to accurately identify the target antigen.

Reference range — Adults. Antibody titer < 1:100

Reference range — Children. See adults

Interpretation. Autoantibodies against Sm have a high specificity for systemic lupus erythematosus. They, as well as autoantibodies against double-stranded DNA, autoantibodies against nucleosomes and autoantibodies against ribosomal phosphoproteins can be classified as pathognomonic for this disease, but only occur in 5-40% of patients (Caucasians 8%, Negroids 30%).

Literature.
Tan EM, Chan EKL, Sullivan KF et al (1988) Antinuclear antibodies (ANAs): Diagnostically specific immune markers and clues toward the understanding of systemic autoimmunity. Clin Immunol Immunopathol 47:121–141

Zieve GW, Khusial PR (2003) The anti-Sm immune response in autoimmunity and cell biology. Autoimmun Rev 2(5):235–240

Autoantibodies against salivary gland excretory ducts

W. STÖCKER

Synonym(s). Salivary gland duct epithelium antibodies; parotid antibodies

Definition. Autoantibodies against antigens of the salivary gland excretory ducts

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Autoantibodies against salivary gland excretory ducts display a smooth to fine granular staining of the cytoplasm of the epithelial cells in the indirect immunofluorescence test (IIFT, immunofluorescence, indirect) with the parotid gland as the substrate (starting dilution 1:10) (Fig. 1).
The parallel use of parotid gland and rat kidney as the substrate serves to rule out autoantibodies against mitochondria (AMA), whose binding to the parotid tissue can simulate the presence of a salivary gland-specific antibody.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Autoantibodies against salivary gland excretory ducts are detected in 40-60% of patients with primary Sjögren's syndrome.

Literature.
MacSween RN, Goudie RB, Anderson JR et al (1967) Occurrence of antibody to salivary duct epithelium in Sjogren's disease, rheumatoid arthritis, and other arthritides.A clinicalandlaboratorystudy. Ann Rheum Dis 26:402–411

Legend

Autoantibodies against salivary gland excretory ducts. Fig. 1. Substrate primate parotid gland

Autoantibodies against the spindle apparatus

W. Stöcker

Synonym(s). Autoantibodies against MSA-1/MSA-2; nuclear mitotic apparatus (NuMA) protein; HsEg5; NuMA antibodies; autoantibodies against the nuclear mitotic apparatus protein

Definition. MSA-1 is synonymous for the inappropriate name NuMA (no cell nucleus exists in mitosis). MSA-2 is the protein HsEg5 ("human spindle kinesin-like protein").

Molar mass. MSA-1 (NuMA): 210 kDa, MSA-2 (HsEg5): 116-130 kDa

Function and pathophysiology. In the interphase, MSA-1 is part of the nuclear matrix, during mitosis it is found on the spindle poles, near the centrioles, and contributes to the formation of the spindles.
MSA-2 plays a key role in the formation of spindle fibres and mitosis.

Sample material. Serum or plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. In the indirect immunofluorescence test (IIFT, immunofluorescence, indirect) (starting dilution 1:100), HEp-2 cells with antibodies against MSA-1, in the interphase, display a fine granular to reticular fluorescence of the nuclear matrix, apart from the nucleoli, in the mitotic cells, in the metaphase, the spindle fibres are displayed as 2 opposing compartments, while the staining is focussed towards the centrioles (Fig. 1). As an alternative to immunofluorescence, a Western blot specifically designed for large molecules can also be used, which displays a band with 210 kDa in the presence of anti-NuMA.
By comparison, only the spindle fibres of the mitotic cells, but not the cell nuclei of the interphase cells, are stained with antibodies against HsEg5 ("NuMA-2", 116 kDa).

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. The analysis is normally not specifically requested; the antibodies are often only detected by accident.

Diagnostic value. Antibodies against MSA-1 (NuMA-1) can also occur with Sjögren's syndrome and various forms of arthritis, occasionally also with antiphospholipid syndrome and SLE.
Antibodies against MSA-2 (HsEg5) occur with various rheumatic diseases, also including systemic lupus erythematosus.
High titers of the autoantibodies against centrioles/centrosomes are an indication of progressive systemic sclerosis or Raynaud's disease.

Literature.

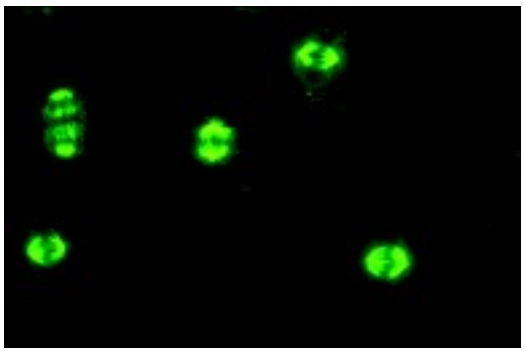
Andrade LE, Chan EK, Peebles CL, Tan EM (1996) Two major autoantigen-antibody systems of the mitotic spindle apparatus. Arthritis Rheum 39:1643–1653

Bonaci-Nikolic B, Andrejevic S, Bukilica M, Urosevic I, Nikolic M (2006) Autoantibodies to mitotic apparatus: Association with other autoantibodies and their clinical significance. J Clin Immunol 26:438–446

Grypiotis P, Ruffatti A, Tonello M, Winzler C, Radu C, Zampieri S, Favaro M, Calligaro A, Todesco S (2002) Clinical significance of fluoroscopic patterns specific for the mitotic spindle in patients with rheumatic diseases. Reumatismo 54:232–237

Mozo L, Gutiérrez C, Gómez J (2008) Antibodies to mitotic spindle apparatus: Clinical significance of NuMA and HsEg5 autoantibodies. J Clin Immunol 28:285–290

Whitehead CM, Winkfein RJ, Fritzler MJ, Rattner JB (1996) The spindle kinesin-like protein HsEg5 is an autoantigen in systemic lupus erythematosus. Arthritis Rheum 39:1635–1642



Autoantibodies against the spindle apparatus. Fig. 1. Substrate: HEp-2 cells

Autoantibodies against SS-A

W. Stöcker, W. Schlumberger

Synonym(s). Ro/SS-A antibodies; anti-SS-A; anti-Ro; anti-Ro60; anti-SS-A antibodies; anti-Ro antibodies; anti-Ro60 antibodies; anti-Sjögren's syndrome antigen A

Definition. Anti-Ro/SS-A antibodies are directed against epitopes of the protein components of the Ro/SS-A ribonucleoprotein complex. This complex consists of one RNA molecule (Y1-, Y2-, Y3-, Y4- or Y5-RNA) and one protein with a molecular weight of 60 kDa (Ro60). The biological function of Ro60 is unclear. Cytoplasmic Ro60 appears to be involved in regulating the translation of correct 5S-rRNA, while nuclear Ro60 seems to be involved in producing correct 5S-rRNA. It is primarily localised in the cell nucleus, but also occurs in the cytoplasm. Statements that another protein with a molecular weight of 52 kDa is also an integral part of the Ro/SS-A antigen have proven to be false. Antibodies against the 52-kDa protein (Ro-52, TRIM21) are not disease-specific and must be considered independent of the antibodies referred to as anti-Ro/SS-A.

Sample material. Serum or plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Antibodies against Ro/SS-A display a fine granular fluorescence of the nuclei in the indirect immunofluorescence test (IIFT, immunofluorescence, indirect) with HEp-2 cells in the interphase. The nucleoli also appear positive, but are somewhat distinct from the nucleoplasm, while, in some of the samples they do not react at all. Mitotic cells also display a granular fluorescence, apart from the chromosomes. The corresponding granular reaction of the hepatocyte nuclei is missing on primate liver, but smooth fluorescing nucleoli are displayed at higher antibody titers (Fig. 1, Fig. 2). By contrast, the autoantibodies against U1-nRNP and autoantibodies against Sm, which are important for discriminatory testing, display an equally strong granular nuclear fluorescence with the hepatocytes as with the HEp-2 cells. Individual cells in the sinusoids of the liver (lymphocytes, monocytes) also provide a strong reaction with anti-SS-A antibodies.
In the case of a positive result in the IIFT, a monospecific enzyme immunoassay (enzyme-linked immunosorbent assay, chemiluminescence immunoassay) or immunoblot with native purified or recombinant SS-A antigen (60-kDa protein) or Western blots with native nuclear antigens can be used to accurately identify the antibody.
Moreover, the immunoblot and Western Blot methods also enable antibodies that react with Ro-52 to be detected in parallel to the antibodies against Ro60.

Reference range — Adults. Negative

Diagnostic value. Anti-Ro/SS-A antibodies are characteristic serological markers of Sjögren's syndrome, with which they generally occur together with anti-La/SS-B antibodies (autoantibodies against SS-B), the prevalence amounts to 40-95%. Moreover, anti-SS-A can also occur with primary biliary cholangitis (formerly: primary biliary cirrhosis). Predominantly without SS-B reactivity, antibodies against SS-A can also be detected with systemic lupus erythematosus (SLE) (20-60%) and lupus neonatorum (neonatal lupus syndrome with congenital heart block, caused by diaplacentally transferred anti-SS-A; 100%).
Antibodies against Ro-52 were originally described in patients with Sjögren's syndrome or SLE (38%), but subsequently also with polymyositis (31%), progressive systemic sclerosis (28%), autoimmune hepatitis (35%), PBC (27%), hepatitis B (10%) and hepatitis C (22%). While they provide an indication of the presence of an autoimmune disease, they have no significance for differential diagnostics. Special significance was formerly ascribed to antibodies against Ro-52 in the pathogenesis and diagnosis of congenital heart block in infants. The probability of a congenital heart block is low with isolated anti-Ro-52, but it rises sharply if they are joined by autoantibodies against SS-A and SS-B. In pregnant mothers, all 3 specificities should therefore be analysed several times during the course of pregnancy.

Literature.

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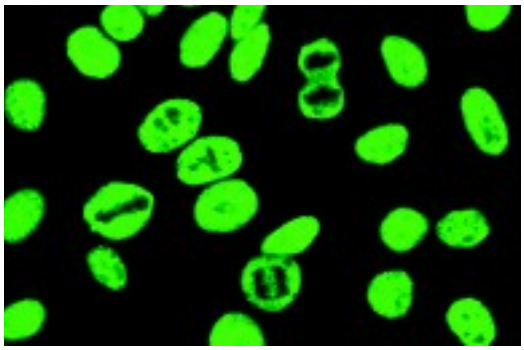
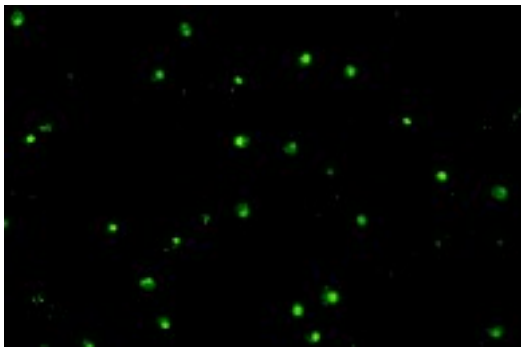


Autoantibodies against SS-A. Fig. 1. Substrate: HEp-2 cells



Autoantibodies against SS-A. Fig. 1. Substrate: primate liver

Autoantibodies against SS-B

W. Stöcker, W. Schlumberger

Synonym(s). Autoantibodies against La' La/SS-B antibodies; anti-SS-B antibodies; anti-La antibodies; anti-Sjögren's syndrome antigen B antibodies

Definition. La/SS-B antibodies are directed against a phosphoprotein with a molar mass of 48 kDa. The antigen is predominantly localised in the cell nucleus, only 10% of the antigen also occurs in the cytoplasm.

