INTEGRATING MDR PROTEINS AND GST POLYMORPHISMS INTO CLINICAL FACTORS FOR LONG-TERM OUTCOME ANALYSIS IN PEDIATRIC PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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INTRODUCTION

The molecular mechanisms underlying chemoresistance in pediatric acute lymphoblastic leukemia (pALL) patients are still poorly understood. Traditional risk factors, such as leukocyte count ≥ 50^9/L, age ≥ 10 years and T-lineage are unfavorable well known factors to obtain complete continuous remission. However, Proliferative index, P-glycoprotein (Pgp) and MRP1 efflux transporter, and glutatione-S-transferases (GSTs) have shown discordant results as prognostic factors in many studies. Therefore, our objective was to develop an accurate analysis encompassing all these factors, associated and/or isolated, in order to get more precise prognostic information.

METHODS AND RESULTS

Here we studied 44 patients with pALL and analyzed proliferation index, P-glycoprotein (Pgp) and MRP1 efflux transporter membrane expression and activity (through flow citometry), and glutatione-S-transferases (GSTs) GSTM1 and GSTT1 polymorphisms (through multiplex PCR) (Figures 1, 2, 3 and 4).

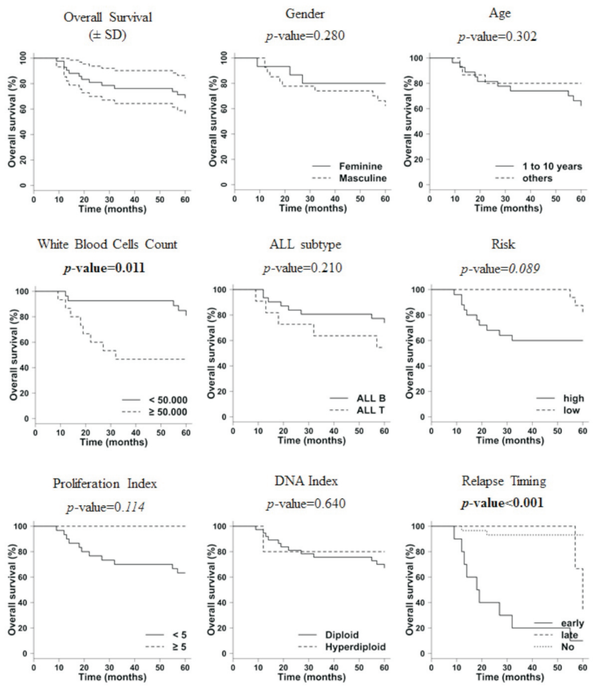

Figure 1: Kaplan-Meyer survival curves displaying pediatric ALL patients survival in 60 months, according to clinical and pathological features. Log-rank test was performed to verify if the curves differences were statistically significant (*p*-values<0.05 are displayed in bold). Borderline p-values are displayed in italic.

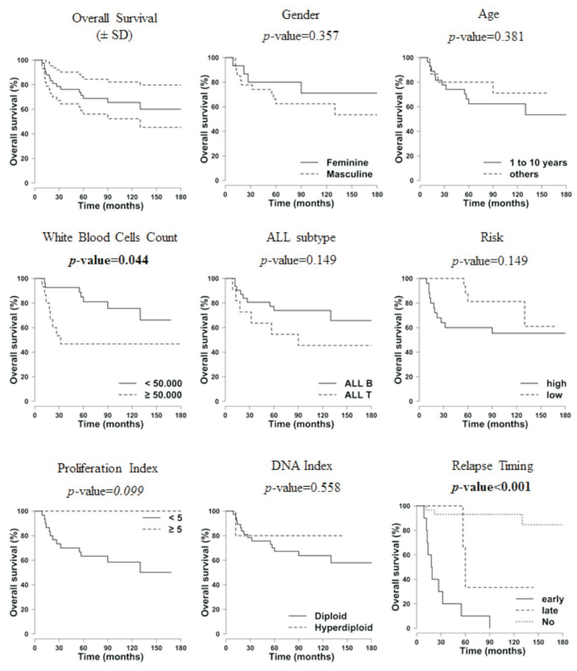

Figure 2: Kaplan-Meyer survival curves displaying pediatric ALL patients survival in 15 years, according to clinical and pathological features. Log-rank test was performed to verify if the curves differences were statistically significant (*p*-values<0.05 are displayed in bold). Borderline *p*-values are displayed in italic.

