



Determining cut-off values to increase selectivity and specificity in X-ALD testing within at-risk populations

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Who we are

Scope of Activities

Medical diagnostics, including:

general diagnostics - thyroid and steroid hormones, biogenic amines, vitamin D metabolites, fat-soluble vitamins, and CoQ10,

inherited metabolic disorders - amino acids, acylcarnitines, purines, pyrimidines, organic acids,

mainly based on microsampling methods, particularly DBS (Dried Blood Spot) and VAMS (Volumetric Absorptive Microsampling, Capitainer).

Toxicology

R&D in the field of metabolomic studies resulting in c.a. 5 original scientific publications/year with an average impact factor (IF) of 4.9.

Equipment

8 (U)HPLC-TQD/QTRAP-MS/MS systems from Sciex/Bruker

1 DART-TQD-MS/MS system from Bruker

Staff

20 staff members, 7 with a PhD degree



Why X-ALD?

Prevalence

The most common peroxisomal disorder - 1:17 000 (male and female).

Description

Onset, symptoms and disease progression are highly variable and unpredictable:

- ♂ adrenal failure (80%, <18y),
rapidly progressing leukodystrophy (40%, <18y),
slowly (years) progressing myelopathy and peripheral neuropathy with complete penetrance.
- ♀ no adrenal failure or leukodystrophy,
slowly progressing (decades) myelopathy and peripheral neuropathy (90%, >40y).

Management and treatment

Myelopathy - no treatment,

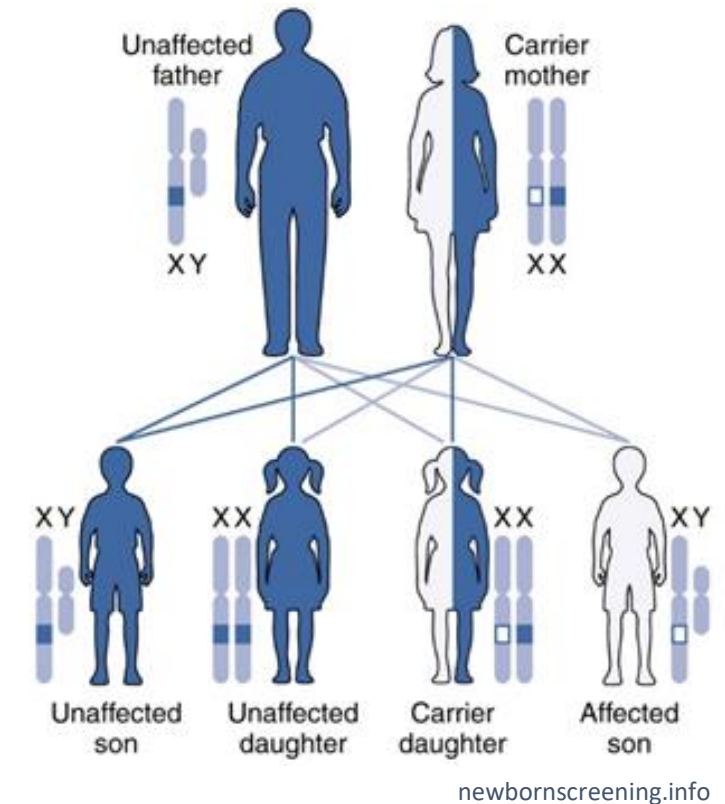
Leukodystrophy - allogeneic hematopoietic cell transplant (HCT) in the early stages.

Prognosis

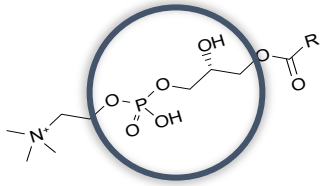
Early diagnosis prevents severe morbidity and mortality and with appropriate supportive care life expectancy is near normal.

Diagnosis

WGS/WES or LC/GC-MS – based assays.



