# The General Practice Guide to Autoimmune Diseases

Edited by Y. Shoenfeld and P. L. Meroni

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# The general practice guide to autoimmune diseases

The development of autoimmunity and autoimmune diseases is believed to involve interactions between genes, hormones, and the environment and was labeled in 1989 as "The mosaic of autoimmunity". This complex interplay between the immune system and various stimuli, that comprise the pebble of the mosaic, is controlled by a wide array of mechanisms [1–2]. In the last decade there have been enormous strides in our understanding of autoimmune mechanisms which enabled us, to some extent, to predict and prevent diseases [2–4]. The relationships between environmental factors such as infectious agents, vaccines, adjuvant and drugs as well as hormones such as vitamin-D, ferritin and prolactin that can shift the immune perpendulum toward autoimmune inflammation have been extensively studied [5–10]. Therefore, nowadays we aspire into an era where we can recommend preventive measurements that will ameliorate or postpone autoimmunity. Of which a proper diet, avoidance of exposure to certain hormones (i.e. oral contraceptive) or UV radiation, climatotherapy, and the consumption of vitamin-D have been reported [11–16].

The diagnosis of autoimmune and auto inflammatory diseases has always been a challenging task. The presences of autoantibodies, such as rheumatoid factor, antinuclear and anti-CCP antibodies, as well as newly recognized as anti-pentraxin antibodies, in combination with diverse genetic markers have become central for early and accurate diagnosis of systemic diseases [17–20].

Last but not least the accumulated knowledge regarding systemic and organ specific autoimmune diseases has opened a new horizon for target oriented therapies. Intriguingly, it seems that once immune modulation is concerned the resemblance between autoimmune diseases outweigh their differences. Thus many of these novel targeted interventions were found to be beneficial in more than one autoimmune condition.

In the current book aimed for general practitioners (GPs) we tried, together with well known rheumatologists and autoimmunologists, to focus on what the GPs need to know and when they better refer the patient to the specialist. The EASI organization aimed for standardization of autoantibodies constructed from rheumatologists and autoimmunologists decided to expand the knowledge to the GPs. This is the first book of its kind and we hope to update it in the future.

We hope that you will enjoy reading the book.

Yehuda Shoenfeld,
Pier Luigi Meroni

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**Dr. Yehuda Shoenfeld (MD FRCP)**

Dr. Yehuda Shoenfeld was the head of a Department of Medicine since 1984 until March 2011, and he has founded in 1985 and is now heading the Zabludowics Center for Autoimmune Diseases, at the largest hospital in Israel — the Sheba Medical Center, which is affiliated to the Sackler Faculty of Medicine in Tel-Aviv University, in Israel. Dr. Shoenfeld is the Incumbent of the Laura Schwarz-Kipp Chair for Research of Autoimmune Diseases in Tel-Aviv University.

His clinical and scientific works focus on autoimmune/rheumatic diseases, and he has published more than 1600 papers. His articles had over 25 000 citations. He has authored and edited 25 books, some of which became cornerstones in science and clinical practice, such as "The Mosaic of Autoimmunity", "Infections and Autoimmunity" and the textbook "Autoantibodies" and "Diagnostic criteria of autoimmune diseases", all of which were published by Elsevier and sold by the thousands.

He is the founder and Editor of the "Autoimmunity Reviews" (Elsevier) (Impact factor 6.4) and Co-Editor of "Journal of Autoimmunity" (Impact factor 9.2). Dr. Shoenfeld has educated a long list of students (> 20) being Professors and heads of departments and institutes.

**Professor Pier Luigi Meroni**

Pier Luigi Meroni is a Full Professor of Rheumatology at the University of Milan and Director of the Department of Rheumatology, Istituto G. Pini, Milan. He received his medical degree in 1972 from the same institution, completing postgraduate training in clinical immunology in 1975, and haematology in 1979.

Professor Meroni received the Roussel Prize in 1987 for studies on age-associated immunodeficiency, and the EULAR Research Prize in 2005 for etiopathogenesis studies of the anti-phospholipid syndrome. He has been Chairman of the IUIS/WHO/AF/CDC Sub-Committee for the standardisation of diagnostic tests for rheumatic diseases, a member of the ACR ANA Task Force, and the Italian representative for the EASI group (European autoimmunity standardisation initiative forum). In addition, Professor Meroni is a member of the Scientific Committee of the AESKU Institute of Autoimmunity, a member of the Henry Kunkel Society, and Director of the postgraduate school of specialisation in Rheumatology at the University of Milan.

Professor Meroni has published widely in his field, with over 400 original papers in the literature, over 44 book chapters, and 2 books. He is a member of the Editorial Board of the journals *Annals of Rheumatic Diseases, Clinical and Experimental Rheumatology, Autoimmunity, Autoimmunity Review, Lupus, Journal of Autoimmunity,* and *Current Rheumatology Reviews.*

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# Autoimmune rheumatic diseases

# Systemic lupus erythematosus

Jaime Solís, Torsten Witte, Falk Hiepe, Gerald Messer, Georges Chyderiotis, Lucile Musset, Bach-Nga Pham, Nicole Fabien, Nils-Olivier Olsson, Ricard Cervera

## 1 Introduction

Systemic lupus erythematosus (SLE) is a chronic, multi-system autoimmune disease of unknown aetiology characterised by the production of non-organ specific autoantibodies and tissue immune-complex deposition which can potentially involve any organ and, therefore, has a wide range of clinical manifestations (Table 1). Renal involvement is frequently seen (30–50 %), and it is considered the most important predictor of the outcome of the disease.

SLE mostly affects young women (female : male ratio is 9 : 1), with an age at onset ranging from 15 to 55 years, and with some ethnic variability, being most frequent in Afro-Caribbean and Asian females.

In order to classify a patient as having SLE, 4 out of 11 criteria defined by the American College of Rheumatology (ACR) should be present at any time of the evolution of the disease (Table 2).

**Table 1.** Most common signs and symptoms in the "Euro-Lupus" cohort ($n = 1000$) during the 10-year prospective study (1990–2000).

| | | | |
|---|---|---|---|
| Arthritis | 84 % | Sicca syndrome | 16 % |
| Malar rash | 58 % | Livedo reticularis | 14 % |
| Fever | 52 % | Thrombosis | 14 % |
| Photosensitivity | 45 % | Lymphadenopathy | 12 % |
| Nephropathy | 39 % | Discoid lesions | 10 % |
| Serositis | 36 % | Myositis | 9 % |
| Raynaud's phenomenon | 34 % | Haemolytic anaemia | 8 % |
| Neurologic involvement | 27 % | Lung involvement | 7 % |
| Oral ulcers | 24 % | Subacute cutaneous lesions | 6 % |
| Thrombocytopenia | 22 % | Chorea | 2 % |

**Table 2.** American College of Rheumatology classification criteria for systemic lupus erythematosus.

1. Malar rash
2. Discoid rash
3. Photosensitivity
4. Oral ulcers
5. Arthritis
6. Serositis
   - Pleurisy
   - Pericarditis
7. Renal disorder
   - Persistent proteinuria
   - Cellular casts
8. Neurologic disorder
   - Psychosis
   - Seizures
9. Haematologic disorder
   - Haemolytic anaemia
   - Leukopenia
   - Lymphopenia
   - Thrombocytopenia
10. Immunologic disorder
    - Anti-dsDNA
    - Anti-Sm
    - Antiphospholipid antibodies
11. Antinuclear antibody

## 2 Diagnostic measurements for experts

In a patient with suspected SLE, laboratory measurements should be performed to detect the presence of non-organ specific autoantibodies, which are the hallmark of the disease. Antinuclear antibodies (ANA) are detected in more than 95 % of SLE patients, although their presence is not specific for the disease, and

they may also appear either in other autoimmune disorders or even in healthy population. Anti-double stranded DNA (anti-dsDNA) antibodies are useful for diagnosis, follow-up and prognosis of the disorder. They are present in 60–80 % of SLE patients, and there is a correlation between anti-dsDNA levels and disease activity, particularly predicting renal involvement. Anti-C1q antibodies are also useful for predicting renal involvement. Anti-Sm antibodies are the most specific antibodies, but are less frequently detected (10 %), and have no relation to disease course. The presence of anti-Ro (SS-A) and anti-La (SS-B) antibodies is related to some clinical features such as neonatal lupus, congenital heart block, subacute cutaneous lupus and leucopenia. Antiphospholipid antibodies, such as lupus anticoagulant (LA), IgG and IgM anticardiolipin antibodies (aCL) and IgG and IgM anti-$\beta_2$-glycoprotein I antibodies, are seen in nearly one third of patients with SLE, and they are associated with an increased risk of arterial and venous thrombosis as well as with pregnancy morbidity. Complement levels (C3, C4 and CH50) should be measured during follow-up because low levels have a strong correlation with SLE activity.

Further evaluation, including renal biopsy, should be performed if significant proteinuria or haematuria is present. The classification of lupus nephritis according to the International Society of Nephrology and Renal Pathology Society (Table 3) provides prognostic and therapeutic information. Diffuse proliferative glomerulonephritis (class IV) is both the most frequent and the most severe lesion, resulting in nearly 10 % of patients having end stage renal disease at 5 years.

**Table 3.** International Society of Nephrology/Renal Pathology Society 2003 classification of lupus nephritis.

| | |
|---|---|
| Class I | Minimal mesangial lupus nephritis |
| Class II | Mesangial proliferative lupus nephritis |
| Class III | Focal lupus nephritis |
| Class IV | Diffuse lupus nephritis |
| Class V | Membranous lupus nephritis |
| Class VI | Advanced sclerosis lupus nephritis |

## 3 Requirements for family practitioners

Because of the wide spectrum of clinical features, many symptoms and signs could be the initial manifestations of the disease. SLE should be suspected mainly in young patients (especially women) with polyarthritis/polyarthralgias, cutaneous lesions (especially in photo-exposed areas) (Fig. 1), recurrent oral ulcers, unexplained anaemia, lymphopenia or thrombocytopenia. The presence of persistent proteinuria or haematuria can be the first manifestation of lupus nephritis.

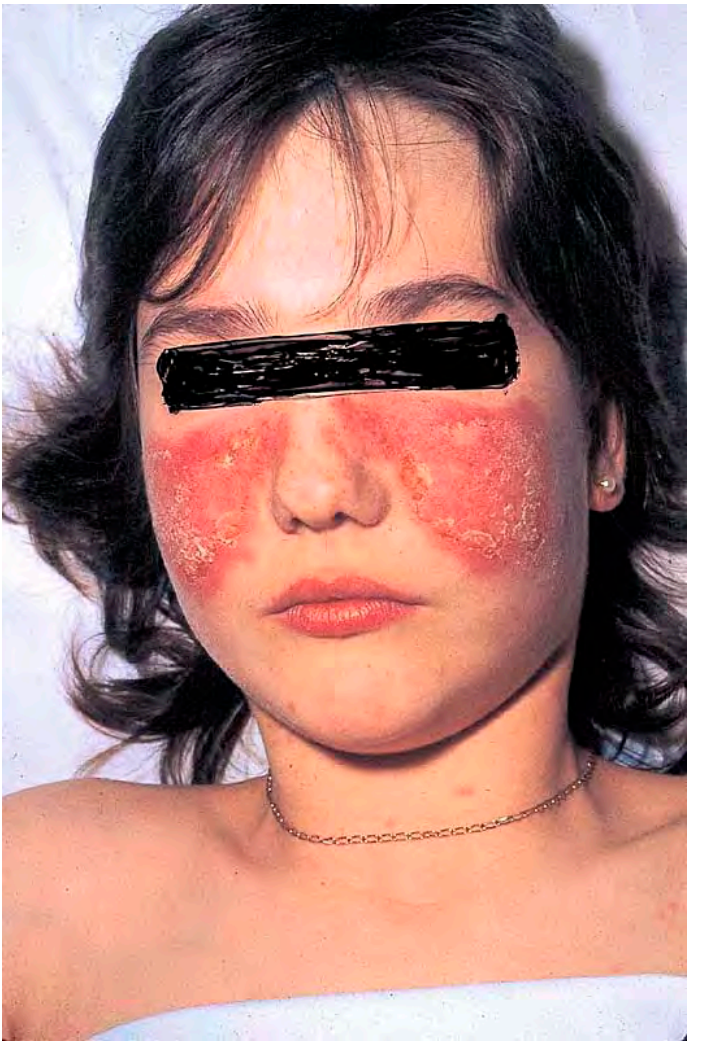

**Figure 1.** Malar rash in a patient with systemic lupus erythematosus.

When SLE is suspected, the patient should be referred to a specialist department for further evaluation in order to confirm the diagnosis, check organ involvement and start therapy.

The role of the general practitioner in SLE has paramount importance because close follow-up allows early diagnosis, recognition of reactivation and manage-

ment of side effects of medications, such as infections, cytopenias, and renal or hepatic toxicity.

Close control of cardiovascular risk factors, such as hypertension, diabetes, hyperlipidaemia, smoking or obesity, is essential for better disease prognosis because accelerated atherosclerosis currently constitutes one of the main causes of morbidity and mortality in SLE.

## 4 Follow up

*Clinical observations*

SLE is a chronic disease whose course is characterised by periods of flares and remissions. Some patients have chronic manifestations and other stay asymptomatic for long periods.

*Expectations*

The long term prognosis for patients with SLE has improved to nearly 90 % survival 10 years after diagnosis due to the better recognition and management of the disease.

*Blood tests*

Routine blood and urine analysis should be performed every 3–6 months, together with the measurement of anti-dsDNA antibodies and C3, C4 and CH50 levels in order to monitor disease activity.

## 5 Management

Because of the multiplicity of clinical presentations, SLE treatment must be individualised according to each patient's features, with special attention given to the presence and severity of renal involvement.

In general, mild manifestations, such as fatigue, cutaneous lesions or oral ulcers should be treated with antimalarial drugs as the first choice. Hydroxychloroquine is preferred over chloroquine because of its lower retinal toxicity, although periodic ophthalmologic controls are still recommended to minimise it.

Non steroidal anti-inflammatory drugs (NSAID) are indicated for arthralgias or arthritis, but it is necessary to monitor renal function to avoid nephrotoxicity.

Corticosteroids have probably been the most useful treatment for control of the disease, but should be prescribed at the lowest possible dose and for the shortest period of time in order to minimise their adverse effects. Nevertheless, many patients require low dose corticosteroids as maintenance treatment for long periods in order to avoid flares.

When high doses are needed, or internal organ involvement (especially renal) is present, other immunosuppressive agents, such as azathioprine, cyclophosphamide or mycophenolate mofetil should be introduced.

In cases of refractory disease in which at least two immunosuppressive drugs have failed, rituximab, a monoclonal antibody directed against B cells, appears to be effective, although no randomised, controlled trials have confirmed this formal indication yet.

In patients with aCL or LA, special care should be taken to prevent thrombosis, usually by the prescription of platelet aggregation inhibiting drugs, such as aspirin. In cases in which thrombosis has already occurred, anticoagulant therapy should be maintained to prevent new recurrences.

Recently, belimumab, a monoclonal antibody directed against soluble B lymphocyte stimulator (BLyS), has been licensed for the use in serologically active patients that do not respond to the standard therapy.

## 6 Diagnostic tests

Indirect immunofluorescence tests are the preferred methods for the detection of ANA. They have been performed on many rodent tissues, but currently are performed on HEp-2 cells, where several patterns have been recognised depending on the predominant autoantibody in serum. The most frequent pattern is the diffuse or homogeneous nuclear staining.

ANA are present in more than 95 % of SLE patients but they can also appear in other autoimmune diseases and in healthy people. Negative ANA test extensively excludes the diagnosis. By contrast, anti-dsDNA and anti-Sm antibodies are rarely seen in conditions other than SLE, and are therefore highly specific.

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# Neonatal lupus erythematosus

Thomas Werfel

## 1 Introduction

Neonatal lupus erythematosus (NLE) is a passively acquired, uncommon autoimmune disease, which is caused by the transplacental passage of maternal immunoglobulin G 52/60-kDa anti-Ro (SS-A) and/or 48-kDa anti-La (SS-B) autoantibodies or, less frequently, anti-U1 ribonucleoprotein (U1-RNP) autoantibodies [1, 2]. These antibodies have been found with high frequency in the sera of women with the rheumatic diseases, Sjögren's syndrome or systemic lupus erythematosus (SLE).

The pathogenesis of NLE probably involves more than simple transplacental passage of these antibodies, since the disease is rare, even in mothers who test positive for anti-Ro and anti-La antibodies. The incidence of NLE in children to mothers with SLE is about 1–2 %, and 15–20 % in children of mothers diagnosed with SLE and Ro antibodies, but also occurs in the children of asymptomatic women [3, 4]. NLE has been reported slightly more frequently in female than in male infants with an onset between birth and a few months of life [2].

The NLE syndrome is characterised most commonly by a transient lupus dermatitis or permanent congenital heart block (CHB). About 50 % of the NLE infants develop CHB, which carries a high mortality risk in the first year of life. The cardiac damage takes place between 18 and 24 weeks of gestational age [1]. During this period, the autoimmune reaction leads to an irreversible fibrotic destruction of the atrioventricular (AV) node in the foetus, which results in a low ventricular rate and, in the worst case, leads to a complete AV block and requires a permanent pacemaker implantation [1].

Skin lesions caused by NLE are present at birth or appear soon after and take the form of annular or circinate erythematous patches (Fig. 1), most often on the face and trunk [5]. Less frequently, NLE is associated with haematological and hepatic abnormalities, such as thrombocytopenia and an increased amount of transaminase enzymes. The noncardiac symptoms of NLE are transient and usually decline in parallel with the maternal antibody levels in the neonatal circulation within 2–6 months postpartum.

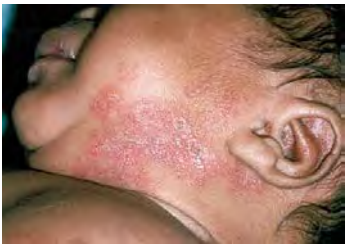

**Figure 1.** Erythematous patches and plaques on the face of a neonate with diagnosed NLE.

## 2 Diagnostic measurements for experts

Because of the potential for serious complications of undiagnosed NLE, a comprehensive evaluation of both child and mother is required and represents a unique challenge for rheumatologists, dermatologists, obstetricians, perinatologists, and paediatric cardiologists to identify pregnancies at risk and to care for the patients. In this context, neonatal and maternal serum should be tested for antinuclear antibodies (ANA), specifically for anti-Ro, anti-La antibodies, and anti-U1 ribonucleoprotein antibodies. Despite being positive for Ro and/or La antibodies, up to 60 % of infants' mothers with NLE may be clinically asymptomatic when their child develops NLE [5]. Mothers, in whom SLE is positively diagnosed by clinical symptoms and laboratory test results, should be monitored closely [2].

In addition to serum tests, a physical examination should be performed including a cardiac examination, an echocardiogram and electrocardiogram, liver function tests and a platelet count [5]. In women with autoimmune disorders, frequent ultrasonographic monitoring of the foetal heart rate is recommended during pregnancy [2]. The very early diagnosis of a foetal heart block by echocardiography is essential for appropriate therapy and the improvement of cardiac symptoms in the foetus [1].

In order to obtain an accurate diagnosis of NLE, skin biopsies for routine histology and direct immunofluorescence microscopy examinations are also recommended [2].

## 3 Requirements for family practitioners

Many women who bear a child with neonatal lupus syndrome have anti-Ro or anti-La autoantibodies, but do not have a diagnosis of lupus or another autoimmune disease at the time of their pregnancy. There is, however, a substantial risk of subsequent development of autoimmune connective tissue diseases [5].

Women with SLE should be referred to a rheumatologist and high-risk obstetrical provider to discuss their desire to have a child and to be informed about the

increased risk for the development of an autoimmune disease in their offspring before trying to become pregnant. The outcome for both mother and child is best when SLE has been under good control for at least six months before the onset of pregnancy. The patient should be regularly observed in order to provide timely prophylaxis. Furthermore, the development of the foetus must be monitored continuously during pregnancy. Postpartum, various examinations should be conducted by a neonatologist in order to confirm or to exclude the diagnosis NLE, which is generally based on clinical findings when maternal and/or neonatal autoantibody titres for anti-Ro (SS-A), anti-La (SS-B), and/or anti-U1-RNP are detected.

## 4 Follow up

*Clinical observations*

Children with NLE need continued follow-up visits, especially prior to adolescence and if the mother herself has an autoimmune disease. Although these children may not be at increased risk of developing SLE, the genesis of any type of autoimmune disease in early childhood may be of concern.

Patients with NLE and cardiac involvement require monitoring to assess the cardiac function and the necessity for a pacemaker. Mothers of neonates with NLE, particularly neonates with CHB, have a two- to three-fold increased risk of further affected neonates. An estimated 25 % of subsequent pregnancies are affected, and thus should be carefully monitored, particularly between 18 and 24 weeks of gestational age [6].

*Expectations*

The neonatal mortality rate of NLE patients with congestive heart failure is 20–30 %. Skin, haematologic and hepatic manifestations usually improve with the disappearance of maternal autoantibodies. In some cases, severe liver failure with a poor prognosis may occur.

## 5 Management

It is recommended that the management of NLE be commenced before or, at the latest, during pregnancy and that both the mother and the child be treated.

*Treatment of the mother*

In general, the management of NLE includes medical treatment of disease flares in mothers with SLE, who are at high risk of bearing an affected child, by using drugs that are effective against the disease but also safe for the foetus. Such an

approach may diminish or reduce the prevalence of the child developing complete heart block in association with NLE. In this context, corticosteroids and some immunosuppressive drugs are sometimes used, but long-term data in children exposed to immunosuppressive drugs *in utero* is lacking [2].

*Treatment of the neonate*

The treatment of the neonate must be individualised according to the manifestation of NLE, which consequently also affects the long-term prognosis of the child.

In patients with NLE that affects the heart, pacemaker placement along with the surgical correction of structural abnormalities in the heart may be necessary.

NLE that affects the skin, blood, spleen, or liver is usually self-limited and resolves without intervention within 2–6 months. In more severe cases, supportive treatment is possible for NLE skin lesions by using mild topical corticosteroids to control cutaneous lesions, antimalarial agents (e. g. hydroxychloroquine) to inhibit chemotaxis of eosinophils and locomotion of neutrophils and, possibly conducting laser treatment for residual telangiectasia. Additionally, photoprotection by avoiding direct sun exposure and applying sunscreens is highly desirable because solar exposure may precipitate skin lesions.

## 6 Diagnostic tests

NLE should be suspected in any infant born with CHB or who develops erythematous cutaneous patches and telangiectases. For the diagnosis of NLE various examinations are necessary. Commonly, antibodies to Ro (SS-A) and La (SS-B) are detected clinically by 'Ouchterlony' immunodiffusion, enzyme-linked immunosorbent assays (Elisa), or Western blot in both the infant and the mother. While the 'Ouchterlony' immunodiffusion test system is used as a screen for these antibodies without indicating their specificity, recombinant antigens have been created for the selective detection of the target antibodies in immunosorbent assays, which are much more sensitive and widely used especially to test for Ro52 antibodies. For a diagram of the indirect competitive Elisa method, using the example of Ro52, see Fig. 2: a buffered solution of recombinant Ro52 antigens is added to the microtitre plate, where they adhere via charge interactions (Fig. 2a), and the remaining free plastic surface is blocked with non-reacting proteins. In the next step, serum which may contain pathologic concentrations of Ro52 antibodies as well as an enzyme-linked competitive antibody is added (Fig. 2b). Both of them compete for binding with the coating antigen on the microtitre plate (Fig. 2c). After washing, the enzyme-linked secondary antibody is activated by adding a specific substrate causing a colour reaction that can be measured photometrically (Fig. 2d). The more intense the colour, the less antibody of interest is present in the serum sample.

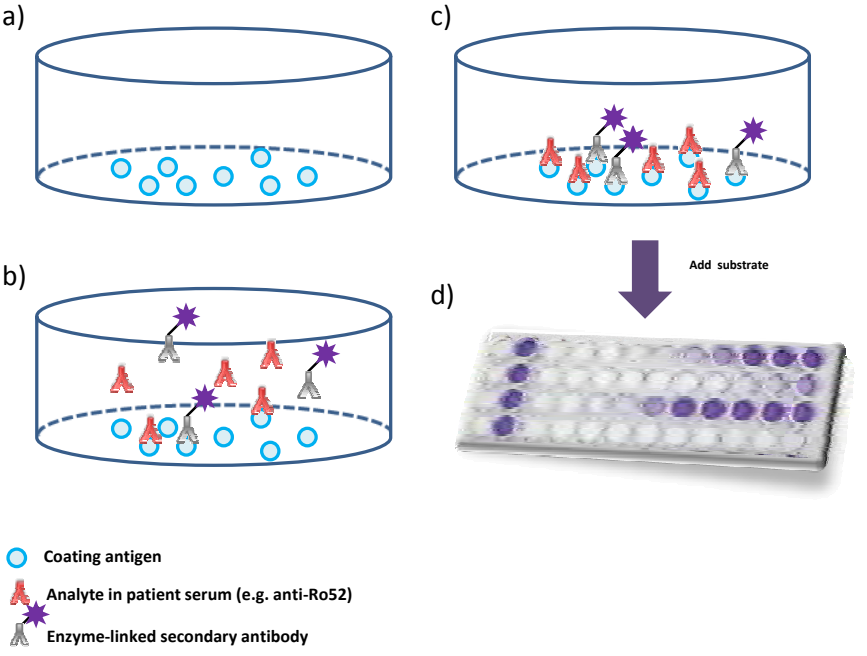


**Figure 2.** Schematic presentation of the indirect competitive Elisa method: a) coating antigen on microtitre plate, b) add serum with target antibody and enzyme-linked competitive antibody, c) competition for binding with the coating antigen d) microtitre plate after addition of activating substrate.

A third serological test used to diagnose NLE is called the immunoblot or Western blot. By means of this test it is possible to distinguish between antibodies to Ro52 and Ro60, as well as La and U1-RNP autoantibodies.

In order to confirm the diagnosis of NLE, especially when skin alterations appear, biopsies are examined histologically. Microscopic examination reveals hyperkeratosis in the affected areas, a thickened basement membrane, and a large number of CD4 T-lymphocytes. Furthermore, it may be useful to perform the Lupus band test by direct immunofluorescence staining to determine the presence and extent of immunoglobulin and complement deposits in skin biopsies from the affected tissue in comparison to non-lesional skin.

## 7 Testing methods

Several serological tests are available for the detection of autoantibodies specific for NLE. Differences appear in their specificity, sensitivity, and their intensity of

labour. 'Ouchterlony' immunodiffusion is generally being replaced by more sensitive enzyme-linked immunosorbent assays, while the Western blot exhibits a broad specificity but is very labour-intensive, and thus is primarily used for research studies. In general, all of these tests permit a safe diagnosis of NLE, especially when combined with the histological assessment of biopsied tissue samples.

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# Systemic sclerosis

Cecilia Chighizola, Karsten Conrad, Pier Luigi Meroni

## 1 Introduction

The first description of Systemic Sclerosis (SSc) was made by Carlo Curzio and dates back to 1753, but the name *scleroderma* was ascribed only in 1847 by Gintrac. SSc is a clinically heterogeneous, multisystemic, autoimmune connective tissue disorder typically involving the skin (Raynaud's phenomenon (RP), digital ulcers, skin thickening, telangiectasias, calcinosis Fig. 1), lung (pulmonary fibrosis, pulmonary hypertension), heart (arrhythmias, myocardial fibrosis, congestive heart failure), musculoskeletal apparatus (arthritis, arthralgias, tendon friction rubs, joint contractures, myopathy), gastrointestinal tract (oesophageal and small intestine hypomotility) and kidney (scleroderma renal crisis).

SSc can be further subcategorised into 4 principal subsets:

- limited SSc (skin sclerosis restricted to the hands and the distal forearms, and to a lesser extent the face and the neck),
- diffuse SSc (sclerotic skin also on the chest, abdomen, upper arms and shoulders),
- SSc sine scleroderma (internal organ involvement only) and
- overlap syndromes (criteria fulfilling SSc occurring concomitantly with features of systemic lupus erythematosus [SLE], rheumatoid arthritis [RA] or inflammatory myopathy).

Limited SSc was formerly identified with CREST syndrome (**C**alcinosis, **R**aynaud's phenomenon, **E**sophageal dismotility, **S**clerodactyly, **T**elangiectasias), a clinical entity described long ago. However, patients with limited SSc do not necessarily have all the features of CREST syndrome, although many of them do. Table 1 summarizes the clinical features of limited and diffuse SSc.

The disease occurs worldwide, and the incidence and the prevalence rates show wide variation, with the higher prevalence being approximately 230 cases per million in the USA and South Australia. The disease is predominant in females, with a 3–5 : 1 ratio; SSc onset is most commonly between 30 and 50 years of age.

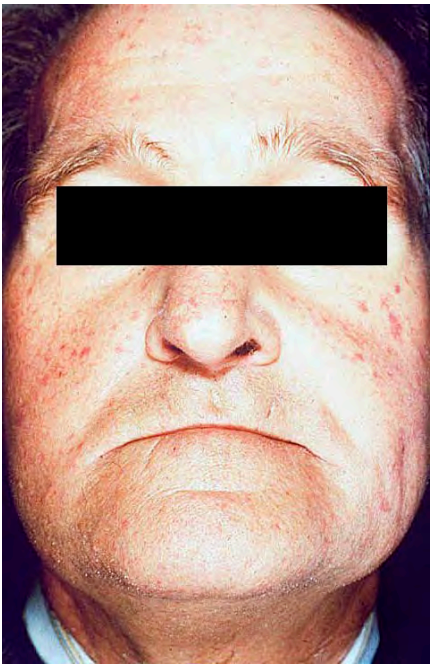

**Figure 1.** A patient suffering from limited SSc with a typical *scleroderma facies*: the skin is thickened, the wrinkles are smoothed but the ones around lips are furrowed, there is a remarkable reduction in the maximum oral aperture ("microstomia"), the nose becomes pinched, many telangiectasias can appear, the face can appear expressionless because of a reduced capacity to smile or to move eyelids or cheeks.

Pathophysiologically, the two leading mechanisms involved in SSc are a massive accumulation of extracellular matrix components leading to fibrosis and a vascular disease characterised by arterial vasospasm, smooth muscle hyperactivity, intimal proliferation and eventual vascular occlusion leading to tissue ischaemia [1].

## 2 Diagnostic measurements for experts

*Skin involvement*

Regarding the extent of cutaneous involvement, the most widely scoring system, named "modified Rodnan thickness skin score", evaluates skin sclerosis at 17 sites, with scores at each site being 0 (normal), 1 (equivocal sclerosis), 2 (definite sclerosis), or 3 (hide bound); the skin score reflects disease severity.

In SSc patients, nailfold capillaroscopy shows a typical scleroderma pattern, with enlarged capillary loops and/or the loss of capillaries or avascular areas.

*Gastrointestinal involvement*

In cases with symptoms of oesophageal dysfunction, a barium swallow, endoscopic and/or manometric investigation are warranted.

*Lung involvement*

The most sensitive method for detecting early lung disease in scleroderma is to perform pulmonary function testing: mild changes in function can be detected

**Table 1.** Signs and symptoms of the disease.

| | **Limited SSc** | **%** | **Diffuse SSc** | **%** |
|---|---|---|---|---|
| **Constitutional symptoms** | Rare | | Severe | |
| **Skin** | RP alone for years | 95 | Delayed RP | 85 |
| | Digital ulcerations on fingertips or distal toes | 15 | Digital ulcerations on fingertips or distal toes | 30 |
| | Skin thickening limited to hands (sclerodactyly) and face (Fig. 1) | 95 | Skin thickening progressing from fingers to trunk rapidly | 100 |
| | Subcutaneous calcinosis at sites of trauma | 50 | Subcutaneous calcinosis at sites of trauma | 10 |
| | Telangiectasias on the face, upper chest, palms, fingertips, and mucous membrane in early stages | 80 | Telangiectasias on the face, arms and trunk in the later stages | 30 |
| **Musculoskeletal apparatus** | Minimal arthralgias | 60 | Arthritis, carpal tunnel syndrome | 80 |
| | Tendon friction rubs | 3 | Tendon friction rubs | 65 |
| | Myopathy | 10 | Myopathy | 20 |
| **Lung** | Inflammatory alveolitis leading to pulmonary interstitial fibrosis | 35 | Inflammatory alveolitis leading to pulmonary interstitial fibrosis | 45 |
| | Pulmonary hypertension | 10 | Pulmonary hypertension | $<1$ |
| **Heart** | Pericarditis | 15 | Pericarditis | 15 |
| | Congestive heart failure | 5 | Congestive heart failure | 15 |
| | Arrythmias | 20 | Arrythmias | 15 |
| | Pericarditis | 20 | Patchy myocardial fibrosis (at autopsy) | 50 |

**Table 1.** *(continued)* Signs and symptoms of the disease.

| | Limited SSc | % | Diffuse SSc | % |
|---|---|---|---|---|
| **Gastrointestinal tract** | Oesophageal hypomotility (dysphagia, dyspepsia, reflux symptoms) | 75 | Oesophageal hypomotility (dysphagia, dyspepsia, reflux symptoms) | 75 |
| | Small intestine hypomotility (intermittent pseudo-obstruction) | 25 | Small intestine hypomotility (intermittent pseudo-obstruction) | 25 |
| | Association with PBC and AIH | 17 | | |
| **Kidneys** | Scleroderma renal crisis (malignant hypertension, rapidly progressive renal failure) | 1 | Scleroderma renal crisis (malignant hypertension, rapidly progressive renal failure) | 20 |

**RP**, Raynaud's Phenomenon **AIH**, Autoimmune Hepatitis **PBC**, Primary Biliary Cirrhosis

before any symptoms develop. The most common changes of pulmonary function testing are either a reduced diffusion capacity ($D_LCO$) or a reduction in lung volumes typical of a restrictive ventilatory defect associated with a reduction in gas exchange. A high-resolution computed tomography scan of the chest is a very sensitive technique for detecting changes in the lung parenchyma, showing, in cases of active alveolitis, a ground-glass opacity of the lung and a honeycombing lung parenchyma in cases of interstitial fibrosis.

Bronchoalveolar lavage is used to detect inflammation and active alveolitis.

Pulmonary hypertension can be detected early and non-invasively by measuring the pulmonary artery pressure with two-dimensional Doppler echocardiography; patients with a pathologic or borderline tricuspid regurgitant jet velocity should undergo a right-heart *catheterisation.*

### *Laboratory investigations*

A positive antinuclear antibodies (ANA) with a centromere (Fig. 2), a speckled and/or nucleolar staining pattern is frequently noted; specific autoantibodies include anti-centromere antibodies (ACA, usually associated with limited SSc) and anti-DNA topoisomerase I (anti-Scl-70, usually associated with diffuse SSc) [1].

## 3 Requirements for family practitioners

Most commonly, SSc patients present to family practitioners complaining of Raynaud's phenomenon, that may have been present alone for years before any other manifestations occur. It is important to distinguish between patients with primary or uncomplicated Raynaud's phenomenon and those with a secondary one.

A secondary cause of Raynaud's phenomenon is suggested by the following findings:

- age at onset of more than 30 years;
- episodes that are intense, painful, asymmetric, or associated with ischaemic skin lesions;
- clinical features suggestive of a connective tissue disease;
- positivity of ANA and antibodies against extractable nuclear antigens (ENA);
- evidence of microvascular disease on microscopy of nail-fold capillaries.

The family practitioner should exclude potential causative or aggravating factors (carpal tunnel syndrome, environmental agents and injury, use of particular drugs such as sympathomimetic agents, cocaine, nicotine, ergotamines etc). Patients should undergo a nailfold capillaroscopy and a superior limb arterial Doppler ultrasonography plus laboratory tests (complete blood count, active phase reactants, serum protein electrophoresis, thyroid function test, cryoglobulins, rheumatoid factor, ANA, anti-ENA, C3 and C4) to rule out diseases such as hypothyroidism, cancer, cold agglutinin syndrome, POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes), cryoglobulinaemia, atherosclerosis, embolic disease, thoracic outlet syndrome.

The presence of ANA displays relatively low positive predictive value for an associated connective tissue disease (30 %), whereas the presence of antibodies against a specific autoantigen is more highly suggestive of secondary disease: scleroderma is more likely in patients with ACA or anti-topoisomerase antibodies.

Skin thickening is another feature commonly present at disease onset; apart from SSc, it may be a manifestation of many other diseases and can result from exposure to drugs (e. g., bleomycin, pentazocine, vitamin K and B12) or harmful environmental factors (petroleum distillates, organic solvents, vibrating tools). Some endocrine disorders (diabetes mellitus and hypothyroidism), renal disease, and infiltrative disorders (amyloidosis, eosinophilic fasciitis, chronic GVH disease) can cause scleroderma-like skin changes [2].

## 4 Follow up

Patients suffering from non complicated limited SSc should undergo a rheumatologic visit once a year, while limited SSc patients with pulmonary involvement and diffuse SSc patients need a tighter follow-up, three times a year.

Patients must be questioned about the appearance of symptoms such as dysphagia, dyspepsia, constipation, diarrhoea, breathlessness on exertion, non-productive cough. At clinical examination, particular attention should be paid to heart and lung auscultation revealing arrhythmias and velcro rales; fingertip ulcers and skin thickening (modified Rodnan thickness skin score) should be strictly monitored. Blood pressure measurement is recommended on a monthly basis.

Periodical routine laboratory tests should be performed, including complete blood count, acute phase reactants, muscular enzymes, hepatic and renal functions, with urine analysis and measurements of the glomerular filtration rate.

A complete assessment to evaluate the internal organ involvement (electrocardiogram, two-dimensional Doppler echocardiography with pulmonary artery pressure measurement, chest X-ray, pulmonary function tests with $D_LCO$ evaluation, barium swallow, nailfold capillaroscopy) is necessary at diagnosis and yearly thereafter; high-resolution computed tomography scan of the chest, gastroenteric tract endoscopic study, a manometric investigation of the oesophagus and a 24-hour pH monitoring are required every two years or if new symptoms appear [1].

## 5 Management (therapeutic principles)

In consideration of the multifaceted nature of the disease, therapeutic management of SSc patients consists of a combination of agents acting upon different clinical aspects. The management of Raynaud's phenomenon should first consider non-pharmacologic strategies such as avoidance of cold, stress, nicotine, caffeine and sympathomimetic decongestant medications. Low-dose acetylsalicylic acid and calcium-channel blockers are first-line options.

In severe Raynaud's phenomenon, it has been demonstrated that iloprost (a prostacyclin analogue given parenterally) reduces the number of weekly attacks and the global Raynaud severity score; moreover, it is effective in healing at least 50 % of digital cutaneous lesions, helping to avoid amputation of the distal tip of a digit. More recently, bosentan (a non selective endothelin antagonist already registered for treatment of pulmonary hypertension) has shown a beneficial effect upon digital ischaemia, reducing the appearance of new ulcers.

In patients with abnormal oesophageal motility, the empiric use of acid reducing agents, particularly proton pump inhibitors, is generally recommended; prokinetic agents may be valuable.

In cases of active inflammatory alveolitis — that is presumed to precede the development of interstitial fibrosis — treatment is recommended. The combination of glucocorticoids (prednisone 10 mg/kg) and cyclophosphamide (administered both orally at a dose of 1 mg/kg increased to 2 mg/kg if tolerated and intravenously at a dose of 600 mg/m$^2$ per month) is the only therapeutic regimen that has shown

a modest clinical efficacy at preventing deterioration of lung function in patients with active alveolitis.

Pulmonary hypertension in SSc may be due to lung interstitial fibrosis, increased pulmonary arterial vasoreactivity or obliterative vasculopathy. Among the therapeutic options for pulmonary hypertension, there is evidence for the efficacy of bosentan, the phosphodiesterase-5-inhibitor sildenafil and various prostacyclin analogs, that may be administered by inhalation, subcutaneous infusion or intravenously.

A mild myopathy with little biochemical or histological change is a common feature of SSc; glucocorticoids alone or in combination with methotrexate or azathioprine are generally employed [3].

## 6 Diagnostic tests and testing methods

The presence of characteristic autoantibodies is supportive of the diagnosis of SSc [4, 5].

ACA are seen mostly in patients with limited SSc and with a greater frequency in women. They are associated with calcinosis, tuft resorption and digital ulcers. ACA are typically detected by the indirect immunofluorescence on HEp-2 cells giving a discrete speckled appearance on interphase nuclei and chromatin of mitotic cells (Fig. 2). Three main centromere/kinetochore-associated proteins (CENP-A of 29 kDa, CENP-B of 80 kDa and CENP-C of 140 KDa, altogether known as "CENPs") are recognised by autoimmune sera. To characterise the reactivity to individual CENP antigens, Elisa or Line immunoassays are necessary. ACA display a sensitivity of 3–12 and 57–82 % for diagnosing diffuse and limited SSc respectively.

Anti-Scl-70 are associated with diffuse SSc with a sensitivity of 34–65 % among patients with diffuse SSc even if 25 % of patients do not have extensive skin, heart or kidney problems, with a clinical course similar to that of limited SSc. Moreover, they are associated with prominent pulmonary interstitial fibrosis and vascular problems, although Scl-70 positive patients are protected from vasculopathy type of pulmonary hypertension. Anti-Scl-70 are directed against an acid nuclear enzyme, DNA topoisomerase I, which catalyzes the conversion of DNA topologic forms mediated through transient single-strand DNA breaks and relegation. Historically, the usual method for detection anti-Scl-70 was immunodiffusion and immunoblotting, but nowadays most laboratories detect them by Elisa or Line immunoassays. The disappearance of anti-Scl-70 is associated with favourable outcomes, and serum levels of anti-Scl-70 may correlate positively with the severity of skin involvement and with global disease activity.

A nucleolar pattern of ANA at high titres is very specific to scleroderma, and several specific antibodies have been identified; they are not very common and commercial assays are still not available for all of them:

- anti-$U_3$-RNP or antifibrillarin antibodies are associated with diffuse cutaneous disease, pulmonary fibrosis and isolated pulmonary hypertension,
- anti-Th/To and anti-Pm/Scl antibodies are more frequent in white patients with limited scleroderma. Pulmonary hypertension and myositis are respectively common features in these patients,
- anti-RNA-polymerase I antibodies are associated with severe, diffuse forms of systemic sclerosis; higher frequency of cardiac, hepatic and renal involvement,
- anti-RNA-polymerase III antibodies are detected in 12–23 % of patients with systemic sclerosis. They are associated with diffuse or extensive skin manifestation and have been detected during a renal crisis in the absence of skin manifestations, i. e., sclerosis sine scleroderma.
- Anti-$U_1$-RNP antibodies are associated with overlap syndromes, mostly with mixed connective tissue disease.

## 7 Diagnostic criteria

There are no universally accepted classification and/or diagnostic criteria for SSc. In 1980, the ACR classification criteria (Table 2) were designed to differentiate SSc from other diseases; unfortunately, they do not include specific tests for ANA and nailfold capillaroscopy (Fig. 2). It has been shown they lack sensitivity, particularly in identifying patients with limited SSc. More recently, Nadashkevich proposed an updated classification set that included the presence of ANA but did not include nailfold capillaroscopy. The validity of these criteria has been tested preliminarily on a population of 99 SSc patients, yielding a 99 % sensitivity and 100 % specificity. However, these criteria have not been widely adopted [6].

**Table 2.** Clinical and laboratory diagnostic criteria.

| Major Criterion | Minor Criteria |
|---|---|
| Proximal scleroderma (Skin involvement extending proximally to metacarpophalangeal joints) | 1. Sclerodactyly<br>2. Digital pitting scars of fingertips or loss of substance of the distal fingerpad<br>3. Bibasilar pulmonary fibrosis |

To make a diagnosis of Systemic Sclerosis, at least the major criterion or two or more minor criteria must be fulfilled.

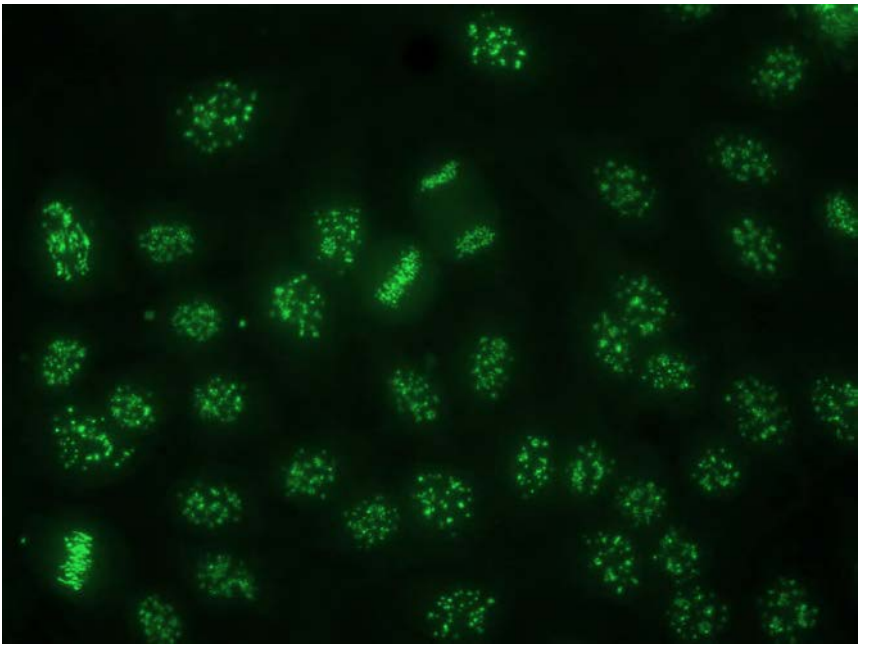

**Figure 2.** Indirect Immunofluorescence test on Hep-2 cells showing a discrete speckled appearance on interphase nuclei and chromatin of mitotic cells, typical of anti-centromere antibodies(ACA).

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# Polymyositis and dermatomyositis

Jiří Vencovský, Ingrid E. Lundberg, Cees Kallenberg, Rudolf Mierau

## 1 Introduction

Polymyositis (PM) and dermatomyositis (DM) are characterized by chronic inflammation of striated muscles leading to altered muscle function. The main clinical symptom is muscle weakness and low muscle endurance, which is localised predominantly in the proximal portions of upper and lower extremities (Table 1). Other muscles may be involved, such as upper oesophageal and breathing muscles causing difficulties in swallowing and respiratory problems. The systemic nature of the disease is underlined by a possible presence of extramuscular involvement including involvement of the skeletal, pulmonary and cardiac systems and constitutional symptoms. DM patients have a typical cutaneous rash (Fig. 1), but also differ from PM by muscle biopsy characteristics, which suggests a possibility of different pathogenic pathways in these two diseases. Autoantibodies are present in up to 80 % of patients and are frequently associated with particular clinical manifestations. The disease may lead to muscle atrophy and permanent damage of different organs and systems.

The annual incidence of PM and DM is reported to be around 7 per million people and latest figures estimate the prevalence at 21.5/100 000. The overall female:male incidence ratio is 2.5 : 1. In about 15 % of cases, the disease is associated with various malignancies, this is particularly true for dermatomyositis. Myositis with inclusion bodies recognized on muscle biopsy (inclusion body myositis, IBM) occurs mainly in men and usually after 50 years of age.

## 2 Diagnostic measurements for experts

Muscle inflammation leads to muscle weakness, which can be measured by manual muscle strength test (MMT). This test uses a standardised grading system for measurement of muscle strength in individual muscles or muscle groups. It is recommended to perform the test serially in order to evaluate disease activity over time.

**Table 1.** Signs and symptoms of polymyositis and dermatomyositis.

| Affected organ | Clinical manifestation | Frequency |
|---|---|---|
| Skeletal muscles | Muscle weakness, particularly proximal extremities | 100 % |
| Oesophageal muscles | Dysphagia | 30 % |
| Pharyngeal muscles | Nasal voice | < 30 % |
| Breathing muscles | Respiratory difficulties | < 30 % |
| Lungs | Alveolitis, interstitial pulmonary fibrosis | 30–80 % |
| Heart | ECG abnormalities, myocarditis, rhythm disturbance | < 30 % |
| Joints | Arthralgia, arthritis | 50 % |
| Skin | Cutaneous rash | 100 % in DM |
| | – Pathognomonic | |
| | – Gottron's papules | 60–80 % |
| | – Heliotrope rash | < 50 % |
| | – Other skin changes include "mechanics hands", "V sign" chest rash, "Shawl sign", erythroderma, nailfold capillary changes and cuticular overgrowth, panniculitis and others. | |
| Skin | Calcinosis | More frequent in juvenile DM |
| Vascular | Raynaud's phenomenon, vasculitis in children | < 30 % |
| Constitutional | Fatigue, fever, weight loss | < 30 % |

Inflammation within the muscles causes oedema which can be visualized by magnetic resonance imaging (MRI). It is necessary to use an MRI technique which suppresses the signal of fat to recognise the changes. Because the inflammation can be only focal, MRI may be used to select the optimal biopsy site. MRI may also show atrophy and fibrosis in the advanced stages of disease and therefore helps in distinguishing between active disease and accumulated damage.

Muscle biopsy is the most valuable tool to confirm the diagnosis of PM or DM [1]. This method is particularly important for a definitive diagnosis of polymyositis and to exclude other myopathies that may mimic polymyositis. Classical PM has endomysial inflammatory cell infiltrate composed particularly of $CD8^+$ T-cells that surround and sometimes invade non-necrotic muscle fibres. Macrophages

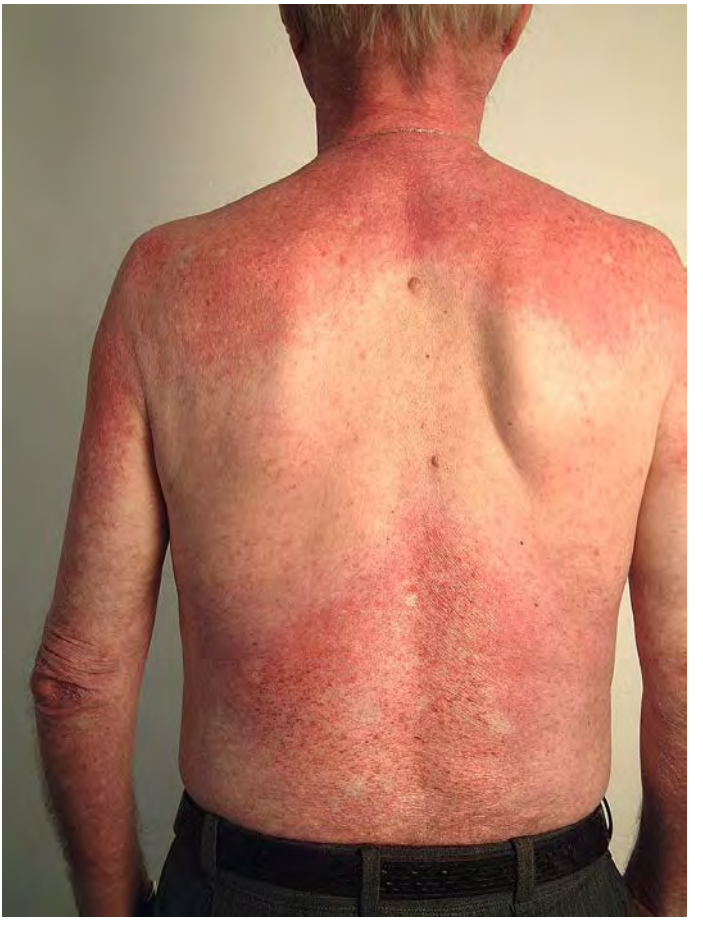

**Figure 1.** Typical rash in dermatomyositis.

and $CD4^+$ T-cells may also be present. Muscle fibres display ubiquitous MHC-I expression, and this is sometimes seen even in the absence of inflammatory infiltrate, which could be helpful in the diagnostic procedure. Inflammatory cells in dermatomyositis are localized mainly in the perivascular and perimysial space and mostly macrophages, $CD4^+$ T-cells and occasional B-cells are present. Frequently membrane attack complex (MAC) depositions are found on small blood vessels. Muscle histology in inclusion body myositis is similar to PM, but rimmed vacuoles, ragged red fibres, and cytochrome oxidase-negative fibres suggest IBM.

**Table 2.** Diagnostic criteria.

| **Clinical criteria** |
|---|
| – Symmetric weakness of limb-girdle muscles and anterior neck flexors progressing over weeks to months, with or without dysphagia or respiratory muscle involvement |
| – Typical skin rash of DM including a heliotrope rash, Gottron's sign, Gottron's papules and involvement of the knees, elbows and medial malleoli as well as the face, neck, and upper torso |
| **Laboratory criteria** |
| – Elevation in serum of skeletal-muscle enzymes (particularly creatine phosphokinase and often aldolase), serum aspartate and alanine aminotransferases, and lactate dehydrogenase |
| – Electromyographic triad of short, small, polyphasic motor units, fibrillations, positive sharp waves and insertional irritability, and bizarre, high-frequency repetitive discharges |
| – Muscle biopsy abnormalities of degeneration, regeneration, necrosis, phagocytosis, inflammatory infiltration and atrophy in perifascicular distribution |

Definite disease requires 4 criteria (three plus rash) for dermatomyositis and 4 criteria for polymyositis; probable disease must include 3 criteria (two plus rash) for DM and 3 criteria for PM; and possible disease requires 2 criteria (one plus rash) for DM and 2 criteria for PM.
For these criteria to be applied, the exclusion of number of situations: central or peripheral neurologic disease, muscular dystrophy, granulomatous myositis, infections, use of toxins or drugs, rhabdomyolysis, metabolic disorders, endocrinopathies, and myasthenia gravis is required.

Most but not all patients with PM and DM have characteristic autoantibodies present in their serum, whereas these autoantibodies are usually lacking in inclusion body myositis and are less frequent — with one exception (see Table 3) — in cancer associated myopathy.

Some of the antibodies are specific for myositis and cannot be found in other diseases, some are myositis-associated and may be detected in other connective tissue diseases [2, 3], but are still helpful in making the diagnosis and categorising patients (Table 3).

## 3 Requirements for family practitioners

Polymyositis and dermatomyositis are chronic inflammatory disorders of striated muscles. The leading clinical symptom is muscle weakness and, in particular, low

muscle endurance and easily fatigued muscles, accompanied by a variety of systemic manifestations. The history often includes difficulties walking up stairs, needing to rest after one set before continuing or walking uphill. Muscle weakness is predominantly seen when testing pelvic muscles and neck flexors. Getting up from a squatting position is a simple test that is often impossible to perform for myositis patients. Muscle pain may be present in some patients, but is usually not the main symptom. Diagnosis of dermatomyositis is somewhat easier than polymyositis owing to presence of the typical skin changes. The onset of disease is usually acute or subacute with weakness and fatigue causing patients to see a general practitioner. Notably some patients may present with predominating pulmonary symptoms such as dyspnoea or cough, and with signs of interstitial lung disease on chest radiography. In such patients an underlying rheumatic disease like myositis should be considered.

ESR and CRP are usually within normal range, although CRP may be elevated in some patients with acute inflammation. Often the first serum chemistry shows highly elevated amino-transferases, which, when CK levels are not measured, may be misinterpreted as hepatic injury. To establish a correct diagnosis it is necessary to verify muscle weakness by an appropriate test, measure serum levels of muscle enzymes and/or myoglobin and perform electromyographic testing. Muscle biopsy should always be done in polymyositis and is highly recommended in dermatomyositis to confirm the correct diagnosis, since many conditions may mimic PM and DM. It is advisable that the biopsy is processed by a pathologist experienced in muscle diseases. Testing for serum autoantibodies is often helpful as well as muscle MRI. When a patient presents with muscle weakness and has elevated muscle enzymes, he or she should be referred for further diagnostic specification to a specialist in inflammatory muscle diseases, which may be rheumatologist, neurologist or dermatologist, depending on the local situation and also on the presentation of the disease.

The severity of disease varies from patients confined to bed or to a wheelchair in the acute stage to patients with more subtle manifestations, such as difficulties in climbing stairs or raising hands. When present, dysphagia and interstitial lung disease are usually associated with a worse prognosis. The majority of myositis patients have a chronic disease with exacerbations and remissions, requiring treatment and regular follow up over many years. In some patients, more often with DM, the disease may improve to the extent that long-term remission without the need for treatment is achieved. Since the association with malignancy exists, patients should be evaluated for a possible tumour occurrence by different screening methods, including chest X-ray, abdominal ultrasonography, laboratory examinations and, in selected cases, by positron emission tomography. If pathology occurs, then this should be thoroughly investigated by appropriate and more sophisticated methods.

## 4 Follow up

*Clinical observations*

Disease activity should be assessed periodically. The use of Core set measures developed by IMACS (International myositis assessment and clinical studies group) is recommended [4]. These include visual analogue scale (VAS) for physician and patient, manual muscle testing (MMT), health assessment questionnaire (HAQ), muscle enzymes and specific tools to assess extramuscular activity. In clinical studies, a positive response to therapy is achieved, when 3 of any 6 measures improve by 20 % or more, with no more than 2 worsened by 25 % or more, one of which cannot be MMT. Similarly, disease damage can be assessed once a year using core set measures which include VAS by patient and physician, MMT, HAQ and myositis damage index for extramuscular involvement [5].

*Expectations*

A majority of patients have relapsing-remitting course or chronically progressive illness, although some may have monophasic disease and go into full and permanent remission. Generally, patients with anti-SRP antibodies have a poor prognosis. Patients with anti-synthetase antibodies, where interstitial lung disease may predominate the clinical features, often respond to immunosuppressive treatment, but they also often have a protracted course with a high risk of relapse when attempts are made to stop treatment.

*Blood tests*

Serum levels of "muscle enzymes" particularly CK, but also LDH, aldolase, ALT, AST are periodically measured. Serum myoglobin levels may also be used. Although frequently helpful, it is accepted that serum levels of these proteins only partially assess the activity, and should not be used as a sole measure of disease activity. Cases of patients with low levels of CK despite active disease exist and other patients may have an elevated CK despite a low degree of disease activity.

## 5 Management

The mainstay for pharmacological treatment is glucocorticoids. Prednisone is usually given at a dose of around 0.75- 1 mg/kg/day and the treatment is continued for a prolonged period of time with tapering over months, guided by disease activity. Prognosis is worse if the effective treatment is delayed and side effects of the high doses of glucocorticoids are common, therefore it is recommended that glucocorticoids are combined with another immunosuppressive drug. The most frequently used are methotrexate or azathioprine. If these are not effective or not tolerated,

there are reports where cyclosporine, cyclophosphamide, mycophenolate mofetil have proven efficacious. Alternatively combinations of methotrexate with azathioprine or cyclosporine could be used when a single therapy is not effective [6]. For patients with interstitial lung disease there are case reports or case series to suggest that cyclophosphamide, cyclosporine or tacrolimus may be effective. Intravenous immunoglobulins are advocated for resistant cases, but not all reports are positive. Plasmapheresis and leukapheresis have not shown efficacy. Several small series or case reports suggest that rituximab may have good potential, but a recent large controlled trial has not reached the primary endpoint. Anti-TNF drugs were initially described as effective, but more recent studies are negative and worsening of disease has even been described. Exceptionally, some patients may benefit from autologous stem cell transplantation. Inclusion body myositis is usually unresponsive to glucocorticoids and also to other immunosuppressive drugs. Pharmacological treatment is combined with exercise, which should be supervised by experienced physiotherapists and individualised to the patient's situation.

Most patients with myositis respond to treatment to a certain extent. When treatment-resistant inflammatory myopathy presents, it is always necessary to reconsider the original diagnosis [7].

## 6 Diagnostic tests

There is no single diagnostic test in myositis, and although detection of autoantibodies is helpful, they are present only in about 60–80 % of cases.

Various techniques are employed in the detection of autoantibodies specific for or associated with myositis. Indirect immunofluorescence on HEp-2 cells detects antinuclear or, frequently, anticytoplasmic autoantibodies (Table 3). These autoantibodies must be subsequently identified by specific tests, e. g., the Elisa technique with purified or recombinant antigens. Line or dot-blot immunoassays with spotted autoantigens on nitrocellulose paper are increasingly popular. Immunodiffusion or counterimmunoelectrophoresis can also be used; these techniques require comparison of a precipitin line with one obtained using standard serum of known autoantibody specificity. Some laboratories use Western blotting for autoantibody detection, where nuclear or cytoplasmic cell extracts are electrophoresed in the polyacrylamide gel and transferred to nitrocellulose paper. Strips of nitrocellulose are then incubated with patients' sera and bound autoantibody detected using enzyme immunoassay (Fig. 2). Several of the myositis autoantibodies do not react in these assays and immunoprecipitation of proteins or nucleic acids is used in their detection. In the protein assay, serum antibodies are bound to protein A-Sepharose beads, which are then mixed with $^{35}$S-methionine-labeled cell extract. Immunoprecipitated proteins on the beads are subjected to polyacrylamide gel electrophoresis and developed by autoradiography. In the case of RNA assay the resulting immunoprecipitates are electrophoresed in the gel and subsequently

**Table 3.** Autoantibodies in myositis.

| | Antigen | Frequency in myositis | Clinical association |
|---|---|---|---|
| **Myositis specific** | | | |
| **Anti-ARS** | | | |
| Anti-Jo-1 | Histidyl-tRNA synthetase | 15–30 % | ASS |
| Anti-PL-7 | Threonyl-tRNA synthetase | 5–10 % | ASS |
| Anti-PL-12 | Alanyl-tRNA synthetase | < 5 % | ASS |
| Anti-EJ | Glycyl-tRNA synthetase | 5–10 % | ASS |
| Anti-OJ | Isoleucyl-tRNA synthetase | < 5 % | ASS |
| Anti-KS | Asparaginyl-tRNA synthetase | < 5 % | ILD, arthritis |
| Anti-Zo | Phenylalanyl-tRNA synthetase | < 1 % | ASS |
| Anti-YRS | Tyrosyl-tRNA synthetase | < 1 % | ASS |
| **Anti-SRP** | Signal recognition particle 6 peptides | 5–10 % | Necrotising myositis |
| **Anti-Mi-2** | 218/240 kDa helicase family proteins | 5–10 % | DM |
| **Anti-p155(/140)** | Transcriptional intermediary factor 1γ | 9–21 % | Only DM, frequently CDM (50–75 %) |
| **Anti-CADM- 140** | RNA helicase encoded by MDA-5 | 19 % of DM | C-ADM (ILD) |
| **Anti-SAE** | Small ubiquitin-like modifier | 4 % (8 % in DM) | Severe skin in DM, ILD |
| **Anti-p140 (Anti-MJ)** | Nuclear matrix protein (NXP-2) | 23 % of JDM | JDM, Calcinosis |
| **Myositis associated** | | | |
| **Anti-PM-Scl** | Nucleolar protein complex of 11–16 proteins | 8–10 % | PM, DM, overlap with Scl |
| **Anti-U1-RNP** | Small nuclear RNP | 10 % | MCTD |
| **Anti-Ku** | 70/80 kDa DNA-PK regulatory subunit | < 20 | Overlap with scleroderma |
| **Anti-Ro (52, 60)** | hY RNA + peptides | 10–40 % | |

**ASS**, antisynthetase syndrome; **PM**, polymyositis; **DM**, dermatomyositis; **CDM**, cancer associated DM; **Scl**, scleroderma; **ARS**, aminoacyl-tRNA synthetase; **SRP**, signal recognition particle; **RNP**, ribonucleoprotein; **DNA-PK**, DNA dependent protein kinase; **hY**, human cYtoplasmic; **ILD**, interstitial lung disease; **MCTD**, mixed connective tissue disease; **C-ADM**, clinically amyopathic dermatomyositis

silver stained. These tests are used only by few specialized laboratories. They can be considered as the most reliable techniques for confirmation. This approach has enabled discovery of several new autoantibodies in myositis sera.

