The Natural History of Adrenal Insufficiency in X-Linked Adrenoleukodystrophy: An International Collaboration

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Context: Primary adrenal insufficiency is an important clinical manifestation of X-linked adrenoleukodystrophy (ALD). Other manifestations include spinal cord disease and/or inflammatory demyelinating cerebral disease. Implementation of newborn screening requires natural history data to develop follow-up recommendations.

Objective: To delineate the natural history of adrenal insufficiency in male patients with ALD and to assess associations between the risk for developing adrenal insufficiency, spinal cord disease, or cerebral disease and plasma C26:0/C22:0 and C24:0/C22:0 ratios, which are diagnostic biomarkers for ALD.

Design: Retrospective review of medical records.

Setting: Two international tertiary referral centers of expertise for ALD.

Patients: Male patients with ALD followed at the centers between 2002 and 2016.

Main Outcome Measures: The primary endpoint was adrenal insufficiency; secondary endpoints were spinal cord and cerebral disease.

Results: Data on 159 male patients was available. The probability of developing adrenal insufficiency was described with survival analysis. Median time until adrenal insufficiency was 14 years (95% CI, 9.70 to 18.30 years). The cumulative proportion of patients who developed adrenal insufficiency was age-dependent and highest in early childhood [0 to 10 years, 46.8% (SEM 0.041%); 11 to 40 years, 28.6% (SEM, 0.037%); >40 years, 5.6% (SEM, 0.038%)]. No association between clinical manifestations and plasma ratios was detected with Cox model or Spearman correlation.

Conclusions: Lifetime prevalence of adrenal insufficiency in male patients with ALD is ~80%. Adrenal insufficiency risk is time-dependent and warrants age-dependent follow-up. Besides on-demand testing if symptoms manifest, we suggest a minimum of adrenal testing every 4 to 6 months for patients age ≤10 years, annual testing for those age 11 to 40 years, and solely on-demand testing for those age >40 years. **(*J Clin Endocrinol Metab* 104: 118–126, 2019)**

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Abbreviations: ALD, adrenoleukodystrophy; AMC, Academic Medical Center; BMI, body mass index; MGH, Massachusetts General Hospital; VLCFA, very-long-chain fatty acid.

Primary adrenal insufficiency is a major clinical manifestation in boys and men with X-linked adrenoleukodystrophy (ALD; Online Mendelian Inheritance in Man number 300100), a progressive neurodegenerative inborn error of metabolism (1). This inborn error of metabolism is readily diagnosed by detecting elevated plasma very-long-chain fatty acids (VLCFAs), in particular the ratios of C26:0/C22:0 and C24:0/C22:0 (2–4). In addition to primary adrenal insufficiency, which is defined by low cortisol production in the adrenal glands despite sufficient levels of ACTH (5), other clinical manifestations include slowly progressive spinal cord disease and/or rapidly progressive inflammatory demyelinating cerebral disease (6, 7). Primary hypogonadism (with decreased androgen levels) has also been reported, but the clinical relevance is unclear (8).

The penetrance and natural history of adrenal insufficiency are unknown because large prospective natural history studies are lacking (1). However, the loss of adrenal function seems gradual and initially starts with elevated plasma ACTH levels before overt adrenal insufficiency with an abnormal cortisol response after cosyntropin stimulation and endocrine symptoms become apparent (1, 9). Average time to adrenal insufficiency or time from initial plasma ACTH elevation to onset of endocrine symptoms are unknown (9). In a prospective study among neurologically presymptomatic boys (n = 49), the adrenal function was impaired in 80% at time of the ALD diagnosis. Of the 14 boys followed longitudinally, one converted to overt adrenal insufficiency during the 2-year follow-up (9). In an additional study among 28 men with ALD, none converted to adrenal insufficiency during 3 years of follow-up (10), highlighting the need for larger cohorts and longer follow-up. Like adrenal insufficiency, cerebral disease is a prominent feature of ALD in childhood, with an estimated relative frequency of 40.5% (n = 2088) (6). Spinal cord disease, however, usually manifests in adulthood with a mean age of onset of 27 ± 11 years (n = 35) (11). Treatment options include adrenal steroid replacement therapy for adrenal insufficiency, supportive therapy for spinal cord disease, and stem cell transplantation for cerebral disease (1, 7, 12). Timely initiation of adrenal steroid replacement therapy and stem cell transplantation is essential. Therefore, ALD newborn screening has been initiated in several U.S. states and will be implemented in the Netherlands (13–16). With the implementation of newborn screening, questions about the follow-up of adrenal function in boys and men with ALD have become urgent. When should caregivers initiate adrenal function assessments, how often, and until what age?

