(46,XX[18]/45,X0[7]). The patient was conditioned with the classical Bu-Cy regimen and rescued with 5.3 million/kg of body weight allogeneic CD34+ peripheral blood stem-cells. He received IV cyclosporine and four doses of methotrexate as GVHD prophylaxis, but on day +11 he manifested a hyperacute cutaneous and oral mucosa GVHD grade II. On day+16 the patient engrafted the WBC completely, while platelets engrafted earlier, on day+13. One month later, he manifested post-transplant thrombotic thrombocytopenic purpura. Cytogenetic analyses of the patient revealed the complete donor karyotype 46,XX/45,X0 two times after transplantation. Chimerism study was 99.5% of donor type. On day +97 he manifested an extensive chronic cutaneous GVHD grade III, with conjuctival and oral involvement. The post-transplant course was further complicated by the development of diabetes mellitus, severe osteoporosis with a pathologic smash of the L2 vertebra and CMV reactivation. Nineteen months later, the patient is in fairly good general condition. He has limited cutaneous GVHD, suffering mainly from his orthopedic problem. *Summary/Conclusions.* Our patient manifested osteoporosis, diabetes mellitus and conjuctival defect after SCT from a mosaic TS. Osteoporosis is a rare phenomenon in male patients after SCT, while it is very common in adults with TS who show a reduction in bone mass and an increased risk of fractures. Diabetes mellitus and ophthalmic disorders are also uncommon after SCT. On the contrary, diabetes mellitus is 2-4 times more frequent in TS compared with the general population and ophthalmic problems are seen in 63% of women with TS. As it is has been accepted, dosage of specific genes located on X chromosome in X0 cells are responsible for such abnormalities in TS. Therefore, the X0 cells could have pathologic consequences in the recipient, especially under immunologic stress.

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LEVELS OF METALLOPROTEINASE-9 (MMP-9) IN PATIENTS WITH POLYCYTHEMIA VERA

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Chronic myeloproliferative disorders are characterized by the progressive remodeling of the bone marrow stroma during angiogenesis and the fibrotic tissue deposition. Angiogenesis shares common mechanistic steps between the cells and the extracellular matrix including proteolysis. The family of metalloproteinases (MMPs) and their inhibitors play a serious role in this proteolysis and their balance determines whether the tissue remodeling goes towards matrix breakdown or increased fibrosis. Matrix metalloproteinase-9 (MMP-9) is produced in haematopoietic tissue by monocytes and mature granulocytes and is stored in the gelatinase granules while pro-MMP-9 is also stored in platelets and acts as an endogenous angiogenic factor. In order to investigate the role of MMP-9 as an angiogenic factor in Polycythaemia Vera (PV) and because only one report exists in the literature showing elevated levels of MMP-9 in the plasma of 17 patients with PV, we investigated serum levels of MMP-9 in 38 polycythaemic patients. A total of 38 patients with PV (18 males and 20 females) with a mean age of 56,1±15,54 (m ± SD) years (range 24-81) were included. Twenty five patients were managed with phlebotomy, four received hydroxyurea, eight were managed with hydroxyurea and phlebotoby and one was treated with interferon. Three had clinically detected spleen enlargement. The control group consisted of 16 healthy subjects (mean age 55,3±6 years). The age difference between the two groups was not statistically significant (p=0,82). Serum levels of total MP-9 were measured by a commercial quantitative sandwich enzyme immunoassay. Serum MMP-9 concentrations did not differ among polycythemic patients and the control group (316±249 pg/ml and 446,2±290,3 pg/ml, respectively, p=0,086). In the patient group and in the control group we found no statistically significant correlation between serum MMP-9 levels and platelet counts, haemoglobin, WBC counts and age. No difference was found between patients on different therapeutic regimens. In conclusion the present report demonstrates no difference among polycythaemic patients and healthy individuals in concern to MMP-9 serum levels in a large group of patients. Future studies are needed to investigate whether serum markers for collagen metabolism reflect the disturbed balance of matrix synthesis and proteolytic degradation that exists in neoangiogenesis that characterizes polycythaemia vera and myeloproliferative disorders.