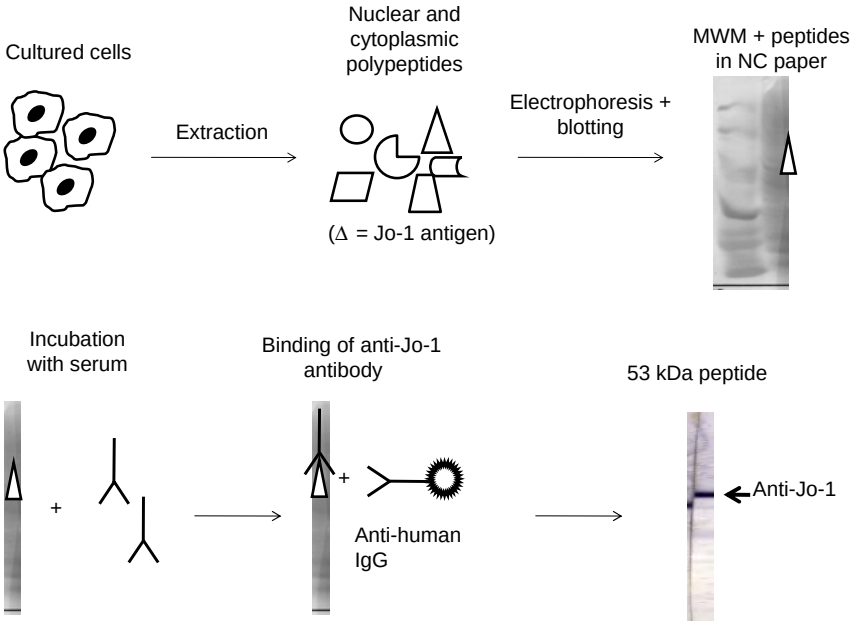


**Figure 2.** Test principle for detection of autoantibodies by Western blotting. Polypeptides are extracted from the cells (e.g. HeLa cells) and then divided by electrophoresis in polyacrylamide gel according to their molecular weight. Peptides are then blotted from gel to nitrocellulose paper, which is cut into strips. Every strip is incubated with patient's serum and antibody bound to peptide is then visualized with labelled anti-human IgG and developed with substrate. As an example, Jo-1 antigen (△) is delineated. The result then shows a positive band on the strip of the molecular weight typical for Jo-1 antigen.
**MWM**, molecular weight markers; **NC**, nitrocellulose.

## 7 Testing methods

*Benefits*

A positive test for an autoantibody which is myositis-specific or associated with myositis greatly contributes to the diagnostic workout and in many cases also helps in prediction of prognosis. Since typical autoantibodies cannot be found in all myositis patients, a negative result for autoantibodies does not, however, exclude the diagnosis.

The fact that several different assays to detect myositis autoantibodies are available, may be considered a limitation, since different tests differ greatly in their sensitivity and, although not formally compared, our experience suggests there may be discrepant results between individual assays. Therefore, for detection of autoantibodies related to myositis, extra caution is recommended in interpretation of the results and comparison of several detection methods should be used for a final declaration of positivity. Immunoprecipitation techniques usually require the use of radioactivity or a sophisticated procedure and therefore are not routinely available in clinical practice.

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# Myositis overlap syndromes

Jiří Vencovský, Ingrid E. Lundberg, Cees Kallenberg, Rudolf Mierau

## 1 Introduction

Some patients with myositis may also have clinical or laboratory signs and symptoms of another, defined, connective tissue disease, such as systemic lupus erythematosus (SLE), scleroderma, rheumatoid arthritis, mixed connective tissue disease (MCTD) or Sjögren's syndrome. The term "overlap syndrome" is then used for such patients to recognise the differences in clinical course, prognosis and management. Classification into overlap syndromes is facilitated by detection of autoantibodies associated with each of these syndromes. The main overlap syndromes, with myositis as one of the important features, include mixed connective tissue disease (MCTD, Sharp's syndrome), antisynthetase syndrome (ASS), polymyositis/scleroderma overlap (PM/Scl) associated with anti-PM/Scl antibody and scleroderma/polymyositis Scl/PM associated with anti-Ku antibody (Tables 1, 2) [1].

The prevalence of overlap syndromes is unknown — for MCTD it is probably around 10/100 000. ASS constitutes about 30 % of polymyositis and dermatomyositis cases. In MCTD the female:male ratio is about 9 : 1, in ASS 2.7 : 1.

## 2 Diagnostic measurements for experts

The MCTD is characterised clinically by Raynaud's phenomenon, so called puffy hands, sclerodactyly, arthritis, oesophageal dysmotility and myositis. A prerequisite for diagnosis of MCTD is the presence of high titre of anti-U1-RNP autoantibodies. This was defined originally in haemagglutination assay, which is no longer employed by most laboratories. Therefore this criterion is not currently associated with a specified titre and requires a statement of clearly positive, high levels of anti-U1-RNP instead. U1-RNP contains A, C and p68 antigens, and in contrast to SLE, MCTD is associated particularly with antibodies against the latter antigen. Occasionally, high levels of anti-p68 are also found in SLE and these patients often have some clinical features of MCTD such as myositis, fibrosing alveolitis, Raynaud's phenomenon and sclerodactyly. The presence of anti-Sm or anti-dsDNA antibodies indicates that SLE — with a much higher prevalence of nephritis and of SLE

**Table 1.** Signs and symptoms of MCTD, ASS and PM+Scl overlaps [1, 4, 5].

| Clinical features | | % of patients |
|---|---|---|
| **MCTD** | Arthritis/arthralgia | 95 |
| | Raynaud's phenomenon | 85 |
| | Decreased oesophageal motility | 67 |
| | Impaired pulmonary diffusing capacity | 67 |
| | Swollen hands | 66 |
| | Myositis | 63 |
| | Lymphadenopathy | 39 |
| | Skin rash | 38 |
| | Sclerodermatous changes | 33 |
| | Fever | 33 |
| | Serositis | 27 |
| | Splenomegaly | 19 |
| | Hepatomegaly | 15 |
| | Neurologic abnormalities | 10 |
| | Renal disease | 10 |
| **ASS** | Myositis | > 85 |
| | Interstitial lung disease | 89 |
| | Arthritis | 94 |
| | Raynaud's phenomenon | 67 |
| | Fevers | 87 |
| | Mechanic's hands | 71 |
| **PM/Scl (Anti-PM/Scl)** | Raynaud's phenomenon | 100 |
| | Arthritis/arthralgia | 97 |
| | Myositis | 88 |
| | Lung fibrosis | 78 |
| | Sclerodactyly | 97 |
| | Sjögren's syndrome | 34 |
| | Dermatomyositis rash | 38 |
| | Dysphagia | 78 |
| | Calcinosis | 47 |
| **Scl/PM (Anti-Ku)** | Raynaud's phenomenon | 86 |
| | Limited scleroderma | 84 |
| | Diffuse systemic scleroderma | 16 |
| | Myositis | 40 |

**MCTD**, mixed connective tissue disease, **ASS**, antisynthetase syndrome, **PM/Scl**, polymyositis/scleroderma overlap syndrome.

typical skin reactions — could be diagnosed. In order to diagnose myositis, muscle weakness should be demonstrated by manual muscle testing, and evidence of elevation of muscle enzymes, electromyographic myogenic changes and inflammation in muscle biopsy should be provided. The major cause of morbidity and mortality in patients with MCTD is pulmonary hypertension, which should be suspected in patients with dyspnoea and should indicate tests for pulmonary arterial pressure.

The antisynthetase syndrome (Table 1) is associated with one of the currently known antisynthetase autoantibodies (see chapter polymyositis and dermatomyositis). The clinical features are characterised by interstitial lung disease, myositis, Raynaud's phenomenon, fevers, non-erosive arthritis and a skin rash on the hands (so called mechanic's hands, Fig. 1). As interstitial lung disease is highly associated with anti-synthetase antibodies and is important for prognosis, all these patients should be evaluated for possible pulmonary involvement. Lung function tests, including diffusing capacity of the lung for carbon monoxide (DLCO), and high resolution computer tomography (HRCT) should be performed. HRCT will show inflammatory alveolitis or advanced changes including fibrosis. In some cases, interstitial lung disease is the presenting symptom and precedes manifestation of muscle disease by several months.

In patients presenting overlap features between scleroderma and myositis, particular attention should be paid to detection of anti-PM/Scl and anti-Ku antibodies.

**Table 2.** Diagnostic criteria for MCTD [3].

| **Clinical criteria** |
|---|
| • Oedema of the hands |
| • Synovitis |
| • Myositis |
| • Raynaud's phenomenon |
| • Acrosclerosis |
| **Laboratory criteria** |
| • Positive anti-nRNP at a high concentration* |

Requirements for the diagnosis: Serologic criterion + at least 3 clinical
(In the case that oedema, Raynaud's phenomenon and acrosclerosis are combined, then 4 clinical criteria are required).
*Original text stated: Positive anti-nRNP at a haemagglutination titre of 1:1600 or higher.

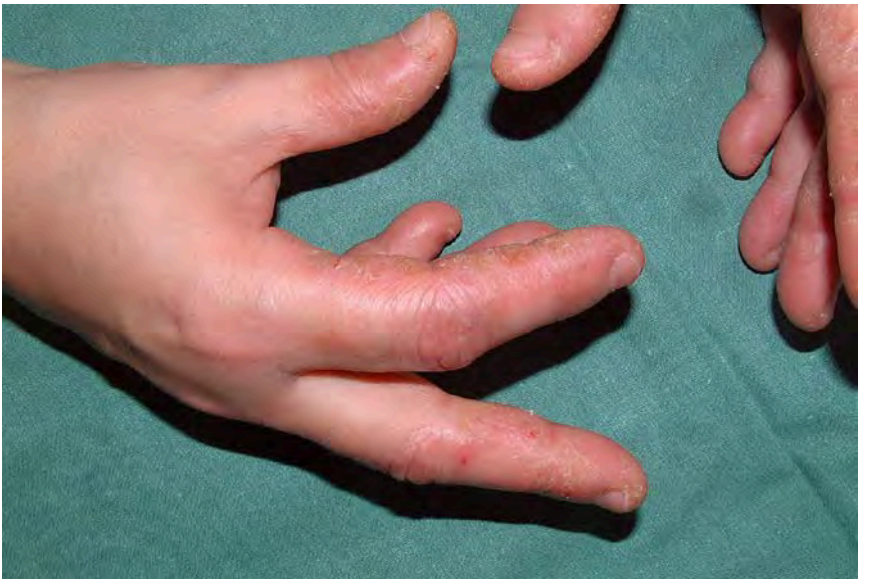


**Figure 1.** Mechanic's hands in a patient with antisynthetase syndrome.

## 3 Requirements for family practitioners

Frequency of individual clinical symptoms in MCTD varies (Table 1) and they are usually not present at the same time, but appear sequentially [2]. The earliest signs are swollen hands with puffy fingers, arthritis, Raynaud's phenomenon, myositis and sclerodactyly. Arthritis is very common and may be quite severe leading to deformities. It is usually non-erosive, but occasionally marginal erosions or even large destructions may develop. MCTD was originally described as a relatively benign disease, but this has changed during the last 20 years, and it is known that some patients have renal, cerebral, pulmonary and cardiac involvement. Particularly significant is pulmonary hypertension, because it is the most frequent cause of death in these patients. Trigeminal neuropathy may also be present. The fact that several different diagnostic criteria sets were proposed for the disease [3] reflects the heterogeneity of the patients and also some controversies about the real existence of the syndrome. The latter fact arises from longitudinal observations showing development of clearly defined rheumatoid arthritis, systemic lupus erythematosus or scleroderma in some patients. However, most of the patients fulfilling the criteria for MCTD have a distinct syndrome.

ASS usually presents acutely, with myositis, fever, dyspnoea, arthritis, mechanic's hands (Table 1) [4]. Interstitial lung disease is sometimes the leading symptom with myositis being found only when specifically looked for or not

present at all. But in many cases myositis is quite severe. Patients usually have symmetrical polyarthritis of hands and wrists, which resembles rheumatoid arthritis, however feet are usually spared and there are no X-ray erosions. The disease has moderate response to therapy and tends to flare after tapering. ASS patients do not usually have renal and CNS disease. Serositis is also rare in ASS.

Patients with anti-PM/Scl antibodies have myositis or scleroderma, mostly with limited cutaneous involvement, or both diseases. Pulmonary involvement is less severe than in ASS and patients usually have a good prognosis [5].

Anti-Ku antibodies may be detected in scleroderma-polymyositis overlap syndrome and these patients represent a group characterized by Raynaud's phenomenon, sclerodermatous skin changes restricted to the extremities, inflammatory myopathy responsive to glucocorticoids, occasional extramuscular inflammation, and good prognosis.

When initial signs and symptoms hinting at a myositis overlap syndrome (muscular weakness, general weakness or fatigue, fever, CK elevation, articular swelling or effusion, Raynaud's phenomenon, dyspnea, skin changes typical for SLE, dermatomyositis or scleroderma, cytopenia, or combinations of these symptoms, particularly when together with antinuclear antibodies) are detected, the patient should be referred to a specialist for further diagnostic steps. Depending on which symptoms are prominent, this preferentially should be a rheumatologist, a neurologist, or a dermatologist.

## 4 Follow up

*Clinical observations*

In MCTD the overlapping features develop sequentially and patients should be investigated for their presence. Particular attention should be given to pulmonary hypertension, which may develop in the absence of interstitial lung disease and may progress rapidly. Other organs may be affected as the disease progresses and occasionally patients may develop symptoms and signs compatible with another defined rheumatic disease entity and therefore renal, cardiac, CNS, and gastrointestinal status should be checked periodically.

In ASS the follow up is similar to PM/DM patients with particular attention to interstitial lung disease.

*Expectations*

For many MCTD cases the prognosis is favourable and patients usually respond to glucocorticoid treatment. Some patients develop pulmonary hypertension, which may lead to death rapidly. More rarely myocarditis, renovascular hypertension, and cerebral haemorrhage appear. Articular disease can sometimes cause significant

deformities. In most cases the disease goes into a remission over time with low inflammatory disease activity and the anti-RNP antibodies may disappear.

Patients with ASS often have relapsing myositis and arthritis. Interstitial lung disease may stabilise with treatment; however, in some patients it may be progressive and the prognosis is somewhat worse than in MCTD.

*Blood tests*

Complete blood count is used to look for leucopenia or thrombocytopenia. Urine is checked for proteinuria. Serum levels of "muscle enzymes" are measured in order to assess muscle involvement.

Patients with MCTD usually have extremely high levels of gammaglobulins and IgG, which may decrease during treatment and increase with flare. Acute phase proteins may be abnormal, particularly when arthritis is present, but frequently there is only mild elevation. Complement levels are usually normal. Rheumatoid factors are found in about 70 % of MCTD patients.

The autoantibodies typical for the overlap syndromes usually persist during disease course, and their specificity doesn't change much. Although anti-Jo-1 has been shown to fluctuate in correlation with disease activity in longitudinal measurements, frequent re-measurements of the myositis overlap typical autoantibodies in general are of no value in the long term management of the patient.

## 5 Management

Treatment of MCTD follows the approaches used in systemic lupus erythematosus, polymyositis, scleroderma, and rheumatoid arthritis and is dependent on the presenting symptoms or pattern of organ involvement. In some patients nonsteroidal antirheumatic drugs may suffice, but in the majority, various doses of glucocorticoids are necessary. When used in appropriate doses the treatment is usually successful. Immunosuppressive drugs may have to be used, such as cyclophosphamide, azathioprine, methotrexate, and hydroxychloroquine depending on the organ involvement and degree of reversibility of the symptoms. Raynaud's phenomenon is difficult to treat; calcium channel blocker nifedipine, angiotensin-II receptor blocker losartan or intravenous prostacyclins may be effective. In pulmonary hypertension endothelin receptor antagonists bosentan or sitaxentan may be used. Some patients benefit from the use of phosphodiesterase-5 inhibitor sildenafil. Long-term anticoagulation is recommended. The tyrosine kinase inhibitor imatinib mesylate has recently been shown to improve pulmonary fibrosis in MCTD.

Treatment of ASS is the same as described in the polymyositis and dermatomyositis chapter.

## 6 Diagnostic tests

There is no single diagnostic test in overlap syndromes and diagnosis in these conditions is dependent on a number of clinical variables and blood tests from which autoantibodies are particularly helpful.

Anti-U1-RNP antibodies produce a speckled nuclear pattern (Fig. 2) in indirect immunofluorescence on HEp-2 cells (IIF) or other substrates and are detected in high titres of around 1 : 1000 or more. They were originally described as antibodies reactive with ribonuclease-sensitive extractable nuclear antigen (ENA), which distinguished them from anti-Sm antibodies that reacted with ENA even after ribonuclease treatment. Anti-U1-RNP may be detected by immunodiffusion or counterelectrophoresis using ENA, by Elisa or LIA assay with purified or recombinant antigens, immunoblotting or immunoprecipitation with $^{32}$P labelled extracts. Line immunoassays usually detect anti-U1-RNP reliably. U1-RNP contains C, A and p68 antigens and MCTD is particularly characterised by anti-p68. Anti-p68 very often is accompanied by antibodies to BB' which should not be interpreted as anti-Sm activity unless a concomitant reaction with anti-Sm-D is observed. The levels of anti-U1 RNP antibodies do not seem to correlate with disease activity, but may decrease or disappear after long disease duration.

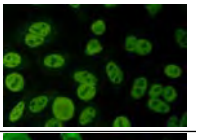
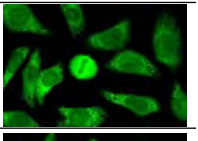
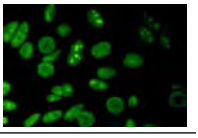
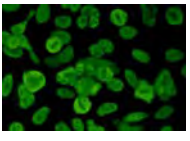
| Autoantibody | Immunofluorescence on HEp-2 cells | |
|---|---|---|
| U1-RNP | speckled, or coarse granular; no staining of nucleoli and metaphase chromosomes; high titer |  |
| Jo-1 | granular cytoplasmic; no nuclear staining (unless other ANA are simultaneously present) |  |
| PM-Scl | nucleolar, plus fine granular staining of nucleoplasm, no staining of metaphase chromosomes; often low titer |  |
| Ku | fine granular, no staining of metaphase chromosomes; mostly high titer |  |

**Figure 2.** Immunofluorescence findings on HEp-2 cells typical for autoantibodies in myositis overlap syndromes.

Antisynthetase antibodies found in ASS produce a fine speckled cytoplasmic pattern in IIF (Fig. 2). There are 8 autoantibodies currently recognised, from which anti-Jo-1 is by far the most frequent (see polymyositis and dermatomyositis chapter). Serum levels of anti-Jo-1 tend to correlate with the activity of the disease.

Anti-PM/Scl produce a homogeneous nucleolar pattern on IIF (Fig. 2). They can be detected by immunodiffusion or immunoprecipitation, but are nowadays detected mostly by line or enzyme immunoassays with native or recombinant antigens or peptides. Autoantibodies are directed predominantly against two molecules of 100 kDa (100 %) and 75 kDa (60 %).

Anti-Ku antibodies produce a speckled nuclear pattern sparing nucleoli on IIF (Fig. 2). Some laboratories use counterimmunolectrophoresis, but line immunoassay is available.

## 7 Testing methods

*Benefits*

For detection of antibodies to U1-RNP, Jo-1, PM/Scl and Ku, commercial assays are available. Indirect immunofluorescence can suggest the type of autoantibody. Anti-U1-RNP and anti-Jo-1 can be measured quantitatively by Elisa.

*Limitations*

For anti-U1-RNP and anti-Jo-1 many assays with usually good reliability are available. There are fewer opportunities to detect anti-PM/Scl and particularly anti-Ku. These assays must be validated in each laboratory with known antibody specificities to ensure reliable performance.

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# Sjögren's syndrome

Torsten Witte, Pier-Luigi Meroni

## 1 Introduction

Henrik Sjögren described the syndrome in 1933 as a combination of dry eyes and mouth in patients with rheumatoid arthritis. Sjögren's syndrome is a frequent autoimmune disorder (prevalence 0.5–2 %) characterised by lymphocytic infiltration of the salivary and lacrimal glands and leads to dry eyes and mouth, the Sicca syndrome. Secondary Sjögren's syndrome is associated with a connective tissue disease or rheumatoid arthritis, whereas primary Sjögren's syndrome is not associated with other disorders.

The differential diagnosis of Sjögren's syndrome and other causes of the Sicca syndrome is difficult. Sicca syndrome may be a consequence of aging, infections (hepatitis C, HIV), sarcoidosis, or iatrogenic (more than 200 drugs such as tricyclic antidepressants or beta-blockers reduce the saliva and tear flow) and affects up to 10 % of the population [1]. The signs and symptoms of Sjögren's syndrome are summarized in Table 1.

**Table 1.** Signs and symptoms of Sjögren's syndrome.

| Signs of glandular manifestation | Signs of extraglandular disease |
|---|---|
| Constant thirst | Arthritis |
| Feeling of dry eyes | Polyneuropathy |
| Recurrent conjunctivitis | Palpable purpura |
| Increased rate of upper airway infections | Raynaud's phenomenon |
| Parotid swelling | |

## 2 Diagnostic criteria

Numerous sets of classification criteria have been proposed, including the San Diego, Copenhagen, Greek or Japanese criteria. In 1993 the preliminary criteria of a European study group formed by members of 26 centres from 12 countries

were proposed [2]. Since then, these criteria have been revised several times. More recently, new American/European consensus criteria were developed from the original criteria and now are widely used [3].

The diagnosis of primary SS requires 4 of the 6 criteria in Table 2, furthermore either criterion 4 or 6 must be included. The diagnosis of SS can be made in patients who have no Sicca symptoms, if 3 out of the 4 objective criteria are fulfilled.

**Table 2.** The classification criteria of Sjögren's syndrome.

| | |
|---|---|
| 1. Ocular symptoms | – Daily feeling of dry eyes for more than 3 months<br>– Recurrent foreign-body sensation<br>– Tear substitutes are used more than 3 times per day |
| 2. Oral symptoms | – Daily feeling of dry mouth for more than 3 months<br>– Recurrently or persistently swollen salivary glands as an adult<br>– Liquids are frequently used to aid swallowing dry food |
| 3. Ocular signs | – Schirmer's I test performed without anaesthesia ($\leq$ 5 mm in 5 min)<br>– Positive vital dye staining results (van Bijsterveld score of more than 4) |
| 4. Positive lip biopsy findings | – Focal lymphocytic sialadenitis with a focus score more or equal 1 [6] |
| 5. Oral signs | – Abnormal salivary scintigraphy findings<br>– Abnormal parotid sialography findings (diffuse sialectasies without obstruction in the major ducts)<br>– Abnormal sialometry findings (unstimulated salivary flow $\leq$1.5 mL/15 min |
| 6. Autoantibodies | – Positive SSA and/or SSB antibody results |

## 3 Diagnostic measurements for experts

Since complaints about dry eyes and mouth are common, even in the absence of objective problems, the verification of dry eyes and mouth is crucial in the diagnostic work-up of Sjögren's syndrome. Various tests have been proposed:

### 3.1 Tests to verify dry mouth

*Salivary gland scintigraphy*

The uptake and secretion of sodium pertechnetate technetium Tc 99m correlates with salivary flow rates and is a good way to measure salivary gland dysfunction. In this test, $^{99m}$Tc-pertechnetate is injected intravenously. 15 min after injection, diluted lemon juice is administered orally as a stimulator of the glands. Subsequently, the uptake, activity and washout of the marker in the parotid and submandibular glands is recorded.

*Sialography*

Diffuse sialectasis may be seen after injection of radiopaque material into the salivary glands. This test is not specific for SS, however sialography using water-soluble media can exhibit sensitivity and specificity ratios similar to that of the biopsy of minor salivary glands. The diagnostic value of parotid sialography for diagnosing SS greatly depends on the skills of the observer. Sialography can exclude obstructions as a differential diagnosis for SS.

*Sialometry*

In this test, the patients have to swallow all the saliva in their oral cavity and then two cotton balls are placed on the mouth floor, close to the gingival border, where they remain for 15 minutes. Before and after, the weight of the cotton balls is compared. The weight difference is changed from g/min to ml/min, and a saliva production of less than 0.1 ml/min is regarded as reduced. Sialometry is a low cost test, a good measure of the degree of decreased salivary flow and helps to establish xerostomia. It does not distinguish Sjögren's syndrome from other causes of dry mouth.

*Saxon's test*

This test is a stimulated variant of sialometry. A sterile 10 × 10-cm gauze sponge is folded twice at 90° angles (final size 5 × 5 cm) and placed in a sterile, screw topped 60-ml plastic tube, so that the dry gauze and tube can be weighed. The patient has to swallow to remove any pre-existing oral fluid, then the saliva is collected by asking the patient to chew on the gauze for 2 minutes. Afterwards, the patient replaces the gauze into the same tube, and the amount of saliva produced in 2 minutes can be determined by subtracting the original weight from the weight obtained after chewing.

### 3.2 Tests to verify the dry eyes

*Schirmer's I test*

In a Schirmer test, a bent piece of Whatman No. 41 filter paper is placed in the outer one-third of the lower lids of both eyes for exactly 5 minutes. The strip can then be removed, and the length of the strip that was moistened by tears can be measured. A definitive positive (pathologic) result is less than or equal to 5 mm after 5 minutes. This test can be useful to help exclude or confirm significant dryness of the eyes, however it is not specific for Sjögren's syndrome. Furthermore, false-positive results occur.

*Rose bengal staining*

Rose bengal is an aniline dye that stains devitalized cells. Slit-lamp examination is performed after rose bengal staining to detect abnormal uptake in the cornea. The observer semi-quantitatively ranks the degree of epithelial defects on a scale from 0 to 9 on each eye. A score (the van Bijsterveld score) of at least 4 points is regarded as pathologic.

### 3.3 Salivary gland biopsy

Minor salivary glands can be removed from an incision of the lower inner lips of the patient and the degree of lymphocytic infiltration can be evaluated histologically. At least 4 salivary gland lobules should be obtained for analysis. The biopsy is the most definitive test for Sjögren's syndrome. Biopsy is not always necessary, but, when the diagnosis is in doubt or if a definitive diagnosis is needed and has a therapeutic consequence, it may be helpful. Biopsy can also help in the differential diagnosis of sarcoidosis. Focal aggregates are the hallmark of Sjögren's syndrome. A focal aggregate consists of at least 50 lymphocytes (predominantly $CD4^+$ cells and, to a lesser extent, plasma cells and macrophages). At least 1 focal aggregate per 4 $mm^2$ is regarded as pathologic.

## 4 Requirements for family practitioners

Complaints of dry eyes and dry mouth are extremely common and indeed, more than a third of elderly persons have Sicca symptoms. A common explanation for Sicca symptoms is the use of medications interfering with the gland function, such as antidepressants, anticholinergics, beta-blockers, diuretics, and antihistamines. In the general population these complaints do not correlate with the objective symptoms of dry eyes and mouth. Complaints of Sicca syndrome are associated with depression and fibromyalgia. It is therefore crucial in the diagnostic work-up of patients complaining of dry eyes and mouth, to confirm the complaints by objective tests (such as Schirmer's test or sialometry). When an autoimmune origin

of Sicca syndrome is suspected, the patient should be referred to a rheumatologist for further evaluation. Here, the autoimmune character of Sjögren's syndrome must be verified either by confirmation of the presence of antibodies against SSA and/or SSB in the serum or by salivary gland biopsy.

In the majority of patients, SS is a benign disorder. However, one third of patients have additional extraglandular complications (arthritis/arthralgia, polyneuropathy, vasculitis, purpura, pneumonitis, interstitial lung disease, haematologic involvement).

In addition, patients with Sjögren's syndrome have a higher prevalence of malignant non-Hodgkin lymphoma, in a recent European study as high as 4.3 %. Cryoglobulins and complement consumption are unfavourable prognostic parameters for the development of lymphoma. Patients with these laboratory abnormalities should be followed for development of lymphoma, in particular when anaemia, fever or weight loss occur.

## 5 Follow up

Most patients can be monitored at follow-up visits every 3 months and, if the patient is stable, up to every 6 months. If acute complications such as vasculitis occur, inpatient care may be appropriate, or follow-up visits must be performed at shorter intervals. During follow-up visits, specific attention must be paid to the efficacy of treatment and to new complications. In general, severe complications such as vasculitis with palpable purpura, leukopenia, renal insufficiency, occur early in the course of the disease, whereas malignancies (non-Hodgkin lymphoma) may develop any time.

Female patients with antibodies against SSA/Ro have an increased risk of complications including neonatal lupus during pregnancy. The risk of congenital heart block is about 2 %, but if one child develops congenital heart block, the risk for congenital heart block during a subsequent pregnancy is approximately 15–20 %.

The prognosis of pSS is generally good, provided there is no malignancy and no severe organ involvement.

## 6 Management

Treatment is mostly symptomatic [4]. In order to treat dry eyes, artificial tears should be applied. If artificial tears are used at least four times per day, the patients should use a preparation free of preservatives to avoid eye irritation. In very severe cases, temporary plugging of the lacrimal puncta can be performed. Patients should avoid rooms with dry air, not work at a computer for extended periods without a break, and avoid medications with anticholinergic or antihistaminic effects. In order to treat dry mouth, patients should always have liquids available. Sugar-free lemon drops or bubble gums help to stimulate the saliva flow.

Patients should visit a dentist frequently and carefully clean the teeth. In order to treat dry skin problems, skin creams or lotions may be applied. Females may use vaginal lubricants, postmenopausal women vaginal oestrogen creams.

## 7 Medication

Pilocarpine and cevimeline can stimulate the salivary and lacrimal glands, but many patients complain of side effects such as sweating, diarrhoea and tachycardia.

Whether or not hydroxychloroquine improves the inflammation of glands and the production of saliva and tears has not been clearly established. According to our own experience, it may be beneficial in the early course of the disease when the glands have not yet been completely destroyed. Hydroxychloroquine and NSAIDs are helpful in arthritis as a complication of pSS. Immunosuppressive agents such as cyclophosphamide or azathioprine in combination with corticosteroids are indicated in major organ involvement (vasculitis with neuropathies, glomerulonephritis, interstitial lung disease), but are not useful against dry eyes and mouth. In a recent placebo-controlled study, B cell depletion by rituximab improved both glandular as well as extraglandular manifestations of Sjögren's syndrome [5]. Rituximab may therefore also be considered in severe extraglandular manifestations of Sjögren's syndrome.

## 8 Diagnostic tests and testing methods

Antinuclear antibodies (ANA), measured by immunofluorescence using HEp2 cells, are present in more than 80 % of pSS patients, but also in up to 20 % of the general population.

ANA are directed against SSA/Ro in approximately 75 % of the patients. These autoantibodies should be identified when Sjögren's syndrome is suspected. Several techniques have been described to detect anti-SSA/Ro (and anti-SSB/La): counterimmunoelectrophoresis (CIE), Western Blot (WB), dot blot (DB) and Elisa. The use of two assays offers the best results in terms of sensitivity and specificity. Antibodies against SSA/Ro are used in the classification of the disorder, but are also present in 50 % of SLE patients and in 1 % of healthy individuals.

Antibodies against SS-B/La are present in 30–50 % of patients with primary SS and in 15–25 % of patients with SLE. Antibodies against SS-B/La rarely occur alone, but usually are observed in patients with antibodies against SSA/Ro.

Rheumatoid factors are frequently found in pSS, but also in 5 % of the population.

## 9 Further laboratory tests

In patients with vasculitic purpura, cryoglobulins should be measured. Sjögren's syndrome is associated with autoimmune thyroid disease in up to a third of patients. When hypothyroidism is suspected, thyroid-stimulating hormone (TSH) should be measured. In addition, S-electrophoresis helps to detect monoclonal gammopathies and complete blood count should be performed periodically to detect leukopenia.

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# Undifferentiated connective tissue diseases (UCTD)

Falk Hiepe

## 1 Definition

UCTD is an oligosymptomatic connective tissue disease with a limited autoantibody repertoire that does not meet the classification criteria of any specific connective tissue disease such as systemic lupus erythematosus (SLE), Sjögren's syndrome (SS), systemic sclerosis (SSc), autoimmune myositis or mixed connective tissue disease (MCTD). Only a minority of patients with UCTD will develop a defined connective tissue disease.

## 2 Epidemiology

Data regarding prevalence and incidence of UCTD are not available although Mosca et al noted that 20 %–52 % of patients in rheumatology clinics with a CTD may have UCTD. Mainly women suffer from this disease (female : male ratio is 20 : 1). The mean age at disease onset is 32 years (range 7–72 years).

## 3 Clinical manifestations

The course of the disease is mild. Each patient exhibits few clinical manifestations, mainly arthralgia (66 %), arthritis (32 %), Sicca symptoms (30 %), Raynaud's phenomenon (30 %), leukopenia (19 %), photosensitivity (17 %), anaemia (15 %), oral ulcerations (14 %) and alopecia (13 %). Severe organ manifestations e. g. of CNS or kidneys are uncommon. Most patients present manifestations similar to SLE, SSc or SS with a related ANA profile but do not meet the corresponding classification criteria.

## 4 Laboratory

Inflammatory parameters (ESR, gamma globulins) may be slightly or moderate increased. CRP levels are often normal. Anaemia, leukopenia and thrombocytopenia may occur. Some patients have low C3 and/or C4 levels.

Autoantibody profile: Antinuclear antibodies (ANA) detected by indirect immunofluorescence on HEp2 cells are positive in all patients. The ANA titre is usually low. Detailed analysis of the ANA antibodies usually identifies Anti-Ro/SSA, anti-La/SSB, anti-centromere or anti-U1-RNP antibodies. A patient will usually present with only one specificity of ANA antibody. Anti-dsDNA antibodies are rarely detectable. Some patients show ANA positivity without any specific antibodies able to be identified.

**Table 1.** Preliminary Classification Criteria for Undifferentiated Connective-Tissue Disease.

| **Inclusion Criteria** | Clinical manifestations which may be considered specific to a defined CTD and are thus excluders of UCTD* | Laboratory markers which may be considered specific to a defined CTD and are thus excluders of UCTD* |
|---|---|---|
| 1. Signs and symptoms suggestive of a CTD but not fulfilling the diagnostic or classification criteria for any of the defined CTDs** for at least 3 years*** | Malar rash<br>Subacute cutaneous lupus<br>Discoid lupus<br>Cutaneous sclerosis<br>Heliotrope rash<br>Gottron's papules<br>Erosive arthritis | Anti-dsDNA<br>Anti-Smith<br>Anti-Scl70 |
| 2. Presence of antinuclear antibodies determined on two different occasions | | |

* Applicable to patients at disease onset

** Using established classification criteria for PM/DM,CTD, SLE SSc, RA and SS (see relevant chapters)

*** If the disease duration is less than 3 years, patients may be defined as having an early UCTD.

Adapted from Mosca et al. and Doria et al.

## 5 Classification criteria

Preliminary classification criteria were proposed by Mosca at al in 1990.

1. Signs and symptoms suggestive of a connective tissue disease, but not sufficient to meet the criteria for a defined connective tissue disease,
2. Positive ANA,
3. Disease duration of at least 3 years. Patients with shorter disease duration (< 3 years) are considered as early UCTD. During these 3 years, some of the so-called "early UCTD" patients develop a defined connective tissue disease.

An amendment has since been suggested to avoid the misdiagnosis of transitory or early, defined CTD (Table 1).

**Differential diagnosis:** defined connective tissue disease (SLE, primary Sjögren's syndrome, SSc, dermatomyositis, polymyositis, MCTD, scleroderma/myositis overlap), ANA-positive rheumatoid arthritis.

**Treatment:** In accordance with the mild clinical manifestations, UCTD patients require symptomatic therapy and sometimes no treatment at all. The therapy includes NSAID, low-dose glucocorticoids and antimalarials. Immunosuppressive drugs are not indicated.

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# The antiphospholipid syndrome

Philipp von Landenberg, José Luis Rodriguez-Garcia, Munther A Khamashta

## 1 Introduction

The antiphospholipid syndrome (APS) represents the most prevalent acquired thrombophilia and causes venous, arterial and small-vessel thrombosis, pregnancy loss, and preterm delivery for patients with severe pre-eclampsia or placental insufficiency. It is associated with the presence of antiphospholipid antibodies (aPL), mainly lupus anticoagulant (LA) and anticardiolipin (aCL) and anti-β2 glycoprotein-I (β2-GPI) antibodies, directed against negatively charged phospholipids (aPL) [1].

In the general population, aPL can be detected in about one in five patients who have had a stroke at less than 50 years of age. About 25 % of patients with venous thromboembolism in whom a thrombophilia test is done have aPL. In addition, 10 %–15 % of women with recurrent miscarriage are diagnosed with APS. Although foetal death is linked to APS, the overall contribution of this syndrome is uncertain. aPL are detected in 11 %–29 % of women with pre-eclampsia [1].

APS is associated with systemic lupus erythematosus (SLE) in about 35 % of the cases, 5 % of APS patients have 'lupus-like' syndrome, 5 % other immune diseases and in 55 % of the patients APS presents alone. Mean age at the onset of symptoms of APS is 31 years with a 5/1 female/male ratio [1, 2].

## 2 How do antiphospholipid antibodies increase the risk of vascular thrombosis and pregnancy morbidity in APS?

β2-GPI has been described as one of the major target antigens for aPL [3] and plays a pivotal role in the pathophysiology of APS. β2-GPI, a protein synthesized in the liver, circulates in plasma in a closed conformation. After a small injury, cells express phosphatidylserine on their surface which binds to β2-GPI and, as a result, its conformation changes from closed to stretched structure. The aPL will bind to β2-GPI so that this protein is then able to interact with receptors on the surface of the cells. It leads to a procoagulant state with effects on the vascular bed and placenta by means of activation of endothelial cells, monocytes and platelets

and the complement cascade. These mechanisms often act in the presence of other cardiovascular risk factors, which are present in more than 50 % of APS patients and may trigger the thrombotic event ('second hit') [3].

Lupus anticoagulant is the strongest predictor of features related to APS. The role of aCL in the absence of LA is more debated, with no associated increased risk for stroke or myocardial infarction. Isolated anti-β2-GPI is weakly associated with clinical manifestations of APS. High-risk aPL profile includes LA positivity, triple positivity (LA + aCL + anti-β2-GPI) or isolated persistently positive aCL at medium-high titers [1].

## 3 Criteria and non-criteria clinical manifestations in APS

The spectrum of clinical manifestations of APS is wide and the prevalence of the clinical features is highly variable [1, 2]:

1. Frequent (> 20 % of cases): deep venous thrombosis, early foetal losses (< 10 weeks), stroke, migraine, arthralgia and/or arthritis, thrombocytopenia and livedo reticularis.
2. Less common (5 %–20 % of cases): superficial thrombophlebitis in legs, skin ulcers, pulmonary embolism, transient ischaemic attack, amaurosis fugax, cognitive dysfunction, mitral or aortic valve thickening or dysfunction, myocardial infarction, haemolytic anaemia, pre-eclampsia, late foetal losses (≥10 weeks) and premature birth.
3. Unusual or rare (< 5 %): arterial thrombosis in legs, venous or arterial thrombosis in arms, subclavian or jugular thrombosis, epilepsy, multi-infarct dementia, chorea, transverse myelitis, pulmonary hypertension, diffuse alveolar haemorrhage, angina, valve vegetations, retinal artery or vein thrombosis, cutaneous necrosis, splinter haemorrhages, avascular necrosis of bone, mesenteric ischaemia, adrenal haemorrhage, Budd-Chiari syndrome, APS nephropathy, renal artery or vein thrombosis, eclampsia and placental abruption.

Thrombosis of deep limb veins (39 %) and pulmonary embolism (14 %) are the most common venous manifestations of APS, and ischaemic stroke (20 %) is the most prevalent arterial thrombotic event. SLE-related APS patients have more episodes of arthritis and livedo reticularis, and more frequently exhibit thrombocytopenia and leucopenia, although show a similar profile regarding vascular thrombosis and pregnancy morbidity when compared with those patients with non SLE-related APS [2].

## 4 Classification criteria for definite APS (Sydney 2006)

Although APS can involve almost any organ, only vascular thrombosis and recurrent fetal loss are included in the revised classification criteria (Sydney 2006)

[4]. Accordingly, APS is present if at least one of the clinical criteria and one of the laboratory criteria (LA, aCL and/or anti-β2-GPI) are met (Table 2). Although other clinical and laboratory features not included in revised classification criteria for APS, such as heart valve disease, livedo reticularis, nephropathy, neurological manifestations, thrombocytopenia, antiphosphatidylserine antibodies (aPS), antibodies against annexin V and vimentin/cardiolipin complex, antiphosphatidylethanolamine (aPE) antibodies, antibodies against prothrombin alone (aPT-A) and antibodies to the phosphatidylserine–prothrombin (aPS/PT) complex are undoubtedly frequent in patients with APS, the committee considered that adoption of these features as independent criteria for definite APS may decrease diagnostic specificity, even though their association with APS is recognized [4].

However, clinical manifestations of APS are highly prevalent in the general population and in many cases there is a coincidental vascular risk factor to explain the vascular event; therefore, the diagnostic value of a positive result in aPL testing may be controversial. Thus, consideration of the non-criteria manifestations of the syndrome may help to establish an accurate diagnosis and therapeutic approach. It is worth mentioning that livedo reticularis is present in about 25 % of patients and represents a physical sign that should make the clinician suspect the diagnosis of the syndrome in the appropriate clinical context [1].

The *catastrophic antiphospholipid syndrome* (CAPS) is the most severe and infrequent variant of the syndrome and is a condition characterized by multiple vascular occlusive events, usually affecting small vessels and evolving over a short period of time, together with laboratory confirmation of the presence of antiphospholipid antibodies. A diagnosis of definite CAPS must fulfil these classification criteria [1, 2]:

1. Evidence of involvement of three or more organs, systems and/or tissues.
2. Development of manifestations simultaneously or in less than a week.
3. Confirmation by histopathology of small vessel occlusion in at least one organ or tissue.
4. Laboratory confirmation of the presence of antiphospholipid antibodies.

In daily clinical practice it is not unusual to find patients with clinical manifestations suggestive of APS who are persistently negative for the routinely used assays to detect LA aCL and anti-$\beta_2$-GPI. Therefore, the term *seronegative APS* (SN-APS) has been coined to include these patients with clinical features suggestive of APS who are persistently negative for aPL [4]. The profile of such patients includes the development of thrombotic events and/or pregnancy morbidity such as recurrent foetal loss, often with non-criteria APS manifestations such as livedo reticularis or thrombocytopenia, in the absence of conventional aPL.

Although APS diagnosis relies predominantly on laboratory results, where the detection of aPL is mandatory, routine screening tests (aCL, anti-$\beta_2$-GPI and LA) might miss some cases of true seropositive-APS by failing to pick up cases

**Table 1.** Revised classification criteria for the antiphospholipid syndrome [1].

Antiphospholipid antibody syndrome (APS) is present if at least one of the clinical criteria and one of the laboratory criteria that follow are met:

*Clinical criteria*

1. *Vascular thrombosis*

   One or more clinical episodes of arterial, venous, or small vessel thrombosis, in any tissue or organ. Thrombosis must be confirmed by objective validated criteria (i.e. unequivocal findings of appropriate imaging studies or histopathology). For histopathologic confirmation, thrombosis should be present without significant evidence of inflammation in the vessel wall.

2. *Pregnancy morbidity*

   (a) One or more unexplained deaths of a morphologically normal foetus at or beyond the 10th week of gestation, with normal foetal morphology documented by ultrasound or by direct examination of the foetus, or

   (b) One or more premature births of a morphologically normal neonate before the 34th week of gestation because of: (i) eclampsia or severe preeclampsia defined according to standard definitions, or (ii) recognized features of placental insufficiency-, or

   (c) Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded.

*Laboratory criteria*

1. *Lupus anticoagulant (LA)*

   Present in plasma, on two or more occasions at least 12 weeks apart, detected according to the guidelines of the International Society on Thrombosis and Haemostasis.

2. *Anticardiolipin (aCL) antibody of IgG and/or IgM isotype*

   In serum or plasma, present in medium or high titer (i.e. > 40 GPL or MPL, or > the 99th percentile), on two or more occasions, at least 12 weeks apart, measured by a standardized Elisa.

3. *Anti-β2 glycoprotein-I antibody of IgG and/or IgM isotype*

   In serum or plasma (in titer > the 99th percentile), present on two or more occasions, at least 12 weeks apart, measured by a standardized Elisa, according to recommended procedures.

[1] Modified from *J Thromb Haemost. 2006*; 4: 295–306.

with other antibodies directed against different phospholipids or protein cofactors (non-criteria aPL), such as prothrombin, phosphatidylethanolamine, annexin V and vimentin/cardiolipin complex. Nevertheless, their main disadvantage is the lack of standardization for such assays.

## 5 Who should be tested for aPL

The main focus for the search for antiphospholipid antibodies should be on patients with a thrombotic event and/or with pregnancy morbidity. In addition, patients with SLE should be tested carefully for these autoantibodies, since the simultaneous presence of SLE and aPL increases the risk for thrombosis.

Most important in the diagnostic procedure of antiphospholipid antibodies is the confirmation of a positive result after 12 weeks [4]. Before this a definitive diagnosis cannot be made. Antiphospholipid antibodies are very closely associated with a variety of infections. Thus, the possibility that the patient has a current infection must be excluded. This leads to this imperative requirement to retest the initial positive detection of aPL (see Fig. 1).

## 6 Treatment and prognosis

Management of APS must be individualized according to the patient's clinical status and history of thrombotic events and pregnancy morbidity. The aPL profile (high or low risk), the coexistence of other thrombotic risk factors and the presence of an underlying autoimmune disease are the most important variables for planning the treatment regimen in APS patients (see Tables 2 and 3) [1, 5].

In a cohort of 1000 patients from the 'Euro-Phospholipid project' the total mortality rate during the 5-year follow-up period was 5.3 %. In addition to severe thrombotic events (i. e., myocardial infarction, stroke or the catastrophic APS), infections and haemorrhages accounted for one-third of deaths [2].

Regarding obstetric APS, with proper management with aspirin and heparin more than 70 % of pregnant women will deliver a viable live infant [1].

Patients with CAPS require intense observation and treatment, often in an intensive care unit. The mortality rate has improved due to the use, as first-line therapies, of full anticoagulation, corticosteroids, plasma exchanges and intravenous immunoglobulins [1, 2].

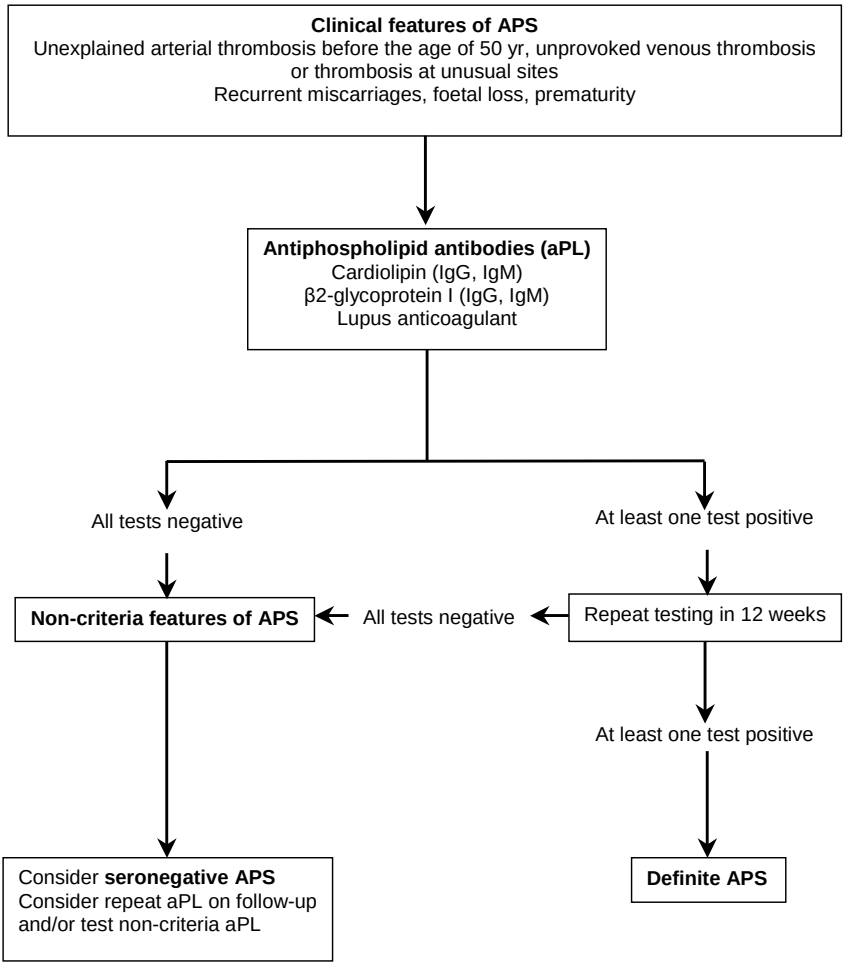


**Figure 1.** Algorithm for the diagnosis of the antiphospholipid syndrome (APS).

**Table 2.** Recommendations for the prevention and long-term management of thrombosis in antiphospholipid antibody-positive patients (Modified from Lupus 2011, 20: 206–18).

| |
|---|
| *aPL carriers* |
| A strict control of cardiovascular risk factors should be accomplished in all individuals with a high-risk aPL profile*.<br>Thromboprophylaxis with usual doses of low molecular weight heparin in high-risk situations, such as surgery, prolonged immobilization and puerperium. |
| *Primary thromboprophylaxis in SLE patients with aPL* |
| These patients should receive hydroxychloroquine and low-dose aspirin. |
| *Primary thromboprophylaxis in aPL-positive individuals without SLE* |
| Long-term primary thromboprophylaxis with low-dose aspirin in those with a high-risk aPL profile, especially in the presence of other thrombotic risk factors. |
| *Secondary thromboprophylaxis* |
| Patients with definite APS and a first venous event should receive oral anticoagulant therapy to a target INR 2.0–3.0.<br>Patients with definite APS and arterial thrombosis should be treated with warfarin at an INR > 3.0 or combined antiaggregant-anticoagulant (INR 2.0–3.0) therapy, although other options such as antiaggregant therapy alone or anticoagulant therapy to a target INR 2.0–3.0 would be equally valid in this setting (lack of consensus).<br>An estimation of the patient's bleeding risk should be performed before prescribing high-intensity anticoagulant or combined antiaggregant-anticoagulant therapy.<br>Non-SLE patients with a first non-cardioembolic cerebral arterial event, with a low-risk aPL profile and the presence of reversible trigger factors could individually be considered candidates to treatment with antiplatelet agents. |
| *Duration of treatment* |
| Patients with definite APS and thrombosis should receive indefinite antithrombotic therapy.<br>In cases of first venous event, low-risk aPL profile** and a known transient precipitating factor, anticoagulation could be limited to 3–6 months. |

* High-risk: LA positivity, triple positivity (LA + aCL + anti-β2-GPI) or isolated persistently positive aCL at medium-high titers.

** Low-risk: isolated, intermittently positive aCL or anti-β2-GPI at low-medium titers.

**Table 3.** Usual recommended treatment of antiphospholipid syndrome during pregnancy (Modified from Lancet 2011, 376: 1498–1509).

| |
|---|
| **APS without previous thrombosis and recurrent early miscarriage (< 10 weeks' gestation)** |
| Low-dose aspirin (i.e., 100 mg/day) alone or together with LMWH (usual prophylactic doses, i.e., enoxaparin 40 mg/day) |
| **APS without previous thrombosis and foetal death (> 10 weeks' gestation) or previous early delivery (< 34 weeks' gestation) due to severe pre-eclampsia or placental insufficiency** |
| Low-dose aspirin plus LMWH at usual prophylactic doses |
| **APS with previous thrombosis** |
| Low-dose aspirin plus LMWH at usual therapeutic doses (i.e., enoxaparin 1.5 mg/kg/day) |

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# Rheumatoid arthritis

Manfred Herold

## 1 Introduction

Rheumatoid arthritis (RA) is a systemic inflammatory disorder causing irreversible joint damage and significant disability. Its aetiology is still unknown but genetic factors, environmental influences as well as lifestyle modalities like smoking all impact on disease susceptibility.

RA is the most common chronic, inflammatory rheumatic disease with a prevalence in developed countries of between 0.5 % and 1 % and an estimated annual incidence of about 40 cases per 100 000 persons. People can be affected at any age but most frequently the onset of disease occurs between the ages of 40 and 70 years; the incidence increasing with age [1]. Women are affected about three times more often than men [2].

## 2 Diagnostic criteria

The diagnosis cannot be established by a single laboratory test or radiographic findings but is the summarised conclusion of a spectrum of disease manifestations.

Until recently, the 1987 RA classification criteria of the American College of Rheumatology (ACR; formerly, the American Rheumatism Association) were widely used. These criteria were developed by evaluating patients with established RA and had a diagnostic sensitivity of 91 % and a specificity of 89 %. The criteria include the presence of morning stiffness, arthritis of three or more joint areas, arthritis of the hand joints, symmetric arthritis, rheumatoid nodules, elevated levels of serum rheumatoid factor, and radiographic changes (Table 1). The 1987 ACR criteria are excellent to differentiate an established RA from a non-RA arthritis but have a lack of sensitivity in early disease. A rethinking of diagnostic classification to allow effective treatment in early RA resulted in the new classification criteria defined in 2009 and published 2010 [3].

Nowadays the classification as 'definite RA' is based on the confirmed presence of synovitis in at least one joint and a total score of 6 or more from a possible 10

**Table 1.** The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis published in *Arthritis Rheum 1988; 31: 315–24.*
For classification purposes, a patient shall be said to have rheumatoid arthritis if he/she has satisfied at least 4 of these 7 criteria. Criteria 1 through 4 must have been present for at least 6 weeks.

| Signs & symptoms | Comments |
|---|---|
| morning stiffness | lasting at least 1 hour before maximal improvement |
| arthritis of 3 or more joint areas | PIP, MCP, wrist, elbow, knee, ankle, and MTP joints |
| arthritis of hand joints | at least 1 area swollen in a wrist, MCP, or PIP joint |
| symmetric arthritis | simultaneous involvement of the same joint areas on both sides of the body |
| rheumatoid nodules | subcutaneous nodules, over bony prominences, or extensor surfaces, or in juxta-articular regions, observed by a physician |
| serum rheumatoid factor | tested by any method for which the result has been positive in < 5 % of normal control subjects |
| radiographic changes | radiographic changes typical of rheumatoid arthritis on posteroanterior hand and wrist radiographs, which must include erosions or unequivocal bony decalcification localized in or most marked adjacent to the involved joints |

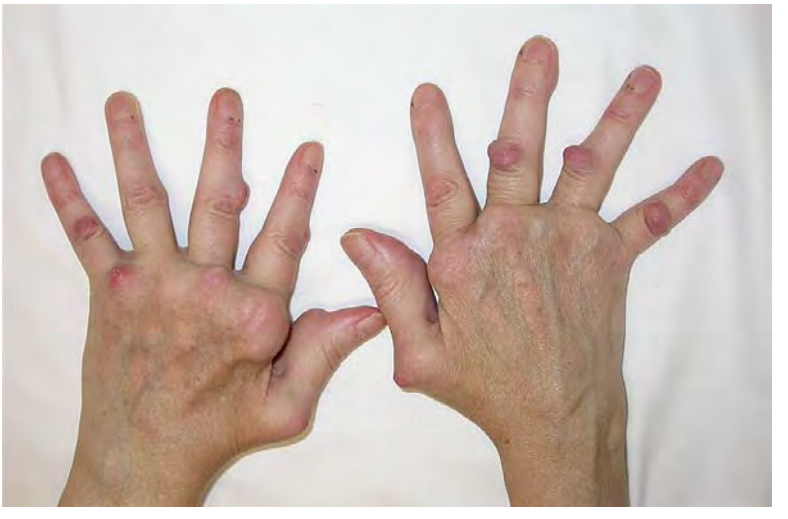

**Figure 1.** Long-standing rheumatoid arthritis with typical signs including swollen MCP joints, ulnar deviation of fingers, atrophy of musculii interossei and rheumatoid nodules (picture M. Herold 2009).

**Table 2.** 2010 ACR/EULAR Rheumatoid Arthritis Classification Criteria [4]. Comparing to the ACR criteria of 1987 classification of symptoms as RA is possible in a very early phase of the disease. A maximum of 10 points is possible. Patients with 6 or more points are classified as RA.

| Signs & symptoms | Points |
|---|---|
| ***Joint involvement*** | ***0–5*** |
| 1 large joint | 0 |
| 2–10 large joints | 1 |
| 1–3 small joints (with or without involvement of large joints) | 2 |
| 4–10 small joints (with or without involvement of large joints) | 3 |
| > 10 joints (at least 1 small joint) | 5 |
| ***Serology*** | ***0–3*** |
| negative RF *and* negative ACPA | 0 |
| low-positive RF *or* low-positive ACPA | 2 |
| high-positive RF *or* high-positive ACPA | 3 |
| ***Acute-phase reactants*** | ***0–1*** |
| normal CRP *and* normal ESR | 0 |
| abnormal CRP *or* abnormal ESR | 1 |
| ***duration of symptoms*** | ***0–1*** |
| < 6 weeks | 0 |
| ≥6 weeks | |

point score being determined as the sum of single scores in four domains (Table 2) which are:

- number and site of involved joints (range 0–5),
- serological abnormality (range 0–3),
- elevated acute-phase response (range 0–1) and
- symptom duration (range 0–1).

## 3 Requirements for family practitioners

A broad range of clinical signs and symptoms (Fig. 1) is seen in patients with RA (Table 3) predominantly pain, stiffness especially in the morning, swelling of peripheral joints and decreased range of motion. But RA is a systemic disease and untreated patients have increased morbidity and mortality compared to the general population. The higher mortality is largely attributed to an increased incidence of cardiovascular diseases with a more than 3 fold higher risk of myocardial infarction. The risk of malignancy from lymphomas and certain carcinomas is

**Table 3.** Signs and symptoms of RA*.

| |
|---|
| ***Symptoms*** |
| – joint swelling |
| – pain & stiffness (commonly in the morning and lasting > 1 hour) |
| – weakness |
| – deformity |
| – general symptoms of sickness (fatigue, malaise, weight loss, depression) |
| ***Articular characteristics*** |
| – palpation tenderness |
| – synovial thickening |
| – erythema & effusion (early on) |
| – decreased range of motion (later on) |
| – ulnar deviation of fingers (later on) |
| – subluxation (later on) |
| – ankylosis (later on) |
| ***Distribution*** |
| – symmetrical (especially later on) |
| – distal more common than proximal |
| – PIP, MCP/MTP, wrist/ankle more common than elbow/knee, shoulder/hip |

* modified from Lee & Weinblatt 2001 [1]

also slightly increased. Early, aggressive and effective treatment is the goal in the management of RA patients.

RA usually begins with the painful swelling of several joints caused by an inflammation of the synovial membrane. Most often the small joints of hands (Fig. 2) and feet such as proximal interphalangeal (PIP) and metacarpophalangeal (MCP) or metatarsophalangeal (MTP) joints are involved, but sometimes also the larger joints of hands and feet, elbows, shoulders and knees. Affected joints are swollen, tender and warm, and stiffness limits their movement. Morning stiffness up to several hours is a commonly mentioned clinical characteristic which affects quality of life and ability to function in the morning. According to an international recommendation [4], rapid referral to a rheumatologist with a noted clinical suspicion of RA is advised in the presence of 3 or more swollen joints, a positive squeeze test indicating inflamed adjacent joints like MTP or MCP and a morning stiffness of more than 30 minutes until major improvement occurs (Table 4).

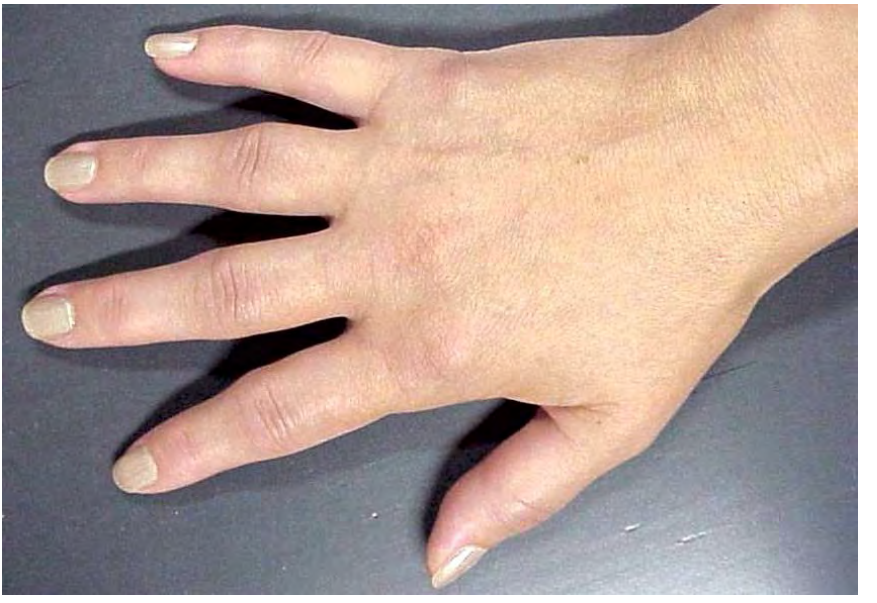

**Figure 2.** Very early RA with swollen and painful PIP joints (picture M. Herold 2003).

**Table 4.** Early signs and symptoms which are highly suspicious for a beginning RA [3].

| |
|---|
| 1. $\geq 3$ swollen joints |
| 2. MTP/MCP involvement, Squeeze test positive |
| 3. morning stiffness of $\geq 30$ minutes |

Early diagnosis is vital as permanent structural damage occurs within the first weeks of active RA and only intervention with disease modifying antirheumatic drugs (DMARDs) slows the progression of structural and irreversible joint damage and improves long term outcome, as well as overall patients' quality of life.

