Notes for the Use of this Book

This reference book on the serological diagnosis of systemic autoimmune diseases is divided into two main sections. The autoantibodies observed in autoimmune diseases are described in alphabetical order in Part 1, and autoimmune disorders as well as symptoms that indicate the possible presence of an autoimmune disease are listed in Part 2. Guide marks (the symbol "➢") were inserted to ensure fast and easy cross-reference between symptoms, a given autoimmune disease and associated autoantibodies. Bibliographic references were omitted due to the broad scope of the subject matter. Only the first authors of historical or some important recent publications have been named.

With some exceptions (e. g., antinuclear antibodies) the prefix "anti-"was omitted for better clarity of alphabetization. Anticentromere antibodies, for example, are listed as "centromere antibodies". In as far as they were known to the authors, synonyms or alternative names for the antibodies were also listed. Obsolete terminology is indicated as such, and the names preferred by the authors are used in the alphabetical index.

The autoantibody description section begins, in some cases, with a brief introduction or historical account. This is followed by information on the target structures (autoantigens), detection methods, clinical relevance, and indications for testing of the autoantibody.

The authors' rating of the clinical relevance of each autoantibody listed in the book is indicated using variable coloring and lettering.

White Letters on Green Background

These are autoantibodies of high diagnostic relevance (markers for diagnosis, prognosis or monitoring) that can usually be determined in all laboratories.

Black Letters on Medium Green Background

These are diagnostically relevant autoantibodies that are not measurable in all laboratories and therefore are determined in specialized laboratories only.

Black Letters on Light Green Background

The clinical relevance of these antibodies is (still) unclear due to their very low frequency of detection, discrepancies between the findings of different studies (lack of comparability due to differences in study design, methodology, ethnic differences, etc.), methodological problems, or preliminary nature of study findings.

Black Letters on White Background

These autoantibodies are currently not clinically relevant, are no longer clinically relevant, or are clinically relevant only in isolated cases. This can also include disease-specific autoantibodies if their testing does not provide any added diagnostic advantages over other parameters.

Autoantibodies – Definitions and Characteristics

Autoantibodies are a very heterogeneous group of antibodies with respect to their specificity, induction, effects, and clinical significance.

Autoantibodies
Definition and Characteristics

- Autoantibodies are immunoglobulins (= antibodies) directed against endogenous antigens (= autoantigens), such as:
  · Proteins (e. g., intracellular enzymes, receptors, structural proteins)
  · Glycoproteins (e. g., beta-2 glycoprotein I)
  · Nucleic acids (e. g., DNA, RNA)
  · Phospholipids (e. g., cardiolipin)
  · Glycosphingolipids (e. g., gangliosides)
- Autoantibodies are detectable in serum and in certain other body fluids (e. g., synovial fluid, cerebrospinal fluid). Depending on the type of target structure recognized, they may also be bound in tissue (e. g., autoantibodies at autoimmune blistering diseases).
- Autoantibodies may be induced by different mechanisms (= non-natural or pathological autoantibodies) or may occur without such induction as part of the natural repertoire (= natural autoantibodies). Natural autoantibodies play a more or less physiological role (e. g., first line of defense against infections, immunoregulation), whereas non-natural autoantibodies can exert pathogenetic effects (e. g., block or stimulate receptors).
- A number of non-natural autoantibodies induced specifically or predominantly in certain diseases are of great clinical relevance regardless of whether they are pathogenetic or not.

Autoantibodies in the Diagnosis of Autoimmune Diseases

The prevalence of autoimmune diseases in the population (3 - 5 %) underlies the importance of autoantibody diagnostics in the public health sector. Autoimmune thyroid diseases are currently considered to be the most common autoimmune diseases, followed by rheumatoid arthritis, antiphospholipid syndrome and autoimmune liver diseases. Nonetheless, the prevalence of most autoimmune diseases defined so far is low. This reference book deals with systemic autoimmune diseases and the autoantibodies associated with them (non-organ-specific and neutrophil-specific autoantibodies). The autoantibodies relevant for diagnosis of organ-specific autoimmune diseases are covered in another volume. The systemic autoimmune diseases include chronic inflammatory rheumatic diseases (connective tissue diseases, rheumatoid arthritis, systemic vasculitides) and antiphospholipid syndrome. Organ-specific autoimmune diseases which may develop systemic manifestations will not be covered in this volume.

Methodological Considerations when Testing for Autoantibodies

The diagnostically relevant autoantibodies are heterogeneous with respect to their fine specificities, i. e., they recognize a variety of epitopes. As a result, different assays often detect different subpopulations of autoantibodies. The test results for a particular autoantibody specificity can therefore vary from one method to another. This has the following consequences in practice:

1. The possibility of false-positive or false-negative test results can never be reliably ruled out if only one detection method is used to determine the presence of a given autoantibody specificity. Combined screening for antinuclear antibodies by way of indirect immunofluorescence (IIF) followed by differentiation of autoantibody specificity via enzyme immunoassay (EIA) and other novel technologies has proven to be an effective approach to serological diagnosis of connective tissue diseases. Control tests should be performed whenever negative nuclear fluorescence is found in combination with a positive result for a given ANA specificity (e. g., ➢ dsDNA, ➢ Sm, or ➢ U1-RNP antibodies) or whenever

negative cytoplasmic fluorescence is found in combination with a positive result for a given anticytoplasmic antibody specificity (e. g., ➢ Jo-1 antibody). When this constellation (IIF negative, EIA positive) is found, the diagnostic value of the EIA result is limited, even when attempting to exclude false-positive results (see also point 3).

