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EVALUATION OF THE USEFULNESS OF SERUM CYTOKINES IL-1β AND sFasL MEASUREMENTS IN THE DIAGNOSIS OF AUTOIMMUNE HYPOTHYROIDISM AND HYPERTHYROIDISM IN CHILDREN

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INTRODUCTION

Autoimmune thyroid disease (AITD) is one of the **most common** organ-specific **autoimmune disorders,** of which Hashimoto's thyroiditis **(HT)** and Graves' disease **(GD)** are 2 of the **most common clinical expressions. Cytokines play** a crucial role in **modulating immune responses** in both these disorders.

The **apoptosis pathway** is up-regulated in chronic autoimmune thyroiditis **(cAIT)** and **destruction** of the thyroid leads to hypothyroidism **(hypoT)**. This phenomenon is also present in Graves' disease **(GD)** manifested with hyperthyroidism **(hyperT)**.

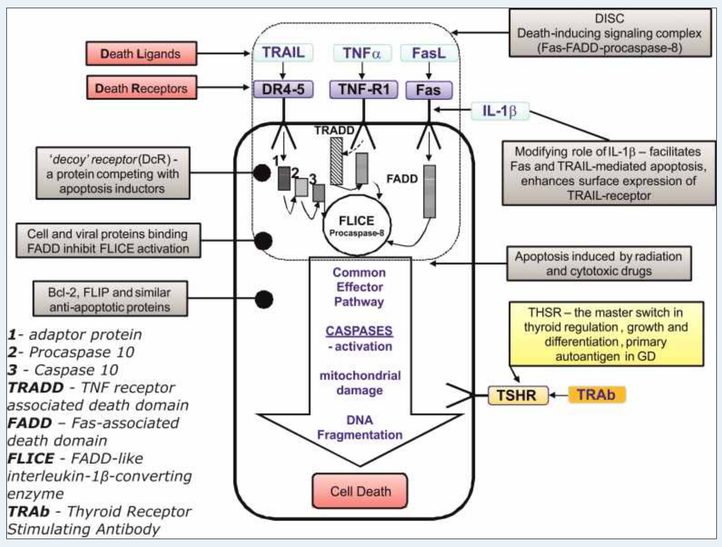

Figure. 1. *Regulation of apoptosis in a thyrocyte, based on Patricia L. Arscott and James R. Baker, Jr., „Short Analytic Review: Apoptosis and Thyroiditis", Clinical Immunology and Immunopathology, Volume 87, Issue 3, June 1998, Pages 207-217, modified: W. Stacha, M. Niedziela, M. Mikoś*

The role of **soluble FasL (sFasL)**, a proteolytic product of FasL, is less clear in **induction** of **apoptosis** in both **thyrocytes** and **lymphocytes**.

IL-1β is an important **mediator** of the **inflammatory response** and is involved in a variety of cellular activities, including **cell proliferation, differentiation** and **apoptosis**.

AIM OF THE STUDY

The aim of this study was to determine the relationship between the concentration of proinflamatory cytokines IL-1ß and sFasL with immune thyroid factors in the serum of children with autoimmune thyroid disease (AITD).

MATERIAL AND METHODS

1. Studied groups and analyzed markers: n=45 children in **3 subgroups**: **n=11 children** with **hypoT, n=19** children with **hyperT** (newly diagnosed patients) and **n=15** healthy subjects as an **euthyroid control.**
Summary of the groups and **descriptive statistics** are presented in **Table 1.:**

