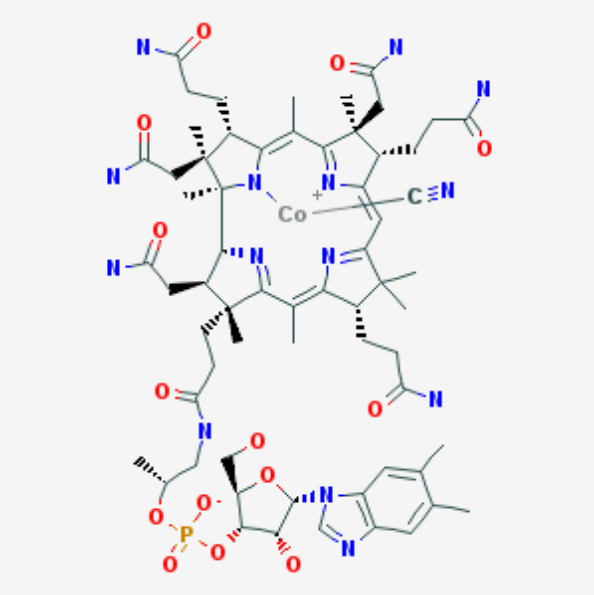
Elévation artéfactuelle de la Vitamine B12



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Introduction

- Vitamin B12 (or cyanocobalamin) is a (not very) small water-soluble molecule
- Play a vital role as coenzyme (methionine synthase and L-methylmalonyl–coenzyme A mutase):

In erythropoiesis, and in the methylation necessary for cell metabolism and DNA synthesis (also in myelin sheet synthesis)

- Exists in several forms & in circulation, B12 is bound to 2 binding proteins, namely holo-haptocorrin (80-95%) and holo-transcobalamin (5-20%)

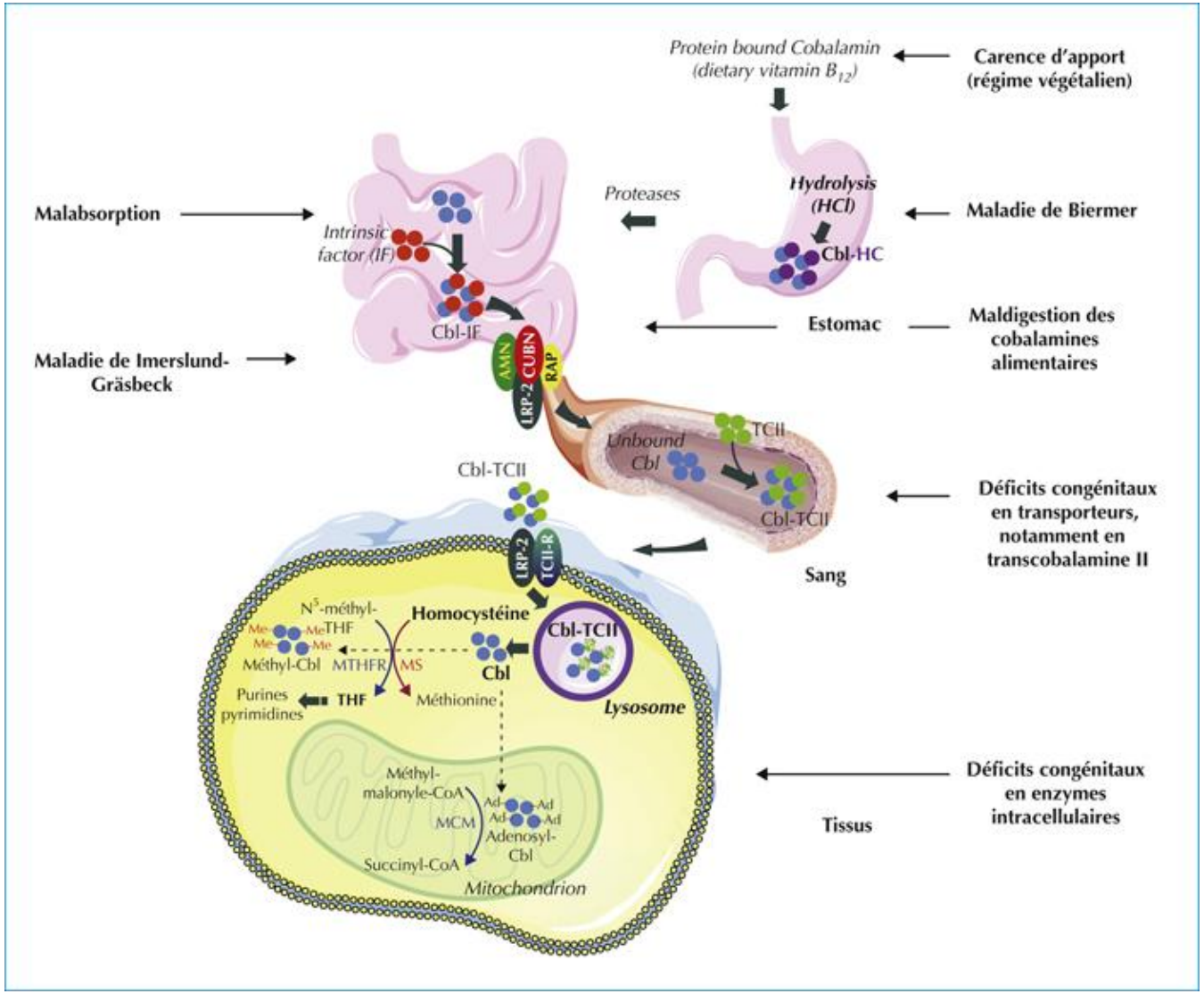
Only the B12 found holo-transcobalamin is bioactive

