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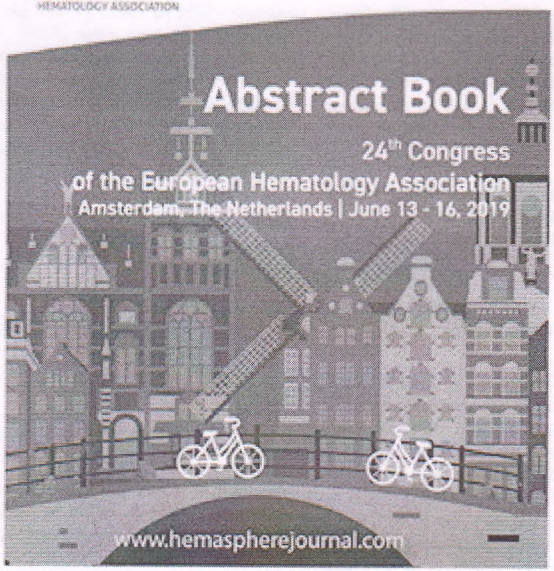

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detected in two ED patients (1 HR and 1 IR patients), which was the same mutation NRAS G12C. FLT3 gene mutations (including FLT3-ITD and FLT3-TKD) were the most frequently found in HR APL patients. 88.33% of HR patients(10/12) harbored FLT3 mutations, while 20% of IR patients (5/25) and 22.22% of LR patients (2/9) were found harbored FLT3 mutations($P < 0.05$). Among the 17 cases of FLT3 mutant patients, only one HR patient (5.88%, 1/17), who carried FLT3-TKD D835Y mutation, died 3 days after induction treatment. Meanwhile, the less common WT1 gene mutations were all found in IR APL patients.

Summary/Conclusion: HR APL patients had the highest frequency of gene mutations, however there were no significant difference between the three risk groups regarding the median mutation numbers. ED patients did not have more gene mutations compared with non-ED patients. FLT3 gene mutations were the most frequently occurred mutations, especially in HR APL patients, though might have no relation with early mortality.

PF266 COMBINING FLOW CYTOMETRY-BASED LEUKEMIC CELL ENRICHMENT AND MUTATIONAL ANALYSIS FOR DETECTION OF RESIDUAL DISEASE IN ACUTE MYELOID LEUKEMIA: A PILOT STUDY

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Background: Persistent minimal residual disease (MRD) is an independent prognostic marker in acute myeloid leukemia (AML) that has become increasingly important for risk stratification and treatment planning. MRD can be evaluated by multi-parameter flow cytometry (MFC) and molecular methods including PCR-based methods detecting leukemia-specific fusion transcripts and targeted deep sequencing of genes recurrently mutated in AML. However, while MFC is highly operator-dependent, PCR-based methods are only available for a minority of AML patients. In addition, targeted deep sequencing approaches are hampered to distinguish between mutations responsible for preleukemic clonal hematopoiesis and true leukemia-specific mutations.

Aims: In this study we aimed for developing a highly sensitive and broadly applicable method for MRD detection in AML by combining MFC-based leukemic cell enrichment followed by mutational analysis.

Methods: To identify markers suitable for leukemic cell enrichment, AML samples from 150 newly diagnosed patients were analyzed for 24 surface markers using MFC with a strictly standardized protocol. In 25 cases, samples were also available at relapse. To assess the normal hematopoietic stem and progenitor cell (HSPC) compartments normal bone marrow (BM) samples were obtained from 12 lymphoma patients without any evidence of disease in the BM. For validation of our method, BM samples were prospectively collected from a total of 42 patients in complete remission (CR) after induction chemotherapy. Mutational analysis of sorted leukemic cell enriched samples was done using targeted deep sequencing of 39 recurrently mutated genes using an Ion Torrent sequencing platform. In some NPM1-mutated samples analysis was performed using a mutation-specific digital droplet PCR assay.

Results: A combination of antibodies against CLL-1, TIM-3, CD123 and CD117 was identified to perform best for leukemic cell enrichment by enabling staining of >90% of AML cells in 137 of 150 diagnostic AML samples (91.3%) and in 25 of 25 (100%) relapse samples. In dilution experiments using NPM1-mutated samples and normal BM, leukemic cell enrichment by these markers followed by mutational analysis showed a sensitivity of 10^{-5} for residual disease detection. In contrast, NPM1-mutations were not detected in cells negative for the marker combination in dilutions higher than 1:100. Using this marker combination for cell sorting allowed a 30- to 250-fold cell enrichment in prospectively collected BM samples of 42 patients in complete remission. In 39 samples DNA quality of sorted cells was sufficient for sequencing. Twenty-one samples tested MRD positive whereas 18 were negative. With a median follow-up of 372 days 71% of MRD positive (15/21) and 28% (5/18) of MRD negative patients relapsed (p = 0.007). Accordingly, median relapse free survival was significantly longer in MRD negative patients (497 vs. 242 days, p = 0.0035). Of note, in 8 out of 10 MRD positive remission samples, in which we also analyzed marker negative cells, mutations were exclusively found in marker positive cells emphasizing the importance of MFC-based cell enrichment prior to sequencing.

