**Materials and Methods**

**Study population and sample collection**

This study was conducted in collaboration with Children’s Hospital Zagreb, University Hospital Centre “Sisters of Mercy” and University Hospital “Dubrava”, Zagreb, Croatia. The informed consent was obtained from all participants of legal age or their parents/legal guardians prior to inclusion into the study and the protocol was approved by the Ethical Committees of collaborating hospitals. Thirteen pediatric patients (9 newly diagnosed, untreated (UC-New) and 4 in remission (UC-Remission)) and 17 adults (9 newly diagnosed, untreated (UC-New) and 8 in remission (UC-Remission)) with UC as well as 15 pediatric and 10 adult age-matched controls were enrolled in the study. Tissue samples from pediatric patients were collected from descending colon and terminal ileum whereas from adults the samples were taken from sigmoid colon and terminal ileum. Healthy control samples were obtained from non-IBD patients who underwent ileocolonoscopy for clinical investigation of rectal bleeding, anaemia or diarrhoea. Colon samples from UC-New patients were taken from inflamed areas whereas tissue samples from patients in UC-Remission were collected from similar locations but without inflammation, confirmed by endoscopy and pathohistological screening. All intestinal samples from heathy controls and terminal ileum samples from UC patients were non-inflamed (macroscopic and histological findings). The diagnosis of pediatric UC was based on modified Porto diagnostic criteria [1] and in adults on established clinical, endoscopic, and histologic criteria [2]. Disease activity in children was assessed by Pediatric Ulcerative Colitis Activity Index (PUCAI) [3] whereas in adults the Truelove and Witts’ severity index was used [4]. Active disease in children was defined as disease with PUCAI >10 and presence of mucosal lesions on ileocolonoscopy and histology and in adults by the presence of clinical symptoms (at least 2–4 soft stools/day and blood in the feces), endoscopic signs of inflammation (granularity, friability, pus, blood, and/or ulcers), and a histologic picture of active inflammation. Disease extension was based on macroscopic findings based on Paris and Montreal classification of the disease for pediatric and adult patients, respectively. Immediately after collection, the samples were immerged into RNAlater solution (Ambion, Foster City, United States).

**Quantitative Polymerase Chain Reaction**

Real-time polymerase chain reaction (qPCR) was used to analyse the target gene expression. Biopsies were homogenised in lysis buffer RLT, a part of the isolation kit, (T25basic, IKA Labortechnik, Germany) and total RNA was isolated using RNeasy Kit (Qiagen, Hilden, Germany). The purity and concentration of extracted RNA was determined with Bio Photometer (Eppendorf, Hamburg, Germany) at A260/280. 2ug of total RNA was reverse-transcribed using High Capacity cDNA Reverse Transcription Kit with RNAse inhibitor (Applied Biosystems, Foster City, United States) using random hexamers as primers. Both RNA isolation and reverse transcription were performed according to the manufacturer’s protocols.Quantitative PCR was performed on 7500 Real-Time PCR System using TaqMan Gene Expression Assays (both Applied Biosystems) for examined genes (IFN-γ Hs00989291\_m1, IL-2 Hs01555413\_m1, IL-6 Hs00985641\_m1, IL-1β Hs00174383\_m1, IL-17A Hs00174114\_m1, SOCS1 Hs00705164\_s1, SOCS3 Hs02330328\_s1, MDR1 Hs00184491\_m1, MRP1 Hs00219905\_m1 and BCRP Hs00184797\_m1) according to established standard protocols (40 cycles of amplification, denaturation at 95oC for 15 seconds and annealing/extension at 60oC for 1 min) . GAPDH (Glyceraldehyde 3-phosphate dehydrogenase) served as endogenous control. The assay for each sample was performed in triplicates using non-template control (NTC) as the negative control for each gene. Data were collected and analysed with the 7500 System SDS Software v.1.4.0.25. (Applied Biosystems, USA).

**Statistics**

Data were analysed using the comparative Ct method (the ΔΔCt method) [5] and the results are presented as transcript levels relative to levels in controls. After checking for data normality, the comparison of average transcript levels was performed by analysis of variance (ANOVA) and subsequent Dunnett’s multi-comparison (controls to each subgroup) and Bonferroni post-test (difference between subgroups and between children and adults) to adjust for the multiple comparisons. The level of significance was set up at P < 0.05. The Spearman rank correlation coefficients were calculated to analyse the correlation between mRNA expression of efflux transporters and that of examined cytokines and SOCS molecules, as well as correlation between efflux transporters/cytokines and disease activity (P < 0.05 was considered statistically significant). All analysis of samples for individual patients were paired. Statistical analyses were performed using GraphPad Prism 5 (GraphPad Software version 5.03, San Diego, CA).

**References**

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