Abstracts

Bone marrow Pathology

001 EXPRESSION OF CD86 IN ACUTE MYELOGENOUS LEUKEMIA IS A MARKER OF DENDRITIC/MONOCYTIC LINEAGE

D Rondelli, F Re, M Arpinati, N Testoni, P Ricci, C Terragna, P Preda[1], D Ruggeri, M Baccarani, S Tura. *Institute of Hematology and Medical Oncology "Seràgnoli"; [1]Division of Pathology, S. Orsola Hospital, University of Bologna, Italy*

Objective: To determine whether expression of the CD86 costimulatory molecule in acute myeloid leukemia (AML) may identify blast cells committed to the monocytic/dendritic lineage.

Methods: One-hundred-ten consecutive AML patients observed at diagnosis were studied by flow cytometry. In selected experiments, in-vitro cultures with $CD34^+CD86^+$ or $CD34^-CD86^+$ blasts were performed in the presence of granulocyte -macrophage colony stimulating factor (GM-CSF) ±tumour necrosis factor α (TNF-α) , or GM-CSF +interleukin 4 (IL-4), respectively, to induce a dendritic differentiation, documented by morphological and immunophenotypic assays. T cell alloreactivity to $CD86^+$ AML cells and leukemic dendritic cells (AML-DC) was tested in mixed leukocyte cultures and anti-leukemic cytotoxic assays.

Results: CD86 was expressed in 54% of cases of AML, while CD80 and CD1a were only occasionally positive. $CD86^+$ AML samples included M5 and M4, but also 47% M0-M1 FAB types, and were more frequently $CD14^+$ ($p<0.00001$) and $CD34^-$ ($p=0.00005$) than $CD86^-$ AML. Six different patterns of $CD86^+$ AML were identified, according to CD34 or CD14 total or partial coexpression. Four samples enriched in $CD34^+CD86^+$ AML cells differentiated into AML-DC $CD86^+$, $CD80^+$, $CD40^+$, $CD11c^+$, $HLA\text{-}DR^{++}$, $CD14^{+/-}$, that were also $CD1a^+$ or $CD83^+$, after 6 days of in-vitro culture with GM-CSF ±TNF-α $CD34^-CD86^+$ AML cells differentiated into AML-DC after 3–5 days (n=5 exps), and trisomy 8 was found in two AML and AML-DC samples by fluorescent in situ hybridisation (FISH) analysis. Finally, AML-DC induced more potent allo-T cell proliferation, cytokine release and anti-leukemic cytotoxicity than $CD86^+$ blasts.

Conclusion: In AML, CD86 is a marker of monocytic/dendritic lineage. Since $CD86^+$ blasts may differentiate into DC rapidly, $CD86^+$ AML patients might be optimal candidates for immunotherapy studies, both in autologous and in allogeneic settings.

002 CLINICAL IMPLICATIONS OF THE WHO CLASSIFICATION

K Foucar. *University of New Mexico, Department of Pathology, Albuquerque, NM, USA*

The newly proposed WHO classification system of hematolymphoid neoplasms is founded upon the delineation of lineage (myeloid, B, T, histiocyte, natural killer cell, etc.) and stage of maturation (precursor vs. mature cell of origin). Within each broad category distinct clinical pathologic entities are identified by the integration of clinical, morphology, immunophenotype and/or genotype. The large number of myeloid disorders are divided into mature (chronic myeloproliferative and myelodysplastic processes) and immature (acute myeloid leukemias). The subclassification of acute myeloid leukemias is particularly problematic because the large number of subtypes generated from a lineage based system of classification generally do not define distinct clinicopathologic entities. Indeed, genotype often is more useful than lineage assessment in defining distinct clinicopathologic subtypes of AML. However, when a strict "entity-based" genetic approach is applied, a large proportion of AML cases are unclassifiable. The resulting compromise for the classification of AML consists of using genotype to identify the four genetic subtypes of AML that are true clinicopathologic entities, while a lineage-based classification is applied to the remaining cases. A survey of oncologists/pathologists will be presented along with illustrative cases. A cost comparison of development of therapeutic agents, development of classification systems and clinical trials will be highlighted.

