Erasmus MC
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Diagnosing vitamin B12 deficiency:

The complexity of vitamin B12 testing

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What is vitamin B12 deficiency?

- Just a low concentration of vitamin B12 in the blood?
- The occurrence of macrocytic anemia (in the absence of folate deficiency)?
- The occurrence of typical glossitis?
- The occurrence of typical neurological symptoms, such as loss of sensibility in the lower extremities?
- The relief of typical, vitamin B12 deficiency-associated symptoms by treatment with vitamin B12?
- The occurrence of increased amounts of methylmalonic acid in serum or urine
- All or a number of those?

Connection between clinical symptoms and metabolism

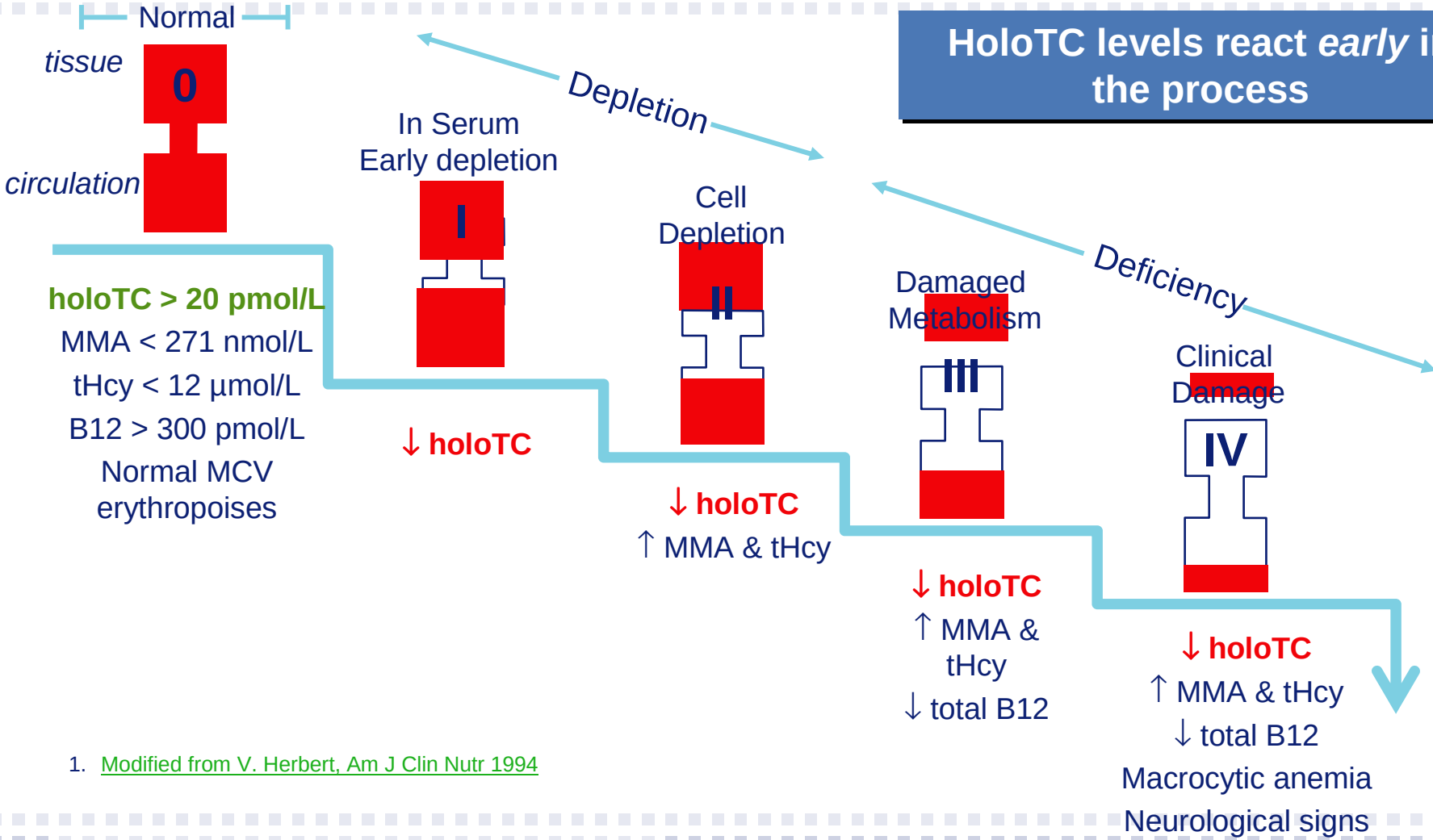
- Vitamin B12 is as coenzyme involved in two important reactions:
  - methylmalonylCoA → succinylCoA
  - Homocysteine → methionine
- Deficiency of vitamin B12 leads to storage of MMA and homocysteine

- The Hcy>Meth – reaction is necessary for cell devision and growth:
  - Hence B12-def. leads to anemia and mucosal damage
- Methionine is necessary as methyl-group donor in many reactions, including the methylation of nerve-isolating lipids and proteins
  - Hence B12-def. leads to nerve damage and loss of tactile sensibility

Vitamin B12 in the blood

- Is bound to transcobalamin and to haptocorrin:
  - Transcobalamin-bound B12 is the biologically available form for the peripheral tissues>>> named holo-TC or ActiveB12
  - Haptocorrin-bound B12 is scavanged from the peripheral tissues to be delivered to and metabolized by the liver
  - Holo-TC has normal refence value concentrations 21- 120 pmol/l
  - Holo-Haptocorrin has normal reference value concentrations from 125 – 500 pmol/l
- *A minority of total serum B12 is responsible for biological function.*

How vitamin B12 deficiency develops (hypothesis)



HoloTC levels react *early* in the process

1. Modified from V. Herbert, Am J Clin Nutr 1994

What may lead to depletion and deficiency?