## 4 Diagnostic measurements for experts

Diagnosis is mainly based on clinical signs and symptoms. In addition, blood tests are useful in classifying the collection of symptoms as RA and helpful in estimating disease activity. Several autoantibodies have been detected in RA patients but two major antibody systems dominate in RA, the rheumatoid factors (RFs) and antibodies against citrullinated peptides or proteins (ACPAs). Rheumatoid factor

is the most common and best known antibody. RFs are immunoglobulins with activity directed to the Fc part of immunoglobulin G (IgG). RFs may be of any immunoglobulin type. For diagnosis of RA usually RF of the immunoglobulin class IgM (IgM RF) is measured. IgM RF is present in about 80 % of RA patients. At the time of first symptoms of RA, patients are often RF negative but develop RF activity within the first year of disease. Up to 20 % of RA patients remain negative for RF throughout the course of their disease. These patients are classified as seronegative RA. The diagnostic sensitivity of RF is around 69 %, the specificity about 85 %. RF is also seen in patients with other autoimmune diseases such as Sjögren's syndrome (present in 70 %) or systemic lupus erythematosus (up to 30 %) and also in patients with chronic inflammation including hepatitis or chronic bacterial and other viral diseases. IgM RF was one of the diagnostic criteria in the ACR criteria of 1987 (Table 1) and is also one of the laboratory markers in the new 2010 EULAR/ACR criteria (Table 2), where not only antibody positivity is considered as a diagnostic feature but also the serum concentration. In the new diagnostic criteria RF is equal to ACPA.

ACPAs are antibodies targeting citrullinated peptide or protein antigens. Several commercially available assay systems are available for ACPA testing. The most frequently used tests are the anti-CCP (anti-cyclic citrullinated peptides) and the anti-MCV (anti mutated citrullinated vimentin) tests both with comparable sensitivity of about 67 % and a specificity of about 95 %. Sensitivity of ACPA is comparable to RF, specificity is significantly higher. High positive RF and/or ACPA predict an erosive course of the disease.

Other autoantibodies are also found in the sera of RA patients. These RA non-specific antibodies include antinuclear antibodies (ANA), antiphospholipid antibodies, anti-neutrophil cytoplasmic antibodies (ANCA), antibodies to type II collagen and others. They have neither diagnostic nor prognostic importance.

Non-specific markers of inflammation such as ESR and CRP are usually elevated in active disease and correlated with the numbers of involved joints and with disease activity.

Imaging plays a key role in diagnosis and management of RA. Standard radiography of hands and feet at the time of diagnosis and in follow-up are the first choice of imaging RA. Juxta-articular osteopenia is an early sign. Erosions characterise established disease and are usually irreversible and untreatable. Increasing number of erosions in follow-up radiographs suggests inadequately controlled RA and that correction and intensifying of drug therapy is necessary. Ultrasound and magnetic resonance imaging (MRI) provide a more accurate assessment, as well as earlier detection of lesions and are used in addition to plain radiography in the early stages of disease when erosions are not seen and RA is suspected.

## 5 Management

The management of RA is based on drug treatment with disease-modifying antirheumatic drugs (DMARDs), glucocorticoids (GCs) and nonsteroidal anti-inflammatory drugs (NSAIDs) as well as non-pharmacological interventions such as physical, occupational and psychological therapeutic approaches.

NSAIDs reduce pain and stiffness, are more efficacious than analgesics and are widely used in times of active disease. NSAIDs relieve symptoms but do not influence the long term course of disease. Non-selective as well as COX-2 selective NSAIDs are used. There are concerns over NSAIDs' gastrointestinal, renal, and cardiovascular side effects. COX-2 selective drugs or the addition of gastroprotective agents (misoprostol, double doses of H2 blockers, and proton pump inhibitors) to non-selective NSAIDs significantly reduce gastrointestinal complications. For some of the COX-2 selective drugs, long term use has been associated with increased cardiovascular risk and for all non-selective NSAIDs the same risk cannot be excluded. Consequently, the US Food and Drug Administration and the European Medicines Agency recommend the shortest possible treatment duration with NSAIDS and contraindications for patients at risk.

Glucocorticoids (GCs) have widespread use in RA. GCs quickly improve symptoms such as pain and stiffness and decrease joint swelling and tenderness. They are given in early disease as bridging therapy until DMARDs exert their anti-inflammatory effects. The usual dose of prednisone is 5 to 10 mg daily. Initial doses up to 25 mg daily may be used, but should be tapered as rapidly as clinically feasible. Short term use of GCs is also indicated to treat acute flare-ups of disease activity. GCs are also useful as chronic adjunctive therapy in patients with severe disease that is not well controlled on NSAIDs and DMARDs. If GC therapy of 3 or more months is required, calcium and vitamin D supplementation should also be prescribed to avoid glucocorticoid-induced osteoporosis. The need for accompanying anti-resorptive therapy with bisphosphonates depends on risk factors including the results of bone-mineral density (BMD) measurement.

Intra-articular glucocorticoid injections are effective for controlling a local flare in a single active joint.

The mainstay of RA treatment is the early and continuous application of DMARDs. The term DMARDs comprises a group of drugs which are defined by their use in RA but are otherwise unrelated. DMARDs slow down progressive joint damage, reduce synovial joint swelling and pain and prevent loss of joint function. DMARDs should be started as soon as the diagnosis of RA is made. Methotrexate (MTX) is the gold standard and is recommended as the first treatment strategy in patients with active RA. MTX is given as a single dose between 7.5 and 30 mg once a week usually orally or subcutaneously. Besides MTX, leflunomide (orally, 20 mg daily) and sulfasalazine (orally, between 2 and 3 g daily) are also widely used. Antimalarials like chloroquine (orally, 250 mg daily) and hydroxychloroquine (orally, 200 mg daily) have DMARD-like properties and may be preferentially given in

milder forms of RA with low disease activity. DMARDs are sometimes combined to increase efficacy.

If synthetic DMARDs have failed, a biological DMARD (so called biological) in addition to the synthetic DMARD may be applied. Biologicals are biotechnologically produced drugs which are administered parenterally either by subcutaneous or intravenous application. Biologicals reduce or suppress inflammation by targeting molecules involved in the inflammatory response (such as pro-inflammatory cytokines) or by blocking pro-inflammatory cellular activity by targeting molecules on lymphocyte surfaces. To date, 9 biologicals have been launched for RA treatment. Infliximab, etanercept, adalimumab, golimumab and certolizumab pegol are so-called tumour necrosis factor (TNF) inhibitors. Other biological agents with different targets are anakinra (interleukin-1 receptor antagonist), tocilizumab (anti-interleukin-6 receptor antibody), abatacept (T-cell costimulation modulator) and rituximab (anti-CCD20 antibody). These biological agents are usually combined with methotrexate or other DMARDs to improve efficacy.

DMARDs have shown their ability to slow disease progression and to prevent joint destruction. They are given as early as possible in the disease process.

## 6 Follow up

The aim of treatment is remission or sustained low disease activity. Monitoring of disease activity should be regularly performed and treatments switched if treatment goals are not attained.

Disease activity is estimated by counting tender and swollen joints and using visual analogue scales (VAS) to evaluate patient and physician global scoring. Questionnaires such as the health assessment questionnaire are also helpful to evaluate treatment success. Measurement of ESR and CRP is necessary to determine inflammation activity.

Various composite scores have been designed for use in studies and in daily routine. The most common are the disease activity score measured on 28 joints (DAS-28), SDAI (simple disease activity index) and CDAI (Clinical Disease Activity Index).

DAS-28 is calculated by a complex mathematical formula, which includes the number of tender (TJ) and swollen joints (SJ) out of a total of 28, the ESR, and the patient's global assessment (PGA) of global health measured on a VAS between 0 ($\hat{=}$ very good) and 10 ($\hat{=}$ very bad). A DAS-28 score greater than 5.1 implies active disease, less than 3.2 well controlled disease, and less than 2.6 remission.

SDAI is a similar estimation of disease activity but calculation is easier. SDAI also includes physician's global assessment (MDGA) of disease activity on a VAS similar to the patients' VAS between 0 and 10 and uses CRP instead of ESR. SDAI is the sum of TJ, SJ, MDGA, PGA and CPR in mg/dl. SDAI value of $> 40$ constitutes

high disease activity, SDAI of 20–40 indicates moderate RA activity, and SDAI of < 20 mild disease.

CDAI is the only index that does not include a measure of acute-phase response. CDAI is the sum of TJ, SJ, MDGA and PGA. CDAI < 10 represents low disease activity, > 22 (up to a maximum of 76) high disease activity.

A special feature of rheumatoid arthritis is Felty's syndrome which is defined as rheumatoid arthritis and the presence an enlarged spleen (splenomegaly), and an abnormally low white blood count. Felty's syndrome is a very rare disease of unknown origin seen in less than 1 % of RA patients who usually have long standing RA. It is supposed that patients with this syndrome are more at risk of infection because of their low white blood cell count.

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# Juvenile idiopathic arthritis

Michael Borte, Karsten Conrad, Veit Krenn, Ulrich Sack

## 1 Introduction

Juvenile idiopathic Arthritis (JIA) is defined as a group of diseases, which starts before the 6$^{th}$ year of life and lasts at least 6 weeks, in the absence of other articular diseases (Table 1: differential diagnoses). Beside arthritis, JIA can take a non-arthritic, systemic course (Still's syndrome).

70 to 80 % of all chronic joint diseases in childhood can be defined as JIA, this represents a prevalence of 2 to 3 per 10 000 children. The disease usually starts between the 2$^{nd}$ and 4$^{th}$ or between the 8$^{th}$ and 12$^{th}$ years of life. Currently, JIA is classified into 5 entities (Table 2).

Nowadays, JIA is considered, at least in some entities, to be an auto-inflammatory rather than an autoimmune disease [1]. In fact, autoantigens are not (yet?)

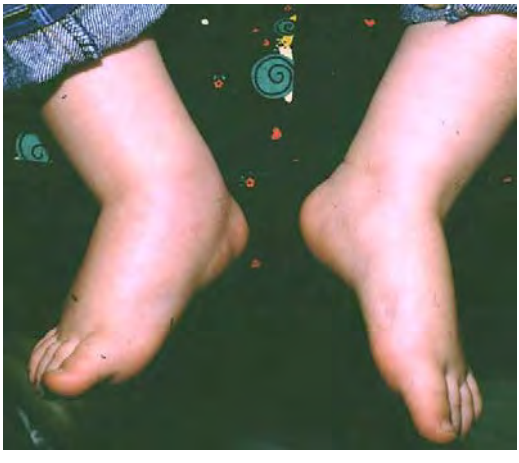

**Figure 1.** Clinical presentation of a swollen, arthritic ankle in a 1 year old boy with an acute JIA.

**Table 1.** Differential diagnoses of JIA.

| Differential diagnoses |
|---|
| – Acute rheumatic fever |
| – Infections (arthritis purulenta, lyme arthritis, osteomyelitis, tuberculosis) |
| – Reactive arthritis (following scarlet fever [β-haemolytic group A streptococci]; urogenital infections [*Chlamydia trachomatis*, *Neisseria gonorrhoea*, *Ureaplasma urealyticum*]; enteral infections [Yersinia, Salmonella, Shigella, *Campylobacter jejuni*, *Clostridium difficile*, *Brucella abortus*]; or viral infections [Parvovirus B19, Rubella, Hepatitis B, HIV, Measles, Varicella, Mumps, EBV, Coxsackie, Adenovirus, Influenza, Parainfluenza, RS-Virus (RSV)) |
| – Connective tissue diseases |
| – Immunodeficiency diseases |
| – Haematological diseases |
| – Neoplasias |
| – Injuries |
| – Foreign body |
| – Orthopaedic diseases |
| – Frostbite |
| – Psychogenic arthralgias |

known. Autoantibodies are not considered to be of diagnostic help in this disease, but have relevance in differential diagnosis.

## 2 Diagnostic measurements for experts

JIA may or may not start with joint pain. Depending on the JIA entity (Table 2), different symptoms may be more obvious. Most important is the exclusion of other diseases.

The patient's history is important to determine infections which may be responsible for acute rheumatic fever, reactive arthritis, or infectious arthritis, e. g. tuberculosis. Previous injuries or accidents can indicate infections causing arthritis purulenta or osteomyelitis, but the history should also check the possibility of foreign bodies or frostbite. Frequently overlooked, systemic diseases such as immunodeficiencv diseases, haematological diseases or neoplasias can cause joint pain. Furthermore, family history gives important information about hereditary background and psycho-social situation.

**Table 2.** Diagnostic criteria of Juvenile idiopathic Arthritis (JIA) entities.

| JIA entities | Characteristics |
|---|---|
| Systemic JIA (Still's syndrome) | – 10–15 % of JIA<br>– Young children, both genders<br>– Sudden onset; high, septicaemia-like fever<br>– Maculo-papulous exanthema<br>– Extra-articular symptoms (e.g. pancarditis, hepatosplenomegalia)<br>– Late-onset destructive arthritis<br>– High mortality (10 %) |
| Seronegative polyarthritis | – 30–40 % of JIA<br>– Mainly female patients<br>– Symmetric polyarthritis, small and large joints<br>– IgM rheumatoid factor negative<br>– Good prognosis for joints |
| Seropositive polyarthritis | – 5–10 % of JIA<br>– Onset around adolescence<br>– Symmetric polyarthritis, small and large joints<br>– IgM rheumatoid factor positive<br>– Poor prognosis similar to adult RA |
| Oligoarthritis in young children | – 25–30 % of JIA<br>– Young children, mostly girls<br>– 75 % ANA positivity<br>– Asymmetric oligoarthritis of large joints<br>– 50 % chronic iridocyclitis (10 % permanently damaging) |
| Oligoarthritis in older children | – 20–25 % of JIA<br>– Older children, mostly boys<br>– 80 % HLA-B27 association<br>– Asymmetric oligoarthritis, common sacroiliitis<br>– Frequently transition into ankylosing spondylitis |

Abbreviations: **RA**, Rheumatoid arthritis; **ANA**, antinuclear antibodies

Examination of affected joints should be supplemented by sonography of the joints as well as the spleen and liver. Cardiac function must be investigated to exclude acute rheumatic fever by echocardiography, electrocardiography and X-ray.

Patients with signs of an antinuclear antibody (ANA)-positive disease must be seen by an ophthalmologist at regular intervals. Immunodeficiency diseases can also cause arthritis; these patients frequently have a high incidence of infections in their histories.

Orthopaedic diseases must be differentiated from "growth pains" and are mostly found in joints taking a heavy burden, by incorrect posture, heavy body weight, exercise avoidance or even incorrect shoes.

Laboratory investigation focuses on the JIA entities and the differential diagnoses to exclude. Primarily, leukocyte count, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), IgM rheumatoid factors (RF), anti-Streptolysin O titres (AST), HLA-B27 detection and autoantibody screening by indirect immunofluorescence help to make a clear diagnosis.

## 3 Requirements for family practitioners

JIA is a highly individual syndrome including distinct entities. The most important task for the family doctor is to not overlook a patient with suspicious symptoms and to consult a specialist as soon as possible. Please note that for most explanations for articular pain there is no need for a rheumatologist but for an oncologist, haematologist, immunologist, or an orthopaedist. To make the right decision, family doctors can examine the patient as shown above and order the first laboratory tests.

There is no confirmatory test for a diagnosis of "Juvenile idiopathic arthritis".

## 4 Follow up

*Clinical observations*

During symptomatic or immunosuppressive treatment or therapy with biological agents, signs and symptoms should gradually improve. This may take any time from weeks to months, and depends on the underlying disease entity.

*Expectations*

JIA can be a chronic or a self limiting disease. Usually, with adequate therapy, most patients will achieve a partial or complete remission. Spontaneous remissions also occur.

*Blood tests*

Clinical improvement is directly associated with an improvement in levels of inflammatory parameters (ESR, CRP). Minimal laboratory testing is required to adequately care for patients. In patients who fail to improve during treatment, additional laboratory and clinical testing can be useful to further refine the clinical diagnosis and to change the therapeutic regimen appropriately.

## 5 Management

The treatment must be individualised according to the JIA entity, the severity of disease, the patient's wishes and the presence of associated diseases. Altogether, the following treatment approaches can be considered [2]:

*Drugs*

1. *Nonsteroidal anti-inflammatory Drugs (NSAIDs)*
   These drugs reduce inflammation and relieve pain. Indomethacin, Ibuprofen, Diclofenac or Naproxen are the most common.
2. *Basic Therapeutics*
   Chronic activity in rheumatic diseases can be modulated by chloroquine or sulfasalazine.
3. *Immunosuppressive drugs*
   Autoinflammatory and autoimmune processes can be treated with drugs such as Azathioprine, Methotrexate, or cyclophosphamide
4. *Corticosteroids*
   Prednisone or intra-articularly-given triamcinolonacetonide are established anti-inflammatory compounds.
5. *Biologicals*
   Anti-TNF therapies are well established in the treatment of JIA. Furthermore, anti-IL1b therapies are a therapeutic option [3, 4].

*Surgery*

In selected cases, synovectomy or surgical corrections may be necessary to manage JIA.

*Accompanying therapies*

Physical therapy, ergotherapy and consideration of social networks must complement medical care.

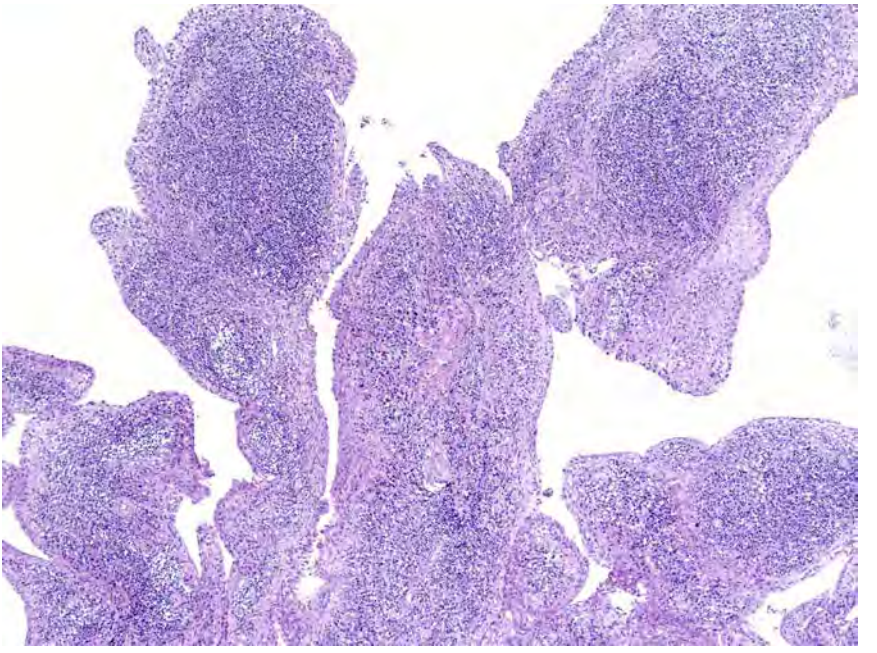

**Figure 2.** Histopathology of a high grade JIA synovialitis with lining cell hyperplasia, dense lymphocytic inflammatory infiltration. HE staining, original magnification 70 ×.

## 6 Diagnostic tests

There is no specific test for JIA. Laboratory investigation is highly dependent on the JIA entities (Table 2) and the differential diagnoses to exclude (Table 1).

## 7 Testing methods

Detection of CCP-antibodies is not indicated in JIA. Leukocyte count, C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) give a fair overview of inflammatory activity and are common in clinical labs or even in some outpatients' departments.

IgM rheumatoid factors (RF) must be detected by using isotype specific detection reagents.

Anti-Streptolysin O titres (AST), serological tests for bacterial and viral diseases and sometimes direct detection of infectious antigens should be performed as indicated by clinical findings. Tuberculosis (Tb) should be excluded by interferon-γ release assay; confirmation must be done in a specialised Tb laboratory.

Screening for antinuclear antibodies must be done by indirect immunofluorescence (HEp-2 cells). If there are any positive ANA titres, an ENA-screen should be done.

HLA-B27 positivity can be confirmed by flow cytometry or by DNA based test systems.

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# Relapsing polychondritis

Manfred Herold

## 1 Introduction

Relapsing polychondritis (RPC) is a rare, chronic, inflammatory, autoimmune disease of unknown origin characterized by recurrent inflammation with the possible destruction of cartilage and neighboring connective tissue. It was first described in 1923 by Jaksch-Wartenhorst [reviewed in 1 & 2]. The name *relapsing polychondritis* (RPC) was suggested by Pearson and coworkers in 1962 (reviewed in [1 & 2]) because of its episodic nature as an active, cartilage-destroying disease. Autoimmune reactions to antigens present in cartilages such as type II collagen [3] and matrilin seem to be triggers for clinical symptoms. Polychondritis may attack cartilages in different parts of the body [1]. A painful inflammation of the ear (Fig. 1) is the most commonly seen symptom (Table 1).

RPC is a rare disease. In Rochester, USA, the estimated prevalence of RPC is about 3.5 cases per million The ratio of female to male cases seems to be equal and the disease has been reported in all races and ages between 13 and 84 years [1]. The mean age of patients at diagnosis is in the late forties.

## 2 Diagnostic criteria

Diagnosis is still based on criteria defined by McAdam et al. (reviewed in [2]) in 1976 (Table 2). Nowadays the diagnosis of RPC can be made on the basis of chondritis in two of three sites (auricular, nasal, laryngotracheal) or on the basis of chondritis in one of these sites (auricular, nasal, laryngotracheal) plus two additional features such as ocular inflammation, audio vestibular damage or inflammatory arthritis [3] or on the basis of one or more clinical signs with histological confirmation of chondritis.

RPC-specific laboratory tests are unknown. Only non-specific laboratory signs of inflammation including elevated erythrocyte sedimentation rate (ESR), increased C-reactive protein (CRP), moderate leukocytosis and thrombocytosis are seen. Antinuclear antibodies (ANA) may be present but no specific ANA-subtypes are known.

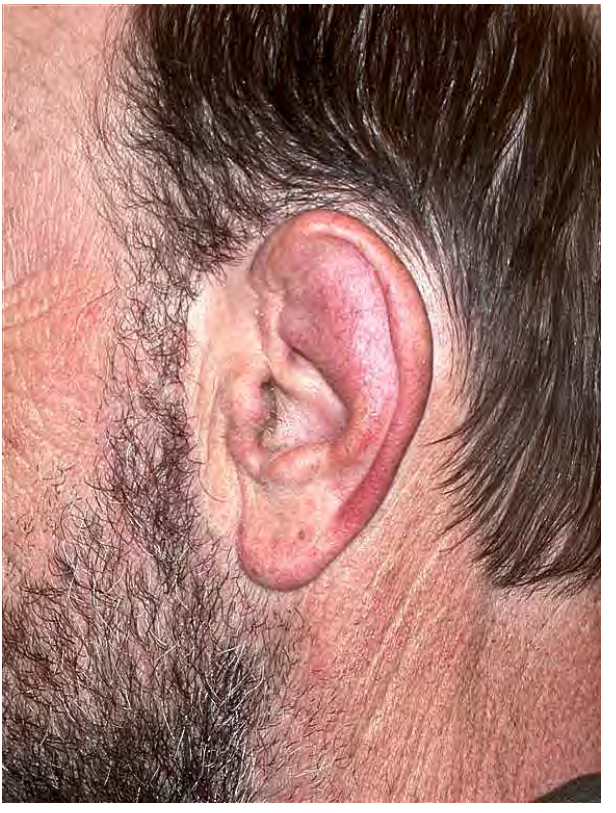

**Figure 1.** Man of 50 years of age who has suffered from relapsing polychondritis in both ears for several years (picture M. Herold 2008).

## 3 Requirements for the family practitioner

The most frequent clinical manifestation is an inflammation of the cartilage of the ear either unilaterally or bilaterally presenting as acute pain with swelling and redness (Fig. 1). Painful joints are the second most common feature in RPC. Parasternal joints including sternoclaviclar, manubriosternal or costosternal may be involved as well as peripheral joints presenting as nonerosive, asymmetrical oligo- or polyarthritis.

RPC, as primarily a disease of the cartilage, also affects the respiratory system where most parts, from the external nares, nasal septum, epiglottis and larynx, to the trachea and bronchioli, contain cartilage. Airway involvement is potentially serious and responsible for the significant morbidity and mortality seen in patients with RPC [4]. Nasal chondritis causes stuffiness, crusting, rhinorrhea,

**Table 1.** Estimated incidence of signs and symptoms in relapsing polychondritis*.

| | Presentation (%) | Cumulative (%) |
|---|---|---|
| ESR increase | 74 | 82 |
| Anaemia | 50 | 53 |
| Auricular chondritis | 40 | 85 |
| Arthritis | 37 | 57 |
| Laryngotracheal symptoms | 25 | 49 |
| Nasal chondritis | 25 | 57 |
| Ocular symptoms | 20 | 52 |
| Saddle nose | 18 | 29 |
| Airway stricture | 15 | 23 |
| Dermatologic | 10 | 28 |
| Hearing loss | 9 | 32 |
| Systemic vasculitis | 3 | 12 |
| Vestibular dysfunction | 0 | 17 |
| Cardiac valve | 0 | 6 |
| Aneurysm | 0 | 5 |

* modified from Staats et al. 2002 with estimated percentages abstracted from Michet et al. 1986.

**Table 2.** Diagnostic criteria for relapsing polychondritis according to McAdam et al. 1976 (reviewed in [5]).

Three or more clinical signs must be present:

1. Recurrent chondritis in both auricles
2. Non-erosive inflammatory polyarthritis
3. Nasal chondritis
4. Ocular inflammation
5. Respiratory tract chondritis
6. Audio vestibular dysfunction and damage

epistaxis and cartilage destruction with saddle nose deformity. Involvement of the larynx results in hoarseness, aphonia, wheezing and inspiratory stridor. Chondritis of the tracheobronchial tree causes effects varying from subtle, asymptomatic inflammation to life-limiting complications. Bronchial inflammation may cause stenosis, wall thickening, obstruction and hyperdynamic airway collapse. Symptoms include cough, dyspnoea and wheezing. In the early stages of the disease,

pulmonary function tests may indicate lower airway involvement of RPC even in asymptomatic patients. In cases of airway manifestations of RCP, it is important to diagnose early and to start treatment before irreversible damage occurs within the tracheobronchial system.

Beside the typical cartilage-including organs, other proteoglycan-rich structures such as eyes, inner ear, blood vessels and heart can also be involved. More than 50 % of patients (Table 1) develop ocular inflammation mainly as scleritis and episcleritis, but also in the form of keratoconjunctivitis Sicca, uveitis, ulcerative keratitis and optic neuritis.

## 4 Management

In regards to treatment of RPC, there are no evidence-based recommendations as randomised, controlled trials have not been published. Treatment is based on empirical clinical observations and usually starts with anti-inflammatory drugs targeting symptoms. In patients with mild symptoms of nasal or auricular chondritis or peripheral arthritis, nonsteroidal anti-inflammatory drugs (NSAIDs) may be sufficient. If NSAIDs are not effective, or a more rigorous anti-inflammatory treatment is indicated, glucocorticoids are the treatment of choice usually starting with 0.5 to 1 mg prednisone equivalents per kg bodyweight with the dose reducing to the minimal required amount. In life-threatening situations, with acute airway obstructions, pulse therapy with 1000 mg methylprednisolone intravenously for 3 days may be used.

Long term glucocorticoid therapy is associated with undesirable side effects but discontinuation of glucocorticoids often results in disease relapse. As in other chronic, inflammatory rheumatic diseases such as rheumatoid arthritis, several immunomodulatory and anti-inflammatory drugs have been used to reduce reliance on glucocorticoids. Disease modifying antirheumatic drugs (DMARDs) including methotrexate, leflunomide, azathioprine, cyclophoshamide, cyclosporine have been tried as has intravenous immungloulin. A treatment of choice has not yet been defined. Within the last few years, in some patients with a catastrophic course or symptoms refractory to all therapeutic regimes, biologicals, as used in refractory, chronic, inflammatory rheumatic diseases, have been tried. Successful treatment of refractory RPC was described with drugs targeting proinflammatory cytokines [5] such as anakinra (blocking IL-1 signalling), tocilizumab (blocking IL-6 signalling) and TNF-inhibitors (blocking TNF-alpha) including infliximab, etanercept and adalimumab. With rituximab (anti-CD20 resulting in B-cell depletion), only a partial response was seen. Abatacept, a T-cell co-stimulation inhibitor, was successfully administered to patients with RPC and is currently being tested in RCP in a phase 1 trial.

RPC is a rare, systemic, inflammatory, autoimmune disease with variable features and which targets cartilage. The clinical symptoms show a wide rage from

mild attacks, easily handled with NSAIDs on demand, up to acute-onset and life-limiting airway-destructing inflammation. Early diagnosis is important to limit irreversible cartilage destruction and fatal complications. Glucocorticoids are the most important medical treatment. Biologicals may be the treatment of choice to minimise glucocorticoids or in cases of refractory disease.

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# Psoriasisarthritis

Manfred Herold

## 1 Introduction

Psoriatic arthritis (PsA) is a chronic, inflammatory arthropathy associated with psoriasis. Joint pain, stiffness and swelling are the main symptoms of PsA which affects peripheral joints, spine, and entheses and is characterized by diverse phenotypic subtypes and a variable clinical course. 5 to 7 % of patients with psoriasis are affected. Untreated, this disorder may result in joint damage with significant functional impairment, disability, reduced quality of life and increased mortality.

## 2 Diagnostic criteria

For many years the Moll and Wright criteria [1], based on five distinct clinical subsets (oligoarticular asymmetric arthritis, polyarticular arthritis, distal interphalangeal joint predominant, spondylitis predominant, and arthritis mutilans), have been used for the classification of PsA. According to these criteria, PsA can be classified in a patient who has psoriasis, an inflammatory form of arthritis, is negative for rheumatoid factor, and shows one of five distinct clinical subsets (Table 1).

**Table 1.** Clinical subtypes for psoriatic arthritis described by Moll and Wright [1]. To diagnose psoriatic arthritis a patient with psoriasis, inflammatory arthritis and rheumafactor negative must present one of the five clinical subtypes. With these criteria specificity is 98 % and sensitivity 91 %. The frequency is estimated according to different publications.

| Type | Clinical Subtypes | Frequency |
|---|---|---|
| I | distal interphalangeal joint predominant as for osteoarthritis | 5 % |
| II | arthritis mutilans | 5 % |
| III | symmetrical polyarthritis as in RA | 15 % |
| IV | oligoarticular asymmetric arthritis, often HLA-B27 positive | 70 % |
| V | spondylitis predominant, HLA-B27 positive | 5 % |

Over the last few years, several classification criteria for PsA have been proposed [2] and used in literature but none of them have been accepted as the best to define patients with PsA. In 2006 the CASPAR (ClASsification criteria for Psoriatic ARthritis) study group developed a new classification scheme based on extensive analysis of over 500 patients with PsA and more than 500 patients with other types of inflammatory arthritis serving as controls [3]. According to the CASPAR criteria, a disease may be classified as PsA in the presence of an established, inflammatory, articular disease with at least 3 points from the following features:

- current psoriasis (assigned a score of 2; all other features are assigned a score of 1),
- a history of psoriasis (unless current psoriasis is present),
- a family history of psoriasis (unless current psoriasis is present or there is a history of psoriasis),
- dactylitis, juxta-articular new bone formation,
- rheumatoid factor negativity, and
- nail dystrophy (Table 2).

Skin involvement has the highest scoring with 2 points indicating that most patients have develop psoriasis before PsA.

**Table 2.** CASPAR criteria for psoriatic arthritis [3].

| Presence of inflammatory articular disease (joint, spine, or entheseal) plus the following features which are validated by points. | |
|---|---|
| • Psoriasis current | 2 |
| history of Psoriasis | 1 |
| family history of Psoriasis | 1 * |
| • Nail dystrophy | 1 |
| • negative rheumatoid factor | 1 |
| • dactylitis current | 1 |
| • history of dactylitis | 1 |
| • Radiographs (hand or foot) | |
| with juxta-articular new bone formation | 1 ** |
| To meet the CASPAR 2006 classification criteria for psoriatic arthritis, a patient must have inflammatory articular disease and $\geq$3 points from the remaining categories. Criteria specificity is 98.7 % and sensitivity is 91.4 %. | |

* patient-reported history in a first- or second-degree relative.
**as recorded by a rheumatologist.

## 3 Requirements for family practitioners

An estimated 5–7 % of people with psoriasis also have psoriatic arthritis and the annual incidence rate is close to 2 PsA cases per 100 psoriasis patients. The incidence seems to be unrelated to the duration of psoriasis but several studies suggest that the severity of psoriasis is associated with a higher risk of developing PsA (reviewed in [4]). Psoriatic arthritis usually begins between the ages of 30 and 55 years and has an equal sex distribution.

Diagnosis is mainly based on clinical symptoms. No specific laboratory tests are known for PsA. Non-specific markers of inflammation such as ESR and CRP may be elevated and correlate with the numbers of involved joints.

In clinical examination the arthritis presents with typical signs of inflammation such as tenderness, warmness, swelling and limitation of motion. In the earliest stage of PsA a monoarthritis of a knee is often reported.

In severe cases the erosive arthritis may cause a complete resorption of entire phalanges resulting in a so called arthritis mutilans with clinical features including "falling joints" or digital telescoping described as "opera glass finger" (Fig. 1). Alternatively, spondylitis with stiffness and pain in lower back and neck resulting from inflammation of the joints and discs in the spine may be the dominant clinical symptom.

Joint pain can also occur without joint inflammation and the clinical sign of swelling. Painful distal interphalangeal (DIP) joints are one of the possible distinct

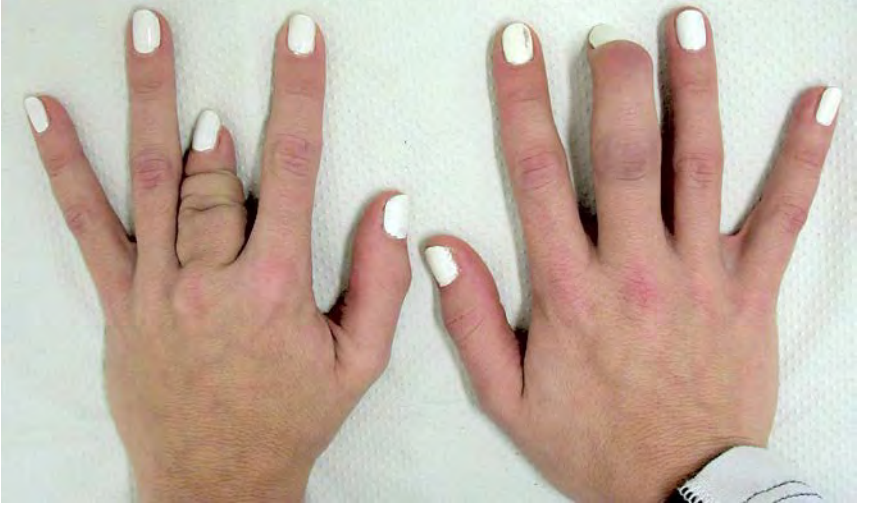

**Figure 1.** Arthritis mutilans of finger 3 on the left hand and dactylitis of finger 3 on the right hand (picture M. Herold 2005).

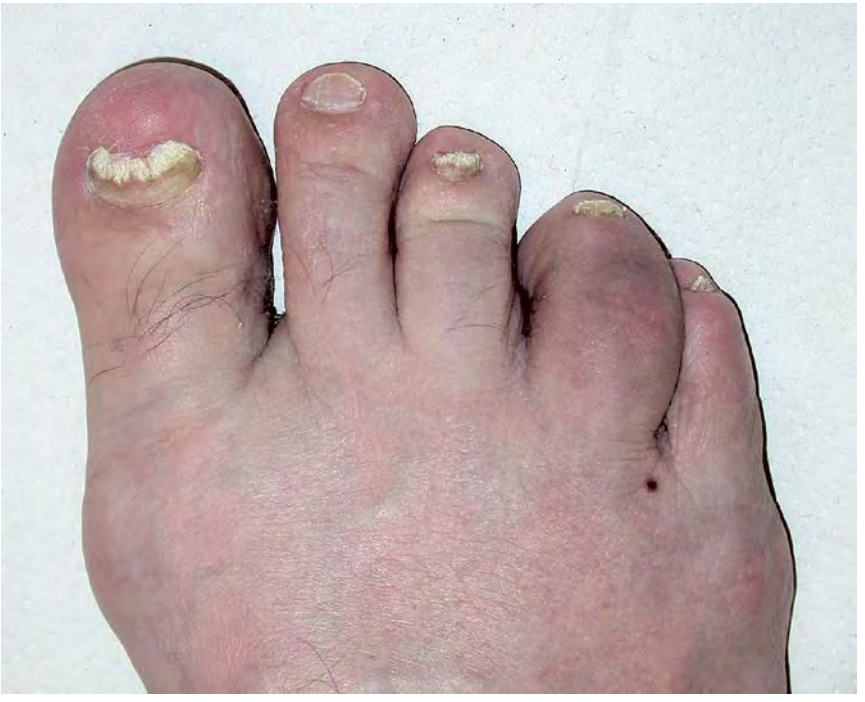

**Figure 2.** Dactylitis of toe 4 and psoriasis associated nail dystrophy (picture M. Herold 2005).

features of PsA and might be misinterpreted as osteoarthritis of the DIP joints. DIP arthritis or DIP arthropathy is usually associated with nail psoriasis.

Tenderness, pain and swelling over tendons may be caused by an inflammatory involvement of the entheses. Achilles tendon and entheses of the lower limbs are most often involved but other locations of tendon insertions (pelvis, thorax, epicondyles) are also possible.

A further characteristic feature of PsA is dactylitis or "sausage-shaped digit" (Fig. 2). Dactylitis is defined as diffuse and usually painful swelling of the entire digit due to a combination of synovitis of interphalangeal joints in line and flexor tenosynovitis.

## 4 Diagnostic measurement for experts

PsA is influenced by genetic factors and associated with human leukocyte antigen (HLA) alleles including HLA-Cw6, HLA-B13, B-17, B-27 and others. Oligoarticu-

lar (4 or fewer involved joints) or polyarticular (5 or more involved joints) asymmetric arthritis are the most frequent patterns observed in patients with PsA.

Typical radiographic features have been described in PsA with signs of destructive and proliferative changes. In peripheral PsA, radiographs show marginal erosions with adjacent bone proliferation, lack of periarticular demineralisation, (sub)luxations, ankylosis and pencil-in-cup phenomena. Paravertebral soft tissue calcifications, asymmetrical paravertebral ossification and signs of asymmetrical sacroiliitis may be seen along the spine.

## 5 Management

Treatment of PsA depends on the symptoms and severity of the disease and should be appropriately customised. A curative treatment does not exist, but, without treatment, PsA may be disabling. In mild forms of the disease, nonsteroidal anti-inflammatory drugs (NSAIDs), analgesics and low-dose glucocorticoids may be used. Alternatively, infiltrative therapy with intra-articular glucocorticoids in single joint involvement or enthesial inflammation may be appropriate. Nonresponders and patients with severe peripheral arthritis should be treated with disease modifying antirheumatic drugs (DMARDs) as used in rheumatoid arthritis. In PsA, methotrexate (MTX) is the most commonly used DMARD with efficacy on symptoms of arthritis and skin. Leflunomide has also shown effectiveness in PsA and treating skin symptoms. Sulfasalazine may improve the symptoms of arthritis but is ineffective on the skin. Cyclosporine can achieve rapid improvement of skin lesions caused by psoriasis but is less effective in musculoskeletal symptoms. Antimalarials such as chloroquine and hydroxychloroquine are ineffective. In patients with ankylosing spondylitis, DMARDs such as MTX, leflunomide or sulfasalazine have been ineffective in treating axial manifestations. From this experience it can be concluded that these DMARDs would also be ineffective in treating spinal symptoms of PsA.

TNF-α seems to play a central role in the pathogenesis of both PsA and psoriasis. The TNF-inhibitors etanercept, infliximab, adalimumab and golimumab have been approved for the treatment of PsA and psoriasis [5]. All TNF-α inhibitors have demonstrated their efficacy in different clinical disease expressions including peripheral arthropathy, axial involvement, enthesopathy and skin manifestations. Several controlled studies also demonstrated that TNF-α inhibitors are able to slow down the radiological progression of PsA.

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# ANCA-associated vasculitides

Julia U. Holle, Elena Csernok, Allan Wiik, Wolfgang L. Gross

## 1 Introduction

The ANCA-associated vasculitides (AAV) are comprised of Granulomatosis with Polyangiitis (formerly Wegener's Granulomatosis, GPA), Microscopic Polyangiitis (MPA) and Churg-Strauss Syndrome (CSS) (see [1] for review and Figs. 1 and 2). They share the features of small vessel vasculitis but are otherwise a heterogeneous group with different preferences of organ involvement and frequency of ANCA positivity. The AAV are classified according to the American College of Rheumatology (ACR) criteria and the Chapel Hill Consensus Conference (CHC) definitions (Table 1) [see 1 for review]. Due to efforts to eliminate eponyms in disease names, Wegener's granulomatosis was renamed in 2011 to Granulomatosis with Polyarteritis (GPA) [2].

In general, GPA and CSS are characterised by granulomatous lesions (especially of the respiratory tract) and small- to medium size vessel vasculitis in biopsy specimens, whereas MPA is a small to medium size vessel vasculitis without granuloma. Moreover, GPA is characterized by space-consuming lesions, e.g. orbital or pulmonary "granuloma" or masses. In CSS, asthma and eosinophilia in peripheral blood and affected tissues are also a hallmark of the disease. Generalised disease in GPA and CSS is usually preceded by a localised phase in GPA (upper and lower respiratory tract involvement, e.g. sinusitis) and a phase of refractory asthma/localized polypoid sinusitis and/or eosinophilia in CSS (Table 2). ENT manifestations/relapses of GPA may be associated with nasal carriage of *Staphylococcus aureus*. Vasculitis manifestations include alveolar haemorrhage, glomerulonephritis, sensorimotor polyneuropathy and represent potentially life-threatening organ manifestations. Virtually any organ can be affected by small vessel vasculitis (Table 2). ANCA are detected in almost all patients with MPA and GPA in the active generalised stage of the disease; in CSS, ANCA are present in only 40 % and their presence is associated with typical vasculitis manifestations such as glomerulonephritis. In GPA, ANCA are mainly directed against the neutrophil serine protease proteinase 3 (PR3), whereas, in MPA and CSS, ANCA mainly target the neutrophil enzyme myeloperoxidase (MPO). ANCA play a major pathogenetic

**Table 1.** Classification criteria of ANCA-associated vasculitides.

| ANCA-associated vasculitis | American College of Rheumatology Criteria | Chapel Hill Consensus Conference Criteria |
|---|---|---|
| **Granulomatosis with Polyangiitis (formerly Wegener's Granulomatosis)** | **nasal or oral inflammation**<br>**abnormal chest radiograph:** nodules fixed infiltrates or cavities<br>**abnormal urinary sediment**: microhaematuria/ red cell casts<br>**granulomatous inflammation on biopsy**<br>*at least 2 of 4 criteria must be present* | granulomatous inflammation involving the respiratory tract, necrotizing vasculitis affecting small to medium-size vessels<br>*necrotizing glomerulonephritis is common* |
| **Microscopic Polyangiitis** | no criteria | necrotizing vasculitis affecting small to medium-size vessels<br>*necrotizing glomerulonephritis is very common, pulmonary capillaritis often occurs* |
| **Churg-Strauss-Syndrome** | **asthma**<br>**blood eosinophilia** (> 10 % on white cell count)<br>**mono- or polyneuropathy**<br>**pulmonary infiltrates**, non-fixed<br>**paranasal sinus abnormality**<br>**extravascular eosinophils in biopsy**<br>*at least 4 criteria must be present* | **eosinophil-rich and granulomatous inflammation of the respiratory tract**, **necrotizing vasculitis affecting small to medium-size vessels**, associated with asthma and blood eosinophilia |

role in the induction of small vessel vasculitis, as they induce neutrophil activation in small vessels by interacting with their target antigens which are expressed on the surface of activated neutrophils [1].

The AAV are rare diseases: GPA is the most frequent AAV with an incidence of 9/Mill/yr. The incidence rates of CSS and MPA are 1–2.4/Mill/yr and 1/Mill/yr, respectively [3].

## 2 Diagnostic procedures for experts

AAV are multi-system disorders often affecting many organs. Therefore, they require a thorough patient inquiry regarding potential organ manifestations. The suspicion of AAV should be raised if a patient presents with refractory sinusitis or asthma, especially if these symptoms occur in the context of massive fever, weight loss, impaired kidney function (crescentic glomerulonephritis!), haemoptysis (alveolar haemorrhage), purpura (leukocytoclastic vasculitis of the skin), or sensorimotor paresis (polyneuropathy). Proptosis of the bulbus may be a sign of

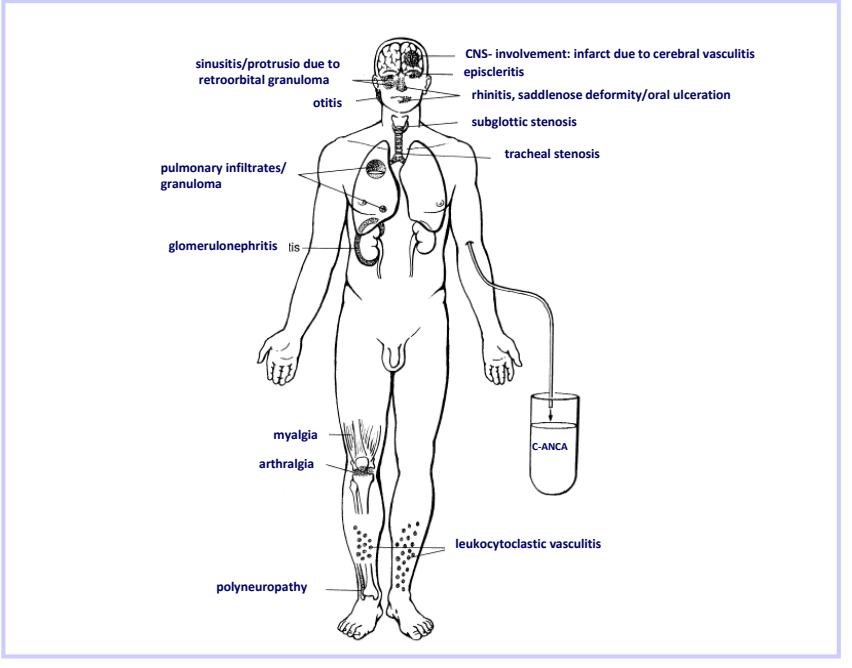


**Figure 1.** Clinical manifestations of Granulomatosis with Polyangiitis.

**Table 2.** Frequency of organ involvement in ANCA-associated vasculitides.

| Organ involvement | GPA (%) | MPA (%) | CSS (%) |
|---|---|---|---|
| Joints | 25 | 50 | 28 |
| Upper respiratory tract (e.g. rhinitis, sinusitis) | 90 | not spec. | 47 |
| Asthma | not spec. | not spec. | 100 |
| Lower respiratory tract (e.g. infiltrates, nodules, alveolar haemorrhage) | 50 | 35 | 38 |
| Kidney (glomerulonephritis) | 50 | 80 | 16 |
| Heart (e.g. myocarditis, coronary arteritis) | 10 | 20 | 30 |
| Skin (e.g. purpura) | 20 | 70 | 31 |
| Peripheral nervous system (mono- or polyneuropathy) | 20 | 60 | 78 |
| Gastrointestinal tract ( e.g. ulcers, bleeding) | not spec. | 30 | 33 |
| ANCA pos. | 80 | 75 | 48 |

retro-orbital granulomatous masses, and compromised respiratory function may be a sign of subglottic inflammation/stenosis in GPA.

Routine work-up includes blood testing (ESR and CRP, blood count, creatinine and serum electrolytes), urinalysis, ANCA testing and chest X-ray. ENT assessment should be done routinely in GPA and CSS.

Creatinine clearance and 24-hr protein quantification need to be performed if serum creatinine and/or urinalysis are pathological. If there are pathological findings on X-ray, high-resolution CT (HR-CT) and/or bronchoalveolar lavage is used to confirm granulomatous lesions within the airways, alveolar haemorrhage or alveolitis.

MRI of the head is a useful technique to detect sinusitis and granuloma formation in GPA, but there is no agreement as to whether an MRI should initially be done as a routine or only performed if the patients present with symptoms. In CSS, routine assessment also includes lung function testing.

Further diagnostic tests should be performed according to the patient's symptoms (e. g. neurological assessment including EMG and ENG or full cardiac examination).

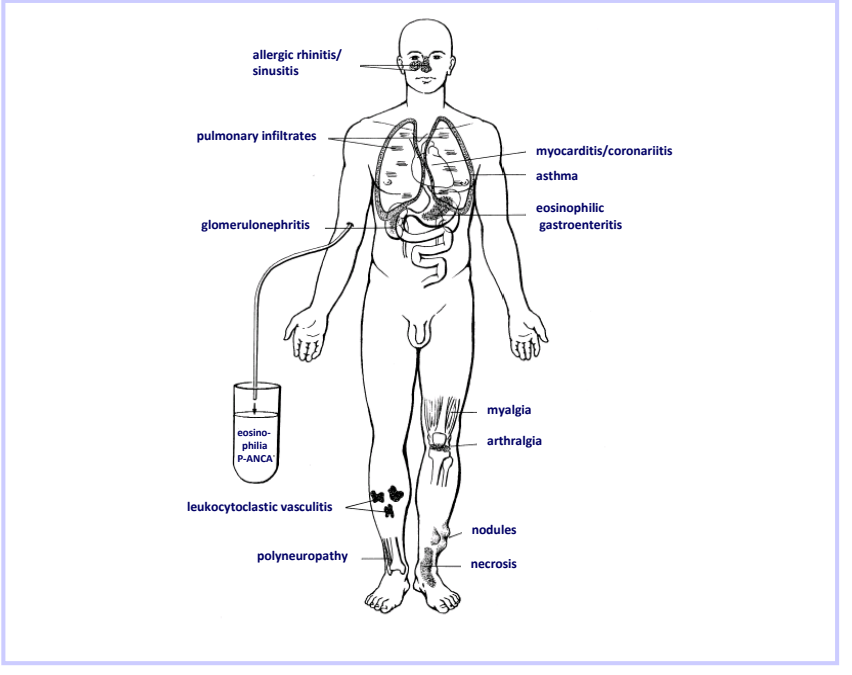


**Figure 2.** Clinical manifestations of Churg-Strauss-Syndrome.

To confirm the diagnosis, a biopsy from an affected area should be sought (e. g. nasal biopsy, kidney biopsy) at first presentation of the patient.

## 3 Requirements for family practitioners

If AAV is suspected, the patient should be referred to a rheumatologist/internist. The rheumatologist should screen the patient for organ manifestations and initiate immunosuppressive therapy according to disease stage and activity. After immunosuppressive therapy is introduced, the patient requires monitoring of disease activity and potential side effects of treatment (see below).

Routine blood tests to monitor disease activity include ESR and CRP, blood count, serum creatinine and electrolytes and urinalysis. Creatinine clearance and proteinuria should be assessed regularly in cases of renal involvement. Immunosuppressive therapy may cause bone marrow toxicity with leucopenia/pancytopenia or hepatotoxicity. Regular screening of blood count and hepatic enzymes is therefore needed under most immunosuppressants. Cyclophosphamide can induce haemorrhagic cystitis and bladder carcinoma via its toxic metabolites (such as acroleine). Patients under cyclophosphamide therapy should therefore receive mesna which binds to acrolein.

Blood tests are usually carried out by the family practitioner on a regular basis as recommended by the respective specialist (ranging from once weekly to once monthly). The role of serial ANCA testing is controversial and is usually done at intervals of several months.

Relapse of the disease occurs in 30 to 60 % of patients. In case of recurrent disease activity, the suspicion of relapse or side effects due to immunosuppressive therapy, the responsible specialist should be contacted.

## 4 Follow up

Patients require immunosuppressive therapy for several years or for life. Patients remain at risk for relapse or opportunistic infections such as CMV reactivation or *Pneumocystis jirovecii*-pneumonia. In some patients, irreversible organ damage occurs if immunosuppressive therapy is introduced too late (e. g. polyneuropathy of haemodialysis due to renal insufficiency).

Follow-up assessments are usually done by the specialist every three to six months and include routine assessment as stated above (blood tests, ANCA, urinalysis, creatinine clearance and assessment of proteinuria) and additional technical diagnostic procedures according to organ involvement and symptoms (e. g. MRI of the skull for retro-orbital granuloma).

**Table 3.** Treatment of AAV modified from EULAR/EUVAS recommendations.

| Disease stage | Recommended treatment |
|---|---|
| **Localised (WG) GPA** | **Cotrimoxazole** 2 × 960 mg/day |
| **Early systemic (induction)** | **MTX** 15 mg /week s.c. or oral, increase to 20–25 mg/week + GC<br>folic acid substitution |
| **Generalised (induction)** | **Cyclophosphamide i.v. or Rituximab (RTX) i.v. + glucocorticoids**<br>**Cyc** 15 mg/kg i.v. for at least 6 times in two to three-weekly intervals<br>**RTX** 375 mg/m2 i.v. 4× in weekly intervals<br>**GC**: prednisolon 1 mg/kg/day for 1 month,<br>taper to <15 mg/day within 3 months |
| **Severe, Crea > 500 µmol/l** | standard therapy for generalized disease + plasma exchange |
| **Maintenance of remission** | **Azathioprine** 2 mg/kg/day and **MTX** 20–25 mg/week (first choice)<br>**Leflunomide** 20 mg/day<br>duration: at least 18 months |
| **Refractory, Relapsing, Persistent** | **IVIG** 2 g/kg for 5 days<br>**Rituximab** 375 mg/m2 weekly for 4 weeks<br>**Infliximab** 3–5 mg/kg i.v. one to two monthly<br>**MMF** 2g/day<br>**15-deoxyspergualin** 0.5 mg/kg/day until nadir; then stop until leucocyte recovery (six cycles)<br>**ATG** 2.5 mg/kg/day for 10 days (adjusted to lymphocyte count) |

## 5 Management

In current treatment recommendations by the EULAR (European League Against Rheumatism), therapy is tailored according to disease stage and activity [4] (Table 3). Life- or organ-threatening disease (e. g. alveolar haemorrhage, extracapillary necrotizing glomerulonephritis) requires remission induction with cyclophosphamide (oral or i. v. pulse) or rituximab and glucocorticoids (initially 1 mg/kg/day). While cyclophosphamide has been the gold standard of remission induction for many years, recent studies suggest that rituximab ($4 \times 375$ mg/m$^2$) at weekly intervals is equally effective [5]. Rituximab has been licensed for remission induction for GPA and MPA in the US in 2011. Remission induction is usually needed for three to six months. During this period of time, glucocorticoids should be tapered to less than 10 mg/day (prednisolone). After successful induction of remission, the therapy regimen is switched to maintenance medication such as azathioprine, methotrexate and leflunomide (plus low-dose glucocorticoids). There are no controlled studies evaluating for how long maintenance therapy is necessary. Current guidelines recommend maintenance therapy for at least 18 months. In the US, glucocorticoids are often stopped early (after several months), whereas in Europe glucocorticoid therapy is kept for longer. In severe disease (defined as renal failure with a creatinine $> 500\mu$mol/l) plasma exchange is recommended in addition to standard therapy.

In cases of systemic disease without threatened organ function (the so-called early systemic phase of disease), MTX (plus glucocorticoids) is recommended for the induction of remission. Localised disease in GPA (defined as disease limited to the upper and respiratory tract with no systemic symptoms) may be treated with cotrimoxazole to reduce relapses of the upper respiratory tract, probably by controlling nasal *Staphylococcus aureus* infection. Therapy options for refractory disease include rituximab, TNF-antagonists, intravenous immunoglobulins (IVIG), deoxyspergualin and Antithymocyte-globulin (ATG) (Table 3).

## 6 Diagnostic tests

Anti-neutrophil cytoplasmic antibodies (ANCA) are used as diagnostic markers for the ANCA-associated vasculitides, especially in generalised GPA and MPA, as ANCA is found in a high percentage of these patients. In CSS, ANCA is detected in only 40 % of cases and seems to be correlated to vasculitic manifestations in CSS (such as glomerulonphritis and polyneuropathy). In GPA, ANCA are mainly directed against the neutrophil serine protease proteinase 3 (PR3), whereas in MPA and CSS, ANCA mainly target the neutrophil enzyme myeloperoxidase (MPO).

An immunofluorescence test (IFT) is used as a screening test for the detection of ANCA (Fig. 3). If IFT is positive, an enzyme-linked immunosorbent assay (Elisa) needs to be performed to identify the target antigen of ANCA. Only pro-

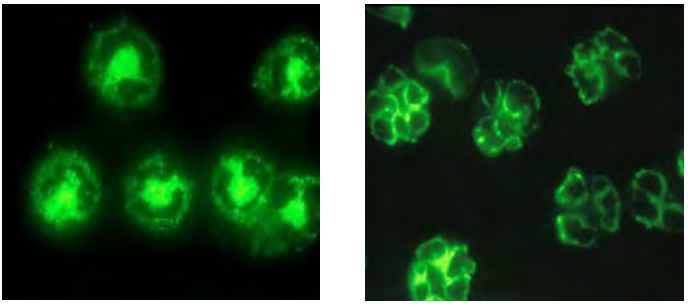

**Figure 3.** IFT with formalin-fixed neutrophils displaying a cytoplasmatic (left) and perinuclear pattern (right).

teinase 3 (PR3) and myeloperoxidase (MPO) represent common specific target antigens for AAV. Consensus guidelines currently recommend performing an IFT together with an Elisa to detect the ANCA-pattern and the target antigen [4].

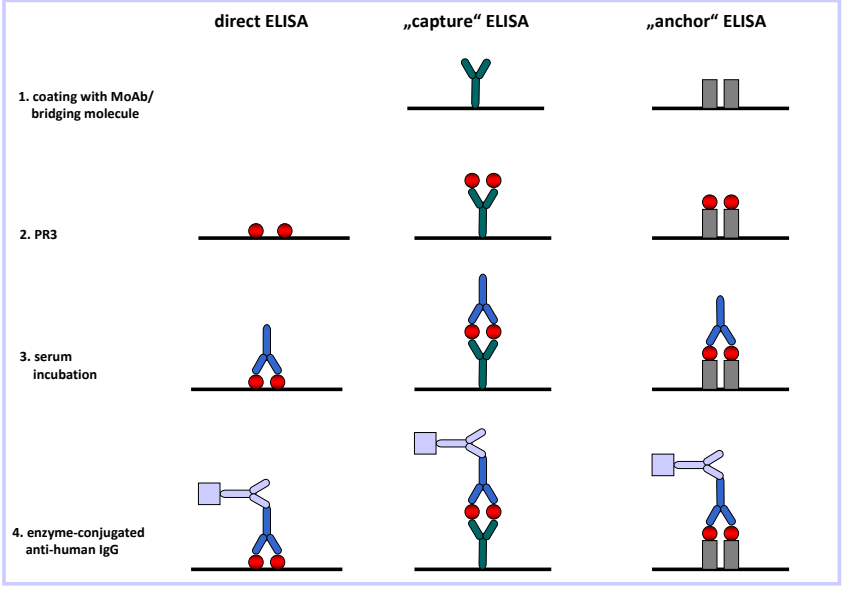


**Figure 4.** Systematic overview on Elisa procedures for the detection of ANCA directed against proteinase 3.

## 7 Testing methods

By IFT, two main fluorescence patterns can be distinguished, a cytoplasmic (C-ANCA) and a perinuclear pattern (P-ANCA). Target antigens are detected by enzyme-linked immunosorbent assay (Elisa). Conventional (direct) Elisas using PR3 immobilised to the surface of the Elisa plate, are not standardised and show a great variation in performance and can lack sensitivity as well as specificity, but are still routinely used for the detection of the target antigen of ANCA.

To reduce the covering of possible epitopes by the plastic plate in conventional Elisas, capture Elisa (sensitivity 72–76 %, specificity 100 %) has been developed and is superior in overall diagnostic performance compared to direct Elisa (sensitivity 58–80 %, specificity 95–100 %), however, the sensitivity of capture Elisa may also be reduced by the capturing antibodies, which may also hide relevant epitopes [6]. High-sensitivity PR3-ANCA Elisa (hsPR3-ANCA Elisa) immobilises PR3 via a bridging molecule to the plastic plate, thus preserving all epitopes for the binding of ANCA, and is superior to direct Elisa and capture Elisa in a study testing for PR3-ANCA in patients with GPA [6] (sensitivities and specificities for direct Elisa: 60 % and 99 % respectively, for capture Elisa: 72 % and 99.3 % respectively, for high sensitivity Elisa: 96 % and 98.5 % respectively) (see Fig. 4 for Elisa procedures).