Let's start with the method



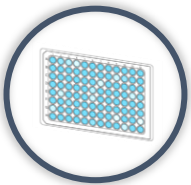
Markers

Lysophosphatidylcholines – LPC - 26:0, 24:0, 22:0



Matrix

DBS



Extraction

30min extraction in MeOH at 37°C



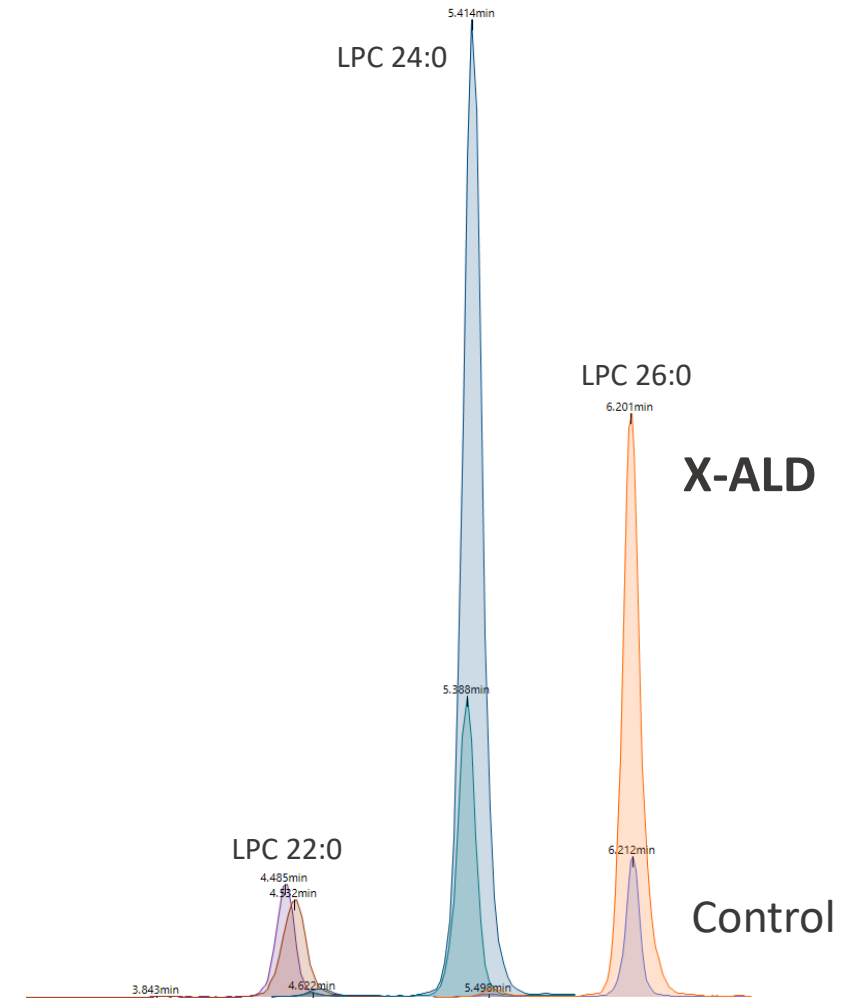
Instrumentation

LC-MS/MS Bruker EVOQ TQ+ coupled with UPLC and PAL3

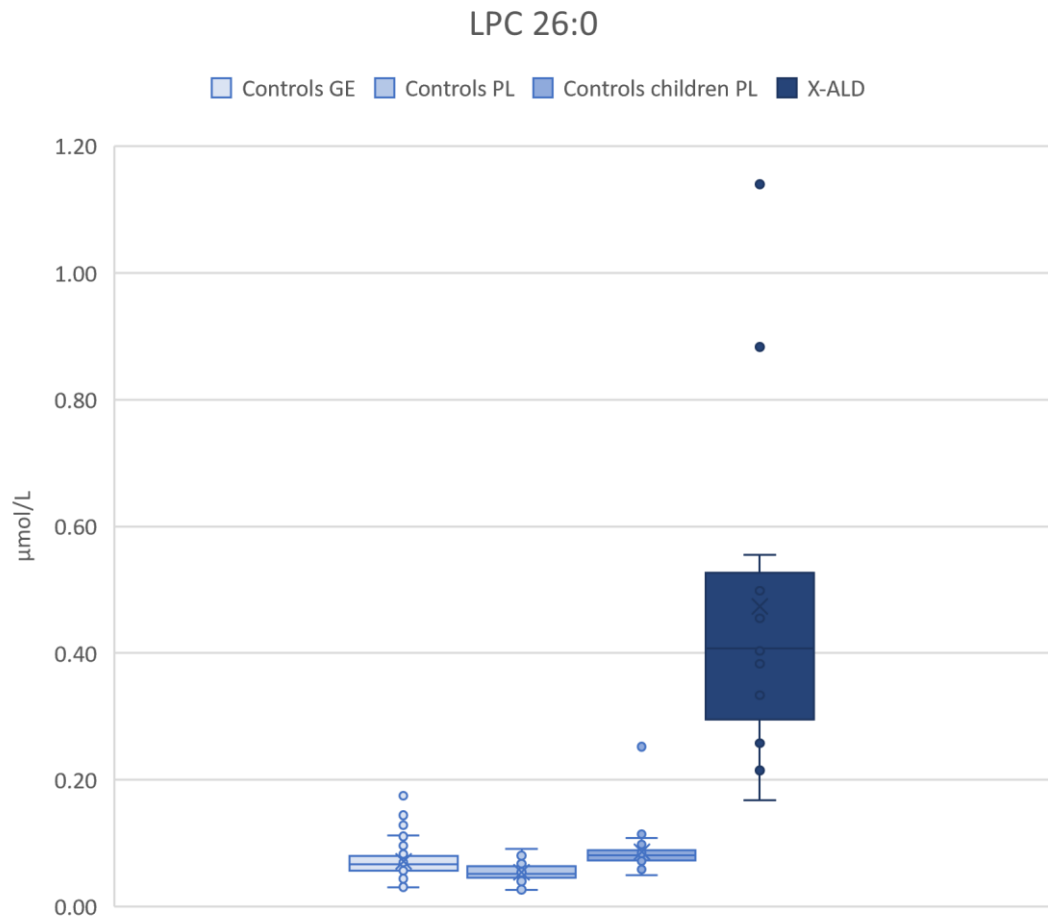


Analytical validation

Accuracy and repeatability <10%,
minimal volcano and volume effects



