Challenges in pediatric reference ranges

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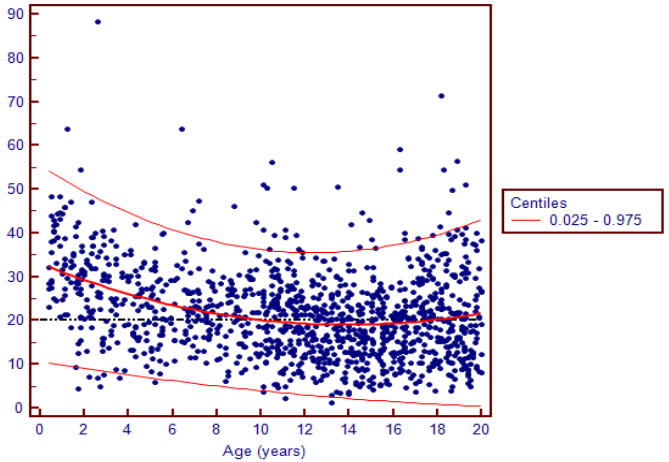
- **Problematic of pediatric reference ranges**
- **Our experience in CHU de Liège**
  - Establishing our own reference ranges
  - Implementing CALIPER data

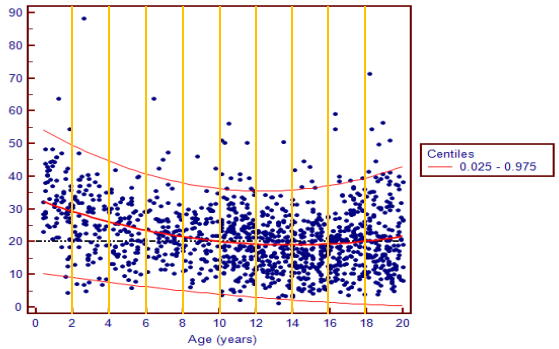
Problematic of pediatric reference ranges

- **Needs age and gender partitioning**
  - Which parameter should be split?
    - Parameters with no need of pediatric reference ranges (Ions,…)
    - Parameters with needs for pediatric reference ranges for newborns and infants (bilirubin, TSH,…)
    - Parameters in constant evolution (Immunoglobulins,…)
    - Parameters with needs for age and gender reference ranges (generally linked to pubertal development: Iron, Bone ALP,…)

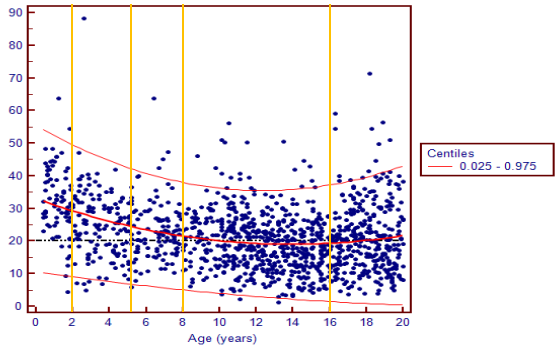
Problematic of pediatric reference ranges

- **Needs age and gender partitioning**
  - Which parameter should be split?
  - How to split the population?





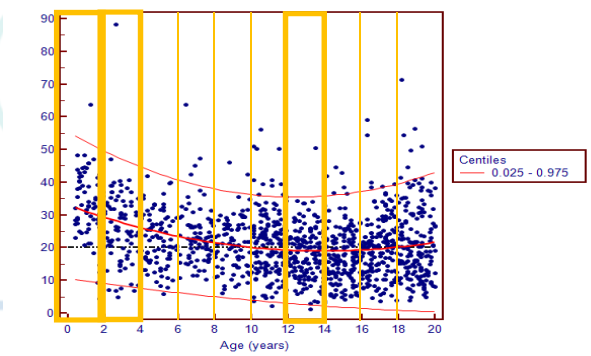
Systematic approach

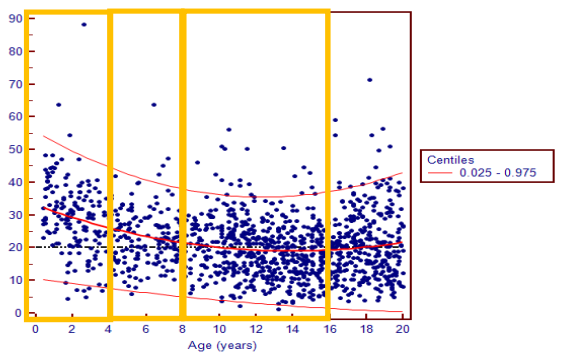


Literature based approach
"Free-style" approach

Problematic of pediatric reference ranges

- **Needs age and gender partitioning**
  - Which parameter should be split?
  - How to split the population?
  - How to evaluate needs for partitioning?
    - Classical statistical tests (Mann-Withney, student t test, Anova,…)





Problematic of pediatric reference ranges

- **Needs age and gender partitioning**
  - Which parameter should be split?
  - How to split the population?
  - How to evaluate needs for partitioning?
    - Classical statistical tests (Mann-Withney, student t test, Anova,…)
    - Harris and Boyd Test
      - Only for homogenous subgroup
      - Only for n ≈ 120
      - Better for gender partitioning
      - Less sensitive

Having two subgroups of reference values, the statistical criteria proposed by *Harris & Boyd* are:

if $0{,}7 \geq s_1^2/s_2^2 \geq 1{,}5$

or $(\bar{x}_1 - \bar{x}_2)/(s_1^2/n_1 + s_2^2/n_2)^{1/2} \geq 5 \cdot \left[\left(\frac{n_1 + n_2}{2}\right)\Big/120\right]^{1/2}$,

for $n_1 \geq 50$ and $n_2 \geq 50$ and $\bar{x}_1$, $\bar{x}_2$, s_1 and s_2 being the means and the standard deviations and n_1, n_2 the number of subjects in subgroups 1 and 2, respectively, it is necessary to estimate reference limits in both subgroups separately.

