**Upregulated expression of FoxP3 and T-bet in severe, but not euthyroid Hashimoto's thyroiditis.**

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**Introduction:** Hashimoto's thyroidits (HT) is Th1-mediated autoimmune disorder characterized by progressive thyroid failure. Recently, Th17 and Treg CD4+cell subset, each with growing number of transcriptional factors have been also implicated; however, the relation of different T-cell subsets and their master regulators with disease severity remains largely unknown.

**Methods and materials:** We investigated the expression levels of Th1/Th17/Treg cell-associated factors (T-bet/ETS1, HIF1A, BLIMP1/FOXP3, CTLA4) in peripheral T-cells of 10 hypothyroid, untreated patients, 10 patients with substituted hypothyroidism due to HT, 12 euthyroid HT subjects and 11 healthy controls by qRT-PCR. NormFinder was used to identify the most stable expressed mRNA (TBP, SD=0.3145) between three reference genes tested across clinical endpoints. Data were normalized and fold difference in expression was calculated by ΔΔCt method, followed by Kruskal-Wallis and Dunn's post-hoc tests.

**Results:** Compared to euthyroid patients and controls, patients with burned-out HT, both hypothyroid (2,25-fold difference vs controls, P<0.01) and rendered euthyroid by hormone therapy (2,3-fold, P<0.01), showed increased FoxP3 mRNA expression in T-cells (global P=0.0013). Similarly, T-bet mRNA levels were up-regulated in burned-out, but not in euthyroid subjects (1,73-fold and 2,46-fold, hypothyroid and thyroxine-substituted patients vs controls, respectively, both P<0.01). No difference in expression between treated and untreated hypothyroid patients was seen.

**Conclusion:** Severe, but not euthyroid HT is associated with up-regulation of T-bet and FoxP3 mRNA in peripheral T-cells, suggesting an unbalanced role for T-bet positive (encompassing NK/CD4+Th1/CD8+ effectors) and FOXP3 positive (protective CD4+Treg) T-cell compartments in advanced HT. No relation to non-canonical Th17 regulators was observed at mRNA level.