	Hypo-thyroidism (hypoT)	Hyper-thyroidism (hyperT)	Control group	Significance
n	**n=11**	**n=19**	**n=15**	
sex	10 girls / 1 boy	15 girls / 4 boys	7 girls / 8 boys	**ns**
Age [years]	12.2 ± 1.9	12.4 ± 4.9	10.5 ± 4.8	**ns**
BMI [kg/m2]	18.69 (5.45)	18.25 ± 3.42	18.17 ± 3.50	**ns**
BMI SDS	0.3 (2.04)	-0.38 ±1.05	-0.55 (1.29)	**ns**
Cole'a index	1.05 ± 0.22	0.95 ± 0.13	0.9 ± 70.14	**ns**
TSH [0.5-5.0 µIU/mL]	37.34 (17.69) ↑	0 (0.01) ↓	2.42 (1.52)	**p<0.001 (K-W)**
fT4 [0.7-1.85 ng/dL]	0.54 ± 0.31 ↓	4.24 ± 1.06 ↑	1.03 ± 0.12	**p<0.001 (ANOVA)**
fT3 [1.7-3.5 pg/mL]	2.10 ± 0.97	19.01 ± 5.30 ↑	2.70 ± 0.56	**p<0.001 (ANOVA)**
ATG [<60 UI/mL]	124 (589) ↑	101 (552) ↑	20 (4.5)	**p<0.001 (K-W)**
ATPO [<60 UI/mL]	3000 (111) ↑	3000 (1536) ↑	10 (29)	**p<0.001 (K-W)**
TRAb [<1 IU/L]	0.7 ± 0.3	16.75 (24.6) ↑	0.5 ± 0.3	**p<0.001 (K-W)**
IL-1β [pg/mL] mean ± SD median (IQR)	**2.58 ± 1.80 2.16 (0.87)**	**1.45 ± 0.67 1.39 (1.27)**	**1.48 ± 0.70 1.88 (1.04)**	**p=0.002 (K-W)**
sFasL [ng/mL] mean ± SD median (IQR)	**0.27 ± 0.11 0.26 (0.14)**	**0.14 ± 0.07 0.14 (0.09)**	**0.09 ± 0.10 0.06 (0.15)**	**p<0.001 (K-W)**

Table 1. *Descriptive statistics and significance of diffrences - hyperT, hypoT and control group (ANOVA - analysis of variance, K-W - Kruskal-Wallis non-parametric test)*

2. Inclusion criteria: clinical, hormonal and autoimmune: TRAb+ in GD: ATPO+ / ATG+ in AITD.
3. Methods: thyroid hormones - MEIA tests (Abbott, AxSym); IL-1β and sFasL - ELISA tests (BenderMedSystem, Vienna, Austria), antibodies TRAb / ATG / ATPO – RIA tests (Brahms, Berlin).
4. Serum concentrations of IL-1β and sFasL in patients with cAIT and GD (vs control) were evaluated at the beginning of disease (before treatment) by ELISA.
5. Statistical analysis was carried out in SPSS 17 and GraphPad Prism 6. Shapiro–Wilk normality test, ANOVA (Newman–Keuls post-test), nonparametric Kruskal–Wallis (Dunn's post-test) and Spearman's rank correlation were used.

RESULTS

1. IL-1β concentration was **significantly higher** in **cAIT** [median (IQR)] 2.16 (0.87) pg/ml **vs control** 1.88 (1.04) pg/ml **(p<0.05)** and in **cAIT vs GD** 1.39 (1.27) pg/ml **(p<0.01) (K-W p=0.002) (Fig. 2.)**
2. sFasL concentration was **significantly higher** in **cAIT** [median (IQR)] 0.26 (0.14) ng/ml **vs control** 0.06 (0.15) ng/ml **(p<0.01)** and in **cAIT** vs **GD** 0.14 (0.09) ng/ml **(p<0.05) (K-W p<0.001) (Fig. 3.)**
3. Significant positive correlations were identified in **GD** group: between **IL-1β** and **ATPO (r=0.47; p<0.05)**, as well as between **sFasL** and **BMI SDS (r=0.48; p<0.05) (Fig. 4. & 5.)**
4. The results of ROC curve analysis enabled determination of **usefulness** of **monitoring cytokine concentrations** in order to **discriminate children** with **autoimmune thyroid disease** from **healthy children (Fig. 6 & 7.)**:
a. IL-1β (AUC=0.77, p=0.003) with low sensitivity (59.1%) and high specificity (95%)
b. sFasL (AUC=0.897; p<0.001) with very high sensitivity (100%) and high specificity of (73.3%)
The **concentrations** of **these markers increase** in **hypothyroidism.**
5. Moreover **IL-1β** and **sFasL** effectively **discriminated** both **clinically opposing states: cAIT** and **GD** children among themselves, with **high sensitivity** and **specificity (Fig. 8. & 9.)**:
a. IL-1β (AUC=0.773; p=0.002) with sensitivity of 72.7% and specificity of 86.4%
b. sFasL (AUC=0.833; p=0.003) with sensitivity of 94.7% and specificity of 72.7%