No consensus

Fuentes-Arderiu et al., Eur J Clin Chem Clin Biochem 1997; 35(9):733

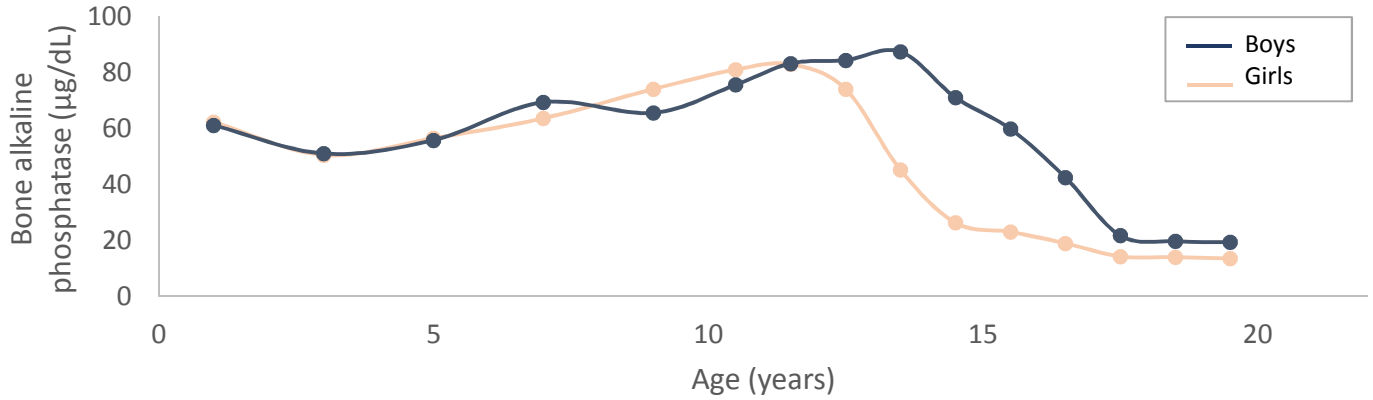
Problematic of pediatric reference ranges

- **Population recruitment**
  - 120 sera / subgroup
  - How to collect the sera
    - Recruitments from collectivities?
    - Sera from leftover out-clinic patients
    - Ethical issue of serum from infants or newborns

- Problematic of pediatric reference ranges
- **Our experience in CHU de Liège**
  - Establishing our own reference ranges
  - Implementing CALIPER data

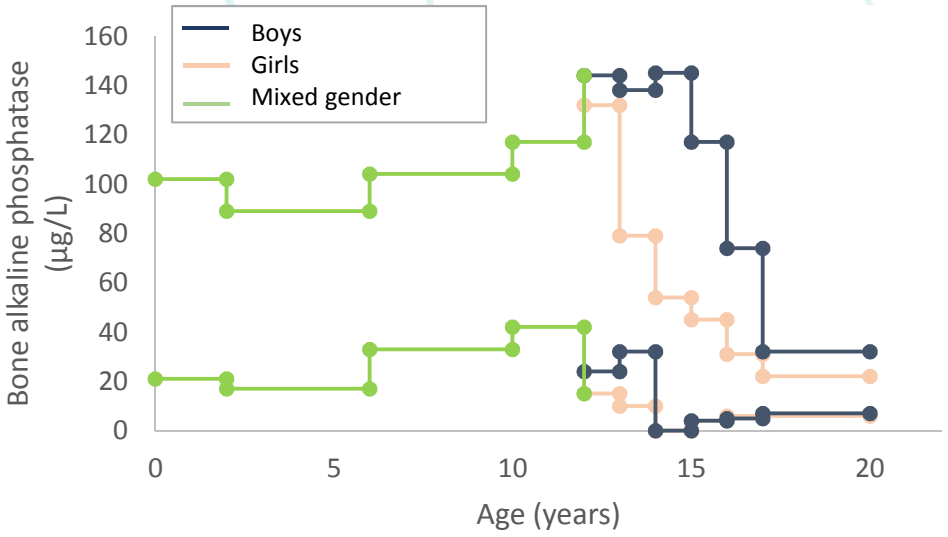
Establishing our own pediatric reference range

- **Bone alkaline phosphatase**
  - Large cohort of 1200 children and teenage
  - Sera of out-clinic patients who underwent allergy exploration
  - 40 girls and 40 boys by age range of 1 or 2 years
  - Liaison XL (Diasorin◦) with LIAISON◦ BAP OSTASE◦ kit



Establishing our own pediatric reference range

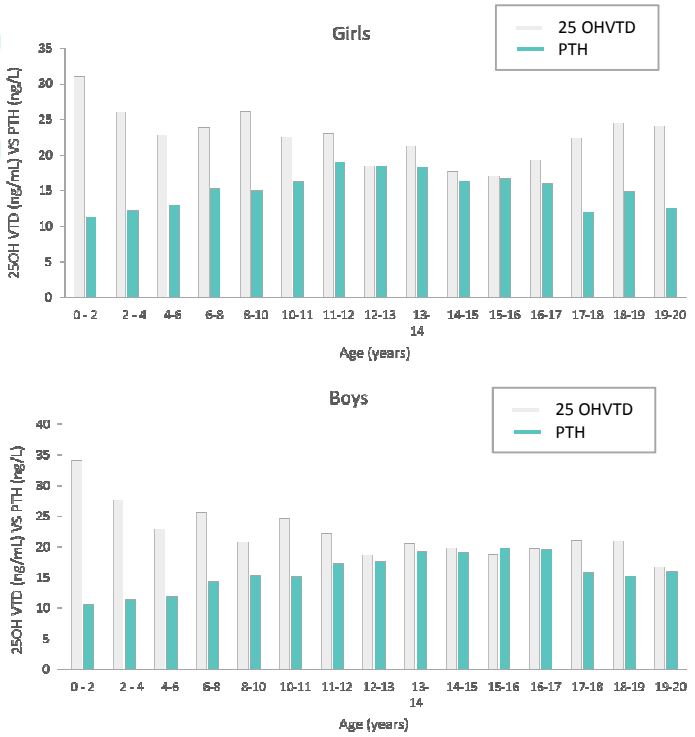
- **Bone alkaline phosphatase**
  - Liaison XL (Diasorin◦) with LIAISON◦ BAP OSTASE◦ kit