003 EXTRAMEDULLARY PRESENTATION OF ACUTE MYELOID LEUKEMIA

J Diebold. *Hôtel Dieu, 1 Place du Parvis Notre-Dame, 75181 PARIS Cedex 04, France*

Extramedullary accumulation of myeloblasts or immature myeloid cells realizes tumors which are called myeloid sarcoma in the WHO classification. Such tumours can develop in lymphoid organs, bone (skull, orbit, etc), skin, soft tissue, various mucosae and organs with glandular parenchyma, and the central nervous system (CNS). They may precede or occur concurrently with acute myeloid leukemia, reveal blastic transformation of chronic myeloproliferative disorders or myelodysplastic syndromes, or relapses in treated patients. The diffuse infiltrate is constituted by medium to large cells that are difficult to recognize. Imprints are very useful. Today, immunohistochemistry allows the diagnosis and distinguishs variants: granulocytic (MPO+, CD68+, lysozyme positive, CD34+/–), monoblastic (MPO–, CD68+, lysozyme positive, CD34–), myelomonoblastic (MPO+/–, CD68+, lysozyme +/–, CD34+/–) or megakaryoblastic (positivity for factor VIII, CD11, CD31). They also often express CD43, CD7, CD79a and CD56 (particularly monoblastic variant with a t(8;21)). The diagnosis is missed in about 50% of the cases in the absence of immunohistochemistry. The myeloid sarcomas should be treated as acute leukemia and they share their prognosis.

004 CONSIDERATIONS IN DIAGNOSIS OF MYELODYSPLASIA

A Orazi. *Indiana University School of Medicine, Indianapolis, Indiana, USA*

Myelodysplastic syndromes (MDS) are a clinically heterogeneous group of clonal hematopoietic disorders characterized by dysplastic changes in one or more myeloid cell lines that may occur as primary diseases or may follow toxic exposures or therapy. MDS have been traditionally subdivided according to the FAB system (1982) into five major categories which show different rates of progression to acute myeloid leukemia and overall survival. However, several of the FAB defined MDS groups are prognostically heterogeneous and a more comprehensive approach that takes into consideration other parameters (e.g. cytogenetics) has been developed by the WHO classification committees. The WHO system distinguishes the following subtypes of MDS:

- Refractory anemia (± ringed sideroblasts).
- Refractory cytopenia with multilineage dysplasia (± ringed sideroblasts).
- Refractory anemia with excess blasts (RAEB-1 and RAEB-2).
- Myelodysplastic syndrome, unclassifiable.
- MDS associated with isolated del (5q) chromosome abnormality ("5q-syndrome").

The morphological classification of MDS is principally based on the percentage of blasts in the BM and PB, the presence of ringed sideroblasts, and the type and degree of dysplasia. These parameters are usually assessed on bone marrow (BM) and peripheral blood smears. The presence of dysplasia is not, however, in itself evidence of a clonal marrow disorder, being observed in numerous other conditions (e.g. B12 and folate deficiencies, exposure to toxic factors or chemotherapy, viral infections, chronic autoimmune conditions). Problems are most commonly encountered in cases of low grade MDS in which only unilineage dysplasia and no increase in blasts are observed. Cytogenetic abnormalities that are found only in a proportion of MDS cases are helful in confirming a suspected diagnosis of MDS and yield prognostic information. Preliminary evidence has also shown a potential role for flow cytometric immunophenotyping in MDS cases. The value of bone marrow biopsy (BMB) in this group of disorders is generally well established. MDS is usually associated with

*Denotes that the author is the presenting author.

characteristic topographic alterations, among which an important finding is the presence of abnormally localized immature myeloid precursors (ALIP) in the central marrow cavities. ALIP positivity is mainly present in the aggressive MDS subtypes and is associated with poor prognosis. Presence of ALIP, however, is not unique to MDS and has been reported in reactive hematologic conditions. Immunohistochemistry (IHC) can be used to increase the diagnostic accuracy of BMB. Both an increase in the percentage of CD34 positive cells and a tendency of positive cells to form aggregates have been shown to be a reliable predictor of leukemic transformation and of poor survival in MDS cases, irrespective of their subtype. IHC can be used to confirm the presence of dysmegakaryopoiesis in MDS by identifying micromegakaryocytes and other abnormal forms and by showing anomalous antigenic expression in these cells. BMB analysis is especially important in three subsets of MDS: MDS with hypocellular marrow (MDS-h); MDS with fibrosis (MDS-f), and therapy-related MDS (TR-MDS). The presence of fatty changes and/or reticulin fibrosis in the BM of these patients, by causing hemodilution and poorly cellular aspirates, can make the disease characterization of BM smears very difficult or impossible and prevent adequate cytogenetics. IHC with antibody reactive with precursor cells and vascular endothelia can be helpful in distinguishing MDS-h from aplastic anemia and MDS-f from chronic idiopathic myelofibrosis, respectively. IHC for p53 protein can help in identifying cases of TR-MDS. IHC with endothelium markers allows assessment of microvessel density in MDS. Preliminary results have suggested a correlation between increased angiogenesis, aggressive MDS subtypes, and rate of progression to acute leukemia.

