# Sera from patients with Hashimoto's disease impair TSH signaling in a TSH receptor bioassay, and coexisting TRAb are identified as stimulating

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**Introduction**: TPO-Ab and TRAb are biomarkers of thyroid autoimmunity. Here we measured biological activity of thyroid autoimmunity (TA) by a TSH receptor bioassay in sera from patients with Hashimoto's disease (HD).

**Methods**: fT4, fT3, TSH, TPO-Ab, TRAb (Thermo Fisher) and sTRAb [1] were tested to confirm the diagnosis. Stimulating (sBioTRAb positive >approx. 1.2–1.3 stimulation index = SI) and blocking (bBioTRAb positive <approx. 0.7 SI) activity were measured by a TSH receptor CRE reporter gene bioassay in HEK cells [2]. Groups of patients with TA: 1. Hypothyroid and only TPO-Ab extremely positive 2. Hypothyroid and TPO-Ab moderately positive, TRAk and sTRAb negative or slightly positive 3. Hypothyroid and TPO-Ab, TRAk and sTRAb clearly positive 4. In an extended collective of G1 and G2 (52 TPO-Ab positive patients) the correlation between TPO-Ab and bBioTRAb values was studied. Statistical distributions were compared with Welch's t test and Pearson's correlation.

**Results**:
Signal tranduction was impaired in sera with high TPO-Ab titers in G1 ($p<0.01$) as compared to G2 and in total there was a significant correlation between TPO-Ab titers and inhibition of TSH signaling in the blocking assay (G4, r=04691, $p<0.001$, n=52). Interestingly, in G1 TRAK and sTRAb were negative, however, in G2 two cases were slightly sTRAb-positive; 7 cases were in grey zone of TRAK. In G3 CRE signaling could be stimulated by sera containing high TRAb ($p<0.01$) showing stimulatory character of coexisting TRAb values.

**Table 1:** Means ± SEM for G1-G4

| | | anti-TPO [U/ml] | TRAK [IU/L] | sTRAb [IU/L] | bBioTRAb [SI] | sBioTRAb [SI] |
|---|---|---|---|---|---|---|
| G1 | n=10 | 7576±1449 | 0.1±0.02 | 0.01±0.00 | 0,52±0.004 | n.d. |
| G2 | n=34 | 2088±201 | 0.4±0.1 | 0.14±0.06 | 0.97±0.07 | n.d. |
| G3 | n=7 | 3829±1707 | 22.5±10.3 | 24.34±6.35 | n.d. | 2.0±0.68 |
| G4 | n=52 | 3427±469 | 0.38±0.06 | 0.11±0.04 | 0.80±0.03 | n.d. |

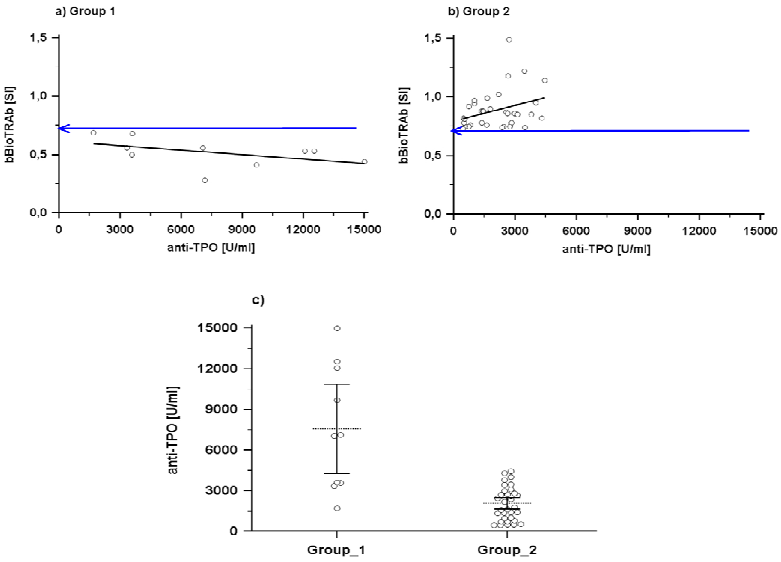


**Fig. 1:** Correlation between TPO-Ab titers and inhibition of TSH signalling in a blocking TSHR bioassay. Blue arrows are depicting the bBioTRAb cutoff. **(a)** G1 with r = - 0,5784 (p=0,08) **(b)** G2 with r = 0,3377 (p=0,05) **(c)** Comparsion of means of G1 and G2 showing a highly significant ($p<0.0001$) difference between the 2 groups.