- Insufficient intake of vitamin B12
- Insufficient release of B12 from food components by gastric enzymes
- Insufficient degradation if haptocorrin by pancreatic enzymes in pancreatic insufficiency
- Competition for ingested B12 by bacterial overgrowth
- Insufficient production of Intrinsic Factor//production of inactive intrinsic factor
- A diversity of extremely rare metabolic diseases, related to B12 transport and metabolism.

Most frequent causes of B12-deficiency

- Insufficient nutritional intake
- Insufficient Intrinsic Factor production by auto-antibodies to gastric mucosa and Intrinsic Factor, as in **pernicious anemia**
- Severe inflammation of ileal mucosa, as in **Crohn's disease**

- Increased utilization or loss?
  - **preganancy**
  - **Malignancy**
  - **Proteinurea?**

Aims of the multicenter study

- To establish analytical validity of the Active B12 assay
- To investigate clinical utility of the parameter
- To compare Active B12 with Total B12
- To establish reference values and clinical decision points in a representative patient population

Establishing reference values

- For ActiveB12 (Abbott AxSym assay) we found

21-117 pmol/l

- To be the reference values in an N=250 population (50% man) of healthy blood bank volunteers.
- We did not find a significant difference between man and women

Study population

Table I: Characteristics of study population

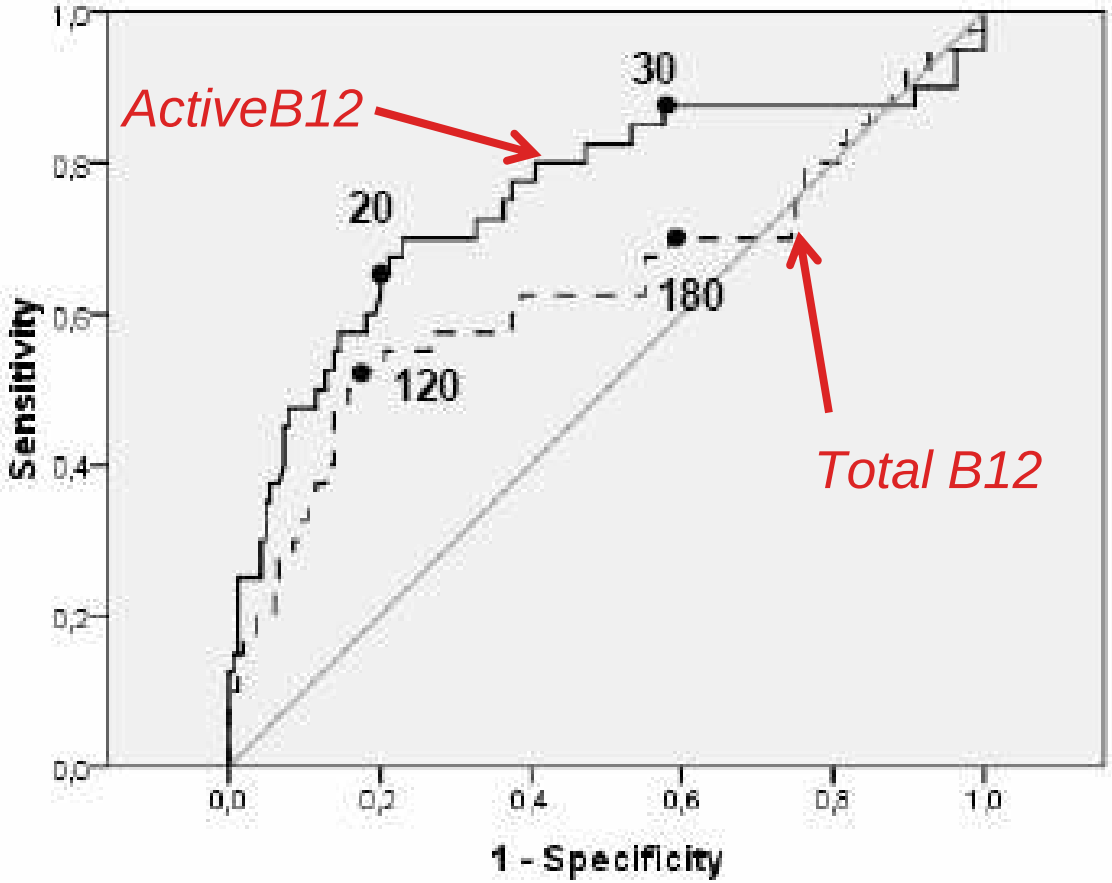
	N	Mean [95% CI]	Percentage abnormal (cut-off value[c])
Age, years	1,696	64.9 [63.9-65.9]	
Male, n (%)	1,693	633 (37.4%)	
Creatinine[a], µmol/L	1,491		
Males	567	107 [102-112]	25% (>115)
Females	921	82 [80-84]	26% (>90)
eGFR[a,b] (ml/min/1.73m^2)	1,488	65 [63.3-67.0]	26% (<60)
Hemoglobin, mmol Fe/L	1,654		
Males	617	8.4 [8.3-8.5]	46%(<8.6)
Females	1,034	7.9 [7.8-7.9]	30%(<7.5)
MCV, fL	1,649	91.0 [90.6-91.4]	7.3% (>100)
WBC, 10^9/L	1,631	7.6 [7.5-7.8]	2.2%(<3.5)
Platelets, 10^9/L	1,600	284 [279-288]	3.5% (<150)
Total B12[a], pmol/L	1,318	249 [242-256]	7.9% (<145)
HoloTC[a], pmol/L	1,593	36.5 [35.5-37.6]	16% (<21)
MMA[a], µmol/L	566	0.30 [0.28-0.32]	21% (>0.45)

Individuals with age < 18 years were excluded from analysis; [a]Geometric mean; [b]According to MDRD guidelines; [c] Reference values depicting either the 2.5% lower value or 2.5% higher value depending on the biomarker.

