PROTEOMIC PROFILE OF HUMAN GUT BIOPSIES FROM CELIAC PATIENTS WITH AND WITHOUT SEVERE COMPLICATIONS

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Background and Aims. The mechanisms underlying the massive expansion of intraepithelial CTLs (IELs) and the destruction of the intestine epithelial cells of celiac patients have remained elusive. IELs can undergo malignant transformation during the course of rare but severe complications: enteropathy associated T-cell lymphomas and refractory sprue. These complications, support the idea that CD-IELs are permanently submitted to stimuli that promote their expansion and ultimately may favour their transformation. To characterize T cells and protein expression in CD in relationship to severe complications, we used 2D-Dige approach. Methods 14 patients were characterized for the presence of anti-transglutaminase, VDJ-TcR and VDJ-BcR genescan, HLA-DQ and KIRs genotypes, villous atrophy. One patient presented a restricted T-cell population, a patient a DLCL. Two patients, with absence of HLA-DQ2 and DQ8, were used as controls. Results Patient with the T-cell-restriction showed a prevalent Tgamma-clone in blood and two Tgamma-clones and a Tbeta-clone in the biopsy. Sequence from these 4 clones were performed. HLA-DQ2+D8 is found in the CD-DLCL, 5 cases were DQ2-homozigote, 6 cases were DQ2-heterozygote. A complete set of KIR with activating function (KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS5 KIR3DS1) was present in two samples. Data from comparative protein expression evidenced difference between sample with a restricted T-cell pattern and other samples. Histological features by Marsh modified criteria is in progress of evaluation. *Conclusions.* The frequency of KIR with activating function was found to be higher in patients than in controls. Different protein-profiles founded may be useful to better understand pathogenetic mechanisms involved in CD-complications.

PROGNOSTIC SIGNIFICANCE OF MRNA EXPRESSION MARKERS IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Although mutational status of immunoglobulin variable region genes (VH-genes) is confirmed as the strongest predictor of long term prognosis in B-CLL, it's assessment has not been adopted as a routine laboratory practice. Several microarray-based studies have demonstrated that a set of genes is differentially expressed in B-CLL with mutated and unmutated VH-genes. In this study we have compared prognostic values of the RT-PCR defined expression levels of four genes previously shown to be differentially expressed in VH-unmutated and mutated B-CLL subtypes: SEPT10, SLAM, ADAM29 and LPL. *Methods.* This study has been performed on samples obtained from 81 B-CLL patients selected from HRC database based on the availability of clinical data. VH-sequencing was carried out for all cases. Real-Time PCR experiments were performed on unpurified peripheral blood mononuclear cells. TBP gene encoding TATA-box binding protein was used as a reference, as it was found in preliminary experiments to have the highest gene stability measure in B-CLL cells. *Results.* The median age of patients is 55,4 (range 28 - 75 years), male to female ratio = 2,7. Fifty five percents of patients had Binet stage A, 32% - B, 13% - C. The median follow-up is 72,7 months (range 11 - 234), 28 patients have died (35%). Forty nine (60%) patients had unmutated VH-genes and 32 (40%) -mutated VH-genes. There was significant correlation with mutational status of normalised expression levels for all tested genes: ADAM (R= - 0,389, p=0,0004), SEPT (R=0,336, p=0,0022), SLAM (R= - 0,373, p=0,0006) and LPL (R=0,607, p<0,0001). In logistic regression analysis expression levels of SLAM (p=0,05) and LPL (p=0,02) were statistically significant predictors of mutational status. The cut-offs for best discrimination of subgroups with different mutational status were found for each gene using Youden index. With these cut-offs correct classification rates were for SEPT10 - 71%, for SLAM - 74%, for ADAM29 - 77% and for LPL - 85%. The median overall survival of patients with unmutated VH-genes was 64 months, while in patients with mutated VH-genes - 205 months (p<0,0001). When analysing overall survival by expression levels of tested genes using defined cut-offs, statistically significant differences were found for ADAM29 (p=0,015), SLAM (0,00034) and LPL (0,0013). When combining assessment of 3 genes (LPL, SLAM and ADAM29) concordance for all 3 genes was observed in 21 patients (26%), for 2 genes in 50 patients (62%), for 1 gene in 10 patients (12%). Based on complete and partial corcondance 71 patients (88%) were correctly classified into unmutated or mutated subtypes. Relation of expression level of LPL to ADAM29 and SLAM gives 86% and 84% concordance rate, respectively. *Conclusions*. Our preliminary data show that combined assesement of mRNA markers can potentially be used to predict survival in B-CLL patients. In this study we have not demonstrated any advantages in predictive values of LPL/ADAM29 or LPL/SLAM ratio above individual gene expression measurements. Futhermore, calculating ratio of an overexpressed gene to an underexpressed gene may not be an optimal approach, because it can obscure cases where one of the genes gives discordant result. A classification score is probably prefferable. *Key words*. B-CLL, mutational status of VH-genes, expression analysis.

