tology) and to know the implication of PAX-5 gene in this pathology. *Methods.* Among a series of 141 SMZL we applied spectral karyotyping (SKY) in 25 cases with complex karyotype. In patients with t(9;14) we studied by FISH the involvement of PAX-5 gene using a split probe (Dako, Denmark). *Results.* In 3 out of 25 cases with t(9;14)(p13;q32) detected by SKY, rearrangement of PAX-5 was confirmed. Our three patients presented a complex karyotype. The most frequent additional abnormalities were gains of chromosome 1 (3 cases) and gains of chromosome 3 (2 cases). They showed morphology and immunophenotype features typical of SMZL. All three cases presented bone marrow involvement and two showed a splenic diffuse pattern uncommon in this pathology. *Summary/Conclusions*. In all patients t(9;14) was found after the application of spectral karyotyping (SKY) technique confirming that complex rearrangements could mask this anomaly when are studied by conventional cytogenetics. Our findings confirm the rare but recurrent involvement of t(9;14) in SMZL cases and that this anomaly is not specific for a subtype of NHL. The prognosis of PAX-5 rearrangements in SMZL remains unclear and a further follow-up of patients is necessary to better understand the role of this aberration. *Acknowledgements.* This work has been partially supported by grants from Instituto de Salud Carlos III, Ministerio de Sanidad y Consumo (PI 051072) and Fundació La Marató de TV3 (Càncer). We want to thank Juan Cruz Cigudosa for his help in the tone-up of SKY technique and Carme Melero for their expert technical assistance.

0593

I-FISH ANALYSIS OF IMMUNOFLUORESCENTLY LABELED PLASMA CELLS IN PATIENTS WITH MULTIPLE MYELOMA

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Background. Early detection of specific chromosomal aberrations in plasma cells of patients with MM may have diagnostic, prognostic and therapeutic implication. One of the most frequent and prognostically most significant clonal aberrations in MM are rearrangements of IgH gene at 14q32 region (generally poor prognosis), deletions of RB1 gene at 13q14 and/or loss of whole chromosome 13 (moderately adverse or medium prognosis). The translocation t(11;14)(q13;q32) is associated with longer overall survival and, in contrast to other IgH rearrangements, it is considered to be a favorable prognostic factor. However, the detection of genetic aberrations involved in MM by conventional cytogenetic and/or classical I-FISH methods may be limited by low proliferative index of plasma cells. The sensitivity and specifity of I-FISH analysis may significantly increase previous immunofluorescent labeling of malignant myeloma cells. This method allows identification of chromosomal changes even in cases with low bone marrow infiltration. *Aims.* The aim of the study was to assess the frequency of the most significant chromosomal aberrations (abnormalities of IgH gene and RB-1 gene) in immunofluorescently labeled non-dividing plasma cells of patients with MM by I-FISH and to evaluate their prognostic significance. *Methods.* I-FISH analyses were done on plasma cells labeled by the Amca Anti-Human kappa-chain, Amca Anti-Human lambda-chain and Amca Anti-goat IgG monoclonal antibodies (Vector Laboratories). For I-FISH directly marked locus specific DNA probes (Abbott-Vysis) were used. Detection of deletion/monosomy of chromosome 13 was performed by LSI 13q14 (RB1) and LSI 13q34 DNA probes. Aberrations of 14q32 region were proved by LSI IgH rearrangement probe. For detection of specific IgH translocations LSI IgH/CCND1 and/or LSI IgH/FGFR3 probes were used. Molecular cytogenetic findings were correlated with different clinical and laboratory parameters. *Results.* Altogether 114 newly diagnosed MM patients were examined by I-FISH. Deletion of RB-1 gene was found in 22 (19%) patients and monosomy 13 was identified in other 34 (30%) of them. Combination of both aberrations was proved in 6 (5%) cases. Aberration of IgH gene was found in 60 (57%) from 106 evaluated patients (deletions, partial trisomies and monosomies and numerical changes involving chromosome 14 were also found). Sixteen out of 33 cases (48%) evaluated for t(11;14)(q13;q32) were positive. Another six patients were examined for t(4;14)(p16;q32) and translocation was proved in four of them. Patients with aberration of 13q had significantly shorter event-free survival (EFS), strong association with advanced clinical stages was also proved. Between IgH positive and IgH negative cases, difference in EFS was not statistically significant due to heterogeneity of IgH positive patients. In most cases t(11;14) is associated with other chromosomal aberrations and prognostic relevance of these findings remains to be cleared. *Summary.* I-FISH on plasma cells detected by the immunofluorescent staining permits to increase yield of number of chromosomal abnormalities in MM patients. This method significantly contributes to the higher sensitivity and specifity of diagnostic procedures and is important for determination of prognosis and treatment of MM patients. Our results confirmed 13q aberrations as a marker of poor prognosis (p=0.008).

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0594

DETECTION OF STRUCTURAL ABERRATIONS OF CHROMOSOME 7 IN MYELOID MALIGNANCIES USING COMBINATION OF MOLECULAR CYTOGENETIC TECHNIQUES

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Background. Complete or partial loss of chromosome 7 is a frequent chromosomal aberration in myeloid disorders such as myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). Allelotypic studies have delineated at least three distinct loci, that are frequently deleted: 7q22, 7q31 and 7q35. It has been hypothesized that there are localized tumor suppressor genes that contribute to the pathogenesis of these disorders. *Aims.* Using combinations of conventional and molecular cytogenetic techniques we have focused on the analyses of deletions and translocations involving chromosome 7 in bone marrow cells of patients with MDS and AML. Correlation of clinical characteristics, outcome and survival of patients according to cytogenetic findings were evaluated. *Methods.* Using classical cytogenetic techniques we examined 32 patients with myeloid malignancies (16 MDS, 16 AML), whose bone marrow cells contained 7q deletion or rearrangements of chromosome 7. Fluorescence *in situ* hybridization (FISH) with locus specific probe for 7/7q31 region (ABBOTT VYSIS), and 7q22/q35 specific probe (QBiogene) were used in all patients to confirm the deletion and to prove the breakpoints. Multicolor banding technique (mBAND) for chromosome 7 was carried out in 16 patients for precise mapping of the extent of deletions (XCyte7 DNA Probe Kit, MetaSystems). Chromosomes involved in complex translocations were identified by multicolor FISH (mFISH) (24XCyte DNA Probe Kit, MetaSystems). *Results.* By using conventional cytogenetic techniques deletion of 7q was found in 5 patients, in two as a sole aberration, in 27 patients translocation of chromosome 7 was ascertained. According to the results of FISH with locus specific probes for 7q22, 7q31 and 7q35 region and mBAND for chromosome 7 five groups of patients were established: patients with deletion 7q as a sole aberration (2x), patients with deletion 7q and complex karyotype (3x), patients with combined translocation and deletion 7q (19x), patients with combined translocation and deletion 7p (5x) and patients with translocation of chromosome 7 without deletion 7p or 7q (3x). Deletions of all three FISH screened regions on the long arms were the most frequent, the breakpoints were heterogenous and varied among patients. On the short arms of chromosome 7 region 7p13.2p15.2 was the common deleted segment. Complex karyotype was confirmed by mFISH in 29 patients. Most of the deletions in patients with complex karyotype were cryptic, not detectable using conventional cytogenetic techniques. *Summary.* Aberrations of chromosome 7 are associated with a poor prognosis, increased risk of infection, rapidly progressive disease and poor response to treatment. Survival time was short in our cohort of patients (median 7 months), 25 patients died. Systematic molecular cytogenetics studies reveal cryptic rearrangements and provide novel information about genes possibly involved in these events.

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