Natural history data of adrenal insufficiency will help in the development of recommendations on adrenal function follow-up in male patients with ALD. Large prospective natural history studies have been initiated (1), but it will take years before sufficient data have been collected because ALD is a rare disorder (incidence, 1:14700 live births) (17) and the annual incidence of new cases of adrenal insufficiency among patients with ALD is low (9, 10). Follow-up recommendations will therefore be initially based on retrospective data. Moreover, because not all boys and men develop adrenal insufficiency, it would be informative for follow-up and counseling to assess whether certain predictors are associated with a higher risk for adrenal insufficiency. To date, associations between the clinical manifestations of ALD and other biological factors have not been identified (1, 18–20). We hypothesized that plasma C26:0/C22:0 and C24:0/C22:0 ratios, which are the diagnostic hallmarks of ALD (2–4), might be associated with the risk for developing adrenal insufficiency, spinal cord disease, or cerebral disease.

The purpose of this retrospective international multicenter study was to describe the natural history of adrenal insufficiency in boys and men with ALD. In addition, we aimed to assess associations between the risk for developing adrenal insufficiency, spinal cord disease, or cerebral disease and plasma C26:0/C22:0 and C24:0/C22:0 ratios.

Materials and Methods

Study design and patients

We (I.C.H., F.K.L., and R.A.B.) retrospectively reviewed medical records from male patients with ALD who have been followed at the Massachusetts General Hospital (MGH) in Boston, USA and the Academic Medical Center (AMC) in Amsterdam, Netherlands, between 2002 and 2016. Both centers are international tertiary referral centers of expertise for ALD. The local institutional review boards reviewed the study and waived the need for informed consent.

Procedures

The primary endpoint was adrenal insufficiency. Adrenal insufficiency was considered present if one of the following criteria was met. The first criterion was an abnormal cortisol response after standard dose (250 μg) intravenous (or intramuscular) cosyntropin stimulation (5). A cortisol response after intravenous cosyntropin stimulation was considered abnormal if the maximum peak cortisol level was ≤500 nmol/L (18.1 μg/dL) after 30 or 60 minutes. The second criterion was a morning plasma cortisol level <140 nmol/L (5.1 μg/dL) and a morning plasma ACTH level twofold above the upper limit of the reference range. The third criterion was receipt of daily glucocorticoid therapy for primary adrenal insufficiency. This last criterion was added because we could not retrieve initial laboratory test results of patients who received their adrenal insufficiency diagnosis decades ago. Moreover, if patients were started on daily glucocorticoid replacement therapy despite laboratory results that did not adhere to the first two criteria at initiation, they were still labeled as having adrenal insufficiency because the exact onset of adrenal insufficiency in these patients could not be established according to our criteria once they were

receiving replacement therapy. Adrenal insufficiency was not considered present if daily glucocorticoid therapy was prescribed for another indication than primary adrenal insufficiency, if stress dosing was prescribed as a precaution, if only morning plasma ACTH levels were elevated, or if the patient had endocrine symptoms with sufficient plasma morning or stimulated ACTH and cortisol test results. Time to adrenal insufficiency was defined as the age at which one of the criteria was met.

Secondary endpoints were spinal cord disease and cerebral disease. Spinal cord disease was considered present if both symptoms and signs were present at neurologic examination. Symptoms considered were sensory complaints, a gait disorder, or urge incontinence for urine or stool. Signs at neurologic examination included muscle weakness, pathological reflexes, sensory abnormalities, or spasticity. Time to spinal cord disease was defined as the age at which both symptoms and signs of spinal cord disease were first documented. Cerebral disease was considered present if there were white matter lesions on brain MRI beyond the corticospinal tracts. Time to cerebral disease was defined as the year at which white matter lesions on MRI beyond the corticospinal tracts were first documented.