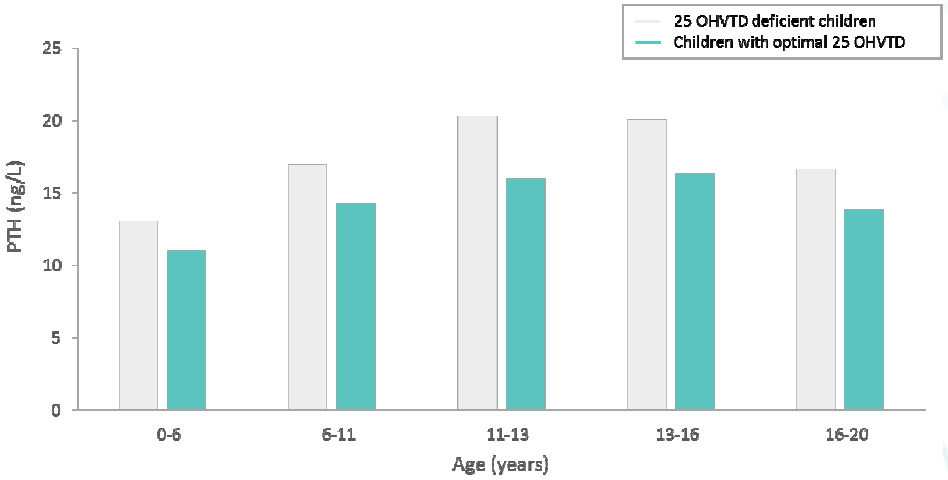


BALP (µg/L)	Mean ± SD	n	Robust method interval without potential outliers
0 - < 2 years	67,6 ± 32,8	80	21 - 102
2 - < 6 years	54,7 ± 19,9	160	17 - 89
6 - < 10 years	69,2 ± 17,8	160	33 - 104
10 - < 12 years	82,8 ± 23,3	161	42 - 117
12 - < 13 years F	75,4 ± 28,4	40	15 - 132
12 - < 13 years M	94,0 ± 34,0	40	24 - 144
13 - < 14 years F	47,2 ± 22,1	40	10 - 79
13 - < 14 years M	91,7 ± 37,9	40	32 - 138
14 - < 15 years F	31,9 ± 17,6	42	0 - 54
14 - < 15 yearsM	74,2 ± 35,1	40	0 - 145
15 - < 16 years F	26,5 ± 12,9	40	4 - 45
15 - < 16 years M	65,0 ± 32,3	40	4 - 117
16 - <17 years F	19,8 ± 7,2	40	6 - 31
16 - < 17 yearsM	45,0 ± 27,1	40	5 - 74
17 - < 20 years F	14,8 ± 4,9	120	6 - 22
17 - < 20 years M	24,8 ± 16,0	121	7 - 32

Establishing our own pediatric reference range

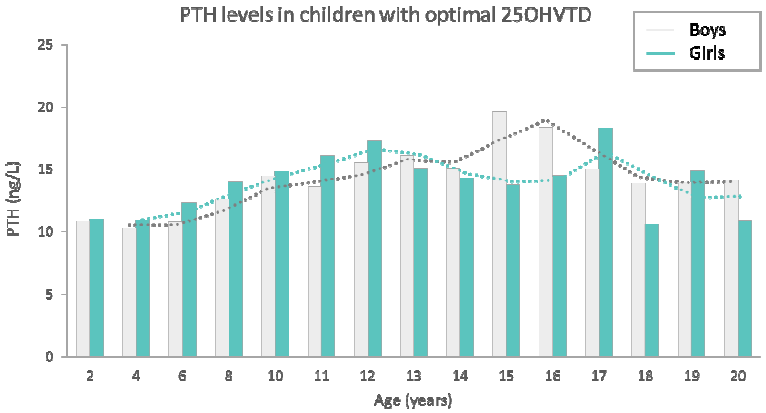
- **PTH**
  - Liaison XL (Diasorin◦) with the LIAISON◦ 1-84 PTH kit (3rd generation PTH).
  - Same cohort
  - Problematic of secondary hyperparathyroidism





Establishing our own pediatric reference range

- **PTH**
  - Liaison XL (Diasorin◦) with the LIAISON◦ 1-84 PTH kit (3rd generation PTH).
  - Based on non 25VTD deficient children (25 VTD > 20 ng/mL)



	n	25OHVTD (ng/ml) Mean ± SD	PTH (ng/L) Mean ± SD	Robust interval method without potential outliers
0 - < 6 years	179	31,7 ± 8,6	11,0 ± 4,0	3,6 - 17,4
6 -< 11 years	156	28,7 ± 7,2	14,2 ± 5,2	5,1 - 20,8
11 - < 13 years	84	26,4 ± 5,1	16,1 ± 6,6	5,2 - 24,4
13 - < 16 years	102	26,4 ± 5,4	16,1 ± 6,8	3,4 - 24,7
16 - < 20 years	159	29,2 ± 8,8	13,7 ± 6,3	0 - 25

