Childhood Acute Lymphoblastic Leukemia (ALL): role of polymorphisms in the GST-μ, GST-θ genes on toxicity and steroid apoptosis

R. Franca[1], A. Houedjakou[2], M. Granzotto[1], A. Zanut[2], G. Stocco[2], P. Rebora[3], M.G. Valsecchi[3], M Rabusin[1] and G. Decorti [2]

[1]IRCCS Burlo Garofolo, Trieste, Italy
[2]Dept. of Life Sciences, University of Trieste, Italy
[3]Center of Biostatistics for Clinical Epidemiology, University of Milano-Bicocca, Monza, Italy

In the AIEOP-BFM-ALL 2000 trial (Associazione Italiana Ematologia Oncologia Pediatrica/ Berlin-Frankfurt-Münster Acute Lymphoblastic Leukaemia Study Group, protocol in force in Italy between September 2000 and July 2006), 15% of paediatric patients treated according to risk-adapted poly-chemotherapeutic regimens relapsed, suggesting that additional criteria to those based mainly on the minimal residual disease are required to better define prognosis and consequently adjust therapy. A previous study on the homozygous deletion (null genotype) of glutathione S-transferase-μ (GST-M1) and -θ (GST-T1) revealed an higher relapse rate for non-null GST-M1 Prednisone Poor Responders (PPR) and for GST-T1 null Prednisone Good Responders (PGR) within this cohort.[1] Furthermore, in a small population of ALL children investigated for chemotherapy side effects (particularly those occurring during the induction and reinduction phases in which glucocorticoids (GCs) are essential drugs), the risk of severe infections was increased in GST-M1 null PGR patients.[2] These results suggest that the deletion of GST-M1 might account for an enhanced GC susceptibility and toxicity.

A larger pharmacogenetic retrospective study on GST-M1 and GST-T1 polymorphisms and grade III/IV gastrointestinal/hepatic/neurological toxicities and severe infections occurring in the induction and maintenance phases in the AIEOP-BFM-ALL 2000 trial has been performed. Patient's inclusion criterion was the enrolment in one of the 13 different Italian AIEOP hospitals that reported at least 30% of grade III/IV toxicities during the induction phase (785 patients eligible. 527 DNA available: 119, 223, 49 and 86 subjects with grade III/IV gastrointestinal/hepatic/neurological toxicities and infections respectively). To date, 447 and 428 DNA were successfully genotyped for GST-M1 and GST-T1 by multiplex polymerase chain reaction (229 (51,2%) and 72 (16.8%) null genotypes respectively). Possible associations between the phenotypes and polymorphisms in each gene was investigated by contingency tables and using two sided Fisher's exact test on available data. No significant effect emerged.

In order to verify the hypothesis of a genetic regulation of GSTs on apoptosis, the association between GST-M1 and GST-T1 variants and methylprednisolone (MP)-induced apoptosis has been investigated in unstimulated peripheral blood mononuclear cells (PBMC) extracted from *buffy coats* of healthy donors (obtained from 'Centro Trasfusionale Ospedale Maggiore di Trieste', Italy); apoptosis was measured after 18 h MP-incubation (range $2x10^{-8}$-$2.67x10^{-3}$M) by Propidium Iodide/Annexin V (Ann) FITC flow citometry. Total apoptotic implement (I_{AP}) was calculated on the basis of total Ann^+ cells, as (y-x/x*100) where y= Ann^+ treated cells (%) and x= Ann^+ untreated controls (%)). Dose-response curves were derived, and I_{AP} were compared between GSTs genotypes using a non parametric t-test. Preliminary results on 24 subjects enrolled did not show any significant difference. However, larger numbers of samples are required before drawing any definitive conclusion on the role of GSTs variants on GC-induced sensitivity.

1. Franca et al., Pharmacogenomics. 2012 Dec;13(16):1905-16.
2. Marino et al., Pediatr Blood Cancer. 2009 Dec;53(6):984-91.