LATE ONSET NEUTROPENIA LIKE A COMPLICATION OF TREATMENT WITH ANTI-CD20

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Introduction. The occurrence of unexplained peripheral blood cytopenia, particularly neutropenia, has been recently reported after rituximab (R). Its prevalence may be underestimated since it may occur late after treatment (Cattaneo C *et al.*, Leuk Lymphoma. 2006 Jun;47(6):965-6, Chaiwatanatorn K *et al.*, Br J Haematol 2003). Apparently the risk is higher when R is associated with Fludarabine in combination therapy, producing a decrease in bone marrow precursors. Patients and methods: observational and retrospective study in order to determine the incidence of WHO grade III - IV neutropenia (neutrophils: 0.6x109/L) and the development of infection complications in patients treated with R-CMF or R-CF in our hospital from 6/8/2003 to 20/3/2006. We have compared these data with the incidence of delayed neutropenia during 621 courses of R or R-CHOP administered as outpatient schedule in the same period. Data source: clinical reports. Variables: demographic data (age, gender), date of diagnosis, histological type, schedule therapy, number of cycles, clinical response (complete remission (CR), partial remission (PR), and non response (NR), prophylaxis with G-CSF, blood counts (leucocytes, neutrophils, hemoglobin and platelets) during all treatment and follow-up, infections and number of admissions in the hospital. Kaplan-Meier survival and Cox regression were calculated. *Results.* From 78 courses of Immuno-chemotherapy in 16 consecutive patients diagnosed of follicular NHL: 10, B-CLL: 5, mantle NHL: 1. R-CMF (14 patients), R-CF (2 patients). (9 males/7 females), mean age 58.75 (20-78). In first line 8, second line 6 and third line 2. Number of courses received: mean 4,8 (1-6). Response: CR:13, NR: 1, Non valuable: 2. G-CSF prophylaxis during therapy in 50%. 9 patients (56.2%) developed neutropenia during the treatment or

in the following year, one of them had also anemia and thrombocytopenia. In 4 patients the combination therapy was administered in first line, 3 as second line and 2 as third line therapy. Patients received 5 (6 courses), 1 (5 courses), 1 (3 courses), 1 (2 courses) and 1 (1 course), 8 developed infections that required admission into hospital (1 died after third course by sepsis). Another two patients developed severe infections without neutropenia. A late and persistent neutropenia after to finish therapy (1-6 months later), was observed in 4 patients (duration: 3, 12, 11 and 4 months); 2 patients got over at 11 and 12 months after and in two of them is present now. They were treated with G-CSF, prednisone and cyclosporine in 2 cases without response and reached normal values when we associated IV immunoglobulins extract. OS: mean 26.4 months (6-49), RFS: 10.6 months (1-30). *Conclusions.* Delayed-onset cytopenia, particularly neutropenia, is a clinically significant complication of rituximab treatment when it is administered in combination with Fludarabine increasing the risk of severe infections.

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A LOW DENSITY DNA MICROARRAY FOR COMPARING GENE EXPRESSION PROFILES IN HEMATOLOGICAL MALIGNANCIES

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High density microarrays (HDM) are powerful tools for simultaneously profiling the expression levels of thousands of genes. The application of this technology to study of neoplastic hematological disorders.has identified new sub groups of disease not related previously and new prognosis markers. However there is a limited experience in the gene expression studies using low density microarrays (LDM) in neoplastic hematological disorders. A gene expression analysis system based on a LDM containing 538 oligonucleotides has been developed. Whole technical process was optimized to improve the analysis of differential expression. We have analyzed mRNA from cell line cultures (Jurkat, U937), whole blood samples from healthy subjects and different hematological malignancies (HM) using this chip. A hierarchical clustering procedure applying Welch t-statistics with Bonferroni correction was used to analysis gene expression data. The LDM generated a linear response of 2 magnitude orders and a CV values less than 20% for hybridization and label replicates. This procedure detects 0,2 fmols of mRNA. We have found genes with statistically significant differences between Jurkatt and U937 cells cultures, and blood samples from 15 healthy donors, 59 lymphocyte leukemia and 13 myeloid leukemia and myelodisplasia syndrome patients. A classification system based on gene expression data was constructed with an accuracy of 97%.to predict healthy or lymphocyte leukemia status. To identify different subsets of patients in the B-CLL group, whole blood samples from 12 B-CLL patients were collected and defined as stables, according to clinical and analytical criteria at the time of diagnosis, stable (n=6) if disease stability was maintained for more than five years after the diagnosis and progressive (n=6) if the disease progressed less than one year after the diagnosis. Applying Welch statistical test without correction and a $p<0.05$ yielded two lists of 29 and 19 probes differentially hybridized from VSN and quantile-robust normalized data, respectively. The supervised hierarchical clustering of B-CLL samples with 29 statistically significant probes shown that samples grouped together based on their stable or progressive behavior. Eighteen probes were statistically significant in both normalized data. In order to confirm the data expression of POU2F2, PSMB4, FCER2, LCP1, and ABCC5 genes represented by 5 of the 18 statistically significant probes, real-time RT-PCR was performed. Three out of 5 genes -POU2F2, PSMB2, and FCER2- were overexpressed in B-CLL stable patients. Differences were statistically significant ($p<0.05$) and, therefore, results obtained from the chip for POU2F2, PSMB2, and FCER2 genes were confirmed. In conclusion, a viable LDM for gene expression analysis and a simple procedure has been developed useful for analysis of whole blood samples, without any cellular or sample manipulation prior to RNA extraction with variability and reproducibility similar to others commercial HDM. The application to different samples is capable to establish significant differences in gene clusters and could be useful for clinical application in HM