2. There are now a number of different assay platforms available to detect autoantibodies. The time-honored indirect immunofluorescence test continues to be widely used as a screening tool. Assays that allow rapid and specific identification of autoantibodies include enzyme immunoassays (EIA), line immunoassays (LIA), chemiluminescence immunoassays (CIA), addressable laser bead immunoassays (ALBIA) and other microbead based assays.
3. The diagnostic value of an autoantibody specificity varies according to the type of detection method used. Higher assay sensitivity usually results in higher diagnostic sensitivity, but often results in a lower specificity of a given autoantibody for the corresponding disease. For example, autoantibodies to various "extractable nuclear antigens" determined by double radial immunodiffusion (Ouchterlony technique) are highly specific for connective tissue diseases though the sensitivity is limited. The use of highly sensitive enzyme immunoassays increases the diagnostic sensitivity, but usually does so at the expense of diagnostic specificity. The optimal sensitivity/specificity ratio of a new assay should therefore be carefully determined before the assay is introduced for routine diagnostics. Such an analysis should also take individual laboratory differences into account (available range of methods or possibilities for cooperation with reference laboratories, experience of the clinical pathologist and degree of cooperation with referring physicians). The limit values recommended or specified by the manufacturers of commercial assays cannot always be used without reservation. Differential grading of reference ranges has proven to be effective (e. g., borderline or weakly positive, positive, strongly positive). This requires close cooperation between the laboratory and the clinician, which can be difficult due to the common practice of transferring the samples to an "industrialized" laboratory.

If only enzyme immunoassays are available for determination of autoantibodies, the following rule of thumb applies: The higher the titer, the more reliable the test result. However, high-titered false-positive results due to reactivities against proteins used to block free binding sites on the microtiter plate must be excluded (β-casein antibodies or other antibodies against milk proteins found in patients with milk intolerance may produce high-titred EIA results if used as blocking agents). Another important consideration is that titers of some autoantibody specificities (e. g., ➢ ANCA and ➢ dsDNA antibodies) can be correlated with disease activity.

All physicians want the results of laboratory tests to be reproducible and comparable with the results of other tests from other laboratories. But the standardization

of the determination of autoantibodies is still in the infancy. A start, and considerable help in respect of standardization, is the production of so-called standard sera and reference sera. Such standard sera are given by definition a certain concentration of international units (IU/ml). In the case that the sera are not suitable for quantitative determinations, the preparation can be accepted by the IUIS and WHO as a reference preparation. Standard sera and reference sera are available at the Center for Disease Control (CDC), USA, the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (CLB), the Netherlands, the National Institute for Biological Standards and Control (NIBSC), UK, and the companies Inova Inc. (USA) and American Diagnostica Inc. (AD), USA. Recommendations for an improved standardization of indirect immunofluorescence as well as enzyme immunoassays are compiled from the Clinical and Laboratory Standards Institute (CLSI) (document I/LA2-A2).

Because the amount of international standards is limited, most of the manufactorers of immunodiagnostic test kits use their own in house standards. These standards should be absolutely calibrated against the international "primary standards". Only in this case the in house standards should be used as "secondary standards". This calibration should be done with each lot of manufacturing immunodiagnostic test kits.

The reference preparations are very helpful to check the sensitivity and specificity of test kits for detection of ANA/ENA-antibodies, and therefore, a necessary tool for manufacturing immunodiagnostic test kits for autoantibody detection and should be used with each lot of manufactory immunodiagnostic test kits.

Clinical Relevance of Autoantibodies

Testing for autoantibodies can be helpful or necessary for the diagnosis, differential diagnosis, prognostication, or monitoring of autoimmune diseases. The diagnostic sensitivity and specificity, the positive and negative predictive values as well as the likelyhood ratios of a given autoantibody for the corresponding disease are important criteria for evaluating its diagnostic relevance for a particular disease. The higher these parameters, the more valuable the antibody is for the corresponding diagnosis. As was already mentioned in the discussion of methodological aspects, these parameters differ when using different tests for detection of a particular autoantibody. The diagnostic sensitivity is the proportion of patients with the disease who are correctly identified by the autoantibody test (quotient of true positive tests and total number of patients with the disease). The diagnostic specificity is the proportion of patients without the disease who are correctly identified by the autoantibody test (quotient of true negative tests and total number of patients/healthy volunteers without the disease). Diagnostic sensitivity and specificity figures provided under the heading "clinical relevance" for many autoantibodies should be regarded as guiding numbers only. These values vary

Table 1. International standards and reference sera.