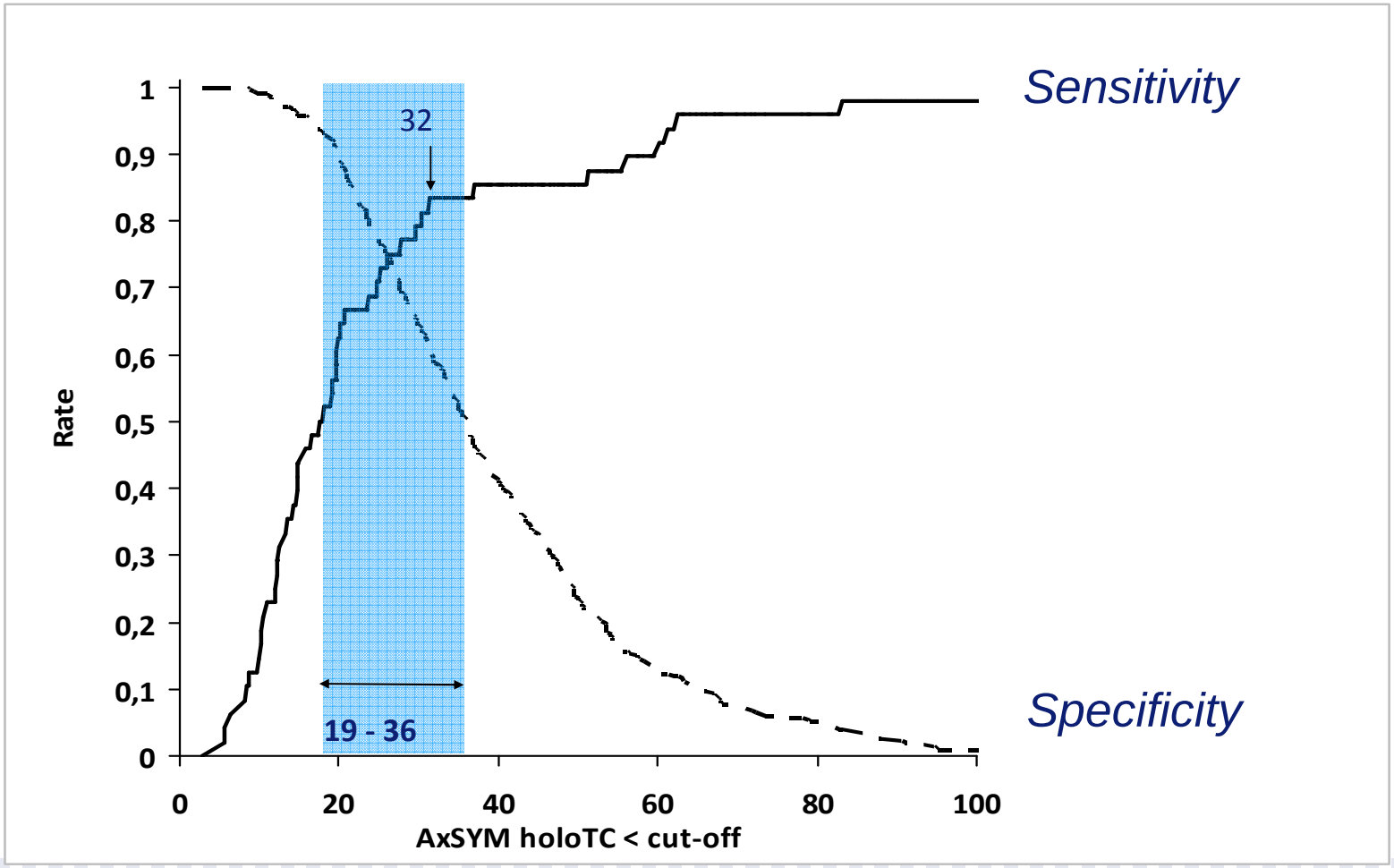
Choosing the reference standard

- There is no generally accepted definition of B12 deficiciency
- Considering that
  - increasing MMA is an early signal of B12-deficiency
  - MMA measurement is complicated but stable and reproducable
  - MMA level is a relatively specific biomarker for B12 status in comparison with homocysteine, Hb, MCV, WBC, platelets
- We have, in this study, taken MMA as a reference standard for defining a patient either B12–sufficient (MMA </= 0.45 µmol/l serum) or B12-deficient (MMA > 0.45 µmol/l serum).