DNA, RNA & … methylation + DNA synthesis

- CBC: Hb? ↘ & MCV? ↗
- B12 level in serum/plasma: Total B12 and/or active B12 levels

To complete metabolic profile

- MMA (Nl < 0.4 µmol/L) & HCY (Nl < 13 µmol/L) levels: early detection of deficiencies; Masked deficiencies
- IF antibodies: Autoimmune screening; Pernicious anemia?
- Schilling test: Checking if the body has enough IF using radio-B12



source of image: Andrès, E., *et al.* (2013). Vitamine B12 administrée par voie orale: indications et modalités pratiques d'utilisation. *Médecine thérapeutique*, *19*(3), 189-195.

Decreased Levels:

- Severe malabsorption *(i.e. pernicious anemia, total or partial gastrectomy)*
- Mild malabsorption *(i.e. acid blocking drugs, mild atrophic gastritis)*
- Dietary deficiency *(i.e. VEGE)*
- Abuse of nitrous oxide

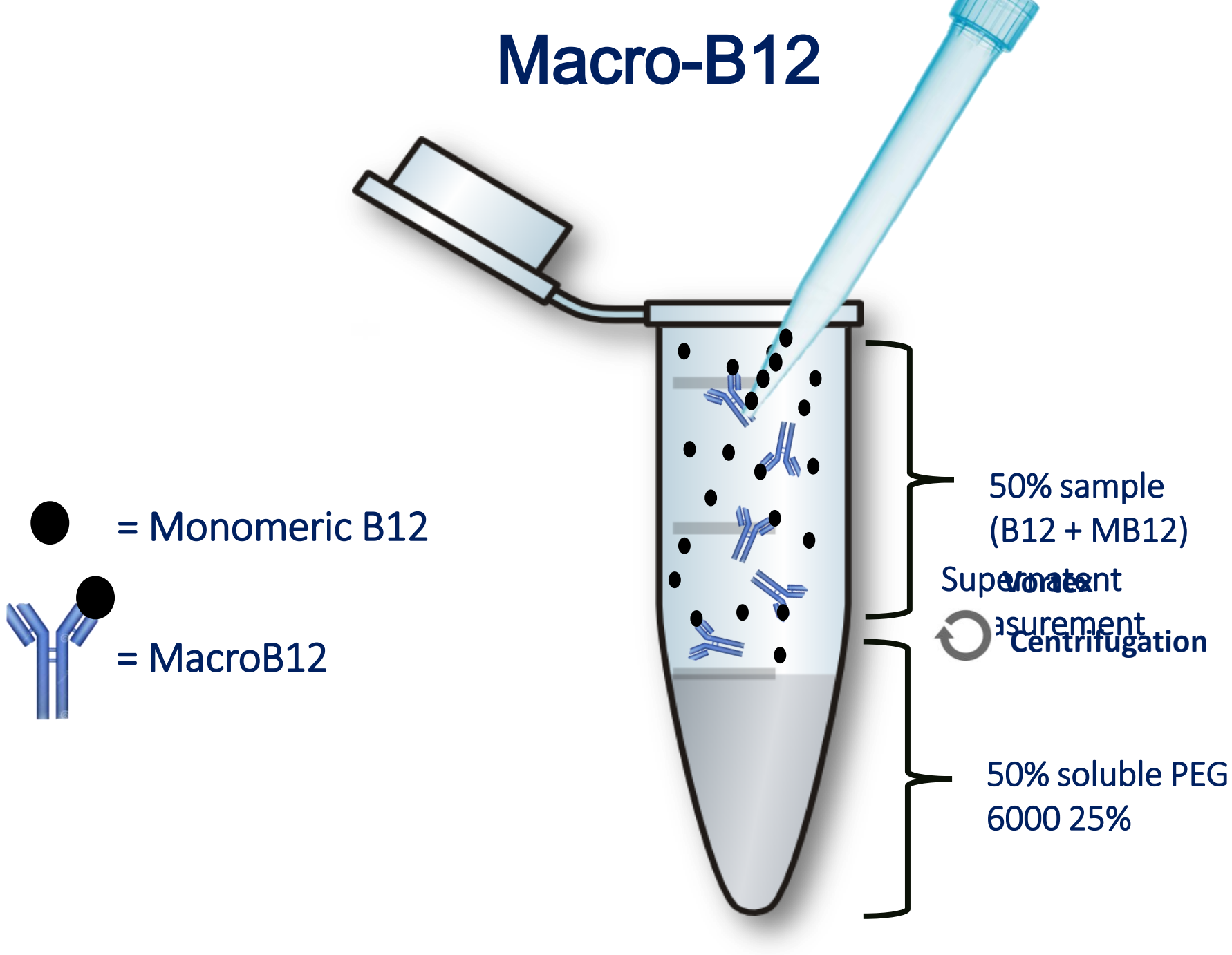
Increased Levels

- Observed in hematologic disorders *(i.e. promyelocytic leukemia, polycythemia vera or hypereosinophilic syndrome)*
- In liver disease
- In subjects taking supplementation
- Black people and Latin-American people
- Due to certain types of interference *(i.e. heterophilic antibodies and macro-B12)*