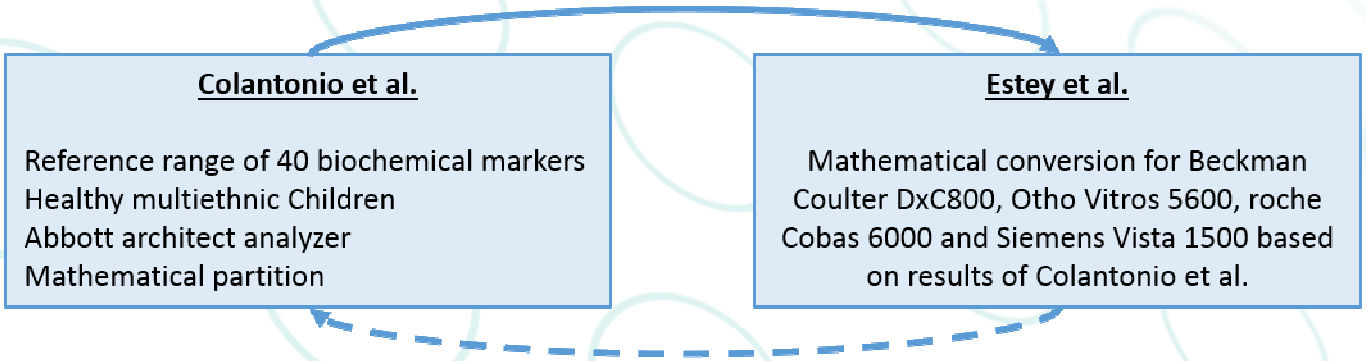
- Problematic of pediatric reference ranges
- Our experience in CHU de Liège
  - Establishing our own reference ranges
  - Implementing CALIPER data

Implementing CALIPER data

- **What are CALIPER studies?**
  - Canadian laboratory initiative on pediatric reference intervals
  - Several papers that establish pediatric reference intervals based on
    - Multicentric Canadian data
    - Mutli-ethnic (Still Canadian Population)
    - Healthy subjects recruited through communities
    - Numerous analyzer tested
    - Largely referenced in Pubmed

Implementing CALIPER data

- **What are CALIPER studies?**
  - Canadian laboratory initiative on pediatric reference intervals
  - Several papers that establish pediatric reference intervals based on
    - Newly established reference intervals (Colantonio et al., Bailey et al.,…)
    - Transference studies (Estey et al,…)