Autoantibody or IIF staining pattern	Name	Supplier
➢ ACPA	CDC 17	CDC
➢ Antinuclear antibodies (ANA)	WHO 66/233	CLB
➢ ANA homogenous/dsDNA	CDC 1	CDC
➢ ANA speckled	CDC 3	CDC
➢ β2 glycoprotein I (IgG)	HCAL/CDC 13	CDC/INOVA*
➢ β2 glycoprotein I (IgM)	EY2C9/CDC 14	CDC/INOVA
➢ Cardiolipin (IgA)	aCL IgA ("Harris-Standard")	AD
➢ Cardiolipin (IgG)	aCL IgG ("Harris-Standard")	AD
➢ Cardiolipin (IgG)	HCAL/CDC 13	CDC/INOVA
➢ Cardiolipin (IgM)	aCL IgM ("Harris-Standard")	AD
➢ Cardiolipin (IgM)	EY2C9 /CDC 14	CDC/INOVA
➢ Centromere	CDC 8	CDC
➢ dsDNA	WHO-Wo/80	CLB
➢ Jo-1/histidyl-tRNA synthase	CDC 10	CDC
➢ La/SS-B (ANA speckled)	CDC 2	CDC
➢ Myeloperoxidase	CDC 15	CDC
➢ Nucleolar	CDC 6	CDC
➢ PM/Scl	CDC 11	CDC
➢ Proteinase 3	CDC 16	CDC
➢ Rheumatoid Factor (RF)	WHO-64/1	CLB
➢ Ribosomal-P	CDC 12	CDC
➢ Ro60/SS-A	CDC 7	CDC
➢ Topoisomerase I/Scl-70	CDC 9	CDC
➢ Sm (U2-U6 RNP)	CDC 5	CDC
➢ U1-RNP	CDC 4	CDC
➢ U1-RNP	WHO-anRNP	CLB

Abbreviations: AD, American Diagnostica Inc., USA; CDC, Center for Disease Control, USA; CLB, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, The Netherlands; PM/Scl, polymyositis-scleroderma exosomal antigen; Sm, Smith autoantibody; WHO, World Health Organization.
* INOVA: INOVA Diagnostics, San Diego, CA, USA

according to the diagnostic kit or platform (see above) and study design used. In addition, ethnic, geographic and genetic differences in the expression of certain autoimmune responses, which can be quite significant, must also be considered.

Ideally, the diagnostic sensitivity and specificity must both be 100%; however, none of the diagnostically relevant autoantibodies achieve this goal. Some autoantibodies (e. g., ➢ PL-7 antibodies used to diagnose polymyositis) achieve almost 100% specificity but have a very low sensitivity. Others (e. g., ➢ Ro/SS-A antibodies used to diagnose Sjögren's syndrome) have a low specificity but high sensitivity. Autoantibodies involved in the pathogenesis of a given disease (e. g., ➢ proteinase-3 antibodies used to diagnose granulomatosis with polyangiitis (Wegener's granulomatosis)) generally achieve very high values for both of the two diagnostic parameters. Since the titers of these autoantibodies are usually associated with disease activity, their high sensitivity applies "only" during the active phases of disease.

Some autoantibodies have been identified as criteria for classification of certain diseases, e. g., the criteria of the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) for classification of ➢ systemic lupus erythematosus (SLE) or the ➢ rheumatoid arthritis. Autoantibodies that are not highly specific for the respective disease (e. g., ➢ rheumatoid factors for rheumatoid arthritis; ➢ phospholipid antibodies for ➢ antiphospholipid syndrome) may also be defined as classification criteria, but are considered to be of diagnostic importance only when they occur in conjunction with typical symptoms of the disease. Fortunately, novel classification criteria have been quite recently developed that include disease specific autoantibodies (e. g., ➢ centromere, ➢ topoisomerase I and ➢ RNA polymerase III antibodies used to diagnose ➢ systemic sclerosis). In addition to "establishing the diagnosis" in patients with typical symptoms, a number of disease-specific autoantibodies are very important for early diagnosis if there is a monosymptomatic, oligosymptomatic (forme fruste), or atypical presentation of the disease (e. g., ➢ aminoacyl-tRNA synthetase antibodies to diagnose systemic autoimmune disorder in case of ➢ idiopathic interstitial lung disease).

Which course of action should be taken when disease-specific autoantibodies are detected in a patient/volunteer without any typical symptoms? The first step is to make sure that the test was not a false-positive (see "Methodological Considerations when Testing for Autoantibodies"). The reporting reference laboratory should be consulted if necessary. If the results are classified as actual positive binding of the autoantibody to the corresponding autoantigen (= correct positive test), the test should be repeated within 3 to 6 months (the ➢ phospholipid antibody test should be repeated after 12 weeks). If the antibodies have disappeared, the positive result may have been due to polyclonal B cell stimulation in the course of an infection; this may result in positive reactivity to certain autoantibodies (usually low titers) when highly sensitive tests are used. One must also be aware that disease-specific autoantibodies can also occur in conjunction with tumors. When

persistence or even a rise of the autoantibody titer is observed, the clinical and serological course of the disease should be monitored in one or two-year intervals (depending on the expected course of the disease).

Part 1

Autoantibodies in Systemic Autoimmune Diseases

ACPA

Anti-citrullinated protein/peptide antibodies (ACPA) is the collective name for all autoantibodies directed against citrullinated proteins or peptides. See ➢ citrullinated protein/peptide antibodies.

Note: ACPA is also a former term of antineutrophil cytoplasmic antibodies (ANCA) and a term for anticytoplasmic antibodies detected by indirect immunofluorescence on HEp-2 cells.

Alanyl-tRNA ($tRNA^{Ala}$) Synthetase Antibodies

See ➢ aminoacyl-tRNA synthetase antibodies, ➢ PL-12 antibodies.