Why studying

- Received several calls from clinicians who were not able to explain high levels of B12
- Goal: Investigating the possible presence of macro forms in elevated B12 samples
- Why? To avoid misdiagnosis and unneeded clinical decisions
- How? PEG procedure and comparing the results to healthy volunteers
- Randomly collected samples: > 1,476 pmol/L (50 samples)
- From Nov2018 to Jan2019

Macro-B12


= Monomeric B12
= MacroB12
50% sample
(B12 + MB12)
Supernatant
Vortex
Measurement
Centrifugation
50% soluble PEG
6000 25%

Macro-B12

Dosage

Dosage

● = Monomeric B12

First Strategy: % Recovery in Healthy Volunteers

HVs: 20 to 60 years old

40  22 

$$Recovery\ \% = \frac{Post - PEG \times 2}{Baseline} \times 100$$

Reference ranges for healthy subjects (ROCHE) pmol/L	LOQ (ROCHE) pmol/L
B12	B12
Min: 145.0	Min: 37.0
Max: 569.0	Max: 1,476.0

Observed Recovery

68.3% (90% CI = 65.5 – 71.6%) to 108.4% (90% CI = 103.3 – 113.1%)

Unpublished data, under review

Second Strategy: Reference interval in HVs

- Idea? Based on Smith & Fahie-Wilson's Study (ClinChem, 2010): Macroprolactin study & advantage of RI to increase diagnostic specificity
- Why? True recovery % in our cohort: Laborious, expensive, time consuming and misleading
- How: Absolute post-PEG values taken into account; using robust data to establish the lower/Upper limits and 90% CI in the HVs
- Based on the post-PEG RI: (1) supraphysiological, (2) normalized, (3) infraphysiological B12 samples

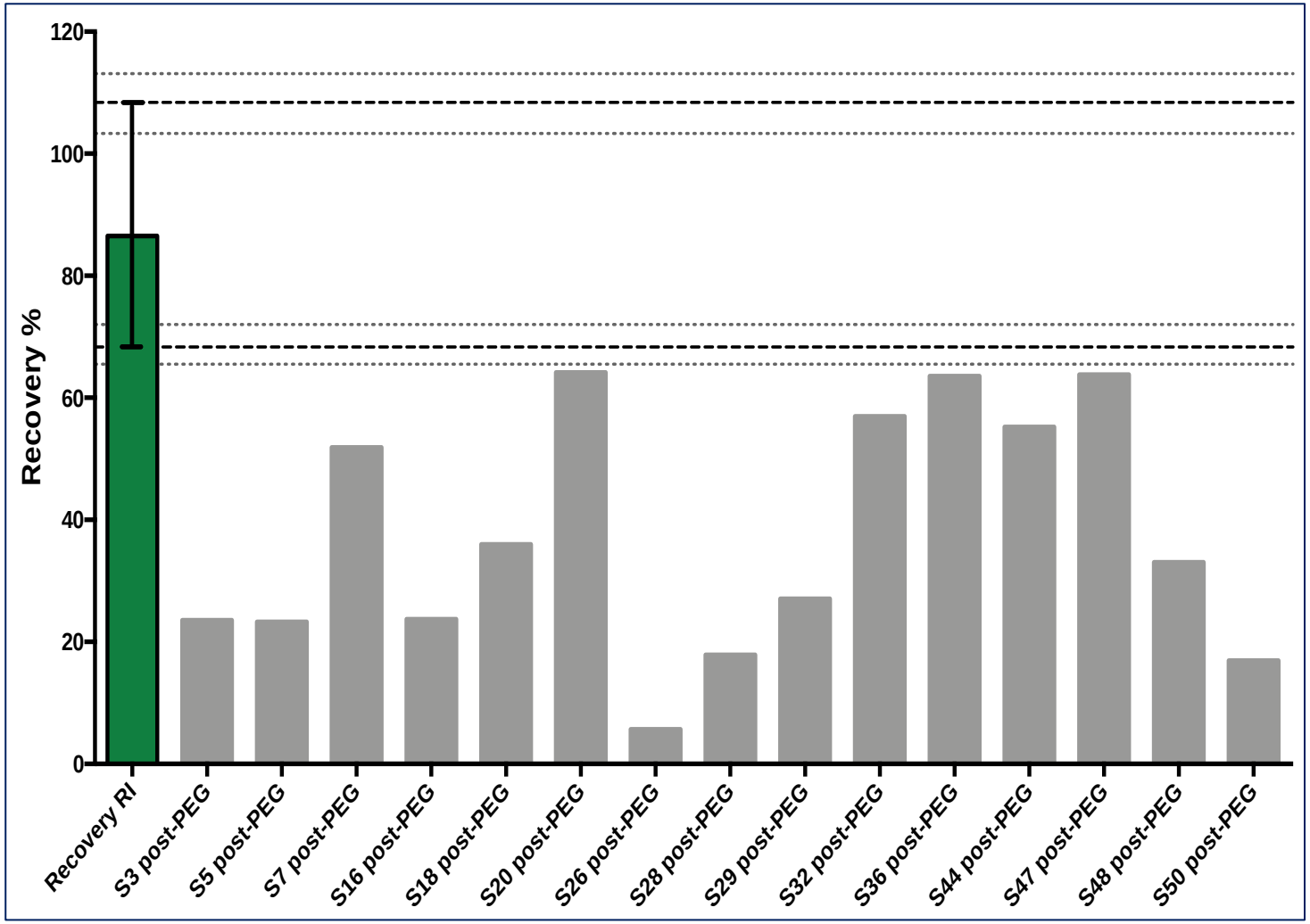
122.1 (90% CI = 106.2 – 142.0) to 514.4 (90% CI = 454.9 – 578.6) pmol/L