Besides the presence and time of onset of endpoints, we collected information on initiation and indication of glucocorticoid and mineralocorticoid replacement therapy, age at diagnosis of ALD, year of last follow-up, and the presence of endocrine symptoms at diagnosis of adrenal insufficiency and at last follow-up. Endocrine symptoms included hyperpigmentation, unintentional weight loss, orthostatic symptoms, diarrhea, salt craving, nausea, and symptoms associated with acute adrenal insufficiency (Addisonian crisis), including unconsciousness and hypotension. In addition, we were interested in possible complications of adrenal insufficiency overtreatment with glucocorticoids and therefore extracted data on last documented blood pressure and body mass index (BMI) to evaluate the frequency of hypertension and obesity. For adults, a systolic blood pressure $\geq$140 mm Hg or a diastolic blood pressure $\geq$90 mm Hg was labeled as hypertension; in children, hypertension was defined as a systolic or diastolic blood pressure $\geq$95th percentile. Likewise, obesity was defined as a BMI $\geq$30 kg/m^2 in adults and as BMI $\geq$95th percentile in children. Moreover, we assessed the frequency of testosterone replacement therapy due to primary hypogonadism and whether patients fathered any children, with or without fertility treatment. In addition to the clinical data, we collected reports of plasma C26:0/C22:0 and C24:0/C22:0 ratios from retrospective medical records.

Statistical analysis

Analyses were performed with SPSS statistical software, version 24 (IBM Inc., Armonk, NY). First, we summarized the clinical and biochemical data. Continuous variables were presented as means with SDs if normally distributed or as medians with ranges if not normally distributed (21). Differences between groups were assessed with Mann-Whitney *U* tests (non–normally distributed continuous data) and χ^2 tests for homogeneity (binary data). Significance was defined as a P value < 0.05. We then used survival analysis to describe the event-free probability distribution of adrenal insufficiency, spinal cord disease, and cerebral disease. Results were visualized in Kaplan-Meier plots. Moreover, for assessment of associations between the risk for developing adrenal insufficiency, spinal cord disease, or cerebral disease and plasma C26:0/C22:0 and C24:0/C22:0 ratios, we used a multivariate Cox proportional hazards model and Spearman rank correlation. Center (*i.e.*, MGH or AMC) was included as a covariate in the Cox models to assess local differences in clinical practice. Visual inspection of the log-log survival plots of center, plasma C26:0/C22:0 ratio, and C24:0/C22:0 ratio confirmed that the proportional hazards assumption was not violated. Center, plasma C26:0/C22:0 ratio, and C24:0/C22:0 ratios were included in the final multivariate Cox proportional hazards model if significant in an univariate model. Subsequently, we used a Spearman rank correlation to assess the correlation between onset of adrenal insufficiency at age $\leq$10.0 years, onset of spinal cord disease at age $\leq$30.0 years, or onset of cerebral disease at age $\leq$10.0 years and plasma C26:0/C22:0 and C24:0/C22:0 ratios. The age categories were chosen on the basis of the presumed predominant onset of adrenal insufficiency and cerebral disease in the first decade of life (6, 9), and spinal cord disease in the third decade (11).

Results

Data collection was completed on November 4, 2016, after which the database was locked for analysis. We included data on 159 male patients with ALD (n = 86 from MGH; n = 73 from AMC). At last follow-up, median age was 24 years (range, 1 to 71 years), and 64 of 159 (40.3%) of patients were age <18 years. Adrenal insufficiency was present in 113 of 159 (71.1%). The youngest patient with adrenal insufficiency was age 7 months, and the oldest patient without adrenal insufficiency was 71 years. Spinal cord disease was present in 65 of 156 (41.7%, three unknown). The youngest patient with spinal cord disease was 17 years, and the oldest without was 64 years. Cerebral disease was present in 82 of 151 (53.3%; eight unknown); the youngest patient with cerebral disease was age 4 years and the oldest without was age 71 years. Adrenal insufficiency was the first manifestation of ALD in 60 of 159 (37.7%) patients, spinal cord disease in 40 of 159 (25.2%) and cerebral disease in 23/159 (14.5%). Adrenal insufficiency and spinal cord disease manifested simultaneously in two patients, and 34 of 159 (21.4%) patients remained asymptomatic. Cohort characteristics and median plasma C26:0/C22:0 and C24:0/C22:0 ratios are listed in Table 1.

Clinical characteristics of the MGH and AMC cohort were not similar (Table 2). Age at last follow-up for the AMC cohort (mean rank, 98.12) was significantly higher than for MGH (mean rank, 64.62; $P < 0.0005$). In addition, in accordance with the difference in age at last follow-up, the proportion of children in the AMC cohort (20.5%) was significantly lower than in the MGH cohort (57.0%; $P < 0.0005$). The frequency of adrenal insufficiency was similar in both cohorts ($P = 0.76$), but spinal cord disease and cerebral disease were more frequent in the MGH cohort ($P = 0.036$ and $P = 0.010$, respectively).

