Apoptotic markers may help in predicting the disease course of pediatric immune thrombocytopenic patients

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Background

Only 70-85% of pediatric immune thrombocytopenia (ITP) patients will enter durable remission and eventually cure, at one year.

Currently, none of the attempts to classify patients on diagnosis to non-chronic and chronic ones, have been successful.

Hypothesis

Different pathophysiology underlines non-chronic versus chronic pediatric ITP.

Objectives

Examine whether apoptotic markers at differ between the non-chronic and those who will turn to have chronic disease.

Mateial and Methods

Patients

Table 1

Clinical data of the ITP patients at diagnosis.

	Non-Chronic ITP n=26	Chronic ITP n=16	All ITP Patients n=42	*p* value
% Female	38%	60%	48%	**0.026**
Age (year)	3.0±2.9	6.1±5.7	4.1±4.3	0.125
Platelet Count (x10⁹/L)	16571±18373	17577±13243	17225±14636	0.843
White Blood Cell (x10⁹/L)	9030±3456	9779±4674	9517±4151	0.602
Hemoglobin (mg/dL)	12.0±1.4	11.8±1.2	11.9±1.2	0.545

Presented is the average ± SD. The differences between chronic and non-chronic ITP patients were analyzed using a T- test. *denotes a statistically significant difference

•We incubated patients' sera with washed platelets and compared the results between healthy controls, chronic and non-chronic ITP patients.

•Posphatidylserine exposure and mitochondrial electrochemical potential were measured using Flow cytometry followed by a 43 markers Human Apoptosis Array.

Results

- We found an increased expression of five apoptotic proteins on platelets incubated with sera of non-chronic pediatric ITP patients, compared to chronic ones' sera, upon diagnosis (table 2).
- No significant difference was found in the apoptotic markers' phosphatidylserine (PS) surface expression and loss of mitochondrial inner membrane potential (ΔΨm), between normal platelets compared to sera from healthy donors.
- An inverse correlation was found between the anti- apoptotic protein IGFBP2 and the pro- apoptotic SMAC protein, or the pro-activation CD40 protein' in accordance with their different effects on platelets. No significant correlations were found between IGFBP2 expression and the other proteins: BIM, p21, and IGF1sR (Table 4).
- Sera taken from chronic ITP patients decreased its expression in the platelet (Figure 1) and displayed a positive correlation with pro-apoptotic SMAC and the pro-activation CD40, which is enigmatic for its function in the platelets.

Table 2

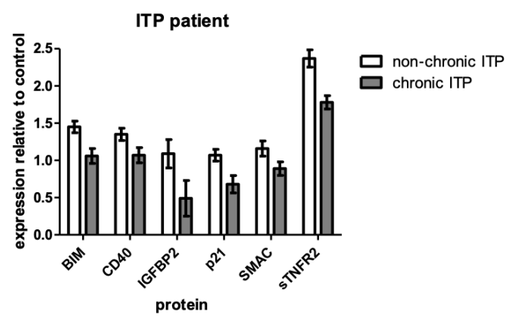
The average expression (Units) level in platelets after incubation with sera taken from ITP patients and healthy controls.

Protein	Non-Chronic ITP	Chronic ITP	Healthy Controls	*p* value (non-chronic/chronic ITP)
BIM	1599	1162	1099	0.025
CD40	579	460	430	0.035
IGFBP2	1968	877	1810	0.001
P21	31710	20212	29746	0.026
SMAC	2237	1709	1927	0.046

Table 3

Pearson correlation coefficient between the expression levels in platelets after incubation with sera taken from ITP patients and healthy controls.

Proteins	BIM	CD40	IGFBP2	P21	SMAC	sTNFR2
BIM		0.776 $p < 0.001$	NS	0.425 $p < 0.01$	0.684 $p < 0.001$	0.482 $p < 0.01$
CD40			-0.492 $p < 0.01$	0.426 $p < 0.01$	0.829 $p < 0.001$	0.715 $p < 0.001$
IGFBP2				NS	-0.505 $p < 0.01$	NS
P21					NS	0.664 $p < 0.001$
SMAC						0.494 $p < 0.01$
sTNFR2						



The expression level in platelets after incubation with sera taken from ITP patients, compared to sera taken from healthy controls, shows a mean±SEM effect of sera from non-chronic ITP patients (white) and chronic ITP patients (gray).

Discussion

- **<u>Two pathways of apoptosis in platelets have been described:</u>**

1. A cell-intrinsic mitochondria-dependent pathway, is the basis for the results presented above.
2. An extrinsic pathway, may be initiated by the interaction between death ligands belonging to the TNF superfamily and the cell surface TNF receptors.

- Altogether, our results suggest that the pathophysiology of non-chronic vs chronic ITP is somewhat different, although they share almost the same clinical presentation.

<u>The limitations of this study:</u>

1. The study tested the effects of sera on normal platelets and not on ITP patients' platelets.
2. We examined the sera at one time point, upon initial diagnosis.
3. The apoptotic protein panel checks the quantitative levels of the markers; however, this does not tell us in what configuration they are found.

Conclusions

Our data may help to stratify ITP patients to non-chronic and chronic ones at diagnosis.

This may enable us to tailor more specific therapy to these seemingly different clinical entities currently being treated in the same way.