Unpublished data, under review

PRV of macro-B12 according to 65.5% cut-off

- 50 patients with serum B12 >1,476 pmol/L were randomly chosen to check out if there is any interferences?
- In 15 macro forms cases: 11♀ & 4♂

  15/50 = 30% of likelihood
- Results obtained after PEG treatment in highly elevated B12 samples, using recovery

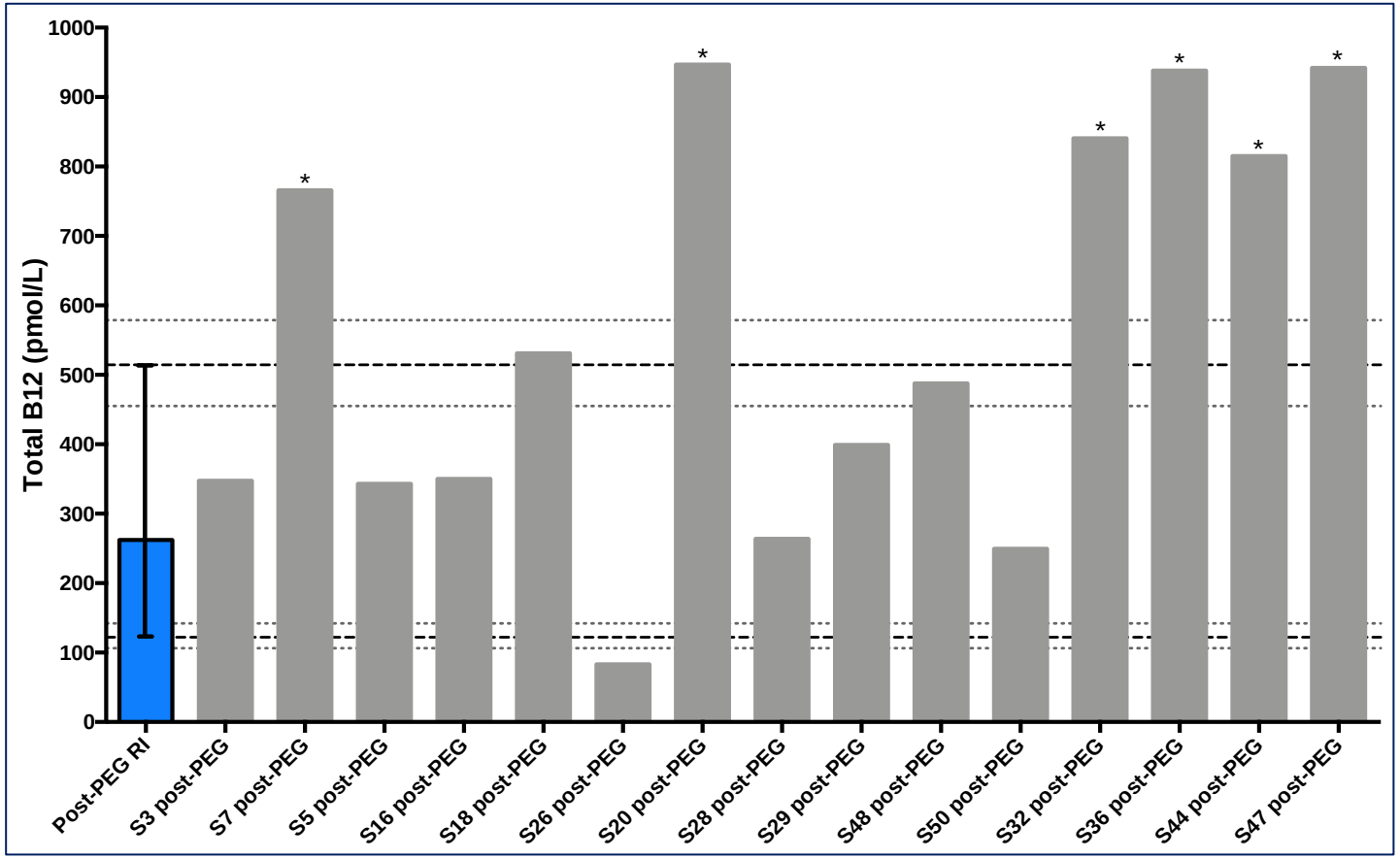


Unpublished data, under review

PRV of macro-B12 according to RI obtained in HVs

- And how were the patients classified according to the post-PEG RI?
- Results obtained following the PEG precipitation procedure in elevated B12 samples, using post-PEG RI