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ARSENIC TRIOXIDE INDUCED MITOCHONDRIAL D-LOOP FRAGMENT MUTATION IN HUMAN T LYMPHOCYTE

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Objective. To understand the differentially expressed of mitochondrial genes (mtDNA) in human T lymphocyte cell line induced by arsenic trioxide (As_2O_3) and to explore the mechanisms of As_2O_3-induced apoptosis and As_2O_3-reduced proliferation. Methods: Human T lymphocyte cell line jurkat E6-1 was treated with 0.5, 1.0, 2.0, and 5.0 micromol /L of As_2O_3 for 48 hrs *in vitro*. Genome DNA was isolated from the As_2O_3treated jurkat cells by salt fractionation. D-loop fragment of mtDNA was isolated and amplified by Polymerase chain reaction (PCR), and the sequencing techniques were applied to identify positive clones. MTT assay, electrophoresis of genomic DNA, and protein/DNA dual parameter flow cytometry were used to examine the effect of As_2O_3 on cell proliferation, cell cycle and apoptosis. *Results.* The single nucleotide mutation on D-loop fragment of mtDNA was found in jurkat cell after 0.5, 1.0, 2.0, and 5.0 micromol /L of As_2O_3 treatment for 48 hrs compared with the baseline. The mutation numbers were 2, 9, 23, and 38 respectively, which showed an As_2O_3 concentration-dependent model. The types of mutation were included base transversion, transition, depletion and insertion. The 2.0 and 5.0 micromol/L As_2O_3-treated samples revealed apoptosis, reduced proliferation and mtDNA mutation, while in the 0.5and 1.0 micromol/L As_2O_3-treated samples, just showed reduced proliferation and mtDNA mutation, no apoptosis was appeared. *Conclusions.* both lower and higher concentration of As_2O_3 induced mtDNA mutation on D-loop region jurkat cell, which might be one of the targets of As_2O_3 on inhibiting proliferation to human T lymphocyte. *Key words.* Arsenic, mitochondrial gene, D-loop fragement, mutation

PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA: RESULTS OF A PILOT AND PHASE II STUDY OF SYSTEMIC AND INTRAVENTRICULAR CHEMOTHERAPY WITH DEFERRED RADIOTHERAPY

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Purpose. To evaluate response rate, response duration, time to treatment failure (TTF), overall survival (OS), and toxicity in primary CNS lymphoma (PCNSL) after systemic and intraventricular chemotherapy with deferred radiotherapy. *Patients and Methods.* From 09/95 to 12/02, 88 patients with PCNSL (median age 62 years) were enrolled onto a pilot and phase II study evaluating chemotherapy without radiotherapy. A high-dose methotrexate (MTX; cycles 1,2,4 and 5) and cytarabine (ARA-C; cycles 3 and 6) based systemic therapy (including dexamethasone, vinca-alkaloids, ifosfamide and cyclophosphamide) was combined with intraventricular MTX, prednisolone, and ARA-C. *Results.* Study accrual was stopped 12/02 and patients were followed until 12/05; 84 of 88 patients were assessable for

response. Of these, 46 patients (55%) achieved complete response (CR), 4 (5%) unconfirmed complete response (CRu), 8 (10%) partial response (PR) (overall response rate 69%) and fourteen (17%) showed progressive disease (PD). Seven of 88 patients (8%) died and in five (6%) chemotherapy was discontinued due to treatment-induced complications. Median follow-up is 42 months (up to 124 months). Kaplan-Meier estimates for median time to treatment failure (TTF) were 19 months, for median overall survival (OS) 55 months, for median response duration 47 months; the 5-year survival fraction was 45%. For patients < 60 years (> 59 years), median TTF was 49 months (9 months), median OS not yet reached (34 months), median response duration not yet reached (24 months) and the 5-year survival fraction 72% (24%). Systemic toxicity was mainly hematologic. Ommaya reservoir infections were seen in 20 (23%) patients. Salvage radiotherapy was applied to 8/39 patients (21%) < 60 years, but to 24/49 (49%) of patients > 59 years, such that radiotherapy added significantly to overall survival in the older age group. *Conclusions.* Primary chemotherapy based on high-dose MTX and ARA-C is highly efficient in PCNSL of patients younger than 60 years. A substantial fraction of these can probably be cured with this regimen.