Then on to the clinical validation



Controls n=280

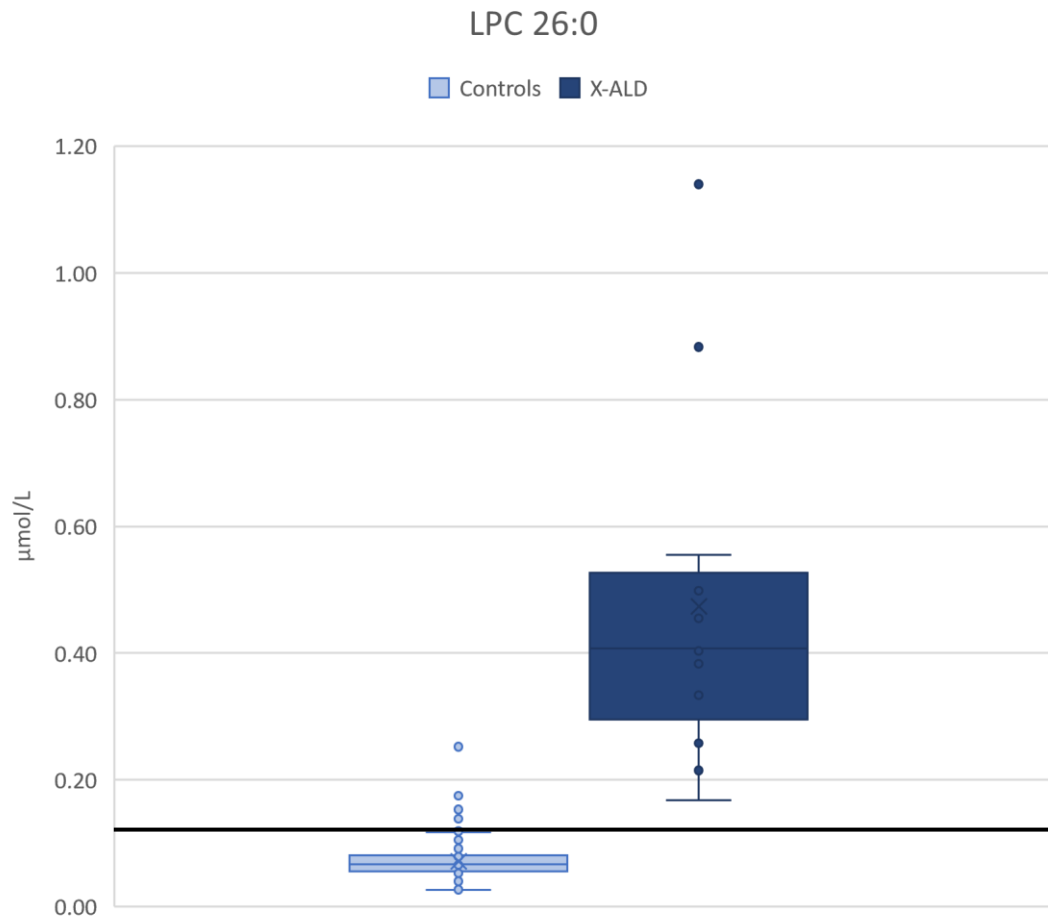
Polish adults
German adults
Polish kids

X-ALD n=13

Male patients
Male patients on Lorentz oil
Male patients after bone marrow transplant
Female carriers



Then on to the clinical validation



Controls n=280

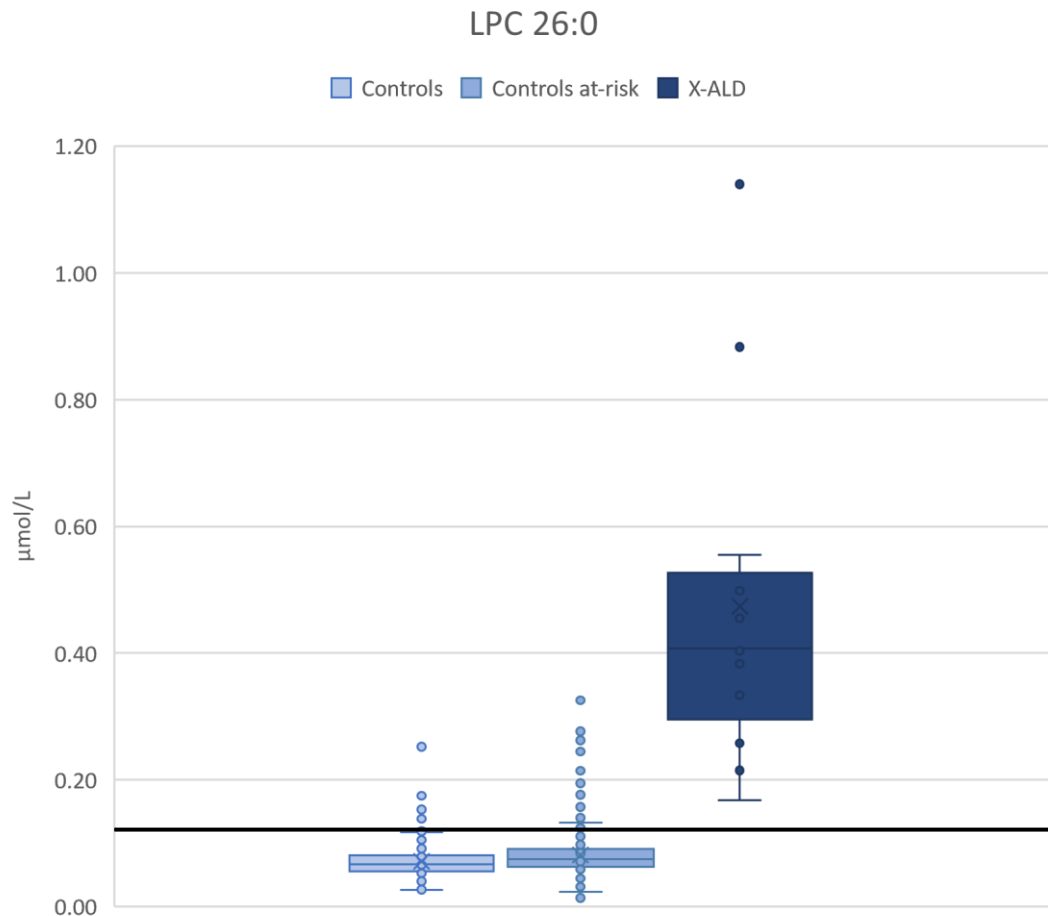
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Then on to the clinical validation