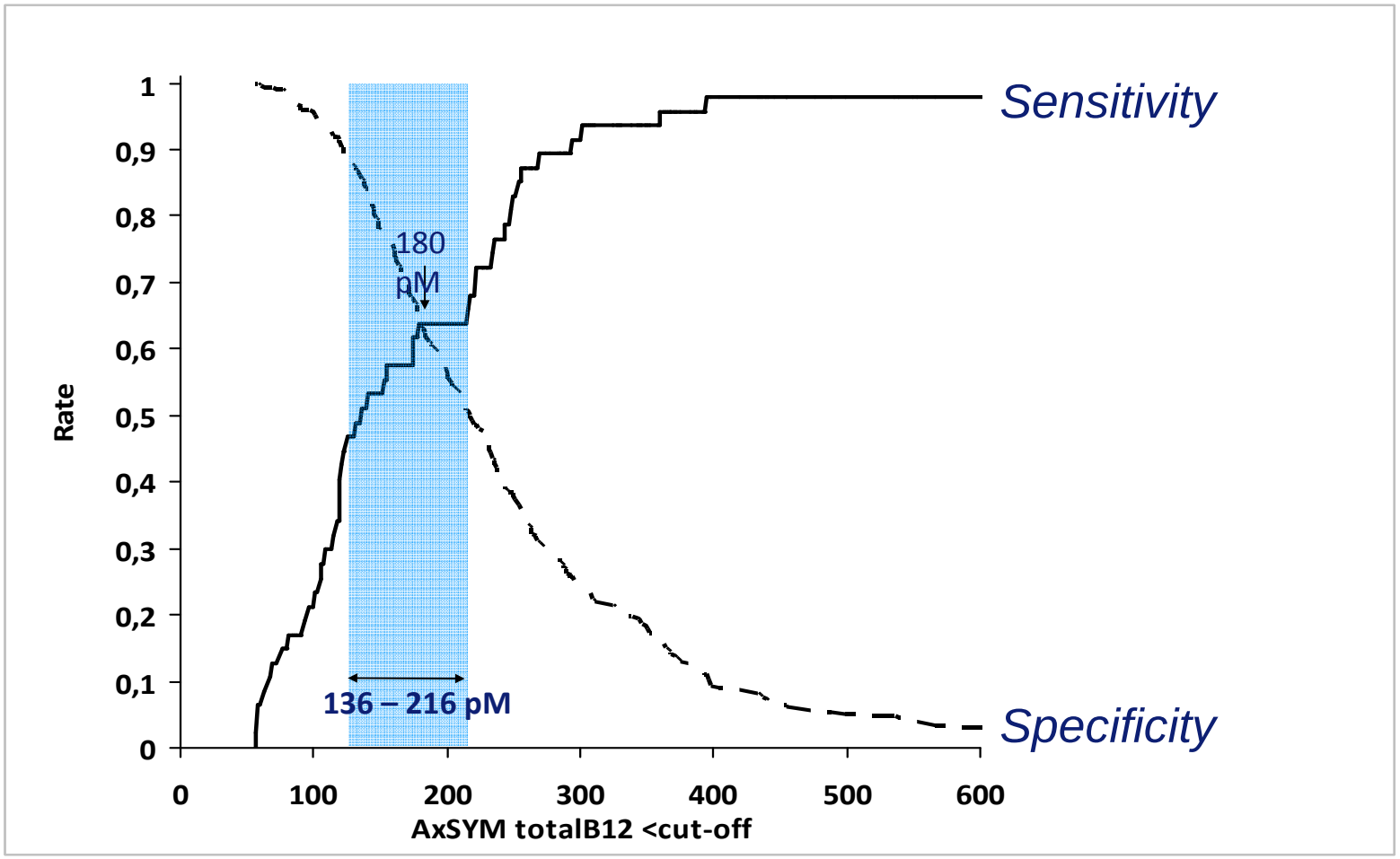
ROC curve for HoloTC(=ActiveB12) and Total B12



Determining Optimal cutoff “Active B12”