Summary/Conclusion: MFC-based leukemic cell enrichment using antibodies against CLL-1, TIM-3, CD123 and CD117 followed by mutational analysis is feasible for MRD detection with high sensitivity and informative on relapse risk in AML patients. Standardization of this method as well as its comparability to other approaches of MRD detection need to be evaluated in a multi-center clinical trial.

PF267 RISK ASSESSMENT OF ANTHRACYCLINE CARDIOTOXICITY IN PATIENTS WITH ACUTE LEUKEMIA AND CONCOMITANT ISCHEMIC HEART DISEASE

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Background: Great attention has recently been focused on the problem of chemotherapy (CT) complications in patients with acute leukemia (AL). Notable among them is the anthracycline cardiotoxicity (AC), the development of which greatly inhibits the carrying out of CT in full doses, which significantly reduces the patients' chances for life. The presence of concomitant ischemic heart disease (IHD) potentiates the formation of AC, increases the risk of myocardial injury development.

Aims: To assess the risk factors of AC development on low cumulative doses of anthracycline antibiotics (AA) and to increase the efficacy of AC prevention in patients with AL taking into account concomitant IHD.

Methods: The study involved 147 patients with newly diagnosed AL, 72 (49%) males and 75 (51%) females, mean age 54.7 ± 9.3 years, ECOG I-II. All patients were treated in hematology department of Poltava Regional Clinical Hospital n.a.M.V.Sklifosovsky. Depending on the IHD presence, the patients were divided into two groups: I (n = 81)–AL pts without concomitant cardiovascular diseases; II (n = 66)–AL pts with IHD. Due to ongoing AC prevention with L-arginine patients of both groups were further subdivided: IA (n = 47) and IIA (n = 36)–pts receiving CT; IB (n = 34) and IIB (n = 30)–pts, who received CT with prophylaxis of AC with L-arginine. The study was approved by the local ethical committee and all patients and all patients signed the inform consent before they were included. The examination was carried out twice: at baseline and after induction CT, when remission was achieved and AA low cumulative doses <200 mg/m^2 were reached. The cardiotoxic effect of AA was evaluated by echocardiography and Holter ECG monitoring and considered to be according to CTCAE 4.03 as reduction of left ventricular ejection fraction (LVEF) more than 10% of baseline and QTc prolongation exceeded 450ms. The episodes of "silent ischemia" were assessed on the basis of ST segment depression by 1 mm or more in the absence of typical pain syndrome.

Results: After induction CT the Holter ECG monitoring has shown an increased number of painless myocardial ischemia periods in 29 (80.5%) pts of subgroup IIA vs 8 (22.2%) before treatment; p<0.001. QTc prolongation was noticed in 14 (38.8%) pts of subgroup IIA vs 7 (14.9%) of subgroup IA without IHD; p<0.05. The presence of IHD was a risk factor for AC development, the manifestations of which are prolongation of QTc interval (OR = 3.636; 95% CI = 1.278–10.349; p<0.05) and QRS voltage decrease (OR = 3.482; 95% CI = 1.270–9.549; p<0.05). Echocardiography showed LVEF reduction more than 10% of baseline in 13 (36%) pts of subgroup IIA, without a significant difference vs subgroup IA. L-arginine in patients with AL and concomitant IHD has reduced the frequency of "silent ischemia", according to Holter ECG monitoring, by 40.5%; p<0.05, QTc interval prolongation–by 28.8%; p<0.05, and according to echocardiography, LVEF decrease more than 10% of baseline–by 22.7%; p<0.05. The absence of L-arginine prophylaxis was a risk factor for AC: painless ST segment depression (OR = 6.214; 95% CI = 2.064–18.710; p<0.05); QTc prolongation (OR = 5.727; 95% CI = 1.458–22.497; p<0.05) and decreased LVEF more than 10% of baseline (OR = 3.674; 95% CI = 1.049–12.865; p<0.05). There was no systolic dysfunction in patients with concomitant IHD, who received L-arginine during induction CT.

Summary/Conclusion: Our results proved that preventive appointment of L-arginine during induction CT reduces the risk of AC development in patients with AL and concomitant IHD.