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# Non-ANCA-associated vasculitides

Julia U. Holle, Elena Csernok, Wolfgang L. Gross

## 1 Introduction

In general, vasculitides are divided according to their manifestation in different vessel beds. Apart from ANCA-associated vasculitides, cryoglobulinaemic vasculitis, Henoch-Schoenlein purpura, cutaneous leukocytoclastic vasculitis and secondary vasculitides due to rheumatoid arthritis, systemic lupus erythematosus (SLE) or Sjögren's Syndrome comprise the group of small vessel vasculitides. In polyarteri-

**Table 1.** Classification criteria of large vessel-vasculitides.

| | **American College of Rheumatology Criteria** | **Chapel Hill Consensus Conference Criteria** |
|---|---|---|
| **Takayasu's Arteritis** | Age < 40 years<br>Claudication of extremities<br>Decreased brachial artery pulse<br>Blood pressure difference > 10 mm Hg<br>Bruit over arteries<br>Arteriogram abnormality | Granulomatous arteritis of aorta and its major branches<br><br>*Usually occurs in patients younger than 50 years.* |
| **Temporal Arteritis/ Giant Cell Arteritis** | Age > 50 years<br>New headache<br>Temporal artery tenderness<br>Increased ESR > 50 mm/h<br>Abnormal artery biopsy: vasculitis with a predominance of mononuclear or granulomatous inflammation | Granulomatous arteritis of aorta and its major branches, with a predilection for the extra cranial branches of the carotid artery<br><br>*Usually occurs in patients older than 50 years and is often associated with polymyalgia rheumatica.* |

**Table 2.** Classification criteria of panarteritis nodosa.

| | American College of Rheumatology Criteria | Chapel Hill Consensus Conference Criteria |
|---|---|---|
| **Polyarteritis nodosa** | Weight loss<br>Livedo reticularis<br>Testicular pain or tenderness<br>Myalgia, weakness or leg tenderness<br>Mono- or polyneuropathy<br>Diastolic blood pressure > 90 mmHg urea > 40mg/dl or creatinine > 1.5 mg/dl<br>Hepatitis B virus<br>Arteriographic abnormality (aneurysms or occlusion of the visceral arteries)<br>Biopsy of medium size vessel (small or medium sized artery) containing PMN | Necrotizing inflammation of medium-sized or small arteries without glomerulonpehritis or vasculitis in arterioles, capillaries or venules |

**Table 3.** Classification criteria of non-ANCA associated small vessel vasculitides.

| | American College of Rheumatology Criteria | Chapel Hill Consensus Conference Criteria |
|---|---|---|
| **Cryoglobulinaemic Vasculitis** | No criteria | Vasculitis, with cryoglobulin immune deposits, affecting small vessels and associated with cryoglobulins in serum |
| **Henoch-Schönlein pupura** | Palpable purpura<br>Bowel angina<br>Age at onset < 20 years<br>Biopsy showing granulocytes in the walls or arteries and venules | Vasculitis, with IgA-dominant immune deposits, affecting small vessels |
| **Cutaneous Leucocytoclastic Vasculitis** | No criteria | Isolated cutaneous leucocytoclastic angiitis without systemic vasculitis |

tis nodosa, medium-size vessels are involved, whereas the large-vessel vasculitides are represented by giant cell arteritis and Takayasu's arteritis. Classification criteria of the vasculitides are given in Tables 1–3, Fig. 1.

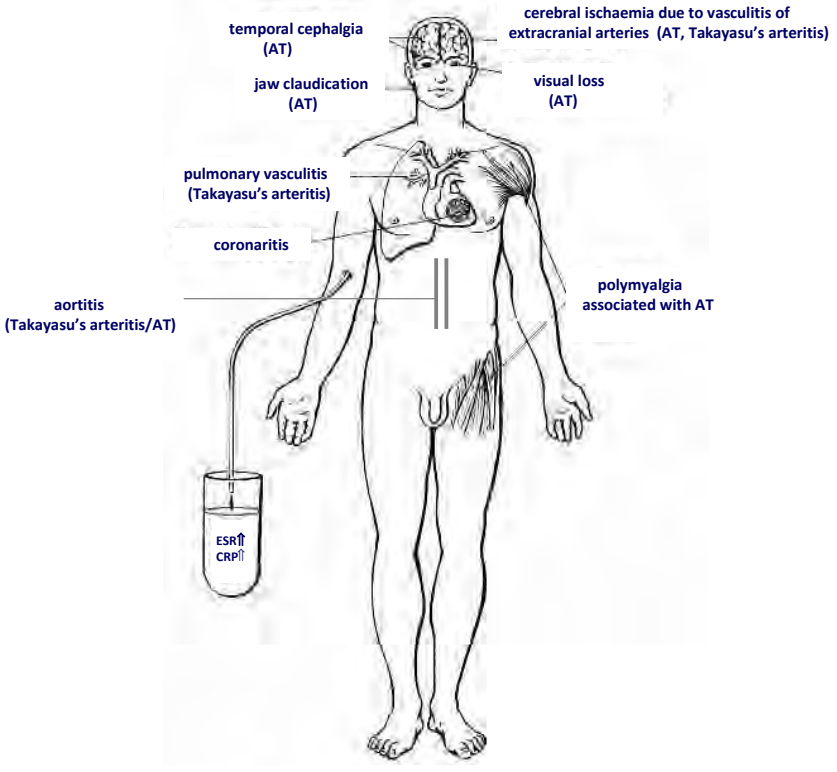


**Figure 1.** Clinical manifestations of temporal arteritis and Takayasu's arteritis.

**Table 4.** Signs and symptoms of GCA/arteritis temporalis.

| Symptoms of GCA/AT | Frequency (%) |
|---|---|
| Cephalgia | >95% |
| Visual disturbance | 30% |
| Visual loss | 10–15% |
| Jaw claudication | |
| Fever | 10–15% |
| Aortitis | 10–15% |

**Table 5.** Signs and symptoms of Takayasu's arteritis (TA).

| Symptoms of TA | Frequency (%) |
|---|---|
| Diminished or absent pulses | 85–95% |
| Vascular bruits | 80–95% |
| Hypertension (renal) | 30–80% |
| Retinopathy | up to 40% |
| Aortic regurgitation due to dilatation of aorta | 20% |
| Pulmonary artery involvement | 15–100% |

Large-vessel vasculitides are characterised by ischaemic symptoms due to stenosis or occlusion of these vessels [1] (Tables 4 and 5). In giant cell arteritis (GCA), temporal arteritis is the typical manifestation, leading to sudden and severe temporal cephalgia. Visual loss may also occur (in around 10 to 15 % of patients) and is usually due to vasculitis of the posterior ciliary artery and subsequent anterior ischaemic opticus neuropathy [1, 2]. Temporal arteritis/GCA is often associated with polymyalgia rheumatica (PMR), which is characterised by severe, proximal myalgia, but PMR also occurs alone. Apart from the temporal artery, other large vessels such as the aorta or brachial/femoral arteries may also be affected and then may lead to claudication of the extremities, which is a hallmark not only of GCA but of Takayasu's arteritis (TA) [1, 2]. TA is a disease of younger people (aged less than 40 years) and tends to follow a more aggressive course. Complications of TA include renal artery stenosis, angina abdominalis

**Table 6.** Signs and symptoms of panarteritis nodosa.

| Symptoms of Panarteriitis | Frequency (%) |
|---|---|
| Weight loss, fever, night sweats | >70% |
| Polyneuropathy | 60% |
| Renal involvement (malignant hypertension, renal artery stenosis) | 40–60% |
| Gastrointestinal involvement (abdominal pain, aneurysmal bleeding) | 40% |
| Skin involvement (livedo, subcutaneous nodules, purpura, digital ischaemia/gangrene) | 40% |
| Arthralgia/myalgia; each: | 30% |
| CNS-involvement (encephalopathy, infarcts, subarachnoidal haemorrhage due to aneurysmal bleeding) | 20% |

due to mesenteric ischaemia, coronary arteritis, aortic regurgitation and pulmonary arteritis. Most frequently affected arteries are the subclavian arteries, the left carotid artery and the abdominal aorta [1, 2]. Temporal arteritis most frequently occurs in Northern Europeans (15–25/100 000/yr), whereas Takayasu's arteritis predominates in Japanese and southeast Asians and rarely occurs in western countries (incidence: 2.6/Mill/yr in North America). There is a female predominance in both GCA (female/male: 4 : 1) and TA (female: male 9 : 1). The female predominance in TA is reported to be lower in Western countries [1, 2].

The typical features of polyarteritis nodosa are aneurysms and/or stenosis of the visceral arteries due to vasculitis of medium-size vessels [3] (Table 6, Fig. 2). Gastrointestinal vasculitis may lead to bowel ischaemia or bleeding. Other vasculitis manifestations include polyneuropathy, vasculitis of skeletal muscles, stenosis of the renal arteries with subsequent hypertension, digital ischaemia/gangrene and CNS involvement. Polyarteriitis nodosa is strongly associated with hepatitis B, especially in countries where this viral infection is common. Its incidence is markedly

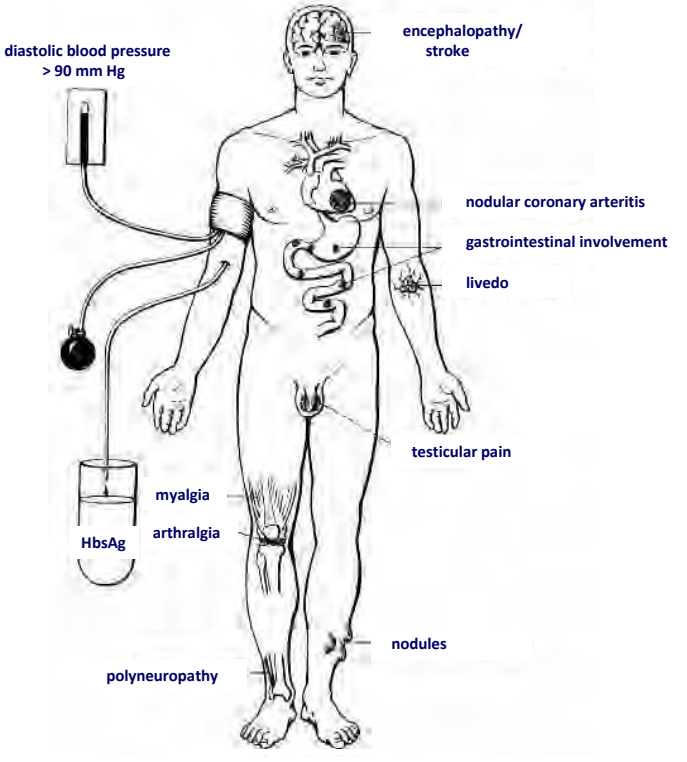


**Figure 2.** Clinical manifestations of polyarteritis nodosa.

higher in an Alaskan Eskimo population with a hyperendaemia for hepatitis B compared to European countries (77/Mill/yr compared to 0.2–34/Mill/yr). Importantly, panarteritis nodosa does, by definition, not affect small vessels, whereas in several small vessel-vasculitides, involvement of medium-size vessels is found (e.g. in granulomatosis with polyangiitis (formerly Wegener's Granulomatosis), microscopic polyarteritis).

In cryoglobulinemic vasculitis (CV), small vessels of skin (purpura), peripheral nerves (polyneuropathy) and kidney (membranoproliferative glomerulonephritis) are frequently affected [4] (Table 7, Fig. 3). CV is strongly associated with hepatitis C infection, especially in Mediterranean countries; if no underlying cause is found, "essential" CV is diagnosed. The definite incidence and prevalence of CV is not known, but is supposed to be higher in Southern Europe compared to Northern Europe and the US. In Southern Europe, 86 % of patients with CV show hepatitis C viraemia and 5 % of patients suffering from hepatitis C virus infection develop CV [4].

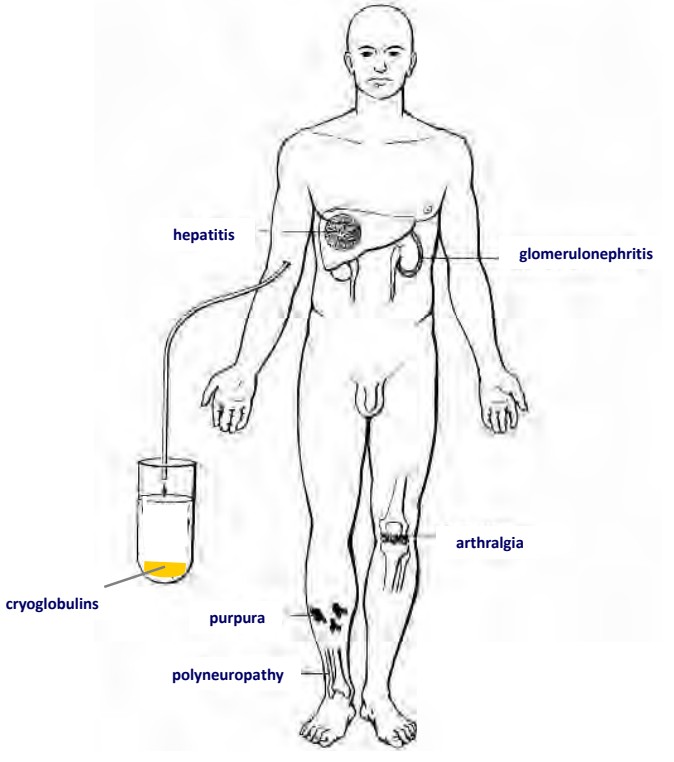


**Figure 3.** Clinical manifestations of cryoglobulinaemic vasculitis.

**Table 7.** Signs and symptoms of cyroglobulinaemic vasculitis (CV).

| Symptoms of CV | Frequency (%) |
|---|---|
| Purpura | 98% |
| Weakness | 100% |
| Arthralgia | 98% |
| Polyneuropathy | 80% |
| Raynaud's phenomenon | 50% |
| Hepatopathy | 80% |
| Renal involvement | 30% |

Henoch-Schoenlein purpura (HSP) mainly occurs in children (incidence 135–180/Mill/yr) and rarely in adults (incidence 13/Mill/yr) and is characterised by cutaneous vasculitis (purpura) with IgA immune complex deposits in the tissue and a decrease of serum complement proteins [5] (Table 8, Fig. 4). HSP may be complicated by renal and gastrointestinal involvement (mesangioproliferative glomerulonephritis, gastrointestinal bleeding due to erosions and ulcers). Prognosis is worse in adults than in children due to a higher frequency of renal involvement. Typically, initial macrohaematuria with subsequent microhaematuria is present in renal involvement. Infections and vaccination are under discussion as triggering factors for HSP.

In disorders such as RA and SLE, secondary vasculitis can occur. In both of these disorders, secondary vasculitis may be due to cryoglobulins. Small vessel vasculitis in RA and SLE predominantly affects skin (purpura) and peripheral nerves (polyneuropathy). In SLE diffuse alveolar haemorrhage due to pulmonary capillaritis and CNS vasculitis has also been described.

**Table 8.** Signs and symptoms of Henoch-Schönlein pupura (HSP).

| Symptoms of PSH | Frequency (%) |
|---|---|
| Purpura | >90% |
| Arthralgia/arthritis | 70% |
| Gastrointestinal involvement | 70% |
| (abdominal pain, nausea, haematemesis, intestinal bleeding) | |
| Renal involvement | 20-80% |

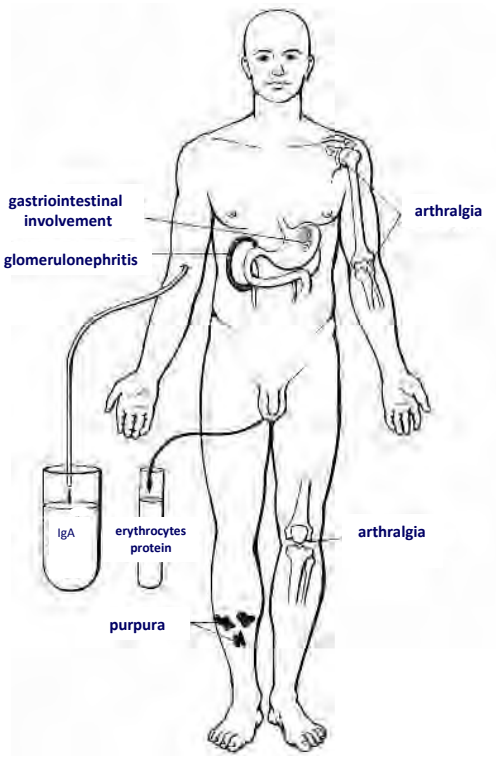


**Figure 4.** Clinical manifestations of Henoch-Schönlein pupura.

## 2 Diagnostic procedures for experts

Routine diagnostic procedures in large vessel vasculitis include palpation of arterial pulses, auscultation of large vessels and assessment of arterial blood pressure. When temporal arteritis is suspected, ultrasound of the temporal artery or cranial high-resolution MRI should be performed to search for inflammation of the vessel wall depicting itself as hypo-ecchogenic "halo". A temporal biopsy should be sought to confirm the diagnosis. Large vessel vasculitis of aorta and arteries of the extremities is detected by MR-angiography and PET (positron emission tomogram) The former visualizes wall oedema and stenosis, the latter shows an enhanced glucose uptake at sites of an inflammatory process, however, both methods have not been thoroughly validated yet for patients under treatment. In polyarteritis, angiography is also frequently used to assess aneuryms and/or stenosis. If possible, a biopsy specimen should be obtained (e. g. muscle biopsy).

The work-up in small vessel vasculitides depends on the suspected organ involvement, e.g. assessment of renal function (urinalysis, creatinine clearance, proteinuria), or neurological evaluation (EMG, ENG). A biopsy should be performed at first presentation to confirm the diagnosis (e.g. biopsy of skin, kidney, skeletal muscle or nervus suralis).

In general, serological markers of inflammation such as ESR and CRP are elevated and represent some of the items in some of the vasculitis classification criteria (e.g. in GCA). There is no specific marker (e.g. an autoantibody) in large vessel vasculitis and polyarteritis; serum should be tested for cryoglobulins, when CV is suspected, and the type of cryoglobulin needs to be assessed if cryoglobulins are detected. CV is characterised by the occurrence of a type II (or sometimes type III) cryoglobulinaemia (Tables 1, 7). The patient should be screened for underlying diseases inducing cryoglobulins such as infections (hepatitis C) and autoimmune disorders (RA, SLE, Sjögren's syndrome). As CV and HSP are immune complex diseases, complement proteins are usually decreased. In polyarteritis, hepatitis B serology needs to be obtained. Serum IgA levels may be elevated in HSP.

## 3 Requirements for family practitioners

In cases of high acute phase reactants and acute temporal cephalgia in patients aged over 50 years, the suspicion of temporal arteritis must be raised. Claudication of extremities, angina abdominalis, stroke or myocardial infarction in young people in conjunction with high acute phase reactants is highly suspicious of Takayasu's arteritis.

Medium and small-vessel vasculitides affect multiple organs and may be more difficult to recognize. Weight loss, fever, arthralgia, high acute phase reactants are common but very unspecific signs of medium- and small-vessel vasculitides. Livedo, purpura, myalgia, polyneuropathy, gastrointestinal bleeding or impaired kidney function may represent signs of an underlying vasculitis in patients with elevated acute-phase reactants.

If any of the diseases are suspected, the patient should be referred to a rheumatologist/rheumatology or internal medicine unit immediately.

## 4 Follow up

Patients require immunosuppressive therapy for several years or for life. Patients remain at risk for relapse or opportunistic infections such as CMV reactivation or *Pneumocystis jirovecii*-pneumonia. In some patients, irreversible organ damage occurs if immunosuppressive therapy is introduced too late (e.g. persistent visual loss in temporal arteritis, persistent polyneuropathy or requirement for haemodialysis due to renal insufficiency).

Patients need to be assessed for signs and symptoms of disease activity on a regular basis by the family practitioner (ideally monthly) and by the rheumatologist (every three months when the disease is stable). Acute phase reactants such as ESR and CRP should also be tested regularly (monthly when the disease is stable). Furthermore, immunosuppressive therapy needs surveillance of certain laboratory parameters as suggested by the specialist (see chapter ANCA-associated vasculitides). In some cases, image guided techniques or other technical diagnostic procedures are needed to document follow-up (e.g. MR-angiography), but may not be validated.

Follow-up laboratory tests (such as cryoglobulins, hepatitis viral load or complement proteins) will be carried out by the specialist.

## 5 Management

Therapy of the vasculitides is adapted according to organ involvement and activity of the disease. Recently, recommendations for the management of small and medium-size vessel vasculitis [6] and large vessel vasculitis [7] have been published by the EULAR (European League Against Rheumatism, Table 9).

In large vessel vasculitis, the early introduction of glucocorticoids is recommended for the induction or remission. Initially, prednisolone should be administered at doses of 1 mg/kg/day and maintained for a month. In case of (early) visual loss, higher doses of glucocorticoids or methylprednisolone pulses may be considered. Adjunctive immunosuppressive therapy is usually needed to control TA (e.g. cyclophosphamide in severe disease and methotrexate (MTX) or azathioprine for less severe disease or maintenance). MTX may also be used in temporal arteritis/GCA for glucocorticoid-sparing. Furthermore, GCA patients should receive low-dose aspirin to avoid arterial occlusion. Arterial reconstruction or bypass-grafting may be needed, especially in TA, but should be performed when the disease is in remission.

Hepatitis B-associated polyarteritis requires antiviral therapy in conjunction with glucocorticoids. Additional plasmapheresis is highly successful in the induction of remission. In non-hepatitis B associated polyarteritis, immunosuppressants such as cyclophosphamide may be used for the induction of remission in organ-threatening disease; MTX is an option in non-organ threatening disease or as maintenance therapy.

CV is treated according to the underlying condition. Anti-viral therapy with ribavirin and interferon-alpha is primarily recommended for hepatitis C-associated CV, whereas essential CV is treated by immunosuppressive therapy in the same way as the other small vessel vasculitides (see chapter ANCA-associated vasculitides). In cases of organ threatening CV, immunosuppressive therapy is introduced in spite of high viral load to reduce organ damage induced by vasculitis, and antiviral therapy is commenced when vasculitis activity is under control. Im-

**Table 9.** Treatment recommendations for non-ANCA-associated vasculitides according to EULAR/EUVAS.

| Vasculitis | Recommended therapy |
|---|---|
| Large vessel vasculitis | Remission induction:<br>- high-dose glucocorticoids (1mg/kg/day) for 1 month<br>- consider additional immunosuppressant as adjunctive therapy<br>Early visual loss:<br>- consider high dose i.v. methylprednisolone<br>Maintenance therapy:<br>- no recommendation<br>- immunosuppressive therapy is usually needed long-term<br>Additional therapy:<br>- aspirin in GCA<br>- reconstructive surgery in TA when disease is in remission |
| Panarteritis nodosa | Hepatitis B-associated:<br>- GC + antiviral therapy + plasma separation<br>Non-Hepatitis-associated:<br>- no recommendation<br>- immunosuppressive therapy needed |
| Cryoglobulinemic vasculitis (CV) | Hepatitis-C-associated CV:<br>- antiviral therapy<br>essential CV:<br>- treat like other small vessel vasculitides<br>rituximab may be an option in HCV-associated and non-viral CV<br>consider plasma separation in life-threatening disease |

munosuppressive therapy is then discontinued or switched to a less toxic agent. Furthermore, rituximab may be an option in hepatitis C-associated and non-hepatitis-C associated CV and should be considered when cyclophosphamide is not successful or contraindicated [8]. It may be useful to combine rituximab with antiviral therapy. Plasmapheresis has been of benefit in life-threatening disease.

## 6 Diagnostic tests

The non-ANCA associated vasculitides are not associated with typical autoantibody profiles. ESR and CRP serve to assess disease activity; in CV and HSP, the decrease of complement may be an additional marker for disease activity.

If CV is suspected, cryoglobulins in serum should be measured and the type of cryoglobulins should be assessed. The patient needs to be tested for hepatitis B and/or C if polyarteritis or CV is diagnosed. In secondary vasculitis, testing for autoantibodies of the underlying disease is necessary (e.g. determination of rheumatoid factor (RF) and anti-CCP-antibodies in rheumatoid arthritis or measurement of antinuclear antibodies (ANA), anti-ds-DNA antibodies and extractable nuclear antibodies (ENA) in connective tissue diseases.

## 7 Testing methods

### Cryoglobulins

Cryoglobulins precipitate in the cold and redissolve on re-warming. To test for cryoglobulins, blood needs to be drawn into a pre-warmed syringe in the absence of anticoagulants. Serum is removed after centrifugation and kept at 4° Celsius for 2–3 days. Cryoglobulins of type I tend to precipitate within 24 hours, whereas cryoglobulins of type III may need up to 7 days to precipitate (Fig. 5). To assess the cryocrit (volume of precipitate as a percentage of original serum volume) the precipitated sample is centrifuged again. The concentration of cryoglobulins can

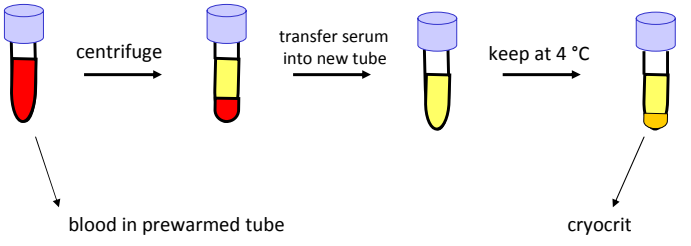


**Figure 5.** Determination of cryocrit.

be determined by spectophotometric analysis. The type of cryoglobulinaemia is specified by immunological assays assessing different cryoglobulin components.

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# Fibromyalgia syndrome

Yoav Arnson, Howard Amital

## 1 Introduction

Fibromyalgia (FM) is a common cause of chronic, diffuse, musculoskeletal pain. It is a disease that affects muscles and soft tissue such as tendons and ligaments. This condition is not associated with genuine tissue inflammation and the aetiology of the disorder remains poorly understood.

The estimated prevalence of FM in the general community ranges between 2 % to 5 % of the population; women are affected almost 10 times more than men. The prevalence increases with age, reaching over 7 % in women aged 60 to 79 years. As mentioned above, the cardinal manifestation of FM is diffuse musculoskeletal pain. Although the pain may initially be localized, often in the neck and shoulders, it eventually involves many muscle groups of upper and lower extremities. Patients typically complain of diffuse pain over the neck, middle, and lower back, chest wall and upper and lower limbs. The pain is chronic and persistent, although it usually varies in intensity. Patients often have difficulty distinguishing joint from muscle pain and also report a burning sensation with swelling, however, the joints do not appear swollen or inflamed on examination. Pain is often aggravated by exertion, stress, lack of sleep, weather changes and shifts of mood. Sensations of numbness, tingling, burning, or a crawling perception are often described.

Patients also may have a variety of poorly understood pain symptoms, including abdominal and chest wall pain and symptoms suggestive of irritable bowel syndrome, pelvic pain and bladder symptoms of frequency and urgency suggestive of the female urethral syndrome or of interstitial cystitis [1].

## 2 Signs and symptoms

Fatigue is present in more than 90 percent of cases and is occasionally the chief complaint. Most patients report light sleep and feeling un-refreshed in the morning, while others report symptoms suggestive of pathologic sleep disturbances such as sleep apnoea or nocturnal myoclonus. Light-headedness, dizziness, and feeling faint are common symptoms. Headaches (either muscular or migraine-type)

are present in a majority of patients. Psychological features presented including mood disturbances, especially depression, anxiety, post-traumatic stress disorder and heightened somatic concern, and cognitive dysfunction especially short term memory loss [2].

Additional symptoms and clinical manifestations may include complaints of ocular dryness, multiple chemical sensitivity and "allergic" symptoms, palpitations, dyspnoea, vulvodynia, dysmenorrhoea, non-dermatomal paresthesias, weight fluctuations, night sweats, dysphagia, dysgeusia, glosodynia, and weakness. FM is often accompanied by other co-morbidities. As many as 80 % of patients with FM also fulfil criteria for chronic fatigue syndrome, up to 80 % have headaches, 75 % have temporomandibular disorders, and up to 60 % may have irritable bowel syndrome.

## 3 Diagnostic criteria

FM diagnosis is problematic because of the difficulty classifying somatic syndromes that lack objective physical or laboratory features or well-characterised pathologic findings. Diagnosing fibromyalgia is based on the combination of patient history, physical examination and exclusion of other causes for symptoms attributed to FM. The clinical diagnosis of FM is based largely upon the patient's history of chronic, generalized pain and associated features. The features include fatigue, sleep disturbances, headache, cognitive difficulties and mood disturbances (Table 1).

FM is currently diagnosed using the American College of Rheumatology (ACR) classification criteria from 1990 (Table 2) [3]. The diagnostic criteria are based on the occurrence of widespread musculoskeletal pain and excess tenderness in of least 11 of 18 predefined anatomic sites (Fig. 1). The existence of both criteria confers an 80 percent sensitivity and specificity differentiating patients with FM from patients with other chronic pain disorders. These ACR classification criteria, performed well in specialty clinics, are very useful in providing some patient homogeneity for clinical trials. However, they have not been widely embraced in primary care and their absence certainly does not exclude the possibility of FM since daily fluctuations might occur.

In recent years the case definition of FM has changed somewhat with increasing recognition of the importance of cognitive problems and somatic symptoms, factors that were not integrated in the 1990 ACR classification criteria. In 2010 Wolfe et al suggested a new set of criteria designed at diagnosing FM [4]. Their criteria are based on two variables: the widespread pain index (WPI) (the number of 19 defined body regions) and the fibromyalgia symptom severity scale (SS) (fatigue, waking unrefreshed, cognitive symptoms) plus the extent (severity) of somatic symptoms in general (Table 3). In their model the WPI strongly correlates with the ACR tender point count, and the SS scale correlates with the other disease

**Table 1.** Clinical features associated with the FM syndrome.

| |
|---|
| **Cardinal signs:** |
| Generalized pain |
| Tender points sensitive to pressure |
| **Characteristic manifestations (more than 75 % of the patients):** |
| Fatigue |
| Non restorative sleep |
| Sleeping Disorders |
| Stiffness (especially in the morning) |
| **Common Manifestation (More than 25 % of the patients):** |
| Irritable colon |
| Raynaud's Phenomenon |
| Headache |
| Sensation of swelling |
| Parasthesia |
| Functional impotence |
| Psychiatric co-morbidities (e. g. anxiety, depression) |
| Symptomatic sensitivity (e. g. cold or stress) |

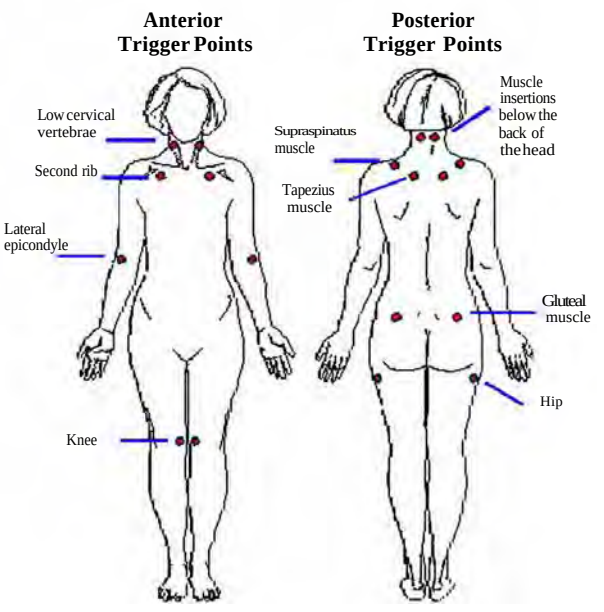


**Figure 1.** Illustration demonstrating the anatomical location of the tender points assessed in Fibromyalgia.

**Table 2.** The American College of Rheumatology 1990 Criteria for the Diagnosis of Fibromyalgia.

| |
|---|
| History of widespread pain, with all of the following present: pain in the left side of the body, pain in the right, pain above the waist and pain below the waist. In addition, axial skeletal pain (cervical spine or anterior chest or thoracic spine or low back) must be present. |
| Pain in 11 of 18 tender point sites on digital palpation:<br>**Occiput:** Bilateral, at the sub occipital muscle insertions<br>**Low cervical:** Bilateral at the anterior aspects of the intertransverse spaces at C5-C7<br>**Trapezius:** Bilateral at the midpoint of the upper border<br>**Supraspinatus:** Bilateral, at origins above the scapula spine near the medial border<br>**Second rib:** Bilateral at the second costochondral junctions just lateral to the junctions on upper surfaces<br>**Lateral epicondyle:** Bilateral, 2 cm distal to the epicondyles<br>**Gluteal:** Bilateral, in upper outer quadrant of buttocks in anterior fold of muscle<br>**Greater trochanter:** Bilateral, posterior to the trochanteric prominence<br>**Knee:** Bilateral, at the medial fat pad proximal to the joint line. |
| Digital palpation should be performed with an approximate force of 4 kg. For a tender point to be considered "positive" the subject must state that the palpation was "painful". "Tender" is not to be considered painful. For classification purposes, patients will be said to have FM if both criteria are satisfied. Widespread pain must have been present for at least 3 months. The presence of a second clinical disorder does not exclude the diagnosis of FM. |

components. In their paper, FM diagnosis was considered with a composite value of WPI $\geq$ 7 and SS $\geq$ 5 or WPI = 3–6 and SS $\geq$ 9. SS scale can be used alone as a measure of FM disease severity. The new set of diagnostic criteria correctly classifies 88.1 % of cases classified by the ACR classification criteria, and *does not require a physical or tender point examination*.

One of the greater drawbacks of both diagnosing systems mentioned here is that disease diagnosis is based on symptom severity. The loss of tender points or painful regions due to any cause, including successful treatment can result in failure to meet diagnostic criteria, unlike other rheumatic diseases like systemic lupus erythematous or rheumatoid arthritis.

## 4 Clinical and laboratory

There are no specific laboratory tests for diagnosing FM. No biomarkers or serologic tests are specific or of diagnostic value in FM. The pathophysiology of FM is considered to be related to aberrant central pain mechanisms. Various central nervous system processes in the brain and spinal cord manifest abnormalities in

**Table 3.** Suggested 2010 Fibromyalgia diagnostic criteria.

| |
|---|
| A patient satisfies diagnostic criteria for fibromyalgia if the following 3 conditions are met:<br>1. Widespread pain index (WPI) $\geq$7 and symptom severity (SS) scale score $\geq$5 or WPI 3–6 and SS scale score $\geq$9.<br>2. Symptoms have been present at a similar level for at least 3 months.<br>3. The patient does not have a disorder that would otherwise explain the pain. |
| WPI: The number of areas in which the patient has had pain over the last week. Score will be between 0 and 19: left shoulder girdle, right shoulder girdle, left hip, right hip, left jaw, right jaw, upper back, lower back, left upper arm, left lower arm, left upper leg, left lower leg, right upper arm, right lower arm, right upper leg, right lower leg, chest, neck, abdomen |
| The SS scale score is the sum of the severity of the 3 symptoms (fatigue, waking unrefreshed, cognitive symptoms) plus the extent (severity) of somatic symptoms in general. The final score is between 0 and 12.<br>SS scale score:<br>1. Fatigue<br>2. Waking unrefreshed<br>3. Cognitive symptoms<br>For the each of the 3 symptoms above, indicate the level of severity over the past week using the following scale:<br>1. No problem<br>2. Slight or mild problems, generally mild or intermittent<br>3. Moderate, considerable problems, often present and/or at a moderate level<br>4. Severe: pervasive, continuous, life-disturbing problems |
| Considering somatic symptoms in general, indicate whether the patient has:<br>1. No symptoms<br>2. Few symptoms<br>3. A moderate number of symptoms<br>4. A great deal of symptoms<br>Somatic symptoms that might be considered: muscle pain, irritable bowel syndrome, fatigue/tiredness, thinking or remembering problem, muscle weakness, headache, pain/cramps in the abdomen, numbness/tingling, dizziness, insomnia, depression, constipation, pain in the upper abdomen, nausea, nervousness, chest pain, blurred vision, fever, diarrhoea, dry mouth, itching, wheezing, Raynaud's phenomenon, hives/welts, ringing in ears, vomiting, heartburn, oral ulcers, loss of/change in taste, seizures, dry eyes, shortness of breath, loss of appetite, rash, sun sensitivity, hearing difficulties, easy bruising, hair loss, frequent urination, painful urination, and bladder spasms. |

patients with FM. Fluctuations of various neurotransmitter concentrations were reported in FM, especially of serotonin and substance P. However, it is far from being clear whether these changes are causative or consequential. The hypothalamic-pituitary-adrenal axis, which is responsible for stress response exhibits mostly diminished response to TRH.

## 5 Management

Given the unclear aetiology of fibromyalgia, and the heterogeneous presentations of the disease, it has become clear that no single therapy is broadly efficacious. Many patients with FM benefit from a multidisciplinary approach in clinical practice. The complex nature of FM suggests that multimodal, individualised treatment programmes that combine pharmacologic and non-pharmacologic therapies may be necessary to achieve optimal outcomes in patients with this syndrome [5].

### *Pharmacotherapy*

A wide range of agents have been employed in the treatment of patients with FM. However, only a small number of these medications have demonstrated effectiveness in controlled clinical trials. Antidepressants, primarily tricyclics, are effective, but they have a relatively narrow therapeutic index, and their use may be limited by poor tolerability. SSRIs have better tolerability than tricyclics, but do not appear to be as effective in relieving the wide range of FM-associated symptoms. Medications that inhibit re-uptake of both norepinephrine and serotonin (SNRI) show promise in treating both pain of FM and associated symptoms of sleep disturbance and fatigue as well as coexistent affective aspects, with fewer side effects than traditional tricyclics. The new antiepileptic pregabalin has been shown to be effective in reducing many of the symptoms associated with FM and is well tolerated. Recently this drug was granted FDA approval for the indication of FM. Few studies support the use of the mixed opiate tramadol for pain management in FM.

Although commonly prescribed, there is little objective evidence to assess the efficacy of nonsteroidal anti-inflammatory drugs (NSAIDs). In one double-blind, placebo controlled trial, ibuprofen was no better than placebo and in another, naproxen led to minor but insignificant symptom improvement. One trial of oral corticosteroid use found no efficacy.

### *Non-pharmacologic Treatment*

A variety of non-pharmacologic treatments have been demonstrated to have at least modest efficacy in patients with FM. A 2004 systematic review found strong evidence for effectiveness of cardiovascular exercise, cognitive behavioural therapy (CBT), patient education, and multidisciplinary interventions that combined

elements of aerobic exercise, CBT and patient education. The same review found moderate evidence for efficacy of strength training, hypnotherapy, biofeedback, and mineral springs or salt baths (balneotherapy). Weak evidence exists for manipulative and manual therapies (chiropractic, massage) and physical modalities including electrotherapy and therapeutic ultrasound. While moderate evidence was also found for acupuncture. Since then, other forms of physical activity, such as tai chi, have been found to be useful treatments in the management of FM.

*Multidisciplinary Treatment*

There is strong evidence that a multidisciplinary approach is effective in treating FM. Five studies of multidisciplinary treatment that combined education, CBT, or both with exercise found beneficial effects on patient self-efficacy and overall FM.

The current guidelines for treating fibromyalgia are that the FM diagnosis must first be confirmed and the condition explained to the patient and family. Any comorbid illness, such as mood disturbances or primary sleep disturbances, should be identified and treated. Medications to consider initially are low doses of tricyclic antidepressants or cyclobenzaprine. Some SSRIs, SNRIs, or anticonvulsants may become first-line FMS medications as more trials are reported.

All patients with FM should begin a cardiovascular exercise program. Most patients will benefit from CBT or stress reduction with relaxation training. A multidisciplinary approach combining each of these modalities may be the most beneficial. Patients with FM who do not respond well to these steps should be referred to a rheumatologist, physical therapy specialist, psychiatrist, or pain management specialist.

## 6 Follow up

There is no single parameter or set of variables that are sufficient for following up on in order to assess disease activity or severity. The complex nature of FM requires assessing and following many variables that are all part of the disease pathology.

Measurement of global sense of well-being, quality of life, and functional capacity in multiple dimensions (physical, vocational, social, emotional) is a key area of assessment and is considered essential by regulatory agencies when contemplating approval of medications for chronic pain states. The Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36) is a generic instrument with 8 subscales. Assessment with the SF-36 has shown that patients with FM have reduced physical functioning, physical role functioning, general health, vitality, and social functioning but increased body pain versus healthy subjects.

The *FM Impact Questionnaire* (FIQ) is a simple instrument specifically designed to reflect changes in the FM patient's general status over time. It includes 10

questions and assessing the disease severity, the clinical course and the response to treatment are pain, fatigue, sleep quality, quality of life and psychiatric assessment.

## 7 Requirements for family practitioners

As most patients with symptoms suggestive of FM are first seen by primary-care physicians, it is imperative that these physicians should be acquainted with the FM construct; and especially with the diagnosis and basic approach to these patients. The significant burden this disorder has on medical services and expenses warrants a proper understanding and management by all medical professionals.

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# Part 2

# Autoimmune skin diseases

# Bullous autoimmune skin diseases

Silke Hofmann, Thilo Jakob

## 1 Introduction

Autoimmune blistering disorders are a heterogeneous group of chronic and severe skin diseases caused by circulating autoantibodies against various structural proteins of the epidermis, the basement membrane zone, or the dermis (Table 1, Fig. 1). The autoantigens play an important role in intraepithelial, epidermo-dermal, or dermal adhesion, and loss of adhesion subsequent to autoantibody-induced inflammation results in blister formation.

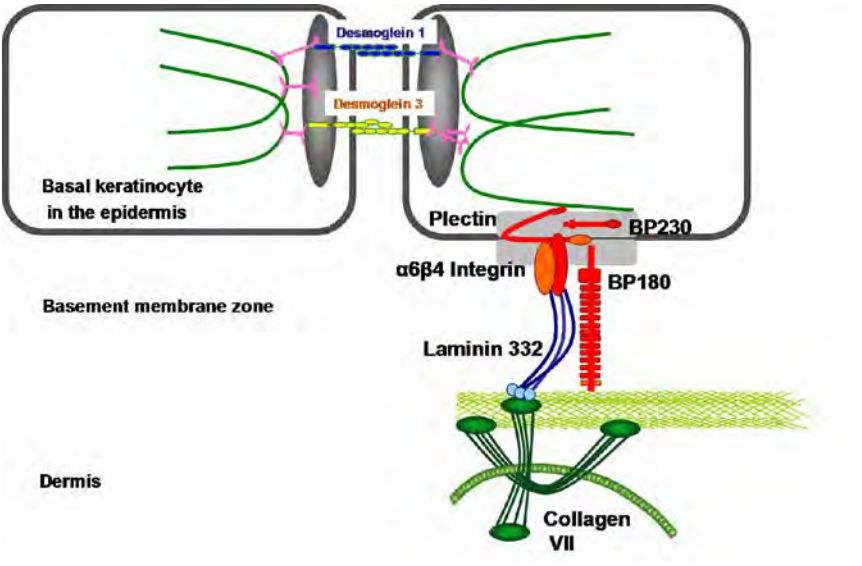


**Figure 1.** Schematic representation of the localisation of the relevant autoantigens for bullous autoimmune disorders. Desmosomes (depicted in dark grey) are adhesion complexes connecting two epidermal keratinocytes, while hemidesmosomes (light grey) are multiprotein adhesive complexes located at the dermo-epidermal junction.

Pemphigus vulgaris, pemphigus foliaceus and bullous pemphigoid represent the most frequent bullous autoimmune skin diseases. In addition, the group of autoimmune bullous disorders includes rare entities such as gestational pemphigoid, mucous membrane pemphigoid, linear IgA dermatosis, epidermolysis bullosa acquisita, or dermatitis herpetiformis.

**Table 1.** Autoantigens of bullous autoimmune disorders.

| Disease | Autoantigen | Localisation in the skin |
|---|---|---|
| ***Pemphigus disorders:*** | | |
| Pemphigus vulgaris | Desmoglein 3<br>Desmoglein 1 | Desmosome (Epidermis) |
| Pemphigus foliaceus | Desmoglein 1 | Desmosome (Epidermis) |
| ***Pemphigoid disorders:*** | | |
| Bullous pemphigoid | BP230 | Hemidesmosome (Basement membrane) |
| | BP180 (collagen XVII) | Hemidesmosome (Basement membrane) |
| Mucous membrane pemphigoid | BP180 (collagen XVII)<br>α6β4-Integrin | Hemidesmosome (Basement membrane) |
| | Laminin 332 | Anchoring filament (Basement membrane) |
| Gestational pemphigoid | BP180 (collagen XVII) | Hemidesmosome (Basement membrane) |
| Linear IgA-Dermatosis | Extracellular domain of BP180 | Hemidesmosome (Basement membrane) |
| ***Other bullous autoimmune disorders:*** | | |
| Epidermolysis bullosa acquisita | Collagen VII | Anchoring fibril (Dermis) |
| Dermatitis herpetiformis | Epidermal and tissue transglutaminase | Dermis |

## 2 Diagnostic criteria

Pemphigus vulgaris and pemphigus foliaceus are intraepidermal bullous disorders, in contrast to bullous pemphigoid, which is associated with subepidermal blister formation. The site of blister formation (intraepidermal versus subepidermal) relates to the clinical presentation, which, in pemphigus, is characterised by superficial and therefore flaccid blisters (that due to their fragility often rapidly result in erosions) and in pemphigoid patients as tense blisters (increased stability due to an intact epidermis as blister roof) (Fig. 2). The clinical features, in combination with histology, direct and indirect immunofluorescence, and detection of autoantibodies to epidermal or dermal autoantigens allow an exact diagnosis to be made (compare Tables 2 and 3).

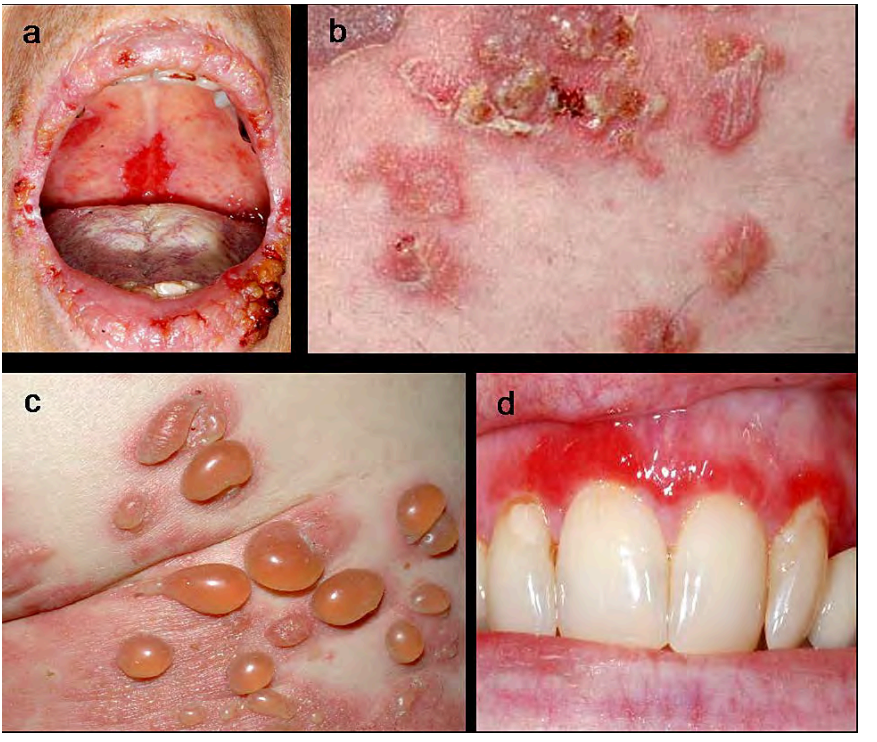


**Figure 2.** Clinical features of pemphigus and bullous pemphigoid. The initial manifestations in pemphigus vulgaris are often mucosal erosions (a). Both, pemphigus vulgaris and foliaceus, manifest with flaccid blisters and erosions of the skin (b), but mucosal involvement is lacking in pemphigus foliaceus. Tense blisters on erythematous skin are a hallmark of bullous pemphigoid (c), while mucosal lesions such as desquamative gingivitis (d) are rare in bullous pemphigoid, but occur in mucous membrane pemphigoid.

**Table 2.** Clinical presentation of pemphigus vulgaris, pemphigus foliaceus and bullous pemphigoid.

| | Pemphigus vulgaris | Pemphigus foliaceus | Bullous pemphigoid |
|---|---|---|---|
| **Symptoms** | Painful mucosal erosions, weight loss | (Pruritus) | Pruritus often as initial symptom |
| **Typical clinical presentation** | Flaccid blisters and erosions | Flaccid blisters and erosions | Tense blisters, urticarial plaques |
| **Mucosal involvement** | Present in 100 % (oral and /or nasal, ocular, genital) | In 0 % | In 10–20 % (most frequently oral erosions, desquamative gingivitis) |
| **Age prevalence** | 30–60 yrs. | 30–60 yrs. | > 60 yrs. |
| **Incidence** | 1–5/million/year | 1–5/million/year | 12/million/year |

**Table 3.** Laboratory findings in pemphigus and pemphigoid.

| | Pemphigus vulgaris | Pemphigus foliaceus | Bullous pemphigoid |
|---|---|---|---|
| **Histology** | Suprabasal acantholysis | Subcorneal acantholysis | Subepidermal blistering, eosinophilic infiltrate |
| **Direct immuno-fluorescence** | Intercellular IgG and C3 deposits in the epidermis | Intercellular IgG and C3 deposits in the upper epidermis | Linear IgG and C3 deposits at the basement membrane zone |
| **Indirect immunofluorescence on monkey oesophagus** | Intercellular IgG deposition within epidermis | Intercellular IgG deposition within epidermis | Linear IgG deposition at the basement membrane zone |
| **Autoantibodies directed to** | Desmoglein 3 in 100 %, Desmoglein 1 may be additionally positive (in 45 %) | Desmoglein 1 in 95 % | BP180 in 90 %, BP230 in 60 % |

## 3 Diagnostic measurements for experts

Pemphigus vulgaris is caused by autoantibodies against proteins of the desmosomes, the intraepithelial intercellular adhesion complexes. The pemphigus vulgaris antigen, desmoglein 3, and the pemphigus foliaceus antigen, desmoglein 1, belong to the cadherin supergene family and compensate for each other functionally. In epithelia of mucous membranes, desmoglein 1 is only expressed at very low levels. Therefore anti-desmoglein 3 antibodies in pemphigus vulgaris lead predom-

inantly to erosions in mucous membranes. Autoantibodies against desmoglein 1 and 3 result in a mucocutaneous type of pemphigus vulgaris with blisters on mucosae and the integument. Patients with pemphigus foliaceus develop antibodies against desmoglein 1 only. This antigen is predominantly expressed in the superficial layers of the epidermis where no compensatory desmoglein 3 is present. This explains why anti-desmoglein 1 antibodies induce loss of cell-cell adhesion in the upper epidermis, but not in mucosal epithelia.

Autoantibodies in bullous pemphigoid target two components of hemidesmosomes (adhesion complexes of the dermo-epidermal basement membrane zone): the transmembrane protein BP180 (bullous pemphigoid antigen with a molecular weight of 180 kDa; syn. collagen XVII) and the intracellular BP230.

Since the 1960s, direct immunofluorescence on perilesional skin biopsies has been the gold standard in the diagnosis of autoimmune blistering disorders. Pemphigus disorders demonstrate intercellular intraepidermal, bullous pemphigoid dermo-epidermal deposition of immunoglobulins and complement. Circulating autoantibodies can be detected by indirect immunofluorescence or western blot using recombinant antigens or keratinocyte extracts. Commercially available test systems using recombinant desmoglein 1, desmoglein 3, BP180 and BP230 allow the detection of specific circulating autoantibodies that are used to confirm the diagnosis and to monitor disease activity. The British Association of Dermatologists has developed guidelines for management of pemphigus and pemphigoid [1, 2].

## 4 Requirements for family practitioners

Pemphigus vulgaris and pemphigus foliaceus are potentially severe, autoimmune blistering skin diseases caused by autoantibodies against adhesion proteins of epidermal keratinocytes (desmogleins 1 and 3). These autoantibodies lead to intraepidermal blisters, which, in pemphigus vulgaris, manifest clinically with painful erosions of the oral mucosa, reduced food intake and weight loss. In addition, fragile skin blisters which may result in widespread, often haemorrhagic erosions on trunk and extremities may be present. The hallmark of pemphigus foliaceus are superficial skin blisters which heal without scarring and an absence of mucosal lesions.

Bullous pemphigoid is the most common autoimmune blistering disease and its incidence rises with increasing age. It is associated with autoantibodies against distinct basement membrane proteins (BP180 and BP230) leading to subepidermal blisters. Clinically, bullous pemphigoid presents as a pruritic eruption with large, tense blisters on normal or inflamed skin and rare involvement of mucous membranes. Of note, itch is often the first symptom and urticarial plaques may precede the blister formation.

When the diagnosis of an autoimmune bullous disorder is suspected, the patient should be referred to a dermatologist. A skin biopsy and serological tests

are essential to confirm the diagnosis of pemphigus or pemphigoid. Histology shows suprabasal acantholysis and direct immunofluorescence shows intercellular IgG and C3 deposits in the epidermis in pemphigus disorders. Serological studies demonstrate circulating autoantibodies that bind to the intercellular substance of the epithelium on monkey oesophagus (indirect immunofluorescence) and the molecular specificity of the antibodies is determined by commercially available test systems (e. g. Elisa) with recombinant desmogleins. Bullous pemphigoid is characterized by subepidermal blister formation in histology and linear C3 and/or IgG deposits at the dermoepidermal junction in direct and indirect immunofluorescence. Circulating autoantibodies against BP180 and BP230 are detectable by commercially available test systems (e. g. Elisa).

## 5 Follow up

*Clinical observations*

Usually, immunosuppressive treatment rapidly prevents new blister formation and pruritus and induces healing of skin and mucosal lesions within weeks or (in severe cases) months. Clinical scores such as the ABSIS (Autoimmune Bullous Skin Disease Intensity Score) or PDAI (Pemphigus Disease Area Index) can help to monitor the clinical improvement during treatment.

*Expectations*

Blistering autoimmune disorders are chronic diseases. Most patients require immunosuppression for years or sometimes life-long to remain in clinical remission.

*Blood tests*

Regular assessment of liver and kidney function, full blood counts, and glucose levels is required to recognise potential side-effects of corticosteroids and immunosuppressants. Determination of autoantibody levels to desmoglein 1 and 3 or BP180 is useful for monitoring disease activity. A rise in autoantibody titres can precede clinical relapse of the disease [3, 4].

## 6 Therapeutic management

Pemphigus disorders are treated with topical and oral corticosteroids, usually in combination with adjuvant immunosuppressive drugs [2]. Prednisolone is administered in an initial dose of 1–1.5 mg/kg/day and gradually reduced when no new lesions develop. Adjuvant immunosuppressive drugs (azathioprine, mycophenolate mofetil, cyclophosphamide, cyclosporine, methotrexate or dapsone) are prescribed because of their potential steroid-sparing effect. Such a combined

treatment is able to induce remission in the majority of patients. In recalcitrant cases, intravenous immunoglobulins, rituximab (a chimeric anti-CD20 antibody directed against B-cells), or immunoadsorption are often successful.

In localized forms of pemphigus foliaceus, superpotent topical steroids or topical calcineurin inhibitors may be sufficient to obtain clinical remission. Similarly, there is good evidence that bullous pemphigoid responds to superpotent topical corticosteroids (clobetasol propionate 10–30 g/day). Severe cases require systemic therapy with oral prednisolone (initially 0.5 mg/kg is usually sufficient) alone or combined with immunosuppressive agents such as azathioprine, dapsone or mycophenolate mofetil [1]. In the future, targeted therapies for blistering autoimmune dermatoses will hopefully improve efficiency of treatment and reduce side effects [5].

## 7 Diagnostic tests

Tissue-bound autoantibodies (IgG, to a lesser extent IgA) against desmosomes or basement membrane proteins are detected by direct immunofluorescence analysis on perilesional skin biopsies in practically all patients (Fig. 3 a, b). However, this initial test does not allow discrimination between e. g. bullous pemphigoid and other, more rare, subepidermal blistering disorders such as epidermolysis bullosa acquisita. This problem can be overcome by using salt-split-skin as a substrate for indirect immunofluorescence to assess circulating autoantibodies from patients' sera. By incubation of normal human skin in 1M NaCl, the proteins of the basement membrane zone are separated, and bullous pemphigoid-autoantibodies bind to the epidermal side (Fig. 3c), while autoantibodies against the antigen of epidermolysis bullosa acquisita, collagen VII, bind to the dermal side of the blister. The optimal substrate for indirect immunofluorescence diagnosis of pemphigus disorders is monkey oesophagus.

Using recombinant forms of the autoantigens, the specificity of circulating autoantibodies can be determined by commercially available test systems (e. g. Elisa). The recombinant protein is attached to a solid surface, and incubated with patient's serum. Antibodies against the respective antigen bind to it, and are subsequently detected by an enzyme-linked anti-human immunoglobulin antibody. Currently assay systems for the detection of antibodies directed against the following autoantigens are on the market: desmoglein 1, desmoglein 3, BP180, BP230, collagen VII, tissue and epidermal transglutaminase (see Table 1 for the different autoantigens and associated disorders).

## 8 Testing methods, limitations and benefits

The available assay systems are accurate, sensitive and specific (see Table 3). Many samples can be simultaneously assessed, and the autoantibody titres measured

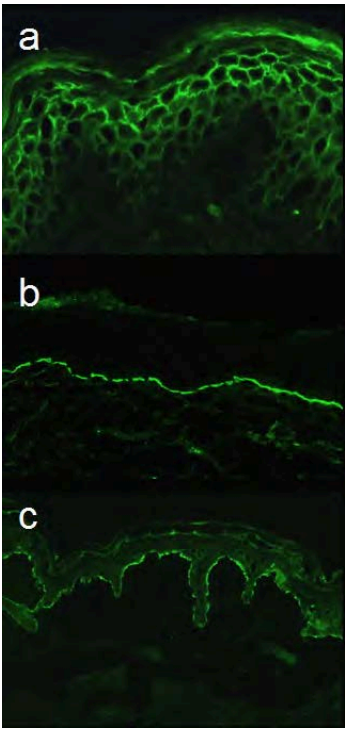


**Figure 3.** Direct immunofluorescence analysis showing intercellular IgG deposits in the epidermis in pemphigus vulgaris (a), and linear IgG deposits at the basement membrane zone in bullous pemphigoid (b). Circulating autoantibodies against the pemphigoid autoantigens BP180 or BP230 bind to the epidermal side of a 1M NaCl-induced artificial split by indirect immunofluorescence (c).

often correlate with the clinical disease activity. For some rare autoantigens (e. g. laminin 332) commercial assays are not yet available. These antibodies can be detected in selected research laboratories by western blot analysis using either keratinocyte extracts or recombinant laminin 332.

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# Discoid lupus erythematosus

Thomas Werfel

## 1 Introduction

Cutaneous lupus is a family of diseases that are classified by the cause of the clinical signs and symptoms into three major groups:

- Acute cutaneous lupus erythematosus
- Cutaneous lupus erythematosus
- Chronic cutaneous lupus erythematosus

Discoid lupus erythematosus (DLE) is the major form of chronic cutaneous lupus erythematous. It is a chronic, photosensitive dermatosis that usually occurs as an independent disorder. However, it may also develop in patients with systemic lupus erythematosus (SLE).

DLE manifests in the shape of reddish discs with adjacent desquamating areas. These flakes do not detach from the skin, and manual removal reveals a keratotic plaque beneath. Tissue atrophies develop in the central region of the disc, which causes scarring and alopecia in hirsute skin. Ultraviolet (UV) light and certain drugs induce and exacerbate these skin defects, and they can arise together with the lesions of subacute cutaneous lupus erythematosus (SCLE) and malar rash.

There are two subclasses of DLE: *localized DLE* is defined as limited to the head and neck, whereas *widespread DLE* targets other areas as well and has the higher potential to progress into full-fledged SLE.

The prevalence of DLE is about 50–85 % in all patients with cutaneous lupus erythematosus (CLE), which occurs as often as SLE, i. e. with an incidence of 17–48 : 100 000. DLE manifests mainly in women (gender ratio 2 : 1) between the ages of 20–40 years with a mean age of 38 years [1]. DLE is slightly more common in African Americans than in Caucasians or Asians.

Currently, the causes of DLE are not understood in detail, but a genetic predisposition is likely. The development of the skin lesions may be due to the autoimmune induction of a heat-shock protein in keratinocytes as a reaction to ultraviolet light (UV) light exposure or stress. This protein may target T-cells, causing epidermal cell cytotoxicity [2].

## 2 Diagnostic measurements for experts

DLE is a disease that primarily manifests in the skin, limiting the clinical diagnostic approaches to physical, histological and serological parameters. While there are clinically asymptomatic patients, some may report mild pruritus or transient pain together with the appearance of lesions. A systemic involvement is rare and occurs in approximately 5 % of DLE patients, leading to arthralgia or arthritis. Hematological and serologic abnormalities most often coincide with the *widespread* variant of DLE.

In order to establish a diagnosis pointing to DLE, it may be useful to perform the lupus band test (LBT): biopsied tissue samples, taken both from skin lesions and non-lesional skin, are compared with each other. Affected samples usually reveal deposits of immunoglobulins and complement factors at the junctions of dermis and epidermis. In about 90 % of cases, lesional skin taken from DLE patients shows a positive direct immunofluorescence. For SLE, the LBT is positive in affected and unaffected skin, whereas for CLE, unaffected skin samples do not fluoresce. Using this test, however, it is not possible to distinguish between different forms of CLE. Hence, it is not specific for DLE, but can lead the expert in the right direction [3].

The most common histological findings characteristic for and indicative of DLE are listed in Table 1, together with the serological parameters. However, these are positive only in the minority of approx. 35 % of patients with DLE. A detailed description of the laboratory tests follows in the sections 'Diagnostic tests' and 'Testing methods'.

## 3 Requirements for family practitioners

Patients usually consult their general practitioner because of changes in the skin. The clinical attributes of the skin lesions are quite characteristic and their pattern is usually photodistributed, although even skin unexposed to sunlight may be affected.

The primary lesion manifests as an erythematous papule or plaque. Initially, scaling is slight, progressing together with lesion size, resulting in a thick, adherent scale with possible changes in pigmentation: hypopigmentation may occur in the center of the lesion, whereas hyperpigmentation tends to be apparent at the active border.

As lesions age, they grow and cause the formation of keratinous plugs which obstruct follicular openings. The final stage of the lesion is inactivation with atrophy and scarring (see Fig. 1), which may lead to permanent alopecia (see Fig. 2). Uncommon manifestations of DLE are hypertrophic or verrucous lesions appearing on the arms and fingers. These features do not necessarily manifest in all lesions.