Alpha-Actinin Antibodies

Cross-reactive ➢ dsDNA antibodies that are associated with lupus nephritis (Renaudineau et al., 2007). The controversial nephritogenic potential of alpha-actinin antibodies is discussed (Kalaaji et al., 2006; Zhao et al., 2006; Manson et al., 2009). The modulation of alpha-actinin expression in/on mesangial cells of the kidney may contribute to the susceptibility for lupus nephritis.

Alpha-Enolase Antibodies

Autoantigen

The ubiquitous glycolytic enzyme α-enolase is 82% homologous with the other two isoforms, β- and γ-enolase, and homologous with tau, a soluble lens protein of the crystalline family.

Detection Methods

Enzyme immunoassay (EIA) or Western blot (WB) using purified α-enolase.

Clinical Relevance

The induction of autoantibodies in various diseases against the ubiquitously occurring α-enolase may be a result of microbial infections or hyperproliferative processes in tissues under certain pathophysiological conditions. A pathogenic effect on endothelial cells via complement activation, inhibition of the binding of plasminogen to α-enolase and induction of apoptosis is discussed.

The occurrence of α-enolase antibodies in systemic autoimmune diseases is summarized (for review see Terrier et al., 2007; α-enolase antibodies in organ specific autoimmune disease: see K.Conrad, W. Schößler, F. Hiepe, M. J. Fritzler: "Autoantibodies in Organ Specific Autoimmune Diseases – A Diagnostic Reference", Pabst Science Publishers, Lengerich 2011):

- Parameter of nephropathy: Detected in 69 % of patients with primary membranous nephropathy and in 58 % of those with the secondary forms, e. g., patients with ➢ systemic lupus erythematosus (SLE) and ➢ rheumatoid arthritis (RA). Detected in 27 % of SLE patients and associated with active renal disease. Alpha-enolase is a target of nephritogenic ➢ dsDNA and non-DNA antibodies (Migliorini et al., 2002). In mixed ➢ cryoglobulinemia, α-enolase antibodies were found in 3 % of patients with nephritis but not in patients without nephritis.
- In patients with ➢ **systemic sclerosis**, α-enolase antibodies were found in up to 30 %. They were associated with diffuse cutaneous scleroderma, pulmonary and vascular involvement (Pratesi et al., 2000).
- Alpha-enolase is a cytoplasmic enzyme of neutrophils and also a minor target of autoantibodies in ➢ **ANCA-associated vasculitides**.
- Alpha-enolase of human dermal microvascular endothelial cells was described as a target of autoantibodies in 37.5% of patients with ➢ **Behçet's disease** (Lee et al., 2003).
- In patients with ➢ **relapsing polychondritis**, α-enolase antibodies were found in 46 % (Tanaka et al., 2006).
- Autoantibodies to alpha-enolase were present in the sera of patients with very early ➢ **rheumatoid arthritis (RA)** and may have prognostic value (Saulot et al., 2002). The binding of those autoantibodies was associated with post-translational modification, e. g., citrullination (Kinloch et al., 2005). Autoantibodies against citrullinated α–enolase were found in 24 – 62 % of RA patients (Lundberg et al., 2008; Fisher et al., 2011). See also ➢ citrullinated protein/peptide antibodies and ➢ CEP-1-antibodies.

Indications

None currently established. It remains to be determined whether testing for these autoantibodies will provide any clinical advantages for diagnosis or prognosis of mixed cryoglobulinemia (renal involvement), retinopathies (paraneoplastic), RA, Hashimoto's encephalopathy or other diseases.

Comments: Alpha-enolase antibodies are a heterogeneous group of autoantibodies that recognize various epitopes of α-enolase proteins (and some of β- and γ-enolase proteins) and perhaps some related proteins as well (e.g., the lens protein tau). Their relationship to ➢ ANCA and ➢ centrosome antibodies (centrosome antibody positive sera detected purified α- and γ-enolase [Rattner et al., 1991; Fritzler et al., 2003]) still needs to be clarified. Further investigations are therefore necessary for differentiation (epitope specificity) and, thus, for improved clinical utility of α-enolase antibodies.

Alpha-Fodrin Antibodies

Autoantigen

The 120 kDa apoptotic cleavage product of alpha-fodrin. Alpha-fodrin is a 240 kDa protein which binds to beta-fodrin or beta-spectrins to form a heterodimer as a part of the eukaryotic cell membrane cytoskeleton. Alpha-fodrin is associated with membrane ion channels and pumps, and appears to be involved in control of secretion from glands.

Detection Methods

WB or EIA using the recombinant antigen or the N-terminal alpha-fodrin peptide RQKLEDSYRFQFFORDAEEL (Shiari et al., 2006; Chen et al., 2007).

Clinical Relevance

- In some studies, alpha-fodrin antibodies were found in patients with primary or secondary ➢ **Sjögren's syndrome** (SjS) and had high sensitivity (> 60 – 93 % depending on classification criteria used) and specificity (93 %) (Witte et al., 2000 and 2003). Other studies, however, showed a lower diagnostic relevance in SjS (Zandbelt et al., 2003; Ruffatti et al., 2004; Sordet et al., 2005; Locht et al., 2008). Different study designs and methodical aspects were suggested for this discrepancy. For example, the antibody titer decreases during immuno-

suppression therapy, explaining lower frequencies in some studies. Furthermore, IgG antibodies in patients with primary SjS are associated with characteristic clinical and immunological features indicative of local (e. g., lymphocytic infiltration of salivary glands) and systemic (e. g., increase of CD71 positive T-helper cells and HLA-DR expressing monocytes) inflammation and thus may serve as markers of disease activity (Willeke et al., 2007). In addition, an increased prevalence of alpha-fodrin antibodies of the IgG isotype in primary SjS patients with shorter disease duration was found suggesting that these antibodies may participate in early pathogenic processes.