Adrenal function was assessed with stimulation testing in 51 of 159 patients (32.1%), morning cortisol levels in

Table 1. General Cohort Characteristics

Characteristic	Value
Total patients, n	159
MGH	86/159 (54.1)
AMC	73/159 (45.9)
At last follow-up	
Age, y	24 (1–71)
Children	64/159 (40.3)
Adrenal insufficiency	113/159 (71.1)
Spinal cord disease	65/156 (41.7)
Cerebral disease	82/151 (53.3)
Biochemistry	
C26:0/C22:0 ratio	0.059 (0.02–0.13)
C24:0/C22:0 ratio	1.47 (0.86–1.89)

Data are expressed as absolute number (percentage) or median (range).

six of 159 patients (3.8%), and random cortisol (with or without ACTH) in 34 of 159 patients (21.4%). In 68 of 159 (42.8%) patients, no laboratory results could be retrieved at diagnosis of adrenal insufficiency (59 of 68, 86.8%) or at last follow-up if patients had not been diagnosed with adrenal insufficiency until then (nine of 46 with no adrenal insufficiency, 19.6%). Of the patients with stimulation testing, 30 of 51 (58.8%) had abnormal test results (maximum peak cortisol level $\leq$500 nmol/L or 18.1 μg/dL) and in six of 51 (11.8%) the results were (borderline) normal, but they were started on glucocorticoid therapy nonetheless. Of the patients with morning cortisol testing, one of six (16.7%) had a cortisol value <140 nmol/L (5.1 μg/dL) and two of six (33.3%) had a normal cortisol value and were still started on glucocorticoid therapy.

Random laboratory testing was not included in our definition of adrenal insufficiency, but 15 of 34 patients (44.1%) with random testing were started on glucocorticoid therapy. In these patients ACTH levels were increased >10-fold the upper limit of the reference range in nine of 15 (60%), >2-fold in one of 15 (6.7%), >1-fold in one of 15 (6.7%) with a cortisol level of 225 nmol/L (8.2 μg/dL), and in three of 15 (20%) cortisol levels were 140 to 176 nmol/L (5.1 to 6.4 μg/dL) with normal or missing ACTH levels.

Of the 113 of 159 patients with adrenal insufficiency, 68 of 101 (67.3%; 12 unknown) had endocrine symptoms at diagnosis of adrenal insufficiency. Median delay between onset of endocrine symptoms and diagnosis of adrenal insufficiency was 3.5 years (range, 0.25 to 21 years).

Daily glucocorticoid replacement therapy was used in 105 of 113 (92.9%) patients. Most patients (90 of 105, 85.7%) used hydrocortisone, but other substitutes included prednisone (n = 11), prednisolone (n = 1), or dexamethasone (n = 3). Median daily hydrocortisone dose was 20 mg (range, 8.0 to 80.0 mg), adjusted for body surface area 10.2 mg/m^2 (range, 3.12 to 41.03 mg/m^2). The indication for glucocorticoid replacement therapy was an acute presentation in 20 of 92 patients (21.7%; 13 unknown). In the remaining patients with a chronic presentation, replacement therapy was initiated because of abnormal laboratory results in the absence of endocrine symptoms in 43 of 92 (46.7%) and abnormal laboratory results with endocrine symptoms in 29 of 92 (31.5%). The youngest age at which glucocorticoid therapy was initiated was 7 months, after abnormal laboratory testing in the absence of endocrine symptoms.

Daily mineralocorticoid replacement therapy was used in 58 of 113 (51.3%) patients with a median daily fludrocortisone dose of 100 μg (range, 31.25 to 200). All patients with mineralocorticoid replacement therapy used fludrocortisone. The youngest age at which mineralocorticoid replacement therapy was initiated was 7 months. Data on the presence of symptoms (orthostatic symptoms or salt craving), hyperkalemia, or elevated plasma renin activity as an indication for initiation of mineralocorticoid therapy were available for 19 patients with mineralocorticoid replacement. Symptoms were an indication for initiation of mineralocorticoid therapy in six of 19 (31.6%), hyperkalemia in three of 19 (15.8%), and elevated plasma renin activity in seven of 19 (36.8%). One patient had both hyperkalemia and elevated renin activity, and in four patients, mineralocorticoid therapy was initiated despite absent symptoms, hyperkalemia, or elevated plasma renin activity. Combination therapy of glucocorticoid and mineralocorticoid

