



21-Hydroxylase-Autoantibodies

for the Differential Diagnosis of

Idiopathic Adrenal Insufficiency

**Isolated Addison's Disease or
Autoimmune Polyglandular Syndrome Typ I and II**

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Etiology and Pathogenesis

Chronic adrenal insufficiency is subdivided in primary insufficiency like Addison's disease and secondary insufficiency caused by dysfunction of hypothalamus and/or pituitary gland. [1, 3].

Addison's disease is the result of a continuing destruction of the adrenal gland. About 90 percent of the gland have to be destroyed before clinical symptoms of adrenal insufficiency appear.

In the past tuberculosis was responsible for Addisons's disease in 70-90 % of cases. Today the most common cause is an autoimmune process leading to an idiopathic atrophy of the gland.

In about one half of the patients with Addison's disease additional autoimmune endocrine disorders are found, especially insulin-dependent diabetes mellitus and thyroid diseases. The occurrence of two or more of these autoimmune diseases in one patient defines the autoimmune polyglandular syndrome (APS) type II. A mucocutaneous candidiasis as the first or leading symptom (70-80 %) determines the autoimmune polyglandular syndrome type I. Table 1 shows the characteristics sorted by frequency of both forms of APS.

Circulating antibodies against the adrenal cortex can be detected in about 70 % of patients with Addison's disease and in almost 100 % of patients with an autoimmune polyglandular syndrome. These antibodies appear many years before the onset of clinical symptoms and are disease specific [8].

The antibodies are directed against steroidogenic enzymes, in particular 21-hydroxylase, 17-alpha-hydroxylase and the P450 side-chain cleavage enzyme (P450_{scc}). Of all examined antibodies only those directed against 21-hydroxylase showed a high specificity for Addison's disease, whether isolated or part of a autoimmune polyglandular syndrome type I/II. 21-Hydroxylase, a protein of 55 kD, is a key enzyme in the steroid biosynthesis (conversion of 17-OH-progesterone or progesterone into 11-desoxycortisol).

According to recent investigations the demonstration of 21-hydroxylase antibodies is also of prognostic value.

808 children with organ-specific autoimmune diseases but without adrenal insufficiency were tested for 21-hydroxylase antibodies [5]. Almost all antibody positive children developed Addison's disease within 10 years (in average after 1 year). The initial concentration of the antibodies showed a clear correlation to the progression of the disease.

None of the antibody negative children developed an adrenal insufficiency.

In an analogous investigation with 8.840 adults Addison's disease developed in the group of initially antibody positive patients in 21 % and further 29 % showed a sub-clinical adrenal insufficiency within the observation period of 10 years. [4].

Table 1: Characteristics of the two types of autoimmune polyglandular syndrome

Type I	Type II
Addison' Disease	Addison's Disease
Hypoparathyroidism	Hashimoto Thyroiditis
Insulin-Dependent Diabetes Mellitus (IDDM)	IDDM
Mucocutaneous Candidiasis (First Symptom)	Hyperthyroidism (Graves' Disease)
Chronic aggressive Hepatitis	Myasthenia Gravis
Malabsorption Syndrom	Vitiligo
Alopecia	Pernicious Anemia
Keratopathy	Hypogonadism
Vitiligo	Alopecia
Pernicious Anemia	Celiac Disease
Hypogonadism	
Autoimmune Thyroid Disease	
Autosomal recessive transmitted (60-65% have 3-5 symptoms)	Associated with HLA-B8/DR-3 (beginning in 3. decade)

Clinical Features

Addison's disease is a rather rare illness with a prevalence of 40 - 60 cases per 1 million population and affects mainly women. The average age at diagnosis is 40 years (range 17 - 72 years) [1,6].

The symptoms are caused by the deficiency of cortisol and aldosterone. Weakness and tiredness are common, but also anorexia, nausea and vomiting, loss of weight, pigmentation of skin and mucous membrane, hypotension and sometimes hypoglycemia.