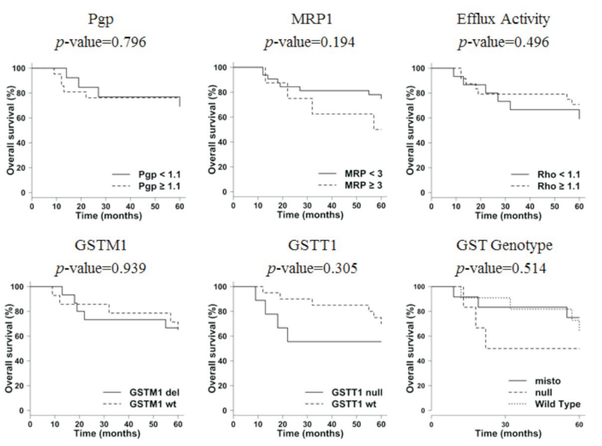

Figure 3: Kaplan-Meyer survival curves displaying pediatric ALL patients survival in 5 years, according to Pgp and MRP1 expression, efflux activity, and GSTM1 and GSTT1 status. Log-rank test was performed to verify if the curves differences were statistically significant (*p*-values<0.05). Borderline *p*-values are displayed in italic.

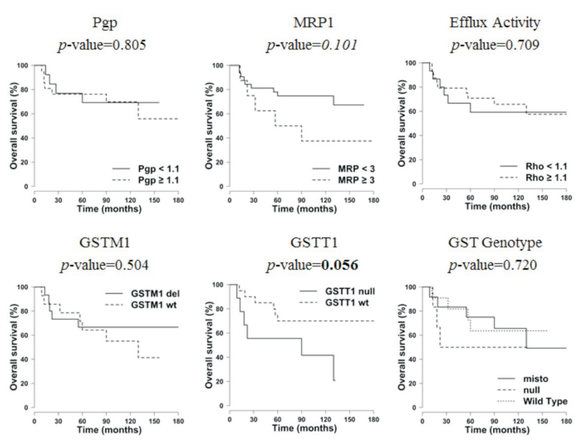

Figure 4: Kaplan-Meyer survival curves displaying pediatric ALL patients survival in 15 years, according to Pgp and MRP1 expression, efflux activity, and GSTM1 and GSTT1 status. Log-rank test was performed to verify if the curves differences were statistically significant (p-values<0.05). Borderline p-values are displayed in italic.

Our main findings were:

(1) Patients with low proliferation index combined with high WBCC had a worse survival than the others, meaning that patients with high proliferation index have a better chance of survival independently of the WBCC (Tables 1 and 2 and Figure 5);

Table 1 : Multivariate analysis of WBCC, ALL subtype, age, gender, DNA Index, and proliferation index impact on prognosis.

	5-years Hazard Risk			15 years Hazard Risk		
Feature	Crude (95% CI)	Adjusted (95% CI)	*p*-value	Crude (95% CI)	Adjusted (95% CI)	*p*-value
WBCC	3.89 (1.26 - 11.96)	4.74 (1.15 - 19.58)	**0.024**	2.74 (0.99 - 7.59)	3.55 (0.98 - 12.79)	***0.051***
ALL subtype	2.01 (0.66 - 6.15)	1.8 (0.42 - 7.79)	0.432	2.1 (0.75 - 5.9)	2.32 (0.63 - 8.5)	0.208
Age	0.51 (0.14 - 1.87)	0.67 (0.16 - 2.85)	0.577	0.6 (0.19 - 1.9)	0.7 (0.2 - 2.46)	0.574
DNA Index	0.62 (0.08 - 4.79)	1.22 (0.12 - 12.91)	0.868	0.55 (0.07 - 4.22)	0.92 (0.1 - 8.75)	0.940
Proliferation Index	0 (0 - Inf)	0 (0 - Inf)	**0.019**	0 (0 - Inf)	0 (0 - Inf)	**0.004**
Gender	2.01 (0.55 - 7.32)	1.11 (0.25 - 5.03)	0.887	1.7 (0.54 - 5.36)	0.86 (0.22 - 3.32)	0.828

CI: Confidence Interval. Differences were considered significant when *p*<0.05 (displayed in bold). Borderline *p* values are displayed in italic.

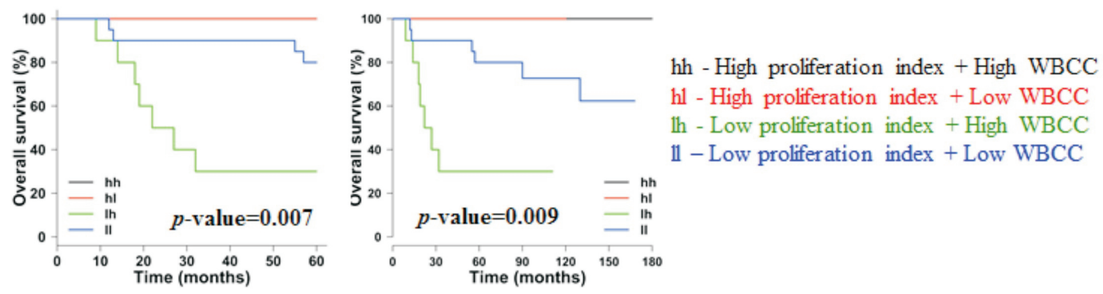

Figure 5: Kaplan-Meyer survival curves displaying pediatric ALL patients survival in 5 and 15 years, according to Proliferation Index and WBCC. Log-rank test was performed to verify if the curves differences were statistically significant (*p*-values<0.05 are displayed in bold).