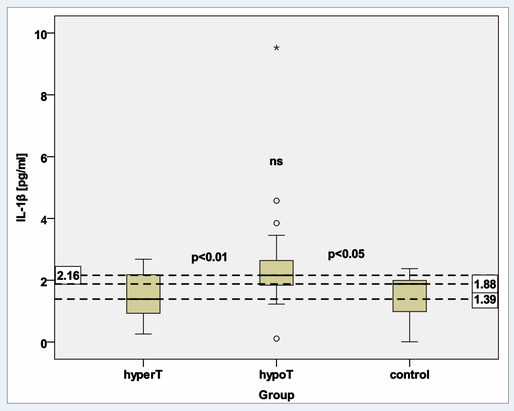

Figure 2. *Boxplot of IL-1ß: hypoT vs control p<0.05; hyperT vs hypoT p<0.01; hyperT vs control ns*

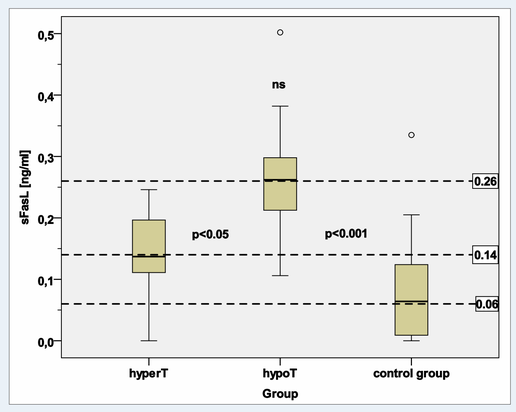

Figure 3. *Boxplot of sFasL: hypoT vs control p<0.001; hyperT vs hypoT p<0.05; hyperT vs control ns*