Table 2. Clinical Differences Between Centers

			MGH		AMC		
Variable	Patients (n)	Test Statistic	Patients (n)	Mean Rank or Proportion (%)	Patients (n)	Mean Rank or Proportion (%)	*P* Value
Age at last follow-up	159	4461.500	86	64.62	73	98.12	<0.0005
Proportion of children	159	21.787	86	57.0	73	20.5	<0.0005
Adrenal insufficiency	159	0.095	86	72.1	73	69.9	0.757
Spinal cord disease	156	4.379	85	34.1	71	50.7	0.036
Cerebral disease	151	6.669	85	63.5	66	42.4	0.010

Differences between centers were assessed with a Mann-Whitney *U* test for non–normally distributed continuous data and the χ^2 test for homogeneity for binary data.

replacement was used in 58 of 113 (51.3%) patients and monotherapy with glucocorticoid replacement in 47 of 113 (41.6%) patients. No patients had mineralocorticoid monotherapy. Seven patients with adrenal insufficiency solely used glucocorticoid replacement stress dosing, and one patient with adrenal insufficiency did not use any kind of replacement therapy at time of the database lock. This patient had an abnormal cortisol response after cosyntropin stimulation without endocrine symptoms and cerebral disease and was thus referred to another center for stem cell transplantation and concomitant endocrine follow-up. Details of the treatment of adrenal insufficiency are summarized in Table 3.

Hypertension at last follow-up was detected in eight of 64 (12.5%) adult patients with adrenal insufficiency and glucocorticoid replacement therapy, and six of 41 (14.6%) boys with adrenal insufficiency and glucocorticoid replacement therapy. Obesity at last follow-up was detected in five of 47 (10.7%, 17 missing) adult patients with adrenal insufficiency and glucocorticoid replacement therapy and 11 of 38 (28.9%, three missing) boys with adrenal insufficiency and glucocorticoid replacement therapy. Logistic regression models were run among patients with adrenal insufficiency to assess the effect of daily glucocorticoid dose on the likelihood of having hypertension and obesity. Glucocorticoid dose was not a statistically significant predictor for hypertension, and an increase in glucocorticoid daily dose was associated with a lower likelihood of having obesity (coefficient, −0.39; odds ratio, 0.68; 95% CI, 0.49 to 0.94; $P = 0.020$), but these analyses were not corrected for duration of glucocorticoid therapy or adherence.

In four of 158 (2.5%; one unknown) patients, the use of testosterone replacement therapy due to primary hypogonadism was documented. In two of these patients, age at initiation of testosterone replacement was noted, namely, 26 and 33 years. There was only one patient who fathered children with the help of fertility treatment and 34 of 95 (35.8%) adult patients fathered children without medical support. There were 58 of 95 (61.1%) adult patients who had not tried to conceive children or for whom data on fertility status were unavailable.

Median time until 50% of patients had adrenal insufficiency was 14 years (95% CI, 9.70 to 18.30 years) (Fig. 1). At 56 years of age, 80% (SEM, 0.037%) had developed adrenal insufficiency. The event-free probability function showed a negative exponential distribution, indicating that the cumulative proportion of patients who developed adrenal insufficiency was highest between 0 and 10 years, at 46.8% (SEM, 0.041%). Thereafter, an additional 28.6% (SEM, 0.037%) developed adrenal insufficiency between 10 and 40 years of age. The remaining 5.6% (SEM, 0.038%) developed adrenal insufficiency after 40 years of age.

Median time until glucocorticoid replacement therapy was 16 years (95% CI, 8.76 to 23.24 years), and median time until mineralocorticoid replacement therapy was 56 years (95% CI, 28.69 to 83.31 years). At 61 years, 80% (SEM, 0.043%) were receiving glucocorticoid therapy, and at 63 years, 57% (SEM, 0.067%) were receiving mineralocorticoid therapy. Median time until spinal cord disease was 35 years (95% CI, 32.86 to 37.14 years). At 60 years of age, >90% (SEM, 0.036%) had developed spinal cord disease. Median time until cerebral disease was 35 years (95% CI, 28.61 to 41.39 years). Up to age 10 years, 30% (SEM, 0.038%) had developed cerebral disease. At 64 years, 80% (SEM, 0.065%) had developed cerebral disease (Fig. 2).