9/50 = 18% of likelihood



Unpublished data, under review

Size exclusion chromatography vs Recovery % and PEG RI

Patient	Recovery (%; Cobas®)	Post-PEG (pmol/L; Cobas®)	SEC
S3	23.5	347.3	Insufficient volume
S5	23.2	343.0	Insufficient volume
S7	51.9	765.6	Insufficient volume
S16	23.7	350.0	Immune complexes
S18	36.0	530.8	Immune complexes
S20	64.2	946.9	No immune complexes
S26	5.6	83.1	Immune complexes
S28	17.9	263.9	Immune complexes
S29	27.1	399.3	Immune complexes
S32	57.0	840.6	No immune complexes
S36	63.6	938.1	No immune complexes
S44	55.2	815.2	No immune complexes
S47	63.8	942.0	No immune complexes
S48	33.1	487.8	Immune complexes
S50	16.9	249.6	Immune complexes

Unpublished data, under review

Comparisons:

- Comparing only **40 samples > 1,476 pmol/L** :
- **Architect® i2000** analyzer classified **11 samples** with macroB12 as compared to 12 on the Cobas® according the Recoveries (27% vs 30%)
- Comparison statistic test between 2 analyzers:

Before (*p* 0.2500) and post-PEG (*p* 0.5791): Not significantly different (weighted kappa was 0.955)

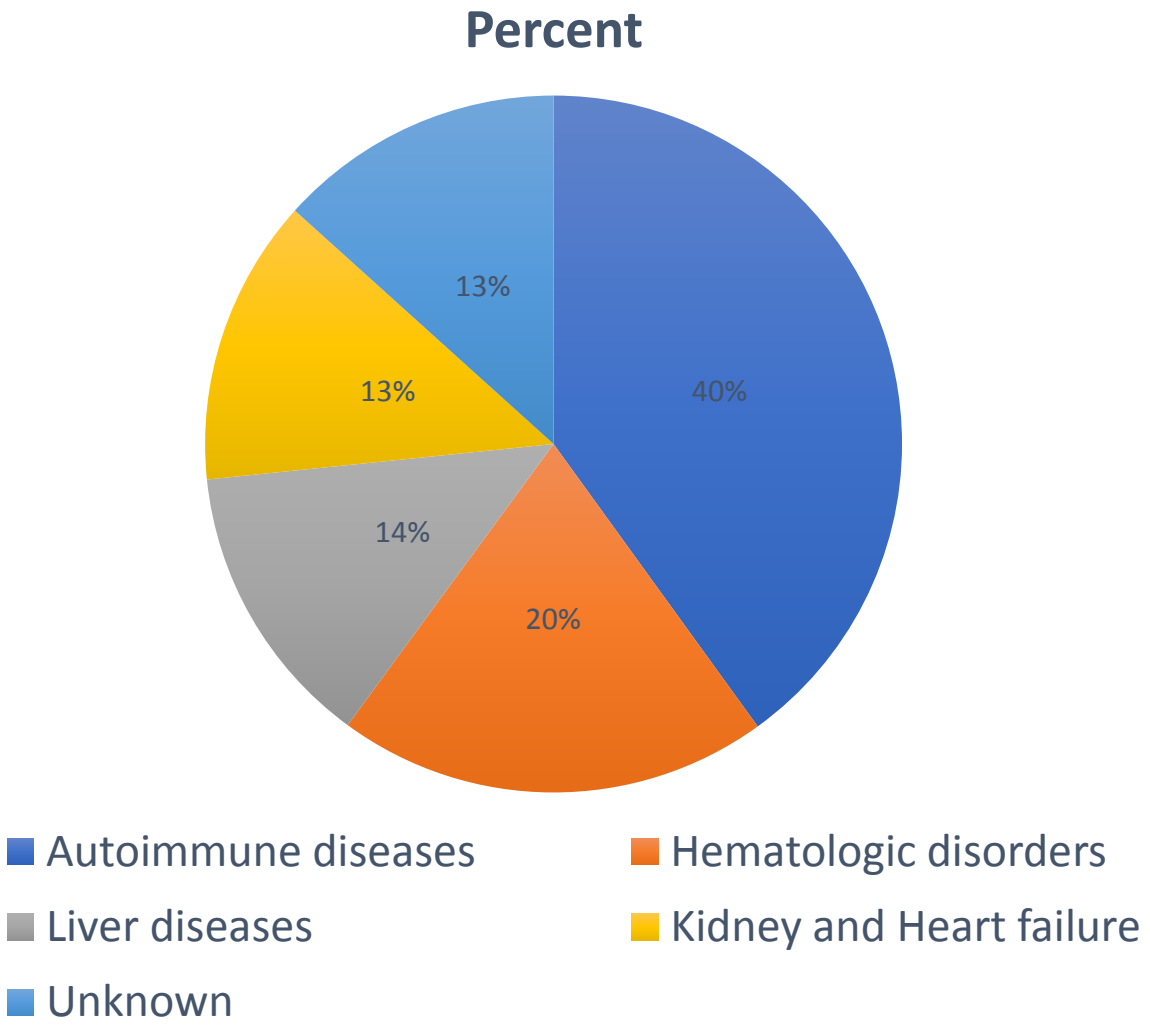
❖ Two cases have also been published with a Siemens analyzer