Sample material. Serum or plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Autoantibodies against La/SS-B display similar patterns to autoantibodies against SS-A in the indirect immunofluorescence test (IIFT, immunofluorescence, indirect): HEp-2 cells display a fine granular fluorescence of the cell nuclei in the interphase. The nucleoli also appear positive, but are somewhat distinct from the nucleoplasm, while, in some of the samples they do not react at all. Mitotic cells also display a granular fluorescence, apart from the chromosomes. The corresponding granular reaction of the hepatocyte nuclei is missing on primate liver, but smooth fluorescing nucleoli are displayed at higher antibody titers. By contrast, the autoantibodies against U1-RNP and autoantibodies against Sm, which are important for discriminatory testing, display an equally strong granular nuclear fluorescence with the hepatocytes as with the HEp-2 cells.

In the case of a positive result in the IIFT, monospecific assays (enzyme-linked immunosorbent assay, chemiluminescence immunoassay, immunoblot) with native purified or recombinant SS-B antigen or Western blot with native nuclear antigens can be used to accurately identify the antibody.

Reference range — Adults. Negative

Reference range — Children. Negative

Diagnostic value. La/SS-B antibodies, together with autoantibodies against SS-A, are the characteristic serological markers of Sjögren's syndrome; the prevalence amounts to 40-95% in each case.
The presence of La/SS-B antibodies is extremely rare in the absence of Ro/SS-A antibodies.

Literature.
Tan EM, Chan EKL, Sullivan KF et al (1988) Antinuclear antibodies (ANAs): Diagnostically specific immune markers and clues toward the understanding of systemic autoimmunity. Clin Immunol Immunopathol 47:121–141

Autoantibodies against steroid hormone producing cells

W. STÖCKER

Definition. Autoantibodies against steroid hormone producing cells are directed against target antigens of the following endocrine organs:

- Adrenal cortex (zona glomerulosa, fasciculata and reticularis),
- Ovaries (theca cells, corpus luteum),
- Testes (Leydig interstitial cells),
- Placenta (syncytiotrophoblast).

Function and pathophysiology. The target antigens of these autoantibodies are multiple enzymes involved in the synthesis of the steroid hormones, especially steroid-21 hydroxylase (21-OH), steroid-17-α hydroxylase (17-OH) and the cytochrome-P450 “side chain cleavage enzyme” (P450scc). 21-OH only occurs in the adrenal cortex where it transforms 17-α progesterone and progesterone into 11-deoxycortisol and deoxycorticosterone. 17-OH is expressed in the gonads and the adrenal glands, while P450scc is expressed in the adrenal glands, gonads and placenta.
The autoantibodies are associated with Addison’s disease and with various forms of autoimmune polyendocrinopathy (APE). Of these, the rare type 1 is a syndrome with mucocutaneous candidiasis, hypoparathyroidism, Addison’s disease and hypergonadotropic hypogonadism. The more common APE types 2 and 3 are defined as combinations of autoimmune thyroiditis, autoimmune diabetes mellitus, vitiligo, pernicious anaemia and (not type 3) Addison’s disease.

Sample material. Serum

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Indirect immunofluorescence (immunofluorescence, indirect) with frozen sections of the adrenal cortex, ovary, placenta and testis is the main method of detecting autoantibodies against steroid hormone producing cells (Fig. 1, Fig. 2, Fig. 3, Fig. 4). The starting dilution is 1:10. All 3 immunoglobulin classes IgA, IgG and IgM are analysed using trivalent FITC-labelled antisera.
In addition, antibodies against the individual enzymes involved in hormone synthesis are detected with radioimmunoassays using immunoprecipitation.
Due to the correlation between autoimmune polyendocrinopathies and other relevant autoantibodies (autoantibodies against parietal cells, autoantibodies against thyroid peroxidase, autoantibodies against pancreatic islets, autoantibodies against striated muscle), the following are often also used in immunofluorescence in addition to the organs mentioned above: Primate stomach, thyroid gland, parathyroid gland and pancreas. The easiest way to analyse these types of antibody profiles is by using modern “BIOCHIP Mosaics”.

Indication. Autoantibodies against steroid hormone producing cells are associated with Addison’s disease (cf. autoantibodies against the adrenal cortex) and autoimmune polyendocrinopathies (APE). These autoantibodies only play a role in exceptional cases in relation to the isolated hypofunction of the gonads unrelated to Addison’s disease or APE.

Interpretation. The focus is on autoantibodies against 21-OH, which are found in a mixed autoimmune adrenalitis group in 64-76% of cases. If only patients who have recently fallen ill to the disease are considered, the prevalence increases to almost 100%. Anti-21-OH can already be detected in the serum before the onset of the disease (Addison’s disease only becomes manifest once 90% of the adrenal gland is lost). Anti-17-OH and anti-P450scc are rare with autoimmune adrenalitis, but if they occur with this disease they indicate the development of an autoimmune polyendocrinopathy (types 1 and 2).
A fifth of anti-17-OH and anti-P450scc-positive sera do not contain any anti-21-OH; if type 2 APE is suspected, relying solely on the anti-21-OH result or on the IIFT with adrenal gland as the substrate is not adequate; testis and ovary must also be included in the fluorescence in addition to the adrenal gland. With regard to the prevalence of autoantibodies against steroid hormone producing cells, see Tab. 1.

Autoantibodies against steroid hormone producing cells. Tab. 1. Prevalence with selected clinical pictures

Clinical picture	Prevalence		
Addison’s disease	64–100	5	9
APE type 1	64	55	45
APE type 2	96	33	42
Singular ovarian insufficiency	0	6	0

Literature.

Anderson JR, Goudie RB, Gray K et al (1968) Immunological features of idiopathic Addison’s disease: an antibody to cells producing steroid hormones. Clin Exp Immunol 3:107–117

Betterle C, Dal Pra C, Mantero F et al (2002) Autoimmune adrenal insufficiency and autoimmune polyendocrine syndromes: Autoantibodies, autoantigens, and their applicability in diagnosis and disease prediction. Endocrine Reviews 23:327–364

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Legends

Autoantibodies against steroid hormone producing cells. Fig. 1. Antibodies against the adrenal cortex. Substrate: primate adrenal cortex

Autoantibodies against steroid hormone producing cells. Fig. 2. Antibodies against theca cells. Substrate: primate ovary

Autoantibodies against steroid hormone producing cells. Fig. 3. Antibodies against Leydig interstitial cells. Substrate: primate testis

Autoantibodies against steroid hormone producing cells. Fig. 4. Antibodies against syncytiotrophoblast. Substrate: primate placenta

Autoantibodies against thrombocytes

W. STÖCKER

Synonym(s). Anti-thrombocyte antibodies; anti-HPA; thrombocyte antibodies; anti-human platelet antigen

Definition. Thrombocyte antibodies (TA) are the antibodies against antigens on the platelet surface (see also antibodies against heparin/PF4)

Synthesis/distribution/decomposition/elimination. The corresponding antigens are inherited codominantly and generally have a biallelic manifestation (Tab. 1).

Autoantibodies against thrombocytes. Tab. 1

Antigen*	Manifestation (%)	Synonym#	Glycoprotein
HPA 1	a (97); b (26)	Zw a/b	IIIa
HPA 2	a (99); b (14)	Ko b/a	Ib
HPA 3	a (90); b (60)	Bak a/b	IIb
HPA 4	a (> 99); b (< 0.1)	Yuk b/a	IIIa
HPA 5	a (99); b (20)	Br b/a	Ia

**HPA* Human Platelet Antigen; #other nomenclatures also exist

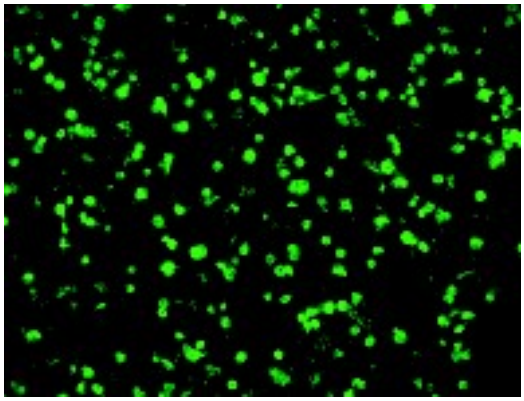
Other antigen systems are also known. Clinically, the most important antibody is anti-HPA-1a.

Function and pathophysiology. Similar to the autoantibodies against erythrocyte antigens, TA can also occur as auto- and alloantibodies. However, many autoantibodies do not display a detectable specificity against specific antigens, so, from a functional perspective, they can be considered alloantibodies in connection with thrombocyte transfusions. The antibodies bond to the surface of the platelets with the corresponding antigen, i.e. either to endogenous thrombocytes (autoantibodies) or to transfused or foetal thrombocytes (alloantibodies). These antibody-laden thrombocytes are broken down by macrophages of the reticuloendothelial system. This typically does not lead to an intravascular reaction with complement activation. As a result, normally no serious systemic symptoms are observed, in contrast to antibodies against erythrocyte antigens.

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Various systems can be used as screening tests [direct and indirect immunofluorescence with platelet smears (immunofluorescence, indirect; Fig. 1.) or using flow cytometers, also enzyme-linked immunosorbent assay-related methods]. However, a positive result only has a reliable diagnostic value, if the specificity of the antibodies against one or more of the defined glycoproteins has been established. The MAIPA (Monoclonal Antibody Immobilisation Platelet Assay) is used for this purpose.



Autoantibodies against thrombocytes. Fig. 1. Substrate: human smear

Reference range — Adults. Negative

Reference range — Children. Negative

Interpretation. Associated clinical pictures:

Autoantibodies:

- Autoimmune thrombocytopenia [idiopathic thrombocytopenic purpura (ITP); Werlhof's disease; systemic lupus erythematosus (SLE)]

Alloantibodies:

- Post-transfusion purpura (alloantibodies formed by thrombocyte transfusions cross-react with endogenous thrombocytes and result in a clinical picture similar to ITP.)
- Neonatal immune thrombocytopenia (severe thrombocytopenia of the foetus and the newborn with a risk of intracranial bleeding; due to the functional disorder of the antibody-laden thrombocytes, the complications are more severe than expected based on the number of thrombocytes.)
- The transfusion of thrombocyte concentrates does not lead to a measurable rise in the number of thrombocytes.

Positive findings are only relevant in connection with the characteristic clinical symptoms. Negative findings do not rule out any of the aforementioned clinical pictures.

Literature.

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Autoantibodies against THSD7A (thrombospondin type-1 domain-containing protein 7A)

Synonym(s). THSD7A autoantibodies; anti-THSD7A antibodies

Definition. Autoantibodies against the thrombospondin type-1 domain-containing protein 7A (THSD7A) are specific markers for primary membranous nephropathy (pMN, synonym: primary membranous glomerulonephritis) in addition to autoantibodies against phospholipase-A2 receptors (anti-PLA2R).

Pathophysiology. Membranous nephropathy is characterised by a chronic inflammation of the renal corpuscles (glomeruli) with increasing restriction of renal function. The primary form of MN is based on autoimmune reactions, which are directed against the transmembrane proteins PLA2R (autoantibodies against the phospholipase A2 receptor) and THSD7A and potentially other, previously unidentified antigens.

The proteins are located on the surface of the podocytes in human glomeruli, which are damaged by the binding of the autoantibodies. Immunocomplexes are deposited “in situ” in the area of the glomerular basement membrane and activate the complement system; this leads to the overproduction of extracellular matrix proteins, the destruction of the cytoskeleton of the podocytes, thickening of the basement membrane and proteinuria.

MN is the most frequent cause of a nephrotic syndrome (symptom complex consisting of proteinuria, hypoproteinemia with hypoalbuminemia, hyperlipoproteinemia, oedema). The more pronounced the proteinuria, the higher the long-term risk of kidney failure, while a high mortality also exists in connection with thromboembolic and cardiovascular complications.

