(54.3%) genes methylation. Methylation of 1, 2, 3, 4 and 5 genes simultaneously was detected in 10.9%, 28.3%, 26.1%, 19.6% and 8.7% of patients, respectively. There was no any difference in the number of patients with *SFRP1*, *SFRP4*, *SOX7* and *p15^{INK4b}* methylation in the groups with different bone marrow blasts counts. Methylation of *SFRP5* gene was more frequently seen in patients with refractory anemia with excess of blasts (RAEB): 43.5% *vs* 13.0% in patients without excess of blasts; OR=5.1, 95%CI: 1.2-22.3, *p=0.047*. The patients without excess of blasts were characterized by methylation of 0-1 genes: 26.1% *vs* 8.7% of RAEB patients, although the difference was not significant. At the same time, there was the tendency to increase of the number of cases with 3-5 methylated genes in patients with 10-19% blasts compared to patients with 5-9% blasts. In the whole MDS group, there was no any correlation between the number of methylated genes and patient's age, number of bone marrow blasts or karyotype. Increased number of methylated genes did not influence on the OS.

Summary/Conclusions: MDS progression is associated with enhancement of epigenetic disturbances leading to increase of the number of methylated genes, in particular, *SFRP5*

E1202

IRON OVERLOAD-ASSOCIATED GENETIC INSTABILITY IN MYELODYSPLASTIC SYNDROME

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Background: Not only clinical experience, but also numerous experimental and animal-modell studies demonstrated that iron overload (IO) negatively impacts on survival outcomes in patients suffering from myelodysplastic syndromes (MDS). However, the molecular mechanisms of IO induced genetic damage have neither been sufficiently demonstrated nor been fully understood.
Aims: We aimed to investigate the association between IO, measured as serum ferritin (SF) as suitable surrogate marker, and genetic instability, considered to be a main factor responsible for MDS progression to secondary acute myeloid leukemia (sAML).
Methods: We included 55 patients (median age 74 years, 62% males). The majority (n=50) had different MDS subtypes, 5 patients had myelodysplastic/myeloproliferative neoplasms (MSD/MPN). The patients were divided into two subgroups with normal and elevated SF levels, using 275 µg/l as cut-off for elevated SF. 26 patients showed normal SF levels (median: 65 µg/l, range: 8-256 µg/l), while 29 patients showed elevated SF levels (median: 1150 µg/l, range: 283-3872 µg/l). Various parameters for genetic instability were analyzed and statistically compared between the two subgroups with normal and elevated SF: a) molecular karyotyping (SNP array analysis) to determine the total genomic alterations (TGA) size, b) immunofluorescent determination of γH2AX-foci for quantifying DNA double-strand breaks (DSB) in CD34+ peripheral blood (PB) cells, c) telomere length (TL) of PB granulocytes and lymphocytes, and d) plasma nitric oxide metabolites as markers for oxidative stress.
Results: Subsequent analyses revealed a positive correlation of SF levels and bone marrow blast counts. Thus, all further analyses were adjusted by a logistic regression model for unevenly distributed blast counts. PB nitric oxide metabolites did not correlate with SF level (N=33 patients with available data). Iron overload measured by SF showed a significant correlation with the number of γH2AX-foci per CD34+ PB cell (r=0.481, p=0.039). The median number of γH2AX-foci per CD34+ PB cells was significantly highlower in 8 patients with normal SF as compared to 10 patients with elevated SF level (1.9 *vs* 5.7 γH2AX-foci/CD34+ cell (adjusted p=0.050). TGA size was positively correlating with SF levels (r=0.397, p=0.026) and with marrow blast counts (r=0.381, p=0.077). TGA size was higher in 28 patients with elevated SF (median: 34 Mbp, range: 0-248 Mbp) as compared to 23 patients with normal SF (median: 0 Mbp, range: 0-155 Mbp, adjusted p>0.5). Telomere length and IO showed a significant negative correlation (r=-0.497, p=0.002). TL in granulocytes was significantly reduced in 13 patients with elevated SF (median: -1.61 kb, range: -4.06-1.31) as compared to 13 patients with normal SF levels (median: 0.48 kb, range: -3.13-5.32 kb, adjusted p=0.024). In contrast, lymphocyte TL was not influenced by the SF level.
Summary/Conclusions: In this study, IO was significantly associated with numerous markers of genetic instability. Elevated SF levels were promoting the TGA size, spontaneous nuclear damage assessed by γH2AX-foci and replicative stress (TL) in granulocytes representing the myeloid compartment, whereas the lymphocyte compartment remained uninfluenced by IO. These findings further support the assumption of IO being closely related to leukemic transformation in MDS. Our results contribute to explain the association of IO and disease progression found in several studies. Whether these results may have consequences for diagnostics and therapeutic decision-making in patients with MDS remains to be further investigated.