- The stringency of criteria used for classification of SjS and disease activity may likely explain the differences in the frequency of alpha-fodrin antibodies between different studies. Using the revised European criteria, the American/European consensus criteria (Vitali et al., 2002) and the highly stringent San Diego criteria (Fox et al., 1986), IgA and/or IgG antibodies against alpha-fodrin were found in 93 % (Witte et al., 2010). IgA antibodies against alpha-fodrin were more prevalent than IgG antibodies (88 % vs 64 %). However, a meta-analysis including 23 studies showed a pooled sensitivity of alpha-fodrin IgG and IgA antibodies of only 28 % and 42 %, respectively (Hu et al., 2013).
- Alpha-fodrin antibodies can be detected in up to 100 % in patients with juvenile onset of SjS, in some cases before ➢ Ro/SS-A or La/SS-B antibody became positive (Kobayashi et al., 2001).
- Alpha-fodrin antibodies were found in high frequency (72 %) in patients with moyamoya disease (Ogawa et al., 2003).
- They are also detectable at higher frequencies in patients with ➢ rheumatoid arthritis (30 – 40 %), ➢ systemic lupus erythematsus (20 %), Graves' ophthalmopathy (31 %), multiple sclerosis (20 %), normal-pressure glaucoma, chronic HCV infection (25%) and Hashimoto thyreoiditis (22 %) (Ulbricht et al., 2003; He et al., 2005; Kahali et al., 2005; Grus et al., 2006 ; Szanto et al., 2008; Potthoff et al., 2009). In conclusion, regarding the specificity for SjS, both IgA and IgG antibodies against alpha-fodrin are present in up to 40 % of patients with active inflammatory diseases, but in only 2 % in blood donors. Therefore, antibodies against alpha-fodrin appear to be helpful in the distinction of SjS from fibromyalgia, the main differential diagnosis in clinical practice, but not from SLE or RA (Witte et al., 2007).

Indications

Suspicion of SjS, if Ro/SS-A and/or La/SS-B antibodies are not detectable. The relevance in early diagnosis and monitoring of SjS should be further evaluated. Interstingly, autoantibodies against alpha-fodrin may indicate early active disease, which is still treatable by immunosuppression. It was shown that hydroxychloroquine treatment of SjS patients significantly improves saliva production only in SjS patients with (but not without) antibodies against alpha-fodrin

(Rihl et al., 2009). Therefore, the presence of these autoantibodies may iindicate a therapeutic „window of opportunity“ in patients with early (or very early) SjS (Witte et al., 2010).

Note: Alpha-fodrin antibodies are not part of the new ACR criteria of the SjS (Shiboski et al., 2012).

Aminoacyl-tRNA Synthetase (ARS) Antibodies

Synonym: Anti-synthetase antibodies.

Collective term for all autoantibodies directed against aminoacyl-tRNA synthetases (ARS). The following ones have been described so far:

- Histidyl-tRNA synthetase antibodies (➢ Jo-1 antibodies),
- Threonyl-tRNA synthetase antibodies (➢ PL-7 antibodies),
- Alanyl-tRNA synthetase antibodies (➢ PL-12 antibodies),
- Isoleucyl-tRNA synthetase antibodies (➢ OJ antibodies),
- Glycyl-tRNA synthetase antibodies (➢ EJ antibodies),
- Lysyl-tRNA synthetase antibodies (➢ SC antibodies),
- Asparaginyl-tRNA synthetase antibodies (➢ KS antibodies),
- Leucyl-tRNA synthetase antibodies,
- Glutaminyl-tRNA synthetase antibodies,
- Phenylalanyl-tRNA synthetase antibodies (Zo antibodies),
- Tyrosyl-tRNA synthetase antibodies (Ha antibodies).

Autoantigens

Aminoacyl-tRNA synthetases. These cytoplasmic enzymes catalyze the binding of a specific amino acid to the corresponding transfer RNA (tRNA) molecule. For instance, the amino acid histidine is bound to $tRNA^{His}$ with the aid of histidyl-tRNA synthetase, leading to the formation of histidyl-tRNA complexes.

Detection Methods

- IIF using HEp-2 cells: some sera with this reactivity produce diffuse or fine granular staining of the cytoplasm.
  Note: The staining pattern is not seen with all sera and when it is seen, it is suggestive of, but not specific for, ARS antibodies.
- Immunodiffusion (Ouchterlony technique) or counter-current immuno-electrophoresis (CIE) using soluble antigens from rabbit thymus or human spleen.
  Note: This technique has a low sensitivity and does not detect all ARS antibodies.

- WB using HeLa or MOLT4 cell extracts or special aminoacyl-tRNA synthetase preparations.
  Note: False-negative results are possible.
- EIA, LIA/DIA, CIA or ALBIA using biochemically purified or recombinant aminoacyl-tRNA synthetases.
- Immunoprecipitation (IP), e. g., radioimmunoprecipitation of tRNA from ^{32}P-labeled cell extracts (HeLa S3 cell lineage).