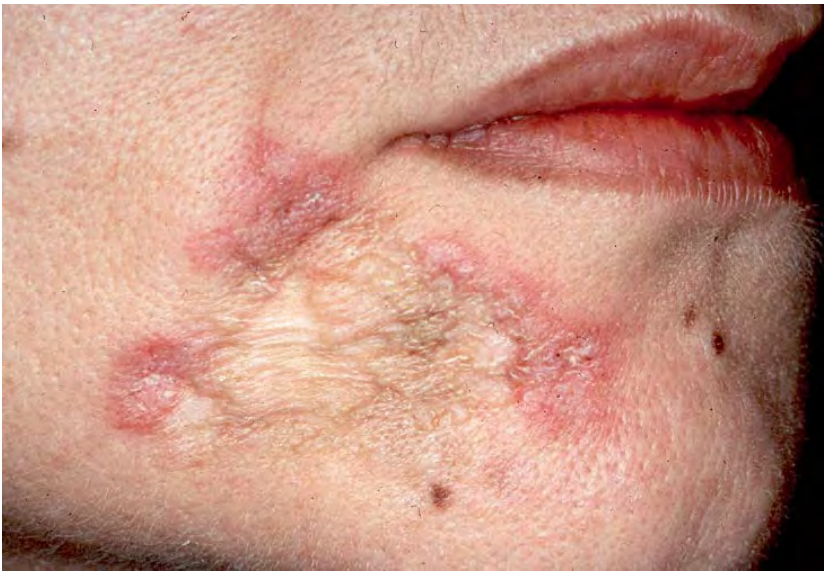

**Figure 1.** Chronic DLE lesion with scarring.

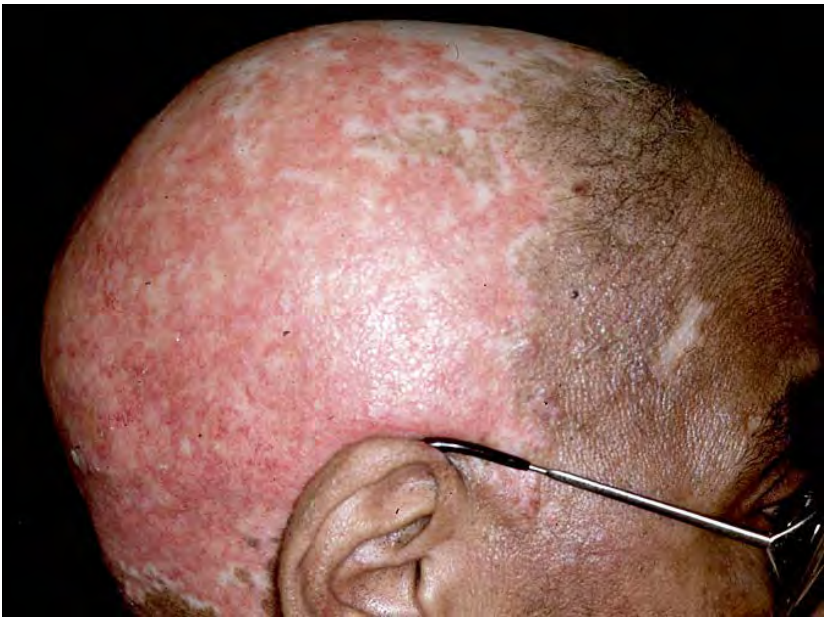

**Figure 2.** Alopecia induced by scarring DLE.

After diagnosis it is advisable to refer the patients to an institution specialized in dermatology.

## 4 Follow up

Generally, patients should be instructed in the importance of sun-protective measures and their effect on the prognosis. Also, patients should be advised to quit smoking as it negatively affects the efficacy of some drugs.

Patients with DLE should be followed at regular intervals since treatments generally take several weeks to months to show any effect. During follow up visits, the practitioner should document any newly developed symptoms in order to recognize a potential systemic dissemination of the disease. The 'Score of Activity and Damage in DLE' (SADDLE) allows the measurement of disease progression

**Table 1.** Histological and serological parameters indicative of discoid lupus erythematosus.

| **Histology** |
|---|
| – Atrophy of the epidermis |
| – Discontinuous distribution of pigments |
| – Follicular plugging |
| – Hyperkeratosis |
| – Presence of inflammatory cell infiltrates |
| – Thickening of the basal membrane |
| – Vacuolar alterations of the basal cell layer |
| **Serology** |
| – Antinuclear antibodies* |
| – Anti-native DNA (double-stranded or nDNA)* |
| – Anti-Ro (SS-A) autoantibodies (in rare cases)* |
| – Anti-Sm* |
| – Anti-annexin 1 antibodies** |
| – Ro52 protein upregulation** |

* In "classical" cutaneous DLE the serology of autoantibodies is negative in most cases, see text.

** Attractive in vitro parameter due to recent findings.

**nDNA**, nuclear DNA.

via a reliable scoring system [4]. Annually, routine laboratory studies should be performed, including complete blood cell counts, renal function and urinalysis. Further antibody testing is only indicated after a change in symptoms.

Early treatment of DLE lesions can prevent scarring and atrophy, otherwise permanent follicular and skin defects may occur. Systemic progression of the disease is rare, but may lead to life-threatening sequelae. Development of malignant neoplasms can occur in rare cases — hence, new growths within inactive lesions should be removed.

While disfigurement — which is the most important long-term problem in this disease — is possible and pain may persist in some lesions, prognosis in terms of mortality for DLE is good.

## 5 Management

The treatment of discoid lupus erythematosus focuses on the improvement of the patient's appearance, the care of existing lesions, the limitation of scarring and on the prophylaxis against the development of additional lesions. Standard therapies include sun protective measures, medication with corticosteroids for the treatment of lesions and antimalarials if a systemic treatment is required.

### 5.1 Sun protection

Generally, the first step in DLE therapy is to protect exposed skin from UV light, both UVA and UVB. Decreased activity during daylight hours with high UV loads between 10 am and 4 pm may help some individuals, while others exhibit an extremely high photosensitivity and require sunscreens and protective clothing. Obviously, sources of intense artificial light (such as solaria) should be avoided as well. Some patients may benefit from additional cosmetic measures to cover especially prominent scar tissue with wigs or makeup.

### 5.2 Corticosteroids

Corticosteroids suppress inflammation and downregulate several components of the patient's immune system. The generation and recruitment of inflammatory cells, such as eosinophils, mast cells and T-lymphocytes is reduced. Corticosteroids are most commonly applied topically and more rarely injected into the lesion, depending on individual conditions. The daily dosage of corticosteroids should be limited to avoid systemic toxicity and to reduce the potential for local atrophy. Topical application of tacrolimus has also been reported to be beneficial in some cases.

### 5.3 Antimalarials

If a systemic agent is required for DLE, the immunomodulatory drug of choice among antimalarials is (hydroxy-)chloroquine, whereas chloroquine should be considered as a second-line therapeutic drug. Both agents limit complement-dependent antigen-antibody reactions and they inhibit chemotaxis of eosinophils as well as locomotion of neutrophils. The efficacy of these drugs is reduced by first- and second-hand smoking.

### 5.4 Surgery

For some patients, it may be necessary to excise scarred lesions in order to counteract especially disfiguring scarring. This may be achieved surgically or via laser therapy. However, both methods can lead to a reactivation of inactive lesions. Hence, it is advisable to treat a test area and to check if the DLE flares before therapy is commenced.

## 6 Diagnostic tests

In general, no single diagnostic tool exists that can detect the presence of DLE in all patients. Instead, a combination of serological tests, immunopathological and histological approaches can be applied for a positive diagnosis.

The commonly employed serological tests include the detection of antinuclear antibodies (ANA), which are positive in approximately 35 % of all patients with DLE. Well defined autoantibodies such as anti-Ro (SS-A) autoantibodies, anti-native DNA (double-stranded or nuclear DNA) and anti-Sm antibodies are more likely positive in DLE variants associated with systemic disease.

Recently, anti-annexin 1 antibodies have been discovered as a viable means to diagnose DLE [5]. On the other hand, anti-native DNA antibodies and ANA have been proven to be characteristic for lupus erythematosus and only occur in low concentrations in patients with the cutaneous forms of lupus erythematosus (CLE).

Proteins of the Ro-family have recently been reported to be specific for intracellular reactions involved in CLE and Sjögren's syndrome [6]. Epidermal keratinocytes taken from lesional skin reveal nuclear and cytoplasmic upregulation of Ro52, especially in layers adjacent to the basement membrane. This protein is also present in endothelial and lymphocytic infiltrates within the dermis. Today, monoclonal antibodies (mAbs) against Ro52 have been created that can be employed in immunohistochemical testing for CLE. Usually, second-level methods, such as indirect immunofluorescence (IIF), counterimmunoelectrophoresis (CIE) or enzyme-linked immunosorbent assays (Elisa) are performed for the detection of antigens in patient sera. In the case of Ro52, though, the overexpression of the protein itself is measured via immunosorbent assays.

A schematic representation of the sandwich Elisa method is shown in Fig. 3: a buffered solution of anti-Ro52 mAbs is added to the microtitre plate, where they adhere via charge interactions (Fig. 3a), and the remaining free plastic surface is blocked with non-reacting proteins. Next, serum is added (Fig. 3b), which may contain the pathologic levels of the Ro52 protein, which binds to the mAbs and forms antigen-antibody complexes (Fig. 3c). After washing (Fig. 3d), a secondary antibody that is enzyme-linked to a detection molecule is added (Fig. 3e). The latter is activated via a specific substrate, causing a color reaction that can be measured photometrically (Fig. 3f).

Annexin 1 suppresses the generation of inflammatory mediators like prostaglandins, thromboxanes and leukotrienes, resulting in an anti-inflammatory reaction. The levels of anti-annexin 1 antibodies are significantly elevated in patients with CLE as compared to healthy subjects, especially for patients with DLE. These are detected by Elisa tailored to annexin 1. The specificity of this test for CLE can be as high as 95 % [5]. However, no correlation between disease progression and antibody levels has been elucidated as yet.

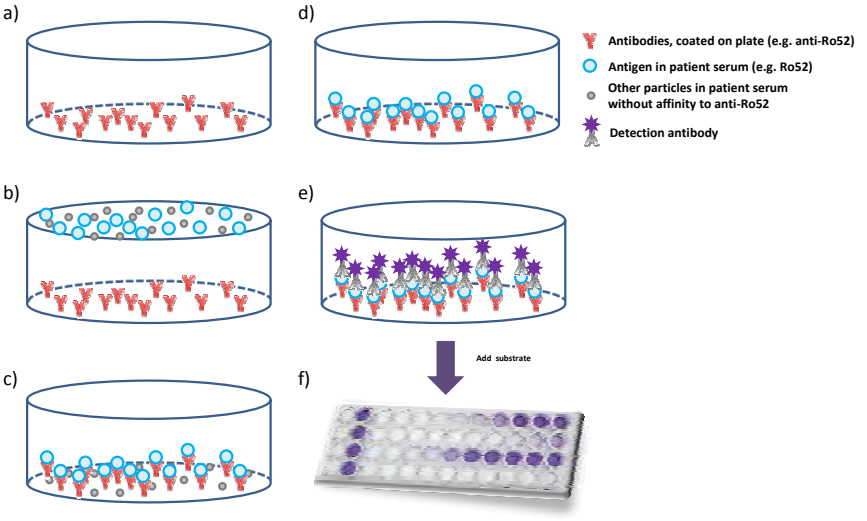


**Figure 3.** Schematic representation of the sandwich Elisa method: a) antibody on microtitre plate, b) add serum with target antigen, c) formation of antibody-antigen complexes, d) washed plate, only antibody-antigen complexes remain, e) add secondary enzyme-linked detection antibody, f) microtitre plate with colored, positive samples after addition of activating substrate.

## 7 Testing methods

Due to the low specificity of the presented serologic testing methods for DLE, their diagnostic value remains unclear. Only very recent methods, targeting specific molecules, such as anti-Ro52- and anti-annexin 1 antibodies, show high sensitivity and specificity for the discoid variant of CLE. However, these methods are relatively new and still need to prove their applicability in daily laboratory routine.

The most efficient method for the diagnosis of DLE remains the physical examination for the clinical manifestations of the disease. The skin lesions are very characteristic and distinct from those found in SCLE and other diseases. Together with the histological assessment of biopsied tissue samples, the attending physician can make a positive diagnosis and may use serology to monitor the progression of the disease.

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# Subacute cutaneous lupus erythematosus

Thomas Werfel

## 1 Introduction

Subacute cutaneous lupus erythematosus (SCLE) is a photosensitive dermatosis, categorized as a cutaneous lupus erythematosus (CLE) variant. It may occur in conjunction with various other disorders, e. g. systemic lupus erythematosus (SLE), Sjögren's syndrome, rheumatoid arthritis, and in patients with deficiencies in the second component of the complement system (C2d). Additionally, it may be induced by certain sun-sensitizing drugs.

SCLE is most common in Caucasian females, with a female-to-male ratio of 4 : 1 and is found in about 50 % of patients that suffer from SLE. The prevalence of SLE ranges from 17–48 : 100 000 with a peak around the ages of 40–60 years [1].

SCLE can coincide with discoid lupus erythematosus (DLE) and can lead to small vessel vasculitis. The skin lesions usually heal without scarring, no atrophy occurs, but a residual dyspigmentation may remain. The same criteria used to classify SLE tend to be positive in patients with SCLE, and serological analysis often helps to establish a diagnosis. A number of human leukocyte antigens (HLA) can be present in SCLE-patients: HLA-B8, HLA-DR3, HLA-DRw52, and HLA-DQ1; additionally, anti-Ro (SS-A) auto-antibodies are associated with SCLE. Together, these factors manifest clinically in an autoimmune response that culminates in keratinocyte apoptosis.

The prognosis of SCLE is generally better than for patients with SLE as the disease is less severe. However, in certain cases, a full systemic manifestation may occur, and end-organ failure is possible.

## 2 Diagnostic measurements for experts

The major elements leading to the diagnosis SCLE are the patient's history (e. g. photo-aggravating factors, hormones as possible trigger factors, family history), a careful clinical evaluation of the presentation of cutaneous symptoms, histological findings and serological markers such as ANA, Ro (SSA) and/or La (SSB) antibodies The histopathological and serological findings associated with SCLE

**Table 1.** Histological and serological parameters indicative of discoid lupus erythematosus.

| **Histology** |
|---|
| – Atrophy of the epidermis<br>– Presence of inflammatory cell infiltrates:<br>  – around blood vessels,<br>  – around appendiceal structures and<br>  – in a subepidermal location.<br>– Vacuolar alterations of the basal cell layer |
| **Serology** |
| – Antinuclear antibodies<br>– Anti-native DNA (double-stranded or nDNA)<br>– Anti-Ro (SS-A) autoantibodies:<br>  – 90 % in patients with annular SCLE,<br>  – 80–85 % for papulosquamous SCLE,<br>  – > 95 % for patients with C2d deficiency, Sjögren's syndrome and vasculitis<br>  – > 90 % in mothers of children with neonatal lupus erythematosus and<br>– 70–80 % in cases of drug-induced SCLE.<br>– Anti-La (SS-B) autoantibodies in < 70 % of cases |

are shown in Table 1. A detailed description of the laboratory tests follows in the sections 'Diagnostic tests' and 'Testing methods'.

Additionally, in order to establish a diagnosis pointing to SCLE, it may be useful to perform the lupus band test (LBT): biopsied tissue samples taken from both skin lesions and non-lesional skin are compared with each other. Affected samples usually reveal deposits of immunoglobulins and complement factors at the junctions of dermis and epidermis. In about 90 % of cases, lesional skin taken from SCLE patients shows a positive direct immunofluorescence. For SLE, the LBT is positive in affected and unaffected skin, whereas for SCLE unaffected skin samples do not fluoresce. But this test does not provide the means to distinguish between different forms of CLE. Hence, it is not specific for DLE, but it can indicate the necessity for further diagnostic tests [2].

SCLE may cause anaemia, leucopenia or thrombocytopenia, which can be detected by full blood cell counts. Additionally, inflammatory skin reactions possibly

result in an elevated erythrocyte sedimentation rate (ESR), whereas complement levels may be depressed and some patients may test positive for rheumatoid factor. Renal involvement of SCLE is tested for with urinalysis and is revealed by red and/or white blood cell casts.

Photoprovocation of uninvolved skin by dermatological experts may elucidate the diagnosis of SCLE in difficult situations.

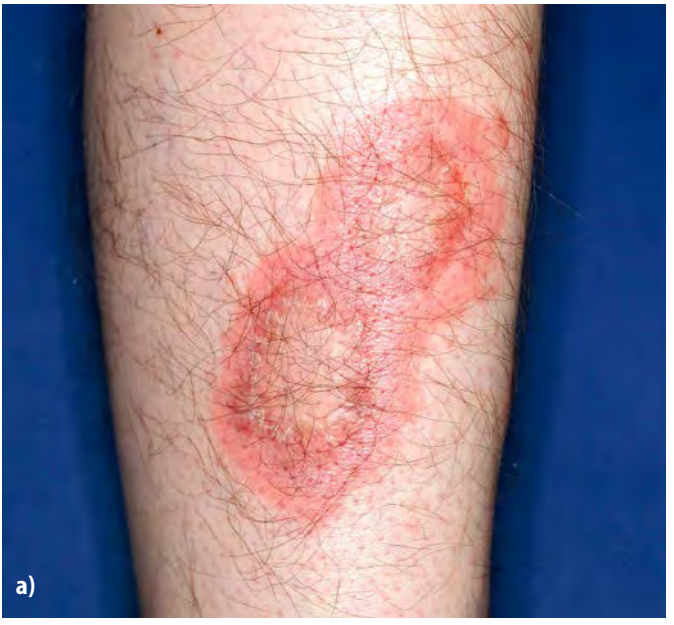
## 3 Requirements for family practitioners

SCLE primarily manifests in the skin, although the joints may also be affected in about half of the patients. In these cases, it is usually the small joints which are afflicted in a symmetrical pattern. In rare cases (< 2 %) arthritis may develop. Additionally, patients may feel fatigue, dryness of mouth and eyes and may manifest symptoms characteristic for SLE, such as neurologic or renal involvement, pericarditis and pleuritis. Hence, these factors should be included in the anamnesis if a practitioner finds SCLE-specific skin lesions.

The skin lesions start as popular eruptions with a photosensitive distribution that may wax and wane, depending on exposure and season. With time, the lesions may grow, conflate and turn into two different forms of skin defects: the papulosquamous or annular variants. The former can be confused with psoriasis (Fig. 1a), while the latter may look like erythema annulare centrifugum (Fig. 1b). Most patients tend towards one or the other variant, but additional DLE-type lesions may occur. Additionally, the unspecific cutaneous changes of lupus erythematosus (LE) may be present: ischaemic changes of the distal fingertips, livedo reticularis, mucosal leukoplakic or ulcerative lesions, palpable purpura or urticaria. An important clinical finding that distinguishes this type of lesion from others associated with cutaneous lupus erythematosus (CLE), such as DLE, is the fact that the lesions in SCLE-patients do not scar or atrophy.

Several unusual subtypes of SCLE have been described, such as tumid lupus erythematosus (TLE), Sjögren's syndrome-associated SCLE and erythema multiforme-like lesions in conjunction with DLE. It is unclear whether these variants are individual entities or consequences of SCLE itself.

Importantly, the entity of neonatal lupus erythematosus must be known by all practitioners who treat pregnant women positive for Ro (SSA) or La (SSB) antibodies. Neonatal lupus erythematosus is an uncommon, maternal auto-antibody-associated disease, characterised by cutaneous, cardiac, hepatic, haematological, neurological, and pulmonary involvement. Annular cutaneous signs manifest during the first month of life in most affected infants. Neonatal lupus erythematosus that affects the heart is usually discovered upon physical examination at birth but may be recognised with ultrasonography in utero.



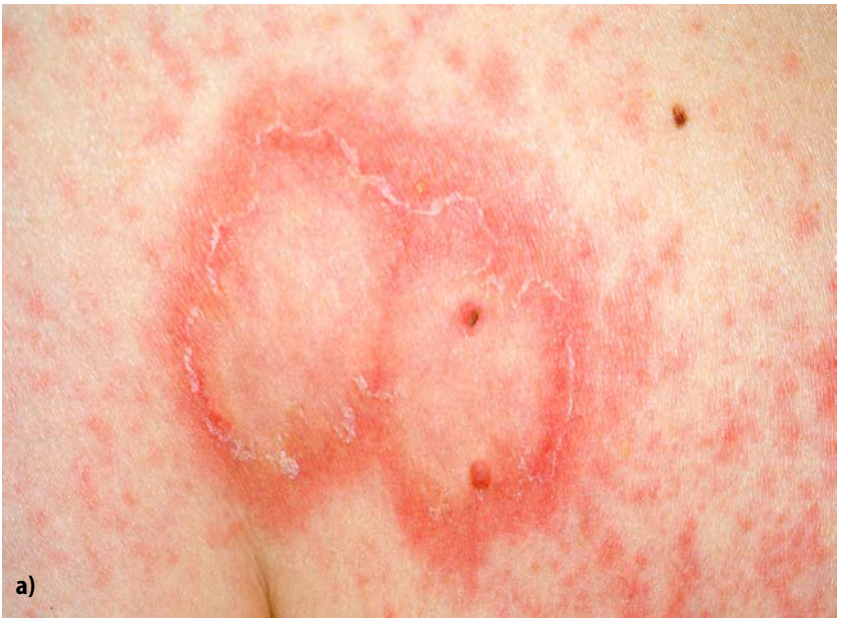




**Figure 1.** a) Annular lesions of SCLE.

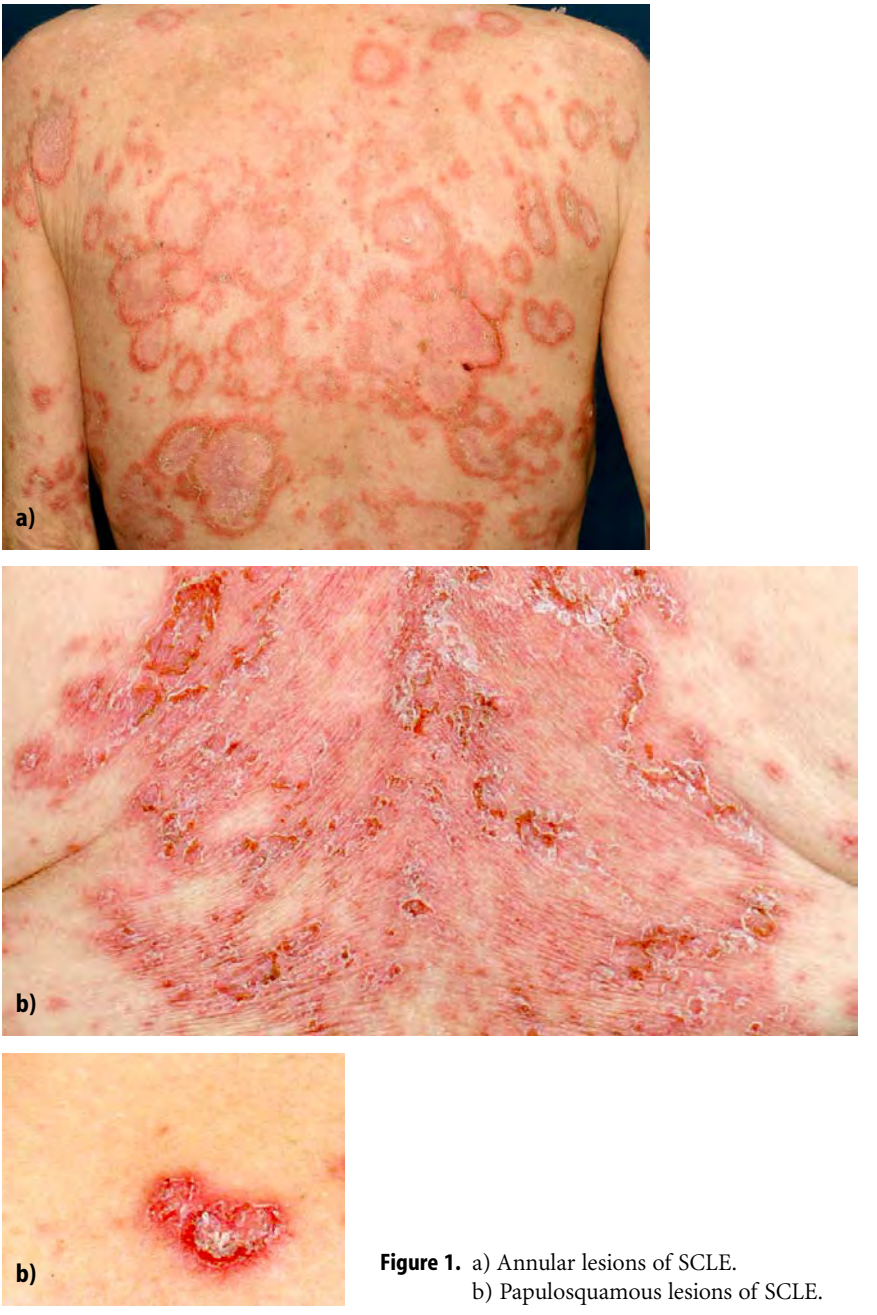



**Figure 1.** a) Annular lesions of SCLE.
b) Papulosquamous lesions of SCLE.

## 4 Follow up

Generally, patients should be instructed on the importance of sun-protective measures and their effect on the prognosis. Also, they should be schooled to recognize the symptoms of SLE as this requires a reassessment of treatment.

Patients with SCLE should be followed at regular intervals since the degree of treatment success varies among individuals and between the administered drugs. Changes in therapeutic strategy should only be made after a sufficiently long period of observation and follow up.

Assessments should be performed once or twice a year with the following laboratory tests: complete blood cell counts, renal function and urinalysis. Also, some SCLE-patients are vitamin D deficient and may require regular supplementation. In patients with SLE as co-morbidity, the sequential assessment of antinuclear antibody (ANA)-and anti-dsDNA antibody levels may be useful as a predictor of the disease's progression.

Without SLE, patients usually have a good prognosis with no persistent skin changes other than occasional dyspigmentation. Spontaneous remission is possible, but a chronic, periodical fluctuation is more common with exacerbation in the spring or summer. In rare cases, the disorder progresses into a severe systemic form with the danger of life-threatening sequelae.

## 5 Management

The lesions caused by SCLE are mostly located on exposed skin, may be viewed as disfiguring and have a detrimental effect on a patient's quality of life. Hence, the primary goal is to improve appearance and to prevent the formation of additional lesions. Topical corticosteroids and calcineurin antagonists are administered to treat local manifestations of the disease. Antimalarials are also given for most affected patients with SCLE. In more severe cases, systemic immunosuppressants are applied.

### 5.1 Sun protection

Generally, the first step in SCLE therapy is to protect exposed skin from ultraviolet (UV) light. Decreased activity during daylight hours with high UV loads between 10 am and 4 pm may help some individuals, while others exhibit extremely high photosensitivity and require sunscreens or protective clothing. Obviously, sources of intense artificial light (such as solaria) should be avoided as well. Usually, no further cosmetic measurements are required.

### 5.2 Corticosteroids

Corticosteroids and topical calcineurin antagonists suppress inflammation and down-regulate several components of the patient's immune system. The proliferation and recruitment of inflammatory cells, such as eosinophils, mast cells and T-lymphocytes, is reduced by corticosteroid therapy. Corticosteroids and calcineurin antagonists (e. g. tacrolimus) can be applied topically to treat single lesions.

### 5.3 Antimalarials

In most cases the immunomodulatory drug of choice is (hydro-)cychloroquine. Antimalarials limit complement-dependent antigen-antibody reactions, and they inhibit chemotaxis of eosinophils as well as locomotion of neutrophils. (Hydro-) cychloroquine can be combined with quinacrine in refractory CLE.

### 5.4 Immunosuppressive drugs

Systemic steroids can be used additionally in exacerbations of the disease. For long-term treatment of severe forms of SCLE, azathioprine, mycophenolate mofetil, cyclophosphamide, cyclosporine, and methotrexate are the established drugs of choice.

## 6 Diagnostic tests

To make a positive diagnosis of SCLE, the most useful serological tests include the detection of ANA, anti-Ro (SS-A) and anti-La (SS-B) autoantibodies and anti-native DNA (double-stranded or nuclear DNA). Most patients with SCLE test positive for anti-Ro autoantibodies with slight differences in the expression rates, depending on the specific variant and patient characteristics (Table 1). Also, anti-Ro antibodies are less frequently found in other types of CLE, such as DLE, and may be employed to distinguish between SCLE and DLE. Other than that, human leukocyte antigens have been associated with SCLE, specifically HLA-B8, HLA-DR3, HLA-DRw52, and HLA-DQ1 [3, 4]. However, HLA typing is not established in the clinical routine diagnosis of SCLE.

Today, anti-Ro antibodies are detected via indirect immunofluorescence (IIF), employing human mitotic epidermoid (HEp-2) cancer cell lines, transfected with multiple copies of the specific DNA sequence that carries the information of the Ro autoantigen. About 15–20 % of these cells over-express the antigen, allowing anti-Ro autoantibodies to bind to cell nuclei, forming stable antigen-antibody complexes. After washing, the cells are incubated with an anti-human antibody conjugated to fluorescein. This three-part complex can be visualized using fluorescent microscopy. Positive samples will emit apple-green fluorescence with a staining pattern characteristic of the particular nuclear antigen distribution within

the cells. If the sample is negative for anti-Ro antibodies, the nucleus will not show a clearly discernible fluorescence pattern, while those positive for anti-Ro antibodies stain as follows: in interphasic cells, a strong nuclear and speckled staining is apparent for Ro positive cells (Fig. 2), while metaphasic cells show no staining in the chromosome region and a variable staining outside the chromosome region [5].

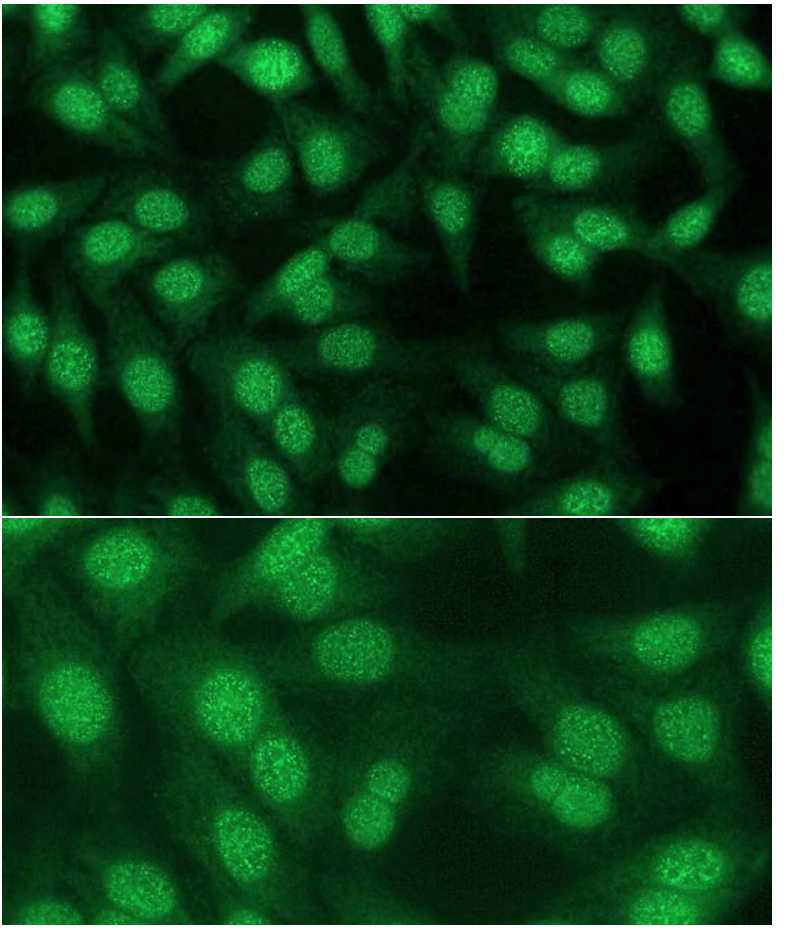


**Figure 2.** Indirect immunofluorescence of cells positive for anti-Ro autoantibodies. Cells show a characteristic speckled staining pattern.

## 7 Testing methods

The most effective serologic tests to diagnose SCLE are immunoassays targeting anti-Ro autoantibodies via IIF.

The established method for the diagnosis of DLE remains the physical examination of the patient's skin for the clinical manifestations of the disease. The skin lesions are very characteristic and distinct from those found in DLE and other diseases. Together with the histological assessment of biopsied tissue samples, the attending physician can make a positive diagnosis and may use serology to monitor the progression of the disease.

## References

[1] Durosaro O, Davis MD, Reed KB, Rohlinger AL. Incidence of cutaneous lupus erythematosus, 1965–2005: a population-based study. Arch Dermatol 2009; 145: 249–53.
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## Part 3

# Autoimmune liver diseases

# Autoimmune hepatitis

Reinhild Klein

## 1 Introduction

Autoimmune hepatitis (AIH) is a chronic, progressive disease which occurs in children and adults of all ages and affects mainly females. Characteristic features are the fluctuating spontaneous course, histologically determined interface hepatitis, as well as the hypergammaglobulinaemia of immunoglobulin G (IgG) type, and the presence of circulating autoantibodies. AIH occurs all over the world with varying incidence and prevalence. Its prevalence is estimated to range between 50 and 200 cases per million in Western Europe and North America among the Caucasian population. In this group, AIH accounts for up to 20 % of cases of chronic hepatitis. In countries in which viral hepatitis B and C are endemic, such as in Asia and Africa, the incidence of AIH seems to be significantly lower [1, 2].

The pathogenesis of AIH is unknown. Loss of tolerance against hepatic tissue is presumed and an underlying genetic predisposition has been suggested. Anti-inflammatory/immunosuppressive treatment induces remission but long-term maintenance therapy is often required. Liver transplantation is generally successful in patients with decompensated cirrhosis unresponsive to or intolerant of medical therapy. Overall, long-term survival and average life expectancy of adequately treated patients are excellent and estimated to be comparable with those of the normal population.

## 2 Diagnostic criteria

The clinical presentation of AIH is very heterogeneous ranging from asymptomatic disease to severe icteric hepatitis, and even fulminant hepatitis which may require liver transplantation. In 1992, the International Autoimmune Hepatitis Group recommended a scoring system for the diagnosis of AIH to allow reliable diagnosis of the disease, and this was further updated in 1999 (Table 1a) [1–3]. The clinical relevance of this scoring system as well as other, more simplified systems (Table 1b) developed in the interim [3] is, however, still a matter of debate.

**Table 1. a)** International diagnostic criteria for the diagnosis of AIH [1–3].

| Parameter | | Score |
|---|---|---|
| gender | female | +2 |
| | male | 0 |
| serum biochemistry | | |
| | ratio of elevation of serum alkaline phosphatase vs. aminotransferase | |
| | > 3.0 | -2 |
| | 1.5-3 | +2 |
| | < 1.5 | +1 |
| | < 1.0 | 0 |
| total serum globulin, -globulin or IgG | | |
| | Times upper normal limit | |
| | > 2.0 | +3 |
| | 1.5-2.0 | +2 |
| | 1.0-1.5 | +1 |
| | < 1.0 | 0 |
| autoantibodies (titres by immunofluorescence on rodent tissues) | | |
| | ANA, SMA or LKM-1 | |
| | > 1:80 | +3 |
| | 1:80 | +2 |
| | 1:40 | +1 |
| | < 1:40 | 0 |
| | antimitochondrial antibody | |
| | positive | -4 |
| | negative | 0 |
| hepatitis viral markers | negative | +3 |
| | positive | -3 |

| | |
|---|---|
| other aetiological factors | |
| History of drug use | |
| yes | -4 |
| no | +1 |
| Alcohol (average consumption) | |
| < 25 g/day | +2 |
| > 60 g/day | -2 |
| Genetic factors: HLA DR3 or DR4 | +1 |
| Other autoimmune diseases | +2 |
| Response to therapy | |
| complete | +2 |
| relapse | +3 |
| liver histology | |
| Interface hepatitis | +3 |
| Predominant lymphoplasmacytic infiltrate | +1 |
| Rosetting of liver cells | +1 |
| None of the above | -5 |
| Biliary changes | -3 |
| Other changes | -3 |
| Seropositivity for other defined autoantibodies | +2 |

## 3 Diagnostic measurement for experts

Autoantibodies are one of the distinguishing features of AIH. The discovery of autoantibodies directed against different cellular targets, including nuclear, cytosolic and microsomal antigens has allowed a suggested subclassification of AIH based on the presence of three specific autoantibody profiles — although there is little evidence to support a role for these antibodies in pathogenesis (Table 2).

According to this approach, AIH type 1 is characterized by the presence of antinuclear antibodies (ANA) and/or anti-smooth muscle antibodies (SMA) directed against F-actin. AIH type 2 is characterized by anti-liver-kidney microsomal antibodies (LKM-1) reacting with cytochrome P450 2D6, and AIH type 3 by autoantibodies against a soluble liver/liver-pancreas antigen (SLA/LP) currently identified as UGA suppressor serine tRNA-protein complex. Although there are some clinical/biochemical differences between these subgroups (Table 2) [4], this serological classification has no implications with respect to therapeutic regimes and is, therefore, still controversial.

An initial liver biopsy for confirmation of diagnosis and for grading and staging is desirable. Biopsies are also helpful for the observation of which degree aminotransferase activities in serum reflect an inflammatory activity in the liver, which is not closely related in all cases. The histological appearance of AIH is the same as that of chronic hepatitis of other aetiology, and although certain changes are characteristic, no findings are specific for AIH [1, 2]. The inflammatory component is characterised by a mononuclear cell infiltrate, which invades the limiting plate surrounding the portal triad and permeates the surrounding parenchyma (periportal infiltrate; piecemeal necrosis; interface hepatitis) and beyond (lobular hepatitis). Eosinophils are frequently present. Fibrosis is present in all but the milder forms of AIH. With distortion of the hepatic lobule and the appearance of regenerative nodules, the result is cirrhosis.

A genetic predisposition is viewed as a prerequisite of AIH, and first degree relatives of AIH-patients are at high risk of also developing AIH or another au-

**Table 1. b)** Simplified diagnostic criteria for AIH according to [3].

| Parameter | Cutoff value | Points |
|---|---|---|
| ANA or SMA | $\geq$ 1: 40 | 1 |
| | $\geq$ 1: 80 | |
| Or LKM | $\geq$ 1: 40 | 2*) |
| Or SLA/LP | Positive | |
| IgG | > upper limit of normal | 1 |
| | > 1.1 × upper limit of normal | 2 |
| Histology (evidence of hepatitis is required) | Compatible with AIH | 1 |
| | Typical for AIH | 2 |
| Absence of viral hepatitis | yes | 2 |

Interpretation of aggregate points: $\geq$ 6 points: probable AIH; $\geq$ 7 points: definite AIH
*) proceed by adding points achieved for all autoantibodies; the maximum is 2 points.

toimmune disease [1, 2]. However, the genetic background of AIH does not follow a Mendelian pattern, and a conclusive role for a single genetic locus capable of explaining the aetiology of AIH has not been identified. Association of HLA A1, Cw7, B8 and DR3 as well as DR4 with AIH and other autoimmune diseases has been conclusively demonstrated in a number of studies. Studies from Europe and the U.S. have identified DRB1*0301 and DRB1*0401 as susceptibility alleles, and DRB1*1501 as a resistance allele. However, immunogenetic findings appear to not apply universally and it has been noted that significant geographic differences exist. While in Caucasian patients those with HLA DR3 and DR4 are independently susceptible to autoimmune hepatitis, DR4 is predominant in Japanese patients, and there are no Japanese patients with DR3.

## 4 Requirements for family practitioners

The clinical presentation of AIH is very heterogeneous ranging from asymptomatic disease to severe icteric hepatitis, and even fulminant hepatitis which may require liver transplantation.

Patients may present with non-specific symptoms of varying severity, such as fatigue, lethargy, malaise, anorexia, nausea, amenorrhoea, abdominal pain, and itching. Arthralgia is quite common. Physical examination may be without pathological findings, but may also reveal hepatomegaly, splenomegaly, jaundice, and signs and symptoms of chronic liver disease. Other autoimmune diseases such as Hashimoto's thyroiditis, ulcerative colitis, type 1 diabetes, rheumatoid arthritis, and coeliac disease as well as other autoimmune liver disorders (primary biliary cirrhosis, primary sclerosing cholangitis) have been described as being associated with AIH.

It is important to identify and treat AIH at its earliest stages, because untreated patients with mild disease progress to cirrhosis within 15 years. If untreated, severe AIH has a very high mortality rate of up to 50 % 3–5 years after diagnosis. Immunosuppressive therapy with corticosteroids, usually in combination with azathioprine is considered the gold standard to induce and maintain remission. Moreover, response to immunosuppressive therapy confirms the diagnosis of AIH. The therapeutic goal should be complete normalization of transaminases because progression to liver cirrhosis may occur in patients with residual inflammatory activity within the liver. However, side-effects of therapy must be taken into consideration. Although some patients remain in remission after drug treatment is withdrawn, most require long-term maintenance therapy. It has been proposed that patients should be in stable remission for at least 4 years before withdrawal of immunosuppressive therapy can be considered.

AIH is not a contraindication to pregnancy. However, patients should be in remission under a maintenance therapy of 5 mg steroids per day. Steroid dose should be increased shortly after delivery because a relapse can occur.

**Table 2.** Classification and characteristics of autoimmune hepatitis (according to [2, 4]).

| **Variable/parameter** | | **AIH type 1** | | **AIH type 2** | **AIH type 3** |
|---|---|---|---|---|---|
| | | ANA positive | anti-actin positive | anti-LKM1 positive | anti-SLA/LP positive |
| | | number patients analysed | | | |
| | | 167 | 218 | 40 | 175 |
| sex | ratio f:m | 2.6 : 1 | 1.9 : 1 | 2.3 : 1 | 5.3 : 1 |
| age | mean | 52.5 | 45.3 | 29.6 | 46.2 |
| | range | 11-88 | 2-83 | 5-73 | 8-86 |
| laboratory parameters at presentation | | | | | |
| AST (normal < 20 IU) | mean | 143 | 137 | 123 | 86 |
| ALT (normal < 20 IU) | mean | 149 | 169 | 125 | 110 |
| IgG (normal < 1.800 mg/dl) | mean | 3.103 | 2.864 | 1.998 | 2.697 |

## 5 Follow up

*Clinical observations and expectations*

Up to 30 % of adult patients already have histological features of cirrhosis at diagnosis. However, the presence of cirrhosis seems not to influence 10-year survival (90 %) and those patients require a similarly aggressive treatment strategy as patients without cirrhosis [1, 2, 5].

| | | | | |
|---|---|---|---|---|
| histopathologic features at presentation | | | | |
| cirrhosis (%) | 25 | 15 | 30 | 6 |
| chronic active hepatitis/ acute hepatitis (%) | 61 | 70 | 63 | 68 |
| chronic persistent hepatitis (%) | 13 | 15 | 8 | 17 |
| geographic variation | worldwide | worldwide | worldwide; rare in North America | worldwide |
| association with other autoimmune diseases | common | common | common | common |
| clinical severity | broad range | broad range | generally severe | broad range |
| treatment failure | infrequent | infrequent | frequent | infrequent |
| relapse after drug withdrawal | frequent | frequent | frequent | frequent |
| need for long-term maintenance | variable | variable | approximately 100% | variable |

In children, about 50 % have cirrhosis at the time of diagnosis. Long-term follow-up reveals that only a few children can completely stop all treatment and about 70 % of children receive long-term treatment. Most of these patients relapse when treatment is discontinued, or if the dose of immunosuppressive drugs is

reduced. About 15 % of patients develop chronic liver failure and are transplanted before the age of 18 years.

The aim of treatment is the induction of remission, i. e. a complete normalization of all inflammatory parameters including histology indices. This can be achieved in 65–75 % of patients after 24 months of treatment. Relapse is characterised by an increase of aminotransferase levels and IgG immunoglobulins and occurs in 50 % of patients within six months of treatment withdrawal and in 80 % after 3 years. It is associated with progression to cirrhosis in 38 % and liver failure in 14 %.

*Blood tests*

During treatment laboratory parameters should be assessed twice a year on patients who are asymptomatic and in remission, in patients with clinical symptoms every three months, and in patients showing inflammatory activity (elevated transaminases, IgG globulins) at least every two weeks until remission.

Autoantibody titres can decrease during immunosuppressive therapy and can even completely disappear in patients in remission. Increase of autoantibody activity together with an increase in IgG globulins in those patients may then indicate aggravation of the disease. However, there are also patients in whom autoantibodies persist despite adequate treatment.

## 6 Management

Independent of the clinically or immunoserologically defined type of AIH, standard treatment is implemented with prednisone (or prednisolone) alone or in combination with azathioprine (Table 3). Both strategies are equally effective. However, depending on a variety of definitions of response, success rates are only in the range of 65–70 %, which leaves a significant number of patients in need of other standard treatment [1, 2, 5]. Adults with cirrhosis at the time of initial biopsy and children, particularly those with AIH type 2, rarely stay in remission when treatment is withdrawn and will almost certainly require life-long maintenance therapy.

No firm guidelines exist for decisions regarding withdrawal of medications because histological changes may lag biochemical responses and a quiescent histological appearance and normal biochemical findings while patients are still receiving therapy, are not necessarily predictive of continued remission once therapy is withdrawn. Therapy is usually administered over a course of at least 2 years. The decision between monotherapy and combination therapy is guided by the side effects of steroid therapy. Cosmetic side effects (Cushing's syndrome), in particular, decrease patient compliance. Serious complications such as steroid diabetes, osteopenia, aseptic bone necrosis, psychiatric symptoms, hypertension and

cataract-formation must be anticipated in long-term treatment, especially when the steroid dose cannot be tapered down to 5 mg per day. Azathioprine can be used to decrease the dose of prednisone [5] but it bears a theoretical risk of teratogenicity. In addition, abdominal discomfort, nausea, cholestatic hepatitis, rashes and leucopenia may be encountered. Toxicity and/or intolerance to azathioprine and its metabolite 6-mercaptopurine can occur and depends upon mutations in the thiopurine methyltransferase genes. Dose reduction is aimed at finding the individually appropriate maintenance dose. Usually, a maintenance dose of prednisone or prednisolone ranges between 10 and 2.5 mg and of azathioprine between 50 and 100 mg per day (Table 3). The use of budesonide is, to date, only recommended for patients with mild inflammation or patients in remission.

**Table 3.** Standard treatment of autoimmune hepatitis in adults.

| Regimen | Single-drug therapy | Combination therapy |
|---|---|---|
| Initial | prednisone or prednisolone 20–60 mg/day | prednisone or prednisolone 15–30 mg/day, azathioprine 50–100 mg/day |
| Maintenance | prednisone or prednisolone 5–15 mg/day | prednisone or prednisolone 15–30 mg/day, azathioprine 50–100 mg/day |

Treatment failure is characterised by a progression of clinical, serological and histological parameters during standard therapy and is seen in about 10 % of patients. In these patients the diagnosis of AIH must be carefully reconsidered. Alternative immunosuppressive therapies have been proposed, mainly on the basis of small series or case reports. These have included cyclosporine, tacrolimus, methotrexate, cyclophosphamide, ursodiol, and mycophenolate mofetil (MMF) [5].

AIH patients who develop decompensated cirrhosis may require liver transplantation. There is no single indicator or predictor for the necessity of liver transplantation. The 5-year survival is up to 92 % and the rate of recurrence of AIH after transplantation ranges between 10 and 35 %.

## 7 Diagnostic test

Autoantibodies are one of the most important diagnostic markers in AIH (Fig. 1), although there is little evidence to support a role for these antibodies in pathogenesis.

AIH type 1 is a classical type, so-called lupoid hepatitis. It is associated with ANA and/or SMA which react with F-actin (Fig. 1A, B). These should be detected by immunofluorescence testing on cryostat sections and not by cell culture

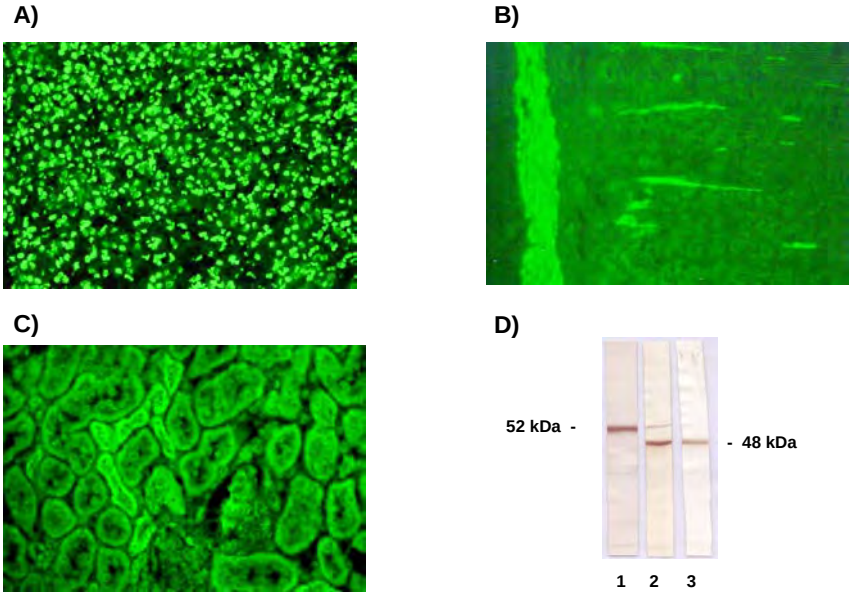


**Figure 1.** Demonstration of AIH-related autoantibodies by immunofluorescence test (IFT) (A–C) and Western blotting (D). A) Demonstration of antinuclear antibodies (ANA) on rat liver. B) Demonstration of antibodies to smooth muscle antigens with anti-actin specificity on rat stomach showing the typical staining of smooth muscle cells and interparietal cell fibers characteristic for anti-actin. C) Demonstration of antibodies to liver-kidney microsomes (LKM) on rat kidney showing the typical coarse granular cytoplasmic staining of tubules. D) Demonstration of anti-SLA/LP antibodies by Western blotting revealing the typical determinants at 52 and 48 kDa (anti-SLA/LP antibodies cannot be detected by IFT!). Three patterns can be observed: 1: sera reacting only with the 52 kDa band, 2: sera reacting with both, the 52 and the 48 kDa bands, and 3: sera reacting only with the 48 kDa band.

slides (for instance Hep2 cells) because the latter tests frequently detect naturally occurring ANA with no clinical relevance e. g. in patients with infectious or drug-induced disorders. Antibody titres may decrease during therapy.

Anti-liver/kidney microsome-1 (LKM-1) and anti-liver cytosol-1 (LC-1) antibodies occurring alone or together characterise AIH type 2 (Fig. 1C). Anti-LKM-1 antibodies are directed against cytochrome P450 2D6. About 10 % of patients belong to this group of AIH, and these are mainly children. Anti-LKM antibodies have also been found in patients with hepatitis B or C or drug-induced hepatitis, but these are directed against either other microsomal antigens or against epitopes of Cyp 2D6 other than the anti-LKM-1 antibodies in AIH type 2.

In contrast to ANA, SMA, or anti-LKM which can also be found in other (liver) disorders, the antibodies to soluble liver/liver-pancreas antigen (SLA/LP) are con-

fined to AIH and have not been found in any other liver disease [4]. They occur in about 30 % of AIH patients and can be associated with ANA or anti-actin. In about 10 % of patients, however, they occur without any other relevant autoantibody and may, therefore, comprise a separate serological group (AIH type 3). However, the antibodies cannot be detected by IFT but only by Enzyme Linked Immunosorbent Assay (Elisa), radioimmunoassay or Western blotting (Fig. 1D). Again, the antibodies can disappear during immunosuppressive therapy. The antigen involved has been identified as human suppressor serine tRNA associated protein, a co-translocation factor which incorporates seleno-cystein in human cells.

Moreover, antibodies to the asialoglycoprotein receptor protein (ASGPR), a membrane protein of hepatocytes, have been described which seem to correlate with disease activity and prognosis.

Antibodies to neutrophils showing an atypical perinuclear staining (pANCA) have also been detected in AIH, and in those patients an association of AIH with primary sclerosing cholangitis and/or ulcerative colitis must be considered.

However, it needs to be clearly stated that patients with AIH exist, in whom currently known autoantibodies are completely undetectable.

The presence of antimitochondrial antibodies in AIH is strongly indicative for an overlap syndrome with primary biliary cirrhosis.

One characteristic laboratory feature of AIH is the elevation of serum immunoglobulins, in particular IgG.

## 8 Testing methods

The benefits and limitations of diagnostic laboratory tests are discussed in the chapter 'primary biliary cirrhosis'.

Using IFT on cryostat sections from rodents the diagnosis of AIH can be made in about 90 % of patients (Fig. 2). Only anti-SLA/LP antibodies cannot be detected by this method. For their measurement, complement fixation, radioimmunoassay and Western blotting have been applied and shown to be the most reliable methods. Since identification of the antigen on a molecular level, Elisas using recombinant antigens are also available.

It is important to state that the demonstration of antinuclear antibodies in a chronic liver disorder is not diagnostic for AIH — especially when IFT on cell cultures (for instance Hep2 cells) is used instead of cryostat sections. In IFT on cell cultures, naturally occurring ANA induced during infectious or toxic processes are quite frequently observed. Furthermore, the ANA should be differentiated. For instance, antibodies to nuclear dots (sp100), nuclear membrane (gp210) or centromeres are rather more indicative of primary biliary cirrhosis than AIH.

For serological diagnosis, several assays are commercially available using recombinant antigens. However, antinuclear antibodies and antibodies to actin can-

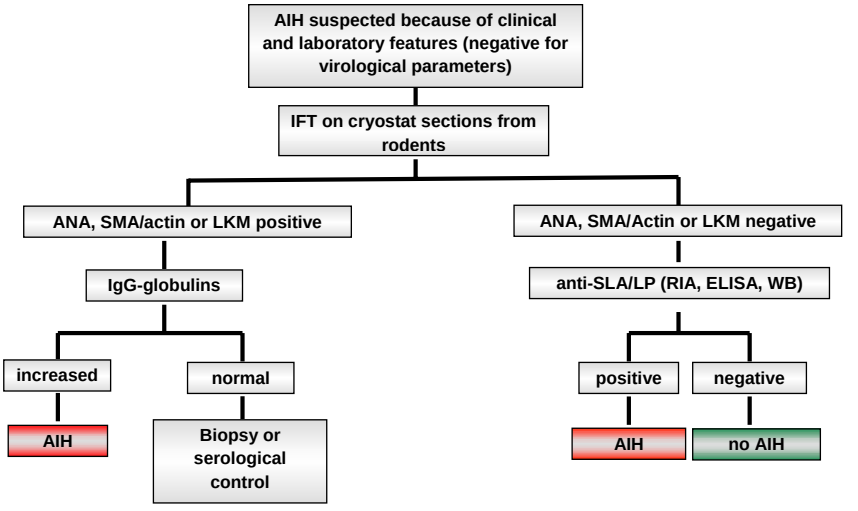


**Figure 2.** Flow chart for the serological diagnosis of autoimmune hepatitis.

not be reliably detected with those tests. The serological diagnosis of AIH should be, therefore, always proven by specialised laboratories.

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# Primary biliary cirrhosis

Reinhild Klein

## 1 Introduction

Primary biliary cirrhosis is a chronic, cholestatic liver disease which affects mainly middle-aged women. It starts with an inflammatory process of the small and middle-sized interlobular bile ducts leading first to a proliferation and then to a loss of bile ducts, to portal inflammation and in late stages to liver cirrhosis [1, 2]. It belongs to the autoimmune disorders because of the presence of antimitochondrial antibodies (AMA) in a high proportion (about 95 %) of patients, although the pathogenetic relevance of these antibodies is not entirely clear.

PBC occurs all over the world but with varying incidence and prevalence. The incidence of PBC ranges from 0.7–49 per million per year. In most recent studies, the point prevalence was estimated to range from 6.7 to 402 per million [2]. The frequency with which PBC is diagnosed increased considerably between 1980 and the present time, the reasons for this change may be complex. Assuming a life expectancy of 20 years after diagnosis, the point prevalence was estimated to be 207 per million, and for women above 45 years, 860 per million.

## 2 Diagnostic criteria

Typical clinical features of PBC are fatigue, pruritus and Sicca-syndrome (Table 1). However, nowadays at diagnosis, the majority of patients are asymptomatic and present for other reasons, e. g. for workup of elevated serum levels of AP or cholesterol. Increased awareness of the condition and the increasing availability of diagnostic tools, in particular serological testing, have led to more frequent and earlier diagnosis of PBC. A diagnosis of PBC is made "with confidence" when biochemical markers of cholestasis, particularly alkaline phosphatase, are elevated persistently for more than 6 months in the presence of serum AMA and in the absence of an alternative explanation (Table 2).

**Table 1.** Signs and symptoms of disease [1, 2].

| Symptoms | Frequency ( %) |
|---|---|
| Fatigue | 80 |
| Pruritus | 20–70 |
| Sicca-syndrome | 20–30 |
| Osteoporosis | 35 |
| Xanthoma | 10–20 |
| Urinary tract infection | 19 |
| Discomfort in the right upper quadrant of the abdomen | 10 |
| Fat-soluble vitamin malabsorption | Rare |
| Association with other autoimmune disorders | 30–40 |

**Table 2.** Diagnostic criteria.

*Clinical criteria*

- fatigue
- pruritus
- upper abdominal pain
- Sicca syndrome

*Laboratory criteria*

- elevation of alkaline phosphatase (AP) and gamma-glutamyltranspeptidase (γ-GT)
- presence of antimitochondrial antibodies (AMA)
- in AMA-negative cases presence of defined ANA-specificities (antibodies to nuclear dots, nuclear membrane, centromeres)
- elevation of IgM-globulins
- hypercholesterolemia
- eosinophilia

## 3 Diagnostic measurements for experts

The major hallmark of PBC is the presence of AMA in serum labelled anti-M2 (Fig. 1). These react with subunits of the 2-oxoacid-dehydrogenase complex (2-OADC) and, in most cases, recognise the E2-subunit of pyruvate dehydrogenase (PDH-E2) (Fig. 2). AMA/anti-M2 positive individuals, even if they have no signs of cholestasis and/or liver inflammation, are very likely to develop PBC. AMA are present in about 95 % of PBC-patients. Of the remaining 5 %, about 2.5 % have PBC-specific antinuclear antibodies (antibodies to nuclear dots

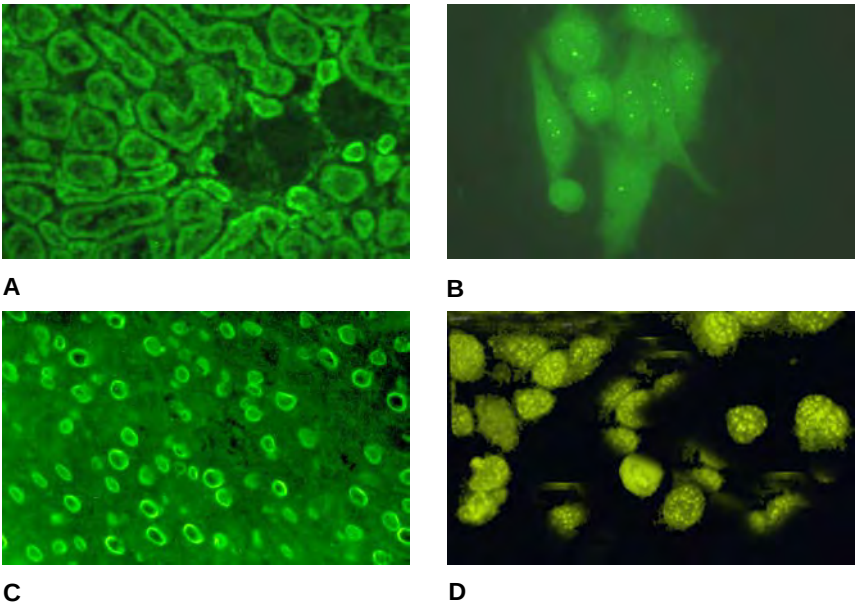


**Figure 1.** Demonstration of PBC-specific autoantibodies by immunofluorescence test (IFT). A) Demonstration of antimitochondrial antibodies (AMA) on rat kidney showing a coarse granular staining of proximal tubules; B) demonstration of antibodies to 'nuclear dots' (sp 100) on cell cultures; C) demonstration of antibodies to nuclear membranes (gp210) on heart muscle from rat; D) demonstration of antibodies to centromeres on cell cultures.

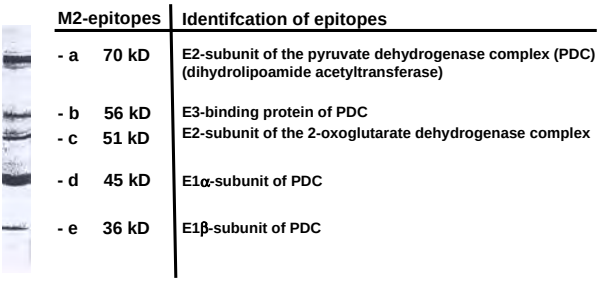


**Figure 2.** Identification of M2-determinants as subunits of the 2-oxoacid dehydrogenase complex.

[sp100], nuclear membrane [gp210], centromeres) (Fig. 1), but there are still about 2.5 % patients who have no relevant autoantibodies but otherwise all the features typical for PBC.

A liver biopsy is no longer regarded as mandatory for the diagnosis of PBC in patients with elevated serum markers of cholestasis and positive serum AMA, but may be helpful in excluding other potential causes of cholestatic disease and in assessing disease activity and stage. A liver biopsy may also be helpful in the presence of disproportionably elevated serum transaminases and/or serum IgG levels to identify additional or alternative processes, especially autoimmune hepatitis.

Histological staging of PBC (stage 1 to stage 4) is determined by the degree of (peri)portal inflammation, bile duct damage and proliferation, and by the presence of fibrosis/cirrhosis (Table 3).

**Table 3.** Histology and staging of PBC.

| |
|---|
| *Stage I (portal stage)* |
| Portal hepatitis, bile duct destruction, granuloma formation |
| *Stage II (periportal stage)* |
| Periportal hepatitis, interface-hepatitis extending to lobules, bile duct proliferation |
| *Stage III (septal stage)* |
| Presence of fibrous septa or bridging necrosis |
| *Stage IV (cirrhotic stage)* |
| Ductopenia, cirrhosis |

## 4 Requirements for family practitioners

Nowadays, most patients are asymptomatic but an elevation of cholestatic enzymes, IgM- and cholesterol levels is observed during laboratory investigations. Many patients also show an eosinophilia. In symptomatic patients, fatigue, pruritus and Sicca-syndrome or symptoms of a collagen disorder such as myalgia or arthralgia are the most common complaints with which patients consult their general practitioner. When the diagnosis is suspected, the patient should be transferred to a hepatologist for further examination and laboratory testing. The presence of AMA confirms the diagnosis. Ultrasound examination of the liver and biliary tree is obligatory in all cholestatic patients in order to differentiate intrahepatic from extrahepatic cholestasis. A normal biliary system is typical for PBC. Abdominal lymphadenopathy, particularly in the hilar region of the liver, is seen in 80 % of patients with PBC.

About 20 % of patients exhibit other simultaneous or consecutive autoimmune diseases, the most frequent being autoimmune hepatitis, CREST syndrome and/or scleroderma, Sjögren's syndrome and thyroiditis.