Table 2: Binomial model of risk of death of pediatric ALL patients according to WBCC and proliferation index combined.

	15 years Hazard Risk	
Low proliferation index + Low WBCC	Odds Ratio (95% CI)	*p*-value
High proliferation index + High WBCC	<0.001 (NA – inf)	0.997
High proliferation index + Low WBCC	<0.001 (NA – inf)	0.996
Low proliferation index + High WBCC	5.4 (1.1 – 32.9)	**0.045**

Low proliferation index: < 5% of cells in S phase. High proliferation index: ⩾ 5% of cells in S phase. Low WBCC: under 50.000/mm^3. High WBCC: above 50.000/mm^3. CI: Confidence Interval. NA: Not Available. Inf: >1000. Differences were considered significant when *p*<0.05 (displayed in bold).

(2) T lineage LLA was associated with high risk (Chi-square; p= 0.049) and borderline with high MRP1 (Chi-square; p= 0.070), although high risk and high MRP1 were not associated with each other (Chi-square; p= 0.135), which suggests that both these features are independent from each other in T lineage ALL;

(3) And patients with high MRP1 and lack of GSTT1 had a worse survival than the others (Figure 6, Tables 3 and 4).

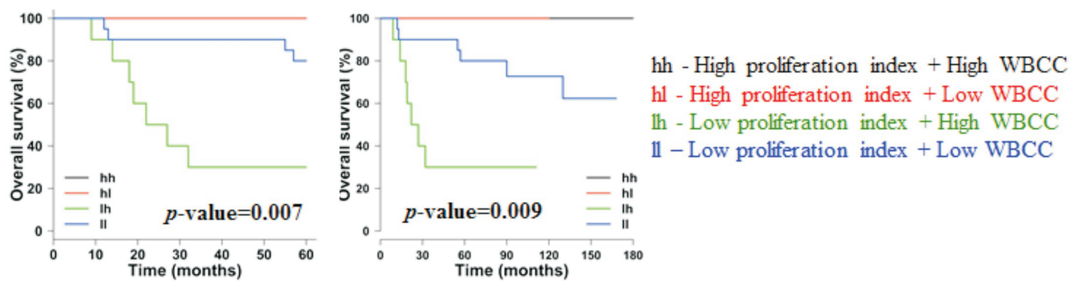

Figure 6: Kaplan-Meyer survival curves displaying pediatric ALL patients survival in 5 and 15 years, according to MRP1 expression and GSTT1 status. Log-rank test was performed to verify if the curves differences were statistically significant (*p*-values<0.05 are displayed in bold).

Table 3: Survival rates of the pediatric ALL patients according to MRP1 expression and GSTT1 status.

	Number of patients	5-year Survival Rate (95% CI)	*p*-value	15-year Survival Rate (95% CI)	*p*-value
Low MRP1 + GSTT1 WT	15	73% (54-100)	0.229	73% (54-100)	**0.020**
Low MRP1 + GSTT1 Null or High MRP1 + GSTT1 WT or	9	67% (42-100)		50% (24-100)	
High MRP1 + GSTT1 Null	3	33% (7-100)		0%	

Low MRP1: MRI < 3. High MRP1: MRI ⩾ 3. CI: Confidence Interval. Differences were considered significant when *p*<0.05 (displayed in bold).

Table 4: Multivariate analysis of WBCC, proliferation index and GSTT1 status impact on prognosis.

	5-years Hazard Risk			15 years Hazard Risk		
Feature	Crude (95% CI)	Adjusted (95% CI)	*p*-value	Crude (95% CI)	Adjusted (95% CI)	*p*-value
WBCC	3.89 (1.26 -11.96)	3.21 (0.81 - 12.79)	*0.096*	2.74 (0.99 - 7.59)	3.08 (0.8 - 11.81)	*0.103*
Proliferation Index	0 (0 - Inf)	0 (0 - Inf)	*0.086*	0 (0 - Inf)	0 (0 - Inf)	**0.05**
GSTT1 status	0.52 (0.15 - 1.85)	0.36 (0.09 - 1.44)	0.159	0.35 (0.11 - 1.08)	0.24 (0.07 - 0.85)	**0.027**

CI: Confidence Interval. Differences were considered significant when *p*<0.05.

These data further suggests that not only MRP1 and GSTT1 display independent prognostic value, as seen in the multivariate analysis (Table 1), but also that the combination between high MRP1 with absence of GSTT1 may additionally worsen survival (Tables 3 and 4).

CONCLUSION

Our data suggests that low proliferation, high MRP1, and lack of GSTT1 play a negative role in pALL treatment response. Moreover, the combination between these features seems to yield an even worse response. Therefore, new strategies that overcome all these features are needed.

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