Comments: Immunodiffusion or CIE, EIA, LIA/DIA, CIA and ALBIA are currently used in routine laboratory testing for determination of ➢ Jo-1, ➢ PL-7, and ➢ PL-12 antibodies. Special myositis-LIAs also allow the determination of EJ and OJ antibodies. However, there is still a strong need for cost-effective and standardized assays for the detection of ARS autoantibodies.

Clinical Relevance

- Anti-ARS autoantibodies are specific markers for the so-called ➢ antisynthetase syndrome (ASS), a subset of the spectrum of the ➢ idiopathic inflammatory myopathies (IIMs). ASS is characterized by multiple organ involvement, primarily ➢ interstitial lung disease (ILD), and is often accompanied by myositis, non-erosive arthritis, Raynaud's phenomenon, "mechanic's hands", skin rashes, sicca syndrome and constitutional symptoms, such as fever. Because ASS lacks diagnostic criteria and can present in different combinations of clinical features, the presence of at least one ARS antibody is required for definite diagnosis. However, diagnosis and management of ASS are still challenging due to often masked and/or non-specific symptoms at the disease onset (Ghirardello et al., 2009).
- Jo-1 antibodies are detectable in 20 – 30 % of patients with IIMs (Selva-O'Callaghan et al., 2006). The were found in 60 – 80 % of ASS patients (Sordet et al., 2006), but also in 10 - 40 % of PM and 2 – 10 % of DM patients (Sordet et al., 2006). PM/DM patients positive for Jo-1 antibodies may have or may develop features of ASS. ARS antibodies other than Jo-1 antibodies are present in less than 5 % of ASS patients (Ghirardello et al., 2011). At least one of the aforementioned ASS antibodies is found in up to 80 % of all patients with myositis and ILD. The ARS antibodies generally do not cross-react or co-exist with others.
- Each ARS antibody seems to be associated with heterogeneous disease expression and severity (Aggarwal et al., 2014), in which lung and joint involvement could be prominent at early disease stages. Patients with ARS antibodies other than Jo-1 antibodies have decreased survival compared to Jo-1 antibody positive patients, probably partly attributable to a delay in diagnosis (Aggarwal et al., 2013).
- The lung manifestation in ARS positive patients may precede myositis (in this clinical subtype ARS antibodies are the most frequently detetable antibodies)

or even may proceed without the development of myopathy (**seropositive amyopathic interstitial lung disease**) (Yoshifuji et al., 2006). ARS autoantibodies have been reported in about 7 % of patients with ➢ idiopathic interstitial pneumonia. Whether such autoantibodies could also have a predictive value for immune mediated ILD has to be further elucidated (Takato et al., 2013).

- Few cases of pulmonary involvement with acute onset and a rapid progression toward respiratory insufficiency (acute respiratory distress syndrome) have been described in ARS antibody positive patients without myositis. It is conceivable that due to the variable clinical forms of IIM, and to the difficulties related to diagnosis, the prevalence of this association is higher than the few reported cases suggest (Bajocchi et al., 2012).
- ARS antibodies other than Jo-1 antibodies can also be associated with necrotizing myopathy or pericarditis (Labirua-Iturburu et al., 2012; Mehndiratta et al., 2012).

Indications

1. Diagnosis/differential diagnosis of myositis.
2. Clinical features indicative for ASS.
3. Interstitial lung disease or acute respiratory failure of unknown origin.

Comments: Even when the conventional tests for Jo-1, PL-7 and PL-12 antibodies are not positive, the detection of immunofluorescence patterns typical of aminoacyl-tRNA synthetase antibodies may still be a valuable diagnostic indicator in patients with suspected idiopathic myositis, especially in those with concomitant ILD. In these cases, the occurrence of other aminoacyl-tRNA synthetase antibodies or KJ antibodies is possible.

Annexin V Antibodies

Autoantigen

Annexin V is a potent Ca^{2+}-dependent anticoagulant protein that is abundant in cells exposed to blood, e. g., platelets, trophoblast (mainly syncytiotrophoblast) and endothelial cells and which activity is based on the high affinity binding to anionic phospholipids and as a result on the inhibition of the phospholipid-dependent coagulation cascade. Annexin V is forming two-dimensional crystals over anionic

phospholipid-containing bilayers, and so a protective shield is created between the coagulation factors which circulate in the blood and the phospholipids. In the presence of antiphospholipid antibodies the annexin V protection shield is disrupted resulting in an increase of the amount of anionic phospholipids available for promoting coagulation cascade (Rand et al., 2010). Annexin V is probably involved in multiple cellular functions, e.g., transmembrane Ca^{2+} channel activity, inhibition of phospholipase A2, regulation of membrane integrity. Annexin V appears to play a thrombomodulatory role in placental circulation where it is necessary for maintenance of placental integrity.

Detection Methods

EIA using purified annexin V immobilized on γ-irradiated polystyrene plates.