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ELEVATED TNF-α AND LDH WITHOUT DISTURBANCE IN PARATHORMONE ASSOCIATED WITH DIFUSE OSTEOLYTIC LESIONS IN LEUKEMIC TRANSFORMATION OF MYELOFIBROSIS

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Backgrounds. Myelofibrosis is a clonal myeloproliferative disorder characterized by splenomegaly, abnormal deposition of collagen in the bone marrow, extramedullary haematopoiesis, dacriocytosis and leukoerythroblastic blood smear. Development and sustainment of fibrosis are mediated by complex network of several cytokines. These cytokines mainly include transforming growth factor α, basic fibroblast growth factor, vascular endothelial growth factor, platelet factor 4, calmodululin and tumor necrosis factor α, TNF-α. *Aims.* Based on role cytokines in myelofibrosis, we present an atypical case of leukamic transformation in myelofibrosis associated with diffuse osteolytic lesions and extremely elevated sera TNF-α and LDH without disturbance in parathormone in a 49-year-old female that firstly developed malaise and abdominal pain at first visit. *Results.* The laboratory analyses showed decrease in hemoglobine, platelets and presence of erytroblast and dacriocytes in peripheral blood. Cytological examination disclosed hypocellularity with the presence of all cell lines without increased blasts cells. Bone marrow biosy disclosed hypocellularity, presence of all cell lineage and bone marrow reticulin and collagen fibrosis. A diagnosis of myelofibrosis was established. After 3 years, her condition deteriorated with malasia and bone pains. Physical examination showed pale skin and mucous membranes with enlarged spleen 270 mm in diameter. The laboratory analyses showed Hb of 54 g/l, WBC of 8.0×10^9/L, platelets of 122×10^9/L, with myeloblasts 39%, myelocytes 7%, metamyelocytes 1%, bands 6% segmented neutrophils 18%, eosinophils 1%, lymphocytes 22%, monocytes 6%, and 13 erythroblasts/100 leukocytes. The biochemical analyses showed extremely elevated sera LDH activity (1339 U/l). Bone marrow aspirate was hypocellular with 72% of blasts mostly with characteristics of myeloblasts and more than 20% of monoblastic type. Cytochemical staining with myeloperoxidase showed that 30% of blasts were positive, and 25% of blasts were α-naphtol-esterase positive. The cytological finding was in accordance with FAB M4 type of acute leukemia. The immunophenotyping of the peripheral blood cells expressed HLA-DR (74.96%), CD34 (77.99%), CD13 (60.36%), CD33 (42.60%), CD14 (39.89%), CD4 (42.40%) markers. Cytogenetic examination of bone marrow cells showed inversion of chromosome 16 [46,XX, inv(16)(p13q22)]. RT-PCR studies/MYH11 fusion geneα confirmed cytogenetic finding and revealed the CBF transcript. PCR analysis disclosed the presence of FLT3 Asp835 mutation. Retrospective analyses of extracted DNA from bone marrow histological specimen at the time of diagnosis, showed that there no presence of FLT3 mutations. X-ray showed the presence of diffuse osteolyic lesions in the pelvis, long bones as well as in vertebra bodies. The global skeletal scintigraphy documented diffusely increased accumulation of the radiopharmaca. The values of parathormon in the sera and supernatants of cultured blast cells were normal. TNF-α determined by sensitive LDH release assay, was extremely increased (1421 pg/ml) in comparison to control values of 700 pg/ml. Summary / Conclusions. We postulated that elevated TNFα can be reason for lytic bone lesions, accompanied with high sera LDH activity indicating high bone turnover. Also continuously elevated TNF-α can contribute for developing of the leukemia growth in this patient, as endogenous promoter.

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FIP1L1/PDGFR-α- NEGATIVE CHRONIC EOSINOPHILIC LEUKEMIA SUCCESSFULLY TREATED WITH IMATINIB CASE REPORT