Sample material. Serum or plasma

Sample stability. Patient samples for detecting antibodies can be stored at +4 °C for up to 2 weeks or at -20 °C for months and years.

Analytics. Indirect immunofluorescence (immunofluorescence, indirect) using THSD7A-transfected HEK293 cells as substrate. The recommended starting dilution is 1:10.

Diagnostic value. Class IgG autoantibodies against THSD7A are specific for the detection of primary MN and enable a distinction to be made from secondary MN (MN as a consequence of another underlying disease). Anti-THSD7A antibodies predominantly occur in anti-PLA2R seronegative patients; the parallel occurrence of both autoantibodies has only been described in isolated cases. While autoantibodies against PLA2R are detected in the serum of up to 75% of primary MN patients, the prevalence of anti-THSD7A is 2-5% and up to 10% in anti-PLA2R-negative MN patients. The detection of anti-THSD7A in additional anti-PLA2r therefore leads to an increase in the serological detection rate in patients suspected of suffering from primary MN.

An association between anti-THSD7A antibodies and malignant tumours could also be found. An expression of THSD7A in the tumours could lead to the formation of autoantibodies and therefore explain the joint occurrence of cancers and MN.

Literature.

Tomas NM, Beck LH Jr, Meyer-Schwesinger C, Seitz-Polski B, Ma H, Zahner G, Dolla G, Hoxha E, Helmchen U, Dabert-Gay A-S, Debayle D, Merchant M, Klein J, Salant DJ, Stahl RAK, Lambeau G (2014) Thrombospondin Type-1 Domain-Containing 7A in Idiopathic Membranous Nephropahty. N Engl J Med 371(24): 2277-2287

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Larsen CP, Cossey LN, Beck LH (2016) THSD7A staining of membranous glomerulopathy in clinical practice reveals cases with dual autoantibody positivity. Mod Pathol 29(4):421-6.

Autoantibodies against thyroglobulin

W. Stöcker

Synonym(s). Thyroglobulin antibodies; anti-TG antibodies

Definition. Thyroglobulin (TG) is a glycoprotein that plays an important role in storing the thyroid hormones T3 (triiodothyronine) and T4 (thyroxin): T3 and T4 are synthesised by the cells of the thyroid gland epithelium and, bound to the thyroglobulin, stored in the follicles of the thyroid gland. To release hormones, T3 and T4 are dissociated from the thyroid globulin and released into the blood.

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Indirect immunofluorescence (IIF, immunofluorescence, indirect) with frozen sections of thyroid gland as a substrate is suitable for detecting autoantibodies against TG and thyroid peroxidase (TPO). Antibodies against TG react with the colloid of the follicles and create a striped or reticular image (Fig. 1).
Monospecific test systems (enzyme-linked immunosorbent assay, chemiluminescence immunoassay, radioimmunoassay, immunoblot) with native purified thyroglobulin can also be used.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Hashimoto thyroiditis, Graves' disease.
Antibodies against TG are analysed in the presence of differentiated thyroid cancer because they can impair the correct detection of the TG concentration in the serum (tumour marker).

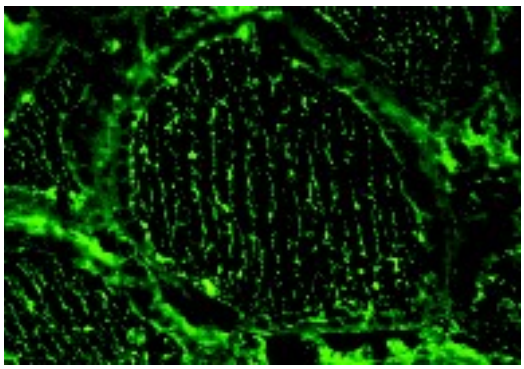
Interpretation. Autoantibodies against TG are associated with autoimmune thyroiditis. This can manifest in the form of hyperthyroidism (hyperthyroidism, e.g. Graves' disease) or in a hypofunction (hypothyroidism, e.g. Hashimoto thyroiditis; see autoantibodies against thyroid peroxidase).
In particular, autoantibodies against TPO and against thyroid stimulating hormone (TSH) receptors are considered relevant diagnostic parameters for diagnosing Graves' disease. In this case, anti-TG antibodies only occur in about 30% of patients. By contrast, its prevalence in Hashimoto thyroiditis amounts to 60%.

Diagnostic value. The diagnostic significance of autoantibodies against thyroglobulin for detecting autoimmune thyroiditis is limited compared to autoantibodies against TSH receptors and thyroid peroxidase antibodies; their detection as part of endocrinological differential diagnostics is no longer recommended by the Thyroid Department of the German Endocrinology Society (DGE).

Literature.

Gentile F, Conte M, Formisano S (2004) Thyroglobulin as an autoantigen: What we can learn about immunopathogenicity from the correlation of antigenic properties with protein structure? Immunology 112:13–25

Iddah MA, Macharia BN (2013) Autoimmune thyroid disorders. ISRN Endocrinol 2013: 509764.



Autoantibodies against thyroglobulin. Fig. 1. Substrate: primate thyroid (unfixed)

Autoantibodies against thyroid peroxidase

W. Stöcker

Synonym(s). Autoantibodies against thyroid microsomes; autoantibodies against thyroid-specific peroxidase; anti-TPO; microsomal antibodies; thyroid peroxidase antibodies

Definition. Antibodies are directed against thyroid microsomes with the key target antigen of thyroid peroxidase, the important iodine accumulation enzyme, which is only expressed by the thyroid gland.

Function and pathophysiology. Autoimmune thyroid diseases can manifest as hyperthyroid and hypothyroid dysfunctions. They are much more common in women (prevalence 2%) than in men (0.2%).
About 60% of all cases of hyperthyroidism are ascribed to Graves' disease. Serologically, autoantibodies against TSH receptors of the thyroid are considered diagnostic markers; however, in cases with normal values, the detection of antibodies against TPO can support the diagnosis. Autoantibodies against thyroglobulin (anti-TG) are also found in up to 30% of patients.
The second important autoimmune disease of the thyroid gland is Hashimoto thyroiditis, the onset of which is often clinically nondescript, but can lead to hypothyroidism over time (Tab. 1).
A special form of autoimmune thyroiditis is postpartum thyroiditis, a temporary hypothyroid dysfunction of the thyroid gland, which is associated with high antibody titers of the anti-TPO antibody. About 5% of women are affected by this disease. The risk is particularly high if insulin-dependent diabetes mellitus is also present. The measurement of anti-TPO antibodies is recommended in all new mothers, as hormone substitution is required in the case of the onset of the disease.
Autoimmune thyroiditis is often combined with other autoimmune diseases (myasthenia gravis, pernicious anaemia, Addison's disease).

Autoantibodies against thyroid peroxidase and thyroglobulin. Tab. 1.

Prevalence	Anti-TPO	Anti-TG
Graves' disease	90	30
Hashimoto thyroiditis	90	60

Sample material. Serum, plasma

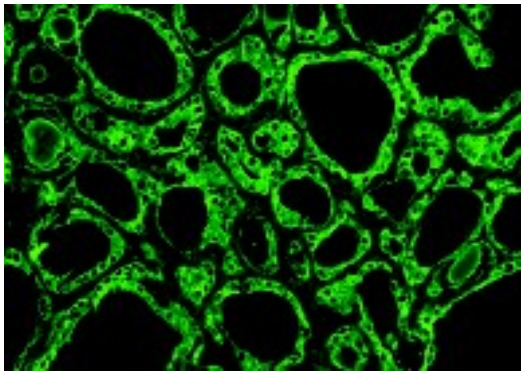
Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Indirect immunofluorescence (IIF, immunofluorescence, indirect) with frozen sections of primate thyroid is suitable for detecting autoantibodies against TG and (TPO) (Fig. 1). Antibodies against TPO display a smooth fluorescence of the cytoplasm of the follicular epithelium cells. The combination of multiple frozen sections ("BIOCHIP mosaic") enables the analysis of antibody profiles, e.g. in order to verify autoimmune polyendocrinopathy. The combination of thyroid and rat kidney facilitates a reliable differentiation from autoantibodies against mitochondria.
Monospecific test systems (enzyme-linked immunosorbent assay, chemiluminescence immunoassay, radioimmunoassay) with native or recombinant TPO can be used for quantification. Detection using immunoblot is also possible. The various test systems available on the market display great consistency between one another.

Indication. Hashimoto thyroiditis, Graves' disease, new mothers, especially with insulin-dependent diabetes mellitus.
Primarily to distinguish between hyperthyroid autoimmune thyroiditis and diffuse autonomy, which is not possible without thyroid detection.

Literature.
Iddah MA, Macharia BN (2013) Autoimmune thyroid disorders. ISRN Endocrinol 2013: 509764.

Saravanan P, Dayan CM (2001) Thyroid autoantibodies. Endocrin Metabol Clin North Am 30(2): 315-337



Autoantibodies against thyroid peroxidase. Fig. 1. Substrate: primate thyroid (unfixed)

Autoantibodies against titin

W. Stöcker

Synonym(s). Titin antibodies

Definition. Autoantibodies against the skeletal muscle structural protein titin.

Structure. Titin is a protein in the striated musculature with a molar mass of about 3,000 kDa, the largest protein in the human body. It forms a filament system in the myofibrils of the vertebrates, which is important for the structural integrity and elasticity of the musculature. The immunogenic region of titin is located on a 30 kDa protein fragment.

Function and pathophysiology. Titin is the first target antigen of the myasthenia gravis-associated autoantibodies against striated muscle, identified in 1990.

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Anti-titin antibodies display a typical horizontal striping in the indirect immunofluorescence test (IIFT, immunofluorescence, indirect) on skeletal muscle and heart. The starting dilution is 1:100. Titres from 1:1,000 are clinically significant. Line blots (immunoblot) with recombinant MGT30 peptide (the immunogenic molecular sections of titin) as the substrate are available for the monospecific detection of the anti-titin antibody.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Myasthenia gravis

Interpretation. Autoantibodies against titin occur in addition to the autoantibodies against acetylcholine receptors (ACHRAB). The presence of autoantibodies against titin generally indicates the existence of a thymoma in addition to myasthenia gravis (Tab. 1).

Autoantibodies against titin. Tab. 1. Prevalence

	Prevalence	
	MG	MG and thymoma
ACHRAB	85	100
Striated muscle	34	75
Titin	34	95

ACHRAB Autoantibodies against acetylcholine receptors; *MG* Myasthenia gravis

Literature.

Aarli JA, Stefansson K, Marton LS et al (1990) Patients with myasthenia gravis and thymoma have in their sera IgG autoantibodies against titin. Clin Exp Immunol 82:284–288

Romi F, Skeie GO, Aarli JA et al (2000) Muscle autoantibodies in subgroups of myasthenia gravis patients. J Neurol 247:369–375

Autoantibodies against Tr /DNER

W. STÖCKER

Synonym(s). Tr autoantibodies; PCA-Tr autoantibodies; anti-Tr/DNER autoantibodies

Definition. Autoantibodies against delta/notch-like epidermal growth factor-released receptors (DNER) in the cytoplasm of the Purkinje cells in the cerebellum

Function and pathophysiology. DNER are expressed in peripheral and central neurons as well as in tumour tissue (in antibody-positive patients).

Analytics. Autoantibodies against Tr/DNER can be detected with the indirect immunofluorescence test (IIFT, immunofluorescence, indirect) with frozen sections of primate cerebellum as the substrate. These antibodies can be identified by the fine fluorescence of the Purkinje cell cytoplasm and a dotted staining of the molecular layer. The indirect immunofluorescence test (IIFT, immunofluorescence, indirect) with transfected HEK cells as the substrate or line blots (immunoblot) with purified defined antigens are suitable for the monospecific detection of anti-Tr/DNER.