Clinical Relevance

- Annexin V antibodies have been detected in patients with ➢ **antiphospholipid syndrome (APS)** and ➢ connective tissue diseases, especially in ➢ systemic lupus erythematosus (SLE), ➢ systemic sclerosis and ➢ rheumatoid arthritis. Patients with annexin V antibodies may have clinical features of APS even in the absence of ➢ phospholipid antibodies (Kaburaki et al., 1997).
- Annexin V antibodies were described especially in SLE-patients as closely related to **fetal loss**. The prevalence and levels of annexin V antibodies were found significantly higher in patients with SLE and fetal loss as well as venous thrombosis than those without these complications. A multivariate logistic regression analysis of risk factors revealed that annexin V antibody may be a significant risk factor for fetal loss in SLE patients: OR 5.9, 95 % CI 1.4–14.8 (Nojima et al., 2001). Other groups confirmed these results (Zammiti et al., 2006; Sater et al., 2011). However, in another prospective study with 1038 healthy pregnant women no association between annexin V antibodies and pregnancy complication was found (Bizzaro et al., 2005). Thus, the association of annexin V antibodies and fetal loss in normal population is still under discussion. On the other hand, this association seems to be sure both concerning the clinical as well as experimental data (disruption of the annexin protection shield by phospholipid antibodies; see above).
- The actions of annexin V antibodies may affect embryo implantation and pregnancy outcome. In pregnant BALB/c mice the infusion of annexin V antibodies leads to the induction of fetal loss, placental thrombosis and tissue necrosis. These autoantibodies can induce endothelial cell and syncytiotrophoblast apoptosis and the inhibition of trophoblast gonadotropin secretion (di Simone et al., 2001).

- Annexin V antibodies of IgG-isotype were detected in 13.9% of patients with cerebral infarction who often had no ➢ cardiolipin antibodies or ➢ lupus anticoagulant (Lee et al., 2011).
- Annexin V antibodies were frequently detected in patients with ➢ **Takayasu-arteriitis.** An association with disease activity and ➢ endothelial cell antibodies was described (Tripathy et al., 2003).

Indications

- The determination may be useful in fetal loss risk assessment in patients with phospholipid antibodies and in patients with connective tissue diseases, especially SLE.
- Recurrent fetal miscarriage of unknown cause.

Antineutrophil Cytoplasmic Antibodies (ANCA)

Collective term for all autoantibodies directed against cytoplasmic antigens of neutrophils. These include:

- ➢ Azurozidin antibodies,
- ➢ BPI antibodies,
- ➢ cANCA (➢ proteinase 3 antibodies),
- ➢ pANCA (➢ myeloperoxidase antibodies).

Autoantibodies against cytoplasmic antigens of human neutrophils were discovered in patients with segmental necrotizing glomerulonephritis and systemic vasculitis in the early 1980's (Davies et al., 1982; Hall et al., 1984). These antibodies initially referred to as anticytoplasmic antibodies (ACPAs) were soon found to be specific for ➢ granulomatosis with polyangiitis (GPA) (van der Woude et al., 1985). Investigators later demonstrated that the antibody titers correlate with disease activity. In addition to the classical ACPA (later named cANCA), a second primary immunofluorescence pattern (pANCA) was found in patients with necrotizing glomerulonephritis and ➢ microscopic polyangiitis (MPA) (Falk et al., 1988). Myeloperoxidase (MPO) was identified as the target antigen of MPA-specific ANCA, and proteinase 3 (PR3) of GPA-specific ANCA (Falk et al., 1988; Jenne et al., 1990).

Autoantigens

Enzymes of the azurophile and specific granules of neutrophil granulocytes, in part also present in monocytes and endothelial cells:

ANCA target antigen	Immunofluorescence pattern on ethanol fixed human neutrophiles
Proteinase 3	cANCA, very rare pANCA
Myeloperoxidase	pANCA, very rare cANCA
Elastase*	pANCA
Cathepsin G	pANCA
Azurozidin	pANCA
Lactoferrin	pANCA
Lysozyme	pANCA
BPI	atypical ANCA or pANCA

*Human neutrophil elastase (HNE)

Ubiquitous proteins such as α-enolase, HMG proteins, ➢ EEA-1 and h-lamp-2 (see ➢ lysosome antibodies) were also identified as target antigens of neutrophil-reacting autoantibodies.

Detection Methods

- IIF using ethanol- (and formalin-)fixed human neutrophils produces different cytoplasmic staining patterns, e.g., granular **c**ytoplasmic (➢ cANCA), **p**erinuclear (➢ pANCA), and **a**typical (aANCA or xANCA).
- EIA, CIA and ALBIA using highly purified or recombinant antigens (➢ myeloperoxidase antibodies, ➢ proteinase 3 antibodies).
- Capture EIA using monoclonal antibodies or PR3 binding proteins.
- Cyto-Bead technology using IIF for simultaneous detection on neutrophils and microbead bound antigens.

Clinical Relevance

- cANCA (specific for proteinase 3) and pANCA (specific for myeloperoxidase) are markers of systemic vasculitides, which are therefore referred to as ➢ ANCA associated vasculitides.
- Apart from these classical ANCA, ➢ neutrophil-specific antibodies (NSA) with perinuclear or nuclear patterns (atypical ANCA; xANCA; GS-ANA) are found

in patients with ➢ rheumatoid arthritis, ➢ Felty's syndrome, chronic inflammatory bowel disease, primary sclerosing cholangitis, and autoimmune hepatitis type 1. These autoantibodies target many different autoantigens except proteinase 3 and myeloperoxidase (see autoantigens) and should be clearly distinguished from cANCA or pANCA.