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Background. Recognition of tyrosin kinases contribution to pathogenesis of idiopathic hypereosinophilic syndrome/chronic eosinophilic leukemia (IHES/CEL) and imatinib treatment tended to rapid improvement in prognosis of significant part of IHES/CEL patients. Imatinib is especially effective in *FIP1L1/PDGFR-α* (F/P) positive patients. Approximately 40% of responding patients lack the F/P fusion gene, suggesting other tyrosinkinase influence. *Patient and methods.* Authors describe case

report of IHES patient diagnosed in 1998. Serious organ damage developed during 2005 and required treatment initiation. Corticoids were quite ineffective as well as cyclosporin A was. Cytogenetic examination of bone marrow cells revealed in 49% of examined metaphases karyotype 45,X,-Y. Fluorescence in situ hybridization (FISH)-based strategy was used to detect F/P in bone marrow cells. Using bacterial artificial chromosome (BAC) probes fusion of F/P was not revealed. Considering the standard treatment ineffectivity, disease progression and clonal hemopoesis finding imatinib treatment was started. Imatinib in dose of 100 mg daily was administered despite the F/P negativity. *Results*. Eosinophils fully disappeared after 6 days of the therapy. Complete hematologic remission was achieved after 2 weeks. Cytogenetic response will be assessed after 3 month treatment. *Conclusions*. The case of IHES/CEL patient with the karyotype 45, X, -Y, F/P-negative but imatinib-sensitive has not been published yet. Identification of imatinib-sensitive target structure responsible for the disease development is a challenge to future research.

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MIXED IMMUNODEFICIENCY, ATYPICAL MYCOBACTERIA AND MYELOFIBROSIS

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Background and Aims. Myelofibrosis can be idiopathic (a chronic myeloproliferative syndrome) or secondary to many kinds of insults, as a reaction to malignancy, infections, endocrinopathies, auto-immune diseases and granulomatous disease. Pancytopenia and hepato-splenomegaly are frequent, and only reversible when the secondary injury can be treated. The authors present a case of myelofibrosis diagnosed in an 11 months old boy; later it was discovered to be secondary to atypical mycobacteria and quadruple antibacillar therapy for one year reverted the clinical status. *Methods/Clinical Case*: A caucasian male infant 4 months old presents with anaemia and pneumonia with pleural effusion. Three months later, he has a peri-anal abscess and presents with hepato-splenomegaly and pancytopenia. The analysis revealed Hb 8,0g/dL (with presence of erythroblasts, frank anisopoikylocytosis, and dacriocytes), leukocytes 1500/mm^3 (absolute neutropenia 150/mm^3, left shift and basophilia), platelets 65000/mm^3; LDH 632 U/L. Bone marrow smear and biopsy showed severe myelofibrosis (without cytogenetic alterations). The thorough studies also demonstrated a mixed immunodeficiency (hypogammalobulinemia, lymphocytopenia, inversion in the relation $CD4^+/CD8^+$) and an auto-immune phenomenon (presence of auto antibodies anti-platelets and anti-granulocytes). The boy was given G-CSF on alternate days (10 μg/kg), immunoglobulin on a mensal basis and co-trimoxazole as prophylaxis for infections. With two years and 6 months old, he had already pericarditis, otitis with tympanic perforation, cutaneous mycosis, several gastro-enteritis and two pyelonephritis. Although he presented palpable lymph nodes in several areas, a liver palpable 3 cm beyond costal grid and a spleen palpable 6 cm beyond costal grid, his physical and somatometric development were normal for his age. The clinical status degrades with a growing spleen (surpassing the iliac crest) and worsening pancytopenia (Hb 6,9g/dL; leukocytes 1000/mm^3; platelets 50.000/mm^3), with muco-cutaneous blood discrasia and signs of extra-medullar hematopoiesis on both kidneys. As he has no brothers, a search was begun in the international panel for bone marrow transplantation. It was stopped by the decision for splenectomy (4 years old). The spleen showed numerous tuberculoid granulomes without caseous necrosis and although the research for typical mycobacteria was negative, he began quadruple therapy (isoniazide, rifampicine, pirazinamide and ethambutol). After splenectomy, there was normalization of haemoglobin and of platelet number; the leukocytes didn't rise as much (2000-3000/mm3), maintaining absolute neutropenia. A bone marrow smear revealed recuperation of the three haematopoietic series. Immunoglobulin therapy was suspended 2 years after splenectomy; with co-trimoxazol and penicillin prophylaxis, at 11 years old, he doesn't have a significative number of bacterial infections. Analytically, he presents Hb 13,7g/dL, leukocytes 2000/mm^3 (neutrophils 120/mm^3), platelets 662.000/mm^3. *Conclusions.* Although one of the causes indicated for secondary myelofibrosis is granulomatous disease, there aren't any published cases reporting myelofibrosis secondary to typical or atypical mycobacteria, whether in immunocompetent or immunodepressed individuals. Also it is not usual to see extra-medullar haematopoiesis on both kidneys, causing enlargement and loss of differentiation cortico-medullar. Finally, both splenectomy and anti-bacillar therapeutic were decisive in the regression of the clinical state, being the remaining neutropenia a manifestation of the immunodeficiency syndrome.