First-degree relatives of patients with PBC are at high risk of PBC or other autoimmune diseases. The patients and their relatives should be informed and evaluated for these conditions.

Attention should focus on the severity or potential severity of the disease.

Because the prognosis of PBC is far better than two or three decades ago, two associated conditions deserve particular attention. Hypercholesterolemia with increased LDL cholesterol is observed in about 20 % of patients. Accordingly, the risk of cardiovascular disease should be evaluated and medical therapy possibly proposed. Osteoporosis and osteopenia might be more frequent in women with PBC so metabolic bone disease should be assessed and prevented.

## 5 Follow up

*Clinical observations and expectations*

There are three major forms of PBC. The typical or classical form is represented by the slowly progressive decline of small bile ducts and parallel increase in liver fibrosis, leading to biliary cirrhosis over a period of about 20 years. These patients may remain asymptomatic for a long time or suffer from fatigue and pruritus.

A second form, which affects 10–20 % of patients, is characterised by the fluctuating or persistent presence of the features of AIH. These patients have a more severe disease course with early development of liver fibrosis and cirrhosis. A third form, which affects 5–10 % of patients, is characterised by a rapid onset of ductopenia and severe icteric cholestasis, progressing very quickly towards cirrhosis in less than 5 years. In these patients the typical signs of portal hypertension (ascites, oesophageal bleeding, encephalopathy, jaundice, etc.) may develop.

*Blood tests*

During treatment, laboratory parameters should be assessed once or twice a year in asymptomatic patients, in patients with symptomatic PBC every three months. An increase of bilirubin is a strong indicator for progression of the disease and serves as marker for the indication of a liver transplantation.

Antimitochondrial antibodies are barely influenced by any therapy. Nevertheless, autoantibodies and quantitative immunoglobulins should be analysed at least once a year in order to exclude or recognise the development of an autoimmune hepatitis (or other autoimmune disorders) as early as possible.

Even after liver transplantation, AMA titres only transiently decrease about one year after the transplant but then again become positive, i. e. they cannot be taken as a marker for the recurrence of PBC in the transplanted liver.

## 6 Management

The cause of PBC is unknown and, therefore, no causal therapy exists. Ursodeoxycholic acid (UDCA) introduced in 1985 into the treatment of PBC, is currently considered the mainstay of therapy at a dosage of 13–15 mg/kg/day, and it is

the only FDA-approved drug for PBC. Randomised, double-blinded, placebo-controlled trials have consistently shown that UDCA improves parameters of liver biochemistry including serum bilirubin. UDCA delays the progression of fibrosis and histological stage, improves quality of life, survival free of transplant and overall survival [3, 4]. It is safe, and side effects are few but it may produce gastric discomfort, a burning sensation or diarrhoea.

The survival rate of UDCA-treated patients in the early stages is similar to that in a control population.

In a subset of patients, the daily dose of 13–15 mg/kg UDCA is not sufficient to achieve the optimal biochemical response. In those patients, a trial with daily doses up to 20 mg/kg/day may be proposed.

About 30–40 % of PBC patients have a suboptimal response to UDCA; these patients need an adjuvant therapy. Currently, glucocorticoids and methotrexate are considered for these patients. Serious side effects of long-term glucocorticoid treatment may outweigh the potential benefit. In this respect, the introduction of budesonide, a nonhalogenated corticosteroid with an extensive first-pass metabolism has been a promising innovation. The effect of methotrexate is still controversial.

Other immunosuppressive agents including azathioprine, cyclosporine, mycophenolate mofetil, and drugs with antifibrotic properties including penicillamine, colchicines, and silymarin have been evaluated and have been shown to be either ineffective or toxic.

Liver transplantation is the treatment of choice in patients with late-stage PBC with decompensated cirrhosis or liver failure. In highly selected patients treatment-resistant pruritus, in the absence of decompensated cirrhosis, or severe osteoporosis may be an indication for transplantation. Survival rates of 80–90 % at 5 years have been reported. The disease recurs in up to 30 % at 10 years after transplantation, but usually displays a mild course under immunosuppressive therapy.

## 7 Therapy of extrahepatic manifestations

UDCA also has a beneficial effect on *hypercholesterolemia*; it induces an average 15–20 % decrease in total and LDL cholesterol at 1 year of therapy. Statins can be given additionally.

The effect of UDCA on *pruritus* in PBC is variable. Cholestyramine is widely used as first-line treatment. Other therapies include glucocorticoids, sertraline, and opiate antagonists.

For *fatigue* the centrally acting modafinil, a drug approved for the treatment of narcolepsy, has been reported to provide significant benefit. The drug, used at doses up to 400 mg/day, seems well tolerated and very effective in those with excessive fatigue.

Current treatments for *osteopenia and osteoporosis*, which affect up to 30 % of PBC patients, include supplementation with calcium (1000–1200 mg/day) and vitamin D (400–800 IU/day). The use of bisphosphonates is controversial.

Management of *portal hypertension* in PBC is the same as that for other cirrhotic patients. Severe portal hypertension, even without any other signs of decompensation, is a good indication for liver transplantation.

## 8 Diagnostic tests

Antimitochondrial antibodies (AMA) are detected primarily by immunofluorescence testing (IFT) using cryostat sections from rat kidney, liver, heart or stomach (Fig. 1). A positive test should be verified by Elisa or Western blotting using the M2-antigen prepared from inner membranes from bovine heart mitochondria or recombinant antigens representing its five components (E2-, E1α- and E1β-subunit and E3-binding protein of pyruvate dehydrogenase complex [PDC], 2-oxoglutarate dehydrogenase complex [2-OGDC]). About 95 % of PBC patients are positive with the M2-antigen, about 85 % react with PDC-E2. In about 10 %, only antibodies to 2-OGDC are observed. However, there are still patients who are AMA positive but anti-M2 negative, i. e. further AMA-subspecificities may exist [5].

AMA can also react with antigens of the outer mitochondrial membrane (anti-M4, -M8), and these antibodies seem to correlate with comparatively active courses.

In about half of the patients with AMA/anti-M2 negative PBC, specific antinuclear antibodies can be observed (antibodies to nuclear dots [sp100], nuclear membrane [pg210] or centromeres) (Fig. 1). These cannot be detected by IFT on cryostat sections but only by IFT on cell cultures (for instance Hep2-cells) or by Elisa using the applicable recombinant antigens (Scheme 1).

Association of AMA with a homogeneous pattern ANA, antibodies to actin or to the soluble liver (liver-pancreas antigen (SLA/LP) are strongly indicative of an association of PBC with autoimmune hepatitis, especially when IgG-globulins are elevated.

## 9 Testing methods

The benefits of the diagnostic laboratory tests, i. e. AMA and anti-M2 and ANA-subspecificities, are the excellent performance characteristics; in particular with specificity (∼100 %) and sensitivity (∼95 %).

Limitations of the IFT are the need for experience in the interpretation of IFT-patterns. Special laboratory equipment (fluorescence microscope) and training of technicians are required. Disadvantages of Elisas are their high sensitivity which may result more frequently in false positive results due to the detection of low-titre,

naturally occurring autoantibodies. The use of recombinant antigens may result in false negative results, because AMA may be directed against conformational epitopes which are not expressed in the soluble phase required for Elisa. Furthermore, in contrast to most other autoimmune disorders, it is important in PBC to look for AMA of the IgG- and IgM-type because some patients have only anti-M2 antibodies of the IgM type.

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# Primary sclerosing cholangitis

Reinhild Klein

## 1 Introduction

Primary sclerosing cholangitis (PSC) is an idiopathic, chronic, cholestatic liver disease characterised by progressive inflammatory destruction of intra- and extrahepatic bile ducts affecting males more frequently than females (60–80 %). Although the disease may affect children and older adults, the median age of onset is in the fourth decade. In 75–90 % of patients, PSC is associated with an inflammatory bowel disease (IBD), primarily with ulcerative colitis (UC) [1]. The prevalence of PSC in Northern Europe and the US is approximately 1/10 000 while 10–100 fold lower frequencies are reported in Southern Europe and Asia. Cholangiography is the most relevant approach to provide essential diagnostic criteria. The natural course of the disease is quite variable with an average time from diagnosis to death or liver transplant requirement of 12 to 15 years. Approximately 10–15 % of PSC patients will develop cholangiocarcinoma (CCA) during their lifetime. Although PSC is associated with multiple autoantibodies, it cannot be considered as a typical autoimmune disease. In childhood, however, PSC is frequently associated with florid autoimmune features, including elevated titres of autoantibodies (especially antinuclear antibodies and antibodies to smooth muscle antigens), elevated IgG and interface hepatitis resembling autoimmune hepatitis [2]. Overall, no therapy has yet proven effective in PSC, and orthotopic liver transplantation remains the only treatment option increasing patient survival.

## 2 Diagnostic criteria

PSC is a disease with a variable course, with progressive obliteration of the biliary tree leading to biliary cirrhosis and its complications such as portal hypertension. At presentation, approximately 15–55 % of patients are asymptomatic [1, 3]. Fatigue, pruritus, jaundice or abdominal discomfort develops in 60 % of cases (Table 1).

The diagnosis of PSC is based on characteristic cholangiographic changes in endoscopic retrograde cholangiopancreaticography (ERCP). The imaging hall-

**Table 1.** Prevalence of symptoms in primary sclerosing cholangitis (according to [1]).

| Symptoms | Frequency ( %) |
|---|---|
| Fatigue | 50–75 |
| Pruritus | 40–70 |
| Jaundice | 9–69 |
| Abdominal pain | 16–60 |
| Weight loss | 10–34 |
| Fevers and chills | 5–28 |
| Hyperpigmentation | 25 |
| Asymptomatic | 15–55 |

marks are multiple segmental intra- and extrahepatic strictures, diverticular outpouchings, beaded ducts, and a pruned appearance of the biliary tree. The strictures can be as short as 1–2 mm or may be several centimetres.

The biochemical hallmark of PSC is an elevation of alkaline phosphatase (AP) — although some patients may have normal AP levels. Bilirubin can be already increased in early stages due to bile duct strictures. AP and bilirubin can fluctuate during the course, and periods of clinical and cholestatic relapses follow periods of clinical remissions with less cholestasis.

Histologically, PSC is characterised by damage, atrophy and loss of medium- and large-sized bile ducts within or outside the liver.

## 3 Diagnostic measurements for experts

As mentioned above, cholangiography is considered to be the gold standard for the diagnosis of PSC. It is used for diagnosis but also therapeutically to dilate or stent strictures and screen for cholangiocarcinoma (CCA) by brush cytology and biopsy. However, magnetic resonance cholangiography (MRC) has emerged as a noninvasive, accurate, and rapid alternative method for the examination of the biliary tree achieving sensitivities of 82–88 % and specificities of 92–97 % in distinguishing PSC from other hepatobiliary diseases. Its disadvantage is that it is purely a diagnostic examination although it can be used to identify patients who would benefit from subsequent therapeutic ERC [1].

Although autoantibodies occur quite frequently in PSC (Fig. 1) they do not contribute to its diagnosis (Table 2) [4]. The prevalent autoantibody reactivity is a perinuclear anti-neutrophilic autoantibody (pANCA or xANCA) present in approximately 80 % of patients but lacking diagnostic specificity. Its target antigen is still unknown although there is some evidence that it may be related to human beta-tubulin isotype 5 [5]. The recent finding of antibodies to recombinant sulfite

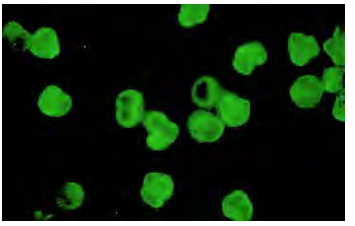

**Figure 1.** Demonstration of antibodies to neutrophils by immunofluorescence test using human neutrophils in a patient with PSC showing the typical perinuclear staining (pANCA).

**Table 2.** Autoantibodies in PSC (according to [4]).

| Antibodies to | Prevalence |
|---|---|
| neutrophils (pANCA) | 26–94 |
| nuclear antigens (ANA) | 8–77 |
| smooth muscle antigens (SMA) | 0–83 |
| liver-kidney-microsomes | 0 |
| soluble liver/liver-pancreas antigen (SLA/LP) | 0 |
| mitochondria (AMA) | 0–9 |
| biliary epithelial cells (BEC) | 63 |
| sulfite oxidase | 33 |
| glutathione S transferase theta 1 | 5 |
| endothelial cells | 35 |
| Saccharomyces cereviseae | 44 |
| cardiolipin | 4–63 |
| immunoglobulin M (rheumatoid factor) | 15 |
| thyroid peroxidase (TPO) | 16 |
| glomerular basement membrane (GBM) | 17 |
| epithelial 40 kDa protein from colon | 67 |

oxidase by Elisa in 56 % of patients with untreated PSC needs to be backed up by larger studies.

The characteristic pathological feature of PSC, i. e. a concentric periductular fibrosis ('onion-skinning'), which progresses to a narrowing and then obliteration of the small bile ducts, leaving a bile duct scar, is found in less than 15 % of PSC patients. Frequently, findings are nonspecific and must be interpreted along with clinical and radiological information [1].

There is more than an 80-fold increased risk of PSC among first-degree relatives suggesting a genetic link. However, it is a 'complex trait' disease, i. e. a

condition not inherited in a Mendelian autosomal dominant, autosomal recessive or sex-linked fashion. Whether there is a primary susceptibility allele is currently controversial, but PSC is probably acquired through inheriting a combination of genetic polymorphisms. An increased frequency of HLA B8 and DR3 (HLA DRB1*0301) in PSC as compared to controls, and also an increase of HLA-DR6 has been observed. The genetics of PSC is still the subject of active research [1, 3].

## 4 Requirements for family practitioners

The clinical presentation of PSC patients ranges between asymptomatic, symptomatic, advanced liver disease and/or malignancy (CCA), which may occur at any time, and patients will require liver transplantation within a short time. In many cases the diagnostic scenario is that of a patient with inflammatory bowel disease (IBD) presenting with elevated liver enzymes, followed by cholangiography and appropriate supplementary biochemical tests and in some cases liver histology. In some patients the development of benign dominant strictures or cholangiocarcinoma may result in a diagnostic setting with rapidly deteriorating cholestasis and attacks of acute cholangitis, sometimes aggravated by intermittent plugging by biliary sludge.

There is no evidence to support a particular temporal relationship between onset of PSC and onset of IBD. In more than half the patients, the diagnosis of IBD precedes that of PSC, in some patients the diagnosis of PSC precedes that of IBD by several years. IBD may even present after liver transplantation for PSC, but PSC may also present in IBD patients after colectomy.

One of the most important factors influencing the course of PSC and its prognosis is the development of CCA. It is the most feared complication of PSC and occurs in 7–15 % of patients. The survival of patients with PSC and CCA is greatly diminished. Early diagnosis of CCA is, therefore, important. Sudden progressive jaundice, weight loss and abdominal pain are frequently associated with the development of CCA, but the majority of patients with these symptoms have extrahepatic metastases at the initial diagnosis of CCA. Thus, the development of CCA is not reliably heralded by symptomatic or biochemical changes. Elevated AP and bilirubin levels are not specific for CCA, and may simply be a reflection of the patient's underlying liver disease. A new dominant stricture in patients with PSC merits both immediate investigation and close surveillance, especially in patients manifesting progression or deterioration of their clinical condition. Also ultrasonography and computed tomography seldom identify CCA. Cytological acquisition during ERC or percutaneous cholangiogram is an advantage over non-invasive imaging. Despite the increased risk of CCA in PSC compared with the general population, serial cholangiographic or radiological imaging alone are not yet recommended for CCA surveillance in PSC patients. Tumour markers also

play a limited role in early detection of CCA in PSC patients. Thus, the sensitivity of CA19-9 in detecting CCA in PSC is only 63 %, and the sensitivity of CEA is even lower (33 %), although the specificity is rather high (CEA 85 % in contrast to 50 % for CA19-9). Furthermore, benign extrahepatic cholestatic disease has been shown to increase the serum levels of CA19-9, with decreasing levels after the resolution of the cholestatic picture, and in benign cholestasis, a correlation has been demonstrated between CA19-9 and serum alkaline phosphate levels [1, 3].

However, patients with PSC are also at increased risk for cancers of the pancreas, gallbladder and liver. Colon cancer risk is increased particularly if the patient has IBD.

## 5 Follow up

*Clinical observations and expectations*

Median survival from time of diagnosis to death or liver transplantation requirement is estimated to be between 9 and 18 years. Asymptomatic patients have a significantly better prognosis than those with symptoms, but up to 17 % of asymptomatic patients present with cirrhosis on liver biopsy at the time of diagnosis. Patients with small-duct PSC seem to have longer survival rates as compared to patients with large-duct disease, and no development of CCA is found in this first group.

For defining a strategy of therapy and timing of liver transplantation, several prognostic models and risk scores have been constructed on the basis of clinical variables proven to correlate independently with prognosis. A high concordance index has been obtained with a novel prognostic model ('PSC score') including cholangiographic changes (distribution of PSC manifestation, presence of dominant bile duct stenosis) together with other clinical parameters [3]. This score has been shown to be superior to other scores, e. g. the Model for End-Stage Liver Disease score, revised Mayo score, and Child-Pugh score. Nevertheless, the major limitation of all prognostic models is the inability to predict CCA development.

*Blood tests*

Blood tests should be regularly performed. An increase of bilirubin and cholestatic enzymes may be indicative for the development of strictures or even CCA. As already mentioned, the determination of autoantibodies plays no major role in the diagnosis of PSC, but may unmask overlap syndromes with other autoimmune conditions especially with autoimmune hepatitis. Tumour markers are not sensitive and specific enough to be recommended for the diagnosis of CCA.

## 6 Management

So far, no treatment for PSC has been proven to be effective in randomised, controlled studies. Medical therapeutic approaches currently in use attempt to target the cholestatic and hepatitic features of PSC. Treatment of cholestatic features includes ursodeoxycholic acid (UDCA) and various means of relieving pruritus. UDCA has been the drug most widely evaluated in the treatment of PSC. Several controlled and uncontrolled studies have consistently demonstrated that UDCA, in a wide dose range from 10 mg/kg/day to 30 mg/kg/day, has beneficial effects on liver biochemistries. However, the relationship between improvement in liver biochemistries and clinically relevant findings such as the development of cirrhosis and its complications, the need for liver transplantation and survival is unknown, and it has not yet been proven to prolong survival or improve the outcome of PSC [1, 3].

Prednisone or immunsuppressive therapy has no beneficial effect in PSC, but may be useful in patients with features of AIH [1–3]. However, progression to cirrhosis occurs in a majority of these patients despite such treatment indicating that some of the pathological processes may be unaffected by immunosuppression.

Strictures of the extrahepatic bile ducts may be amendable to endoscopic or radiological dilation with or without a biliary drainage procedure such as sphincterotomy or stenting.

For disease-associated complications of PSC such as pruritus, fatigue, steatorrhoea and vitamin deficiencies, metabolic bone disease, bleeding peristomal varices, bacterial cholangitis, biliary strictures, gall bladder stones and polyps, and CCA symptomatic treatment is required (Table 3). The medical treatment of IBD in PSC follows the same guidelines as for IBD without PSC.

For patients with end-stage disease, liver transplantation is the treatment of choice. It should be considered before the disease becomes too advanced to enhance the long-term survival rates after OLT. Timing of liver transplantation in PSC does not differ from that of other indications for liver transplantation (consideration of MELD score and local waiting time). Additional circumstances that require evaluation for possible liver transplantation include recurrent bacterial cholangitis, severe extrahepatic biliary obstruction, uncontrolled peristomal variceal bleeding, intractable pruritus, and findings of biliary dysplasia in brush cytology specimens. PSC is among the indications for liver transplantation with the best patient survival with survival rates of 90 % to 97 % at one year, and 83–88 % at five years. However, a major complexity in the pre-transplant evaluation of PSC patients is related to the increased risk of malignancy.

Recurrence of PSC in the liver graft occurs in 2–40 % of the transplanted grafts [1, 3]. This wide range depends upon the rather vague diagnostic criteria. Proposed risk factors for recurrent PSC include recipient age, male sex, sex mismatch, coexistent IBD, cytomegalovirus infections, biologically related living donor liver transplantation, and recurrent and steroid-resistant acute cellular rejection.

**Table 3.** Disease-associated complications of primary sclerosing cholangitis and their treatments [1].

| Complication | Treatment |
|---|---|
| Pruritus | cholestyramine |
| | rifampicin |
| | opioid antagonists |
| | sertraline |
| | ondansetron |
| | liver transplantation (for refractory pruritus) |
| fatigue | no specific treatment available |
| vitamin deficiencies | vitamin supplementation |
| metabolic bone disease | calcium and vitamin D supplementation |
| | bisphosphonates? |
| bleeding peristomal varices | local control |
| | liver transplantation |
| | transjugular intrahepatic portosystemic shunt |
| bacterial cholangitis | antibiotics |
| | prophylactic antibiotics before ERCP |
| dominant biliary strictures | endoscopic treatment |
| | surgical treatment |
| gallbladder stones | cholecystectomy for symptomatic stones |
| gallbladder polyps | consideration for cholecystectomy due to malignant potential |
| cholangiocarcinoma | surgical resection |
| | liver transplantation protocols with neoadjuvant chemoradiation |
| | palliation with endoscopy and photodynamic therapy |

## 7 Diagnostic test and testing methods

As stated above, there is no single test for the diagnosis of PSC. The demonstration of pANCA may be taken as an additional parameter but is neither sensitive nor specific enough to serve as diagnostic marker. The gold standard for the diagnosis is endoscopic cholangiography.

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# Part 4

# Autoimmune gastrointestinal diseases

# Inflammatory bowel diseases

Nathalie Vermeulen, Xavier Bossuyt, Paul Rutgeerts, Severine Vermeire

## 1 Introduction

Inflammatory bowel disease (IBD) is a general term for a heterogeneous group of gastrointestinal diseases, including Crohn's disease (CD) and ulcerative colitis (UC). Both disorders are life-long with periods of remission and relapse. CD is characterized by an asymmetric and segmental transmural inflammation which may affect any part of the gastrointestinal (GI) tract. In 30 % of cases, the site of inflammation is the small bowel (Crohn's ileitis). Twenty percent of cases show inflammation of the colon only (Crohn's colitis). In 50 % of cases, inflammation of the ileum and the colon is found (Ileocolitis). Upper GI involvement in the oesophagus, stomach, duodenum or jejunum can coincide with all 3 locations. The disease behaviour can be stricturing, penetrating or neither [1].

UC, on the other hand, is characterized by a diffuse mucosal inflammation which is limited to the colon. Depending on the extension, the sub phenotypes of UC are proctitis, left-sided colitis and pancolitis, with the inflammation limited to the rectum, extending to the flexura sinistra, and involving the total colon, respectively. Many similarities exist between CD and UC, leading to the lack of a definite diagnosis in approximately 10 % of patients with colon-limited IBD. These patients are (temporarily) diagnosed with colitis-type unclassified or indeterminate colitis [2] (Table 1).

IBD is most often diagnosed in patients between 15 and 30 years, with a second incidence peak at ages above 40.

The pathogenic causes of IBD are still unknown. It is hypothesised that IBD is an immunologically mediated disorder in a genetically susceptible host. IBD is thought to result from an inappropriate and ongoing immune response and loss of tolerance to the normal luminal flora. This aberrant response leads to chronic inflammation of the gut and is most likely facilitated by defects in barrier function of the intestinal epithelium and the mucosal immune system.

IBD occurs worldwide, but a markedly higher incidence is observed in the industrialised areas of the world (Europe and the USA). The average annual incidence of CD in Europe and North America is rising and is estimated at

**Table 1.** Structural distinctions between ulcerative colitis and Crohn's disease.

| Ulcerative colitis | Crohn's disease |
|---|---|
| Rectum ± colon | Mouth to anus |
| Continuous | Discontinuous |
| Mucosal | Transmural<br>*(fissure, abscess, fistula)* |
| Muscular thickening | Fibrosis (stenosis) |
| Mucin depletion | Lymphoid ulcers, aggregates |
| Glandular damage | Granuloma (50–70%) |
| pANCA antibodies | ASCA antibodies |

5–10/100 000. The annual incidence of UC is estimated at 10–20/100 000. The prevalence of CD and UC is between 200 and 500 per 100 000.

## 2 Diagnostic measurements for experts

Diagnosis of IBD is mainly based on eliminating other possible causes of the symptoms including (bloody) diarrhoea and severe abdominal pain. There is no gold standard, but the diagnosis mainly depends on a combination of endoscopic, histological, radiological and/or biochemical examinations.

Initial laboratory investigations usually include markers for acute or chronic inflammation (erythrocyte sedimentation rate (ESR), C-reactive protein (CRP)), anaemia (haemoglobin level, complete blood count), fluid depletion and signs of malnutrition/malabsorption (electrolyte abnormalities). Stool samples should be collected for microbiological testing. IBD-specific antibody tests include the detection of antibodies to autoantigens and microbial antigens. Perinuclear anti-neutrophil cytoplasmic antibodies (pANCA) are antibodies directed to neutrophils that are detected in the serum of 60 to 80 % of UC patients, but also in 5–25 % of CD patients. Antibodies against *Saccharomyces cerevisiae* (ASCA) are detected in 50 to 80 % of CD patients, and in less than 10 % of UC patients. However, at present, these autoantibodies are not routinely screened for in patients suspected of IBD because of their moderate sensitivity and specificity.

To establish the diagnosis in patients suspected of CD, ileocolonoscopy with biopsies of the ileum and colon for microscopic examination is the preferred pro-

cedure. In case of severe, active disease, flexible sigmoidoscopy is safer and better to prevent bowel perforation. A plain abdominal radiograph is valuable in the initial assessment of possible bowel dilatation, calcified calculi, sacroiliitis or the impression of mass in the right iliac fossa. Fluoroscopic examinations (small bowel follow-through, small bowel enema) are the current standard for assessing the small intestine. Barium studies can be helpful, but they are subject to several factors that can influence the quality of the result. Computed tomography (CT), mostly performed in severe cases, provides additional information on bowel thickening, changes in vascularity and mesentery. In case of obstruction or bowel narrowing, small bowel enema and double contrast enema are the procedures of choice to assess disease extent and location. For detection of extramural complications (fistula or abscess), ultrasound, CT and magnetic resonance imaging (MRI) can be performed. Histological examination of endoscopic biopsies searches for signs of patchy chronic inflammation, focal crypt irregularity and granulomas, as these are the generally accepted microscopic features of CD. In ileal samples, irregular villous architecture can be detected [1].

To establish the diagnosis in patients suspected of UC, colonoscopy, preferably with ileoscopy and segmental biopsies, is the procedure of choice. In case of a severe attack, abdominal radiography and sigmoidoscopy are recommended. Other techniques that can be used to asses (the severity of) UC, including hydrocolonic ultrasound, Doppler ultrasound, virtual colonography, leukocyte scintigraphy are of secondary value in the diagnosis of UC. Histological examination of endoscopic biopsies reveals basal plasmacytosis (presence of plasma cells around or below the crypts), an increase in heavy, diffuse transmucosal lamina propria cells and widespread distortion of the mucosa or crypt architecture. These features indicate UC [2].

## 3 Requirements for family practitioners

IBD are chronic diseases with periods of active disease and remission. Symptoms heavily depend on disease activity (remission or active disease), but also on the subtype of IBD (UC or CD), and the severity of the disease (Table 2).

Medical history of a patient should include questioning about the onset and recurrence of symptoms, including rectal bleeding or bloody diarrhoea, abdominal pain, urgency, nocturnal diarrhoea. Furthermore, smoking habits, recent travel, food intolerance, recent medication, and family history should be explored.

Physical examination should evaluate general well-being, pulse rate, body temperature, blood pressure, body weight, abdominal examination for distension and tenderness, oral inspection and check for extraintestinal manifestations, including ocular, oral, joint, or skin lesions. However, physical evaluation may be normal in case of mild or moderate disease. Strongly suggestive symptoms include bloody

**Table 2.** Signs and symptoms of the disease.

| | Crohn's Disease | Ulcerative Colitis |
|---|---|---|
| **Intestinal symptoms** | Abdominal pain and cramping | |
| | Persistent diarrhoea | |
| | Perianal disease | Blood in the stool |
| | Loss of appetite | Rectal tenesmus |
| | Fissures* | Faecal urgency/ incontinence |
| **Non-Intestinal Symptoms** | Fever | |
| | Malaise | |
| | Anorexia* | |
| | Arthropathy* | |
| | Weight loss | Episcleritis* |
| | Delayed growth in children | Erythema nodosum* |
| | Eye irritations* | |

* Symptom found in a minority of cases

diarrhoea lasting for more than 1 week, non-bloody diarrhoea lasting for more than 3 weeks, or severe abdominal pain with significant weight loss.

Initial laboratory testing should include complete blood count, electrolyte, blood urea nitrogen, creatinine, liver enzymes, iron studies, and CRP. Furthermore, examination of stool samples could eliminate the presence of infectious agents.

For definite diagnosis, medical history and physical examination should be complemented with endoscopy and/or histological findings in segmental biopsies. Rapid awareness of possible IBD and referral to a specialist for endoscopy can significantly decrease the time to diagnosis and therefore improve the prognosis of the patient [1, 2].

## 4 Follow up

*Clinical observations*

During treatment, symptoms gradually improve and patients reach clinical remission. Treatment is, if possible, gradually decreased to avoid dependence and/or intolerance.

*Expectations*

IBD patients have variable prognosis; some patients reach remission and remain in remission for several months or years, while others never reach a state of remission. If treatment fails to induce remission, surgery can be an option. Most CD patients will eventually have surgery. One in 4 UC patients will have surgery within 10 years of diagnosis. Patients with extensive disease (pancolitis) have a higher risk for surgery. Patients with severe disease have increased risk for developing colon cancer.

*Blood tests*

Routine laboratory tests, including C-reactive protein determination, can be used to evaluate the response to treatment and to assess clinical improvement. Normalisation of routine laboratory test values and relief of symptoms are indicative of remission. However, complete clinical remission is defined by complete resolution of symptoms and endoscopic mucosal healing in UC patients, and as a drop in Crohn's disease activity index (CDAI) to $<150$ in CD patients. Complete clinical remission must be assessed by a thorough clinical exam and endoscopy.

## 5 Management

The main treatment for IBD aims at inducing and maintaining a state of remission. For each patient, the most effective treatment is determined by considering the disease activity, site of inflammation, disease behaviour, response to previous medications and the preferences of the patient. IBD is mostly treated with aminosalicylates (mesalazine, sulfasalazine), corticosteroids, immunomodulators (thiopurines (azathioprine, mercaptopurine), methotrexate, cyclosporine, tacrolimus) and/or biological therapies (anti-TNF antibodies (Infliximab, Adalimumab)).

Budesonide, a corticosteroid, is the preferred treatment for mildly to moderately active CD. Severe disease should be treated with systemic corticosteroids, possibly complemented with azathioprine/mercaptopurine in case of a relapse, or methotrexate in case of azathioprine/mercaptopurine intolerance. In case of dependence or intolerance to corticosteroids and/or immunomodulators, Infliximab or adalimumab can be added, but surgery can also be an option [3].

In mild to moderate UC, mesalazine is the preferred initial treatment, topical and/or oral. Severe UC should be treated in the hospital with intravenous corticosteroids. Immunomodulators should be started in steroid-dependent or steroid-refractory patients. Patients dependent or intolerant to corticosteroids and/or immunomodulators could be treated with biological therapies. If the disease persists, surgery is an option [4].

The treatment options described here are considered the standard treatment. However, treatment has to be evaluated for each patient.

## 6 Diagnostic tests

The presence of pANCA antibodies in the serum of patients is evaluated by means of indirect immunofluorescence with neutrophils as a substrate. Three distinct staining patterns can be detected; a cytoplasmic staining pattern, a perinuclear staining and an atypical perinuclear staining, characterized by a broad inhomogeneous labelling of the nuclear periphery along with multiple intra-nuclear fluorescent foci. The atypical perinuclear staining pattern (atypical pANCA) is found in 60–80 % of UC patients and in 5–25 % of CD patients.

The presence of ASCA antibodies in the serum of patients is evaluated by means of Enzyme-linked immunosorbent assay (Elisa). These antibodies are detected in 50–80 % of CD patients, compared to less than 10 % of UC patients and less than 5 % of the controls.

Other antibodies described in IBD are antibodies to pancreas, anti-OmpC (E. coli) antibodies, anti-I2 (pseudomonas fluorescens) antibodies, anti-CBirI (Clostridium) antibodies and several anti-glycan antibodies (ACCA, ALCA, AMCA). These antibodies still need confirmation and are currently only used in experimental settings [5].

## 7 Testing methods

Several limitations are associated with pANCA/ASCA testing for IBD. Both antibodies have relatively low sensitivities and specificities, which makes them less accurate in diagnosis of IBD. Furthermore, pANCA is detected with indirect immunofluorescence, which is associated with high interassay and interobserver variability. Therefore, pANCA and ASCA are not routinely tested in every patient suspected of IBD [5].

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# Coeliac disease

Nils Olivier Olsson, Lucile Musset, Georges Chyderiotis, Bach-Nga Pham, Alain Lachaux, Hugues Rousset, Nicole Fabien

## 1 Introduction

Coeliac disease is an autoimmune enteropathy related to gluten intolerance and linked to a strong genetic susceptibility background: DQA1*05-DQB1*0201 (HLA-DQ2) or DQA1*301-DQB1*302 (HLA-DQ8). This disease is characterised by inflammation of the intestinal mucosa causing total or subtotal villous atrophy.

Coeliac disease is usually considered to be rare but its prevalence needs to be re-evaluated in the light of recently developed screening tests. In reality, the majority of patients are either asymptomatic or with few or atypical symptoms. Coeliac disease mostly affects the populations of Northern Europe, the Maghreb countries, Australasia and the United States. It is very rare in Asia and sub-Saharan Africa. Epidemiological studies have shown that 0.5 to 1% of individuals suffer from coeliac disease in Western European and North American populations.

The sex ratio of coeliac disease in children is 1/1. In adults, coeliac disease is 2 to 3 times more frequent in women than in men.

The disease is diagnosed at any age with two frequency peaks. Classically, the onset is in childhood, between the ages of six months and two years, and after gluten has been introduced into the diet (baby cereals containing gluten, pasta, bread …), or in adulthood, mainly between the ages of 20 and 40 years. However, late-onset forms, after the age of 65, are not exceptional. The first clinical signs appear before the age of one year in 73% of cases. The diagnosis is established before the age of two years in 58 to 77% of cases.

## 2 Diagnostic measurements for specialised physicians

The Federation of International Societies of Paediatric Gastroenterology, Hepatology and Nutrition (FISPGHAN) proposed guidelines for the diagnosis and treatment of coeliac disease which have been re-evaluated in 2011 by the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) [1, 2].

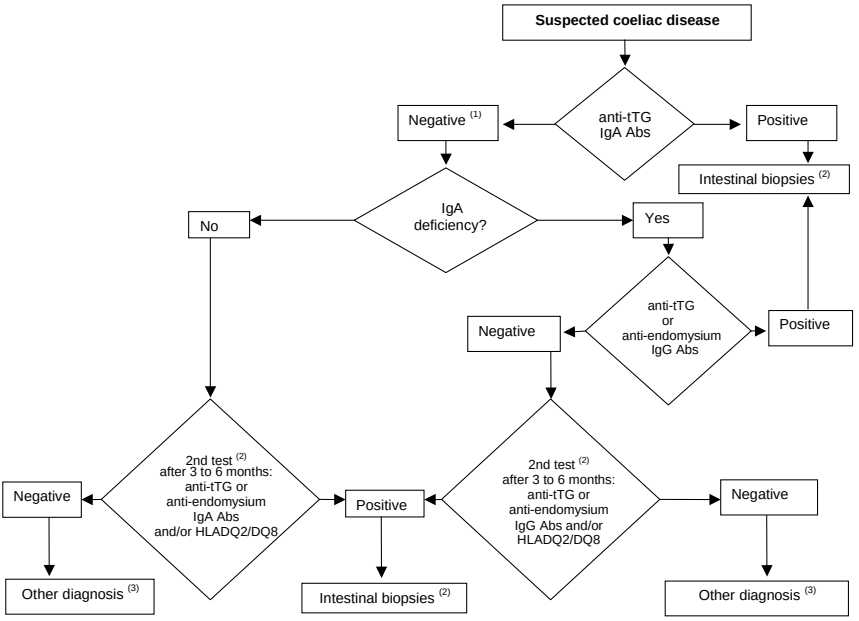
When coeliac disease is suspected in adults and children, serum should be tested for anti-tissue transglutaminase (tTG) IgA antibodies by a technique which uses human recombinant tissue transglutaminase as the antigen. Antibody and histological (biopsy) testing should only be carried out when the patient is on a gluten inclusive diet or the results may be falsely negative. When an anti-tTG IgA antibody test is negative or close to the threshold of positivity, testing for anti-endomysium IgA antibodies is recommended. After a first negative test for anti-tTG IgA antibodies in children suspected of coeliac disease who are not following a gluten-free diet and who do not have IgA deficiency, a second screening for anti-tTG IgA antibodies or anti-endomysium IgA antibodies is recommended within 3 to 6 months. In subjects with IgA deficiency, screening for anti-tTG IgG antibodies or anti-endomysium IgG antibodies is recommended in the same way as for IgA.

The formal diagnosis of coeliac disease is established when total or subtotal villous atrophy accompanied by cryptic hyperplasia and lymphocytic infiltration of the surface epithelium is demonstrated on small intestinal biopsies. Because these histological lesions may be patchy, it is recommended that multiple biopsy specimens be obtained. Due to the high sensitivity and specificity of anti-tTG and anti-endomysium tests, it is no longer necessary to perform control biopsies after initiation of a gluten-free diet. These second biopsies are reserved for patients who have an unsatisfactory response to a strict gluten free diet.

A summary of the diagnostic criteria of celiac disease and an algorithm for the diagnosis are presented on Table 1 and Fig. 1 respectively.

**Table 1.** Diagnostic criteria of coeliac disease.

| |
|---|
| **Clinical signs** |
| Chronic diarrhoea |
| Abdominal pain |
| Malabsorption syndrome |
| Complete resolution after treatment with a strict gluten-free diet |
| **Histological criteria (small intestinal biopsy)** |
| Villous atrophy |
| Cryptic hyperplasia |
| Intraepithelial lymphocytosis |
| **Serological criteria** |
| IgA anti-tissue transglutaminase or IgA anti-endomysium antibodies (IgG anti-tissue transglutaminase or IgG anti-endomysium antibodies in patients with IgA deficiency) |




(1) Interpretation depending on the gluten-free diet compliance;
(2) Medical decision according to clinical context;
(3) Small intestinal biopsy may however be requested in certain circumstances, in the adult and if there is a strong suspicion of coeliac disease.

**Abs**, antibodies; **tTG**, tissue transglutaminase

**Figure 1.** Algorithm for the diagnosis of coeliac disease.

## 3 Requirements for family practitioners

Symptoms may arise from the gastrointestinal involvement with malabsorption of nutrients and vitamins, or may be related to the immune dysfunction which is responsible for extraintestinal symptoms. The clinical manifestations differ from one patient to another (Table 2) [3–5].

Three clinical forms are found:

- asymptomatic, completely silent form, which is detected from serological or histological findings;
- presenting few symptoms, or sub-clinical form;
- symptomatic, the classic form of the disease.

The asymptomatic forms or those with few symptoms are more frequent than the symptomatic forms. The severity of the disease is not necessarily proportional to

**Table 2.** Main signs and symptoms of coeliac disease.

| | |
|---|---|
| **Infant** | Diarrhoea or constipation |
| | Anorexia |
| | Vomiting |
| | Extraintestinal manifestations (Table 3) |
| | Abdominal distension |
| **Child** | Chronic diarrhoea or constipation |
| | Abdominal pain |
| | Vomiting |
| | Extraintestinal manifestations (Table 3) |
| **Adult** | Chronic diarrhoea |
| | Steatorrhoea |
| | Abdominal pain |
| | Extraintestinal manifestations (Table 3) |

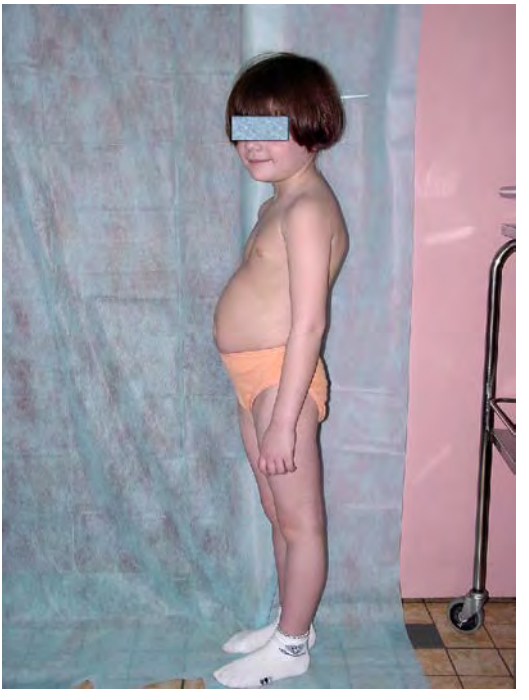


**Figure 2.** Abdominal distension in a child with coeliac disease.

the severity of the intestinal mucosal lesions, as a patient with total villous atrophy may be asymptomatic.

**In the infant,** the most classic presentation associates chronic diarrhoea with malabsorption and signs of malnutrition of varying severity and abdominal distension (Fig. 2). Anorexia is almost always present. Vomiting is frequent. When gluten has been introduced into the diet, any slowing of weight gain should suggest the diagnosis of coeliac disease.

**In the child,** the symptoms are misleading, as diarrhoea often plays a secondary role. Gastrointestinal problems such as abdominal pain, vomiting or constipation may be observed. Sometimes, only fatigability, growth retardation or delayed puberty, or extraintestinal manifestations may be observed (see following paragraph).

**In the adult,** coeliac disease is easily diagnosed when clinical gastrointestinal signs are present (chronic diarrhoea with steatorrhoea and abdominal pain), but diagnosis is much more difficult when the symptoms are minor or when they are related to extraintestinal manifestations.

Extraintestinal manifestations secondary to the malabsorption syndrome are presented in Table 3: anaemia, delayed growth or puberty in the child, bone and joint pain related to osteopenia and osteoporosis, neurological disorders such as peripheral neuropathy, muscular disorders such as muscle cramp or tetany, weight loss or even malnutrition, sometimes with oedema, fatigue, bleeding and haematomas.

Extraintestinal manifestations which are probably not secondary to the malabsorption syndrome, observed in the atypical forms of the disease, are also presented in Table 3: neurological disorders, dermatitis herpetiformis, liver dysfunctions, reproductive disorders, aphthosis, IgA nephropathy, myocarditis, haemorrhagic alveolitis, arthritis.

Lastly, coeliac disease may be associated with other non-intestinal diseases: organ-specific autoimmune diseases such as insulin-dependent diabetes (3.6 to 6.2 %), autoimmune thyroiditis (3 %), primary biliary cirrhosis (2 %), or systemic autoimmune diseases (systemic lupus, Sjögren's syndrome …). There is also an increased risk of coeliac disease in first-degree relatives of coeliac disease patients (5 to 10 %).

When the diagnosis of coeliac disease is suspected, serological testing should be performed. In case of positivity, the patient should be referred to a paediatric or adult gastroenterology unit for a small intestinal biopsy. Most recent guidelines indicate that these biopsies may be avoided in symptomatic children with high level of anti-tTG or anti-endomysium IgA antibodies especially if they are HLA-DQ2/DQ8 positive.

**Table 3.** Main extraintestinal manifestations of coeliac disease.

| **Secondary to the malabsorption syndrome** | |
|---|---|
| Iron, folates, vitamin B12 deficiency | Anaemia |
| Vitamin D and calcium deficiency | Delayed growth or delayed puberty |
| | Bone and joint pain related to osteopenia and osteoporosis |
| Vitamin B12 and B1 deficiency | Neurological disorders such as peripheral neuropathy |
| Magnesium and calcium deficiency | Muscular disorders such as muscular cramp or tetany |
| Malabsorption of the majority of nutrients | Weight loss |
| Hypokalaemia and electrolyte depletion | Fatigability |
| Vitamin K deficiency | Bleeding and haematomas |
| **Probably not secondary to malabsorption** | Neurological disorders: depression, epilepsy, migraine… |
| | Dermatitis herpetiformis |
| | Liver dysfunctions: elevated transaminases… |
| | Reproductive disorders: infertility, amenorrhoea, recurrent miscarriage… |
| | Aphtosis |
| | IgA nephropathy |
| | Myocarditis |
| | Haemorrhagic alveolitis |
| | Arthritis |

## 4 Follow up

*Clinical observations*

The response to gluten withdrawal from the diet is generally rapid: gastrointestinal symptoms improve within 2 to 3 weeks. Gluten-free diet allows restoration of the intestinal villi in patients with coeliac disease and healing of skin lesions in patients with dermatitis herpetiformis. Children with coeliac disease should be monitored for assessment of symptoms, growth, physical examination and adherence to a gluten-free diet. Lack of improvement of symptoms within a few (six to eight) weeks after initiation of a gluten-free diet should prompt the physician to look for involuntary or deliberate gluten ingestion.

*Expectations*

Early detection of coeliac disease and subsequent initiation of a gluten-free diet reduce the risk of developing some important complications such as osteoporosis, vitamin deficiency, spontaneous abortions, low birth weight infants, intestinal lymphoma and cancer.

*Blood tests*

Total adherence to a gluten-free diet usually induces a steady decrease of tissue transglutaminase and endomysium antibodies within 6 to 18 months, and they eventually disappear. So, testing for anti-tTG IgA or anti-endomysium IgA antibodies (anti-tTG IgG or anti-endomysium IgG antibodies in patients with coeliac disease and IgA deficiency) is recommended after 6 and 12 months of a gluten-free diet in patients whose first test was positive. Persistently high antibody levels are suggestive of poor compliance to the gluten-free diet. A decrease or disappearance of antibodies should encourage the patient to continue to adhere to the gluten-free diet.

## 5 Management

At the time of diagnosis, several biological parameters should be screened to detect possible deficiencies: full blood count, electrolytes, serum iron, vitamin B12, phosphocalcic profile, protein electrophoresis, magnesium, liver function tests and prothrombin rate. Diet supplementation with iron, folates, vitamin D and calcium is sometimes necessary at the beginning of treatment to correct a deficiency.

Coeliac disease is not curable but can be treated effectively by lifelong adherence to a gluten-free diet. This is based on the withdrawal of wheat, rye and barley. Corn, rice and potato are allowed. Complete compliance to the diet is difficult as, in addition to products containing flour, gluten is also used to bind foods and as an additive in industrial meals and in certain drugs.

## 6 Diagnostic tests

Initial testing for coeliac disease should be performed by measurement of anti-tTG IgA or anti-endomysium IgA antibodies [1, 2].

Anti-tTG IgA antibodies tests have very good diagnostic performances both in children and in adults: sensitivity is more than 90 % and specificity is more than 95 %. Anti-tTG antibodies are mostly detected by enzyme linked immunosorbent assay (Elisa). These quantitative tests are automated, and now widely available. The nature of the antigen used is important. Tests using human recombinant antigen have shown the best performance.

Sensitivity and specificity of anti-endomysium IgA antibodies detection are similar to anti-tTG IgA antibodies detection. This reference test, despite the inherent limitations of the indirect immunofluorescence method (time consuming, non automated test, observer dependent results) is recommended the first time anti-tTG antibodies are found to be positive. Indeed, some false positive anti-tTG antibodies have been described, especially in adults [2].

When an anti-tTG IgA antibody test is negative or close to the threshold of positivity, testing for anti-endomysium IgA antibodies is also recommended. The overall performances of both tests are equivalent, but their results are not entirely identical.

Measurement of anti-tTG IgG or anti-endomysium IgG antibodies should be limited to patients with IgA deficiency. Serum IgA levels, when unknown, should be measured at the same time as screening of anti-tTG IgA antibodies to exclude IgA deficiency [2].

During follow-up, all tests should be carried out in the same laboratory since variations of antibody levels cannot be interpreted when different reagents are used.

Tests for anti-reticulin and anti-native gliadin antibodies have low sensitivity and specificity respectively and should no longer be used in routine practice. Although a new generation of serological tests using antigen derived from gliadin or synthetic deamidated gliadin peptides are now available, the benefit of these new tests in patients without anti-tTG IgA or anti-endomysium IgA antibodies needs to be evaluated on large populations. Finally, adding HLA-DQ2/DQ8 typing into the diagnosis of coeliac disease may be useful to avoid biopsies in some cases.

## Acknowledgment

We would like to thank Nina Crowte and Catherine Butler for helpful comments.

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# Autoimmune gastritis

Marilda Santos, Helena Silva, João Pedro Ramos, Carlos Dias

## 1 Introduction

Pernicious anemia was first described by Thomas Addison in 1849 and was related to gastric disease by Austin Flint in 1860. Autoimmune gastritis, as a name, appeared later, when the identification of antibodies to anti-parietal cells and to intrinsic factor made the immunological pathogenesis clear.

Autoimmune gastritis which progresses with the loss of zymogenic and parietal cells of the gastric mucosa, mainly affects the gastric fundus and body, sparing the antrum. It is often manifested by the presence of pernicious anaemia, associated with cobalamin deficiency. While frequently remaining silent for 20 to 30 years, it can be detected early and before the development of anaemia, by the presence of anti-parietal cell and/or anti-intrinsic factor antibodies.

In spite of significant controversy several published studies favour a relationship with *Helicobacter pylori* infection, since antibiotic treatments induce an improvement in anaemia and cobalamin levels in nearly half the patients.

## 2 Clinical manifestations

Autoimmune gastritis can remain asymptomatic for many years or present solely by some sort of dyspeptic symptoms. The average age at diagnosis is 60 yo, and a higher prevalence in females is expected. Since pernicious anaemia is the main manifestation of this disease, signs and symptoms overlap significantly in both diseases (Table 1).

Neurological complications that also arise as a result of cobalamin deficiency are more common in advanced stages of the disease and range from peripheral neuropathy to spinal cord and cerebellum injuries, which progress to demyelination and axonal degeneration, and neuronal death.

Intestinal metaplasia in gastric mucosa is a risk factor for developing adenocarcinoma and in these patients it can reach an incidence of approximately 3 times that in the general population.

**Table 1.** Signs and symptoms of pernicious anemia at presentation (adapted from ref. 1).

| **Clinical haematologic manifestations** |
|---|
| Anaemia with pallor and fatigue. |
| **Constitutional symptoms** |
| Loss of appetite<br>Atrophic glossitis (sore, smooth, and red tongue) |
| **Clinical gastrointestinal manifestations** |
| Diarrhoea (cobalaminmalabsortion and intestinal changes) |
| **Clinical neurologic manifestations** |
| Peripheral numbness<br>Muscle wasting<br>Diminishing tendon reflexes<br>Loss of perception to light touch and vibration<br>Spastic ataxia |

Haematologic manifestations: the inadequate production of intrinsic factor leads to malabsorption and hence to a cobalamin megaloblastic anaemia (Table 2).

Biochemical manifestations: typically gastric hypochlorhydria appears as a consequence of parietal cell loss and diminished concentration of pepsinogen. Hypergastrinaemia, due to over-stimulation of gastrin-producing cells because of the low amount of acid produced, is also frequently detected.

Gastric biopsy: histologically the gastric mucosa is characterized by a submucosal mononuclear cell infiltrate, together with parietal and zymogenic cell degeneration.

**Table 2.** Haematologic manifestations.

| Megaloblastic anaemia |
|---|
| Macrocytosis |
| Neutrophil hypersegmentation |
| Leukopenia |
| Thrombocytopenia and púrpura |
| Megaloblastic bone marrow transformation |

## 3 Diagnostic criteria

No definitive diagnostic criteria are internationally accepted for autoimmune gastritis. However, asymptomatic non-anaemic patients, parietal-cell and/or anti-intrinsic factor antibodies can be considered a sign of impending disease. In symptomatic patients the criteria of pernicious anaemia (macrocytic anaemia (MCV > 100 fL), cobalamin deficiency, confirmed cobalamin malabsorption with a positive Schilling test) should be complemented by the detection of anti-parietal cell or anti-intrinsic factor antibodies and the detection of hypergastrinaemia and/or low serum pepsinogen I.

Biopsies typically reveal a pattern of atrophic gastritis together with various stages of a lymphocytic-mononuclear infiltrate. However biopsies can be difficult to evaluate and are of limited use in the diagnosis of autoimmune component involvement.

## 4 Diagnostic tests

Autoimmune gastritis is characterized by the presence of circulating autoantibodies against parietal cells and/or intrinsic factor. Anti-parietal cells are present in virtually 100 % of patients with autoimmune gastritis and anti-intrinsic factor in 60 to 70 % of those.

Cobalamin assays are widely available and at least one determination should be performed. The Schilling test (even if outdated) confirms cobalamin deficiency by intestinal malabsorption as caused by lack of intrinsic factor. There is also an increased cobalamin urinary fraction excretion, when the vitamin is orally administered with intrinsic factor.

Gastrin and pepsinogen determinations can be used to check for hypergastrinaemia and low serum pepsinogen I.

The clinical significance of all these results is uncertain in the absence of anaemia or macrocytosis. In those circumstances, a re-evaluation can be suggested, at 6 month to 1 year interval, with a full blood count, serum gastrin and serum cobalamin.

## 5 Diagnostic measurements for experts

Autoimmune gastritis can be found associated with other endocrine pathologies, such as type 1 diabetes and Hashimoto's disease. Seldom, Addison's disease, primary ovarian failure and hyperparathyroidism can also be found.

20–30 % of relatives can have detectable antibodies to parietal cells or intrinsic factor and a concordance has been found between monozygotic twins. Several HLA susceptibility markers have been suggested but no clinical usefulness has been established so far for those determinations.

## 6 Requirements for family practitioners

Autoimmune gastritis should be considered in older patients whenever macrocytosis is detected, with or without anaemia. Approximately 2 % of the population over 60 is considered to have undiagnosed pernicious anaemia.

Dyspeptic history should be carefully evaluated but can be expected to be either trivial or very variable and so should be considered of limited usefulness. The association with gastric cancer and concomitant autoimmune diseases should be remembered.

Autoimmune markers can easily be performed, but should only be of clinical implication in the presence of haematologic abnormalities.

## 7 Management/treatment

Steroids and other immunomodulatory drugs have been used with some success in decreasing parietal cell and intrinsic factor antibodies, thus increasing the available intrinsic factor and cobalamin absorption and reversing gastric mucosal atrophy with parietal and zymogenic cell regeneration. However, no clear protocols are established and the preferred treatment is cobalamin replacement. Monthly intramuscular 1000 μg of cobalamin is the standard maintenance treatment, whereas oral surcharge can also be considered but has a more unpredictable outcome. 1000 to 2000 μg daily dose can be considered in this situation (Table 3).

**Table 3.** Replacement therapy for pernicious anaemia.

| **Reposition of body stores** |
|---|
| - 6 intramuscular 1000 μg injections of cobalamin at 3 to 7 days interval (or)<br>- Daily oral doses (1000 to 2000 μg) cobalamin |
| **Maintenance treatment** |
| - 1000 μg intramuscular cobalamin every 3 months (or)<br>- 1000 μg intramuscular cobalamin monthly (poorer retemption) (or)<br>- 1000 to 2000 μg oral doses |

## 8 Follow up

Anaemia correction and cobalamin serum levels normalization are the best evidence of treatment efficacy. In those cases where oral treatment has been chosen, a re-evaluation should be considered 1 to 2 months afterthe first approach, with a full blood count and fasting serum cobalamin testing. With parenteral treatment, laboratory testing should be considered after a 6 to 12 months interval.

No significant value has been established for the reappraisal of autoimmune markers in clinically diagnosed patients.

In those patients where autoimmune markers have been found in the absence of anaemia or macrocytosis, a re-evaluation should be suggested, at a 6–12 month interval, of a full blood count, serum gastrin and serum cobalamin levels.

Since autoimmune gastritis has been associated with an increased risk of gastric carcinoma and carcinoid tumour, a periodic endoscopic evaluation should be considered.

## 9 Prognosis

Prognosis has not been established for non-anaemic patients with autoimmune markers. In symptomatic patients, replacement therapy when started before the onset of neurologic complications carries a good prognosis, when patient compliance is achieved.

Gastric neoplastic complications should not be forgotten but are beyond the scope of this review.

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# Part 5

# Autoimmune diseases of the nervous system

# Multiple sclerosis

Stephanie Knippenberg, Jan Damoiseaux, Raymond Hupperts

## 1 Introduction

Multiple Sclerosis (MS) is a chronic, invalidating disease of the central nervous system (CNS), characterized by focal inflammation, demyelination, and loss of neurons within the CNS. The inflammation causes areas of scars within the CNS, giving the disease its name: multiple areas of hard scars (sclerosis). The inflammation is believed to be of autoimmune origin. In MS, white blood cells are able to cross the blood brain barrier. Inflammation, demyelination and loss of neurons is caused by the release of soluble factors by infiltrating leukocytes and resident microglial cells [1].

Clinically, the inflammation within the CNS causes a variety of neurological complaints depending on the location of the inflammation. Muscle weakness and sensory loss are the most common first symptoms of MS, especially in the limbs. Other common first symptoms are optic neuritis (ON) and double vision (Table 1) [2]. Most patients start with a relapsing remitting form of MS (RRMS) that generally becomes progressive overtime, while patients become more severely disabled. In this progressive phase, the disease is referred to as secondary progressive (SP) MS. A small proportion of patients experiences progressive disability from disease

**Table 1.** Common first symptoms of MS.

| Presenting symptom | Percentage of patients |
|---|---|
| Motor weakness of the limbs | 43–46 % |
| Sensory problems | 41–49 % |
| Optic neuritis | 22.5–36 % |
| Double vision | 13–19 % |
| Ataxia | 8 % |
| Bladder dysfunction | 1.25 % |
| Cranial nerve dysfunctions | 1.25 % |

onset onwards without relapses and remissions, and in these patients the disease is called primary progressive (PP) MS [1].

Globally the estimated median incidence of MS is 2.5 per 100 000 (minimum-maximum range is 1.1–4.0) and the median prevalence of MS is 30 per 100 000 (minimum-maximum range of 5–80). MS is more prevalent in countries further from the equator. Women are affected twice as frequently as men and this ratio may be increasing. The age at disease onset is typically between 20 and 50 years of age, although MS can occasionally have its onset during childhood or in the elderly [3].

## 2 Diagnostic measurements for experts

MS can be hard to diagnose since it can have a heterogeneous first presentation (Table 1). The diagnosis is made clinically, based upon the appearance of MS lesions in different parts of the CNS that have occurred at different points in time. To facilitate and standardize the diagnostic process, diagnostic criteria were defined. Historically, the Schumacher and Poser criteria were both popular. Nowadays, the McDonald criteria are most recommended. The McDonald diagnostic criteria were inaugurated in 2001, making a diagnosis possible after a first clinical attack. These criteria were revised in 2005 and are updated again in 2010. The McDonald criteria focus on the demonstration of the dissemination of MS lesions in time and space by predominantly clinical and radiologic data (Table 2).

**Table 2.** Diagnostic criteria according to McDonald criteria 2010.

| Clinical presentation (neurological attacks) | Objective clinical evidence (MRI lesions) | Additional data needed to confirm MS diagnosis |
|---|---|---|
| Two or more | Two or more<br>**or**<br>Objective clinical evidence of 1 lesion with historical evidence of another prior attack | None |
| Two or more | One | Dissemination in space<br>– verified by MRI [a]<br>**or**<br>– verified by second clinical neurological attack implicating a different CNS site |
| One | Two or more | Dissemination in time<br>– verified by MRI [a]<br>**or**<br>– verified by second clinical neurological attack |

| Clinical presentation (neurological attacks) | Objective clinical evidence (MRI lesions) | Additional data needed to confirm MS diagnosis |
|---|---|---|
| One | One | Dissemination in space:<br>– verified by MRI [a]<br>**or**<br>– verified by second clinical neurological attack implicating a different CNS site<br>**and**<br>dissemination in time:<br>– verified by MRI [a]<br>**or**<br>– verified by second clinical neurological attack |
| Neurological progression suggestive of MS without attacks (PPMS) | | One year of neurological disease progression (either retrospectively or prospectively determined) and two of the following points:<br>– Evidence for dissemination in space in the brain based on ≥ 1 MRI brain lesions [a]<br>– Evidence for dissemination in space in the spinal cord based on ≥ 2 MRI spinal cord lesions [a]<br>– Positive CSF [b] |

[a] Verification by MRI must fulfil specific MRI criteria

[b] CSF is determined positive if oligoclonal bands are found in CSF

Abbreviations: CSF, cerebrospinal fluid; CNS, central nervous system; MS, multiple sclerosis; MRI, magnetic resonance imaging; PPMS, primary progressive multiple sclerosis.

Clinically, two distinct episodes of neurological impairment, for which different inflammatory or demyelinated lesions within the CNS are presumed, can be sufficient for the diagnosis of MS, provided that the neurological impairment has been objectively observed for at least 24 hours [4]. Since many people seek medical attention after one episode, additional testing is often necessary. The most commonly used additional diagnostics are magnetic resonance imaging (MRI) and analysis of the cerebrospinal fluid (CSF). MRI of the brain and spine may show areas of inflammation or demyelination (Fig. 1). Gadolinium can be administered intravenously as a contrast to highlight active inflammatory lesions and demonstrate the existence of older lesions not associated with symptoms at the moment

of the evaluation. The sensitivity of MRI criteria for MS is between 35 % and 100 %, and specificity is between 36 % and 92 %. CSF obtained by lumbar puncture can provide information about inflammation of the CNS by testing it for oligoclonal bands of immunoglobulin G (IgG). This is preferentially tested by isoelectric focusing, which is considered to be the gold standard. Oligoclonal bands are found in 75–85 % of subjects with MS. Combination of MRI and CSF criteria for MS enhance sensitivity (56–100 %) and specificity (53–96 %) [4, 5]. Furthermore, the nervous system of a person with MS responds less actively to stimulation of the optic nerve and sensory nerves due to demyelination. These diminished responses can be examined using visual and sensory evoked potentials [4].