005 CLINICOPATHOLOGIC CORRELATIONS IN MYELODYSPLASTIC SYNDROMES

A Tichelli. *Hematology, University Hospitals, Basel, Switzerland*

Myelodysplastic syndromes (MDS) represent a large spectrum of clonal disorders characterized by peripheral cytopenia and an increased risk of transformation in acute myeloid leukemia (AML). The natural history of the MDS ranges from rather indolent forms of disease to diseases with a rapid evolution either to AML or severe pancytopenia. For optimal treatment decision, understanding of the pathophysiologic mechanism as well as knowledge of prognostic factors are mandatory. Part of the clinical manifestation of the MDS is due to the clonal stem cell disorder, leading to a progressive de-differentiation of myeloid lineage and ultimately unregulated blast proliferation. However, immunological mechanisms in the microenvironment contribute to the pancytopenia. The MDS transformed stem cell induces antigenic changes leading to an autoimmune T-cell response directed against the marrow. Both MDS and normal marrow cells are inhibited by cytotoxic T-cells causing various degrees of marrow failure. MDS cells may be more resistant to this immune attack and therefore have a selective growth advantage. The persistent autoimmune response results in overproduction of pro-apoptotic cytokines, which may contribute to dysplasia and ineffective hemopoiesis. In addition, the production of vascular endothelial growth factor by the MDS cells may stimulate angiogenesis, which could favor disease progression. Based on this knowledge, the following treatment options are offered to the patient. Allogeneic stem cell transplantation, which is the only curative treatment, should be applied in younger patients with a matched donor. Transplantation replaces the abnormal clonal stem cell and its consequent microenvironment defects with healthy marrow. The outcome is dependent on the stage of the disease, the age of the patient and the type of donor. For patients where transplantation is not available, the treatment strategy depends on the prognostic factors. Patients with predominantly cytopenic features might profit from immune modulators such as ATG, Cyclosporine or thalidomide, or the use of cytokines. In contrast, patients with primarily leukemic progression (high blast counts; cytogenetic anomalies) should be treated with intensive chemotherapy with or without autologous stem cell transplantation.

006 THE DIFFERENTIAL DIAGNOSIS OF EOSINOPHILIA

BJ Bain. *Department of Haematology, Imperial College Faculty of Medicine, London*

Often the clinical history makes the cause of eosinophilia readily evident. When this is not so, elucidating the cause can be difficult. It may require not only a detailed history (including a travel history and details of drug exposure), but also serology and other specific tests for various parasitic infections, examination of the peripheral blood film, bone marrow aspirate cytology, histology of a trephine biopsy specimen or other tissue biopsy, and cytogenetic, molecular genetic and immunophenotypic analysis. Even with the application of all these diagnostic modalities the cause may remain obscure. If the eosinophilia persists for 6 months, causes tissue damage and remains unexplained despite full investigation the designation "idiopathic hypereosinophilic syndrome" is arbitrarily applied. Some such patients actually represent chronic eosinophilic leukaemia but only the subsequent progress of the disease reveals this to be so. When eosinophilia is not readily explained, the diagnoses that the pathologist must consider include an occult T-cell lymphoma, systemic mastocytosis and eosinophilic leukaemia. A bone marrow aspirate and trephine biopsy may reveal a lymphoma or an increase of blasts cells, the latter leading to a diagnosis of eosinophilic leukaemia. A trephine biopsy and, less often, a bone marrow aspirate may reveal systemic mastocytosis. However, immunophenotyping and T-cell receptor analysis may reveal a clonal proliferation of T lymphocytes, even when there is no overt cytological or histological evidence of a lymphoma. Similarly, cytogenetic analysis may provide evidence of eosinophilic leukaemia even in patients with no increase of blast cells. Cytogenetic analysis is of considerable importance for the diagnosis of several uncommon cytogenetically definable subtypes of chronic eosinophilic leukaemia, the diagnosis of which has prognostic and therapeutic importance. Patients with t(8;13)(p11;q12) and variant translocations have such a poor prognosis that stem cell transplantation is generally indicated whereas those with t(5;12)(q33;p13) may respond to imatinib mesylate.