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PLATELET FUNCTION EXAMINATION IN ESSENTIAL THROMBOCYTHEMIA

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Backgrounds. Basic diagnosis of essential thrombocythemia is proved by estimation of elevated platelets count and corresponding findings of activated megakaryopoiesis in bone marrow with contemporary excluding their reactive changes. The therapy is mainly focused on correction of high platelets count in the blood. The treatment is different in young and elderly patiens, in cardiovascular or thrombotic risk and non-risk patiens. However, the treatment influences not only the count, but also the function of platelets and so can lead to influencing of clinical symptoms. The function of platelets is not currently investigated before drug adminstration and in the course of the treatment. Aim of the study was to evaluate clinical and laboratory importance of platelet functional characteristics in essential thrombocythemia. *Methods.* 30 patients have been included in our observation and we performed (beside the basic laboratory tests) platelets aggregation according to Born, PFA-100 examination and flow-cytometric estimation of CD36, CD42a, CD61, CD62, CD63 markers. The laboratory testing was done before and six months after the treatment. (The platelet aggregation was tested using ADP in two concentrations, colagen and cationic propylgalat as inductors, and three parameters - percentil of aggregation, slope and desaggregation remark - were evalutated). *Results.* the decreasing of aggregation response (in 16 cases after all inductors used) was not accompaned by statisticaly significant changes in other examinations of platelet function tests. Moreover, there were not observed statisticaly significant changes in repeated examinations after six month of the treatment. There was even no correlation between functional examination of the platelets and clinical symptoms of the disease. *Conclusion*: Functional disorder of the platelets seems to be the part of clinical findings of the disease, but does not correspond with biological activity of the disease or with its clinical symptoms and/or with the answer to the therapy. Although, the treatment (especially with acid acetylosalicylic-ASA) can widely modify platelet function, it was not even observed to be significantly different in our ASA-treated vs. ASA-nontreated patients.

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POLYCYTEMIA VERA INITIALLY DIGNOSED AS ESSENTIAL THROMBOCYTEMIA

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Backgrounds. The differentiated diagnosis as part of the myeloid proliferate chronic disease Ph- remains afterwards difficult, in spite of the assays to identify a sensitive and reproductive set of clinic and biologic parameters (ex. WHO). *Aims.* The aims of this piece of work are to present our experience concerning the difference between ET and PV at diagnosis. *Method*: The study contains a number of 38 patients with PV diagnosed in our clinic during the period of 1988-2005. 3 of those cases whose age was 46, 54, and 60 years old were diagnosed initially with ET. *Results.* The value of Hb was 15, respectively 16,5g/dl with Ht 44 respectively 46% for the 2 female cases and 17g/dl respectively 51% for male patients. 2 patients presented at diagnosis, leukocytes under 10.000/mm, only a case from the three of them presented more than 12.000/ mm^3. The spleen varied between 1, 5 and 2, 5 cm under the costal board. At the beginning, the value of Hb and Ht did not allow the diagnosis of PV and the high count of the platelets (650 0000, 74 0000 and 82 0000/mm^3) imposed the diagnosis of ET. The bone marrow examination was applied (after 2002) for only a patient, releasing on bone marrow biopsy: hypercellularity with hyperplasia of all marrow elements, with left deviation of the erythrocyte clusters, a polymorph megakaryocytic aspect, and that's why it was considered unclassified myeloproliferative disorder. The evolution of those three patients was in 8-18 months towards a classic PV with high levels of Hb and Ht who needed phlebotomy. *Conclusions.* At the point of prognostic and the therapeutically view of the integration in TE or PV has a low clinical importance taking into consideration that the similitude of the therapeutically approach for these 2 chronic diseases. Recently the difference becomes more important at point of molecular view (TH V617FC), even possible prognostic (ET after 10 years of evolution). The histological