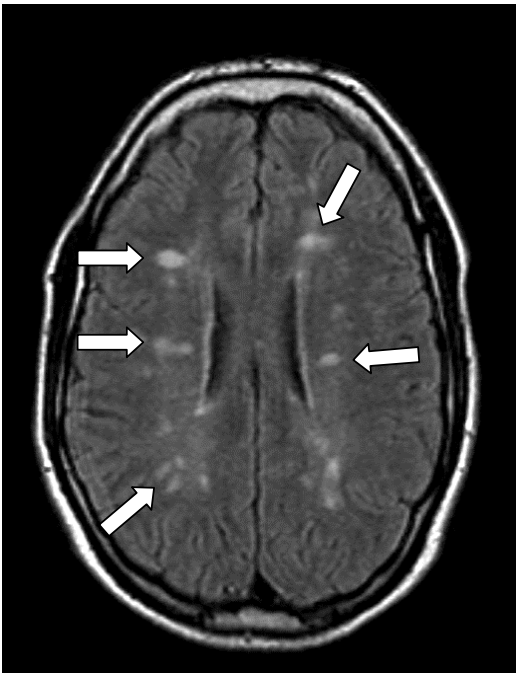

**Figure 1.** MRI showing multiple periventricular white matter lesions, consistent with multiple sclerosis.

## 3 Requirements for family practitioners

MS patients can suffer from a wide variety of neurological complaints, which are diverse in quantity and quality, and can arise from dysfunction of each component of the CNS. Although loss of motor function is the most well recognized symptom, symptoms can also include sensory impairment, visual impairment,

balance disorders, bowel and bladder dysfunction and sexual dysfunction. Cognitive impairment of varying degrees and emotional symptoms of depression or unstable moods are also common. Lifetime prevalence of fatigue is reported by 92 % of patients with MS. Patients suspected of having MS typically consult their general practitioner with muscle weakness, sensory symptoms or optic neuritis. Other neurological complaints may have occurred before the initial presentation. This should be carefully investigated as part of the medical history. When MS is suspected, the patient should be referred to a neurologist for further examination. This ensures that required treatments are started, and reduces anxiety and uncertainty. The presence of 2 clinical neurological attack and 2 or more MRI-detected lesions consistent with MS confirms the diagnosis, according to the McDonald criteria. If this is not the case, dissemination in space and time should be demonstrated by MRI or by a second clinical attack to confirm the diagnosis [4].

## 4 Follow up

*Clinical observations*

Patients with RRMS, experience episodic periods of worsening of neurological functions, called relapses. The relapse rate hardly ever exceeds 1.5 per year. The occurrence of relapses is unpredictable. However, viral infections and stress may increase the risk of a relapse. Other patients, SPMS or PPMS patients, experience a gradually progressive deterioration of neurological functions, or a combination of relapses and progressive deterioration. To rate the neurological deterioration in MS patients, the Kurtze expanded disability status scale (EDSS) is frequently used. The EDSS score is based upon neurological testing and examination of 7 areas of the CNS; pyramidal (motor), cerebellar (coordination), brainstem (speech and swallowing), sensory (touch and pain), bowel and bladder functions, visual, and mental functions (mood and fatigue).The EDSS is an ordinal scale in half-point increments ranging from 0 (normal neurologic examination) to 10 (death due to MS).

*Expectations*

MS is a chronic disease and there is no cure. The reduction in life expectancy is 5 to 10 years, with a median time to death about 30 years from onset. Prognosis depends on the subtype of the disease, gender, and initial symptoms. Relapsing remitting onset of the disease, optic neuritis as initial symptoms, and female gender are associated with a better prognosis [3].

*Blood tests to be done*

There are no blood tests available for monitoring disease activity.

## 5 Management

Since there is no cure for MS, current treatments attempt to prevent relapses and disability progression. Acute relapses are treated with high-dose intravenous corticosteroids, such as methylprednisolone. The registered maintenance therapies for MS are either immune modulating therapies or immune suppressive therapies. First-line maintenance therapies are beta-interferons and glatiramer acetate. Both therapies are administered by subcutaneous injections, varying from once a day to once a week. The first-line therapies are immune-modulating drugs; they skew the balance between a pro-inflammatory and anti-inflammatory immune response towards an anti-inflammatory immune response. Both beta-interferons and glatiramer acetate reduce the number of relapses by 30 %. Common side effects are irritation at the injection site and symptoms similar to influenza.

Non-responders to first-line drugs need more aggressive therapy to prevent increasing disability. Second-line therapies include Mitoxantrone and Natalizumab. Mitoxantrone is an immuno-suppressive drug developed to treat malignancies. It is used for the treatment of very active RRMS or SPMS and gives a significant reduction in relapses, an overall clinical improvement, and a reduction in active lesions on MRI. It is administered intravenously once per month. However, due to rare but serious side effects as cardiotoxicity, infertility, and acute myeloid leukaemia, it is not a physician's first choice. Natalizumab is the most recent drug in the group of disease modifying drugs available for the treatment of MS. It is a monoclonal antibody directed to an adhesion molecule expressed by white blood cells. Natalizumab is administered intravenously once per month. Its primary function is to inhibit migration of leukocytes towards the site of inflammation, i. e., the CNS. Natalizumab has shown a great efficacy, both in terms of relapse rate reduction and halting disability progression. However, long term effects are unknown and it is linked to the development of progressive multifocal leukoencephalopathy in a few patients. Currently, numerous new drugs are being tested for their efficacy in MS treatment [1].

## 6 Diagnostic tests

The presence of oligoclonal bands in CSF has long been considered an important supportive criterion for the diagnosis of MS. The term oligoclonal was coined because a restricted number of intrathecal B cells are triggered to produce immunoglobulins (Ig) of the IgG class. The IgG have a restricted heterogeneity with respect to their mobility in an electric field as is preferentially tested by isoelectric focusing. Isoelectric focusing is a technique which separates molecules based on their difference in isoelectric point. The charge of a protein is dependent on the sum of its ionisable acidic and basic amino acids. When protein is placed in a gel with a linear pH gradient and subjected to an electrical field, it will initially move

to the electrode with the opposite charge. During migration through a changing pH, the protein will either lose or pick up protons, thereby changing its net charge. Eventually, the protein will be uncharged, and will stop migrating. It has reached its isoelectric point. After isoelectric focusing, separated proteins are passively transferred to a cellulose nitrate membrane. Next, the membrane is incubated with sheep anti-human IgG antiserum as primary antibody, and anti-sheep IgG peroxidase conjugate as secondary antibody. The IgG bands are then visualized by the addition of 3-amino-9-ethylcarbazole. The IgG patterns seen in healthy individuals reveal a polyclonal smear which is similar in CSF and corresponding serum. In contrast, the patterns observed in patients with MS reveal discrete bands of IgG in CSF which is not reflected in the serum (Fig. 2).

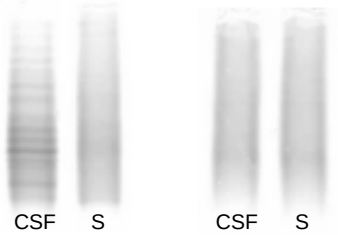


**Figure 2.** Patterns from isoelectric focusing of paired serum (S) and concentrated cerebrospinal fluid (CSF) adjusted to the same amount of IgG applied. A patient with multiple sclerosis (left) and a healthy control (right) are displayed.

## 7 Testing methods

The great benefit of the diagnostic tests and the McDonald criteria is the possibility of diagnosing MS at an early stage, which enables the MS patient to start treatment as soon as possible.

Limitations are the necessity of combining the different diagnostics, which puts extra burden on the patient. Lumbar puncture, in particular, is experienced as unpleasant and stressful by MS patients. In addition, tests run in the laboratory require expertise and are subjective. Currently there is an ongoing search for biomarkers which may facilitate the diagnosis of MS.

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# Guillain-Barré syndrome

Avraham Unterman, Joab Chapman, Yehuda Shoenfeld

## 1 Introduction

Guillain-Barré syndrome (GBS) is an autoimmune acute peripheral neuropathy, causing limb weakness that progresses over a period of days and up to 4 weeks. The syndrome was described in 1916 by three French neurologists: Guillain, Barré, and Strohl, and is considered to be the most common cause of acute generalized paralysis. The four most common subtypes of GBS are acute inflammatory demyelinating polyneuropathy (AIDP), acute motor axonal neuropathy (AMAN), acute motor sensory axonal neuropathy (AMSAN) and the Miller Fisher syndrome (MFS), which is clinically distinct from the other three and is characterised by a triad of ophthalmoplegia, ataxia and areflexia. The four types differ in their pathophysiology and immunological profiles, as well as in their worldwide incidence. In Western countries AIDP accounts for about 90 % of all GBS cases, and AMAN accounts for most of the remaining 10 % [1].

GBS occurs throughout the world, affecting children and adults of all ages, with a median incidence of 1.3 cases/100 000 population (range, 0.4–4.0). Men are affected approximately 1.5 times more than women [1]. About two-thirds of GBS patients have had an infection within a 6-week period prior to the diagnosis, generally either a flu-like episode or gastroenteritis. The most frequent identifiable antecedent infectious organisms are Campylobacter jejuni (23–32 %), Cytomegalovirus (8–18 %), Epstein-Barr virus (2–7 %) and Mycoplasma pneumoniae (9 %) [2].

## 2 Pathogenesis

There is considerable evidence supporting an autoimmune mediated mechanism in GBS [3], though the pathophysiology is different in the various subtypes.

In AIDP the neuropathy is mainly demyelinating: macrophages invade the myelin sheaths and denude axons [1]. Axonal damage can occur secondarily when the inflammation is severe. The exact role of T-cell-mediated immunity in AIDP

remains unclear and there is also some evidence for the involvement of antibodies and complement [2].

In the axonal subtypes, AMAN and AMSAN, the main pathology is axonal injury rather than demyelinative one, and the pathophysiology is better understood. Strong evidence now exists that these axonal subtypes are caused by autoantibodies to gangliosides on the axolemma. An interesting observation is that the lipo-oligosaccharide from the Campylobacter jejuni bacterial wall contains ganglioside-like structures, thus promoting an immune response in some patients by the mechanism of molecular mimicry [1, 2]. There is also evidence indicating a small increase in the risk of GBS following vaccination, especially with the influenza vaccine.

**Table 1.** Asbury and Cornblath's clinical diagnostic criteria for Guillain-Barré syndrome [4] (Modified).

| **I. Features Required for Diagnosis** |
|---|
| Progressive motor weakness of two or more limbs[1)] |
| Areflexia |
| **II. Clinical Features Strongly Supportive of the Diagnosis (in order of importance)** |
| Progression of symptoms over days, up to 4 weeks |
| Relative symmetry |
| Mild sensory symptoms or signs |
| Cranial nerve involvement |
| Recovery (usually begins 2–4 weeks after progression ceases) |
| Autonomic dysfunction |
| Absence of fever at the onset of symptoms |
| **III. Features Casting Doubt on the Diagnosis** |
| Marked, persistent asymmetry of weakness |
| Persistent bladder or bowel dysfunction |
| Bladder or bowel dysfunction at onset |
| Sharp sensory level |
| **IV. Features That Rule Out the Diagnosis** |
| Volatile solvents abuse |
| Acute intermittent porphyria |
| Recent diphtheria infection |
| Lead intoxication |
| Purely sensory syndrome, without motor weakness |
| A definite diagnosis of a condition such as poliomyelitis, botulism, or toxic neuropathy (e.g organophosphates) |

[1)] Excluding Miller Fisher and other variant syndromes.

## 3 Diagnostic criteria

Guillain-Barré syndrome is a clinical diagnosis, supported by laboratory tests and requires exclusion of other mimics. Asbury and Cornblath's clinical criteria for the diagnosis of the Guillain-Barré syndrome [4] are widely accepted. A modified and simplified version of these criteria is listed in Table 1. Laboratory features supporting the diagnosis of GBS are listed in Table 2.

**Table 2.** Laboratory features supporting the diagnosis of Guillain-Barré syndrome.

| **I. Typical cerebrospinal fluid (CSF)** |
|---|
| Normal pressure |
| High concentration of protein |
| $< 50$ mononuclear leuckocytes/mm$^3$ (typically $< 10$/mm$^3$) |
| No polymorphonuclear leukocytes in CSF |
| **II. Typical electrophysiologic diagnostic features** |

## 4 Diagnostic measurements for experts

The most important laboratory aids to the clinical diagnosis, are the electrophysiological studies and the CSF examination. The CSF is typically under normal pressure, contains an increased protein content, and is acellular or contains only a few lymphocytes (usually less than 10, rarely more than 50 mononuclear leukocytes/mm$^3$) [1]. The protein content in the CSF may not be raised until 10 days after the onset of the disease and lumbar puncture may need to be repeated if the diagnosis remains doubtful.

Electrophysiological studies of both motor and sensory peripheral nerves play an important role in supporting the diagnosis, and help differentiate between the main subtypes of GBS — i. e. between the demyelinating form (AIDP) and the axonal forms (AMAN and ASMAN). However, electrophysiological studies are frequently normal or non-diagnostic at the onset of the disease and may need to be repeated after 1–2 weeks.

## 5 Serologic diagnostic tests

Several anti-ganglioside antibodies are associated both with AMAN (GM1, GM1b, GD1a and GalNac-GD1a in 64 %, 66 %, 45 % and 33 % of patients respectively) and with AMSAN (GM1, GM1b, GD1a) but not with AIDP [1, 2].

The Miller Fisher syndrome is associated with anti-GQ1b, a specific and sensitive anti-ganglioside antibody, present in more than 90 % of patients with MFS and absent in other forms of inflammatory neuropathy [2]. Anti-GQ1b have been

shown to damage the motor nerve terminal in vitro by a complement-mediated mechanism [2].

Anti-ganglioside antibodies may be tested in GBS, but their absence does not exclude the diagnosis. Of special diagnostic value are anti-GQ1b antibodies, which are sensitive and specific to MFS [1].

## 6 Requirements for family practitioners

Since GBS is a rapidly evolving and potentially life-threatening condition, family practitioners should be familiar with the symptoms and signs of GBS, and should immediately refer suspected patients to hospital. Paresthesias and slight numbness in the toes and fingers are the earliest symptoms of GBS. The major clinical manifestation is weakness that evolves more or less symmetrically, and reaches its nadir 2–4 weeks after onset of symptoms. The symptoms progress with an ascending pattern from the lower to the upper limbs in 56 % of patients, involve the four limbs simultaneously in 32 % of patients, and spread from the upper to the lower limbs in 12 % of patients [1]. The proximal as well as distal muscles of the limbs are involved. Involvement of the facial muscles is common, whereas the ocular motor muscles are usually spared, except with MFS. The weakness of the respiratory muscles may be severe enough to require assisted artificial ventilation in about 25 % of the patients. More than half the patients complain of pain and an aching discomfort in the muscles, mainly those of the hips, thighs and back. Autonomic involvement is common and may cause ileus, sinus tachycardia, hypertension, cardiac arrhythmia, and postural hypotension.

## 7 Follow up

*Clinical observations*

After a variable plateau phase, recovery begins with return of proximal, followed by distal strength over weeks or months.

*Expectations*

Most patients with GBS recover functionally within 6 to 12 months. Between 4 % and 15 % of patients die, and up to 20 % are left with a disabling motor deficit after a year, despite modern treatment [1, 2]. Poor prognostic factors include older age; severe, rapidly progressive disease; and electrophysiological features that suggest axonal involvement in AIDP [1]. Relapse may occur in a small percentage of patients.

*Follow up studies*

During recovery, improvement in clinical parameters such as muscle strength and ability to walk should be assessed. Commonly patient are treated and followed up in a rehabilitation facility for many months. Reports from these facilities help neurologists in the assessment of recovery and can be useful as reference points if a relapse is suspected. Electrophysiological studies may be used for follow up, especially if recovery is impaired or relapse is suspected. Blood tests are not routinely indicated.

## 8 Management

Treatment of GBS consists of both supportive care and specific therapy. All patients with GBS should be admitted to a hospital for close observation, in order to identify progression to respiratory failure necessitating endotracheal intubation and mechanical ventilation, as well as cranial nerve dysfunction, and autonomic instability. Prophylaxis for deep vein thrombosis should be provided because of prolonged immobilization. Intravenous immunoglobulins (IVIg) and plasma exchange (PEx) have been shown in randomised controlled trials to be similarly effective in accelerating the recovery, but do not significantly reduce mortality. IVIg has been found to be somewhat safer than PEx, having a lower frequency of multiple complications [5]. Thus, its efficacy, safety, and availability make IVIg the treatment of choice in many centers [1, 5]. A combination of PEx and IVIg does not seem to produce significant extra benefit. Corticosteroids are not effective in GBS. A recent Cochrane review [6] examined the evidence for the use of pharmacological agents other than steroids, IVIg and PEx, and found only very low quality studies that were unable to support their use.

Following discharge from the hospital, most patients are candidates for rehabilitation. A multidisciplinary rehabilitation program, with both occupational and physical therapy, is considered very important for recovery [2].

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# Myasthenia gravis

Jan Damoiseaux, Marc de Baets

## 1 Introduction

Myasthenia gravis (MG) is an autoimmune disease associated with antibodies directed to the postsynaptic nicotinic acetylcholine receptor (AChR) at the neuromuscular junction [1, 2]. These antibodies reduce the number of AChR, which leads to muscle weakness. Antibodies have been found to block receptor function or cause local damage to the muscle resulting in interference with neuromuscular transmission. The resultant muscle weakness usually starts with the eye muscles (Fig. 1), and results in ptosis and double vision. MG may also involve other limb, bulbar and respiratory muscles (Table 1).

The annual incidence of MG is 3–7/million and the prevalence is about 60–120/million. The prevalence has appeared to increase in recent decades; probably as a result of the increased sensitivity and frequency of testing in combination with a decrease in mortality rates. In general, women are affected twice as often as men. For patients presenting between the ages of 20–40, the female/male ratio reaches 3 : 1. In patients over the age of 40 years at the time of presentation, men and women are equally affected.

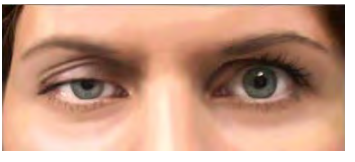

**Figure 1.** Patient with ocular myasthenia gravis. Ptosis due to weakness of the eye muscles is often the presenting clinical manifestation of myasthenia gravis. Typically, the ptosis may be asymmetric.

**Table 1.** Signs and symptoms of disease.

| Affected Muscle | Clinical Manifestation |
|---|---|
| Ocular | |
| Diplopia | External eye muscle paresis |
| Ptosis | Drooping of one or both eye lids |
| Ptosis and diplopia | |
| Bulbar | |
| Articulation | Nasality of speech |
| Face | Weakness, sensation of stiffness of the mouth, inability to whistle, myasthenic snarl |
| Chewing | Difficult chewing |
| Swallowing | Regurgitation of fluids through the nose, choking |
| Neck muscles | Inability to keep the head in balance |
| Combined | |
| Oculobulbar | |
| Limbs | Sudden loss of power during sustained exertion |
| Arms | |
| Hands and fingers | |
| Legs | Sudden falls |
| Combined | |
| Generalised | |
| Respiration | Respiratory difficulties |

## 2 Diagnostic measurements for experts

MG is a disease of progressive muscle weakness during exercise. This can be made obvious by testing muscle stamina, for instance by sustained up-gaze for about 1 minute, making the eyelids droop. Next, the diagnosis can be confirmed by detecting anti-AChR antibodies and, if negative, anti-muscle specific kinase (MuSK) antibodies. Details of the relevant autoantibody tests are described below. Although both autoantibodies are highly specific for MG, about 15 % of patients with generalized MG are seronegative. In these patients the diagnosis of MG can be confirmed either by measuring an increase in muscle strength after treatment with an ACh-esterase inhibitor (e. g. edrophonium or pyridostigmine), or by repetitive nerve stimulation. The most sensitive (95–99 %) and specific ($\sim$ 100 %) electrodiagnostic test for MG is single-fibre electromyography (EMG), measuring action potentials from a small number of muscle fibres innervated by a single motor unit [3]. Despite the excellent association with MG, single-fibre EMG is not often performed because it is dependent on operator skills. The American Association of

**Table 2.** Diagnostic Criteria.

| |
|---|
| **Clinical Criteria** |
| – Muscle weakness during exercise |
| – Positive pyridostigmine test |
| **Laboratory Criteria** |
| – Presence of autoantibodies to AChR[a] |
| – Presence of anti-MuSK antibodies (only in absence of anti-AChR antibodies) |
| – Abnormal EMG (progressive decrease in electrical discharge) |

Abbreviations: **AChR**, acetylcholine receptor; **EMG**, electromyography; **MuSK**, muscle specific kinase.

Neuromuscular & Electrodiagnostic Medicine has developed guidelines for electrodiagnostic testing for evaluation of MG [2, and references therein].

Finally, once MG is diagnosed, the possible presence of a thymoma should be evaluated by scanning of the chest. MG patients at risk for thymoma can be selected by the presence of autoantibodies to skeletal muscle (see below).

## 3 Requirements for family practitioners

MG is a neuromuscular transmission disorder. Typically, signs and symptoms fluctuate: aggravating upon exertion and improving after rest. However, clinical manifestations may also spontaneously vary in time.

The disease usually starts with ptosis and diplopia and stays confined to the ocular muscles in about 15 % of patients. In the majority of patients the disease generalises and affects ocular, bulbar, limb and, in the end- stage, respiratory muscles.

Patients typically consult their general practitioner with fatigue and, at that time, the ocular symptoms may be minimal because of rest during the preceding night. The diplopia is usually intermittent and thus must be specifically asked about.

When the diagnosis is suspected, the patient should be referred to a neurologist for further examination and laboratory testing. The presence of anti-AChR antibodies confirms the diagnosis. If the serum antibody tests for AChR or MUSK is negative further electrophysiological tests are necessary including repetitive nerve stimulation and, if negative, stimulated single fibre electromyography [3].

## 4 Follow up

*Clinical observations*

During symptomatic or immunosuppressive treatment, signs and symptoms gradually improve, over a period varying from weeks to months.

*Expectations*

MG is a chronic disease with variable prognosis. However, with current immunosuppressive therapy, most patients can achieve a partial or complete remission. Spontaneous remissions also occur.

*Blood tests*

During treatment clinical improvement can be assessed by the quantitative (Q)MG score and no laboratory testing is necessary. In patients who fail to improve during immunosuppressive treatment, anti-AChR antibody titre can be measured. If the titre fails to drop after 6–12 months a change in the immunosuppressive regimen is desirable.

## 5 Management

The treatment must be individualised according to the severity of disease, the patient's wishes and the presence of associated diseases. Altogether, two distinct treatment approaches can be considered [4]:

### 5.1 Cholinesterase inhibitors

Cholinesterase inhibitors (e. g. edrophonium or pyridostigmine), which increase the amount of acetylcholine in the synaptic cleft, are the initial treatment in all patients with MG. The dose used should be about 3 to 5 tablets of 60 mg a day. The effect is variable and lasts for about 4 hours. The cholinergic side effects (salivation, abdominal cramps and diarrhoea) can be treated with anti-muscarinic drugs.

### 5.2 Immunosuppressive treatment

If treatment with cholinesterase inhibitors alone is insufficient to control the signs and symptoms of the disease, immunosuppressive treatment is started. The cornerstone of this treatment is the combination of prednisone and azathioprine. Azathioprine has a steroids sparing effect. If this combined treatment is not effective, other immunosuppressive drugs are available, including cyclosporine and

mycophenylate. In severe forms of MG, plasmapheresis is performed in combination with immunosuppression. Finally, in patients under the age of 50, a thymectomy may be performed if anti-AChR antibodies are present. In thymoma cases a thymectomy is always performed irrespective of the age of the patient.

## 6 Diagnostic tests

Autoantibodies to AChR are detected by radioimmunoassays (RIA) as originally described by Lindstrom et al. [5]. In contrast to the classical RIA it is not the autoantigen itself that is radiolabelled, but the snake toxin α-Bungarotoxin (*Bungarus multicincus*). Since α-Bungarotoxin shares high affinity and high specificity for AChR there is no need for extensive purification of the autoantigen from muscle extracts. If autoantibodies are present in the serum, these antibodies will form small immune complexes with the α-Bungarotoxin/AChR complex. These immune complexes are next enlarged by the addition of anti-human IgG enabling precipitation of the immune complexes by centrifugation (Fig. 2). The amount of radiolabel in the precipitate is directly related to the amount of autoantibodies in the serum. Values below 0.25 nmol/L are considered negative. Anti-AChR antibodies are detected in ∼85 % of patients with generalised MG and ∼50 % of patients with ocular MG. Importantly, anti-AChR antibodies are highly specific for MG.

More recently another antibody associated with MG has been discovered. These antibodies are directed to the muscle specific kinase (MuSK), a protein also found at the neuromuscular junction. These antibodies can be detected by a classical RIA, since the autoantigen has been cloned and sequenced and the extracellular domain is readily available as purified recombinant protein. Anti-MuSK antibodies are only detected in patients with generalized MG that are negative for anti-AChR antibodies.

About 15 % of MG patients have a thymoma. These patients are always positive for anti-AChR antibodies, but 80–100 % also have antibodies to skeletal muscle antigens. However, ∼30 % of non-thymoma MG patients also have anti-skeletal antibodies. These antibodies are detected by indirect immunofluorescence. In this test serum is incubated on skeletal muscle slides (monkey) and antibody binding is visualized by a second incubation with fluorochrome-labelled anti-human IgG (Fig. 2). Bands of cross striations can be observed under a fluorescence microscope. The autoantigen recognized is thought to be titin, a protein in the I-band of the myocyte.

## 7 Testing methods

The benefits of the diagnostic laboratory tests, i. e. anti-AChR and -MuSK antibodies, are the excellent performance characteristics, in particular with respect to specificity (∼100 %).

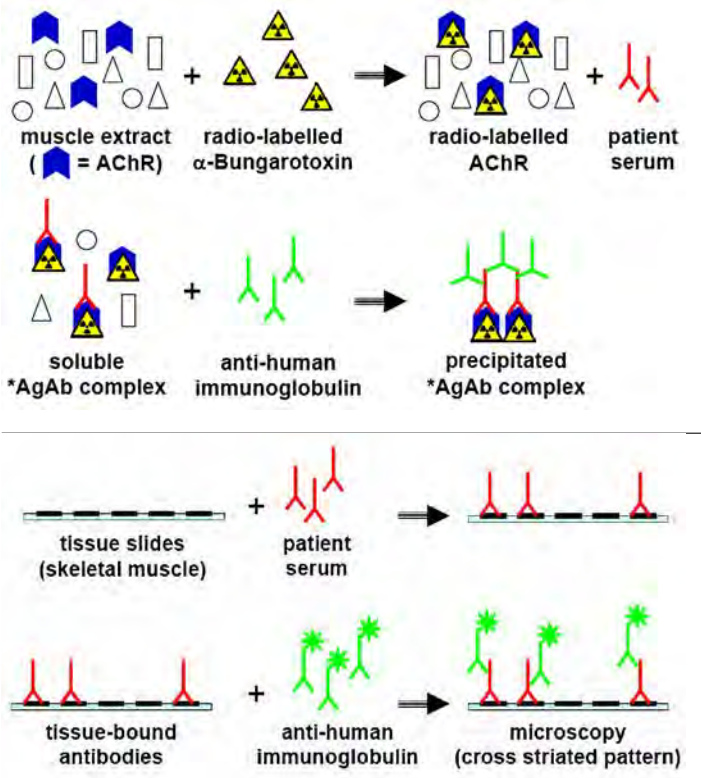


**Figure 2.** Test principles for autoantibody detection in myasthenia gravis. Anti-acetylcholine receptor (AChR) antibodies are classically detected by radioimmuno assay (upper panel). Radiolabelled α-Bungarotoxin (yellow triangles) specifically binds AChR (blue symbol) in muscle extract. Anti-AChR antibodies in the serum will bind the radiolabelled complex. The formed immune complexes are precipitated by addition of anti-human immunoglobulin and subsequent centrifugation. The amount of radiolabel in the precipitate corresponds to the amount of anti-AChR antibodies in the serum.

Anti-skeletal muscle antibodies are detected by indirect immunofluorescence (lower panel). Slides of monkey skeletal muscle are incubated with patient serum and visualized by FITC-labelled anti-human immunoglobulin. Fluorescent microscopy reveals a classical cross-striated staining pattern.

Limitations of the assays concern the need for radiolabels in combination with the low prevalence of disease. This indicates that the number of tests run in a laboratory is relatively low, while the half-life of the reagents is short. Furthermore, special laboratory equipment, facilities, and training of technicians are required. These issues significantly raise the cost per test, unless the tests are restricted to a

few reference laboratories. There is a continuous search for alternatives that solve these shortcomings.

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# Autoimmune encephalitis

Albert Saiz, Francesc Graus

## 1 Introduction

Until recently, autoimmune encephalitis was restricted to the syndrome described as paraneoplastic limbic encephalitis (LE), an infrequent paraneoplastic neurological syndrome (PNS) mainly associated with lung cancer [1]. Paraneoplastic LE used to run a severe clinical course that rarely improved despite removal of the tumour and intensive treatment with immunotherapy. However, in the last five years, the characterization of different antibodies against neuronal surface antigens has led to the identification of different types of LE and other encephalitic syndromes. These are important to recognize because they usually improve with immunotherapy and some of them are associated with tumours that can be diagnosed at an early stage when the chances of cure are highest. Taken together, these encephalitides are not as unusual as previously believed. In a retrospective analysis of encephalitis of unknown origin admitted to an intensive care unit, 1 % were finally identified as autoimmune.

## 2 Diagnostic measurements for experts

In a patient with suspected autoimmune encephalitis, the first step is to identify if the symptoms and imaging studies are compatible with LE [2]. LE presents with a diversity of symptoms including confusion, depression, agitation, anxiety, memory disturbance, and dementia. The typical picture is the subacute onset of confusion with markedly poor short-term memory. Seizures are not uncommon and may be the presenting symptom. Brain MRI shows bilateral high intensity lesions in the amygdala and hippocampus in FLAIR sequences that rarely enhance after gadolinium administration (Fig. 1). In any patient with the diagnosis of LE, detection of anti-neuronal antibodies is critical to support the possibility of a paraneoplastic origin and guide the work-up for the detection of the underlying tumour [3]. Onconeural antibodies Hu (ANNA-1), CV2 (CRMP5) and amphiphysin are associated with lung cancer, almost always small cell lung cancer (SCLC). Anti-Ma2 antibodies indicate the presence of an underlying testicular

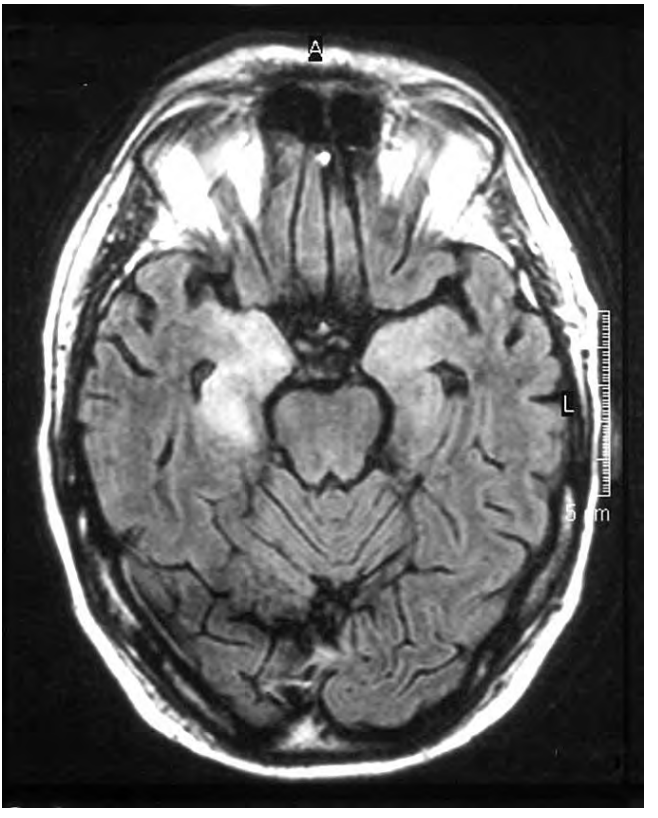


**Figure 1.** Brain MRI of a patient with LE, Hu antibodies and SCLC: Diffuse hyperintensities in FLAIR sequences along bilateral mesial temporal lobes.

**Table 1.** Onconeuronal antibodies and paraneoplastic LE.

| Antibody | Tumour | Antibody positive patients without cancer* | Frequency in cancer without LE* |
|---|---|---|---|
| Hu (ANNA1) | SCLC | 2 % | 16 % |
| CV2 (CRMP5) | SCLC, thymoma | 4 % | 9 % |
| Amphiphysin | SCLC | 5 % | 1 % |
| Ma2 | Testicular | 4 % | 0 % |

* For review see reference [2].

seminoma (Table 1). Antibodies against neuronal surface antigens are reported in patients with LE but they do not indicate if the LE is paraneoplastic. At least 50 % of LE patients with antibodies against AMPA or GABA receptors have SCLC. Anti-AMPAR are also seen in patients with LE and breast cancer. Lastly, in patients with antibodies against proteins of the potassium channel-complex (LGI1, and less frequently CASPR2) or with anti-GAD antibodies, the LE is almost never paraneoplastic (Table 2) [4]. It is important to emphasize that the presence of circulating anti-neuronal antibodies is not necessary for the diagnosis of LE but a work-up to rule out an underlying cancer is mandatory in every patient with LE even in the absence of anti-neuronal antibodies [2].

A severe, but treatment-responsive encephalitis has been associated with antibodies to NR1, a crucial subunit of the NMDA receptors [5]. Most patients are children or young women who do not develop the classical picture of LE. They are initially seen by or admitted to psychiatric wards for acute anxiety, behavioural change, or psychosis, followed in a few days by seizures, decline of consciousness, aphasia, and abnormal movements. Patients may develop hypoventilation and autonomic imbalance that requires admission to intensive care units. The encephalitis is paraneoplastic in some patients who have an ovarian teratoma. The frequency of ovarian teratomas is higher (56 %) in women older than 18 years than girls under the age of 14 years (9 %).

**Table 2.** Antibodies against neuronal surface antigens in LE.

| Antibody | Tumours ( %) | CSF pleocytosis ( %) /IT synthesis | Comments |
|---|---|---|---|
| LGI1* | none | 41/no | Male predominance; Associated rapid eye movement (REM) sleep behaviour disorder; frequent hypernatremia (> 70 %) |
| NMDA receptor | Ovarian teratoma (9–56) | 91/yes | Female predominance; MRI normal in 45 %, frequency of tumour higher in patients >18 years old. Relapses in 20 %. |
| AMPA receptor | SCLC, breast, thymoma (70) | 90/yes | Female predominance; frequent relapses (60 %) |
| $GABA_B$ receptor | SCLC (47) | 80/yes | Seizures in 86 %. Concurrent GAD antibodies in 3 patients |

* Fewer than 5 % present CASPR2 antibodies instead of LGI1 antibodies.

## 3 Requirements for family practitioners

Autoimmune encephalitides are unusual but many of them are potentially treatable. Therefore, a high degree of awareness is important in order to detect the patients at an early stage and to start treatment as soon as possible. The possibility of an anti-NMDA receptor encephalitis must be raised in children and young women who rapidly develop psychiatric symptoms that cannot be classified as typical psychosis, particularly if the patient develops associated seizures, dyskynesias, or language dysfunction characterized by dramatic drop in verbal output and dysarthria. A normal brain MRI does not rule out the diagnosis as it may be normal in up to 50 % of patients. However, CSF examination is almost always abnormal showing variable degrees of pleocytosis [5].

The possibility of an LE must be suspected in young men (who are at risk of LE associated with Ma2 antibodies and testicular cancer) and older patients of both sexes who rapidly develop an amnestic syndrome characterized by short-term memory loss and variable degrees of confusion, behaviour problems and seizures [2]. Seizures may be the predominant symptom in LE associated with GAD or GABAR antibodies. Idiopathic LE associated with antibodies against proteins of the potassium channel-complex (LGI1, and less frequently CASPR2) is more prevalent in men and usually presents as a classical picture of LE. Rapid eye movement sleep behaviour disorder develops at the onset of LE and is rarely reported unless the physician specifically addresses the issue. Hyponatraemia is a frequent finding whereas CSF analysis shows mild pleocytosis in only 41 % of the patients. Some patients may develop prominent myoclonus and the syndrome can sometimes be misdiagnosed as Creutzfeldt-Jakob disease.

## 4 Follow up

*Clinical observations*

Autoimmune encephalitides run a subacute clinical course and patients must be admitted in hospital for vital support, to perform a brain MRI, whole body CT or PET scan to look for an underlying tumour, lumbar puncture and analysis of anti-neuronal antibodies in serum and CSF, and to start immunotherapy.

*Blood tests*

In patients with LE and positive onconeural antibodies, there is no need to repeat the antibody studies because the antibody levels do not correlate with the clinical evolution. In patients with encephalitis associated with antibodies against surface antigens the level of the antibodies tends to decrease in association with the clinical recovery. However, low levels may persist for years. At present, there are no

guidelines for the clinical value of repeated evaluation of antibody levels in these encephalitides.

*Expectations*

Prognosis will depend of the type of encephalitis. Patients with LE, positive onconeural antibodies and cancer rarely improve but the LE stabilizes, usually with severe deficits, after several weeks despite appropriate immunotherapy and treatment of the tumor. Patients with encephalitis associated with antibodies against surface antigens, that are probably responsible for the syndrome, usually improve with immunotherapy. Clinical recovery is particularly significant in patients with idiopathic LE associated with anti-LGI1 antibodies and with anti-NMDA receptor encephalitis provided early treatment is started and the underlying ovarian teratoma, if present, removed [4, 5]. In patients with LE associated with anti-AMPAR antibodies or with anti-NMDA receptor encephalitis, relapses are not uncommon particularly in patients without cancer.

## 5 Management

Early detection and treatment of the underlying tumour is the approach that offers the greatest chance for neurological improvement or symptom stabilization. Therefore, a work-up for cancer must be done in every patient with suspected autoimmune encephalitis. In patients with LE and onconeural antibodies, where the chances of an underlying cancer are highest, imaging studies must include a whole body PET scan if other studies are negative. In patients with Ma2 antibodies elective orchyectomy and serial examination of the testicle to rule out in situ carcinomas is indicated in patients at high risk of testicular cancer such as those with calcifications or undescended testicle(s). In women with anti-NMDA receptor encephalitis, the ovarian teratomas are frequently small and asymptomatic. Although oophorectomy is not recommended if the tumour screening is negative, any small cystic and persistent lesion of the ovary must be viewed with a high index of suspicion and its removal is recommended.

There are no firm guidelines as to which kind of immune therapy should be used in these patients. However immunotherapy should be started early while the screening of the tumour is conducted and without waiting for the results of the antibody determinations. Many patients are initially treated with one or more of the following, intravenous immunoglobulin, corticosteroids or a combination of them. Patients with onconeural antibodies rarely improve with these therapies but some stabilize. Whether these patients require more aggressive immunotherapy is questionable and should depend on the functional status of the patient at the time. Up to 80 % of patients with encephalitis associated with antibodies against neuronal surface antigens respond to first line treatment. For non-responders, second-line

immunotherapy, with rituximab, cyclophosphamide or both, is recommended by experts. There is no data on the value of long-term immunotherapy to prevent relapses in anti-NMDA receptor encephalitis.

## 6 Diagnostic tests

Onconeural antibodies (Ma2, Hu (ANNA-1), CV2 (CRMP5) and amphiphysin) are detected by immunoblot of purified recombinant antigens. Several commercial assays are available. These antibodies may be seen in patients with cancer without PNS. Therefore, a positive result must be put in the context of the clinical picture. Conversely a negative result does not rule out the possibility of a paraneoplastic LE.

Anti-GAD antibodies are detected by RIA. Only high levels support that the neurological syndrome, in this case LE, is related to the antibody. Low levels of GAD antibodies are present in patients with type I diabetes mellitus and autoimmune polyendocrine syndromes without associated neurological syndromes.

Antibodies against neuronal surface antigens are detected by immunofluorescence on HEK293 cells transfected with the appropriate antigen. There is currently a commercial kit that allows the simultaneous measurement of antibodies against NMDAR, GABAR, AMPAR, LGI1, and CASPR2. In patients with anti-NMDA receptor encephalitis, antibodies may be present in the CSF when the serum is negative. Conversely, in patients with non-paraneoplastic LE LGI1 antibody levels are usually higher in the serum and they may be negative in the CSF.

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# Part 6

# Autoimmune diseases of heart and blood

# Autoimmunity in myocarditis and dilated cardiomyopathy

Udi Nussinovitch, Yehuda Shoenfeld

## 1 Introduction

Myocarditis and dilated cardiomyopathy (DCM) are considered by some investigators as the acute and chronic stages of the same disease. The reported prevalence of idiopathic DCM in the USA is 36 cases per 100 000 persons. The annual incidence of idiopathic DCM was reported to be 5–8 new cases per 100 000 persons. In most idiopathic DCM cases, clinical presentation first appears between the ages of 20–50. DCM is the leading cause of cardiac transplantation requirement in young adults. Indeed, some patients with myocarditis will ultimately develop DCM, although others may completely recover without chronic complications.

Myocarditis is an acquired inflammatory condition involving the myocardial tissue. Although myocarditis is largely associated with viral infections, some cases remain idiopathic, while in others there is convincing evidence of autoimmune pathophysiology. It is unknown as to why specific individuals are more susceptible to developing autoimmune heart diseases. The male to female ratio has been reported as 1.2–2 : 1 and 2.5 : 1 in autoimmune myocarditis and idiopathic DCM, respectively. Autoimmunity is influenced by genetic, immune, hormonal and environmental factors. Myocardial injury (due to infection, ischaemia, inflammation, toxins or other cardiotoxic factors) may trigger exposure to autoantigens, subsequently, initiating an autoimmune response, causing myocarditis and DCM. Nevertheless, in some myocarditis/DCM cases no specific trigger is found.

## 2 Diagnostic measurements for experts

Clinically, myocarditis may be asymptomatic or present with chest pain, palpitations, ECG changes, syncope, arrhythmias, and in some cases, sudden death. Early diagnosis may be extremely challenging since signs and symptoms may be unspecific (Table 1). Clinically, DCM is most commonly characterized as symptomatic heart failure. Prior to confirmation of a diagnosis of autoimmune my-

ocarditis/DCM, proof of autoimmunity may be required. Evidence of autoimmune myocarditis/DCM may be found in a mononuclear cell infiltrate presenting with an abnormal human leukocyte antigen (HLA), presence of circulating anti-heart autoantibodies (AHA) or autoreactive lymphocytes in patients, and in unaffected family members, and in in-situ evidence of autoreactive lymphocytes and/or autoantibodies in cardiac tissue.

**Table 1.** Signs and symptoms of myocarditis and dilated cardiomyopathy.

| |
|---|
| – History of an upper respiratory illness or recent viral infection in some patients (in myocarditis) |
| – A number of myocarditis cases are subclinical |
| – Asymptomatic cardiomegaly |
| – Symptomatic left- and right- heart failure |
| – Physical examination findings consistent with heart failure |
| – Chest pain on exertion, or at rest |
| – Dyspnoea on exertion, or at rest |
| – Fatigue |
| – Palpitations and arrhythmias (both ventricular and supra-ventricular) |
| – Peripheral pitting oedema |
| – Systemic and pulmonary embolisms |
| – Syncope |
| – Elevated serum levels of myocardial enzymes (in myocarditis) |
| – Electrocardiographic changes |
| – Sudden death |

## 3 Requirements for family practitioners

Diagnosis should be made in the early stages of the disease in order to identify, control, and treat possible complications. Distinguishing autoimmune myocarditis/DCM from non-autoimmune diseases has limited practical implications currently. Nevertheless, we believe that in the future, specific immuno-modulating therapies will be available for proven autoimmune cases. Diagnosis of autoimmune DCM may require clinical, echocardiographic and laboratory findings (Table 2). Also, exclusion of other causes of myocardial inflammation and cardiomyopathy is important before autoimmune pathophysiology can be concluded. In light of

familial clustering in some cases, the physician should evaluate whether other family members were or are currently affected. Clinical courses of exacerbation and remission may provide supportive evidence of autoimmunity. Despite extensive evaluations, approximately 50 %–80 % of DCM cases remain idiopathic.

**Table 2.** Diagnostic criteria for autoimmune dilated cardiomyopathy.

*Clinical criteria for diagnosing dilated cardiomyopathy (all criteria must be fulfilled).*

1. Ejection fraction < 45 % and/or fractional shortening < 25 %
2. Left ventricular end diastolic dimension (LVEDD) > 112 % than expected according to age and body surface area. Cutoff of LVEDD > 117 % is preferred in familial presentation
3. Exclusion of the following: blood pressure > 160/100 mmHg, intravascular obstruction of main coronary artery lumen exceeds 50 %, alcohol intake > 80 g/day for males, or > 40 g/day for females, persistent supraventricular tachy-arrhythmias, systemic disease, pericardial disease, congenital heart disease and cor pulmonale

*Proposed laboratory criteria for autoimmune dilated cardiomyopathy (diagnosis requires fulfilment of at least one criterion)*

1. Proven mononuclear cell infiltrate with abnormal human leukocyte antigen (HLA) presentation
2. Circulating anti-heart autoantibodies or autoreactive lymphocytes in patients and in unaffected family members
3. In situ evidence of autoreactive lymphocytes and/or autoantibodies in cardiac tissues
4. Disease induction in animals following transfusion of the patient's serum, antibodies, or lymphocytes
5. Proven clinical or echocardiographic improvement following immunoadsorption or immunosuppressive therapy

*Supporting evidence for autoimmune dilated cardiomyopathy, not considered criteria*

1. Clinical course of exacerbations and remissions
2. Positive HLA DR4
3. Familial clustering of autoimmune diseases and/or family history of dilated cardiomyopathy (two or more affected individuals, or sudden cardiac death in a first-degree relative < 35 years old)

Clinically, signs of heart failure might be found upon physical evaluation, including liver congestion, hepatomegaly, lower limb oedema, jugular venous distension, pulmonary oedema, etc. Third and fourth heart sounds are common in DCM. Pericardial friction rub may be found in patients with peri-myocarditis. Chest X-rays may reveal cardiomegaly and pulmonary congestion.

ECG changes may be non-specific and include ST-T changes, Q-waves, atrioventricular conduction delay, bundle branch block, supraventricular arrhythmias and occasionally low voltage (Fig. 1).

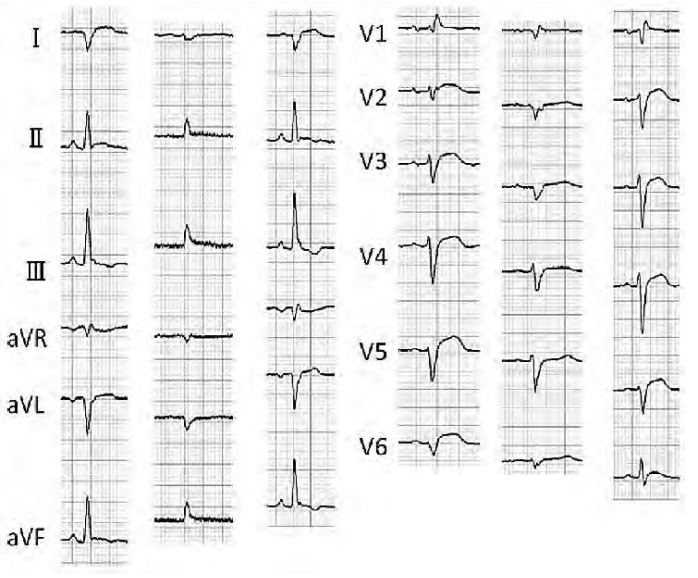


**Figure 1.** Electrocardiogram at admission, acute phase, and recovery phase of myocarditis (Case Study). At admission, convex ST-segment elevations in the precordial leads were seen. Although the voltage of all leads were reduced at the acute phase, the ECG completely returned to the prior findings. (*Adopted with permission from Yuya Matsue, Leon Kumasaka, Wataru Nagahori, et al. 2010. A case of fulminant myocarditis with three recurrences and recoveries. Int Heart J 51: 218–219.*)

Echocardiography might demonstrate cardiac dilatation and poor systolic function. Fulfilment of echocardiographic criteria (Table 2) is crucial in diagnosing DCM.

Clinical investigation in cases of myocarditis should endeavour to identify a trigger, such as isolation of a cardiomyopathic viral agent or identification of sero-conversion against a known cardiac pathogen.

Diagnosis of myocarditis may require referral to a medical facility to perform a cardiac biopsy, which would prove cellular infiltration and cardiomyocyte necrosis.

Absence of angiographically significant coronary artery disease may also assist in excluding ischaemic pathophysiology.

Fulminant myocarditis and other decompensating conditions should be treated in specialized hospital units.

## 4 Follow up

*Clinical observations*

The clinician should observe the patient's clinical performance and screen for contractile deterioration and increased risk of arrhythmias via extended ECG monitoring, and electrocardiographic arrhythmogenic markers. The presence of specific circulating AHA (such as anti-β1 adrenergic receptor autoantibodies) may also be associated with arrhythmias and poor prognosis.

*Expectations*

The majority of myocarditis patients will clinically improve and survive. Approximately 25 % of DCM patients will stabilize or spontaneously improve. Nevertheless, once DCM has developed, particularly in older patients, prognosis may be unfavourable. Heart transplantation may be offered as a definitive treatment.

*Blood tests*

Anticoagulation effectiveness should be monitored by periodic INR measurement to prevent embolisms. Serologic investigations for infectious agents (most of which are viral) may assist in diagnosing an infectious trigger. Moreover, serological investigations of AHA may provide further evidence of autoimmunity. It remains to be explored whether changes in the AHA titre throughout follow-up has any prognostic implications.

## 5 Management

In the acute phase of myocarditis, physical exercise should be avoided. In cases where contractile dysfunction has evolved, appropriate therapy for heart failure should be initiated (i. e., salt-restriction, renin-angiotensin-aldosterone system blockers, diuretics, β-blockers, and digitalis). Patients should avoid exposure to cardiotoxic agents and alcohol. Warfarin should be taken in cases of atrial fibrillation, severe ventricular dysfunction, and past history of thromboembolism. Anti-arrhythmic drug therapy has resulted in conflicting outcomes, and in some cases has triggered or aggravated arrhythmias. Therefore, clinicians usually discourage their use in preventing arrhythmias. Severe heart failure may ultimately require left ventricular assisted devices and heart transplantation. Some patients

will require defibrillator implantation in an attempt to control recurrent ventricular arrhythmias. Cardiac resynchronization therapy may be beneficial in heart failure and intra-ventricular conduction delay.

Specific and non-specific immune-modulating therapies represent possible future treatment strategy regarding autoimmune myocarditis and DCM. Such therapeutic approaches include immunosuppression, immunoadsorption, intravenous immunoglobulins, cytokines-altering therapy, immunisation against autoantigens, or treatment with other specific peptides that modulate a specific immune response. Nevertheless, most of these therapeutic approaches remain experimental and theoretical. The effectiveness of non-specific immunosuppression is unclear.

## 6 Diagnostic tests

Cardiac magnetic resonance (CMR) imaging in myocarditis may demonstrate diffuse patchy mid-myocardial and epicardial late gadolinium enhancement, and sparing of the subendocardium.

Immunological evaluation may require a myocardial biopsy to evaluate whether cardiac deposition of auto-reactive lymphocytes and/or autoantibodies exists. In addition, circulating AHA should be evaluated. Autoimmunity may also be established by induction of cardiac disease in a laboratory animal following transfusion of the patient's sera, antibodies or lymphocytes.

## 7 Testing methods (benefits, limitations)

Definitive diagnosis of autoimmune cardiac myocarditis/DCM is mainly limited by the necessity for invasive procedures such as an endo-myocardial biopsy. There are several techniques available for detecting AHA (i. e., Elisa, immunoassay, surface plasmon resonance measurements, and functional assays), although a gold standard is lacking. In addition, there are no strict criteria as to the definition of abnormal autoantibodies titre. Therefore, future research should focus on laboratory tests' standardisation in the detection of autoimmune markers. The possible benefits from such standardisation may be earlier diagnosis of autoimmune illnesses and earlier treatment with targeted therapy.

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## Abbreviations

**DCM**, dilated cardiomyopathy; **AHA**, anti-heart antibodies; **CMR**, cardiac magnetic resonance.

# Pernicious anemia

Helena Silva, Marilda Santos, João Pedro Ramos, Carlos Dias

## 1 Introduction

Pernicious anemia (PA) is a form of megaloblastic anaemia secondary to poor cobalamin (Cbl) absorption associated with severe lack of intrinsic factor (IF) due to gastric atrophy. Pernicious anaemia was first introduced by Thomas Addison in 1849, who described it as "a very remarkable form of anaemia" later called pernicious (fatal) by Anton Biermer. PA is an autoimmune disease based on the presence of the autoantibodies anti-gastric parietal cells or anti-IF, supported by the presence of mononuclear cell infiltration into gastric mucosa with loss of parietal cells and by the regeneration of these cells under immune suppression with corticosteroids. In 1934, George Hoyt Whipple, George Richards Minot and William Parry Murphy shared the Nobel Prize in Physiology or Medicine for their work on finding a cure for PA, by including liver in the patient's diet. The active ingredient in the liver extracts remained unknown until 1948, when Cobalamin (Cbl) ("the extrinsic factor") was isolated by two chemists, Karl A. Folkers and Alexander R. Todd. With that discovery, it became possible to treat PA, in a cheap and effective way, by injecting Cbl into muscle. In nature, Cbl exists in 3 major chemical forms in different food resources: methylcobalamin (MeCbl), deoxyadenosylcobalamin (AdoCbl) and hydroxycobalamin. Once metabolised, Cbl is a cofactor and coenzyme in many biochemical processes, including DNA synthesis. As MeCbl, it acts as a cofactor for methionine synthesis from homocysteine. As AdoCbl, it contributes to propionyl conversion into succinyl coenzyme A from methylmalonate. The deficient purine and aminoacid synthesis is responsible for the observed megaloblastic anaemia and other haematological, neurological and multi-organ manifestations.

## 2 Clinical manifestations

Clinical manifestations are highly polymorphic and range in severity from milder conditions to severe. In asymptomatic patients, PA can be detected on routine blood analysis as a raised mean corpuscular volume (MCV). Symptoms such as

anorexia, fatigue and other symptoms related to anaemia are very common. The most frequent manifestations are sensory neuropathy with isolated macrocytosis, in milder Cbl deficiencies. Haemolytic anemia, pancytopenia and sclerosis of the cord are rare manifestations presenting in severe forms of PA. The classic manifestations related to PA are Hunter's glossitis (lingual papillae atrophy) and the neuroanaemic syndrome (combined sclerosis of the spinal cord and megaloblastic anaemia) (Table 1).

**Table 1.** Major clinical manifestations related to cobalamin deficiency and present in pernicious anaemia. Adapted from [1].

| **Clinical manifestations** | | **Frequency** |
|---|---|---|
| Haematologic | Megaloblastic anemia, macrocytosis, hypersegmentation of neutrophils, medullary megaloblastosis | Frequent |
| | Isolated thrombocytopenia and neutropenia; pancytopenia | Rare |
| | Haemolytic anaemia, thrombotic microangiopathy | Very rare |
| Neuropsychiatric | Degeneration of the spinal cord | Classic |
| | Peripheral neuropathy, ataxia, Babinsky's phenomenon | Frequent |
| | Cerebellar syndromes involving cranial nerves, including optic neuritis, optic atrophy, urinary or faecal incontinence | Rare |
| | Dementia, Parkinsonian syndromes, depression | Under study |
| Digestive tract | Glossitis, angular queilosis, jaundice, lactate dehydrogenase and bilirubin elevation | Classic |
| | Diarrhoea, constipation, dyspepsia, abdominal pain | Debatable |
| | Type A chronic gastritis, atrophic gastritis or gastric atrophy | All patients |
| | Intestinal metaplasia, gastric neoplasmas: adenocarcinoma, lymphoma, carcinoid tumour; resistant and recurring mucocutaneous ulcers | Rare |
| Cutaneous | Reversible melanin skin hyperpigmentation | Frequent/Debatable |
| Cardiovascular | Thromboembolic disease: angina, stroke (hyperhomocysteinaemia) | Under study |
| Gynaecological | Vaginal mucosa atrophy, chronic vaginal and urinary infections, hypofertility and repeated miscarriages | Under study |

## 3 Diagnostic criteria

There are no definitive diagnostic criteria for PA. However, this disease is diagnosed by clinical manifestations, macrocytic anaemia (MCV >100 fL) (Fig. 1) deficiency of vitamin B 12, confirmed Cbl malabsorption with a positive Schilling test, demonstration of an autoimmune process by specific antibody identification (anti-gastric parietal cells and anti-IF) and type A chronic gastritis, atrophic gastritis or gastric atrophy.

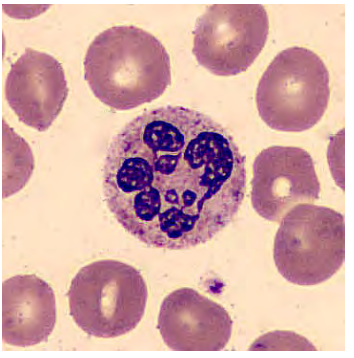

**Figure 1.** Anisocytosis, macrocytosis and neutrophil hypersegmentation are quite obvious in the blood smear.

## 4 Diagnostic measurements for experts

Alcohol abuse, oncological background, medication history and patient diet should be well evaluated. Several medical conditions are associated with macrocytic anaemia, with or without detectable vitamin deficiency, and should be suspected after a careful clinical history is obtained. Folate deficiency should also be ruled out. If nutritional deficiencies are excluded or their presence is doubtful, primary bone marrow disease must be carefully evaluated.

## 5 Requirements for family practitioners

It is important to acknowledge that Cbl deficiency is very prevalent in the general population and occurs frequently among elderly. The same is true for PA and for polyneuropathy (PN), as the frequency of both disorders increases with age. Considering that PA is secondary to parietal cell destruction by autoimmune mechanisms, the consequence is failure to produce IF and a state of achlorhydria (and secondary hypergastrinaemia). Since gastric acid production is very important in food iron absorption, iron deficiency is also a very common complication in PA

and may result in different presentations of anaemia: macrocytic, normocytic or microcytic anaemia. These findings lead to an obvious discussion about iron and Cbl deficiencies overlap, about atrophic gastritis and PA as different entities or different stages in the spectrum of the same autoimmune disease, sharing the same antibodies.

For family practitioners, the challenges are different considering the different settings as PA can be presented.

**Pernicious anaemia presenting as isolated macrocytosis:** these are asymptomatic patients that, on routine haematologic evaluation, present an elevated MCV without anaemia. Commonly this finding may represent a milder form of vitamin B 12 deficiency with normal values of serum Cbl. The determination of plasma levels of Cbl metabolites (pHC and MMA), if available, may be important for identifying Cbl deficiency probably secondary to PA.

**Pernicious anaemia presenting as megaloblastic anaemia:** pernicious anaemia is the most common cause of megaloblastic anaemia in Western countries and its diagnosis poses relatively few diagnostic problems in this setting. In this condition, macrocytic anaemia is associated with hypersegmented neutrophils and abnormal nuclei maturation can be detected on several organs. Megaloblastosis is a generalised process where bone marrow, gastrointestinal and gynaecological

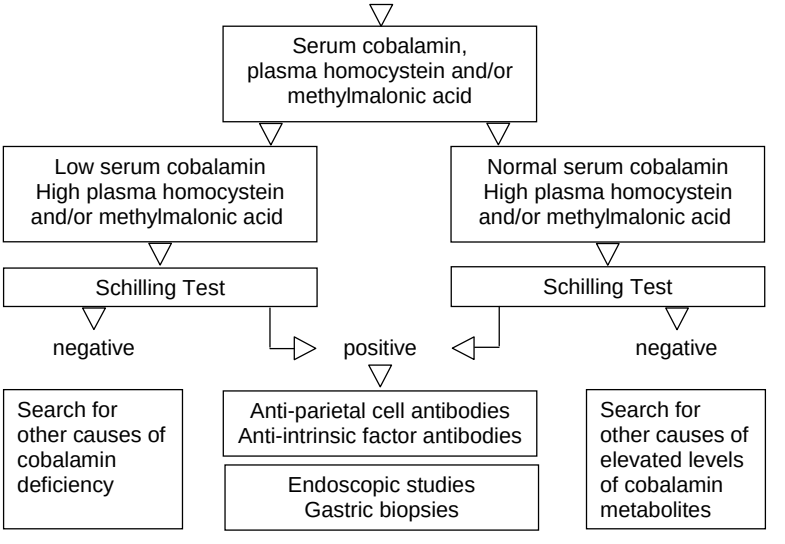


**Figure 2.** Diagnostic approach for pernicious anaemia.

smears or biopsies present characteristic abnormalities consequent to nuclei delayed maturation.

**Pernicious anaemia presenting as polyneuropathy:** as both diseases are very frequent in the general population and more frequently among elderly, it is difficult to establish an aetiologic correlation between both disorders, since the cobalamin deficiency has not been proved as the cause of the neuropathy, even in the absence of other causes. Polyneuropathy in Cbl deficiency frequently presents as sensory or sensorimotor polyneuropathy, usually involving upper and lower extremities concomitantly. It usually has a sudden onset and a shorter illness duration and is less likely to present as pain or lower limb weakness compared to cryptogenic polyneuropathy. Patients commonly experience symptom onset in the hands or in the hands and feet simultaneously, traducing small and/or large-fibre sensory involvement. Patients presenting PN associated with cobalamin deficiency have a low incidence of haematologic abnormalities. They are more likely to have erythrocytes with elevated mean corpuscular volumes, but the incidence of anaemia is the same as in cryptogenic neuropathy.

In patients with PN, with or without haematologic abnormalities, it is mandatory to investigate cobalamin deficiency, perform electrodiagnostic studies (such as electromyography, nerve biopsy or lumbar puncture) and expert evaluation (Internal Medicine or Neurology) to investigate other causes.

## 6 Follow up

Once diagnosis is well established, the follow up procedure will be mainly the clinical monitoring of laboratory abnormalities (serum Cbl) after due treatment. Chronic gastritis (CG) can be classified in clinical stages based on histological findings. Initial stages may present a superficial gastritis with inflammatory changes limited to *lamina propria* on the surface mucosa. In the second stage, designated as atrophic gastritis, inflammation extends deeply into the mucosa with progressive destruction of glandular structures, and progresses to severe gland destruction, gastric atrophy and intestinal metaplasia that ultimately can lead to gastric tumour. It is important to perform regular endoscopic studies (every 3 to 5 years) and, if no lesion is macroscopically identified, several random gastric biopsies must be done.