Depending on duration and extend of the adrenal insufficiency the symptoms can vary from complaining about persisting weakness to a most severe shock at acute massive destruction of the gland.

Table 2: Frequency of findings and symptoms with Addison's disease [1]

Weakness, Tiredness	99%
Pigmentation of Skin	98%
Weight Loss	97%
Anorexia, Nausea and Vomiting	90%
Hypotension (<110/70)	87%
Pigmentation of Mucous Membrane	82%
Stomach Ache	34%
Salt Hunger	22%
Diarrhea	20%
Constipation	19%
Vertigo, Syncope	16%
Vitiligo	9%

Hyperpigmentation can be well pronounced but its lack does not exclude the diagnosis Addison's disease (so-called "light-skinned Addison's Disease" in about 8 % of autoimmune adrenal insufficiency [2]). Addison's disease without hyperpigmentation is mainly seen in fair- or sandy-haired persons, who get no or little tan through sunlight.

Diagnosis

If primary adrenal insufficiency is suspected a rapid ACTH test (determination of plasma cortisol before and 60 minutes after iv bolus of synthetic ACTH) should be performed immediately. Alternatively, basal levels of both plasma cortisol and ACTH can be measured. In addition, aldosterone and plasma renin activity can be determined.

With increasing destruction of the adrenal cortex the plasma cortisol levels decrease. Free cortisol in urine is low or not detectable. Aldosterone concentrations are low or in the lower normal range.

However, in the beginning of hypoadrenalism the steroid concentrations are still within the normal range. Therefore, low normal cortisol levels do not exclude the diagnosis of adrenal insufficiency. In this case the rapid ACTH test is decisive for the diagnosis.

In overt adrenal insufficiency the basal ACTH level are elevated due to the negative feedback mechanism (in secondary adrenal insufficiency ACTH levels are low). The plasma renin activity is raised in almost all cases.

According to Betterle [4,5] the course of an adrenal insufficiency can be classified into 4 stages with the help of endocrine parameters.

Stage 0	normal adrenal function
Stage 1	raised plasma renin activity and normal to decreased serum aldosterone concentration
Stage 2	small or no increase of plasma cortisol after administration of ACTH (ACTH test)
Stage 3	elevated basal ACTH values
Stage 4	decreased basal plasma cortisol levels and appearance of typical symptoms of adrenal insufficiency

Differential Diagnosis

After the diagnosis of an idiopathic adrenal insufficiency the underlying disease has to be searched for. In table 3 the most frequent causes for idiopathic hypoadrenalism are listed.

In more than 70 % of cases an autoimmune process (Addison's disease) is responsible for the disorder. For the detection of the causing antibodies indirect immunofluorescence on adrenal cortex slices is used today in normal routine. This method includes all antibodies directed against adrenal antigens.

Table 3: Causes of idiopathic adrenal insufficiency [3]