Controls n=654

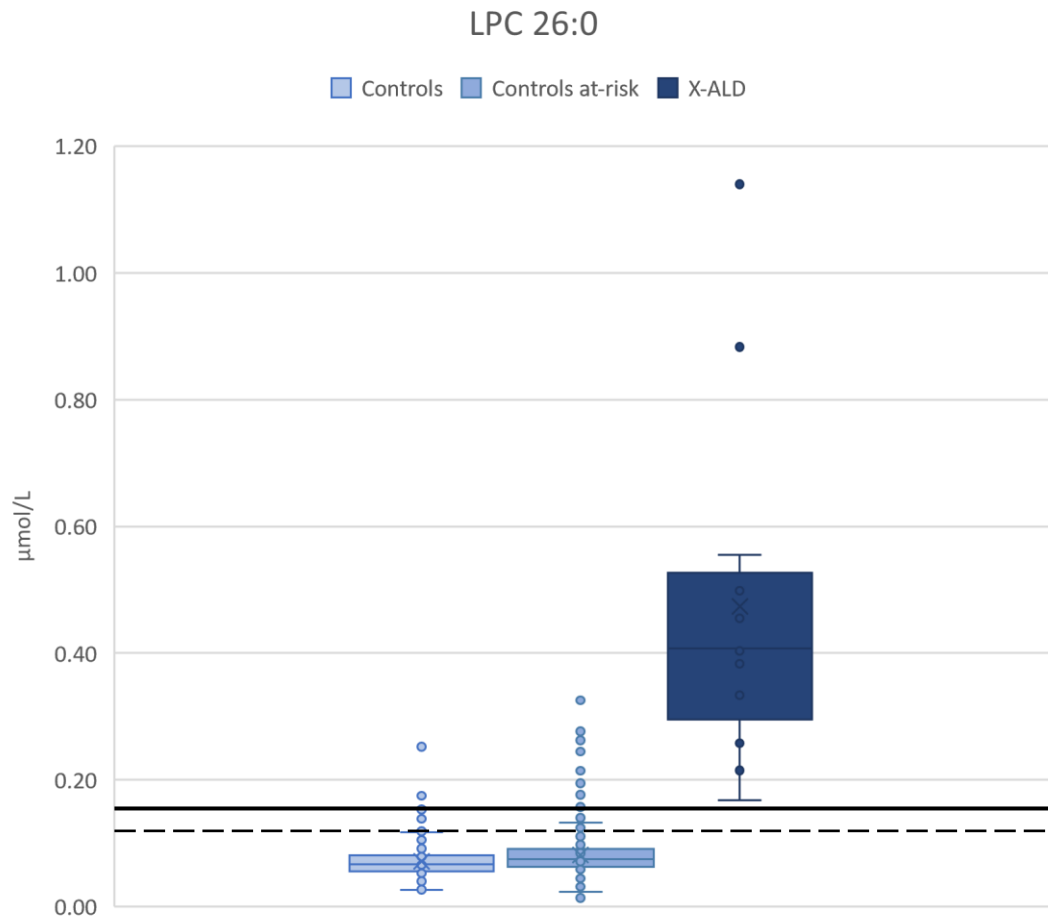
Polish adults
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Children from at-risk population

X-ALD n=13

Male patients
Male patients on Lorenz oil
Male patients after bone marrow transplant
Female carriers