Determining optimal cutoff Total B12



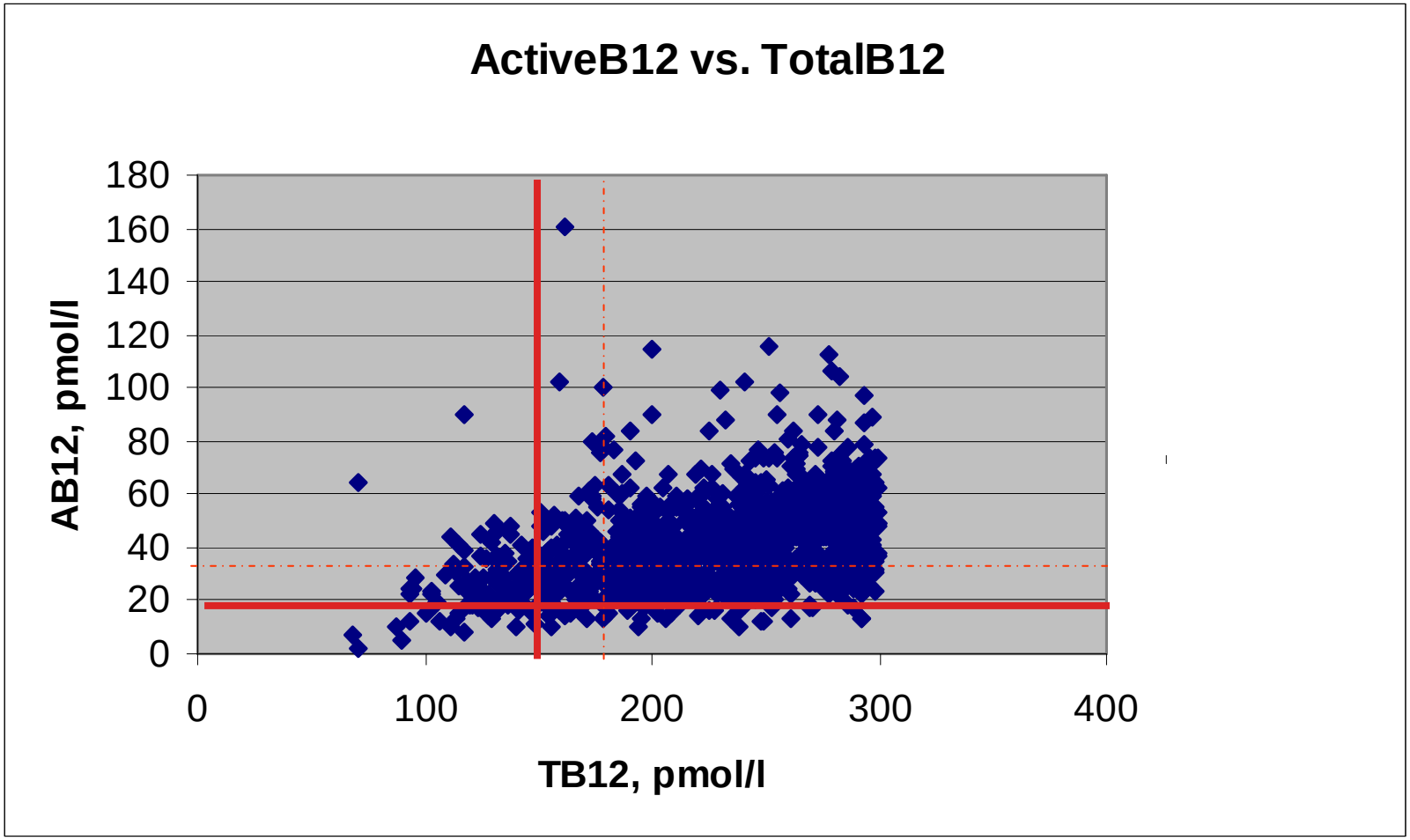
Test characteristics overview

Table II: Test characteristics of total B12 and holoTC assays

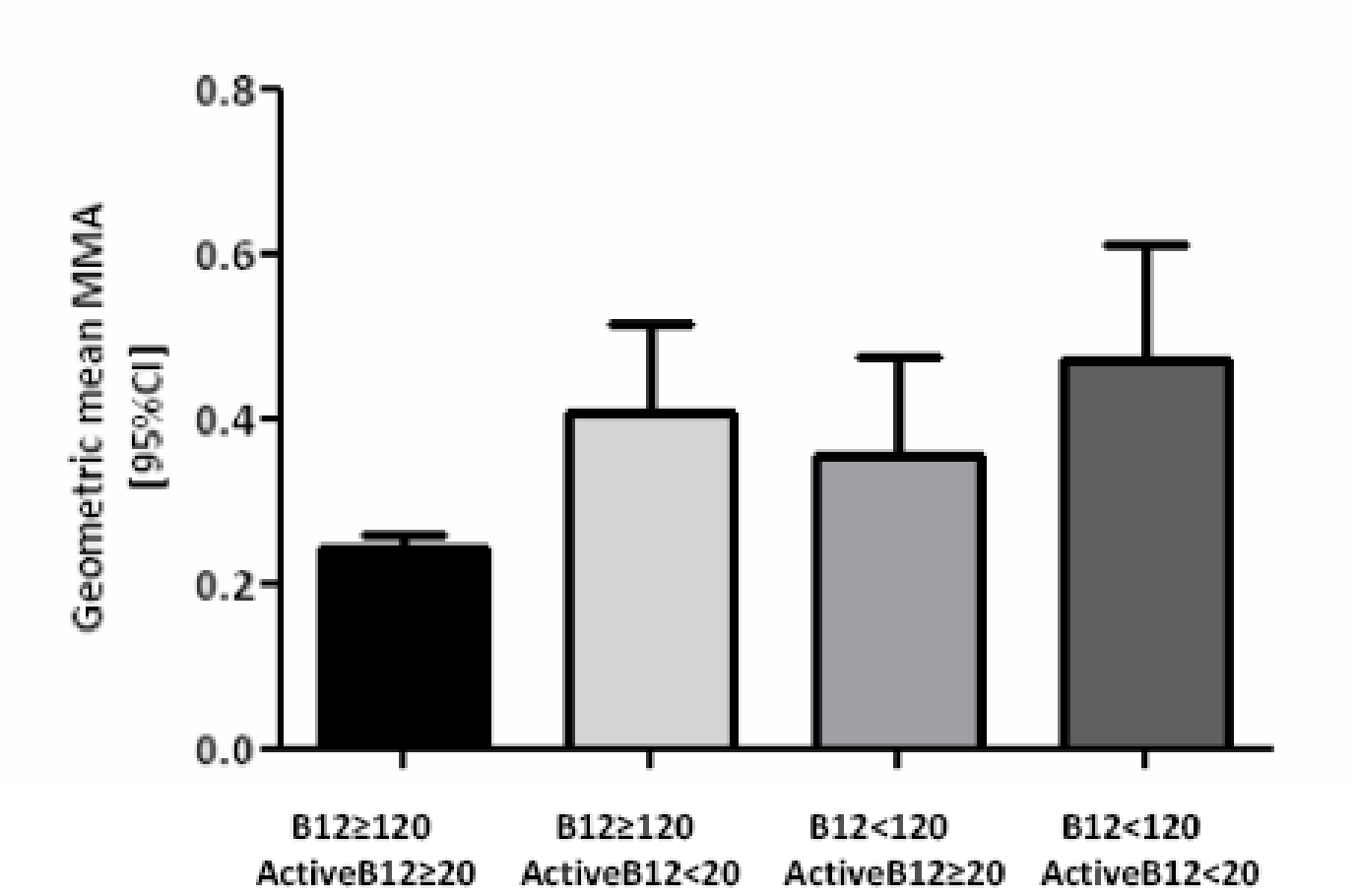
	HoloTC		Total B12	
Cut-off	<21[1]	<32[2]	<145[1]	<180[2]
TP	30	39	25	30
FN	17	8	22	17
TN	274	188	253	201
FP	39	125	60	112
Sensitivity (%)	63.8	83.0	53.2	63.8
Specificity (%)	87.5	60.1	80.8	64.2
PPV (%)	43.5	23.8	29.4	21.1
NPV (%)	94.2	95.9	92.0	92.2
LR+	5.12	2.08	2.77	1.78
LR-	0.41	0.28	0.58	0.56