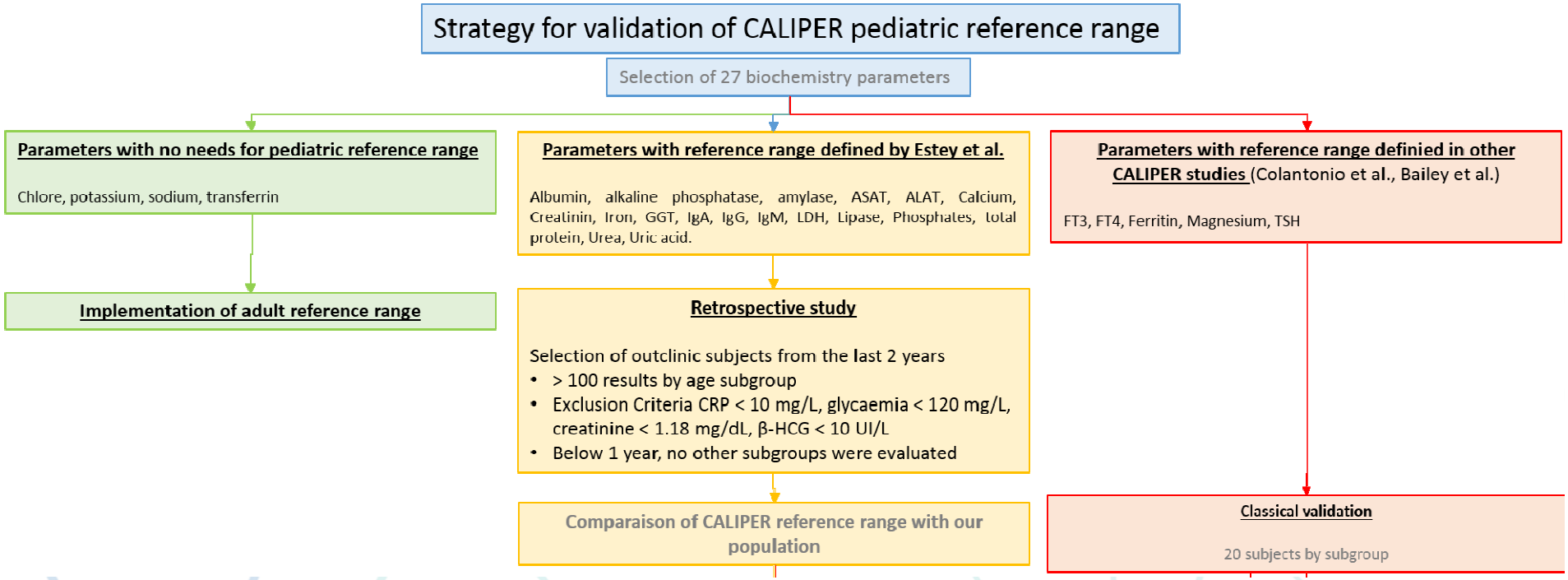


  - Many reviews that promote CALIPER studies…

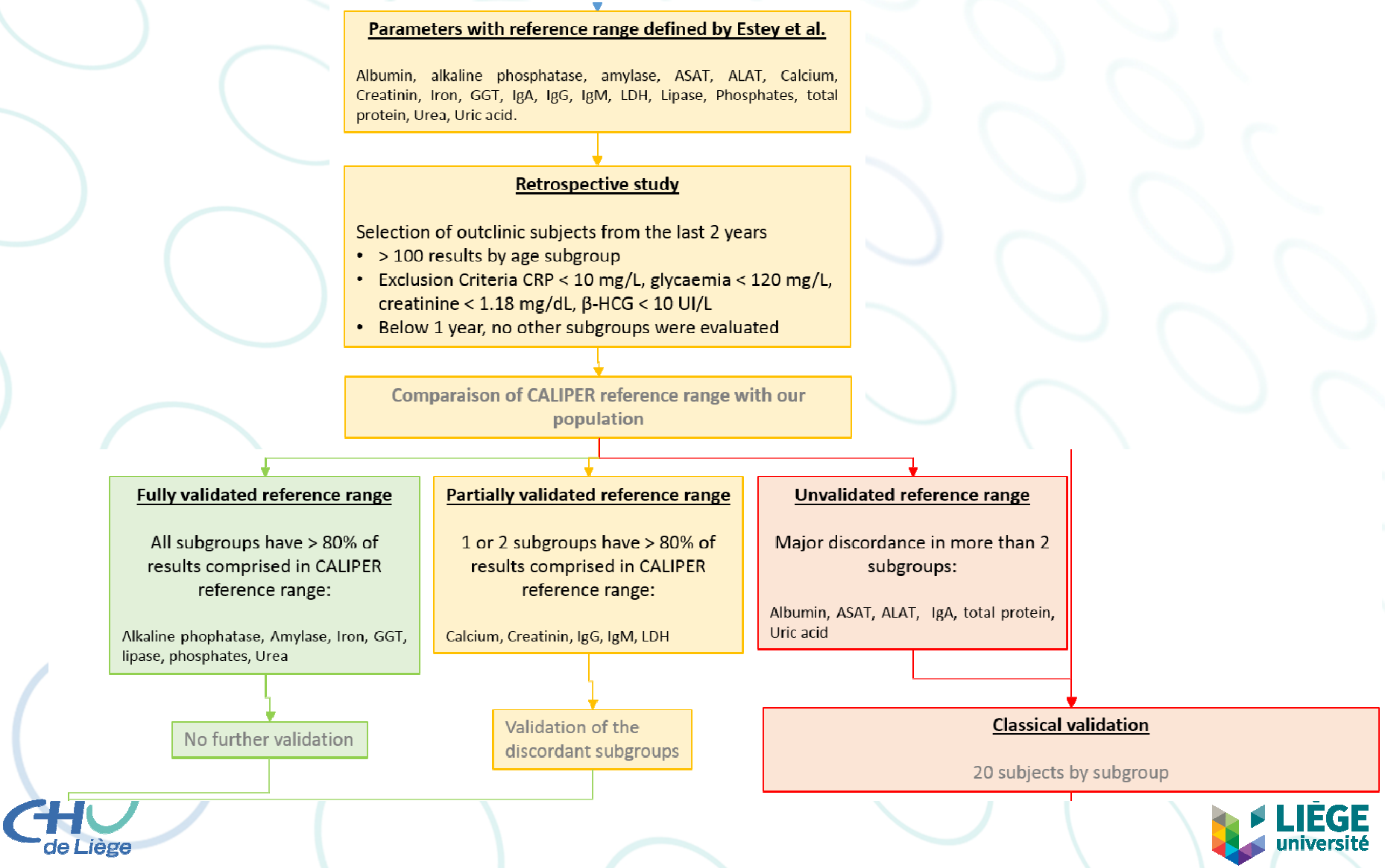
Colantonio et al. : Clin Chem. 2012 May;58(5):854-68
Estey et al.: Clin Biochem. 2013 Sep;46(13-14):1197-219

Implementing CALIPER data

- **Our Strategy to implement CALIPER ranges:**
  - Combination of :
    - analysis of retrospective data
    - classical validation according CLSI guidelines



Implementing CALIPER data: Retrospective study



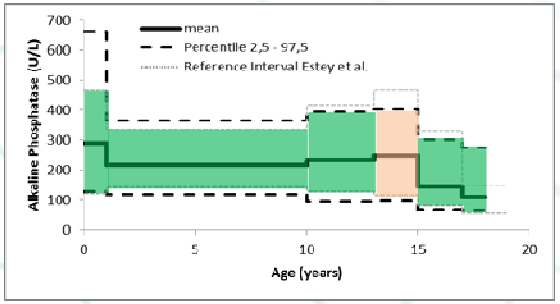
Implementing CALIPER data: Retrospective study

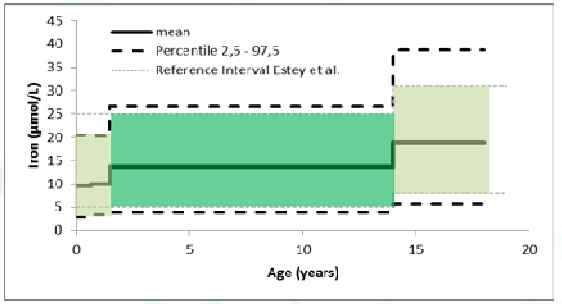
- **Parameters fully validated by the retrospective study**
  - All subgroups have more than 80% of results comprised in CALIPER reference ranges
  - Implementation of CALIPER data

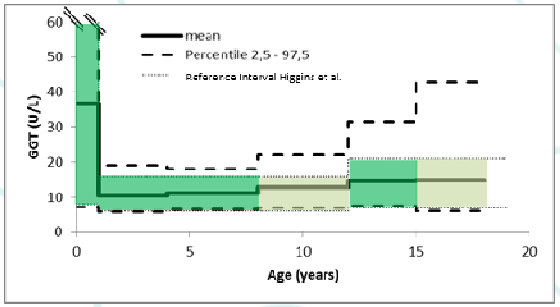
Implementing CALIPER data: Retrospective study

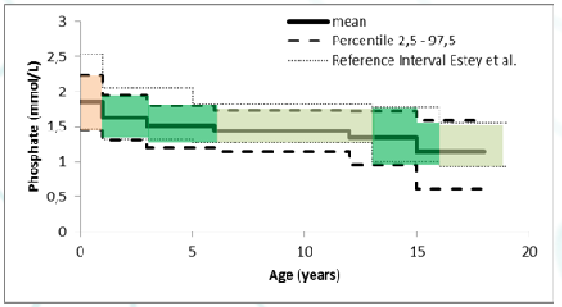
- **Parameters fully validated by the retrospective study**

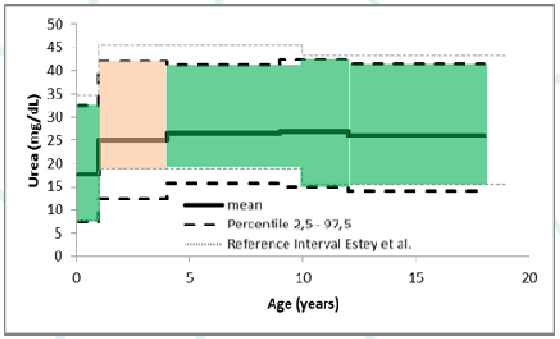
  - ALP
  - Iron
  - GGT
  - Phosphates
  - Urea