Then on to the clinical validation



Controls n=654

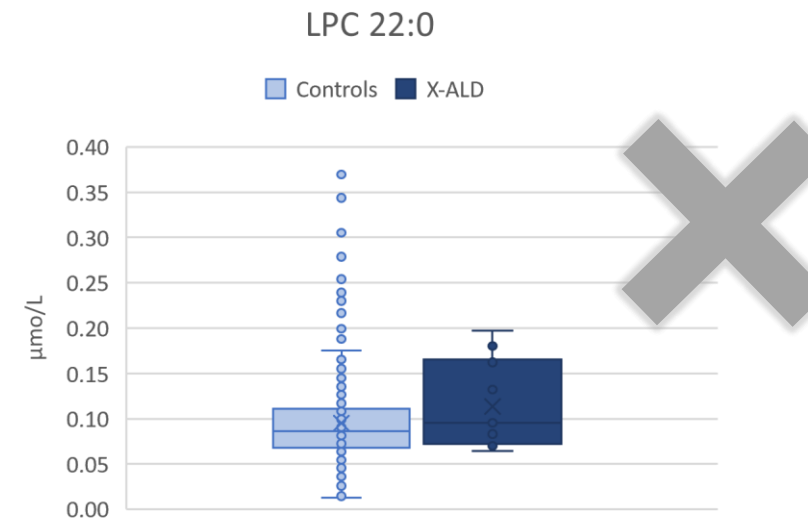
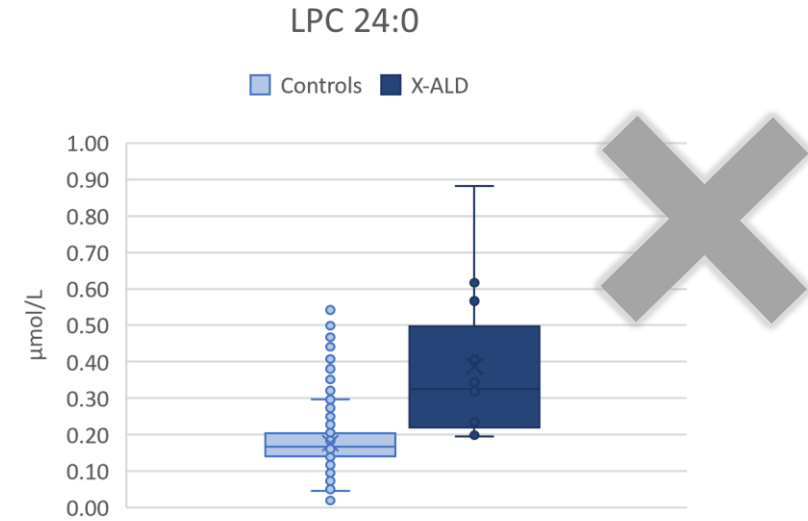
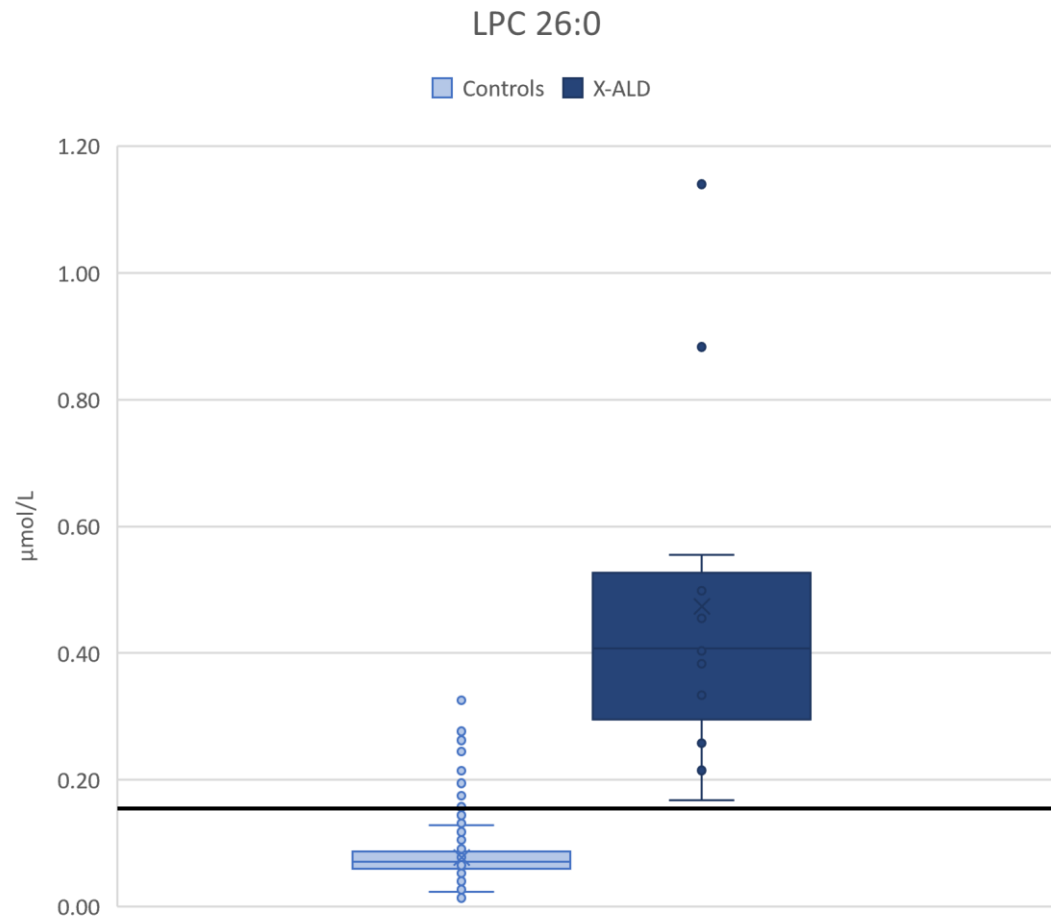
Polish adults
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Children from at-risk population

X-ALD n=13

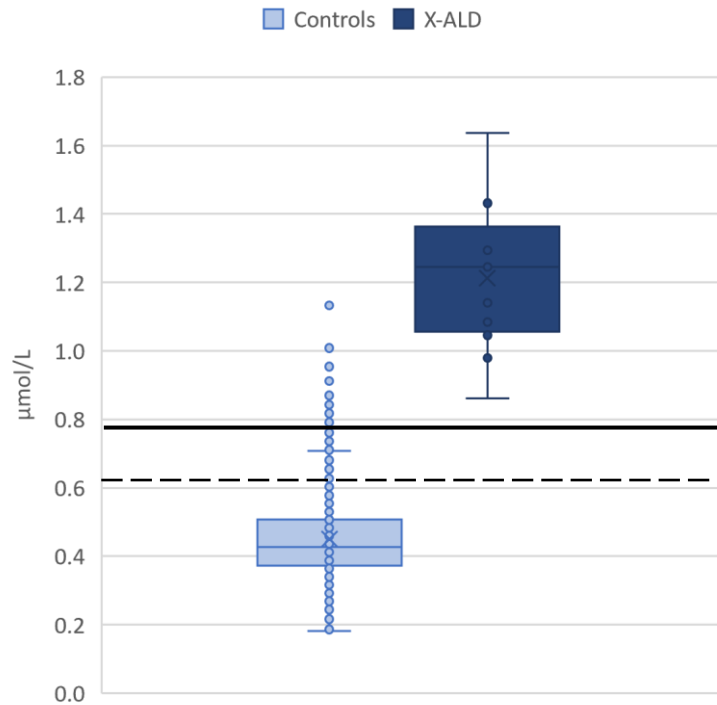
Male patients
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...through the clinical validation...