007 PROGNOSTIC FACTORS IN PH-NEGATIVE CHRONIC MYELO-PROLIFERATIVE DISORDERS: A CLINICOPATHOLOGICAL STUDY ON 1034 PATIENTS

*HM Kvasnicka, J Thiele. *Institute of Pathology, University of Cologne, Germany*

Background: A wealth of data has accumulated in recent years about the considerable heterogeneity of survival patterns in Ph-negative MPDs applying univariate and multivariate evaluation methods to identify independent prognostic features. However, this large body of prognostic data has been partly obscured by ill-defined series with different diagnostic criteria.

Patients and Methods: A total of 1,034 patients with a clinically as well as histological established diagnosis of idiopathic myelofibrosis (IMF, n=676), polycythemia vera (PV, n=218) and essential thrombocythemia (ET, n=140) were enrolled in this observational study with a mean follow-up period of 6.2 years. Contrasting with other studies, the full spectrum of disease stages of IMF was included, starting from early, prefibrotic cases to advanced full-blown stages. In extension of the PVSG criteria for ET, diagnosis of this subtype was performed according to the new WHO criteria.

Results: Regarding the three considered subtypes of MPDs, a disease-specific loss of life expectancy ranging from 8% to 38% could be calculated. Impact of disease was significantly higher in elderly patients, especially in IMF and PV. However, the ET group reveals no relevant reduction of life expectancy. Early prefibrotic stages of IMF showed a more favorable outcome than advanced stages of disease. On the other hand, in ET and PV features of bone marrow histology did not contribute to the patient's prognosis. Multivariate risk stratification revealed age, degree of anemia, leukocytes and platelet counts as the most important parameters in IMF. Risk classification of PV included age at diagnosis, liver size and also leukocyte counts as important clinical predictors. Prognosis of ET was only influenced by the occurrence of thromboembolic and hemorrhagic episodes.

Conclusions: Our results are in keeping with the assumption that hematological features signaling bone marrow insufficiency have a prodigious impact on survival in IMF and PV. Generalization of disease indicated by myeloid metaplasia can occur at every stage, even in so-called hypercellular phases of IMF. On the other hand, risk status of ET patients depends mainly on secondary complications of disease.

008 TUMORS OF HISTIOCYTES AND ACCESSORY DENDRITIC CELLS. A PROPOSED CLASSIFICATION FROM THE INTERNATIONAL LYMPHOMA STUDY GROUP (ILSG)

SA Pileri, TM Grogan[1], on behalf of ILSG *Department of Pathology, Bologna University, Italy; [1]Arizona University, Tucson, Arizona, USA*

Background: Neoplasms of histiocytes and putative immune accessory cells (dendritic cells) are rare, and co-operative studies

based on large series of cases are lacking. The ILSG collected and reviewed 61 of these tumours.