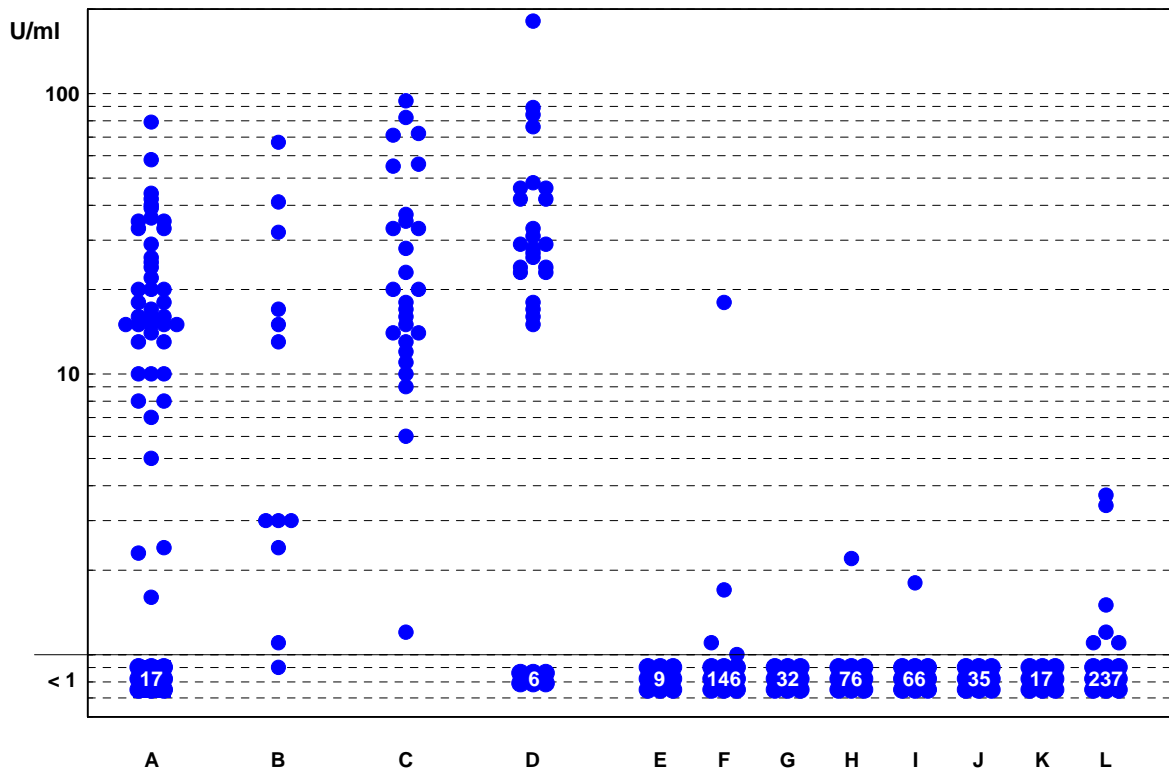
chronic	acute
<ul style="list-style-type: none">• Autoimmune Adrenalitis (Addison's, APS Type II, rarely Type I)• Tuberculosis• Mycosis (Histoplasmosis, Cryptococcosis, Blastomycosis, Coccidioidomycosis)• AIDS (Adrenal atrophy) (Cytomegaly, Bacteria, Protozoa)• Neoplasia (Metastases from Mamma-, Bronchial-, Kidney-Carcinoma, Non-Hodgkin-Lymphoma)• seldom: Sarcoidosis, Hemochromatosis	<p>Hemorrhage, Thrombosis, Necrosis in</p> <ul style="list-style-type: none">• Meningococcal Sepsis• Coagulation Disorder• Antiphospholipid Syndrome (SLE)

The clinical significance of the antibody measurement can be greatly enhanced by detecting specifically the 21-hydroxylase autoantibodies, which are solely responsible for Addison's disease [8,9].

For the test ¹²⁵Iodine labelled recombinant human enzyme (21-hydroxylase) is used. The labelled enzyme is incubated with patient serum and the corresponding antibodies bind to the protein. In a second step the formed immune complexes are precipitated with Protein A. The radioactivity in the resulting pellet is directly proportional to the amount of antibodies in the patients serum.

For establishing sensitivity, specificity and reference values samples from normal subjects (group L) and the following patient groups were measured [7]:

Group A	Isolated Addison's Disease	N = 60
Group B	Polyglandular Autoimmune Syndrome Type I	N = 12
Group C	Polyglandular Autoimmune Syndrome Type II	N = 27
Group D	Immunofluorescence positive	N = 30
Group E	Addison's Disease due to Tuberculosis	N = 9
Group F	Insulin-Dependent Diabetes Mellitus (IDDM)	N = 150
Group G	Non-Insulin-Dependent Diabetes mellitus (NIDDM)	N = 32
Group H	Graves' Disease	N = 77
Group I	Hashimoto's Thyroiditis	N = 67
Group J	Myasthenia Gravis	N = 35
Group K	Premature Ovarian Failure	N = 17
Group L	Healthy Blood Donors	N = 243



From these measurements the following values result:

Reference Range < 1 U/ml	Sensitivity		
	Isolated Addison's	(Group A)	72 %
	APS Type I/II	(Group B - C)	97 %
	Specificity	(Group E - L)	98 %

Therapy

All patients with Addison's disease have to receive a specific hormone substitution. The substitution has to compensate the lack of glucocorticoids and mineralcorticoids which are of utmost clinical importance. Like diabetics these patients need a close and careful follow-up of therapy.