E1203

CONVENTIONAL CYTOGENETICS, ACGH, AND PCR AS INTEGRATED WORKUP FOR A CORRECT DIAGNOSIS OF MDS

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Background: Conventional cytogenetics continues to have a fundamental role in the classification and risk scoring of myelodysplastic syndromes (MDS). Nevertheless, non-informative karyotypes represent up to 20% of cases. Some different molecular methods, such as FISH, aCGH or mutational analysis, could detect new abnormalities and improve the correct subtyping of MDS patients.
Aims: The aim of the study was to adopt an integrated diagnostic work-up for better characterizing MDS patients.
Methods: We analyzed 61 patients (71% male, with a median age of 74 years). According to the WHO classification, 33% were affected by multilineage refractory cytopenias, 12% by refractory anemia, 15% by refractory anemia with ring sideroblasts, 21% by refractory anemia with excess of blasts, 3% by 5q- syndrome, and 12% by chronic myelo-monocytic leukemia; 67% of patients were at low/intermediate-1 IPSS risk. All patients were assessed by conventional cytogenetics, FISH for chromosomes 5, 7, 8, PDGFRalpha and beta rearrangements, aCGH, and PCR for ASXL1, EZH2, TP53, and TET2 mutations. WT1 and RPS14 gene expression levels were also measured by quantitative PCR.
Results: In our series, conventional cytogenetics analysis failed in only 12% of cases: indeed, the sampling for this analysis was the first one during the bone marrow aspiration. Overall, 39% of patients showed at least one chromosomal aberration, with complex karyotypes in 7% of cases. FISH allowed to correctly classify two cases as affected by the 5q- syndrome and one as affected by deletion of chromosome 7; two patients carried PDGFRbeta rearrangement; these abnormalities had not been detected by the conventional cytogenetics.
The aGCH allowed to detect chromosomal aberrations in 38% of cases: aCGH detected 10 "new" mutated cases in respect of the conventional cytogenetics, including alterations of the ETV6 and GATA2 genes; After the mutational analyses, 28% of patients resulted mutated, with highest frequency for TP53 (mutated in the 16% of the overall series). Eight of these TP53-mutated patients showed normal karyotype, and resulted wild-type by FISH and aCGH. Four low/intermediate-1 risk patients (8%) showed the ASXL1 gene mutation. Two cases showed the TET2 mutation. The statistical analysis confirmed the prognostic role of poor cytogenetics either on overall survival (OS) or progression-free-survival (PFS). Also deletions detected by aCGH resulted to play a negative prognostic impact on OS. WT1 and RPS14 gene expression was assessed: over-expression of WT1 was found in one third of all patients, while 70% showed RPS14 values lower than those measured in healthy donors. Statistical analysis showed that the WT1 over-expression had statistical significance on survival, also in multivariate analysis.
Summary/Conclusions: Our study supports the feasibility and the utility of the introduction in the routinary workup of MDS of FISH and aCGH, in order to better stratify MDS patients and correctly design *ab initio* a patient-tailored treatment.

E1204

CLONAL DYNAMICS OF TWO DISTINCT CLONES IN LEUKEMIC TRANSFORMATION FROM MDS WITH ISOLATED DEL(5Q) HARBORING TP53 MUTATION

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Background: Although myelodysplastic syndromes (MDS) with isolated del(5q) represents a favorable prognosis, the presence of TP53 mutation has been associated with transformation to secondary AML (sAML) accompanied by the emergence of a complex karyotype (CK). The demethylating agent azacitidine (AZA) is currently the standard therapy for patients with high-risk MDS. However, some reports showed AZA treatment cannot improve survival in high-risk