[1]Reference value; [2]Clinical decision point based on this study; TP = true positives; FN = false negatives; TN = true negatives; FP = false positives; PPV = positive predictive value; NPV = negative predictive value; LR+ = positive likelihood ratio; LR- = negative likelihood ratio.

Diagnostic samples Erasmus MC (N=1100)



MMA values in different subgroups



Effect of combining both assays

Table III: Single- and combined testing of holoTC and total B12 in screening for vitamin B12 deficiency

	TP (N)	FP (N)
Total B12 (<180 pmol/L)	30	112
HoloTC (<32 pmol/L)	39	125
Combined testing		
HoloTC after total B12[1]	+10	+7
HoloTC before total B12[1]	+1	+7

[1]Individuals who are negative for the primary test and whom are identified as positive in the secondary test;

A focus on the discrepancies between “A” and “T”.

From about diagnostic 3500 samples with total B12 < 300 pmol/l
140 discrepant results were found:

- Group I: Total B12 ≤ 120 but Active B12 > 20 pmol/l:

  16% methylmalonic acid > 0.45 µmol/l

- Group II:Total B12 > 120 but Active B12 ≤ 20 pmol/l:

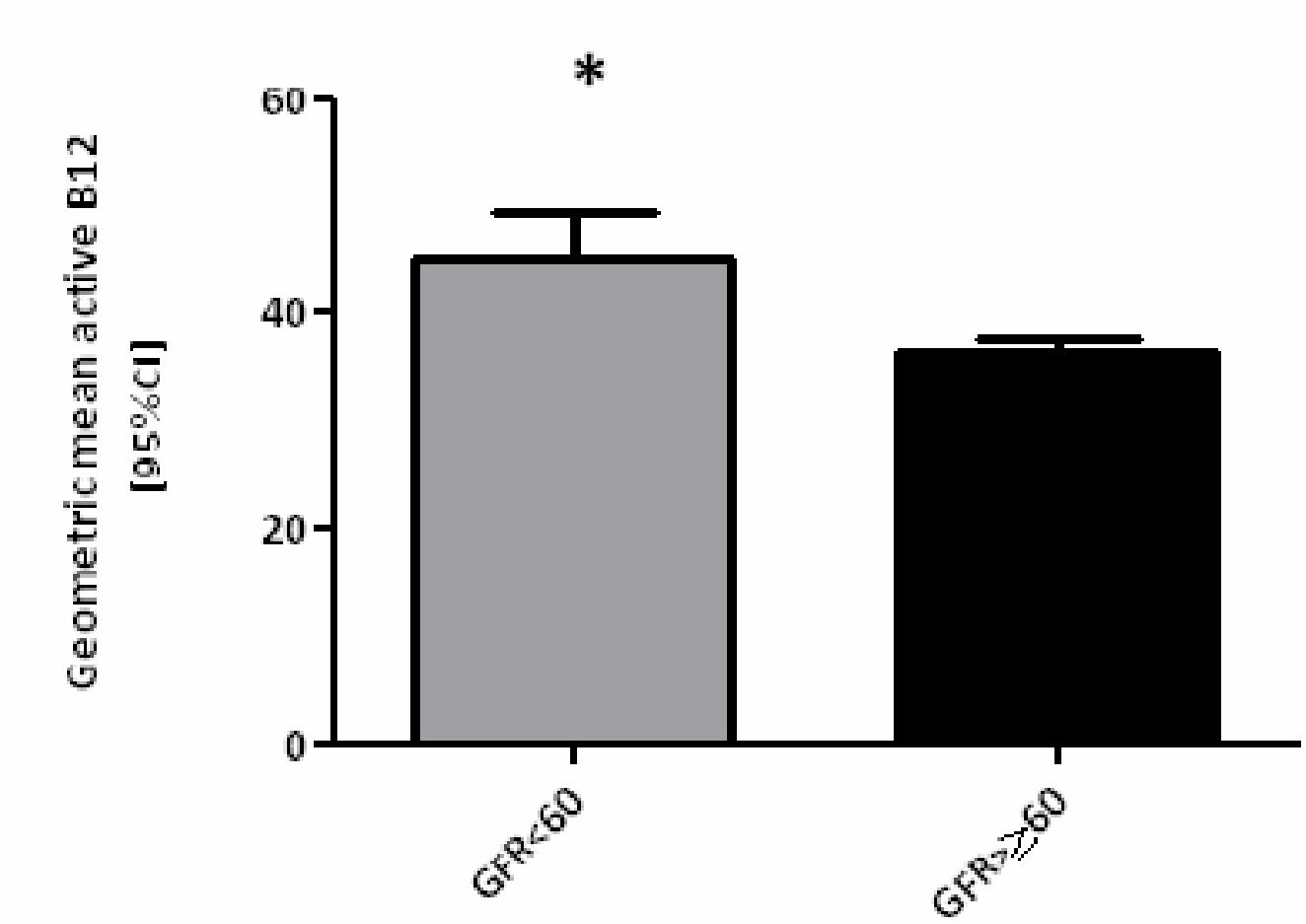
  70% methylmalonic acid > 0.45 µmol/l

Conclusion: Active B12 appears a better predictor of disturbed B12-dependent metabolism than Total B12.