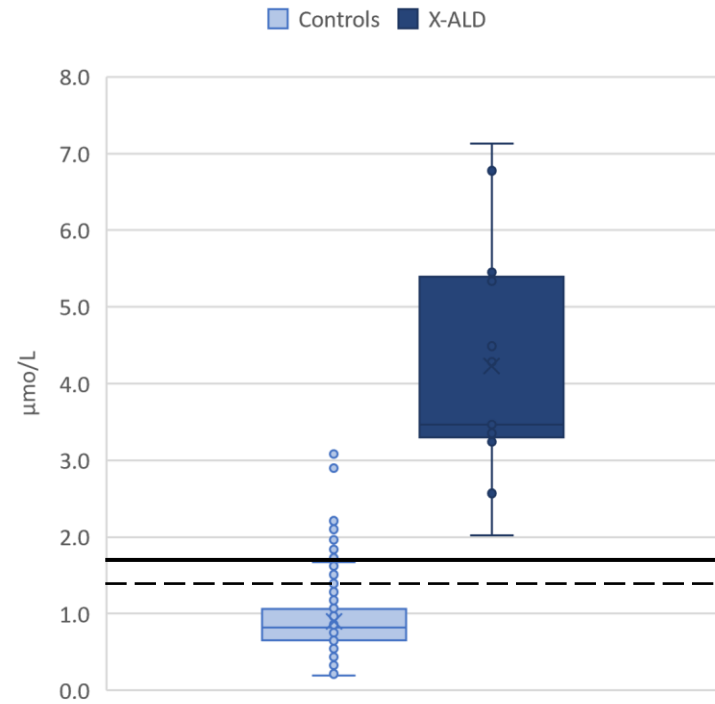


...and on the happy ending.