Sample material. Serum, plasma or cerebrospinal fluid

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Diagnostic value. Anti-Tr/DNER are found in patients with cerebellar degeneration and can provide an initial indication of underlying Hodgkin's disease; see also autoantibodies against neuronal antigens.

Literature.

Bernal F, Shams'ili S, Rojas I, Sanchez-Valle R, Saiz A, Dalmau J, Honnorat J, Sillevis Smitt P, Graus F (2003) Anti-Tr antibodies as markers of paraneoplastic cerebellar degeneration and Hodgkin's disease. Neurology 60:230–234

Graus F, Delattre JY, Antoine JC, Dalmau J, Giometto B, Grisold W, Honnorat J, Smitt PS, Vedeler CH, Verschuuren JJ, Vincent A, Voltz R (2004) Recommended diagnostic criteria for paraneoplastic neurological syndromes. J Neurol Neurosurg Psychiatry 75:1135–1140

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Autoantibodies against TSH receptors

W. Stöcker, Chr. Krüger

Synonym(s). TSH receptor antibodies (TRAb)

Definition. Autoantibodies against the receptors for thyroid stimulating hormone (TSH) (thyrotropin)

Synthesis/distribution/decomposition/elimination. The TSH receptor is a member of the subfamily of the G-protein-bonded glycoprotein hormone receptors. Every thyrocyte has 103–104 TSH receptors. A receptor consists of an extracellular α-subunit with a molecular weight of 53 kDa and a transmembrane β-subunit with a molecular weight of 38 kDa. Porcine, rat and human TSH receptors display a homology of 85-90%. In the region of the binding sites for the TSH, the homology amounts to almost 100%. Anti-TSH receptor antibodies bind to the extracellular domain of the receptor.

Function and pathophysiology. TSH receptor autoantibodies (TRAb) are heterogenous with regard to their biological effect and stimulate (TSAb, “thyroid stimulating antibody”) or block (TBAb, “thyroid blocking antibody”) the TSH receptor. TRAbs that are directed directly against the binding site of the TSH and prevent TSH from binding to the receptor are also referred to as TBII (TSH “binding inhibitor immunoglobulin”). TRAbs have a 90% association with Graves’ disease. In Graves’ disease, the effect is stimulating, i.e. the TRAbs act as TSH agonists. The antibody binding leads to a stimulation of the cAMP cascade, an increased formation and secretion of the thyroid hormone, hyperfunction and proliferation of the thyroid.

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. The current revised test methods of the first generation are radioreceptor assays (RRA), which are based on the displacement of radioactively labelled TSH molecules from solubilised thyrocyte membranes by the TRAb of the patient serum. The patient serum and the labelled TSH are incubated together with the solubilised membranes (receptors) in a single step. The labelled TSH binds to the receptors not occupied by TRAb. The unbound part of the reaction mixture is separated with a precipitating agent and centrifuged. The amount of radioactivity contained in the sediment is inversely proportional to the TRAb concentration in the sample.
In the second-generation test methods of enzyme-linked immunosorbent assays, radioreceptor assays (RRA) and electrochemiluminescence immunoassays, porcine or human TSH receptors are immobilised on the wall of a reaction vessel, to which the TRAb-positive samples bond. Excess sample components are removed by washing the reaction vessel. The bound TRAb inhibits the binding of labelled TSH, which is added in a second incubation step. The amount of labelled TSH detected on the solid phase by photometry, measuring the radioactivity or luminescence is inversely proportional to the TRAb concentration in the sample. The second-generation test systems have almost identical analytical characteristics to one another, independent of the receptor species used (human or porcine: a broad-based consistency exists in the receptor region). In the third-generation ELISA, the bound TRAb-positive samples inhibit the bonding of labelled, thyroid-stimulating, monoclonal antibodies (M22). The measured absorbance is also inversely proportional to the TRAb concentration in the sample. Third-generation ELISA are more sensitive than second-generation tests with the same specificity. In contrast to all other methods, the ELISA is easiest to automate, these days it is generally preferred compared to the radioimmunoassay.

International standard. The first international standard for thyroid-stimulating antibodies (WHO, 1995, Standard 90/672, National Institute for Biological Standards and Control, Hertfordshire, England) contains 0.1 IU per vial as defined.

Reference range — Adults. Negative to marginal: 2 < IU/L (second-generation tests)

Reference range — Children. Negative to marginal: 2 < IU/L (second-generation tests)

Indication. Confirmation or exclusion of Graves’ disease and therapy control.

Interpretation. TRAb are considered a serological marker for diagnosing Graves’ disease, as they are detected in over 90% of untreated patients. In addition, the monitoring of the TRAb concentration during the course of Graves’ disease enables a prognosis to be provided and provides important support in treatment management decisions. High TRAb concentrations after extended thyrostatic therapy are an indication of an increased risk of regression. TRAb are rarely found with Hashimoto thyroiditis and primary myxoedema (possible function-inhibiting antibodies). TRAb can also trigger hyperthyroidism in the foetus of pregnant mothers who suffer from Graves’ disease.

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Autoantibodies against U1-RNP

W. Stöcker

Synonym(s). U1-RNP antibodies

Definition. The corresponding antigens are a group of small nuclear ribonucleoproteins (snRNP), which consists of low-molecular-weight RNA with a high uridine content (U-RNA) and various proteins (molecular weights of 9-70 kDa). The RNA part is referred to as U1-U6 depending on the chromatographic behaviour. The U-n(nuclear)RNP particles each have 6 different core proteins (B, B', D, E, F, G) in addition to the relevant RNA. U1-nRNP also contains particle-specific proteins (70K, A, C) against whose epitopes the antibodies are directed.

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

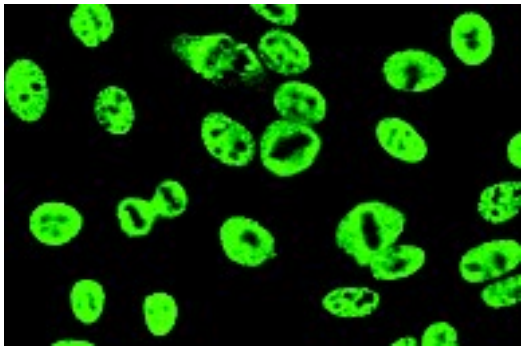
Analytics. Autoantibodies against U1-RNP generally display a coarse granular, sometimes also a medium to fine granular fluorescence, which is distributed across the entire cell nucleus except for the nucleoli, in the indirect immunofluorescence test (IIFT, immunofluorescence, indirect) on HEp-2 cells (Fig. 1). In mitosis cells, the condensed chromosomes are dark, while the periphery displays an almost homogenous, smooth fluorescence. Tissue sections of primate liver also display a granular fluorescence, apart from the nucleoli (Fig. 2). Autoantibodies against U1-nRNP and autoantibodies against Sm react with the primate liver just as strongly as with HEp-2 cells, in contrast to antibodies against Ro/SS-A autoantibodies against SS-A and autoantibodies against SS-B.
In the case of a positive result in the IIFT, a monospecific test (enzyme-linked immunosorbent assay, chemiluminescence immunoassay, line blot) with native, purified, U1-RNP or a Western blot with nuclear antigens can be used to precisely identify the target antigen.

Reference range — Adults. Negative

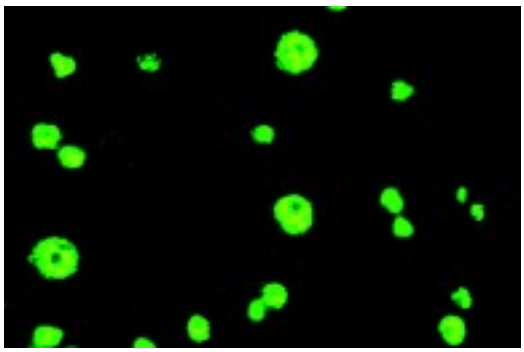
Reference range — Children. Negative

Diagnostic value. U1-nRNP antibodies are characteristic of mixed connective tissue disease (MCTD, Sharp's syndrome). The prevalence amounts to 95-100%. The antibody titer correlates with the disease activity. Antibodies against U1-nRNP also occur in 15-40% of patients with systemic lupus erythematosus, but almost always combined with autoantibodies against Sm.

Literature.
Tan EM, Chan EKL, Sullivan KF et al (1988) Antinuclear antibodies (ANAs): Diagnostically specific immune markers and clues toward the understanding of systemic autoimmunity. Clin Immunol Immunopathol 47:121–141



Autoantibodies against U1-RNP. Fig. 1. Substrate: HEp-2 cells



Autoantibodies against U1-RNP. Fig. 2. Substrate: primate liver

Autoantibodies against vasopressin-producing cells

W. Stöcker

Synonym(s). Anti-VPC, autoantibodies to arginine vasopressin producing cells; AVPcAb

Definition. Autoantibodies against vasopressin-producing cells occur with idiopathic central diabetes insipidus. Autoimmune processes in the hypothalamus and the pituitary gland lead to reduced synthesis and secretion of the hormone vasopressin.

Function and pathophysiology. The antidiuretic hormone (ADH, vasopressin) is produced in the supraoptic and paraventricular nuclei of the hypothalmus and released from the posterior lobe of the pituitary gland depending on the plasma osmolality. In the collecting ducts of the kidney, aquaporins are activated by the hormone, which make the collecting ducts permeable for water and so increase the concentration capacity of the kidney. A lack of ADH leads to diabetes insipidus.
Autoimmune reactions against the hypothalmus and against the pituitary gland contribute to half of the cases with diabetes insipidus. They are associated with autoantibodies against vasopressin-producing cells.

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Autoantibodies against vasopressin-producing cells (VPC) are detected by indirect immunofluorescence (immunofluorescence, indirect). Frozen sections of the hypothalmus (primate tissue, suprasellar region: there is no need to dissect out the supraoptic and paraventricular nuclei) and the posterior lobes of the pituitary gland are used as substrates. The starting dilution of the serum is 1:10, antibodies in immunoglobulin classes IgA, IgG and IgM are analysed.
No anti-VPC can be detected in some of the patients with autoimmune diabetes insipidus. The disease's association with various forms of autoimmune polyendocrinopathy means that it is sensible to also detect the autoantibodies of relevance for these clinical pictures: Frozen sections of the adrenal gland, ovary, placenta and testis, thyroid gland, primate stomach, pancreas and striated muscles are also used for indirect immunofluorescence. The easiest way to create these types of antibody profiles is by using modern "BIOCHIP Mosaics".

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Anti-VPC are analysed in case of suspected autoimmune diabetes insipidus centralis. The detection of anti-VPC in patients with autoimmune polyendocrinopathy is recommended to detect latent diabetes insipidus with the possibility of initiating prophylactic therapy with desmopressin to delay or prevent the onset of the disease.

Interpretation. The detection of autoantibodies indicates an autoimmune background of the central diabetes insipidus.

Diagnostic value. The detection of VPC antibodies can help to distinguish an autoimmune central from renal diabetes insipidus and between various other causes of central diabetes insipidus: Tumours, tuberculosis and sarcoidosis in the region of the pituitary gland, inherited forms, hypoxia, ischaemia, intracranial injuries and others. The prevalence and antibody titer of the VPC antibodies are very high in the case of the onset of autoimmune diabetes insipidus; if they are not detected at this time, an autoimmune pathogenesis can virtually be ruled out.
A small number of patients with endocrine autoimmune diseases without manifest diabetes insipidus develop these symptoms over a few years in which case the anti-VPC have a high predictive value.
The autoimmune pathogenesis of central diabetes insipidus is supported by the presence of anti-VPC in patients less than 30 years of age as well as the simultaneous presence of other endocrine autoimmune diseases and a thickening of the pituitary stalk, which can be detected by nuclear magnetic resonance tomography – the visible sign of the existence of lymphocytic infundibuloneurohypophysitis.

Literature.