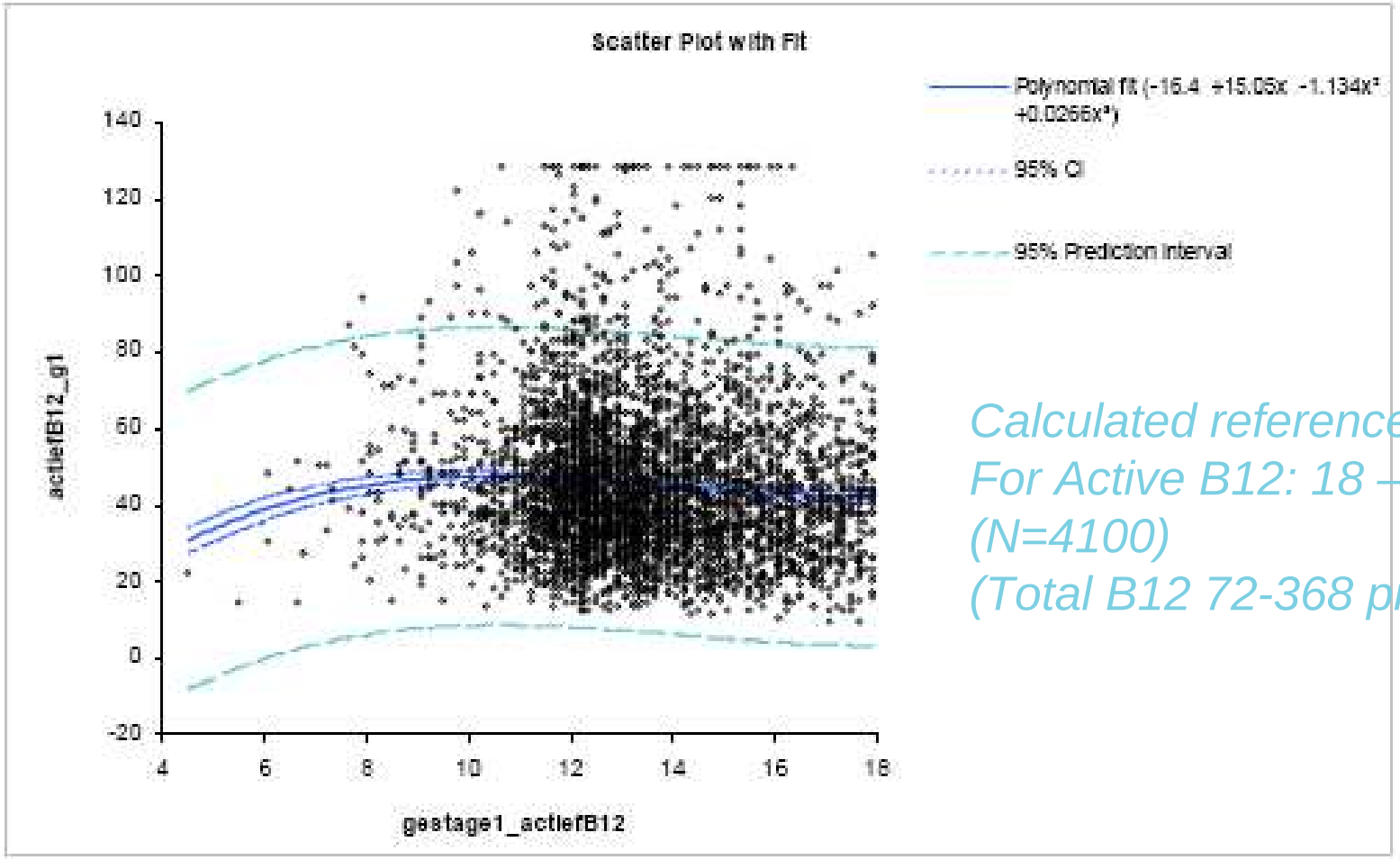
Discrepancies in specific patient groups:

- Normal *Total B12* with a subnormal *Active B12* in particular seen in patients with:
  - inflammatory bowel disease s.a. Crohn's disease
  - Cancer
- Subnormal *Total B12* with normal *Active B12* in particular seen in
  - pregnancy
  - Individuals with a congenital shortage of the B12-binding protein Haptocorrin