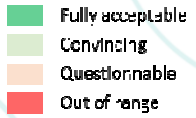










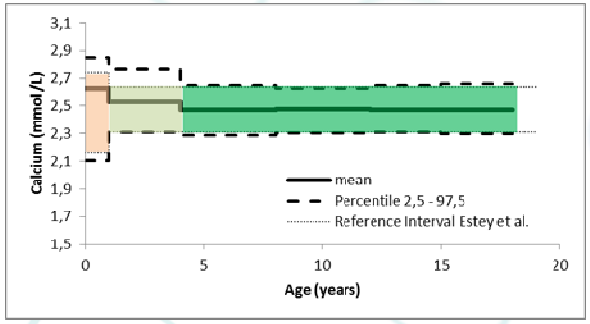


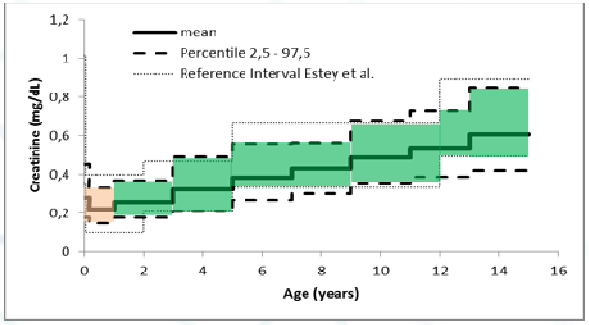
Implementing CALIPER data: Retrospective study

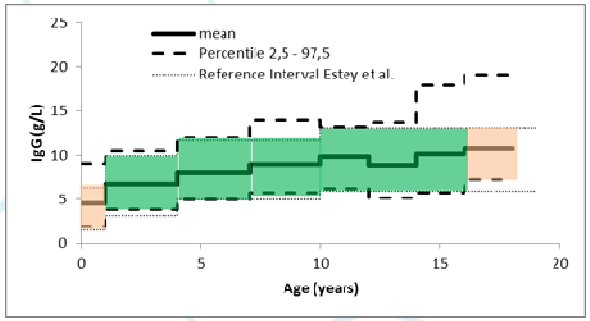
- **Parameters partially validated by the retrospective study**
  - 1 or 2 subgroups have more than 80% of results comprised in CALIPER reference ranges
  - Classical validation of discordant subgroup

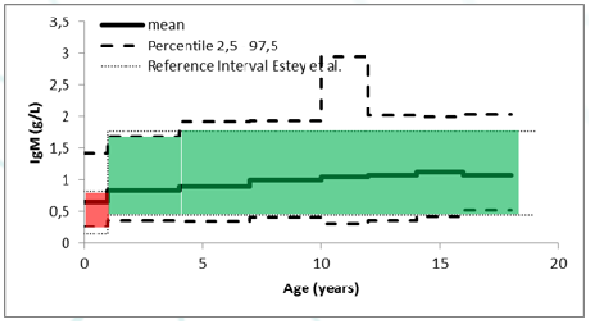
Implementing CALIPER data: Retrospective study

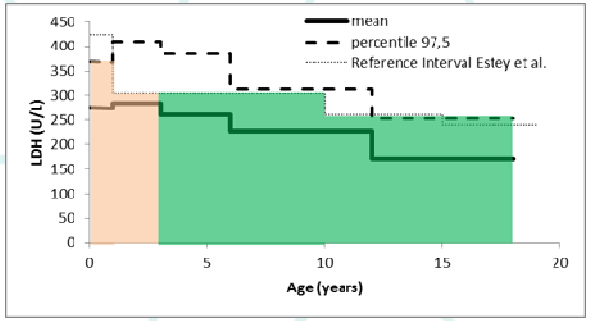
- **Parameters partially validated by the retrospective study**
  - Calcium
  - Creatinine
  - IgG
  - IgM
  - LDH

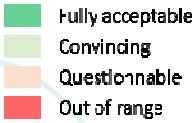












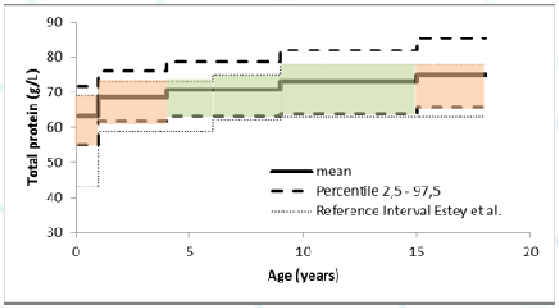
Implementing CALIPER data: Retrospective study

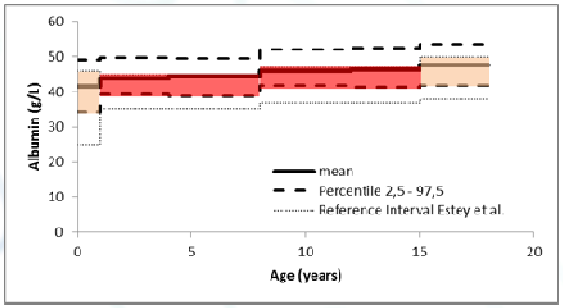
- **Parameters not validated by the retrospective study**
  - Major discordance in more than 2 subgroups
  - Classical validation of all subgroups

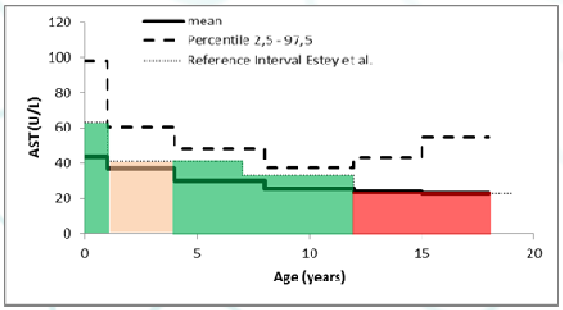
Implementing CALIPER data: Retrospective study