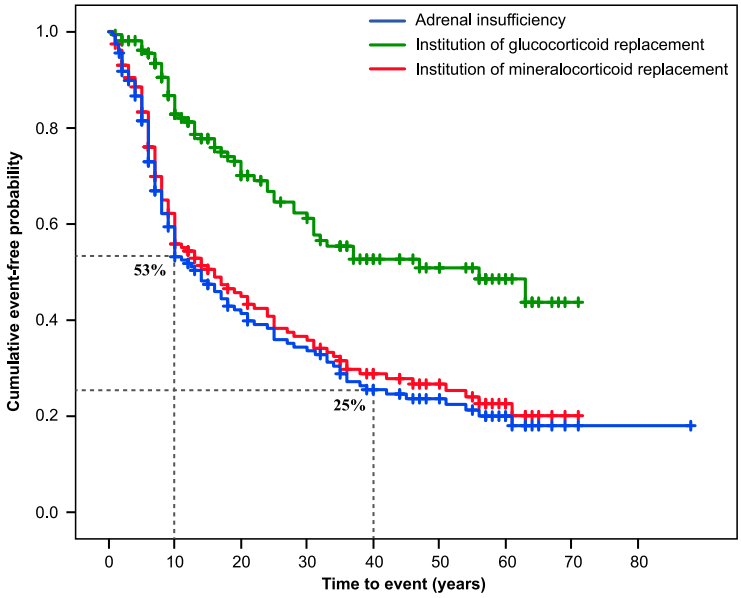
The Cox proportional hazards models detected a significant effect of center on the risk for developing adrenal insufficiency. Patients at MGH had an increased risk for adrenal insufficiency of 54% (95% CI, 1.06% to 2.25%; $P = 0.025$) compared with patients from the AMC. A similar association was found between hospital and spinal cord or cerebral disease. There was no significant effect of plasma C26:0/C22:0 and C24:0/C22:0 ratios on the risk for adrenal insufficiency. Nor was there an association between the ratios and the risk for spinal cord or cerebral disease (Table 4). Likewise, no significant correlations were found between onset of adrenal insufficiency at age ≤10.0 years, onset of spinal cord disease at age ≤30.0 years, or onset of cerebral disease at age ≤10 years and plasma C26:0/C22:0 and C24:0/C22:0 ratios (Table 5).

Table 3. Adrenal Insufficiency Details

Variable	Adrenal insufficiency (n=113)
Endocrine symptoms at diagnosis	68/101 (67.3)
Diagnostic delay, y	3.5 (0.25–21)
Glucocorticoid replacement therapy	105/113 (92.9)
Glucocorticoid dose, mg/m^2/d	10.2 (3.12–41.03)
Mineralocorticoid replacement therapy	58/113 (51.3)
Mineralocorticoid dose, mcg/d	100 (31.25–200)
Hypertension at last follow-up[a]	14/105 (13.3)
Children	6/41 (14.6)
Adults	8/64 (12.5)
Obesity at last follow -up[a]	16/85 (18.8)
Children	11/38 (28.9)
Adults	5/47 (10.6)

Data are expressed as absolute number (percentage) or median (range). Diagnostic delay indicates the time between onset of endocrine symptoms and the diagnosis of adrenal insufficiency.

[a]In patients with adrenal insufficiency and receiving glucocorticoid replacement therapy.



Numbers at risk										Censored
Adrenal insufficiency	159	85	54	43	29	20	11	2	1	46
Glucocorticoid replacement	159	87	55	43	29	20	10	1	0	54
Mineralcorticoid replacement	157	111	73	54	34	25	12	1	0	101

Figure 1. Event-free probability functions of adrenal insufficiency, institution of glucocorticoid replacement therapy, and institution of mineralocorticoid replacement therapy.

