C-67: Direct epitope recognition assay for TSH receptor autoantibodies causing Graves’ disease demonstrates higher diagnostic accuracy than indirect assays based on TSH displacement

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Objectivew

In Graves’ disease (GD) hyperthyroidism is caused by stimulating TSH receptor (TSHR) autoantibodies (sTRAb). TSHR is a seven transmembrane receptor with a large extracellular domain presenting epitopes for stimulating as well as blocking TSHR autoantibodies. Current TSH displacement assays (TDA) quantify indirectly all TSHR autoantibodies (Ab) including blocking Ab. Our novel direct epitope recognition assay (DERA) was developed for direct detection of sTRAb (Fig. 1, right).

Relevance:

GD is a common cause for hyperthyroidism. In routine practice values determined by TDA conflict in several cases with clinical diagnosis.

Methodology:

DERA is performed by bridge technology on microplates using recombinant chimeric hTSHR-LHCG receptors (chim-hTSHR) where the major epitope for blocking Ab is replaced by the corresponding neutral sequence of the LHCG receptor. An antibody anchors chim-hTSHR to microplates. One arm of the sTRAb binds to chim-hTSHR, the second arm of the Ab bridges to a chim-hTSHR fused with alkaline phosphatase. Applying chemiluminescent substrate sTRAb were quantified using a plate luminometer (Fig. 1, right). DERA is performed at ambient temperature with incubation of the second chim-hTSHR at 37°C. Chim-hTSHR showed prolongedthermostability beyond assay duration.

Validation:

The range of detection was established using WHO standard 90/672 for thyroid stimulating autoantibodies showing between-run precision (different lots and days included) of CV <20 % from 0.3 – 50.0 IU/l at within-run CV <10 % (Fig. 2). The power of DERA to distinguish between sera positive or negative for sTRAb related to clinical diagnosis was tested via ROC plot analysis using 182 sera (79 patient, 103 sera GD negative). Autoimmune activity was indirectly quantified by clinical diagnosis (e.g. tachycardia), laboratory parameters (TSH, T3, T4) and treatment. With a decision limit of >0.45 IU/l DERA showed 100.0 % diagnostic sensitivity and 100.0 % specificity. Assuming 10 % imprecision the grey zone is only 0.4 – 0.5 IU/I.

Conclusion:

DERA (direct epitope recognition assay) is a new high sensitive and specific in vitro assay suitable for routine clinical diagnostic in GD. Measuring directly only GD causing TSHR autoantibodies (sTRAb) its diagnostic accuracy exceeds that of currently used TSH displacement assays with indirect quantification. Differences between direct and indirect assay values are suggested to be due to I) the measurement of blocking and stimulating antibodies as well as to II) only partial sharing of epitopes between labeled TSH and TSHR antibodies in the TSH displacement assay. The sTRAb assay is suitable for full automatisation. Substantial decrease of sTRAb values after retrobulbar irradiation support hypothesis of an active autoimmune process in orbital tissue.