

## Supplementary materials to:

# Diagnostic accuracy of holotranscobalamin, vitamin B12, methylmalonic acid, and homocysteine in detecting B12 deficiency in a large, mixed patient population

Running title: Markers of B12 deficiency in a mixed cohort

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In the following tables we report on the results on a per patient analysis of the dataset. Only the data obtained at the occasion from the first blood drawing were taken for this analysis, which included a total of 9,464 patients.

**Table S1.** Baseline characteristics of the 9,464 patients. IQR=interquartile range for continuous variables; CI= confidence interval for proportion. Both are given in [brackets].

Parameter (units)	Median, IQR or 95%-CI
Patient age (years)	54 [39,68]
Female gender (n)	59.3% [58.3,60.3] (5616)
Holotranscobalamin (pmol)	64 [45,93]
B12 (pmol/L)	286 [217,381]
MMA (nmol/L)	177 [137,237]
Hcy ( $\mu$ mol/L)	12.2 [9.7,15.3]
HoloTC/B12 (%)	21.6% [16,28.4]
Haptocorrin-bound B12 (pmol/L)	220 [160,300]
4cB12	0.23 [-0.12,0.58]
-Elevated B12 (4cB12>1.5) (n)	0.89% [0.72, 1.1] (84)
- B12 adequacy (4cB12: -0.5 to 1.5) (n)	89.7% [89,90.3] (8486)
- Low B12 (4cB12: -1.5 to -0.51) (n)	8.9% [8.3,9.5] (841)
- Possible B12 deficiency (4cB12: -1.51 to -2.5) (n)	0.5% [0.37,0.66] (47)
- Probable B12 deficiency (4cB12 < -2.5) (n)	0.06% [0.03,0.14] (6)

**Table S2.** Marker cut-offs for detecting subclinical B12 deficiency (4cB12  $\leq$ -0.5 and  $>$ -1.5) with a sensitivity or specificity of 99% at a given specificity and sensitivity, as well as the optimum decision point in 9,464 patients.

Marker	Cut-off 99% sensitivity	Corresponding specificity (%)	Cut-off 99% specificity	Corresponding sensitivity (%)	Optimum decision point	Corresponding Sensitivity/ Specificity (%)
HoloTC (pmol/L)	<72.4	44.2	<24.8	25.9	<45	85.2 / 81
B12 (pmol/L)	<352	34.4	<141	27.6	<229	87 / 76.6
MMA (nmol/L)	>153	39.6	>485	27.4	>238	83.7 / 82
Hcy ( $\mu$ mol/L)	>8	12.6	>29.5	11.1	>15	67.9 / 77.3

**Table S3.** Marker cut-offs for detecting possible or probable B12 deficiency (4cB12<sub>≤</sub>-1.5) with a sensitivity or specificity of 99% at a given specificity and sensitivity, as well as the optimum decision point in 9,464 patients.

<b>Marker</b>	<b>Cut-off 99% sensitivity</b>	<b>Corresponding specificity (%)</b>	<b>Cut-off 99% specificity</b>	<b>Corresponding sensitivity (%)</b>	<b>Optimum decision point</b>	<b>Corresponding Sensitivity/ Specificity (%)</b>
HoloTC (pmol/L)	<38.5	83.3	<19.5	76	<27	94 / 95.8
B12 (pmol/L)	<197	82.8	<114	56	<167	96 / 91.7
MMA (nmol/L)	>157	38.1	>736	74	>532	96 / 97.5
Hcy (μmol/L)	>6.1	2.6	>34.2	28	>16,4	90 / 80.7

**Table S4.** Diagnostic accuracies of the different markers for detecting low B12 (4cB12 <-0.5 and >-1.5) in 9,464 men and women over or under 50 years. \* Significant difference between the AUC of B12 and HoloTC ( $p<0.05$ );<sup>†</sup> significant difference between HoloTC and MMA ( $p<0.05$ ); <sup>‡</sup> significant difference between Hcy and all other markers ( $p<0.01$ ); <sup>°</sup>significant difference between MMA and B12 ( $p<0.05$ ). The difference in the AUC of HoloTC and B12 ( $p=0.056$  in women) and HoloTC and MMA ( $p=0.087$  in men  $\geq 50$  years) was not significant in either subgroup.

<b>Marker</b>	<b>Women</b>	<b>Men</b>	<b>&lt;50 years</b>	<b><math>\geq 50</math> years</b>	<b>Women, &lt;50 years</b>	<b>Women, <math>\geq 50</math> years</b>	<b>Men, &lt;50 years</b>	<b>Men, <math>\geq 50</math> years</b>
(N whole group)	(N=5589)	(N=3822)	(N=3876)	(N=5537)	(N=2630)	(N=3959)	(N=1246)	(N=2576)
(n with low 4cB12)	(n=475)	(n=344)	(n=244)	(n=675)	(n=171)	(n=304)	(n=73)	(n=271)
HoloTC	0.911	0.912	0.914	0.914 *	0.918	0.919 *	0.914	0.909
B12	0.896	0.906	0.924	0.887	0.921	0.882	0.931	0.894
MMA	0.915 °	0.9	0.924	0.898 †	0.927	0.909 °	0.916	0.889
Hcy	0.784 ‡	0.825 ‡	0.769 ‡	0.80 ‡	0.769 ‡	0.799 ‡	0.851 ‡	0.808 ‡

**Table S5.** Diagnostic accuracies of the different markers for detecting potential or probable B12 deficiency (4cB12 <-1.5) in men and women over or under 50 years. \* Significant difference between the AUC of B12 and HoloTC ( $p < 0.05$ ); # significant difference between Hcy and B12 and HoloTC ( $p < 0.05$ ); ^ significant difference between Hcy and B12 and MMA ( $p < 0.05$ ); ‡ significant difference between Hcy and all other markers ( $p < 0.05$ ).

<b>Marker</b>	<b>Women</b>	<b>Men</b>	<b>&lt;50 years</b>	<b>≥50 years</b>	<b>Women, &lt;50 years</b>	<b>Women, ≥50 years</b>	<b>Men, &lt;50 years</b>	<b>Men, ≥50 years</b>
(N whole group) (n with 4cB12<-1.5)	(N=5616) (n=26)	(N=3848) (n=24)	(N=3884) (n=5)	(N=5580) (n=43)	(N=2634) (n=4)	(N=2982) (n=22)	(N=1250) (n=3)	(N=2598) (n=21)
HoloTC	0.993 *	0.98	0.971	0.99*	0.99	0.995 *	0.956	0.983
B12	0.975	0.978	0.988	0.972	0.988	0.972	0.994	0.971
MMA	0.973	0.989	0.998	0.972	1	0.96	0.997	0.985
Hcy	0.895 #	0.932 ‡	0.831	0.905 ‡	0.748	0.893 #	0.94 ^	0.924 ‡