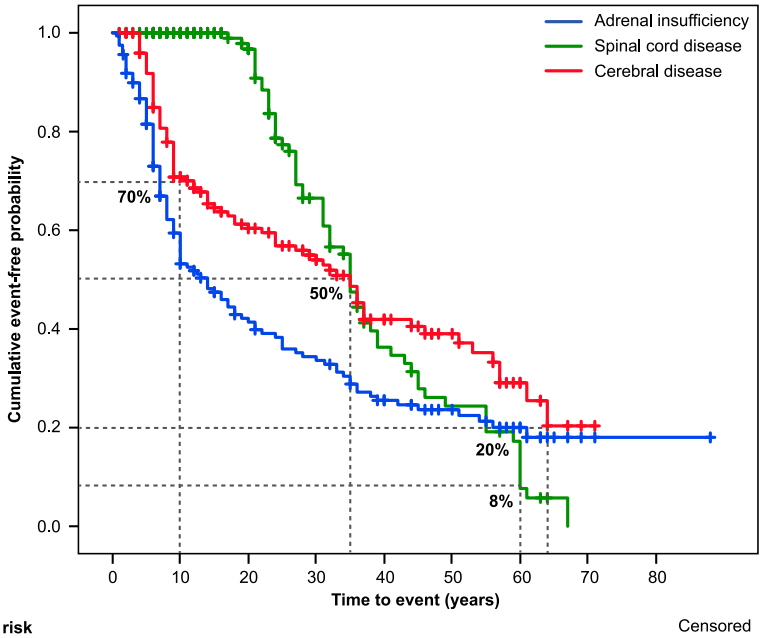
Discussion

This retrospective, international, multicenter study suggests that the lifetime prevalence of adrenal insufficiency in ALD is ~80%. The risk for adrenal insufficiency varies throughout life and is highest in the first decade. Plasma C26:0/C22:0 and C24:0/C22:0 ratios, although diagnostic for ALD, are not associated with the (age-dependent) risk of developing adrenal insufficiency, spinal cord disease, or cerebral disease.

Our results have implications for the follow-up of boys and men with ALD. In accordance with findings of others, abnormal plasma ACTH and cortisol levels preceded endocrine symptoms in most patients (43 of 92, 46.7%), warranting the need for regular assessments of the adrenal function in boys and men without endocrine symptoms (9). The use of survival analyses allowed us to describe the development of adrenal insufficiency over time, while including a heterogeneous cohort of boys and men of all ages. The cumulative probability of adrenal insufficiency was highest until the age of 10 years (46.8%), remained prominent until 40 years of age (an additional 28.6%), and decreased substantially thereafter (an additional 5.6%). This age-dependent risk helps determine the follow-up frequency for adrenal monitoring.

Because our study did not allow for approximation of time between (minor) biochemical abnormalities and overt adrenal insufficiency, the exact frequency of adrenal testing remains controversial. However, we suggest—besides on-demand testing if endocrine symptoms manifest—routine adrenal testing every 4 to 6 months until 10 years of age, annual testing thereafter until 40 years of age, and solely on-demand testing in case endocrine symptoms manifest from age 41 years onward. The youngest age at which adrenal insufficiency was diagnosed in this cohort was 7 months, but a baby age 5 months with biochemical abnormalities of adrenal insufficiency and no endocrine symptoms has been described by others (9). We therefore recommend initiating adrenal testing in the first 6 months of life. Although our data do not warrant a different screening frequency for infants and children age >1 year, we urge physicians to remain vigilant when following infants because symptoms of adrenal insufficiency may be especially vague in this age group.

Importantly, the mineralocorticoid function often remains intact, and



Numbers at risk										Censored
Adrenal insufficiency	159	85	54	43	29	20	11	2	1	46
Spinal cord disease	156	123	86	47	22	14	8	0	-	91
Cerebral disease	151	97	71	54	32	21	9	1	-	69

Figure 2. Event-free probability functions of adrenal insufficiency, spinal cord disease, and cerebral disease.

Table 4. Univariate Cox Proportional Hazards Models

Variable	Hazard Ratio (95% CI)	*P* Value
Adrenal insufficiency		
Center[a]	1.541 (1.057–2.248)	0.025
C26:0/C22:0	113.191 (0.000–4.066 × 10^4)	0.469
C24:0/C22:0	0.498 (0.161–1.538)	0.225
Spinal cord disease		
Center[a]	2.631 (1.541–4.492)	<0.0005
C26:0/C22:0	1.072 × 10^5 (0.000–4.823 × 10^{14})	0.307
C24:0/C22:0	1.079 (0.176–6.615)	0.934
Cerebral disease		
Center[a]	2.504 (1.558–4.025)	<0.0005
C26:0/C22:0	109.261 (0.000–1.495 × 10^8)	0.515
C24:0/C22:0	3.156 (0.924–10.785)	0.067

[a]Reference center is AMC in the Netherlands.

therefore mineralocorticoid replacement therapy should not be initiated unless abnormal signs become manifest. In addition, if adrenal insufficiency has become manifest and treatment has been initiated, clinicians must remain cautious for signs of over- (or under-) treatment.

The frequency of hypertension in adults with adrenal insufficiency and glucocorticoid replacement therapy was low (12.5%) in comparison with the 37.4% to 48.6% reported in the general population (22, 23). In contrast, the frequency of hypertension in boys (14.6%) seemed relatively high in comparison with the general population aged 12 or older (~5% between ages 12 and 18 years) (24). However, the frequency of hypertension in our cohort should be interpreted with caution because we used the blood pressure documented at last follow-up for the assessment of hypertension, which is likely biased by measurement methods and/or anxiety in the medical environment ("white coat syndrome"). Likewise, the frequency of obesity in men with adrenal insufficiency and glucocorticoid replacement therapy was low (12%) in comparison with the 34.9% to 60% reported in the general population (22, 25). Moreover, the frequency of obesity in boys (28.9%) also seemed relatively high in comparison with the 13.3% to 17.3% reported in the general population (26, 27). The frequency of primary hypogonadism for which testosterone replacement therapy was initiated was low in our cohort, at 2.5%, but this is most likely an underestimation because the gonadal function was not routinely checked in all patients.