**Table 2:** Correlation between bBioTRAb and TPO-Ab titers. G4 was classified in groups depending on the anti-TPO titers (> 100 / > 1000 / > 2500 / >4000 U/ml). An increasing negative linear correlation can be seen with increasing anti-TPO titers.

| | | | r | p |
|---|---|---|---|---|
| anti-TPO [U/ml] | > 100 | n=52 | -0,4691 | =0,0005 |
| | > 1000 | n=42 | -0,5338 | =0,0003 |
| | > 2500 | n=27 | -0,5459 | <0,01 |
| | > 4000 | n=12 | -0,6008 | <0,05 |

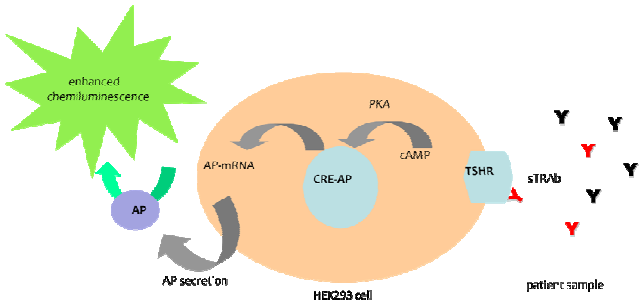


**Fig. 2:** Principle of the BioTRAb assay. The binding of autoantibodies (or TSH) to the TSH receptor in genetically engineered HEK cells induces a cAMP signalling cascade, which ultimately results in secretion of a stable reporter enzyme (AP) for signalling.

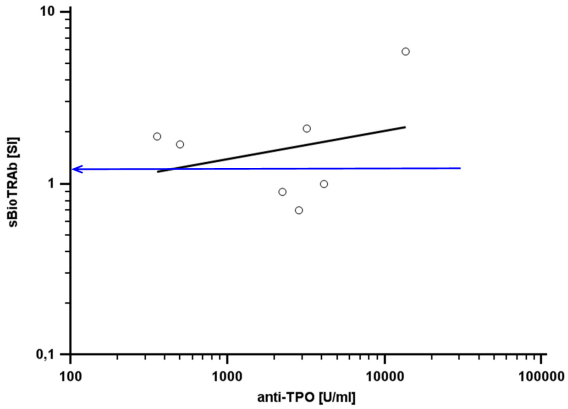


**Fig. 3:** In G3 with clearly positive TPO-Ab, TRAK and sTRAb titers from clinically hypothyroid patients (n=7) there is a positive correlation between TPO-Ab titers and stimuation of TSH receptor in a TSHR bioassay, r=0.8686. (p=0.01). The blue arrow is depicting the sBioTRAb cutoff.

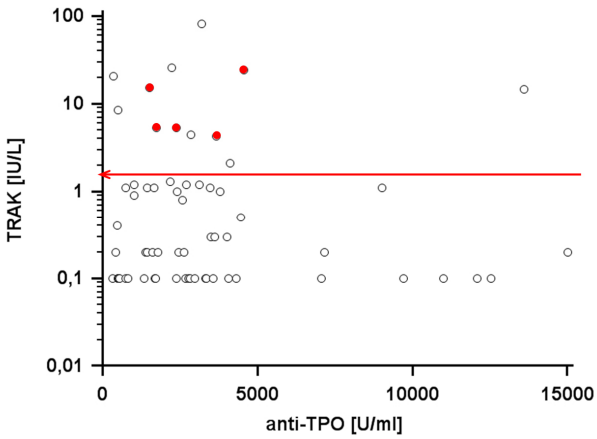


**Fig. 4:** TPO-Ab titers and corresponding TRAK values. The red arrow is depicting the TRAK cutoff. Unselected samples from patients with positive TPO-Ab titers (n=64); n=12 (19%) samples were TRAK and sTRAb positive, 5 of them were hyperthyroid and needed thyrostatic treatment (red dots). Out of these TRAb positive samples, 8 (66%) had a sBioTRAb > 1.2 SI up to 5.9 SI; 9 out of 52 (17%) TRAb negative samples had a bBioTRAb < 0.7–0.28 SI.

**Conclusion**: Sera in HT inhibit cAMP accumulation increasingly with the height of TPO-Ab titers in none thyroidal cells harboring the TSHR and CRE-AP suggesting cAMP pathway may be involved in the pathogenesis of hypothyroidism in HD. Increasing TRAb may lead to hyperthyroidism.

References:
1. Horm Metab Res. 2015 Nov;47(12):880-8
2. Poster ITC Paris 2010 P-0020: https://b-com.mcigroup.com/Abstract/Statistics/AbstractStatisticsViewPage.aspx?AbstractID=32320