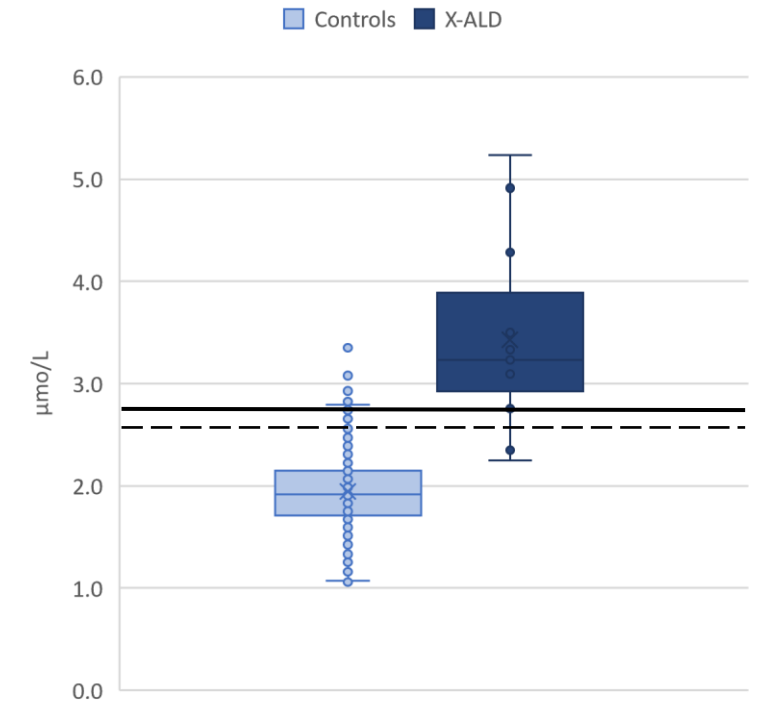
LPC 26: LPC 24



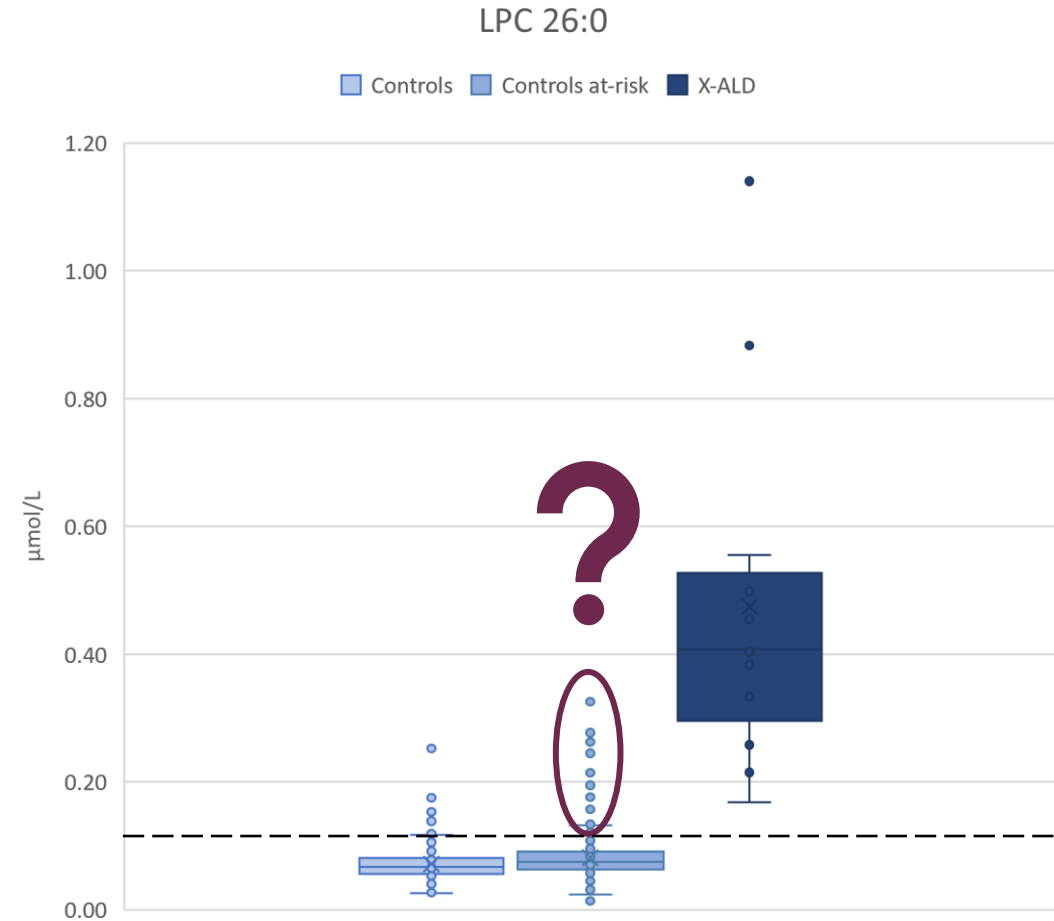
LPC 26 : LPC 22



LPC 24 : LPC 22



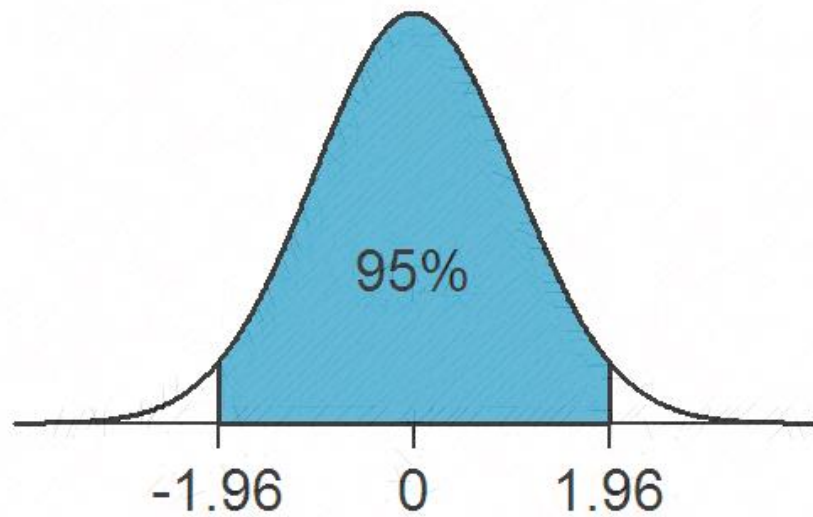
But wait...



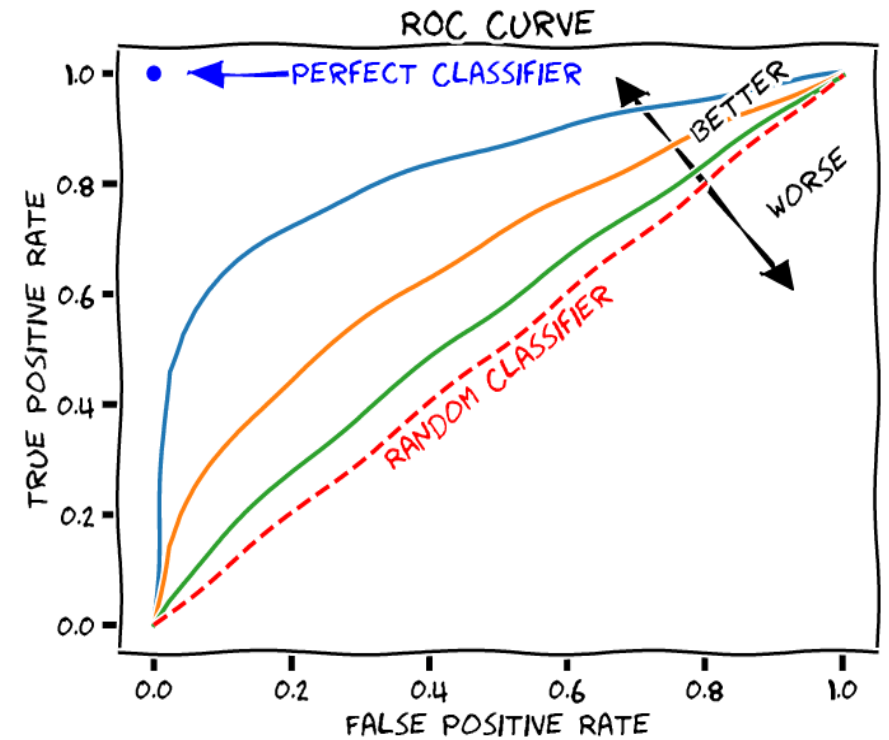
Can we do better?