- ANCA are also found in patients with cystic fibrosis (➢ BPI antibodies).
- Treatment with anti-thyroid drugs (e.g., propylthiouracil, methimazole) may induce ANCA of different specificities (Gao et al., 2004; Slot et al., 2005). An association with systemic vasculitis was observed in the presence of ➢ proteinase 3 or ➢ myeloperoxidase antibodies. Propylthiouracil treated patients with ANCA against multiple antigens might be at increased risk of developing overt clinical vasculitis (Gao et al., 2007).
- Furthermore, associations between ANCA and hydralazine or minocycline therapy, cocaine abuse and HIV infection were described (Jansen et al., 2005 among others).
- ANCA (mainly BPI and elastase antibodies) were significantly more often detected in subjects with homozygous alpha 1-antitrypsin deficiency compared to subjects without this deficiency (Audrain et al., 2001). Fortunately, these ANCA positive persons do not develop systemic vasculitis.

Indications

1. Suspicion of ANCA-associated vasculitis.
2. Differential diagnosis of nephritis.
3. Suspicion of chronic inflammatory bowel disease, especially ulcerative colitis.
4. Suspicion of primary sclerosing cholangitis.
5. Hemorrhagic alveolitis.

Comments: A variety of infectious and noninfectious diseases result in false positive ANCA tests by immunofluorescence. Most of these conditions are associated with negative specific ELISA testing for PR3 or myeloperoxidase antibodies (Harris et al., 1999). Several infectious diseases, particularly infective endocarditis (subacute bacterial endocarditis), have been reported to exhibit positive ANCA tests and to mimic AAV, which may lead to a misdiagnosis and inappropriate treatment (reviewed in Chirinos et al., 2007; Ying et al., 2014). Hence, infective endocarditis is of particular importance in the differential diagnosis of AAV because the misdiagnosis of an infectious disease as AAV and the administration of immunosuppressive therapy could worsen the infection and lead to disastrous consequences.

Antinuclear Antibodies (ANA)

Collective term for all autoantibodies directed against conserved nuclear antigens (e. g., those occurring in all cell types). These include:

- Centromere antibodies,
- Coilin antibodies,
- DEK antibodies,
- DFS70 antibodies,
- dsDNA antibodies,
- Histone antibodies,
- IFI 16 antibodies,
- Ku antibodies,
- La/SS-B antibodies,
- LEDGF/DFS70 antibodies,
- Mi-2 antibodies,
- Nucleolar antibodies,
- Nucleosome/chromatin antibodies,
- PCNA antibodies,
- PM/Scl antibodies,
- Ro/SS-A antibodies,
- Scl-70 (topoisomerase I) antibodies,
- Sm antibodies,
- Sp100 antibodies,
- ssDNA antibodies,
- U1-RNP antibodies.

In addition, various other proteins of different nuclear compartments (coiled bodies, PML bodies, nuclear matrix, nuclear envelope/nuclear pore complexes) may be targets of antinuclear antibodies.

In the narrower sense, ANA are those autoantibodies that produce nuclear staining in tissue specimens (e. g., rat liver) or human tumor cell monolayers (e. g., HEp-2 cells) used in IIF.

Autoantigens

These (non-organ-specific) antigens are ubiquitous in cell nuclei. They may be located in chromatin or in the nucleolus, nucleoplasm, nuclear matrix, or nuclear envelope. The major target antigens of antinuclear antibodies include double-stranded deoxyribonucleic acid (dsDNA) and DNA-associated proteins (histone,

centromere proteins) as well as cytobiologically important enzymes (splicing enzymes, RNA polymerases, DNA topoisomerase I). Nuclear compartments and autoantigens include:

Localization	Antigens
Chromatin	dsDNA, ssDNA, histones, high mobility group (HMG) proteins
Centromere	centromere-associated proteins (CENP-A, -B, -C, -F, -H, -O)
Nucleolus	nucleolar RNA or nucleolar proteins (fibrillarin, RNA polymerase I, Th/To, PM/Scl-100)
Nucleoplasm/ Nuclear matrix	proteins of U-snRNPs (Sm- and U1-RNP antigen) or Y-snRNP complexes (Ro/SS-A and La/SS-B antigen)
Coiled bodies	p80 coilin
PML bodies	Sp100, PML
Nuclear envelope	lamins, lamin-B receptor, gp210, p62, Tpr

Detection Methods

- IIF using monolayers of human tumor cells show nuclear staining patterns that vary according to the localization of the nuclear antigen.
  Note: HEp-2, a human laryngeal carcinoma cell line, is most commonly used. All diagnostically relevant antinuclear antibody specificities (dsDNA, histone, nucleosome, Sm, U1-RNP, Ro/SS-A, La/SS-B, Scl-70, Mi-2, Ku, PCNA, Sp100, anticentromere and antinucleolar antibodies) should test positive by this technique. Caused through the used cell fixation method on the slides Ro60 antibodies are insufficiantly detected by some manufactorers.
- EIA using whole nuclei, nuclear extracts, or mixtures of purified or recombinant nuclear proteins.
- Microbead-Assays (ALBIA) with mixtures of purified or recombinant nuclear proteins.
  Note: The results of different assays may vary depending on the type of antigen mixture used (nuclear extracts, mixtures of purified and/or recombinant antigens). A good assay should allow the detection of all diagnostically relevant ANA specificities. However, most EIA, LIA or ALBIA cannot replace IIF for ANA screening.
- The CytoBead-Assay combines ANA screening and corresponding confirmatory testing using IIF on HEp-2 cells and microbead bound autoantigens.