Pharmacological strategies for prediction of glucocorticoid response in children with inflammatory bowel disease

S. De Iudicibus[1], M. Lucafò[1], A. Di Silvestre[2], A. Ventura[1], G. Decorti[2]

[1] Institute for Maternal and Child Health, IRCCS Burlo Garofolo, Trieste
[2] Dept. of Life Sciences, University of Trieste, Italy

Despite the recent introduction of biological drugs, glucocorticoids (GCs) are still used as powerful anti-inflammatory agents in inflammatory bowel diseases (IBD). The effectiveness of these drugs is very variable and side effects are particularly severe in pediatric patients; therefore, the identification of subjects that are most likely to respond poorly to therapy seems extremely important in childhood IBD. The mechanisms of steroid resistance are however scarcely understood and there is presently no marker to predict the response in advance.

The intronic *Bcl*I polymorphism in the GC receptor (NR3C1) gene has been studied for its relation with GC response. 154 young patients with IBD treated with GCs (82 Crohn's disease and 72 Ulcerative Colitis) were divided in three groups on the basis of their response to GC therapy (84 GC-responder, 55 GC-dependent, and 15 GC-resistants). All patients were genotyped with TaqMan® genotyping technologies. *Bcl*I polymorphism was related to an increased response to steroid therapy in pediatric patients with IBD: indeed, the mutated *Bcl*I genotype was significantly more frequent in responder patients (21.47%) than in dependents (7.3%; O.R. 0.29, C.I. 0.09-0.90, P=0.03). These results were confirmed by a pharmacodynamic assay performed on peripheral blood mononuclear cells (PBMCs) separated from 42 buffy coats of healthy donors: the effect of GCs on cell proliferation was determined by labelling metabolically active cells with [methyl-3H] thymidine. Non linear regression of dose-response data was performed for computing IC_{50}, the GC concentration required to reduce proliferation to 50%. An increased GC *in vitro* sensitivity was observed in lymphocytes with the mutated *Bcl*I genotype (median $2.39x10^{-09}$M, range $7.43x10^{-10}$M - $1.46x10^{-07}$M) compared to non mutated carriers (wild-type and heterozygous; median $2.76x10^{-07}$M, range $2.03x10^{-09}$M - $2.94x10^{-04}$M, p=0.0058 Mann Whitney test). These results suggest that *Bcl*I polymorphism associated with a lymphocyte proliferation assay could represent a tool for predicting the clinical response to steroid treatment in IBD patients.

In order to further understand the mechanism of the variability in GC response, in particular in a complex disease like IBD, a prospective study is ongoing; for each patient enrolled blood samples will be obtained at diagnosis, after 30 days of therapy with GCs, and after GC withdrawal, and disease activity will be assessed by means of validated clinical scores.

Inhibition of proliferation by GC in stimulated patient PBMCs will be evaluated using 5-ethynyl-2'-deoxyuridine (EdU) and Cu(I)-catalyzed cycloaddition 'click' reaction assay by flow cytometry. These results will be integrated with pharmacogenomic analyses: in particular, the role of microRNA (miRNA) in the variability of GC response will be investigated in patients samples obtained during GC treatment. These non-coding RNAs suppress gene expression at the post-transcriptional level and the identification of miRNAs differentially expressed during treatment could allow to better understand the molecular mechanism involved in response to GC.

In conclusion, this innovative panel of pharmacodynamic and pharmacogenomic assays should allow to personalize GC therapy in IBD, improving response and reducing side effects.