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Autoantibodies against Yo

W. STÖCKER

Synonym(s). Yo antibodies; PCA-1; autoantibodies against Purkinje cell cytoplasm

Definition. Autoantibodies against cytoplasmic antigens of the Purkinje cells with paraneoplastic cerebellar syndrome. The name is derived from the index patient Young. Anti-Yo antibodies can provide an initial indication of an underlying tumour (autoantibodies against neuronal antigens).

Sample material. Serum, plasma, cerebrospinal fluid

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. The indirect immunofluorescence test (IIFT, immunofluorescence, indirect) with frozen sections of primate cerebellum is suitable as the standard method for detecting autoantibodies against Purkinje cell cytoplasm (Fig. 1). Autoantibodies against Yo often have a high antibody titer, sometimes up to 1:100,000. In the case of a positive result in the IIFT, a Western blot with cerebellum antigens or a line blot (7immunoblot) with purified defined antigens can be used to confirm the finding. An indirect immunofluorescence test with transfected cells as the substrate is also available for the monospecific detection of anti-Yo antibodies.

Reference range — Adults. Negative

Reference range — Children. Negative

Diagnostic value. The rare autoantibodies against Yo indicate a symptomatic (paraneoplastic) cerebellar syndrome. The antibodies are generally associated with certain tumours, most frequently with ovarian, breast and uterine cancer, but they have also been observed with prostate cancer and adenocarcinoma of the oesophagus. In many cases, the cerebellar syndrome clinically precedes the tumour; the detected autoantibodies against Yo provide an incentive to screen for tumours.

Literature.

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Legend

Autoantibodies against Yo. Fig. 1. Autoantibodies against Yo. Substrate: primate cerebellum

Autoantibodies against cell nuclei

W. STÖCKER

Synonym(s). Autoantibodies against cell nuclei; ANA; ANF (antinuclear factors)

Definition. Autoantibodies that are directed against antigens of the cell nucleus. The names for these autoantigens are either based on the biochemical properties (DNA, histones, ribonucleoproteins: RNP) or on diseases associated with the autoantibodies (SS-A, SS-B: Sjögren's syndrome, antigens A and B; PM-Scl: polymyositis, progressive systemic sclerosis) and sometimes also on the names of the patients in which the antibodies were first described (sm, Ro, La) (Tab. 1).

Function and pathophysiology. While the importance of cell nucleus antibodies has been established for diagnosing many autoimmune diseases, in most cases, their role in the pathogenesis remains unclear, apart from that of autoantibodies against double-stranded DNA for example.

Sample material. Serum, plasma, cerebrospinal fluid

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. The gold standard for detecting autoantibodies against cell nuclei (ANA) is the indirect immunofluorescence test (IIFT, immunofluorescence, indirect) with human epithelial cells (HEp-2) (Fig. 1) and primate liver (Fig. 2), which is renowned for its high specificity – positive and negative samples provide a large difference in signal, because the microscopic assessment enables a precise analysis of how an indicator dye (generally fluorescein) is distributed in a tissue or in cells. A typical fluorescence pattern is generated for every bound antibody, depending on the localisation of the individual autoantigens.
In the case of a positive result, methods such as enzyme immunoassays (enzyme-linked immunosorbent assay, chemiluminescence immunoassay) or immunoblots (line blot) are used with test substrates with defined individual antigens to provide a final distinction. The exclusive use of these monospecific test methods is not sufficient to detect the autoantibodies against cell nuclei, as not all relevant antigens are currently available in purified form. An IIFT must also always take place in parallel to monospecific tests to check their plausibility.

Reference range — Adults. Negative

Diagnostic value. Autoantibodies against cell nuclei (ANA) in the serum of patients are a characteristic finding with many diseases, especially (but not exclusively) of the rheumatic form. Tab. 2 provides an overview of the main diseases.
The detection of ANA is a key diagnostic tool for many autoimmune diseases. Antibodies against nuclear antigens are directed against various nuclear components (biochemical substances of the nucleus). These include the nucleic acids, nuclear proteins and ribonucleoproteins. The prevalence of antinuclear antibodies with inflammatory rheumatic diseases is between 20 and 100%. Therefore, differential antibody diagnostics against nuclear antigens is indispensable in the identification of individual rheumatic diseases and to distinguish between other autoimmune diseases.

Systemic lupus erythematosus

For systemic lupus erythematosus (SLE; synonym: lupus erythematosus disseminatus, LED), the detection of autoantibodies against double-stranded DNA is an important criterion for diagnosis (Tab. 3). Immunocomplexes consisting of double-stranded DNA and corresponding autoantibodies cause tissue damage in the subcutis, kidneys and other organs. The antibody titer correlates with the disease activity. Autoantibodies against Sm are also considered pathognomonic for SLE. In addition, autoantibodies against other polynucleotides, ribonucleotides, histones and other nuclear antigens can be detected with this disease.
Autoantibodies against histones constantly occur in the case of drug-induced lupus erythematosus with symptoms of arthralgia, arthritis, exanthema, serositis, myalgia and enlargement of the liver and spleen. This reversible form of SLE can be triggered by antibiotics (e.g. penicillin, streptomycin, tetracycline), chemotherapeutics (e.g. INH, sulfonamide), antiepileptics (e.g. phenytoin, hydrantoin), antiarrhythmics (e.g. procainamide, practolol), antihypertensives (e.g. reserpine, hydralazine), psychotropic drugs (e.g. chlorpromazine), thyrostatics (thiouracil derivatives), antirheumatic basic therapeutic drugs (e.g. gold, D-penicillamine) and others, such as contraceptives and allopurinol.

Sharp's syndrome

High titers of autoantibodies against U1-RNP are characteristic of Sharp's syndrome ("mixed connective tissue disease", MCTD). The antibody titer correlates with the disease activity (Tab. 4).

Rheumatoid arthritis

In rheumatoid arthritis, autoantibodies against histones are detected in up to half of the patient sera, while titers against U1-nRNP are also occasionally found. Antibodies against RANA ("rheumatoid arthritis nuclear antigen") cannot be detected with HEp-2 cells (Tab. 5).

Progressive systemic sclerosis

Progressive systemic sclerosis (progressive systemic scleroderma, PSS; scleroderma) can manifest in 2 forms which cannot be definitively differentiated from one another. Previously, primarily autoantibodies against Scl-0 and autoantibodies against RNA polymerase III (RP11, RP155) and fibrillarin have been observed in the diffuse form. Autoantibodies against centromeres (CENP-A, CENP-B) are associated with the limited form of PSS (Tab. 6, Fig. 3).

Polymyositis/Dermatomyositis

Autoantibodies against PM-Scl occur with polymyositis and dermatomyositis. Other cell nucleus antibodies (Mi-2, Ku) and autoantibodies against Jo-1 (autoantibodies against aminoacyl-t RNA synthetase) can also be detected with these diseases (Tab.).

Sjögren's syndrome

Autoantibodies against SS-A and autoantibodies against SS-B occur with primary Sjögren's syndrome, predominantly together. Autoantibodies against salivary gland excretory ducts may also be present in 40-60% of the cases (Tab. 8).

Primary biliary cholangitis (formerly: primary biliary cirrhosis)
Besides autoantibodies against mitochondria, a range of autoantibodies against cell nuclei are associated with primary biliary cholangitis, some of which can be considered pathognomonic; see also PBC-associated antinuclear autoantibodies (PBCNA). Moreover, 7autoantibodies against SS-A and autoantibodies against centromeres are often found with PBC, which both, as well as autoantibodies against glycoprotein 210, indicate an unfavourable prognosis (Tab. 9).
Autoantibodies against cell nuclei can also be detected in subjectively apparently healthy persons with a prevalence of 5%, mostly with low titers. The most important diseases associated with autoantibodies against cell nuclei are summarised in Tab. 10.

Autoantibodies against DFS0 also react with HEp-2 cells in the IIFT and generate a dense, fine granular fluorescence pattern in the nucleoplasm, which must be distinguished from the homogeneous and granular patterns of other ANA. The antibodies are not disease-specific and also occur in healthy persons. But, a positive anti-DFS70 finding can at least explain a part of the ANA pattern in the indirect immunofluorescence test, which cannot be assigned to any disease-related ANA.

Antibodies against parts of the cytoplasm of the HEp-2 cells can no longer be clearly differentiated in the immunofluorescence pattern. Only a few

cytoplasm-reactive antibodies can be assigned to a specific disease, including autoantibodies against mitochondria with primary biliary cholangitis and against proteins PL-7 and PL-12 with polymyositis and dermatomyositis. Additional rare antibodies with polymyositis are also directed against OJ, EJ and signal recognition particles. Other cytoplasmic antibodies, such as against ribosomes, the Golgi apparatus, lysosomes and parts of the cytoskeleton, such as actin (autoantibodies against smooth muscles), vimentin (autoantibodies against Sa) or cytokeratins are of secondary clinical importance. The diagnostic uses of mitosis-associated antigens (autoantibodies against mitosis-associated antigens) have also not been completely clarified. The overall view of the arguments made demonstrate the exceptional immunological relevance and the associated diagnostic value of autoantibodies against cell nuclei.

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Autoantibodies against cell nuclei. Tab. 1. Nuclear autoantigens

Polynucleotides	Double-stranded DNA, single-stranded DNA, RNA
Histones	H1, H2A, H2B, H3, H4, H2A-H2B complex
Ribonucleoproteins	U1-nRNP, Sm, SS-A (Ro), SS-B (La)
Antigens of the nucleolus	U3-nRNP/fibrillarin, RNA-polymerase I, PM-Scl (PM-1), 7-2-RNP (To), 4-6-S-RNA, NOR-90 (nucleolus organiser)
Centromeres	Kinetochore proteins
Other proteins	Scl-70, PCNA (cyclin I), nuclear granules, Ku, Mi-2, lamins, lamin-B receptors

Autoantibodies against cell nuclei. Tab. 2. ANA prevalence with selected clinical pictures

Autoimmune disease	Prevalence of autoantibodies against cell nuclei
Systemic lupus erythematosus (SLE) active inactive	 95–100 60–80
Drug-induced lupus erythematosus	100
Mixed connective tissue disease (MCTD, Sharp's syndrome)	100

Rheumatoid arthritis	20–40
Other rheumatic diseases	20–50
Progressive systemic sclerosis	85–95
Polymyositis/Dermatomyositis	30–50
Sjögren's syndrome	70–80
Chronic-active hepatitis	30–40
Ulcerative colitis	26

Autoantibodies against cell nuclei. Tab. 3. Autoantibodies with systemic lupus erythematosus

Antigen	Prevalence
Double-stranded DNA	60–90
Nucleosomes	50–70
Single-stranded DNA	70–95
RNA RNA-helicase A	50 6
Histones	50–80
U1-nRNP	15–40
Sm	5–40
SS-A (Ro)	20–60
SS-B (La)	10–20
Cyclin (PCNA)	3
Ku	10
Ribosomal P proteins	10
(Hsp-90: heat shock protein, 90 kDA	50)
(Cardiolipin	40–60)
Ro-52	38

Autoantibodies against cell nuclei. Tab. 4. Autoantibodies with

Sharp's syndrome (mixed connective tissue disease, MCTD)	
Antigen	Prevalence
U1-nRNP	95–100
Single-stranded DNA	20–50
Ro-52	19

Autoantibodies against cell nuclei. Tab. 5. Cell nucleus autoantibodies with rheumatoid arthritis	
Antigen	Prevalence
Histones	15–50
Single-stranded DNA	8
U1-nRNP	3
(RANA	90–95)

Autoantibodies against cell nuclei. Tab. 6. Cell nucleus autoantibodies with progressive systemic sclerosis	
Antigen	Prevalence
Diffuse form	
Scl-70	25–75
RNA-polymerase III (RP11, RP155)	5–20
Ku (with overlap syndrome with polymyositis/dermatomyositis)	< 5 (25–50)
Fibrillarin	5–10
PM-Scl (PM-1) (75-kDa/100-kDa main antigen)	13 (10/7)
Ro-52	28
NOR-90 (nucleolus organiser region)	Rare
PDGFR (platelet-derived growth factor receptor)	Rare
Limited form	
Centromeres	80–95
7-2-RNP (Th/To)	Rare

Autoantibodies against cell nuclei. Tab. 7. Nuclear autoantibodies with polymyositis and dermatomyositis.