Results: Four major groups were defined based primarily on immunophenotype: these were further refined by histologic and electron-microscopic examination. Eighteen neoplasms were CD68$^+$, lysozyme$^+$, S-100 protein$^{+/-}$, CD45+/$^-$, CD1a$^-$, and FDC antigen$^-$, consistent with histiocytic tumors (HT); all were composed of large, polygonal cells with moderate cellular pleomorphism. Three were nodal, 2 nodal and extranodal, 10 extranodal, and 3 systemic. The mean age of the patients was 43.6 years (6 months to 74 years); of 12 patients with follow-up, only 3 were disease-free and 7 were dead of disease. Twenty-six cases were CD1a$^+$, S-100 protein$^+$, CD68$^{+/-}$, lysozyme$^{+/-}$, CD45$^{+/-}$, and FDC antigen$^-$; all had morphologic features consistent with Langerhans cell tumors (LCT), with convoluted nuclear membranes and abundant, pale cytoplasm, and 8/11 cases with EM had Birbeck granules. Eleven cases had little or no nuclear atypia (LCT.A/typical); six had conspicuous nucleoli, more cellular pleomorphism, and higher mitotic activity (LCT.B/atypical); nine had large, pleomorphic cells with frankly malignant cytologic features (LCT.C/LC sarcoma). All 3 types had a broad age range and included children. However, the mean age of patients with LCT.A, B and C differed, being 11.2 years (2 days to 62 years), 29.8 years (2–72 years) and 40.1 years (10–72 years), respectively, as did the incidence of stages C and D (50% in LCT.A, 83% in LCT.B, and 89% in LCT.C). In all the 3 groups, extranodal and systemic presentations were frequent, while the purely nodal ones were rare (1/11, 0/6, and 2/9, respectively). Four of 8 LCT.C patients with follow-up available died of their disease; among patients with LCT.A and B only 4/13 died: one of a metastatic adenocarcinoma and 3 of disseminated disease: the latter had LCT.A, but with the typical presentation of Letterer-Siwe disease. Seventeen cases lacked CD1a and showed variable expression of CD68 and S-100 protein. All 17 cases were composed predominantly of spindle and fusiform cells, with a whorled or storiform pattern and an admixture of lymphocytes. On the basis of the immunohistochemical and ultrastructural findings, they were further subdivided into two groups. Thirteen cases were regarded as follicular dendritic cell tumors (FDCT)/sarcomas, since they all expressed FDC-associated antigens and 8/9 with EM had desmosomes. Four were considered as putative interdigitating dendritic cell tumors (IDCT)/sarcomas, as they lacked FDC antigens, were S-100 protein$^+$, and on EM (performed in two cases) showed complex interdigitating cellular junctions. All FDCT patients were adults (mean 58.6 years, range 40–90) with nodal (6), extranodal (6) or systemic (1) disease; 7/11 subjects with follow-up were disease-free; 3/11 were alive with disease and 1 was dead of disease. IDCT patients were also adults (mean 72 years, range 60–83) with usual nodal involvement; both the individuals with follow-up available were alive and in complete remission.

Conclusions: Neoplasms of histiocytes and accessory cells can be divided into four main groups, using a combination of immunophenotype, light and electron microscopy: histiocytic tumors, Langerhans cell tumors, follicular dendritic cell tumors, and interdigitating dendritic cell tumors. Histiocytic tumors appear to be highly malignant, while dendritic cell tumors appear to be more indolent, although they may recur or cause death.

009 ESSENTIAL THROMBOCYTHEMIA WITH RINGED SIDEROBLASTS: A HETEROGENEOUS SPECTRUM OF DISEASES, BUT NOT A DISTINCT ENTITY

*A Schmitt-Gräff, J Thiele[1], I Zuk, HM Kvasnicka[1]. *Institute of Pathology, University of Freiburg, Germany; [1]Institute of Pathology, University of Cologne, Germany*

Background: According to the WHO-classification essential thrombocythemia with ringed sideroblasts (ET/RS) still remains an ambiguous category which may be considered as myelodysplastic/myeloproliferative disease, unclassifiable.

Purpose: Because until now only case reports or very small series of patients were described we performed a retrospective evaluation of 38 patients with the diagnosis of ET/RS and ⩾15% ringed sideroblats on smears and bone marrow biopsies.

Results: Based on cytological and histological bone marrow findings three different patterns could be determined which were associated with disparate clinical features and in particular prognosis. Group I included six patients who were consistent with ET, group II comprised 21 patients revealing findings of prefibrotic and early fibrotic chronic idiopathic myelofibrosis (CIMF) and 11 patients (group III) with myelodysplastic syndromes (MDS). Follow-up studies revealed that no patient with ET showed a fiber increase but 8 CIMF patients developed overt myelofibrosis and 4 of the MDS group evolved into AML. In comparision with a control group of 40 patients with (true) ET prognosis was significantly different by displaying a median survival of 100 months (true ET 170 months).

Conclusion: Ringed sideroblasts are not a pathognomic feature of MDS, but probably indicate a disturbance in iron metabolism in a variety of disorders. For this reason, a more accurate classification of so-called ET/RS is warranted by evaluation of smears and in particular bone marrow biopsies. By following the WHO-criteria these patients belong to either ET, CIMF or MDS and show a significantly different survival pattern.

