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Novel in vitro chimeric sTRAb assay measures thyroid stimulating autoantibodies (TSI) in serum of Graves' disease patients

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Introduction:

Graves' disease (GD) is caused by thyroid stimulating autoantibodies (TSI) targeting the thyrotropin receptor (TSHR). The novel chimeric TRAb assay, which directly measures these autoantibodies by double epitope recognition (sTRAb) [1], was evaluated via comparison with the stimulatory index (SI) of a bioassay (bioTRAb) [2] and serum T4 as well as for its clinical significance.

Methods:

In the sTRAb assay a human chimeric TSHR fixed to microtiter plates binds one arm of the sTRAb. The second arm bridges to a human TSHR (aa 21–261) fused with secretory alkaline phosphatase (SEAP) for chemiluminescence signalling. In the bioTRAb assay using wildtype TSHR, cAMP stimulation was measured as SI by SEAP/CRE reporter gene construct. Also TRAb (TRAK human assay, Brahms) and total T4 (Ortho Clinical Diagnostics) were tested. Relations between continuous values were quantified with linear regression analysis of log-transformed values.

Recuitment of samples:

Samples for correlation studies were taken from a collection of 47 untreated GD patients. Treated GD patients presented during usual transfer practice (PDUTP) to thyroid clinics; inclusion criteria were following the guidelines of the ATA and AACC. Patients with active endocrine orbitopathy (EO) were classified according to the EUGOGO guidelines. Waste serum was used for all samples.

Results:

Correlation studies: For untreated GD patients, correlation was determined for sTRAb titers vs. bioTRAb SI (n=32, r=0,66, p<0,0001); sTRAb titers vs. serum T4 (n=47, r=0,74, p<0,0001); bioTRAb SI vs. serum T4 (n=32, r=0,39, p<0,03). For treated GD patients correlation was determined for sTRAb titers vs. bioTRAb SI values (r= 0,71, p<0,001).
Special uncertain cases could be clarified: Positive TRAb values persisted in 3 GD cases succeeding in remission in contrast to negative sTRAb values (present among 37 cases: 8%). In 3 hypothyroid patients (PDUTP of 468 GD patients to thyroid clinics, 0.62%) sera were positive in the TRAb assay, whereas autoantibodies were not detected by the sTRAb and the bioTRAb assays. One euthyroid subject (PDUTP of 252 GD patients: 0,4%) was repeatedly TRAb positive (22 IU/L) but sTRAb and bioTRAb negative. Concerning endocrine orbitopathy (EO) we found that sTRAb titers differentiate between GD (A) and GD with active (B) and inactive EO (C). Corresponding statistical analysis showed the following medians±SD: A: 2,96±9,37 IU/L, n=150; B: 22,77±15,46 IU/L, n=24, and C: 6,6±15,23 IU/L, n=77; p<1e-5.

Conclusions:

The chimeric sTRAb assay, introducing Bridge technology, measures TSI evidenced by bioTRAb SI and thyroid secretion product serum T4 levels. It delivers high diagnostic accuracy for GD, may clarify special cases and assist in monitoring EO. Finally, the robustness of the Bridge Assay will allow high throughput by performance on a suitable automated platform.

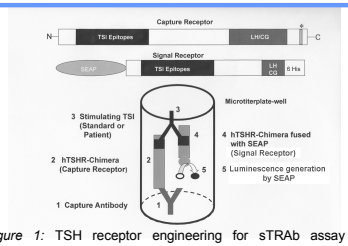

Figure 1: TSH receptor engineering for sTRAb assay demonstrated by linear structure and illustrating the direct detection of TSHR stimulating autoantibodies (sTRAb) with chimeric hTSHR constructs.

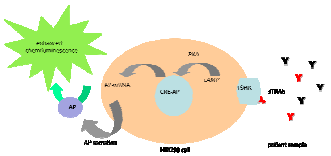

Figure 2: Principle of the bioTRAB assay. The binding of autoantibodies (or TSH) to the TSH receptor in genetically engineered HEK cells induces a cAMP signaling cascade, which ultimately results in secretion of a stable reporter enzyme (AP).

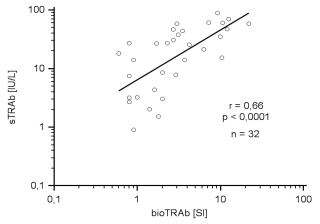

Figure 3a: Correlation between sTRAb and bioTRAb with samples of 32 untreated patients.

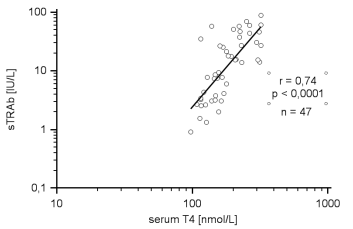

Figure 3b: Correlation between sTRAb and serum T4 with samples of 47 untreated patients.

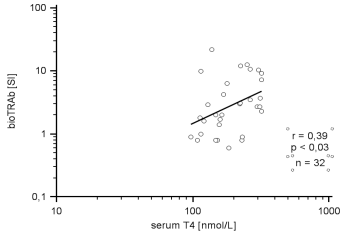

Figure 3c: Correlation between bioTRAb and serum T4 with samples of 32 untreated patients.

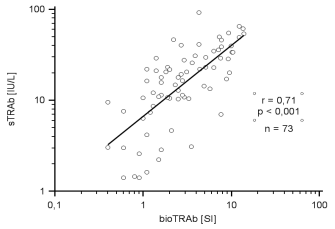

Figure 4: Correlation between sTRAb and bioTRAb with samples of 73 treated patients.

Figure 5a: GD patient with hyperthyroidism, goiter and exophthalmos (written consent).

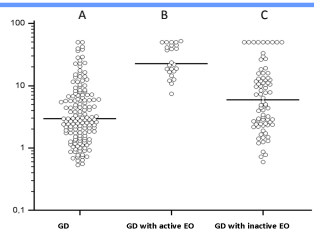

Figure 5b: Scatter plot showing values of 150 GD positive patients without eye disease (A: median 2,96 IU/L), 24 patients with active EO (B: median 22,77 IU/L) and 77 patients with inactive EO (C: median 5,96 IU/L)

Table 1. Special cases

	TRAb [IU/L]	sTRAb [IU/L]	bioTRAb [SI]
D1825	2,1	1,32	0,8
R0834[a]	1,7	0,44	-
R7992	2,3	0,53	0,6
A001a	13,0	0,00	0,5
A002	7,0	0,53	0,6
PL02[a]	18,8	0,0	-
B1344	22	0,00	0,2

3 GD patients in remission (a) with persisting positive TRAb values and negative sTRAb and bioTRAb values; 3 hypothyroid patients (b) with positive TRAb assay, negative sTRAb assay and negative bioTRAb. One euthyroid subject (c) was TRAb positive but sTRAb and bioTRAb negative.
[a] No serum left for determination of bioTRAb

References:

1. Frank CU, Braeth S, Wanjura D, Dietrich JW, Loos U. Bridge Technology with TSH receptor chimera for sensitive direct detection of TSH receptor antibodies causing Graves' disease: Analytical and clinical evaluation. Horm Metab Res 2015 DOI 10.1055/s-0035-1554662
2. Loos U, Schmitt T, Frank C, Büsselmann I. Development of a novel bioassay for the detection of thyroid-stimulating autoantibodies in Graves' disease (GD) facilitating routine measurement by reduced handling steps and high stability of cAMP monitoring reagent. Poster presentation at the ITC Paris 2010, P-0020 Available at: https://b-com.mci-group.com/Abstract/Statistics/AbstractStatisticsViewPage.aspx?AbstractID=32320