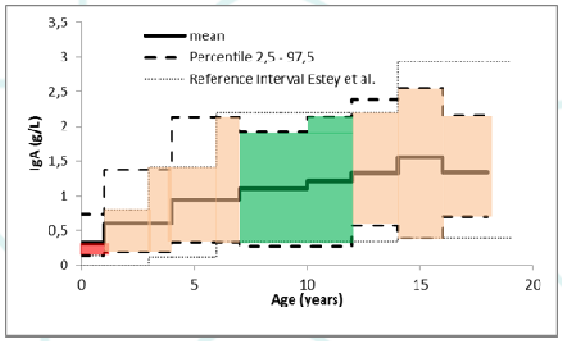
- **Parameters not validated by the retrospective study**

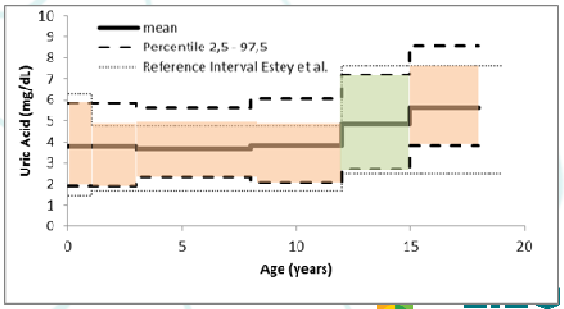
  - Albumin
  - Proteins
  - ALT
  - AST
  - IgA
  - Uric acid





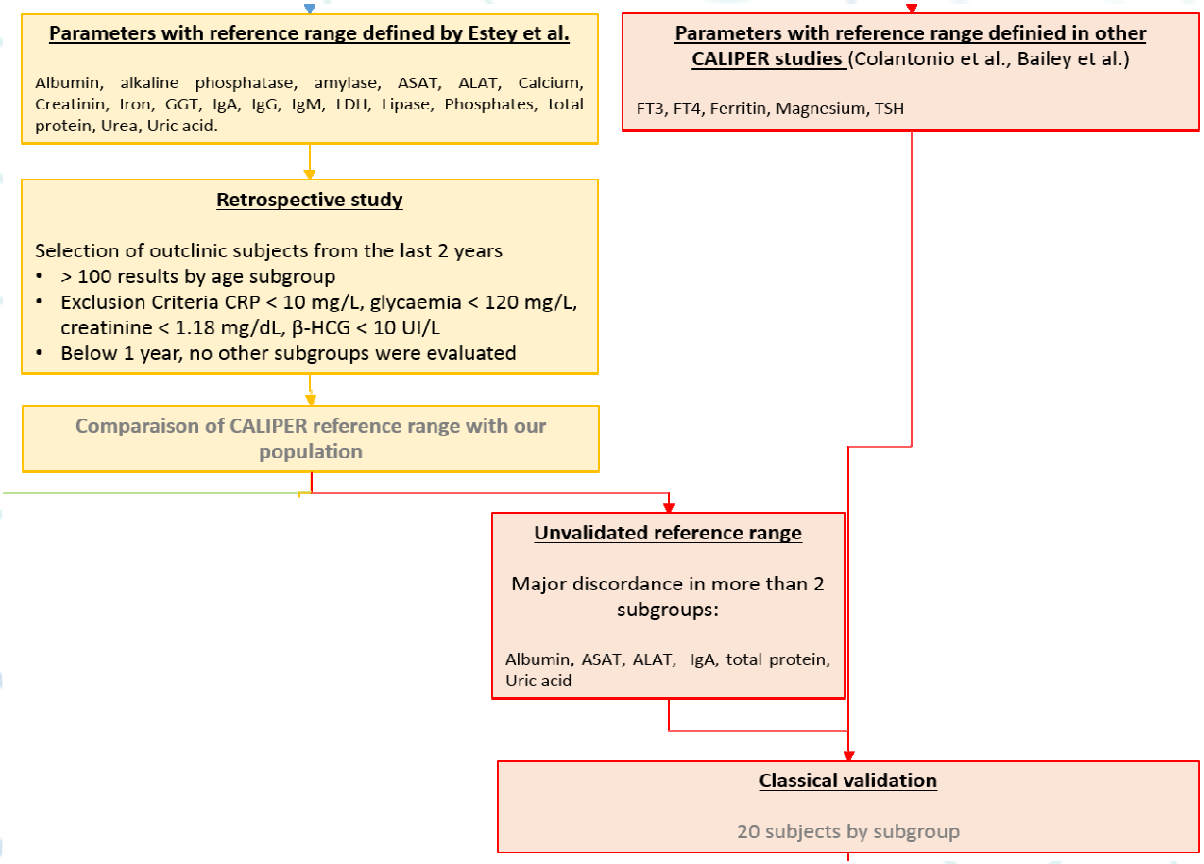






Implementing CALIPER data: Classical validation phase

- **Parameters not validated by the retrospective study**
- **Parameters without transference study**



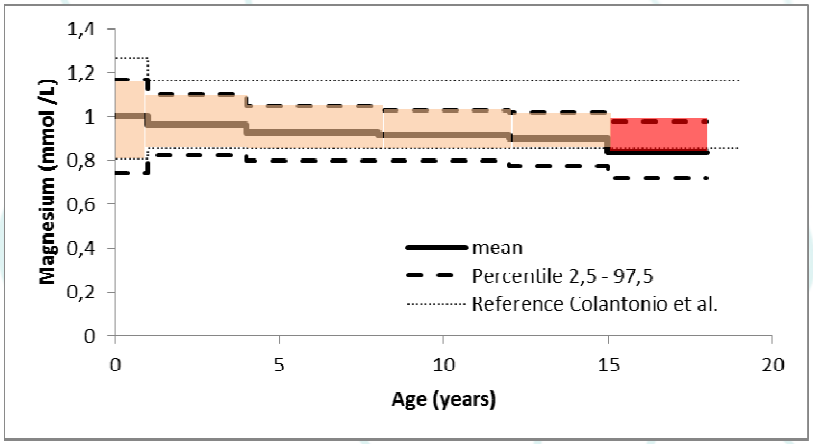
Implementing CALIPER data: Classical validation phase