010 EXPRESSION OF FIBROGENIC CYTOKINES IN BONE MARROW CELLS FROM CHRONIC MYELOPROLIFERATIVE DISEASES

*O Bock, J Schlué, D Reising, H Kreipe. *Institute of Pathology, Medizinische Hochschule Hannover, Carl-Neuberg-Strasse 1, 30625 Hannover, Germany*

Chronic myeloproliferative diseases (CMPD) comprise heterogenous stem cell diseases that differ in their potential to evolve to blast crisis or development of bone marrow fibrosis. In order to elucidate whether the different entities vary in the production of fibrogenic factors, bone marrow cells of CMPD patients from either total trephines or laser-microdissected cell populations were analyzed for the mRNA expression of basic fibroblast growth factor (bFGF) and transforming growth factor beta-1 (TGFbeta-1) using quantitative real-time PCR. mRNA levels of fibrogenic factors in CMPD were correlated with the expression in total bone marrow cells of reactive bone marrow trephines. Using immunocytochemistry, the distribution of fibrogenic factors was correlated with the molecular levels in CMPD and reactive bone marrow. For bFGF, highest levels of transcript were found in idiopathic myelofibrosis with demonstrable fiber increase and in essential thrombocythemia. Cases of polycythemia vera and idiopathic myelofibrosis in the cellular phase exhibited lower transcript levels, but significantly higher levels than reactive bone marrow. Compared with other CMPD and reactive bone marrow, cases with chronic myeloid leukemia exhibited the lowest level of bFGF. In contrast, the chronic myeloid leukemia exhibited significant higher levels of TGFbeta-1, compared with other CMPD entities and the reactive bone marrow. In total bone marrow cells of patients with essential thrombocythemia, the lowest levels of TGFbeta-1 were found. Cellular phase of idiopathic myelofibrosis differed from essential thrombocythemia in an enhanced expression of both cytokine mRNAs, bFGF as well as TGFbeta-1. In laser-microdissected megakaryocytes, highest levels of bFGF were found in idiopathic myelofibrosis. Taken together, quantification of fibrogenic cytokine transcripts gives new insights into the pathogenesis of fibrosing CMPD and can contribute to a more accurate subtyping.

011 LAT (LINKER FOR ACTIVATION OF T CELLS): A USEFUL MARKER FOR MEGAKARYOCYTE EVALUATION ON BONE MARROW BIOPSIES

*M Ungari, W Pellegrini, E Borlenghi, D Marocolo, C Agazzi, F Facchetti. *Departments of Pathology and Hematology, University of Brescia, Spedali Civili, Brescia, Italy*

Immunohistochemistry improves the evaluation of bone marrow biopsies (BMB); in particular, detection of atypical megakaryocytes (MKS) in myelodysplastic syndromes (MDS), chronic myeloproliferative disorders (CMPD) and acute leukemias is facilitated by anti-MKS markers such as von Willebrand Factor (vWF) and CD61, the former being considered the most reliable (*Am J Clin Pathol* 2000;113:506). Recently, LAT (linker for activation of T cells), a molecule involved in T-cell activation and platelet aggregation, was found to stain MKS and platelets on tissue sections (*Am J Pathol* 1999;154:1037). We compared vWF and LAT immunoreactivity on MKS in 60 BMB, including normal controls (NC; 12), MDS (18), CMPD (21) and acute megakaryoblastic leukemia (AMKSL; 9). Immunostaining was performed on paraffin sections with polyclonal antibodies anti-vWF and LAT; immunoreactivity was evaluated by counting positive MKS in 10 cellular HPF and values compared using the Student's t-test. In all cases, no significant differences were noticed between the mean values of vWF+/LAT+ MKS (NC: 44,50/47,00; MDS: 115,22/114,61; CMPD: 139,71/148,62; AMKSL: 379,89/529,44). However, in 20 cases (5 NC; 5 MDS; 6 CMPD; 4 AMKSL) the number of LAT+ MKS was at least 30% higher than vWF+ MKS, while in 3 cases the opposite was found. In 2 AMKSL cases, LAT identified very numerous MKS, but vWF was practically negative; furthermore, in 5 AMKSL cases LAT labelled MKS much stronger than vWF, whereas in most BMB the

immunoreactivity was quite similar. LAT and vWF are good immunohistochemical markers for MKS in normal and pathological conditions; however, LAT seems to identify a higher number of MKS in most cases and, particularly, in AMKSL; this might be related to an altered functional status of neoplastic MKS in this condition. We would recommend the use of LAT in the study of BMB in cases of hematopoietic disorders.