Post-PEG (pmol/L; Cobas®)	Post-PEG (pmol/L; Architect®)
347.3	Insufficient volume
343.0	Insufficient volume
765.6	Insufficient volume
350.0	307.0
530.8	522.5
946.9	909.2
83.1	187.5
263.9	277.5
399.3	405.9
840.6	764.6
938.1	888.6
815.2	606.6
942.0	903.3
487.8	433.9
249.6	252.4

Unpublished data, under review

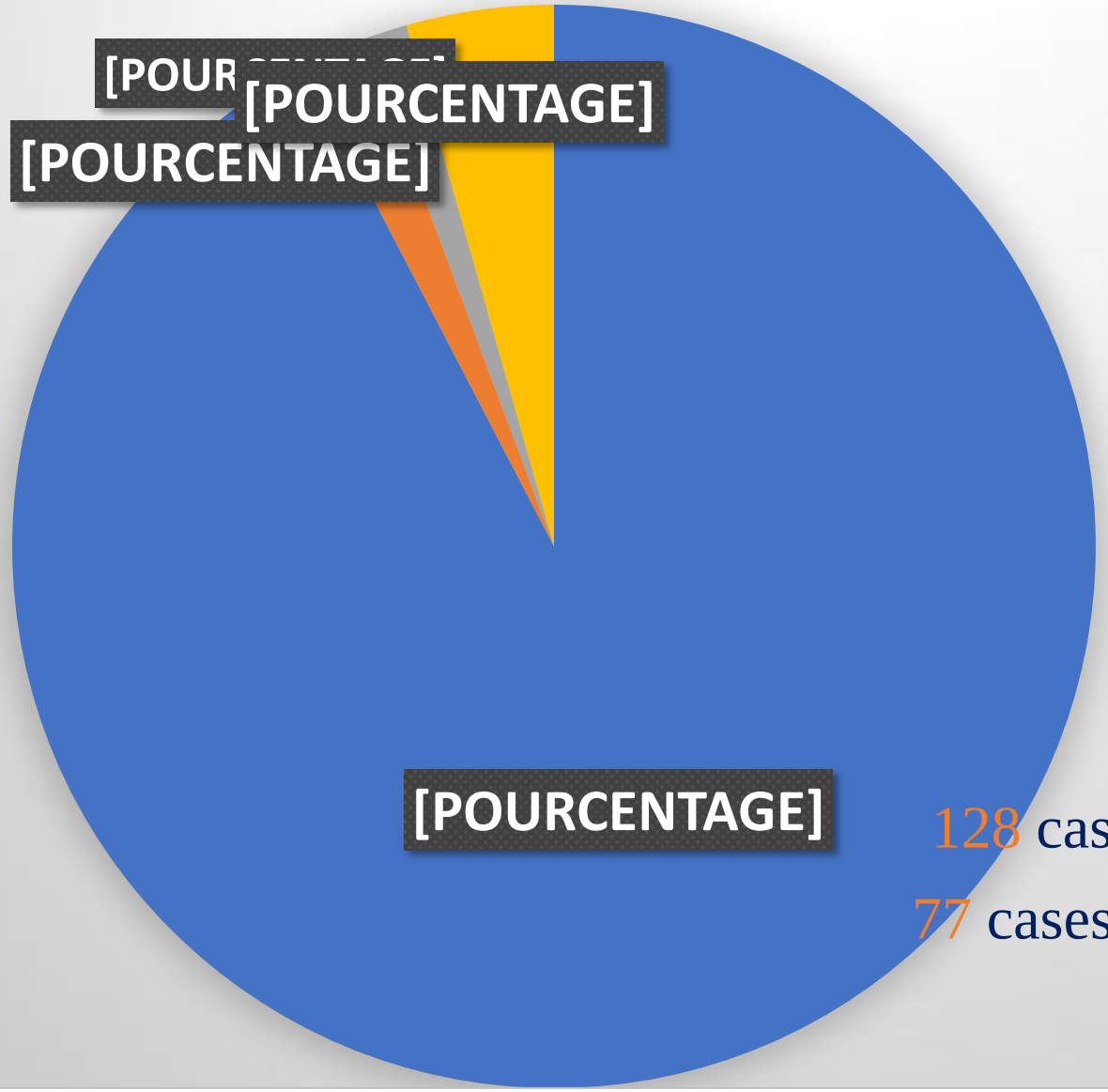
What do they suffer from?