Active B12 and GFR

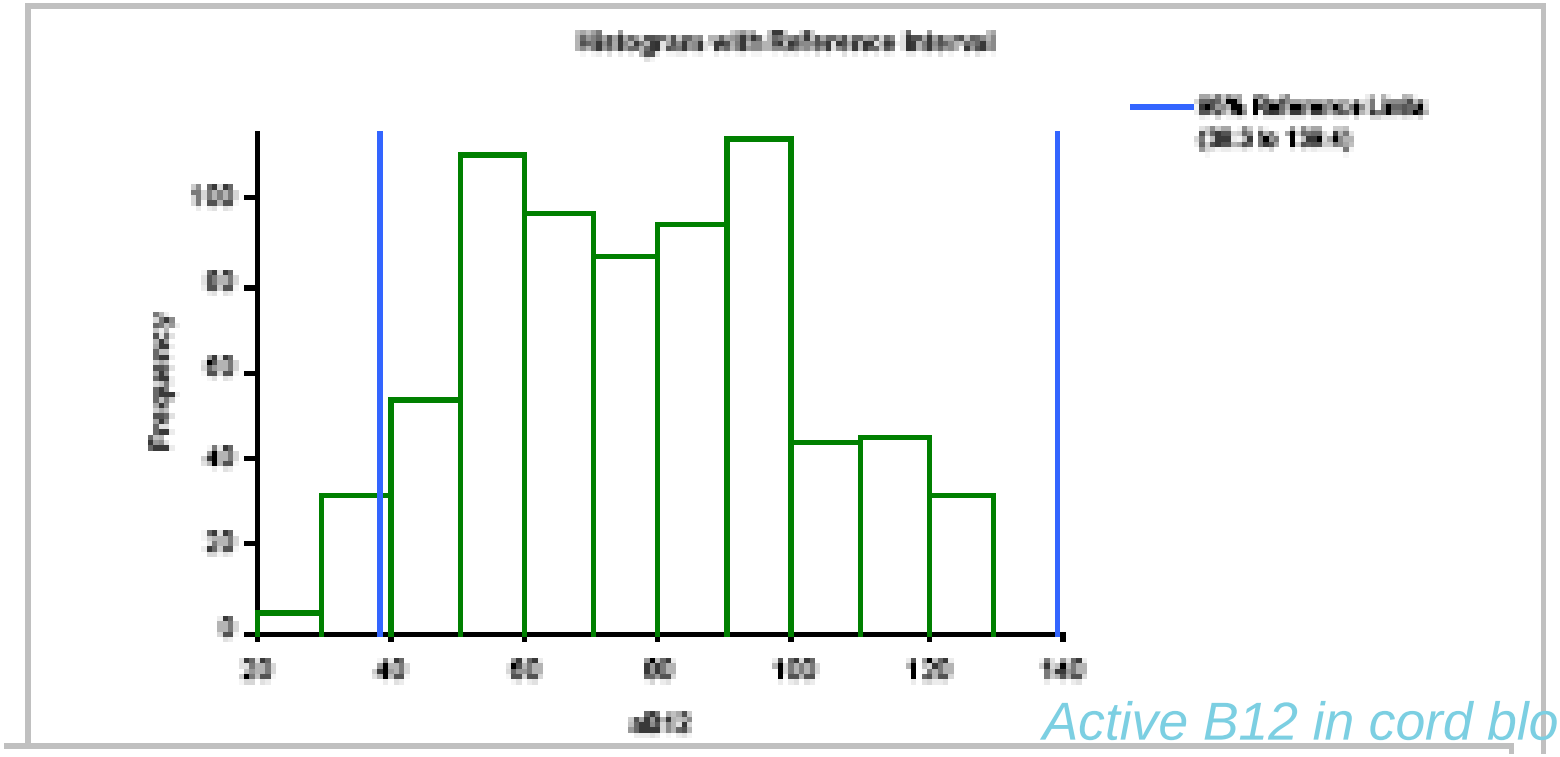


B12 data in 1st trimester pregnancy



Calculated reference interval
For Active B12: 18 – 95 pmol/l
(N=4100)
(Total B12 72-368 pmol/l)

B12 data in cord blood



Active B12 in cord bloods;
Reference Intervals:
Active B12: 39 -138 pmol/l, N=713
Total B12: 38 -820 pmol/l. N=574

Particular case: Haptocorrin deficiency

- Typical case, Patient H.:
  - Total B12 98 pmol/l.
  - No anemia, no macrocytosis
  - No neuropathy
  - No gastro-intestinal disease
  - Normal p-homocysteine
  - Methylmalonic acid normal (0.24 µmol/l; Ref.v. < 0.46)

What is the explanation?

- Additional data: Active B12 46 pmol/l, Holo-Haptocorrin 52 pmol/l, Apo-Haptocorrin 54 pmol/l. >> Total HC 106 pmol/l, which is far below the lower reference value (>175 pmol/l).
- Conclusion: patient with partial deficiency of haptocorrin, which appears to be of no clinical consequence

Conclusions:

- **Accepting MMA > 0.45 µmol/l as a reference standard for vitamin B12 deficiency the *Active B12* assay demonstrates a better sensitivity and specificity in detecting vitamin B12 deficiency than the *Total B12* assay in a mixed collection of diagnostic samples.**
- **Sensitivity can be improved by a higher cut-off but only at the expense of a substantially lower specificity. Total test efficiency decreases. We recommend 32 pmol/l as cutoff.**
- **Between 21 and 32 pmol/l deficiency might be confirmed by MMA determination.**

Conclusions(2)

- **For the detection of B12-deficiency *Active B12* can replace *Total B12* as a first line diagnostic aid; no reason for combination with *Total B12***

Conclusions (3)

- Active B12 is increased in renal insufficiency; this appears to be a physiological phenomenon, not an interference in the assay.
- Active B12 is rather normal in 1st trimester pregnancy whereas total B12 is generally low; Active B12 is relatively high in cord blood.
- By measuring Active B12 and Total B12, holo-Haptocorrin can be calculated. After measuring also apo-Haptocorrin a specific group of patients with (partial) deficiency of Haptocorrin can be diagnosed. This is relevant in view of the question whether treatment is indicated or not.