Antigen	Prevalence
PM-Scl (PM-1), (with overlap syndrome with progressive systemic sclerosis)	8–15 (24–55)
Jo-1 (histidyl-tRNA synthetase)	25–35
Mi-2	5–30
Ku (with overlap syndrome with progressive systemic sclerosis)	5–10 (25–50)
Single-stranded DNA	40–50
PL-7 (threonyl-tRNA synthetase)	4
PL-12 (alanyl-tRNA synthetase)	3
Ro-52	30

Autoantibodies against cell nuclei. Tab. 8. Nuclear autoantibodies with Sjögren's syndrome

Antigen	Prevalence
SS-A (Ro)	40–95
SS-B (La)	40–95
Single-stranded DNA	13
(RANA	70)
(Rheumatoid factors	60–80)
Ro-52	81

Autoantibodies against cell nuclei. Tab. 9. Nuclear autoantibodies with primary biliary cholangitis

Antigen	Prevalence
Nuclear dots	25–40
Nuclear membrane (GP 210)	20–40
SS-A	20
Centromeres	20–30

Ro-52	27

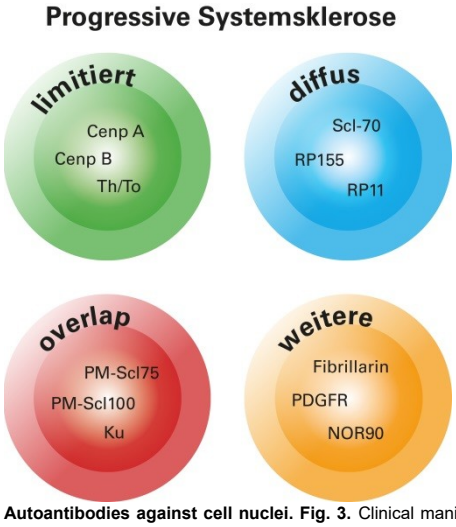
Autoantibodies against cell nuclei. Tab. 10. Nuclear autoantibodies with the most important associated diseases

Antigen	Disease	Prevalence
Double-stranded DNA	Systemic lupus erythematosus	60–90
Single-stranded DNA	Systemic lupus erythematosus Drug-induced lupus erythematosus Mixed connective tissue disease (MCTD, Sharp's syndrome) Polymyositis/Dermatomyositis Scleroderma, Sjögren's syndrome, rheumatoid arthritis	70–95 60 20–50 40–50 8–14
RNA	Systemic lupus erythematosus Scleroderma, Sjögren's syndrome	50 65
Histones	Drug-induced lupus erythematosus Systemic lupus erythematosus Rheumatoid arthritis	95 50-80 15–50
U1-nRNP	Mixed connective tissue disease (MCTD, Sharp's syndrome) Systemic lupus erythematosus Rheumatoid arthritis	95–100 15–40 3
Sm	Systemic lupus erythematosus	5–40
SS-A (Ro)	Sjögren's syndrome Systemic lupus erythematosus Neonatal lupus syndrome	40–95 20–60 100
SS-B (La)	Sjögren's syndrome Systemic lupus erythematosus	40–95 10–20
Fibrillarin	Progressive systemic sclerosis, diffuse form	5–10
RNA-polymerase III	Progressive systemic sclerosis, diffuse form	5-20
RNA helicase	Systemic lupus erythematosus	6
PM-Scl (PM-1)	Polymyositis/Dermatomyositis Overlap syndrome (poly/dermatomyositis and progressive systemic sclerosis) Progressive systemic sclerosis (diffuse form)	8–15 24–55 13
Centromeres	Progressive systemic sclerosis (limited form)	80–95
Scl-70	Progressive systemic sclerosis (diffuse form)	25–75

Cyclin (PCNA)	Systemic lupus erythematosus	3
Ku	Systemic lupus erythematosus Overlap syndrome (poly/dermatomyositis and progressive systemic sclerosis)	10 25–50
Mi-2	Dermatomyositis	5–30

Autoantibodies against cell nuclei. Fig. 1. Substrate: HEp-2 cells (homogeneous pattern)

Autoantibodies against cell nuclei. Fig. 2. Substrate: primate liver (homogeneous pattern)



Autoantibodies against cell nuclei. Fig. 3. Clinical manifestations of progressive systemic sclerosis and associated autoantigens. See also myositis-specific autoantibodies

Autoantibodies against centrioles/centrosomes

W. STÖCKER

Synonym(s). Centriole/centrosome antibodies

Definition. Autoantibodies against centrioles (centrosomes) which play an important role in cell division

Function and pathophysiology. Two centrioles are present before mitosis and position themselves at opposite poles of the cell. They are involved in the development of spindle fibres in all directions. Part of the spindle fibres towards the dividing plane attaches to the centromeres of the chromosomes and stops their growth, the remaining spindle fibres growth over the edge of the dividing plane towards the opposite side where they encounter the spindle fibres growing in the opposite direction and repel one another. This separates the compartments from one another and the parts of the cells are evenly divided to both daughter cells, including the chromosomes. During the interphase, the centrioles duplicate once again.

Sample material. Serum or plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. The indirect immunofluorescence test (IIFT, immunofluorescence, indirect) with human epithelial cells (HEp-2) is suitable for detecting autoantibodies against centrioles (Fig. 1).
The starting dilution is 1:100; immunoglobulin classes IgA, IgG and IgM are analysed with a trivalent serum.
In the case of a typical positive finding, the centrioles are visible in the cytoplasm of the cells with either one or two centrioles per cell. In mitotic cells, the centrioles are positioned at two opposite poles.
Various isolated or recombinant centriole proteins can be used to establish enzyme-linked immunosorbent assays or blot techniques (immunoblot), which report a higher sensitivity than is achieved by indirect immunofluorescence on HEp-2 cells.

Indication. The analysis is normally not specifically requested; the antibodies are often only detected by accident.

Interpretation. High titers indicate a progressive systemic sclerosis or Raynaud's syndrome. Only titers over 1:1,000 are classed as potentially relevant to a disease, as 5% of healthy blood donors also provide a positive reaction up to a dilution of 1:320. Including low titers, the prevalence of antibodies against centrioles amounts to 43% with progressive systemic sclerosis.

Diagnostic value. The antibodies have a high sensitivity for progressive systemic sclerosis, but only a low specificity; see also autoantibodies against mitosis-associated antigens.

Literature.
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Legend

Autoantibodies against centrioles/centrosomes. Fig. 1. Substrate: HEp-2 cells

Autoantibodies against centromeres

W. Stöcker

Synonym(s). Centromere antibodies; anti-centromere antibodies; ACA; CENP antibodies

Definition. The target antigens of autoantibodies against centromeres are the 4 different proteins of the kinetochore centromere protein-A (17 kDa), -B (80 kDa), -C (140 kDa) and -D (50 kDa) (CENP-A, -B, -C, -D). The main antigen is the centromere protein-B, which is detected by all sera with centromere antibodies.

Function and pathophysiology. Directly before a cell division, every chromosome consists of two genetically identical halves, the chromatids, which are connected to one another in the region of the centromere. Every centromere contains a kinetochore, to which the spindle fibres attach during mitosis and pull the chromatids to the respective centriole. The centromeres are the target of autoimmune reactions with progressive systemic sclerosis.

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Antibodies against centromeres can already be detected before the onset of progressive scleroderma. In the indirect immunofluorescence test (IIFT, immunofluorescence, indirect), a very specific fluorescence pattern is displayed on HEp-2 cells, which is characterised by fine, evenly-sized granules (generally 46 or 92 centromeres per nucleus) (Fig. 1). The granules of the interphase cells are evenly distributed over the nucleus, in mitotic cells they form a band in the median plane (metaphase) or arranged in two parallel bands in the vicinity of the centrioles (anaphase) depending on the stage. On tissue sections of primate liver, 10-20 granules distributed over the nucleus are displayed, which have a much weaker fluorescence compared to the picture with HEp-2 cells, and which can easily be overlooked (Fig. 2). Mitotic cells are only rarely identified on the liver. The starting dilution is 1:100.
Detection of the antibodies against centromeres with a monospecific test system (enzyme-linked immunosorbent assay, immunoblot) is recommended for various superimposing fluorescence patterns as well as for confirmation.

Reference range — Adults. Negative

Reference range — Children. Negative

Diagnostic value. With a high specificity and a prevalence of 80-95%, antibodies against centromeres are pathognomonic for the limited form of progressive systemic sclerosis. In the limited form, the acra are preferred and the internal organs are only slightly affected. This includes the variants currently referred to as the CREST syndrome: calcinosis cutis, Raynaud phenomenon, oesophageal dysmotility, sclerodactyly, and telangiectasia. In addition, antibodies against centromeres occur with a prevalence of 20-30% in primary biliary cholangitis (PBC, chronic nonsuppurative destructive cholangitis, formerly: primary biliary cirrhosis).

Literature.

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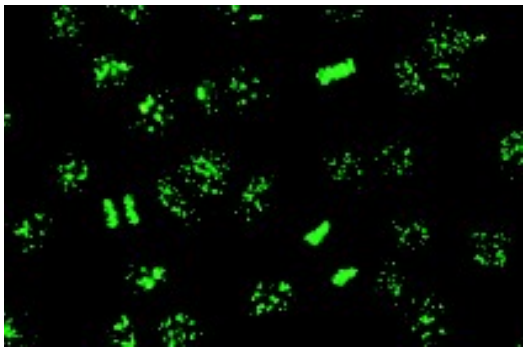
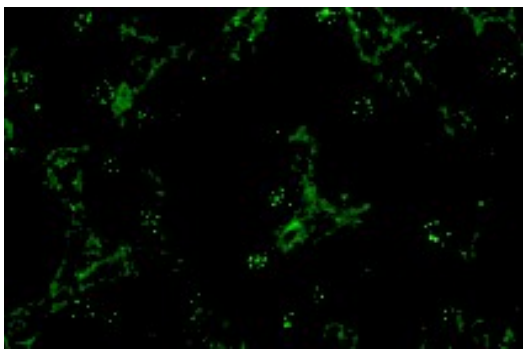


Autoantibodies against centromeres. Fig. 1. Substrate: HEp-2 cells



Autoantibodies against centromeres. Fig. 2. Substrate: primate liver

Autoantibodies against zinc transporter ZnT8

W. Stöcker, Chr. Krüger

Synonym(s). Autoantibodies against ZnT8

Definition. The pancreas-specific zinc transporter ZnT8 is primarily expressed in the β cells of the Langerhans islets where it mediates the transport of cytoplasmic zinc in intracellular vesicles, which are required for the maturation and storage of insulin.
The detection of autoantibodies against zinc transporter ZnT8 is used to diagnose insulin-dependent diabetes mellitus (IDDM).