- Hemochromatosis;
- Rheumatoid Arthritis;
- Juvenile rheumatism;
- Crohn's disease;
- Autoimmune hepatitis;
- Horton (autoimmune steatosis)
- Liver diseases (Steatosis, Fibrosis)
- Non-dietary and aplastic normocytic anemia;
- T-ALL (under chemo + Neupogen);
- Diverticulosis;
- RF, severe CF;
- Neurological disorders
- Unknown etiology

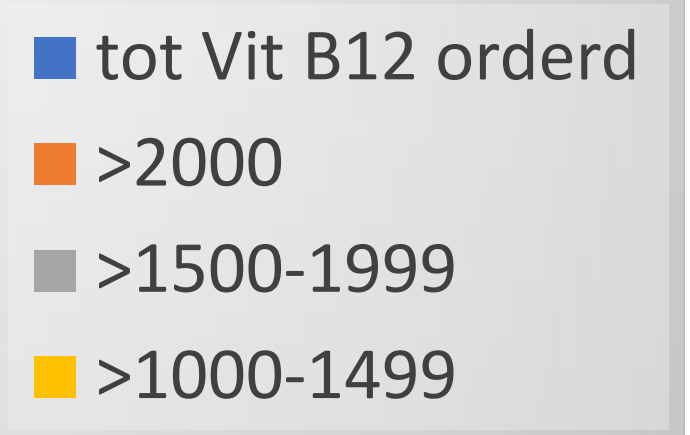


Unpublished data, under review

Vitamin B12: 2017's DATA (Hypothesis)



tot Vit B12 ordered	20158
>2000 pg/mL	427
>1500-1999 pg/mL	280
>1000-1499 pg/mL	959

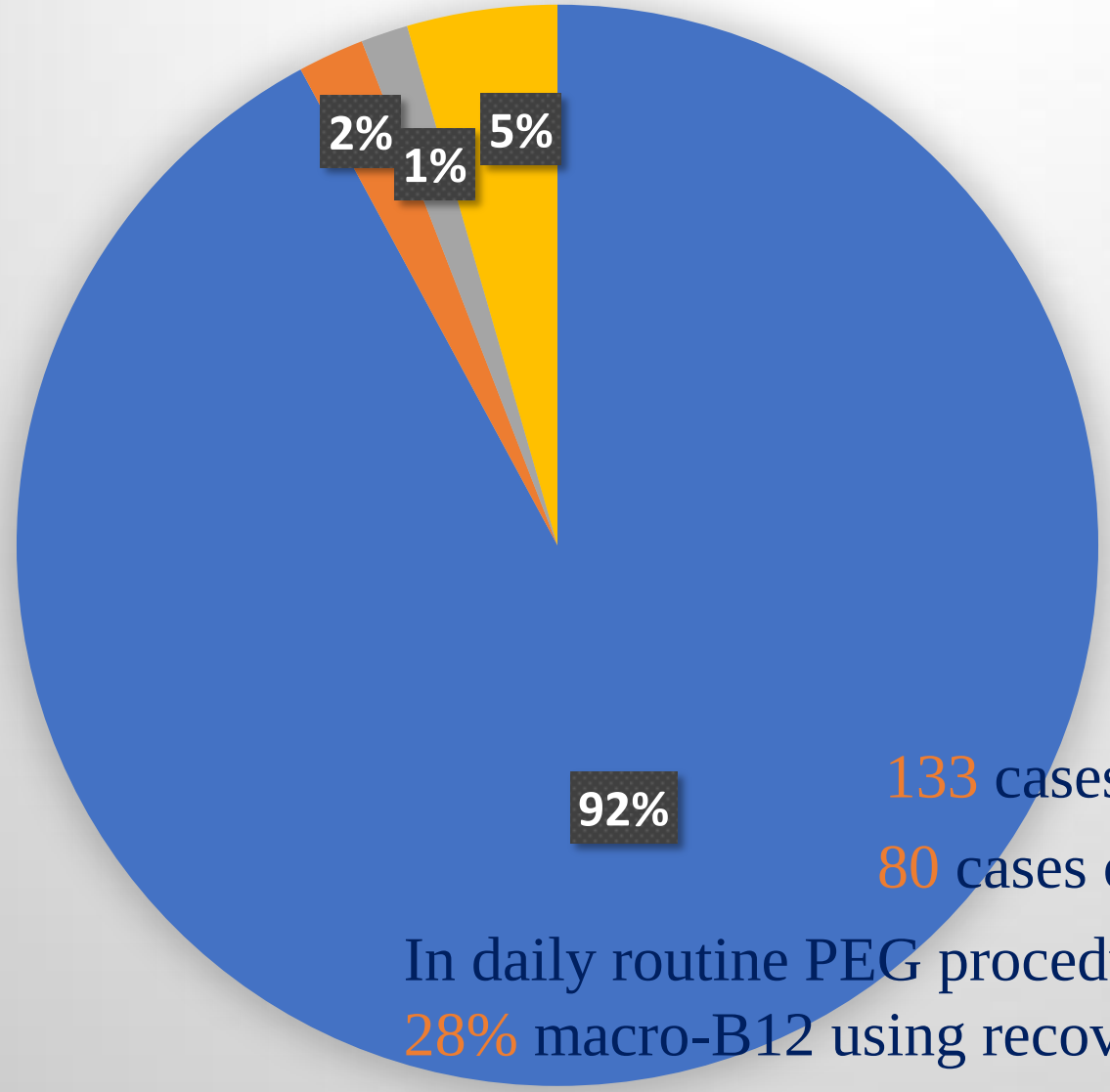


128 cases of macro B12 using % cut-off

77 cases of macro B12 using post-PEG RI

Unpublished data, under review

Vitamin B12: 2018 DATA(Hypothesis)



tot Vit B12 ordered	20568
>2000 pg/mL	443
>1500-1999 pg/mL	314
>1000-1499 pg/mL	1001