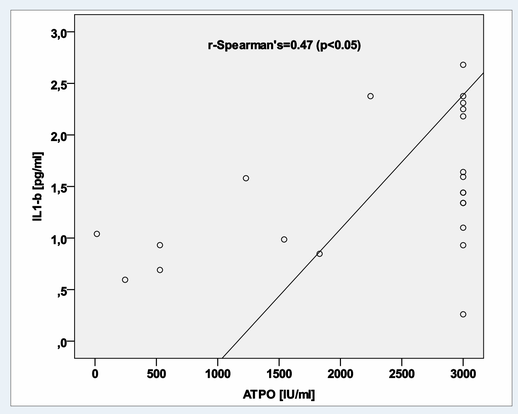

Figure 4. *Positive nonparametric correlation in GD: IL-1ß and ATPO*

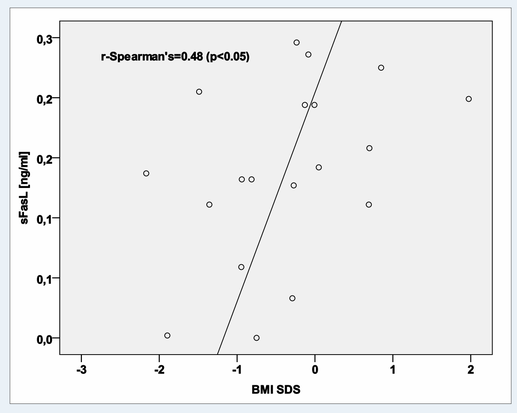

Figure 5. *Positive nonparametric correlation in GD: sFasL and BMI SDS*

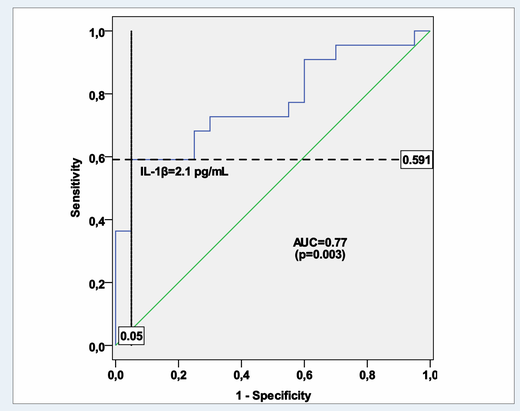

Figure 6. *ROC of IL-1ß: cAIT versus control group (AUC=0.77, p=0.003, cut-off=2.1 pg/ml; sens.: 59.1%, spec.: 95%)*

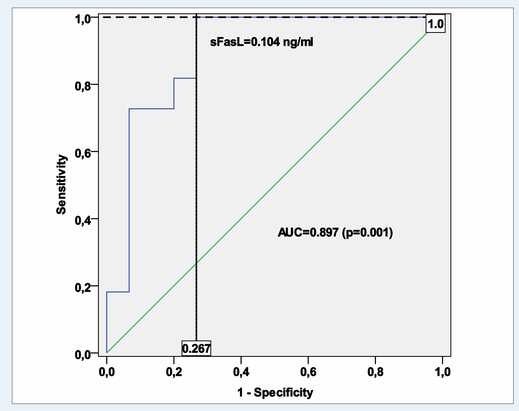

Figure 7. *ROC of sFasL: cAIT versus control group (AUC=0.897, p=0.001 cut-off=0.104 ng/ml; sens.: 100%, spec.: 73.3%)*

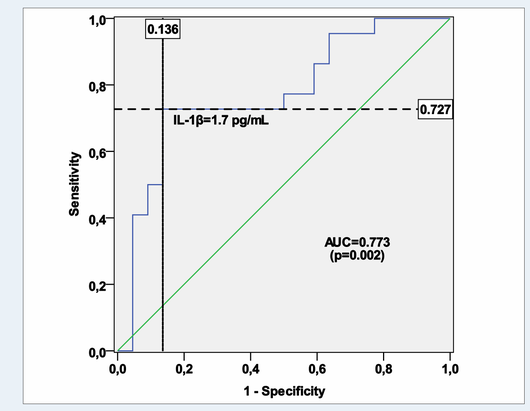

Figure 8. *ROC of IL-1ß: cAIT versus GD (AUC=0.773, p=0.002, cut-off=1.7 pg/ml, sens.: 72.7%, spec.: 86.4%)*

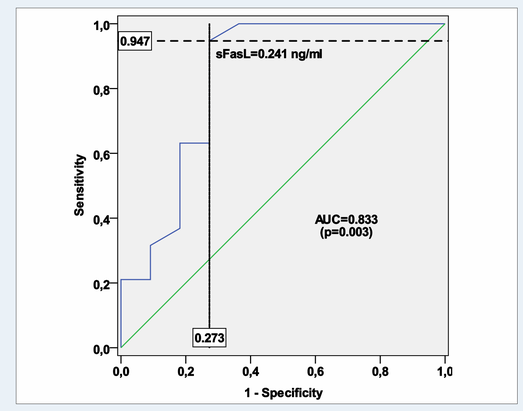

Figure 9. *ROC of sFasL: cAIT versus GD (AUC=0.833, p=0.003, cut-off=0.241 ng/ml, sens.: 94.7%, spec.: 72.7%)*

CONCLUSION

We suggest that both cytokines IL-1β and sFasL may be useful markers in the assessment of thyroid dysfunction of autoimmune hypothyroid and hyperthyroid children.

There was no conflict of interest related to this study.
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