012 LEUKEMIC CELLS OF ADULT T-CELL LEUKEMIA/ LYMPHOMA (ATLL) EXPRESS HTLV-1 TAX PROTEIN IN THEIR ENTERING RE-PROLIFERATION

*K Hasui, A Utsunomiya[1], XS Jia[2], T Hayata[3], F Murata, E Sato. *Kagoshima University, Kagoshima, Japan; [1]Imamura Bun-in Hospital, Kagoshima, Japan; [2]China Medical University, Shenyang, China; [3]Kagoshima Women's Junior College, Kagoshima, Japan*

ATLL is a human T cell leukemia virus (HTLV-1)-related T-cell neoplasm. It has been clarified that HTLV-1 Tax protein (Tax) plays a role in ATLL-oncogenesis. But leukemic cells of ATLL have been found not to express Tax. We succeeded in detecting Tax protein in lymphoma cells in many cases of ATLL by means of modified ImmunoMax employing anti-Tax rat monoclonal antibody supplied by Prof. Tanaka Y (Ryukyu University, Okinawa, Japan). This study investigated whether ATLL leukemic cells expressed Tax or not, by means of the modified ImmunoMax applied to paraffin-embedded tissue sections of naturally sedimented and coagulated peripheral blood (peripheral blood tissue specimen: PBTS) including leukemic cells in five cases of acute type and four cases of chronic type ATLL. Immunological phenotype of leukemia ATLL cells and the other blood cells could be labeled in the sections of the PBTS. Labeling proliferating cells in the leukemic cells in the PBTS sections by anti-Ki-67 antigen antibody, there were cases with high and low rates of proliferating cells. In the both the acute and chronic type of ATLL, the expression of Tax in the leukemic cells paralleled the proliferation of ATLL leukemic cells. Consequently, it was suggested that ATLL leukemic cells expressed Tax when proliferating. PBTS is thought to be a new material for hematopathology, because the re-proliferation capacity of leukemic cells could be evaluated by Ki-67 labeling in the PBTS.

013 DIFFUSE PATTERN OF BONE MARROW INVOLVEMENT IN SPLENIC MARGINAL ZONE LYMPHOMA

*H Abd El, M Hassan[1], M Taha[2]. *Pathology Dept, Faculty of Medicine, Suez Canal University, Ismailia, Egypt; [1]Central Laboratory, Maadi Armed Forces, Cairo, Egypt; [2]Head Central Laboratory, Maadi Armed Forces, Cairo, Egypt*

A 52 year old male patient presented with severe anemia and splenomegaly. Bone marrow aspirate showed atypical lymphoid cells. The marrow trephine biopsy revealed a diffuse interstitial infiltrate formed of medium sized cells with rounded slightly irregular nuclei and abundant cytoplasm, a pattern identical to a hairy cell leukemia (HCL) infiltrate. There were no intra-sinusoidal lymphoid cells. Immunostaining highlighted the B cell nature of the cells, CD20+, CD79a+. HBA44 and CD103 were negative as well as CD5, CD23 and cyclin D1. Peripheral blood examination showed the presence of villous lymphocytes. This report describes an unusual case of diffuse pattern of infiltration of the bone marrow in splenic marginal zone lymphoma (SMZL) and emphasises the importance of complete clinical and laboratory data in the differential diagnosis between HCL and SMZL.

014 PATTERNS OF MINIMAL RESIDUAL DISEASE IN BONE MARROW BIOPSIES AFTER 2CDA FOR HAIRY CELL LEUKEMIA USING IMMUNOHISTOCHEMISTRY

Swiss group for clinical cancer research (SAKK); P Mhawech, M Oberholzer[1], S Aschenafi[2], AS Baur[3], M Kurrer[4], A Von Rohr[5], S-F Hsu-Schmitz[6], B Wagner[2], F Delacretaz[3], N Hurwitz[1]. *Institute of Pathology, University of Geneva, Switzerland; [1]Institute of Pathology, University of Basel, Switzerland; [2]Institute of Pathology, University of Luzern, Switzerland; [3]Institute of Pathology, University of Lausanne, Switzerland; [4]Institute of Pathology, University of Zürich, Switzerland; [5]Institute of Pathology, University of Hirslanden Onkozentrum; Switzerland; [6]Coordination Center of the SAKK, Switzerland*

Background: Immunohistochemistry (IHC) is an accepted method to detect minimal residual disease (MRD) in hairy cell leukemia (HCL) after treatment. No correlation between level of MRD and outcome has been attempted hitherto.

Purposes: (1) Finding a method for MRD quantification; (2) comparing the reliability of IHC in detecting MRD with other methods; (3) correlating the level of MRD with clinical data.