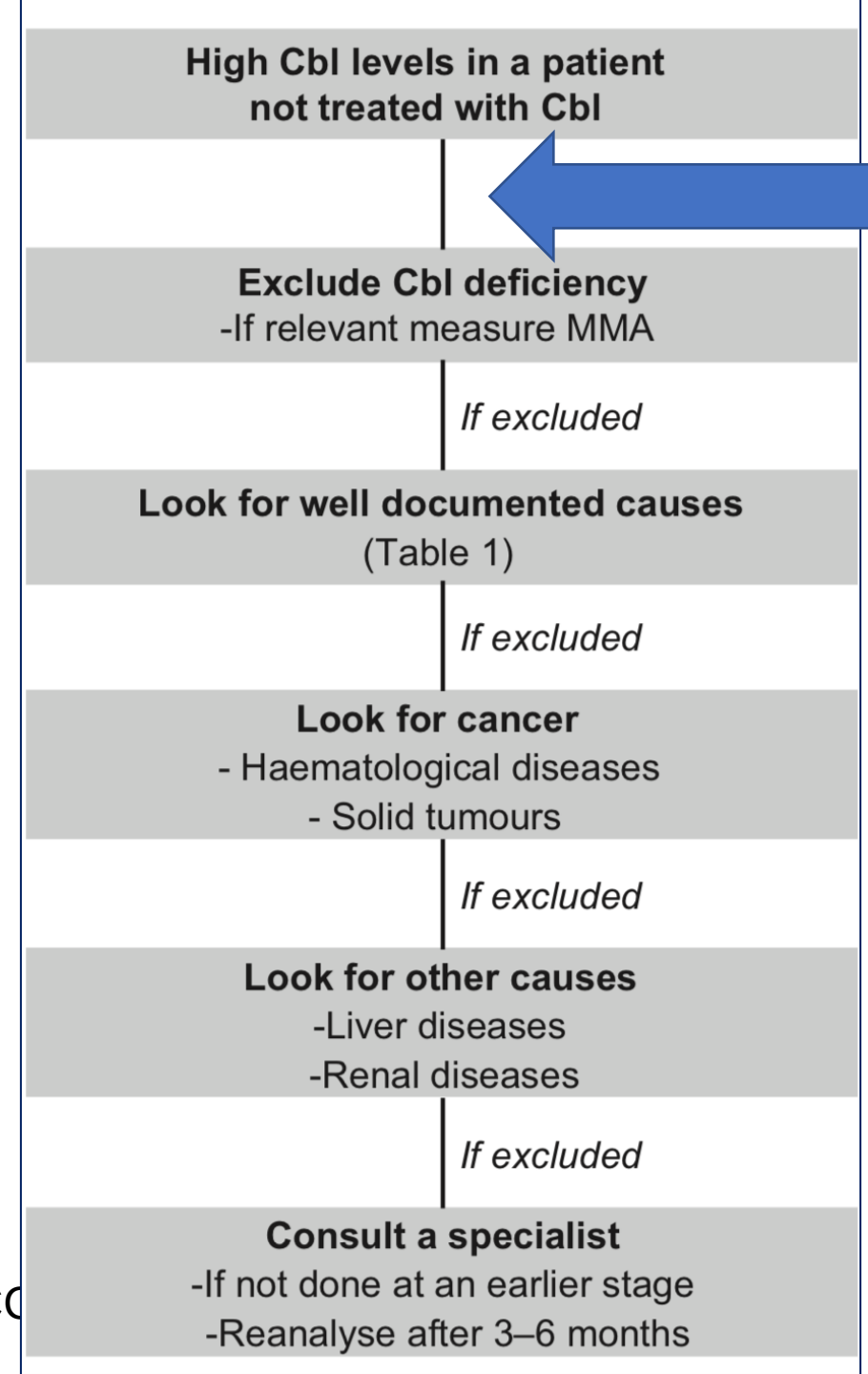
133 cases of macro B12 using % cut-off

80 cases of macro B12 using post-PEG RI

In daily routine PEG procedure for samples > 1500 pg/mL:
28% macro-B12 using recoveries and 15% using PEG RI

Unpublished data, under review

Conclusion



Interferences?

Johan F.B. Arendt* and Ebba Nexo, CC
2012

Conclusion

- 1- The prevalence is may be to high (18%)
- 2- At least, all B12 samples > 1,476 pmol/L have to be subject of PEG treatment
- 3- Dilution is not recommended
- 4- It’s not analyzer dependent
- 5- To be continued…

Sources

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Thanks for your Attention

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