(Hydro)cortisone is the major component of therapy. The dosage of cortisone varies between 20 and 30 mg/day. In stress situations the dosage has to be increased 2fold to 4fold. The glucocorticoids cortisol (30 mg/day) or prednisone (7.5 mg/day) given in several doses can also be used for substitution. These amounts of cortisone or cortisol do not replace the mineralcorticoid component of the adrenal gland. Therefore, the additional use of fludrocortisone (0.05-0.2 mg/day) is necessary as replacement for aldosterone.

Patients with acute adrenal failure need an immediate intravenous dose of hydrocortisone (100 mg bolus followed by infusion of 100 - 200 mg over 24 hours). Patients with hyponatremia and hypovolemia need additionally higher doses of intravenous isotonic saline and glucose solutions.

Literature

1. G.H. Williams, R.G. Dluhy (1994)
Endocrinology and Metabolism, Diseases of the Adrenal Gland
in Harrison's Principles of Internal Medicine
McGraw-Hill, Inc.
2. C.M. Brosnan, N.F.C. Gowing (1996)
Addison's Disease, Lesson of the Week
B.M.J. **312**: 1085-1087
3. W. Oelkers (1996)
Review Article: Adrenal Insufficiency
N. Engl. J. Med. **335**: 1206-1212
4. C. Betterle, M. Volpato, B. Rees Smith, J. Furmaniak, S. Chen, N.A. Greggio,
M. Sanzari, F. Tedesco, B. Pedini, M. Boscaro, F. Presotto (1997)
I. Adrenal cortex and steroid 21-hydroxylase autoantibodies in adult patients with organ-specific autoimmune diseases: markers of low progression to clinical Addison's disease
J. Clin. Endocrinol. Metab. **82**: 932-938
5. C. Betterle, M. Volpato, B. Rees Smith, J. Furmaniak, S. Chen, R. Zanchetta,
N.A. Greggio, B. Pedini, M. Boscaro, F. Presotto (1997)
II. Adrenal cortex and steroid 21-hydroxylase autoantibodies in children with organ-specific autoimmune diseases: markers of high progression to clinical Addison's disease
J. Clin. Endocrinol. Metab. **82**: 939-942
6. M-F. Kong, W. Jeffcoate (1994)
Eighty-six cases of Addison's disease
Clin. Endocrinology **41**: 757-761
7. H. Tanaka, M.S. Perez, M. Powell, J.F. Sanders, J. Sawicka, S. Chen,
L. Prentice, T. Asawa, C. Betterle, M. Volpato, B. Rees Smith, J. Furmaniak (1997)
Steroid 21-Hydroxylase Autoantibodies: measurements with a new immunoprecipitation assay
J. Clin. Endocrinol. Metab. **82**:
8. S. Chen, J. Sawicka, C. Betterle, M. Powell, L. Prentice, M. Volpato, B. Rees Smith, J. Furmaniak
(1996)
Autoantibodies to steroidogenic enzymes in autoimmune polyglandular syndrome, Addison's disease, and premature ovarian failure
J. Clin. Endocrinol. Metab. **81**: 1871-1876
9. A. Falorni, a. Nikoshkov, S. Laureti, E. Grenbäck, A-L. Hulting, G. Casucci, F. Santeusano, P.
Brunetti, H. Luthman, A. Lernmark (1995)
High diagnostic accuracy for idiopathic Addison's Disease with a sensitive radiobinding assay for autoantibodies against recombinant human 21-Hydroxylase
J. Clin. Endocrinol. Metab. **80**: 2752-2755

Further literature available upon request