## 7 Management

The standard treatment aims to correct body stores and to maintain daily needs. Most patients are treated with intramuscular vitamin B 12, which is time consuming, can be painful, can be inconvenient for anticoagulated patients and, rarely, presents toxic reactions. Effective oral treatment is available in clinical practice,

presenting equal efficacy, similar costs, safety and adherence compared to parenteral administration. The efficacy of oral treatment in PA patients depends on both mechanisms of vitamin B 12 absorption, mainly passive absorption (without IF), but also active absorption of free-vitamin B 12 (associated with IF). In most countries, doctors do not prescribe oral formulations because they are either unaware of this option or have concerns about efficacy due to unpredictable absorption. In the literature, there is limited evidence from randomised control trials (RCTs) that oral vitamin B 12 is an effective treatment for cobalamin deficiency in the short term and no evidence for its efficacy for long term treatments in PA patients. High doses of oral vitamin B 12 (1000 to 2000 μg) initially daily, then weekly and then monthly are as effective as intramuscular administration in achieving haematological and neurological responses (Table 2).

**Table 2.** Replacement therapy for pernicious anaemia.

| | |
|---|---|
| Replacement of body stores | Six intramuscular 1000 μg injections of hydroxycobalamin given at 3 to 7 day intervals or |
| | Daily oral doses (1000 to 2000 μg) of cyanocobalamin |
| Maintenance treatment | 1000 μg of intramuscular hydroxycobalamin every three months |
| | 1000 μg of intramuscular cyanocobalamin monthly (because of poorer retention) |
| | Daily oral doses (1000 to 2000 μg) of cyanocobalamin |

Treatment efficacy is synonymous with reversal of the haematological and neurological manifestations and correction of body stores that should be assessed routinely.

Considering that iron deficiency frequently overlaps Cbl deficiency, oral iron supplementation should be given. Folate deficiency should also be corrected if detected.

## 8 Diagnostic tests

The first diagnostic approach aims to identify cobalamin deficiency, by the determination of serum cobalamin, which is the screening test. The presence of elevated levels of plasma homocystein (pHC) and methylmalonic acid (MMA) can support the diagnosis and are more sensitive indicators of cobalamin deficiency than cobalamin serum levels alone, especially for the diagnosis of milder forms of vitamin B 12 deficiency. Normal values of pHC and MMA can rule out cobalamin deficiency. It is important to acknowledge that hyperhomocysteinaemia is present in folate and pyridoxine deficiencies (or improper collection and processing of blood samples), and that both pHC and MMA levels are raised in conditions such as renal insufficiency, volume contraction and various enzyme polymorphisms.

The diagnosis of ileum malabsorption requires a Schilling test. This test will confirm vitamin B 12 malabsorption by determining urinary radioactivity after an oral dose of radioactive Cbl is given. Urinary radioactivity is lower when radioactive Cbl is administered along with IF, confirming IF deficiency (or abnormality). After confirming Cbl malabsorption related to IF deficiency, it is necessary to identify antibodies related to the pathological process that defines PA. Sixty to ninety percent of PA patients present with antibodies to gastric parietal cells, but those are also very prevalent in simple atrophic gastritis (60 %) and thyroid disease. Antibodies to IF are less sensitive (found in 50 to 70 % of PA patients) but more specific for PA.

Finally, the evidence of organ disease associated with autoantibodies requires confirming the presence of type A chronic atrophic gastritis or gastric atrophy by endoscopic procedures and gastric biopsy.

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# Autoimmune thrombocytopenic purpura

Dana Yehudai, Vadasz Zahava

## 1 Introduction

Immune thrombocytopenic purpura (ITP) is a relatively common, immune-mediated disorder affecting approximately 5–10 adults per 100 000 in the western world. It is characterised by isolated thrombocytopenia ($< 100 \times 10^9$/L), and the absence of any obvious initiating and/or underlying cause for the thrombocytopenia [1].

The mechanisms of thrombocytopenia are increased platelet destruction mediated primarily by autoantibodies but also by direct T-cell cytotoxicity against platelets, and decreased platelet production.

The disease can be classified by duration into acute, persistent (3–12 months), and chronic (>1 year), and by patient age (adult or childhood) [2].

*Paediatric ITP* is usually an acute, self-limiting disease preceded by a viral infection or rarely, following immunisation (e.g. MMR). More than 60 % of paediatric patients recover spontaneously within 6 months. In contrast, *adult ITP* exhibits an insidious onset and normally follows a chronic course; spontaneous remissions rarely occur. The median age at diagnosis is 56 years; it is more prevalent in women aged 30–60 years, and equally prevalent in both sexes above the age of 60 [3].

Signs and symptoms vary widely, ranging from asymptomatic or minimal bruising to bleeding episodes including gastrointestinal, skin, mucosal or rarely, intracranial haemorrhage (Fig. 1).

Several factors can contribute to the risk of bleeding and should be evaluated before the appropriate management is determined: the severity of the thrombocytopenia (correlates to some extent with bleeding risk), age, lifestyle factors and uraemia.

Mortality rate is low, ranging from 1–2 %, and can be attributed equally to severe bleeding and infections secondary to immunosuppressive therapy.

The investigation and management of ITP patients vary widely, in part because of the understanding that ITP is a more benign disease than previously thought, and should be treated conservatively, reserving aggressive treatment for patients with severe and symptomatic thrombocytopenia [2].

## 2 Diagnostic criteria

### 2.1 Clinical Criteria

- Mild skin and mucosal purpuric rash/ecchymoses.
- Bleeding tendency (see Table 1).

### 2.2 Laboratory Criteria

- Isolated thrombocytopenia ($< 100 \times 10^3$/ml).

**Table 1.** Signs and symptoms of ITP.

| System | Symptoms |
|---|---|
| | None (asymptomatic) |
| Skin or mucosa | Easy bruising, petechiae, nose bleeds, gum bleeding |
| Genitourinary | Gynaecologic bleeding, haematuria |
| Gastrointestinal | Abdominal pain, upper or lower GI bleeding |
| CNS | Headache, intracranial haemorrhage |
| General | Fatigue, sleep disturbances |

## 3 Diagnostic measurements for experts

ITP is a disease characterised by thrombocytopenia that may accompany a purpurial rash and bleeding tendency. The diagnosis is made when the patient's history, physical examination, laboratory results (including complete blood count) and

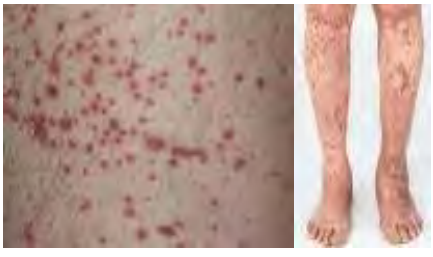

**Figure 1.** Typical purpuric rash of ITP.

peripheral blood smear do not raise another possible aetiology for the thrombocytopenia. Response to ITP-specific therapies could be also supportive of the diagnosis [1].

After ruling out possible alternative causes for thrombocytopenia by a review of the patient's history and physical examination (discussed later), evaluation of peripheral blood smear by haematologist must be made in order to look for blood cell abnormalities not characteristic of ITP (e. g. schistocytes in thrombotic thrombocytopenic purpura).

A bone marrow examination (aspiration and biopsy) may be considered in the following cases: patients over 60, when a splenectomy is considered, and in patients presenting with systemic symptoms not typical of ITP (e. g. fever, weight loss, lymphadenopathy).

Other relevant laboratory tests are anti-phospholipid antibodies which may be present in up to 40 % of ITP patients (recommended in the presence of anti-phospholipid syndrome only), thyroid function tests which include TSH and anti-thyroid antibody owing to the fact that a substantial percentage of ITP patients will develop hypo/hyperthyroidism.

Evaluation of blood group Rh (D) typing and direct anti-globulin test is needed when considering treatment with anti-D immunoglobulin [1].

## 4 Requirements for family practitioners

Many cases of ITP are diagnosed first by the family practitioner incidentally after a routine complete blood cell count. As previously mentioned, patient presentations vary from asymptomatic to history of bleeding episodes, the most serious being intracranial. The patients may consult their general practitioner due to easy bruising, nose and gum bleeding or fatigue. A thorough history and physical examination (should be normal aside from possible purpura; moderate or massive splenomegaly excludes ITP) should be completed, taking into consideration the differential diagnosis of ITP (Table 2). The family doctor should ask about bleeding history (dental procedures, surgeries) to differentiate between the acute and chronic disorders.

The complete blood count should show isolated thrombocytopenia with otherwise normal laboratory results. The practitioner should ask about recent immunisation and transfusions, infectious status (H. pylori, HCV, HIV, CMV and Parvovirus evaluation is recommended), inherited and congenital platelet disorders, exposure to drugs, alcohol and toxins, history of other haematological, autoimmune/immunodeficiency diseases and liver and thyroid disorders [1–2]. In paediatric ITP cases a history of previous infection must be sought.

If the diagnosis of ITP is established, the family practitioner needs to assess relative and absolute contraindications for corticosteroid therapy.

## 5 Follow up

*Clinical observations*

Adult ITP is usually a chronic disease requiring long-term follow-up. Treatment for ITP is considered appropriate only for symptomatic patients and those at risk for bleeding (age > 60 years, platelet count < $20 \times 10^9$/l, history of bleeding episodes, mandated anticoagulant therapy, predisposing profession or lifestyle). As long as the patient is asymptomatic with mild thrombocytopenia (s)he should not be treated, since the main goal of therapy is not to maintain a normal platelet count, but to maintain a safe one [2, 3].

The family practitioner should be aware of any change in platelet count — this requires routine complete blood count monitoring, asking the patient about signs of bleeding, planning of any elective surgery or any other scheduled invasive procedure.

Any change in clinical or laboratory status of the patient requires consultation with a haematologist.

*Expectations*

ITP is a chronic disease with variable prognosis; spontaneous remissions are uncommon. Many patients are asymptomatic or report only minimal bruising, others can experience serious bleeding. The estimated rate of fatal haemorrhage is 0.0162–0.0389 cases per adult patient per year at risk.

Paediatric ITP is usually short-lived with more than 60 % recovering spontaneously within 6 months [1].

**Table 2.** Differential diagnosis of ITP.

| |
|---|
| – Infectious diseases (HIV, HCV, HBV, EBV etc.) |
| – Autoimmune disorders (SLE, Evans syndrome) |
| – Malignancy (e.g. lymphoproliferative disorders) |
| – Liver diseases |
| – Drugs and other toxins |
| – Bone marrow abnormalities (myelofibrosis, aplastic anaemia, myelodysplastic syndrome |
| – Recent immunisation |
| – Inherited thrombocytopenia (e.g. Wiskott-Aldrich syndrome, Bernard-Soulier syndrome etc.) |

*Blood tests*

The main necessary blood test during follow up (in both treated and untreated patients) is CBC — platelet count and haemoglobin should be monitored.

In patients treated with corticosteroids — blood pressure, glucose and potassium values should be monitored and ophthalmologist assessment should be carried out.

## 6 Management

Treatment decision-making should be shared between the clinicians (family doctor and haematologist) and the patient, and should be individualised according to the severity of the disease, patient's age, co-morbidities and presence or absence of current bleeding.

In the uncommon cases of life-threatening haemorrhage or before surgical procedures, immediate therapy must be started. This includes prednisone and IVIG (intra venous immunoglobulins). Other rapid treatment options are high-dose methylprednisolone, platelet transfusion, anti-fibrinolytics and emergency splenectomy [1].

Surprisingly, only a limited number of randomised controlled trials (RCT) using traditional therapies to guide treatment management decisions are known in the literature, in contrast to the new ITP treatments (including thrombopoietic growth factors) for which some evidence-based RCT data already exists.

Nevertheless, once a decision to start therapy has been made, *corticosteroids* are the initial standard of care. Prednisone, prednisolone, methylprednisolone or high-dose dexamethasones (HDD) are commonly used. Approximately two-thirds of patients will respond (partially or completely) during the first week, but only 10–15 % will enjoy a lasting remission [3]. There is some evidence suggesting HDD has an advantage in achieving a sustained response.

If glucocorticosteroids treatment fails, other treatment options (also classified as first-line treatment) includes *IVIG* and *anti-D* in RhD-positive non-splenectomized patients. The beneficial effect of both these treatments is transient (mostly 2–4 weeks), but anti-D can be infused in a shorter time compared to IVIG, may reduce the need for splenectomy and has a potentially longer positive response [1–3].

*Second-line therapy*

Traditionally, *splenectomy* (open or laparoscopic) is considered to be the second-line treatment after first-line therapy has failed. Nevertheless, because spontaneous remissions or improvement may occur 6–12 months after the diagnosis, splenectomy is usually postponed for at least 6 months [1].

Approximately 25 % of patients will relapse after splenectomy and will be defined as having chronic refractory ITP. In these patients the development of accessory spleens should be ruled out [2]. Patients are usually given vaccination against encapsulated bacteria one month before or two weeks after the surgery.

A variety of second-line medical treatment alternatives are available today, both prior to or after splenectomy (with no preference for particular therapy). These therapies include:

1. anti-CD20 monoclonal antibody **Rituximab** — 60 % of patients respond, 40 % have a complete response, [4];
2. **Danazol**, an attenuated androgen response rate > 60 % for > 2 months;
3. **Dapsone**. a corticosteroid-sparing agent — may delay a splenectomy for up to 32 months [1];
4. **Azathioprine** — 45–55 % response rate;
5. **Cyclophosphamide** — 25–85 % response rate with mild–moderate toxicity;
6. **Cyclosporine-A** — clinical improvement in more than 80 % of patients resistant to first-line therapy, 42 % achieved complete remission [1];
7. **Mycophenolate mofetil** (immunosuppressant) and Vinca alkaloids — approximately a 40–50 % response rate, but not a sustained one;
8. **Thrombopoietin receptor agonists**, a novel therapeutic approach intended to stimulate platelet production rather than modulating the immune system — two agents, **Eltrombopag** (non-peptide TPO mimetic, given orally once daily) and **Romiplostim** (peptide TPO mimetic, given subcutaneously once weekly) are FDA-approved for the treatment of ITP: 80–89 % response rate lasting between 1.5 years (Eltrombopag) to 4 years (Romiplostim) with continual administration [5].

*Third-line therapy (for adult failing first-and second-line therapies)*

Approximately 30 % of patients will not achieve satisfactory improvement or will relapse after a splenectomy or after first- and second-line therapies. For this group of patients there are only limited medical options. These need to be discussed with the patient who should be made aware of their toxic side effects: combination chemotherapy (cyclophosphamide, prednisone and vincristine plus azathioprine etoposide); Campath-1H and haematopoietic stem cell transplantation — reserved only for patients with severe chronic refractory ITP with bleeding complications unresponsive to other treatment modalities [1].

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# Part 7

# Autoimmune diseases of endocrine glands

# Autoimmune thyroid diseases

Joana Rema, João Pedro Ramos, Carlos Dias

## 1 Introduction

Autoimmune thyroid diseases (AITD) include a number of conditions that have in common cellular and humoral immune responses which are aberrantly directed against the thyroid gland. Classically, AITD includes Hashimoto's and, Graves' diseases, both of which involve a significant infiltration of the thyroid by T or B cells with the production of thyroid-reactive autoantibodies, and the resulting clinical manifestations of dysfunctional hypo or hyper thyroid function. Other clinical variants of AITD include atrophic thyroiditis, postpartum thyroiditis, drug-induced thyroiditis (such as interferon-induced and amiodaron-induced), polyglandular autoimmune syndromes, and the so-called subclinical thyroiditis, defined by the presence of thyroid antibodies (TAbs) with no obvious clinical disease.

Autoimmune thyroid diseases (AITDs) are the most prevalent autoimmune diseases, affecting up to 5 % of the general population in western countries. Hashimoto's thyroiditis (HT) and Graves' disease (GD) are amongst the most common endocrine disorders in childhood and adolescence. They tend to be familial and are up to six times more frequent in women than in men.

## 2 Etiology and pathogenesis

The etiology of AITD includes the interaction between genetic and environmental susceptibility or triggering factors. It has been postulated that about 80 % of the susceptibility to develop AITD is attributable to genetic factors, while environmental factors would contribute to about 20 %. Whereas iodine intake, stress, infection and food or ambient toxins constitute environmental factors, genetic factors play an important role in the development of AITD as shown by twin and family studies. Although there is a strong genetic basis, the pattern of inheritance seems complex. Human HLA antigens have been associated with these diseases. Genome-wide screening and linkage analyses have identified several chromosomal regions that are linked to AITD.

## 3 Clinical manifestations and diagnostic criteria

The AITD may present with a wide spectrum of clinical symptoms and can be associated with a euthyroid, hypothyroid or thyrotoxic status. The most clinically significant forms of AITD are Hashimoto's and Graves' diseases, and their most relevant clinical signs and symptoms are summarized in Table 1. Hashimoto's disease is usually an insidious syndrome where the clinical presentation is marked by hypofunction, whereas Graves' disease is of a more sudden onset where palpitations and nervousness are common first symptoms, but in both conditions thyroid dysfunction can appear more or less noticeably and with a more or less perceivable goitre, usually painless and without pressure symptoms.

Systemic manifestations are mostly related to thyroid dysfunction and a detailed medical history should provide the necessary clinical clues. The basic evaluation is the same as recommended for the diagnosis of hypo or hyperthyroid function and patients should be asked for those symptoms as mentioned in Table 1, as well as about their previous prescription drug treatment. Whenever the diagnosis of thyroid dysfunction has been previously defined, it is fundamental to confirm it by history and by documenting pre-treatment analytical abnormalities. It should always be remembered that patients who have been under treatment for a long time have often forgotten their clinical past, the reasons for the therapy and their response to it.

Basic analytic evaluation should start with thyroid-stimulating hormone (TSH) and free thyroxine (fT4) measurements. TSH assay should be sensitive enough to accurately differentiate euthyroid from a hypo or hyperfunctional status. Mild hypothyroidism can be detected by a normal fT4 and a slightly elevated serum TSH.

**Table 1.** Main signs and symptoms of Graves' and Hashimoto's Diseases.

| **Graves' Disease** (symptoms of hyperthyroidism) | **Hashimoto's thyroiditis** (symptoms of hypothyroidism) |
|---|---|
| Anxiety | Decreased concentration ability |
| Irritability | Depression |
| Sleeping difficulty | Excessive sleepiness |
| Fatigue | Leg swelling |
| Rapid or irregular heartbeat | Bradychardia |
| Heat sensitivity | Cold intolerance |
| Weight loss, despite normal food intake | Modest weight gain |
| Goitre | Goitre |
| Brittle hair | Coarse hair |
| Diarrhoea | Constipation |

If a dysfunction is detected thyroid antibody levels should be determined to either microsomal (anti-TPO) or thyroglobulin (anti-TG). The first is usually more sensitive and specific, but both can be detected in the general population, and so great care should be considered when evaluating positive results without clear clinical findings. Anti-TSH receptor antibodies (TRAb) can be detected in Graves' disease, with or without simultaneous positivity for anti-TPO or anti-TG antibodies. A radioactive iodine uptake test (RAIU) is a very informative assay, even if not readily available to the general practitioner.

Hashimoto's disease most frequently presents with a low fT4, increased TSH, presence of thyroid autoantibodies and a decreased RAIU. But whilst autoantibodies are a hallmark, all the other signs can be of variable presence; the subclinical thyroiditis syndrome being specifically one condition where antibodies can be present with no other clinical findings. In doubtful situations biopsy can be an ultimate approach.

Graves' disease findings typically include thyrotoxicosis, goitre and exophthalmos, with raised fT4, suppressed TSH, positive TRAB detection and an elevated RAIU. Probable Graves' disease should be considered when at least one of these clinical findings is present with at least the first three laboratory conditions, and it should be suspected when at least one clinical finding is associated with raised fT4 and suppressed TSH. It should be emphasised that no positive TRAB test is necessary for a strong diagnostic probability.

In older patients it should be remembered that clinical symptoms and signs, including goitre, may be difficult to assess. For that reason TSH routine evaluation has been suggested as a cost-effective health screening strategy to be implemented every 5 years starting at age 35 on the average population, or more frequently in individuals at higher risk of developing thyroid dysfunction (personal or familial history of autoimmune thyroid disease, vitiligo, pernicious anaemia, diabetes mellitus or primary suprarenal insufficiency).

## 4 Diagnostic measurements for experts

The TSH assay is an internationally well standardized assay and so different manufacturers should provide comparable results, provided the sensitivity is of similar magnitude. The same applies reasonably to fT4 but significant variability can be expected between different manufacturers' results when the serum protein level is depleted as in severely ill patients. Total T3 is the most difficult assay to standardize and this is one of the reasons why it should be used with care when evaluating a dysfunctional thyroid status.

Autoantibodies assays were, up to some years ago, highly unreliable and results from different manufacturers were incomparable, both in interpretation and in quantification. Whilst recent standardization procedures have significantly removed the poor performance issue relating to positive/negative interpretation,

physicians should bear in mind that the current knowledge about the performance of older determinations can limit their use in the AITD diagnosis as well as in follow-up. There are no available universal standards for these autoantibodies and so different manufacturers can still produce quantifications which cannot be legitimately compared.

Other parameters such as serum cholesterol, triglycerides and alkaline phosphatase are often either increased in hypothyroidism or decreased in the hyperfunctional thyroid status.

## 5 Requirements for family practitioners

AITD are one of the most prevalent autoimmune diseases, and they should be especially considered in the elder population, due to their frequent partial or atypical presentation.

Functional thyroid assays (TSH and fT4) are one of the most sensitive forms of screening, and serum TSH is the single most reliable test to diagnose AITD induced forms of hypo or hyperfunction, and has been suggested to be considered a standard procedure for the general population every 5 years after the age of 35. Autoantibody test results should be considered very carefully when no dysfunctional status is present.

Occasionally goitre can be clinically undetectable and an ultrasound evaluation may be indicated when a strong suspicion remains after a negative physical examination.

Patients usually consult their general practitioners with vague symptoms of fatigue and depression or anxiety and irritability. Referral to the endocrinologist of suspected patients should provide fast and efficient management.

## 6 Management

Treatment of these diseases is addressed at the dysfunctional status. Only very exceptionally should an immunological approach be considered. Treatment includes adrenergic beta-blockers, antithyroid drugs, radioiodine (radioactive iodine 131) and thyroidectomy for the thyrotoxic status. Since surgery in a hyperthyroid patient is dangerous, preoperative treatment with antithyroid drugs is usually mandatory. Antithyroid treatment must be given for between six months and two years. Even then, on cessation of the drugs, the hyperthyroid state may recur. Therapy with radioiodine is the most common treatment in the United States, whilst antithyroid drugs and/or thyroidectomy are used more often in Europe, Japan and the rest of the world. For patients with a large goitre, thyroidectomy is often preferred because of its high efficacy in restoring euthyroidism, although hypothyroidism can result when most of the thyroid is removed. Graves' ophthalmopathy

is treated with steroids, local radiation or surgery and anti-CD20 monoclonal antibodies have been used with some success.

Hypothyroidism must be treated with replacement thyroid hormones. Levothyroxine sodium is the treatment of choice for the management of hypothyroidism with extra care being recommended for those patients older than 50 years or in younger patients with a history of cardiac disease.

## 7 Follow up

Periodic monitoring is essential for the adequate management of hyper or hypo thyroid diseases. Since the treatment of these pathologies may last for several years, a follow-up protocol must be established in strict cooperation with the endocrinologist. Patient compliance with prescription must be adequately monitored and dosage adjustment due to drug interaction and changes in body weight or advancing age must be considered.

Initial evaluation should be repeated every 4 to 8 weeks until stabilization of the functional status is achieved. TSH normalization is the single most useful test to determine that the euthyroidism status has been achieved and that a decrease in patient visit frequency can be considered. It should be taken into account that serum TSH may remain suppressed in hyperthyroid treated diseases for a period of several months after fT4 normalization, so potentially inducing wrong interpretations. In these clinical conditions it is recommended that at least fT4 should also be monitored.

Treatment with anti-thyroid drugs, radioactive iodine and surgery usually require more extensive follow-up procedures that are not the scope of this short review.

If clinical (check weight at home) and laboratory euthyroid function persists patients can be re-evaluated yearly for 2 to 3 years and then at increasing intervals.

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# Diabetes mellitus type 1

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## 1 Introduction

Type 1 diabetes mellitus (T1D) is, in the majority of cases, an autoimmune disease caused by the cell-mediated destruction of insulin-producing pancreatic islet cells. Despite the central pathogenic role of T-cells, autoantibodies, as secreted by auto-reactive plasma cells, are diagnostic indicators of immune processes [1]. In rare cases, especially in non-Caucasian populations, a subtype of T1D without any evidence of autoimmunity can be observed (classification as "Type 1 idiopathic" according to WHO).

In contrast to Type 2 diabetes, which is the result of insulin resistance and decreasing β-cell function, more affecting adults and the elderly, T1D starts suddenly with acute symptoms, mostly in childhood and adolescence. A special form of T1D is the slowly progressive Latent Autoimmune Diabetes in Adults (LADA), starting after the $30^{th}$ to $40^{th}$ year of age and where symptoms are of intermediate seriousness, mimicking Type 2 diabetes.

In T1D, immune reactions reduce the number of insulin producing beta cells in the islets of Langerhans (Fig. 1). Since glucose uptake in many tissues depends on insulin, the absolute lack of insulin is responsible for increasing blood glucose values and lipolysis. This results in symptoms caused by the high concentration of blood glucose, insufficient utilization of glucose and accumulation of lipolytic products (ketone bodies) (Table 1).

The incidence of T1D has been rising from 9 to 16/100 000 over the last 20 years. The prevalence has increased to 0.8 % in recent decades and differs regionally and socially. Men and women are equally affected.

## 2 Diagnostic measurements for experts

T1D is an autoimmune disease with a clear genetic background (e. g. HLA-DQ/DR, IDDM2, PTPN22), however, the concordance rate in identical twins is less than 50 %. Despite some clinical, epidemiological and pathological data, there is no clear evidence for a defined viral trigger. Early metabolic signs of the ongoing process

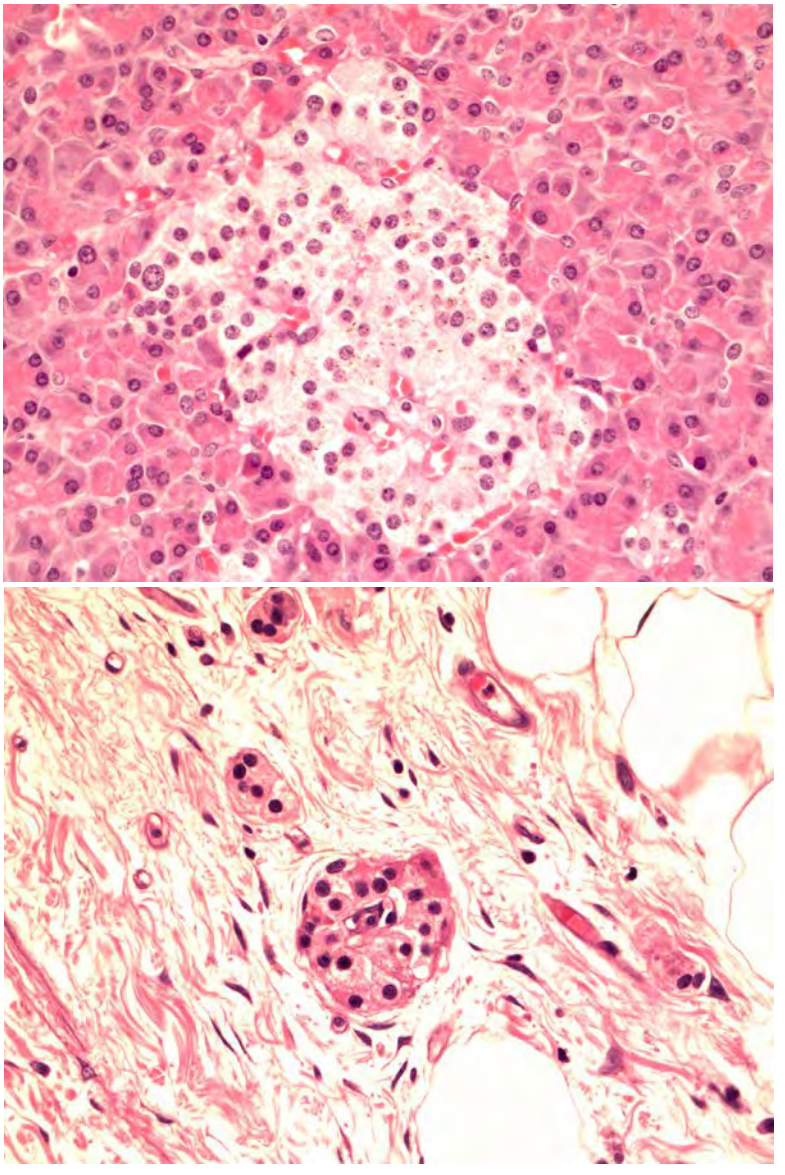


**Figure 1.** Histopathological pancreas specimen of a healthy (a) and a diabetic (b) person. In a healthy pancreas, islets are formed by bright cells with regular size and shape. Under diabetic conditions, small islets are surrounded by fibrous connective tissue. HE staining, magnification 140 ×.

**Table 1.** Symptoms of disease.

| **Acute onset** | |
|---|---|
| high blood glucose concentration | polyuria |
| | polydipsia |
| | blurred vision |
| insufficient utilization of glucose | weight loss |
| | impaired wound repair |
| | fatigue |
| | loss of performance |
| acute complications | diabetic ketoacidosis |
| | hypoglycaemia (under treatment) |
| **Chronic disease** | |
| | neuropathy |
| | nephropathy |
| | retinopathy |
| | cardiovascular disease |

of β-cell destruction are the loss of the oscillatory pattern of insulin secretion, followed by a missing first phase insulin response after iv glucose stimulation.

It is possible to identify humans with a high risk for T1D by combining family history, other risk factors, antibody screening and repeated metabolic testing. Since no approved preventative therapy is available, screening is recommended only in the setting of clinical trials.

Sometimes associated autoimmune diseases such as thyroiditis, coeliac disease, Addison's disease, or autoimmune polyglandular syndromes facilitate early diagnosis.

In most cases, it is clinical symptoms which will promote further diagnostic evaluation. Diagnosis of diabetes mellitus is confirmed by blood glucose testing (usually either fasting or random, in rare cases after oral glucose load). Metabolic acidosis and ketonuria are indicative for T1D. Insulin, proinsulin, and C-peptide concentrations will be very low, however their estimation is of limited value.

The detection of autoantibodies is a typical laboratory finding in T1D (Table 2). Detection of autoantibodies to islet cell-related antigens is a hallmark of T1D confirmation, but can also be used as an early indicator of diabetic risk. Specificity depends on the quality and characteristics of tests applied in the diagnostic laboratory.

– Islet cell antibodies (ICA) are detected by indirect immunofluorescence on unfixed cryostat sections of human pancreas. They represent the most general in-

**Table 2.** Diagnostic criteria.

| **Clinical findings** | |
|---|---|
| | Not defined |
| **Laboratory findings** | |
| Clinical chemistry | Plasma glucose ≥ 11.1 mmol/l (≥200 mg/dl) |
| | Fasting plasma glucose ≥ 7 mmol/l (≥123 mg/dl) |
| | Oral glucose tolerance test: ≥ 11.1 mmol/l (≥200 mg/dl) after 2 h |
| | Ketoacidosis |
| | Insulin, Proinsulin, C-peptide |
| | HbA1c (in follow-up) |
| Immunology | Islet cell antibodies (ICA) |
| | GAD antibodies |
| | IA2 antibodies |
| | Insulin antibodies |
| | ZnT8 antibodies |
| | not yet available: islet-specific T cells |

Abbreviations: **HbA1c**, glycated haemoglobin; **GAD**, glutamate decarboxylase; **IA2**, insulinoma associated antigen; **ZnT8**, Zinc transporter 8

dicator as they are directed against several autoantigens of pancreatic endocrine cells such as glutamate decarboxylase (GAD) and the tyrosine phosphatase, IA2.

- GAD autoantibodies (GADA) in the sera of T1D patients specifically bind the isozyme GAD65 of the enzyme glutamate decarboxylase. They are analysed by radioimmunoassay (RIA) or enzyme immunoassays (EIA) using recombinant or highly purified native GAD65 as the test antigen. The results obtained with latest generation EIAs are comparable with those of RIAs.
- IA2 (the so called insulinoma associated antigen), a pancreas-specific tyrosine phosphatase, is the second main target of autoantibodies in T1D, detectable by RIA or EIA.
- Insulin autoantibodies (IAA) are detectable by RIA or EIA and are often found as the first T1D-specific autoantibody during disease development, especially in children. Other types of insulin antibodies may be induced by insulin therapy.
- ZnT8 antibodies are directed against the cationic efflux transporter protein Zinc T8. These antibodies seem to be the most specific ones for T1D and can also be found in patients without ICA, GADA, IAA or IA2 antibodies [2].
- Insulin receptor autoantibodies can be found in diabetes of different types. They can induce symptoms of insulin resistance (type B Insulin resistance) or hypoglycaemia. They may indicate a different type of autoimmune diabetes (type 3G) associated with systemic autoimmune diseases (mainly Systemic Lupus Erythe-

matosus) or paraneoplastic syndromes [3]. They are important in differential diagnosis.

Islet cell destruction is dominated by cytotoxic T cells. Although detection of such autoreactive cells can be done experimentally, it is not yet a routine method.

Indications for autoantibody assessment are risk estimation in healthy probands and confirmation of the autoimmune pathogenesis of Type1 diabetes and differential diagnosis of Maturity Onset Diabetes of the Young (MODY) and LADA in cases of overt diabetes. This latter is important since up to 10 % of patients classified as Type 2 diabetes actually have Type 1 diabetes of the LADA-type.

Finally, once T1D is diagnosed, the possible association with other autoimmune diseases such as Graves' disease, Hashimoto's thyroiditis, coeliac disease, Addison's disease or autoimmune polyendocrine syndromes should be excluded [4].

## 3 Requirements for family practitioners

The preclinical period of T1D is mostly invisible, but clinical presentation starts when insulin production is no longer sufficient and metabolic complications occur.

Type 1 diabetes can be an acute disease with presentations including abdominal pain, nausea, pseudoperitonitis shock and coma, and it requires immediate therapy. Without insulin therapy, the progress of T1D is rapid and life-threatening. As well as acute symptoms, longstanding hyperglycaemia induces a large number of chronic complications (Table 1).

In families with a high genetic burden of T1D, screening for relevant autoantibodies can help to detect the disease in relatives as early as possible.

Typically, patients contact their general practitioner with rather general symptoms such as polyuria, polydipsia, weakness, weight loss, blurred vision and infections. In a reasonable number of cases, severe metabolic ketoacidosis can be the initial event. Determination of glucose levels is the first and most relevant laboratory test.

When the diagnosis is suspected, first measures must be directed at preventing life-threatening complications and include fluid, electrolyte and insulin substitution. Next, the patient should be referred to a specialised diabetologist for further examination and laboratory testing. After starting regular sc insulin replacement therapy with an intensified insulin regimen, the patient should return for a continued supervision program.

## 4 Follow up

*Clinical observations*

The goal of insulin therapy is to normalise all acute symptoms and signs (Fig. 2).

**Figure 2.** T1D patient before and under insulin therapy.
Image reprinted with permission from Eli Lilly, Indianapolis, USA, 2011.

*Expectations*

T1D is a chronic disease, the prognosis for which depends on the success of maintaining a normal carbohydrate metabolism and avoiding high glucose peak concentrations and hypoglycaemia. Long-term prognosis depends on the development or prevention of secondary complications, i. e. nephropathy, neuropathy, retinopathy, and angiopathy (Table 1).

*Blood tests*

The success of insulin replacement therapy can be monitored by repeated blood glucose testing (self monitoring). Continuous glucose monitoring is now technically possible but is not yet part of routine care. Measurement of HbA1c allows assessment of intermediate-term stability (3 month period) of glucose metabolism.

## 5 Management

The treatment must be individualised according to the severity of disease, the patient's wishes and the presence of associated diseases. All patients with Type1 diabetes should participate in structured training and teaching programs.

1. Insulin replacement therapy is the only routinely applied therapeutic approach. This can be done with intensified conventional insulin injections using short

and long acting insulin preparations or insulin pumps (continuous subcutaneous insulin infusion — (CSII) using short acting insulin). Closed loop systems are under development.
2. Pancreas transplantation: Due to the side effects of immunosuppressive therapy, pancreas transplantation is not a routine procedure; it is mostly performed in combination with renal transplantation in cases of renal failure. Severe cases of neuropathic complications with repeated hypoglycaemia are also an indication.
3. Islet and islet cell transfer: Transfer of human islets or islet cells has been under investigation for years. At present, it is not a real therapeutic option.
4. Immunosuppressive treatment: A lot of immunosuppressive and immunomodulating protocols have been tested during the last 30 years and are still under investigation. Due to the side effects and the lack of long-standing islet cell protection, there is, as yet, no approved immunosuppressive therapy for T1D.

## 6 Diagnostic tests

Laboratory tests for T1D can be divided in two groups: clinical chemistry and immunology.

For detection of blood sugar, HbA1c, insulin, and further parameters relevant for differential diagnosis, clinical chemistry offers standardised test systems.

This is also true for autoantibody detection in immunological labs. To detect the antibodies mentioned above, several methods can be applied. The indirect immunofluorescence test for detection of ICA is a well-established method, standardised by international collaboration. For all singular autoantibodies, there are commercially available, immune-binding assays using recombinant or purified native proteins as antigens. Although RIAs have several advantages, non-radioactive tests are becoming more and more common in diagnostic labs. Different test formats are found at manufacturers' homepages.

## 7 Testing methods

The benefits and usefulness of the diagnostic laboratory tests are related to their specificities and sensitivities [5]:

- **ICA** are detected with a diagnostic sensitivity at the time of T1D clinical manifestation in 80–90 % of children and 70–80 % of adults. ICA can be found also in up to 40 % of patients with LADA and in 5–10 % of patients with gestational diabetes. Over the course of the disease, the frequency of ICA will decrease continuously.
- **GADA** are detectable with a frequency of 80–90 % in newly manifested T1D, in about 40 % of LADA patients and 5–10 % of gestational diabetes patients. They too decline over the course of the disease but persist longer than ICA.

- **IA2 antibodies** are less frequent than ICA or GADA being demonstrable in 50–70 % and 30–50 % of children/adolescents and adults with newly manifested T1D, respectively.
- **IAA** show the highest sensitivity in children younger than 5 years (90–100 %). In children older than 12 years, IAA are detectable only in about 40 % and in adults in 20–30 %.
- **ZnT8** antibodies are detectable in 60–80 % of T1D patients. They can also be found in patients negative for all other T1D specific autoantibodies. The combined presence of detectable autoantibodies against ZnT8, GAD65, IA2, and insulin increases the likelihood of T1D to about 98 % [6].

Limitations of the assays relate to the general characteristics of assays for the detection of specific antibodies. The specificity may vary depending on the antigens used in the assay. Frequently, quality control is hampered by the absence of well-characterised control samples. Special laboratory equipment, facilities, and training of technicians are required. These issues significantly raise the cost per test, unless the tests are restricted to a few reference laboratories. There is an ongoing search for alternatives that will solve these shortcomings.

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# Autoimmune Addison's disease or autoimmune adrenalitis

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## 1 Introduction

Autoimmune adrenalitis (AAD) is currently the most common cause of primary adrenal insufficiency or Addison's disease [1]. It is characterized by deficient production of glucocorticoids and/or mineralocorticoids by the adrenals due to an autoimmune process. Addison's disease is a rare disorder, however it is more common than 30 years ago; its prevalence in the general population having increased three fold since 1970 [1]. Primary adrenal insufficiency is clinically evident in 1 in 8 000 individuals in Western countries [3, 4] and AAD is the most common cause in these territories, accounting for 68–94 % of cases in the different studies [2]. The symptoms and signs of adrenal insufficiency depend upon the rate and extent of loss of adrenal function (Table 1).

## 2 Diagnostic measurements for experts

The first step is the confirmation of the clinical diagnosis of primary adrenal insufficiency demonstrating [2]:

1. Low basal cortisol and high ACTH secretion [basal cortisol < 3 μg/dL (83 nmol/L) and/or ACTH > 100 pg/mL (22 pmol/L), at 8:00 to 9:00 am], or
2. A rise in serum cortisol level up to 18 mcg/dL (500 nmol/L), 30 or 60 minutes after injecting 250 μg IV of ACTH

The second step is to define the autoimmune nature of this process; however there are no diagnostic criteria available. The main point in the differential diagnosis is to exclude secondary conditions that can cause adrenal insufficiency, such as tuberculosis, HIV, drugs, and genetic disorders. After excluding these conditions, it is important to have an image of the adrenal glands; the finding of an enlarged gland makes the autoimmune process less probable. On the other hand, the presence of autoantibodies to adrenal tissue or against steroid enzymes practically confirms

**Table 1.** Clinical manifestations of Addison's disease.

| Symptoms | Frequency |
|---|---|
| Weakness and fatigue | 95–100 % |
| Anorexia | 95–100 % |
| Weight Loss | 95–100 % |
| Dehydration | 80 % |
| Hypotension and tachycardia | 88–94 % |
| Abdominal pain or cramps | 31 % |
| Nausea, vomiting | 75–86 % |
| Diarrhoea | 16 % |
| Salt craving | 16 % |
| Postural symptoms | 15 % |
| Skin or mucosal hyperpigmentation | 90–94 % |
| Lethargy | 90 % |
| Amenorrhoea and reduced libido | (frequency not reported in most series) |

the diagnosis of autoimmune adrenal insufficiency. In the absence of these antibodies but with concomitant autoimmune conditions, the probable diagnosis of AAD can also be supported. We have previously suggested some elements that can lead to AAD diagnosis (Table 2) [2].

## 3 Requirements for family practitioners

Signs and symptoms of adrenal insufficiency depend on the extent and rapidity of loss of adrenal function, mineralocorticoid production, and the degree of stress. The onset of adrenal insufficiency is often very gradual and it may go undetected until an illness or other stress precipitates adrenal crisis. Patients may have dehydration, hypotension, or shock disproportionate to the severity of the current illness; abdominal pain; nausea and vomiting; weight loss and anorexia; hypoglycaemia; fever; hyponatraemia, hyperkalaemia, azotaemia, hypercalcaemia, or eosinophilia; hyperpigmentation or vitiligo. Definite diagnosis of primary Addison's disease is determined by cortisol and ACTH measurements that show inappropriately low cortisol secretion with high ACTH levels. Secondary conditions such as tuberculosis or tumour should be excluded by adrenal imaging techniques. The presence of autoantibodies against adrenal components confirms the autoimmune nature, and is seen in 80 % of the cases.

**Table 2.** Proposed diagnostic criteria for Autoimmune Addison's disease.

1. Basal cortisol < 3 μ g/dL (83 nmol/L) and/or ACTH > 100 pg/mL (22 pmol/L), at 8:00 to 9:00 am

   *or*

   Short ACTH stimulation test with 250 μg IV leading a rise in serum cortisol level after 30 or 60 minutes to a peak of at least 18 mcg/dL (500 nmol/L).
2. Normal or reduced adrenal gland volume on computed tomography (CT) and MRI and absence of calcifications on abdominal X-ray or CT.
3. Anti-cortex adrenal antibodies or high titres of anti-21-hydroxylase antibodies.
4. Exclusion of other causes of primary adrenal insufficiency: genetic (clinical signs or symptoms: achalasia, alacrimia, deafness, or hypogonadotropic hypogonadism in males or genotyping); adrenoleukodystrophy (levels of very long chain fatty acids within normal range); infectious diseases (tuberculosis, paracoccidiomycosis, histoplasmosis, HIV, CMV); drugs (mitotane, ketoconazoles, rifampin, etc); adrenal haemorrhage or thrombosis; neoplasias; infiltrative (sarcoidosis, amyloidosis, haemochromatosis).
5. Other(s) concomitant auto-immune condition(s) (Hashimoto's thyroiditis, pernicious anaemia, rheumatological autoimmune disease, autoimmune haemocytopenia and others)

Definitive diagnosis 1, 2, 3 and 4;
Probable diagnosis 1, 2, 4 and 5

## 4 Follow up

*Clinical observations*

After corticosteroids therapy is initiated, a strikingly progressive improvement of the clinical pictures is observed. Hypertension, bradycardia, suppressed renin levels, and retardation in growth rate are clinical signs of over-treatment with mineralocorticoids.

*Expectations*

The survival of adequately diagnosed and treated patients is the same as for the normal population. Before the availability of steroid replacement, the survival rate was usually two years or less.

*Blood tests*

Serum potassium, glucose, and plasma renin activity should be monitored as part of treatment follow-up.

## 5 Management

The standard initial therapy is replacement with glucocorticoids. During an acute crisis, therapy should not be delayed for diagnostic studies or laboratory results. Hydrocortisone, 100 mg intravenously every 6 hours for 24 hours, should be given for all patients with strong clinical suspicions of AAD, together with physiologic saline (1 litre in the first hour is appropriate in most cases). After cardiovascular stabilization, the hydrocortisone dose should be reduced to 50 mg every 6 hours and subsequently tapered to oral maintenance in 4 to 5 days. In case of complications or persistence of the symptoms, maintain or increase the dose to 200 to 400 mg/day. The correction of the haemodynamic and metabolic disturbances with large volumes of intravenous saline and glucose is mandatory. Look for precipitating factors and particularly for infections.

Glucocorticoid chronic replacement is usually given in two to three doses, with a half to two thirds of the dose in the early morning to mimic the physiologic secretion pattern. Dosage is equivalent to the oral administration of 15–25 mg of hydrocortisone or 25–37.5 mg of cortisone acetate. Mineralocorticoid replacement is accomplished with fluorohydrocortisone (fluorinef, 0.05–0.2 mg daily).

Education is important and a personal card or bracelets/necklace carrying the diagnosis should be recommended.

In periods of stress, increasing cortisol dosage is strongly recommended for all patients. Patients undergoing surgical procedures also need to adjust the glucoccorticoid dose. For major surgery, administration of intravenous hydrocortisone 100 mg/m$^2$ per day is necessary for 24 h peri and postoperatively, before tapering the dosage over several days to a maintenance one [2]. Patients should also learn when and how to inject dexamethasone during emergencies.

## 6 Diagnostic tests

For many years, the best marker for the identification of AAD was high titres of cortex adrenal autoantibodies (ACA), detected by indirect immunofluorescence on cryostatic sections of adrenal glands [3]. These antibodies bind all three zones of the adrenal cortex. Low titres of ACA have been describe in unequivocal post tuberculosis adrenalitis. More recently, the identification of the enzyme steroid-21-hydroxylase as the relevant antigen has allowed the development of highly sensitive and specific radiobinding assays for steroid-21-hydroxylase (CYP21A2 or P450c21) autoantibodies detection [4]. The antigen targets are the steroidogenic

enzymes: P450scc (CYP11A1, side-chain cleavage enzyme), P450c17 (CYP17, 17-alpha-hydroxylase), and P450c21 (CYP21A2, 21-hydroxylase). These antibodies may be present in 80 % of cases [3]. Anti-adrenal antibodies are more common in women. People with autoimmune disorders who carry these autoantibodies develop adrenal insufficiency at a rate of up to 19 % per year [5]. In fact, the presence of ACA in polyglandular autoimmune syndrome type 1 patients has a predictive value for the development of adrenal insufficiency of 92 % in this population.

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# Autoimmune polyendocrine syndromes

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## 1 Introduction

Polyendocrine autoimmune syndromes, as a concept, include a large variety of diseases, ranging from non-organ specific auto-immunity (such as systemic lupus erythematosus, SLE ) associated with antibodies addressed to insulin receptors or others, through to organ tumours associated with subsequent endocrinopathy, Graves' disease associated with anti-insulin syndrome, and a significant number of other non-endocrine pathologies with effects in the endocrine environment.

Nonetheless, the designation of autoimmune polyendocrine syndromes (APS) (or polyglandular autoimmune syndromes or PGA) is usually reserved to very rare genetically mediated diseases with a constellation of multiple endocrine gland failures, secondary to immune mediated mechanisms of glandular cell destruction resulting in gland dysfunction or atrophy. APS result from a breakdown in tolerance to several organ-specific antigens that can be either monogenic or a result of a complex genetic background, with an eventual environmental trigger. Four main syndromes have been described based on clinical findings alone and designated as APS-1, 2, 3 and 4. APS-1, or Whitaker syndrome, is a very rare disease that usually appears before 20 years of age and is characterized by the association of at least two of the following: chronic candidiasis, chronic primary hypoparathyroidism and/or Addison's disease. Hence the reason it is also known as autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy or APECED. APS-2, or Schmidt syndrome, affects mostly adult women and represents the combination of Addison's disease with autoimmune thyroid disease and/or type 1 Diabetes mellitus. APS-3 is the most common of all four and includes those patients that present autoimmune thyroid disease associated with autoimmune diseases other than Addison's disease or hypoparathyroidism. Other combinations of autoimmune diseases not included in the previous groups are classified as APS-4.

## 2 Clinical manifestations

The main clinical presentation of APS-1 includes:

1. Chronic candidiasis (CC), usually occurring as the first manifestation and in the first months of life; it may affect nails, skin, tongue, mucous membranes and, less frequently, the oesophagus with oesophagitis and oesophageal strictures.
2. Chronic hypoparathyroidism (CH) frequently preceding Addison's disease (AD) and most commonly before the age of 15; the most frequent features of CH are those related to chronic hypocalcaemia as described in Table 1. The most apparent sign is tetany, which may vary from a latent form (demonstrated by Chvostek's and Trousseau's signs or electromyography), to painful muscle spasms (usually starting distally in the limbs with centripetal progression, from carpopedal spasms and facial grimacing, up to the trunk), to laryngeal spasm and convulsions in severe cases (no loss of consciousness distinguishing these from tonic-clonic seizures); and
3. Addison's disease (AD) with clinical manifestations due to the combined deficiency of glucocorticoids, mineralocorticoids and androgens. The most frequent symptom is asthenia, and common manifestations include hypoglycaemia, hypotension and gastrointestinal dysfunction.

Other immune or non-immune mediated diseases, so called minor clinical manifestations, may be associated with APS-1 (Table 2).

In APS-2, the major clinical manifestations include:

1. Addison's disease, always present in these patients, with the same symptoms and signs as described earlier,
2. Thyroid autoimmune diseases (TAD), such as Graves' disease (usually present before AD) and chronic thyroiditis (usually simultaneously or after AD) and
3. Type 1 Diabetes mellitus (DM). As for APS-1, APS-2 may be associated with other minor autoimmune diseases (Table 3).

Incomplete APS-2 should be considered in two scenarios: (1) patients that present positive thyroid antibodies and/or ICA or GAD antibodies as well as AD; or (2) patients that present positive ACA/21-OH as well as TAD or type 1-DM. APS-3 is defined by the association of TAD with other autoimmune disease but not AD or CH (Table 4). This is the most common APS since TAD is the most frequent autoimmune diseases in the general population.

In 2001, Betterle proposed a new classification that distinguishes 4 subgroups of TAD related to disorders of four different main systems (Table 4). An incomplete APS-3 can be considered when patients present TAD associated with organ and non-organ specific autoantibodies and with no clinical evidence of other autoimmune disease.

Clinical manifestations associated with APS-4 are miscellaneous combinations of clinical presentations associating endocrine and non-endocrine autoimmune

**Table 1.** APS-1 Major Clinical Manifestations Description and Prevalence [1].

| | | | |
|---|---|---|---|
| Chronic candidiasis | Mucocutaneous candidiasis | | 18–100% |
| | Chronic oesophagitis, oesophagus stenosis | | |
| Chronic hypo-parathyroidism | Neuromuscular | Paresthesias, tetany, irritability, depression, psychosis, cerebral calcifications and intracranial hypertension with papilloedema | 76–100% |
| | Cardiovascular | Prolonged QT interval on ECG, arrhythmias and hypotension | |
| | Gastrointestinal | Intestinal cramps, malabsorption and steatorrhoea | |
| | Cutaneous | Dry skin, thick hair and nail dystrophy | |
| Addison's disease | Constitutional symptoms | Asthenia, fatigue, weakness, weight loss and anorexia | 22–100% |
| | Gastrointestinal | Nausea, vomiting, abdominal pain and diarrhoea (sometimes alternating with constipation) | |
| | Cardiovascular | Orthostatic hypotension and syncope | |
| | Metabolic | Hypoglycaemia | |
| | Cutaneous | Skin hyperpigmentation | |
| | Neuro-psychiatric | Depression, psychosis, confusion, delirium, stupor and pseudotumour cerebri | |
| | Sexual | Axilary and pubic hair decreased in women, reduced libido and erectile dysfunction | |

**Table 2.** APS-1 Minor Clinical Manifestations and Prevalences [1].

| | |
|---|---|
| Endocrinopathies | Hypergonadotropic hypogonadism (24–60%), thyroid autoimmune diseases (4–36%), type 1 diabetes mellitus (0–12%), lymphocytic hypophysitis (7%) |
| Gastrointestinal autoimmune diseases | Atrophic gastritis (13–27%), pernicious anaemia (0–15%), coeliac disease, autoimmune hepatitis (5–31%), malabsortion (18–22%) |
| Cutaneous autoimmune diseases | Vitiligo (0–25%), alopecia areata (13–72%) |
| Systemic autoimmune diseases | Sjögren's syndrome; rheumatoid arthritis |
| Immunological alterations | IgA deficiency, polyclonal hypergammaglobulinaemia |
| Others | Ectodermal dystrophy (10–52%); asplenia (very rare); malignant neoplasias (1–7%); calcification of basal ganglia, membrane tympani and sublenticular cataract; vasculitis; nephrocalcinosis. |

**Table 3.** APS-2 Clinical Manifestations and Prevalence [1].

| | | | |
|---|---|---|---|
| Major | Addison's disease | Same as for APS-1 (Table 1). | 100% |
| | Thyroid autoimmune diseases | Graves' disease, Hashimoto's thyroiditis, idiopathic myxoedema, asymptomatic thyroiditis, endocrine ophtalmopathy, pretibial myxoedema | 69–82% |
| | Type 1 Diabetes mellitus | | 30–52% |
| Minor | Hypergonadotropic hypogonadism (4–9%), vitiligo (4,5–11%), alopecia (1–4%), autoimmune hepatitis (4%), chronic atrophic gastritis (11%), pernicious anaemia (1–4,5%), hypophysitis, neoplasias (2%) | | |

**Table 4.** APS-3 Clinical Manifestations [1].

| **Thyroid autoimmune diseases** | | | |
|---|---|---|---|
| Hashimoto's thyroiditis<br>Idiopathic myxoedema Endocrine exophthalmos Graves' disease<br>Asymptomatic thyroiditis | | | |
| 3A<br>Endocrine diseases | 3B<br>Gastrointestinal apparatus | 3C<br>Skin/ haematopoietic/ nervous system | 3D<br>Connective tissue diseases/vasculitis |
| Type 1 DM<br>Hirata's syndrome<br>Premature ovarian failure<br>Lymphocytic hypophysitis<br>Neurohypophysitis | Atrophic gastritis<br>Pernicious anaemia<br>Coeliac disease<br>Chronic inflammatory bowel diseases<br>AIH<br>Primary biliary cirrhosis<br>Sclerosing cholangitis | Vitiligo<br>Alopecia<br>Autoimmune thrombocytopenia<br>Autoimmune haemolytic anaemia<br>Antiphospholipid syndrome<br>Myasthenia gravis<br>Stiff-man syndrome<br>Multiple sclerosis | SLE<br>Mixed connectivitis<br>Rheumatoid arthritis<br>Reactive arthritis<br>Scleroderma<br>Sjögren's syndrome<br>Vasculitis |

diseases not included in the previous groups. For example, AD associated with hypogonadism, chronic gastritis, etc, or type 1 DM with coeliac disease, Myasthenia gravis, etc.

## 3 Diagnostic criteria

Diagnosis is based on clinical criteria, since no specific laboratory test has been described to date. Hypoparathyroidism, Addison's disease or Diabetes mellitus with associated endocrine failure or malfunction can easily be detected by direct serum assays. But the proposed classification by Neufeld and Blizzard from 1980 is based on clinical criteria, describing four main syndromes (Table 5).

**Table 5.** Classification Criteria for APS. Adapted from [1].

| | |
|---|---|
| APS-1 | Chronic candidiasis, chronic hypoparathyroidism, Addison's disease (*at least two present*) |
| APS-2 | Addison's disease (*always present*) *with* autoimmune thyroid disease *and/or* type 1 Diabetes mellitus |
| APS-3 | Autoimmune thyroid disease *with* other autoimmune diseases (*excluding* Addison's disease *and/or* hypoparathyroidism) |
| APS-4 | Other combinations not included in the previous groups |

## 4 Diagnostic measurements for experts

The presence of immunological abnormalities or confirmed lymphocytic infiltration of the target-organ is not required for APS diagnosis. Although limited, there is a role for autoimmune and genetic tests. The presence of circulating, tissue-specific autoantibodies may be associated with or precede the clinical manifestations and serve as diagnostic markers — with the exception of ICA and/or GAD antibodies which seem to have low value for predicting type 1 DM. Several other autoantibodies are related to minor clinical manifestations as detailed in Table 6. Most of these tests cannot be performed on a routine basis. Non-organ specific autoantibodies are relatively common in patients with TAD, mostly common in APS-3 and are pivotal for the diagnosis of systemic autoimmune diseases such as SLE. Besides immunologic mechanisms, known genetic abnormalities or patterns are associated with APS. APS-1 is unique as a monogenic disease inherited as an autosomal recessive trait. The defective gene AIRE (Auto Immune Regulator) has been identified and is also the most representative mutation of all the APS. APS-2 has an autosomal dominant inheritance with incomplete penetration and correlates to different HLA alleles (increased frequency of HLA-DR3 and/or DR4). Genetic screening could be considered in high risk populations or close relatives of APS patients to allow early diagnosis and replacement treatment.

## 5 Requirements for family practitioners

The APS are very rare syndromes. For family practitioner, all that should be required is the identification of the main clinical manifestations and awareness of possible associations that should raise the suspicion of an APS for appropriate evaluation, follow up and reference to experts or differentiated centres. This knowledge will allow an adjusted approach and the identification of APS in a pre or subclini-

**Table 6.** APS and antibodies — adapted from [1].

| APS | Disease | Autoantibody to |
|---|---|---|
| APS-1 | CH | Parathyroid antibodies (11–38% patients), calcium-sensing receptors* |
| | AD | Adrenal cortex (ACA) (84% of patients show positivity for at least one of these autoantibodies): 21-OH*; P450 side chain cleavage (SCC) enzyme; 17α-OH |
| | Hypogonadism | Steroid-producing cells antibodies (StCA): 17α-OH and P450scc |
| | TAD | Peroxidase, thyroglobulin* ( positive in most patients) |
| | AIH | Anti-LKM*, P450-IA2, P450-2A6 |
| | Alopecia | Tyrosin |
| | Vitiligo | Melanocyte (complement-fixing), aromatic aminoacid decarboxylase (AADC), transcription factors Sox9 and Sox10* (63% of patients) |
| | Type 1 DM | Islet cell (ICA), glutamic acid decarboxylase (GAD), second islet antigen (IA-2) – high frequency in APS-1 patients but low correlation to type 1-DM |
| | Atrophic gastritis | Parietal cells*, intrinsic factor (if also pernicious anaemia), H+K+-ATPase |
| | Malabsorption | Tryptophan hydroxylase* (48% of patients) Endomysium (related to coeliac disease) |
| | Hypophysitis | Anti-pituitary (rare), prolactin-secreting cells |
| APS-2 | AD | ACA/21-OH (91% patients) |
| | Type 1 DM | High frequency of positive ICA, GAD or IA2 Abs |
| | Minor AID | Less frequent, usually associated to positive Abs |
| * The major autoantibodies related to clinical findings | | |

cal phase. When a child presents CC that can be the first manifestation of APS-1, it is important to maintain close observation and re-evaluation. Usually, endoscopic evaluations are not necessary and should be reserved for selected cases. The subsequent presence of symptomatic or asymptomatic hypocalcaemia may identify CH. In the initial evaluation, it is necessary to evaluate calcium serum levels, phosphate, parathyroid hormone (PTH) and 24 h urine calcium and phosphate. PTH serum levels should be low or undetectable with calcium serum levels low and phosphate levels high. Hypercalciuria is associated with low phosphate urinary elimination. About 50 % of APS-1 patients will present all three clinical criteria: CC, CH and AD. Routine laboratory abnormalities in AD may be absent in the early stages. In APS-1, as in APS-2, AD is typically associated with hyponatraemia, hypochloraemia, hyperkalaemia and reduced plasma osmolarity. Other possible findings are hypoglycaemia, mild eosinophilia with lymphocytosis and micro or macrocytic anaemia. For the diagnosis of AD, morning levels of ACTH are increased and cortisol reduced. Reduced levels of aldosterone (with increased plasma renin activity) and dehydroepiandrosterone are also present. The evaluation of TAD, in both APS2 and 3, implies determination of TSH, free T3 and T4 levels, anti-thyroid antibodies and thyroid ultrasound. Practitioners should also be aware of minor clinical manifestations.

## 6 Follow up

Life-long monitoring is important for all diagnosed patients. Persistent Candida infection can lead to epithelial carcinoma of the oral mucosa, tongue or oesophagus. In particular in APS-1, close follow-up of children with CC is mandatory for the early recognition of other features and for the risk of epithelial carcinoma.

All patients should be screened for a broad range of autoantibodies and regular re-evaluation should be considered (every 1–2 years). Special emphasis should be given to ACA/21-OH (diagnostic marker for AD) and thyroid antibodies (those with positive results should be monitored for the development of TAD). Considering APS-2, incomplete forms should be screened for subclinical diseases.

## 7 Management

Hormonal replacement therapy is mandatory in primary hypothyroidism and adrenal insufficiency. In APS-1, the standard treatment for CC is periodic administration of itraconazol, usually more effective for nail infections than mucosal infections. Insulin should be administered for type 1 DM. For CH, treatment is based on long term administration of calcium and vitamin D (already hydroxylated forms) *per os*. For acute treatment of symptomatic hypocalcaemia, intravenous calcium administration is required on an emergency basis with continuous electrocardiographic monitoring.

## 8 Diagnostic tests

Regarding the clinical manifestations of gland insufficiency, routine laboratory evaluation aims at assessing endocrine organ function. As mentioned earlier, in the presence of those clinical and laboratory findings, diagnosis is based on clinical criteria alone.

Diagnosis of an autoimmune disease includes the demonstration of serum autoantibodies and/or *in vitro* cell-mediated events, or the demonstration of lympho-monocyte infiltration in the target organ. But those findings are not required for diagnostic purposes.

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