- **Parameters not validated by the retrospective study:**
  - Validation passed: Albumin, AST, Uric acid
  - Needs to be rechecked: Protein, IgA
  - Validation failed: ALT

- **Parameters without transference study:**
  - Validation passed: Ferritin, Magnesium, TSH
  - Validation failed: FT3, FT4

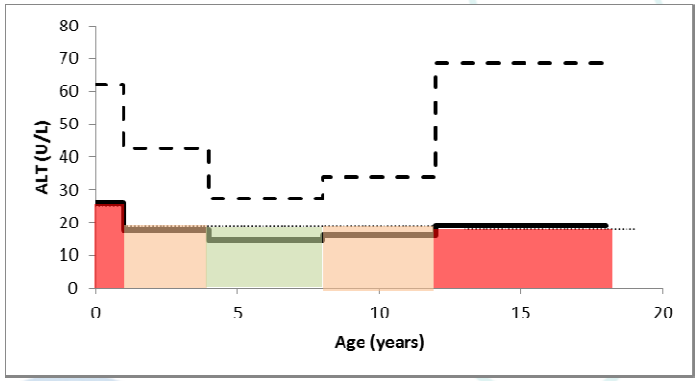
Implementing CALIPER data: Special cases

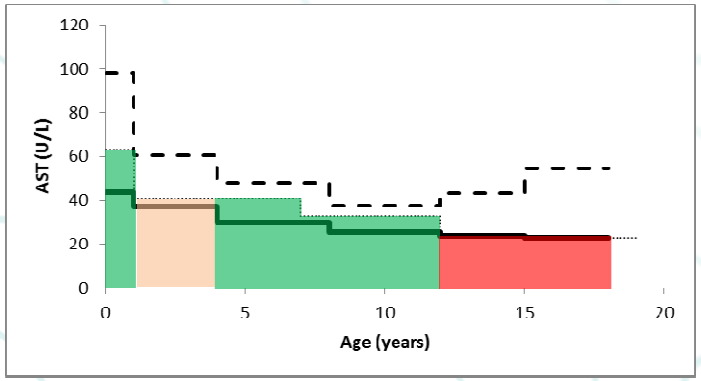
- **Magnesium**
  - Reference values established in Colantonio et al. for Abbott°
  - No transference to Cobas° (no algorithm found)
  - => References intervals do not apply to all analyzers



Implementing CALIPER data: Special cases

- Magnesium
- **ASAT and ALAT**
  - Use of pyridoxal phosphate in American countries
  - No use of Pyridoxal phosphate in Europe

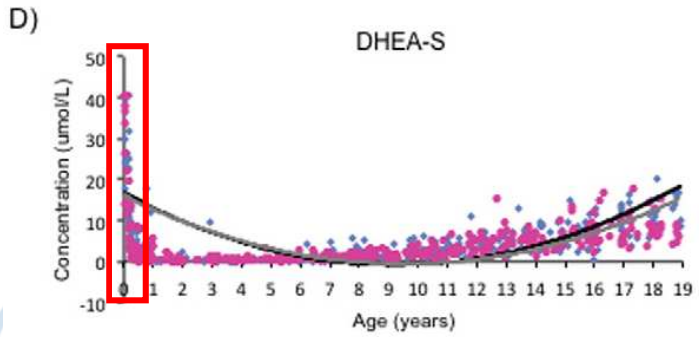




Implementing CALIPER data: Special cases

- Magnesium
- ASAT and ALAT
- **DHEA-S**

DHEA-S (µmol/l)	0–<2 months	115	28.9	>40.7	(15, 33.1)	–	115	28.9	>40.7	(15, 33.1)	–
	2–<6 months	69	0.65	15.6	(0.50, 0.85)	(13.2, 18.1)	69	0.65	15.6	(0.50, 0.85)	(13.2, 18.1)
	6 months–<1 y	77	0.15	4.79	(0.12, 0.19)	(3.95, 5.6)	77	0.15	4.79	(0.12, 0.19)	(3.95, 5.64)
	1–<6 y	78	0.07	3.03	(0.06, 0.09)	(2.38, 3.71)	75	0.07	0.76	(0.06, 0.08)	(0.65, 0.85)
	6–<9 y	108	0.14	4.14	(0.09, 0.21)	(3.65, 4.66)	108	0.14	4.14	(0.09, 0.21)	(3.65, 4.66)
	9–<13 y	133	0.90	7.30	(0.50, 1)	(6.6, 11.2)	133	0.90	7.30	(0.50, 1)	(6.60, 11.20)
	13–<16 y	98	1.50	12.5	(1.09, 1.98)	(11.4, 13.5)	98	1.50	12.5	(1.09, 1.98)	(11.4, 13.5)
	16–<19 y	**45**	**3.96**	**15.5**	**(3.55, 4.41)**	**(13.6, 17.3)**	**46**	**3.36**	**18.2**	**(1.80, 4.83)**	**(16.5, 19.7)**



!!!! Interference !!!

	Abbott° Alinity	Mass Spectrometry
♀ 1 month	> 40,71 µmol/L	1,9 µmol/L
♀ 1 month	> 40,71 µmol/L	0,53 µmol/L

Take home message

- **Pediatric values still represent a challenge**
  - Many difficulties in establishing and validating ranges according to CLSI guidelines
  - Disparities due to partionning
  - Disparities due to ethnicity
- **Many other pediatric studies than CALIPER studies**

Take home message

- **Implementing CALIPER data**
  - Globally trustable
  - Applicable to many analyzers (transference studies)

  - But
    - Better to validate data on your population
    - Special validation for < 1 year old infant
    - Ensure same analyzer and method
    - Be critical, this is not the Bible…

What's your experience?

Thank you