Function and pathophysiology. During an immune reaction, this quickly results in the formation of autoantibodies against various islet cell antigens, whose detection has become highly significant for diagnosing type I diabetes and its prediction in first-degree relatives of diabetics: Autoantibodies against glutamic acid decarboxylase (GAD), autoantibodies against tyrosine phosphatase (insulinoma associated antigen IA2; autoantibodies against insulinoma-associated antigen 2), other cytoplasmic islet cell components (autoantibodies against pancreatic islets) and autoantibodies against insulin. One or more of these autoantibodies against GAD (GADA), IA2 (IA2A), cytoplasmic islet cell antigens (ICA) and insulin (IAA) can be detected in almost all patients at the time of diagnosis of type I diabetes.
In 2007 it was able to be shown that the majority of freshly diagnosed type I diabetics also displayed autoantibodies against zinc transporter ZnT8 (ZnT8A), which was characterised as a useful and independent marker for an autoimmune reaction. These antibodies expand the spectrum of autoimmune diagnostics for type I diabetes, as they can be detected in addition to the previously analysed autoantibodies or even alone in many type I diabetics. An initial epitope mapping of the autoantibodies against ZnT8 that occur in diabetics showed that 70% of these antibodies are directed against the C-terminus and 10% against the N-terminal end of the ZnT8 protein. Three classes of conformation epitopes were detected within the C-terminus, which are due to the exchange of an individual amino acid (Arg325, Trp325, Gln325). The prevalence of anti-ZnT8 antibodies correlates with the age at the time of manifestation of the type I diabetes.

Sample material. Serum

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Enzyme-linked immunosorbent assays are available to detect autoantibodies against ZnT8.

Diagnostic value. Autoantibodies against ZnT8 are found in 60-80% of newly diagnosed type I diabetics and in 26% of sera of type I diabetics previously classified as “autoantibody (GADA, IA2A, IAA, ICA)-negative”. The detection of autoantibodies against ZnT8 improves the predictability of type I diabetes: In the case of a combined detection of autoantibodies against GAD, IA2, insulin and ZnT8, one or more of these autoantibodies were positive in 98% of cases at the time of manifestation of the type I diabetes.
In most cases, the antibodies are already positive before the manifestation of the disease and are therefore considered a marker in the prediabetic phase. A combination of all of the diabetes mellitus-related autoantibodies (GADA, IA2A, IAA, ICA, ZnT8) should be tested in order to accurately assess a possible risk of diabetes in the individual case. If one of the parameters is positive, appropriate measures can be taken to prevent the onset of diabetes mellitus: immunosuppression or a diabetic diet over several years (unoccupied pancreatic islets expose fewer autoantigens); see also autoantibodies against pancreatic islets.

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Autoimmune liver disease-associated autoantibodies

W. Stöcker

Definition. Autoimmune diseases of the liver include autoimmune hepatitis (AIH), primary biliary cholangitis (PBC, formerly: primary biliary cirrhosis) and primary sclerosing cholangitis (PSC).

Function and pathophysiology. In Western Europe, the incidence of autoimmune hepatitis (AIH, former names: lupoid hepatitis, chronic-active hepatitis) amounts to 1.9 cases per 100,000 inhabitants per year. Left untreated, autoimmune hepatitis soon transitions to cirrhosis of the liver. However, prompt and consistent low-dose immunosuppressive therapy until the end of the patient's life gives a patient a normal life expectancy. This is the benefit of a good serological diagnosis and the specific value of detecting the associated autoantibodies. Discriminatory testing must take place to rule out an infection with hepatitis viruses by investigating the corresponding serological parameters.
Some authors classify autoimmune hepatitis according to their autoantibody status: Subtype I (autoantibodies against cell nuclei,autoantibodies against SM), subtype II (autoantibodies against LKM) and subtype III (antibodies against SLA/LP; autoantibodies against SLA). This classification is probably of neither clinical nor therapeutic and prognostic importance.
Autoantibodies against SLA provide the highest diagnostic accuracy for autoimmune hepatitis of all antibodies. Anti-SLA/LP occur with AIH alone or together with other autoantibodies. However, their prevalence only lies between 10 and 30%, but the predictive value is almost 100%: But, every positive finding essentially provides evidence of autoimmune hepatitis (if the corresponding clinical symptoms are present). The serological detection of autoantibodies against SLA/LP therefore enables a precise differentiation from viral hepatitis in many patients with AIH. This enriches the autoimmune serology of hepatitis by a parameter, whose importance must be assessed as higher than that of the other antibodies. A positive anti-SLA result has significant consequences for the hepatologic treatment: The incorrect treatment of AIH with interferon would also have fatal consequences, similar to an immunosuppressive treatment of the viral infection.
Besides antibodies against SLA, the following are also associated with AIH: Autoantibodies against cell nuclei (ANA), autoantibodies against double-stranded DNA, autoantibodies against smooth muscles (SMA), with the key target antigen of F-actin; autoantibodies against F-actin, liver-kidney microsomes (LKM, target antigen: cytochrome P450 IID6), cytosolic liver antigen type 1 (autoantibodies against LC-1, target antigen: formiminotransferase cyclodeaminase) and granulocytes (pANCA; autoantibodies against granulocyte cytoplasm).Autoantibodies against cell nuclei (ANA) and against smooth muscles (SMA) are common with AIH; but they also occur in 10-20% of patients with chronic viral hepatitis and with other diseases. Autoantibodies against LKM can only be detected in about 1% of adult AIH patients, while it is more common in children. But, antibodies against LKM are also found in 1-2% of patients with chronic hepatitis C serology.
The detection of autoantibodies against mitochondria (AMA) is highly significant for the diagnosis of primary biliary cholangitis (PBC). PBC is an immune-mediated, chronic inflammatory, cholestatic liver disease with an unknown cause. It primarily occurs in women (> 90%) aged between 40 and 60. The global incidence of PBC amounts to approximately 4-31 cases/million per year. PBC is characterised by an infiltration of the intrahepatic bile ducts (canaliculi biliferi) by lymph cells and an accumulation of bile (cholestasis). Although PBC is currently incurable, some symptoms can be soothed by treatment with ursodeoxycholic acid and cholestyramine (to bind the bile) so that most patients can lead a normal life with an average life expectancy. Ursodeoxycholic acid is an economical substance with minimal side effects, which soothes cholestasis and improves the liver function. Cholestyramine absorbs the bile acid in the intestine and therefore reduces the itching caused by the bile acid in the bloodstream.
The diagnosis of PBC involves the detection of autoantibodies against mitochondria and cell nuclei as well as the differentiation from other chronic-inflammatory liver diseases, such as chronic viral hepatitis, autoimmune hepatitis and primary sclerosing cholangitis.
Besides AMA, PBC-associated antinuclear autoantibodies can be detected in a third of PBC patients using indirect immunofluorescence (immunofluorescence, indirect). Specific ANA target antigens with PBC are Sp100 and promyelocytic leukaemia proteins (PML proteins), which display a nuclear dot pattern in indirect immunofluorescence, as well as two components of the nuclear pore complex (CP201 and p62), which are specifically associated with a nuclear membrane pattern (PBC-associated antinuclear autoantibodies).
About 10-20% of patients with PBC develop a secondary autoimmune hepatitis (overlap syndrome). In these cases, autoantibodies similar to those identified with AIH can often be detected. Here, antibodies against SLA/LP are an indication of immunosuppressive therapy.
The incidence of primary sclerosing cholangitis (PSC) is defined as 4 cases of the disease per 100,000 inhabitants per year. Men are primarily affected and half of patients also suffer from ulcerative colitis (by contrast, the prevalence of PSC with ulcerative colitis is 4%). Most patients with PSC display autoantibodies against granulocytes (pANCA) in serological tests, while the main target antigen is DNA-bound lactoferrin. In 2017, IgA against the pancreatic antigen glycoprotein 2 (anti-GP 2) was also identified as a possible new marker for PSC with a severe course of the disease and an associated cholangiocarcinoma.

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Autoantibodies against single-stranded DNA. Tab. 1. Prevalence

of autoantibodies against single-stranded DNA with selected diseases

Disease	Prevalence
Systemic lupus erythematosus	70–95
Mixed connective tissue disease	20–50
Sjögren's syndrome	13
Polymyositis/Dermatomyositis	40–50
Rheumatoid arthritis	8
Healthy blood donor	5–10

Lupus anticoagulant

W. Stöcker, W. Schlumberger

Synonym(s). Anti-prothrombinase

Definition. Lupus anticoagulant consists of antibodies in various immunoglobulin classes (IgG, IgM, IgA), which bind to phospholipid protein complexes and play a large role in coagulation.

Function and pathophysiology. Lupus anticoagulant only includes antibodies that delay the phospholipid-dependent conversion of prothrombin to thrombin. This means that they can lead to an extended, in particular, partial thromboplastin time (PTT) as well as thromboplastin time (TPT) in vitro. However, from a clinical perspective, they rarely result in a tendency to bleed; rather arterial or venous thrombosis can be identified if these antibodies are present. If the placenta is the site of the thrombosis, this leads to recurrent abortions.

Analytics. Lupus anticoagulant is detected using coagulation tests. The screening test is the detection of phospholipid-dependent PTT (partial thromboplastin time). A confirmation test (DRVVT, Dilute Russell Viper Venom Test) is carried out if the screening test is positive.
The PTT includes the endogenous activation of the coagulation system as well as the final common path. Extension by a lack of factors I, II, V, VIII, IX, X, XI, XII, XIV and XV.
In the DRVVT, Russel viper venom leads to the direct activation of FX. If the phospholipid concentration of the test solution is reduced, coagulation does not occur in the presence of lupus anticoagulant.
Lupus anticoagulant must be confirmed via appropriate follow-up analyses, while a transient lupus anticoagulant must be ruled out by follow-up checks.

Sample material/sampling conditions. Citrate plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for deep-freeze preservation of IgM.

Diagnostic value. Lupus anticoagulant is detected for the serological diagnosis of the antiphospholipid syndrome (APS). A lupus anticoagulant diagnosis is always required to clarify the cause of thrombosis, a clinically inexplicable thrombocytopenia or repeated abortions. See also autoantibodies against phospholipids.

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Myositis-specific autoantibodies

W. Stöcker, W. Schlumberger

Synonym(s). Myositis-associated autoantibodies

Definition. Autoimmune myositis (idiopathic inflammatory myopathy) refers to a group of systemic autoimmune diseases with inflammation of the skeletal muscles and is associated with various serologically identifiable autoantibodies (Fig. 1).

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. Various techniques are used to detect myositis-associated autoantibodies in serum or plasma: Indirect immunofluorescence (immunofluorescence, indirect) with tissue sections or cell culture substrates, enzyme immunoassay, immunoblot and others.
These primarily investigate autoantibodies in immunoglobulin class IgG, the most important known target antigens are: PM-Scl-75, PM-Scl-100, Ku, Mi-2, SRP, HMGCR, Jo-1, PL-7, PL-12, OJ, EJ, TF1gamma, MDA5, NXP2, SAE, cN-1A.