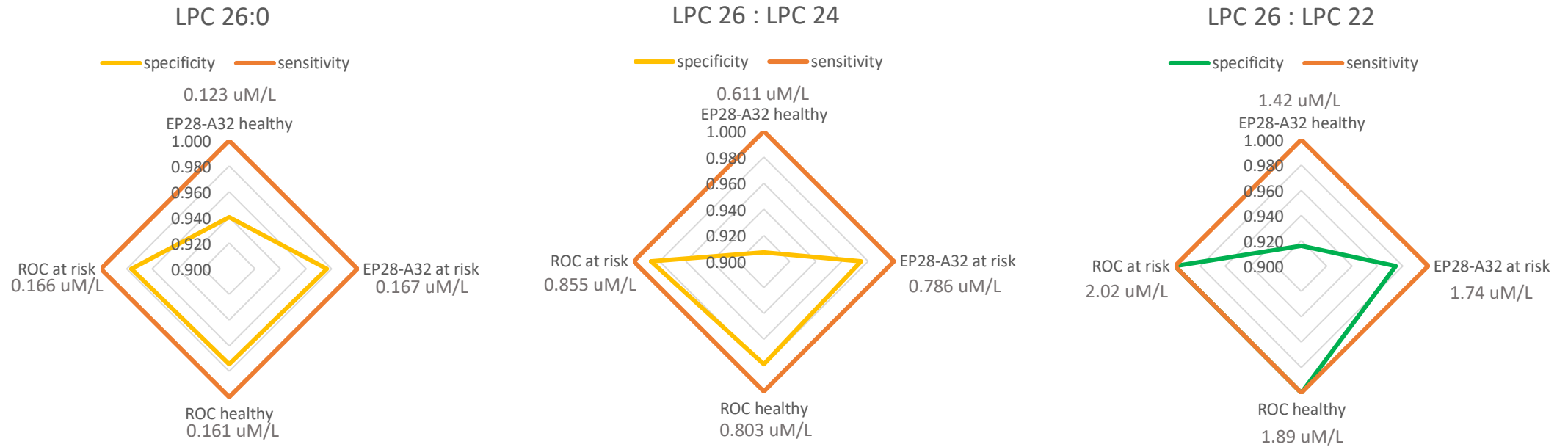
What about a binary classifier model?



VS

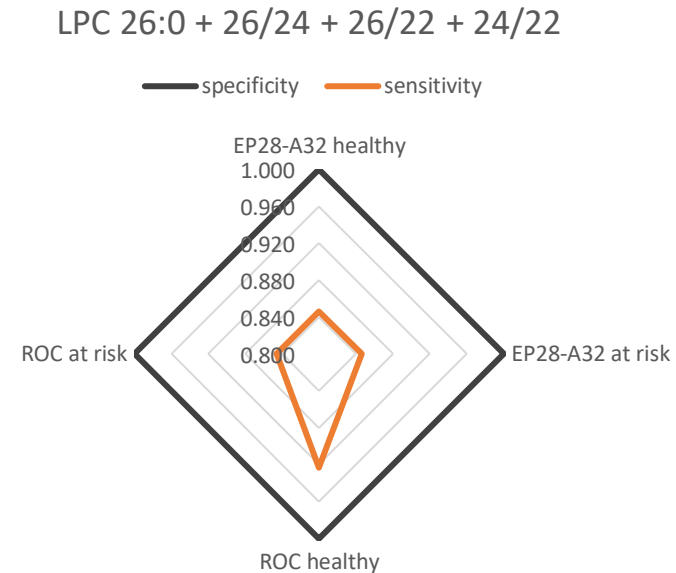
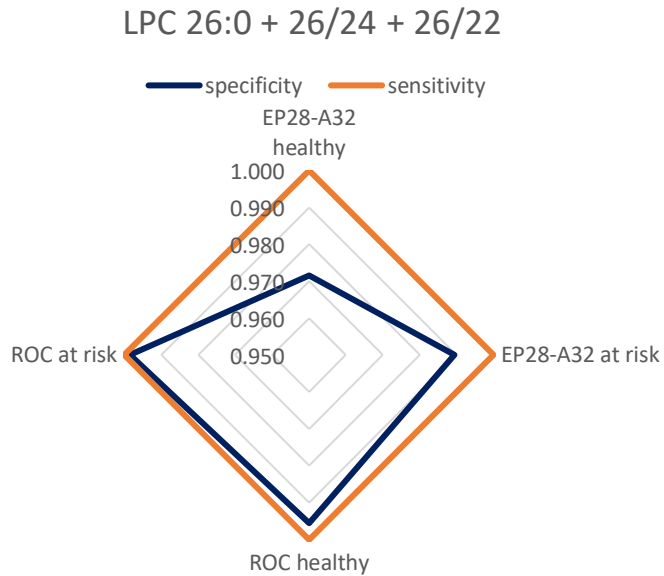


How do they compare?



ROC analysis seems to be more resilient to unpredictable variability offering better diagnostic performance from the get-go.

How do they compare?



However, it still requires further supervision and revision in the highly unpredictable world of rare genetic diseases.

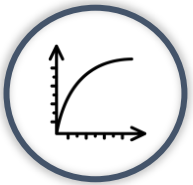
So what do we take from this story?



Population choice matters



Explore your options with biomarkers



ROC-based approach improves performance



Do guidelines reflect well the realities of RGDs?



Thank you for your attention!

X-ALD community in Poland
All M.D. collaborators from Polish pediatric centers