Table 5. Event and Plasma C26:0/C22:0 and C24:0/C22:0 Correlations

Variable	C26:0/C22:0	C24:0/C22:0
Onset adrenal insufficiency ≤10.0 years		
r	−0.086	−0.113
P value	0.538	0.424
Patients, n	54	52
Onset spinal cord disease ≤30.0 years		
r	0.117	0.109
P value	0.525	0.560
Patients, n	32	31
Onset cerebral disease ≤ 10.0 years		
r	−0.023	0.060
P value	0.890	0.720
Patients, n	39	38

Correlations were assessed with Spearman rank-order correlation. The significance level was set at 0.01 (Bonferroni correction for multiple comparisons).

Because not all boys and men develop adrenal insufficiency, we sought to assess whether C26:0/C22:0 and C24:0/C22:0 ratios were associated with the risk for adrenal insufficiency, or with the secondary outcome measures spinal cord disease and cerebral disease. Like other researchers, we could not identify such a relationship (19, 20), again highlighting that the pathophysiology of adrenal insufficiency in ALD remains poorly understood. All patients with ALD have a genetic defect in the *ABCD1* gene (http://www.adrenoleukodystrophy.info) (28). The *ABCD1* gene encodes the ALD protein, a protein essential for the transport of acylated VLCFA across the membrane of the peroxisome, where VLCFAs are degraded (29, 30). *ABCD1* deficiency subsequently results in accumulation of VLCFAs in both men with ALD and women heterozygous for the gene defect. How does VLCFA accumulation result in adrenal insufficiency? VLCFAs accumulate in the zona reticularis and zona fasciculate of the adrenal cortex. The relative sparing of the zona glomerulosa coincides with the finding by us and others that the mineralocorticoid function remains intact in most patients (31, 32). Moreover, *in vitro*, cortisol release is diminished after stimulation with ACTH in the presence of VLCFA (33).

Although VLCFA accumulation is present in all patients, not all men develop adrenal insufficiency, and adrenal insufficiency is extremely rare in heterozygous women (34, 35). Besides the observation that not all

male patients develop adrenal insufficiency, we found an age-dependent risk for developing adrenal insufficiency that was highest until age 10 years and decreased thereafter. Interestingly, this time frame falls into the period with highest susceptibility for conversion to cerebral disease (36), although adrenal insufficiency appears to develop even earlier than cerebral disease (Fig. 2).

Naturally, some caution is advised in the interpretation of our results. Despite our international collaboration, the cohort is still relatively small. The small sample size might have caused the controversial association between a higher daily glucocorticoid dose and a lower likelihood of obesity among patients with adrenal insufficiency. In addition, it is also possible that patients with obesity were previously exposed to higher glucocorticoid doses and their glucocorticoids were subsequently downtitrated to limit weight gain. Moreover, we did not correct for severity of spinal cord disease in this analysis. More disability could result in more weight gain. Admittedly, retrospective data are not perfect, and missing data were prominent. Determining the time of onset of adrenal insufficiency according to current laboratory thresholds was possible only for a subset of patients; therefore, presumed onset of adrenal insufficiency in the remaining patients could differ from onset of adrenal insufficiency if diagnosed according to current standards. Unfortunately, it would take years or even decades to gather enough data on adrenal insufficiency to describe the natural history prospectively because patient numbers are small and incidence is low. The MGH and AMC cohorts were not similar, and center was a significant predictor in the Cox models; this suggests that local practice management is likely different and the cohorts were subject to selection bias. Selection bias has been a major limitation for all studies on ALD, but newborn screening finally provides the means to overcome this bias as all boys in participating states and countries will be diagnosed.

In conclusion, most, but not all, male patients with ALD develop adrenal insufficiency. The risk for adrenal insufficiency varies over time, warranting an age-dependent follow-up frequency of adrenal function. Over time, long-term prospective follow-up of babies diagnosed through newborn screening will elucidate the true natural history of adrenal insufficiency in ALD. Meanwhile, the development of treatment strategies toward reduction of VLCFA accumulation or even restoration of the genetic defect continues, and in the future these might be able to prevent onset of adrenal insufficiency all together.

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