Reference range. Negative

Interpretation. Autoantibodies against PM Scl (PM-1) are directed against several proteins of the nucleolar PM Scl macromolecular complex. The two main antigen-protein components are PM Scl-75 and PM Scl-100, which are distinguished by their molecular weights. The antibodies are detected in up to 15% of patients with idiopathic myositis, most frequently in patients with overlap syndrome. This combines the symptoms of polymyositis, dermatomyositis and progressive systemic sclerosis. Patients with systemic sclerosis alone primarily display antibodies against PM Scl-75, while in patients with the clinical picture of the overlap syndrome, the autoantibodies are directed against PM Scl-75 and PM Scl-100. In tests, which exclusively detect anti-PM Scl-100, some patients with systemic sclerosis remain unidentified.
Autoantibodies against Ku often occur with overlap syndrome of poly-dermatomyositis and systemic sclerosis (prevalence of 25-50%), often in association with primary pulmonary hypertension. They are also detected with myositis, systemic sclerosis and systemic lupus erythematosus.
7Autoantibodies against Mi-2 are observed in about 5-30% of patients with idiopathic myopathy and are considered specific for dermatomyositis, often with nail fold hypertrophy.
Autoantibodies against SRP occur with polymyositis and dermatomyositis, in approx. 5% of cases. They are also markers for necrotising myopathy. Their symptoms are acute, severe, proximal, symmetric weakness of the skeletal muscles, muscular pains, occasionally also including the heart muscle. Extramuscular signs of the disease may be interstitial pulmonary diseases. Other putative markers of necrotising myopathy include antibodies against HMGCR, which occur with a prevalence of about 6%. The suspicion that anti-HMGCR were specifically associated with statin-induced myositis can apparently not be confirmed.
Anti-Jo-1 antibodies are found with polymyositis with a prevalence of 25-35%. They are often associated with the simultaneous existence of other autoimmune diseases, such as systemic lupus erythematosus, systemic sclerosis, interstitial pulmonary fibrosis, Raynaud's syndrome and polysynovitis.
Anti-PL-7 antibodies occur with a prevalence of approx. 3-6% with myositis, in some cases overlapping with systemic lupus erythematosus, systemic sclerosis or interstitial pulmonary fibrosis.
Anti-PL-12 antibodies are detected with a prevalence of up to 3% with myositis.
Anti-OJ antibodies are associated with myositis (prevalence of 3%) and interstitial pulmonary fibrosis (prevalence of 3%). In addition, anti-OJ are also found with Raynaud's syndrome and with overlap syndrome with rheumatoid arthritis. The main symptoms are myasthenia, in some cases in connection with polyarthritis.
Anti-EJ antibodies are diagnostic markers for polymyositis. They can also be detected with interstitial pulmonary fibrosis, with overlap syndrome with systemic lupus erythematosus, arthritis and Raynaud's syndrome.
Anti-TIF1γ antibodies are primarily associated with dermatomyositis (prevalence of approx. 20-30%), while a tumour disease often exists in parallel.
Anti-MDA5 antibodies can be found in patients with dermatomyositis with an average prevalence of about 20% (more commonly in Asian populations) and are characterised by severe skin changes and pulmonary involvement.
Anti-NXP2 antibodies occur in up to 25% of juvenile dermatomyositis as well as in about 5-10% of adult dermatomyositis and polymyositis; tumour associations are possible.
Anti-SAE antibodies are found with a syndrome that starts with severe skin changes, with myasthenia occurring in the further course of the disease; the prevalence amounts to 6-8%.
Antibodies against cN-1A (autoantibodies against cN-1A (Mup44)) are particularly associated with sporadic inclusion body myositis (sIBM), where they occur with a prevalence of 30-40%.
Anti-Ro-52 antibodies occur with a prevalence of approx. 30% with myositis. However, they are not disease-specific and also occur with some rheumatic and non-rheumatic diseases, such as with neonatal lupus erythematosus with a congenital heart block.

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Myositis-specific autoantibodies. Fig. 1. Clinical manifestations of autoimmune myositis and associated autoantigens.

PBC-associated antinuclear autoantibodies

W. Stöcker

Synonym(s). PBC-associated ANA; PBCNA; PBC associated anti-nuclear antibodies; in the broader sense also: autoantibodies to the nuclear pore complex, SS-A and centromeres

Definition. Primary biliary cholangitis-associated antinuclear antibodies, not to be confused with autoantibodies against PCNA ("proliferating cell's nuclear antigen"). Various autoantibodies, some with pathognomonic significance, can be found in the serum of patients with primary biliary cholangitis (PBC, chronic non-suppurative destructive cholangitis, previously: primary biliary cirrhosis). This primarily includes 7autoantibodies against mitochondria, but a range of different nuclear antigens are also the target of autoaggression with PBC:

- [5] Nuclear dots, PML-NB (promyelocytic leukaemia nuclear bodies), PML nuclear dots and nuclear domain 10 (ND10). This relates to high molecular, nuclear matrix-bound multiprotein complexes consisting of at least four autoantigenic components – the proteins Sp100 ("speckled protein", 100 kDa), PML (48-97 kDa), SUMO-1 and SUMO-2 ("small ubiquitin-related modifiers", each ~11 kDa). The antigens Sp100 and PML (autoantibodies against PML) were first found in the tumour cells of patients with acute promyelocytic leukaemia. They occur in different isoforms (splice variants), both protein families are present on the SUMO proteins, in some cases with covalent bonds.
- [5] Antigens of the nuclear membrane: These include glycoprotein 210 (GP 210, autoantibodies against glycoprotein 210), p62 and lamin receptors (autoantibodies against lamin-B receptors).
- [5] In addition, autoantibodies against SS-A and autoantibodies against centromeres also belong to the PBCNA in the broader sense.

Function and pathophysiology. Sp100 and PML play a role in proapoptotic signal transmission. They modulate the activities of transcription factors, while they themselves are regulated by interferons. SUMO-1 and -2 are ubiquitin-related modifiers, which establish a covalent bond with proteins to prevent their decomposition (in contrast to ubiquitins). GP 210 and p62 are integral components of the nuclear pore complex.

Sample material. Serum, plasma

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Analytics. In immunofluorescence (immunofluorescence, indirect), five to twenty splinter-like granules of different sizes, which are distributed laterally across the nuclei ("nuclear dots"), are displayed if autoantibodies against Sp100, ND10, SUMO-1, SUMO-2 and PML are present in the nuclei of the interphase with HEp-2 cells (Fig. 1). The cytoplasm is dark, unless antibodies against mitochondria, which are also associated with PBC, are also present. The nuclear dots were previously wrongly considered to be mitochondria-containing chips of the cytoplasm. The dots are dissolved during mitosis, while only isolated granules fluoresce outside the (non-reacting) chromosomes.
Autoantibodies against PML nuclear dots react with primate liver at least as strongly as with HEp-2 cells (Fig. 2). They can also be identified in the case of the parallel use of both substrates, if autoantibodies against centromeres are present at the same time, which is often the case with PBC: In this case, the nuclear dots of the HEp-2 cells are no longer conspicuous, but they can be seen in the nuclei of the hepatocytes, where the fluorescence of antibodies against centromeres is 10 times weaker. Antibodies against Sp100 can usually not be detected, or with an insufficient sensitivity, with rat tissue, while human autoantibodies against PML, SUMO-1 and SUMO-2 also react with rat tissue in some cases.
The serum is initially used in dilutions of 1:100 and 1:1,000 simultaneously because the antibodies (especially against centromeres) often only become visible at a higher dilution. Antibodies in immunoglobulin class IgG are primarily analysed.
Sp100 and PML are precisely colocalised: enzyme-linked immunosorbent assays or various blot techniques (immunoblot) must be used to distinguish between the corresponding antibodies, using, if necessary, recombinant Sp100 antigens extracted from cell culture or relevant subsections.

Reference range — Adults. Negative

Reference range — Children. Negative

Indication. Suspicion of primary biliary cholangitis

Diagnostic value. Autoantibodies against nuclear dots (see also autoantibodies against cell nuclei) are found in 25-30% of patients with primary biliary cholangitis. Antibodies against PML and Sp100 often occur together, but can also be present independently (Tab. 1). Antibodies against SUMO-1 occur in 15% of (anti-nuclear dot-positive) PBC patients, while antibodies against SUMO-2 occur in 42% of (anti-nuclear dot-positive) PBC patients, always only together with anti-Sp100 and/or anti-PML.
Autoantibodies against glycoprotein 210 occur in 20-30% of patients with PBC; they indicate a severe course of the disease, similar to autoantibodies against SS-A. If autoantibodies against centromeres are also present with PCB, partial hypertension often exists. Anti-GP 210 antibodies are occasionally also observed in autoimmune hepatitis or hepatitis B and C. The joint detection of autoantibodies against PML, Sp100, autoantibodies against glycoprotein 210, AMA-M2 and M2-3E increases the diagnostic sensitivity for PBC from 75% (AMA, analysed on rat kidney) to over 95%. Tab. 1 provides an overview of the autoantibodies associated with primary biliary cholangitis.

PBC-associated antinuclear autoantibodies. Tab. 1. Autoantibodies with primary biliary cholangitis

Antigen	Prevalence
Sp100	20
PML	13
Sp100 alone	5
PML alone	3
SUMO-1	15
SUMO-2	42
GP 210	20–30
p62	23–32
Lamin-B receptors	1–3
AMA M2 – rat kidney	75
AMA M2 – whole antigen (BPO)	90–98
SS-A	20
Centromeres	20-30
Ro-52	27

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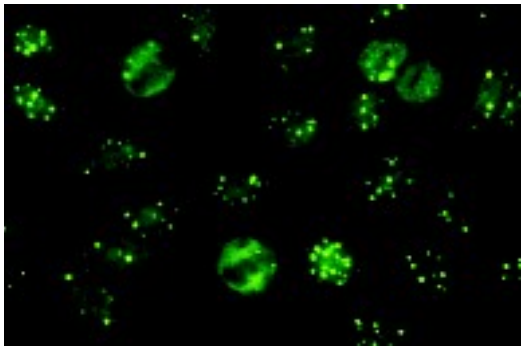
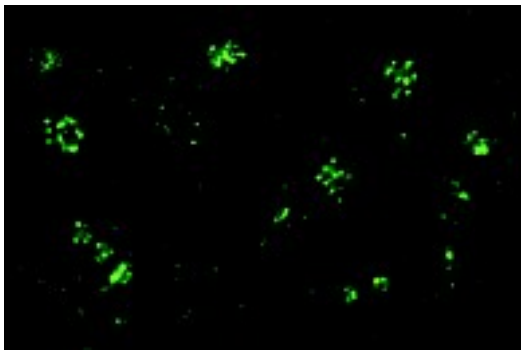


PBC-associated antinuclear autoantibodies. **Fig. 1.** Autoantibodies against mitochondria for primary biliary cholangitis. Substrate: HEp-2 cells



PBC-associated antinuclear autoantibodies. **Fig. 2**. Autoantibodies against mitochondria for primary biliary cholangitis. Substrate: primate liver

Rheumatoid factors

W. Stöcker, W. Schlumberger

Synonym(s). RF

English term. rheumatoid factor(s)

Definition. Rheumatoid factors are antibodies that react with the patient's endogenous immunoglobulins.

Function and pathophysiology. Class G (IgG) immunoglobulins become antigens themselves by conformational changes, e.g. as a result of abnormal glycosylation. The epitope most often detected by the RF are located in the region of the CH2 and CH3 domains of the Fc fragment of IgG. Other RF react with Fab fragments or immunoglobulins, which have been partially digested with pepsin. The RF can belong to the classes IgM, IgG, IgA or IgE. IgM RF are most commonly found in patients with rheumatoid arthritis (RA). They are primarily formed by plasma cells of the synovial membrane, so they can be found earlier and in a higher concentration in the synovial fluid than in the serum. The RF are not pathognomonic for RA, they also occur with other inflammatory connective tissue diseases, such as with systemic lupus erythematosus (SLE) and Sjögren's syndrome as well as with some infectious diseases, such as rubella, leprosy and malaria or also tumours.

Analytics. RF can be detected by enzyme immunoassays (enzyme-linked immunosorbent assays [ELISA], chemiluminescence immunoassays [ChLIA]), nephelometry or agglutination test with sheep erythrocytes or latex particles. ELISA and ChLIA can be used to distinguish between RF in classes IgM, IgA and IgG.

Sample material. Serum

Sample stability. Autoantibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for deep-freeze preservation of IgM.

Diagnostic value. The detection of RF is significant for diagnosing RA, where the IgM isotypes are predominantly detected. Although RF are not limited to RA, their presence in a patient supports the diagnosis of RA, especially with a high titer. RF are generally detected together with antibodies against CCP (autoantibodies against citrullinated peptides), which are almost exclusively found with RA. Both parameters can therefore complement one another. Compared to antibodies against CCP, rheumatoid factors have the same sensitivity (80%), but a lower specificity (62% vs. 97%) for RA. Moreover, a positive RF finding indicates a poor prognosis with joint destruction.

Literature.

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