Methods: 107 follow-up bone marrow biopsies (BMB) from 18 patients (pt), 6,44 BMB/pt mean, were incubated with CD20 and CD72. Morphologically unequivocal hairy cells (HC) CD20/CD72+/ mm^2 were counted and expressed as % of nucleated cells (NC). The mean follow-up was 47.16 month (m). Depending on % of HC, 3 groups of MRD were defined, I: <1%, II: 1–5%, III: >5%. Tumor loads detected by IHC, flow cytometry (FC) and aspirate were compared.

Results: Group I (n=7): all pt were in complete remission (CR) at a mean follow-up of 62 m. Group II (n=6): 3/6 were in CR at a mean of 62m, 2/6 had active disease (AD) at 34 and 39 m, 1/6 relapsed at 49 m. Group III (n=5), 3/5 had AD at a mean of 20 m, 2/5 relapsed at 27 and 39m.

Conclusion: (1) IHC detected the highest tumor load in most cases. (2) A correlation between the level of MRD detected by IHC could be demonstrated. (3) The proposed classification into 3 groups may be used for standardization of MRD and may be useful to identify patients at risk for relapse. These results should be verified on a larger series.

015 MYELOID SARCOMA OR CHLOROMA OF THE INTESTINE AND THE TESTES

A Janssens, P De Paepe[1], J Philippé[2], F Offner, L Noens. *Department of Hematology, [1]Department of Pathology, and [2]Laboratory of Hematology, Ghent University, Belgium*

Myeloid sarcoma (MS) or chloroma is a tumor mass of myeloblasts or myeloid precursors occurring in an extramedullary site. It occurs in patients with acute myeloid leukemia (AML), chronic myeloproliferative disorders or myelodysplasia in transformation. Chloromas are most frequently seen at the time of diagnosis of the bone marrow disorder or at relapse. When the tumor mass precedes the myeloid disease the diagnosis may be difficult.

Case 1: A 51 year old male was admitted with symptoms of small bowel obstruction. Laparotomy revealed an infiltrative mass obstructing the jejunum. A partial enterectomy was performed. The diagnosis of enteropathy-associated multifocal T-cell lymphoma was made. Staging showed no other nodal or extranodal invasion. The patient was referred to our institution and treated with 6 cycles of CHOP chemotherapy. Fourteen months after diagnosis, the patient presented with abdominal pain and signs of subobstruction; a CT scan showed diffuse thickening of the peritoneum. The cytological examination of the biopsy imprints showed a blastic population with moderately vacuolated basophilic cytoplasm and some azurophylic granules. The granules showed positive for myeloperoxidase (MPO) and flow cytometry confirmed the myeloblastic nature (CD34, CD13, CD45, DR positive). Histology showed a diffuse proliferation of blastic cells with a weak immunoreactivity for CD45 and MPO. There was no reactivity for T-cell markers. The initial biopsy specimens were reviewed and re-diagnosed as MS. No bone marrow or peripheral blood invasion could be documented. The patient is currently treated according to an AML protocol.

Case 2: A 71 year old male was admitted with a tumor of the testis. An orchidectomy was performed. The diagnosis of a T-cell lymphoma was made. Staging showed no nodal or other extranodal spread. CHOP chemotherapy was given for 6 cycles. A new retroperitoneal mass was seen at restaging. Biopsies taken during laparotomy seemed to be infiltrated by the known T-cell lymphoma. At the same time the diagnosis of an acute myeloid leukemia was made. The bone marrow aspirate revealed 50% myeloblasts with some Auer rods and the peripheral blood showed a leukocytosis of 24000/µl due to 86% myeloblasts. The patient was referred to our institution with the diagnosis of a chemotherapy-induced AML. Review of the initial biopsies of the testis showed blastic cells positive for MPO, CD68, CLA and negative for B- and T-cell markers. Re-diagnosis of MS was made. Therapy was started according to an AML protocol.

Conclusion: Myeloid sarcoma or chloroma can precede the diagnosis of the hematological disorder by several months. Although any extramedullary tissue can be invaded, infiltration of the intestine and the testes especially by granulocytic precursors is infrequent. In these cases an erroneous diagnosis of lymphoma is often made. Therefore histological examination of adequately fixed material and cytological morphology together with histochemistry and cytochemistry and flow cytometry are necessary to diagnose this rare entity.

016 ERYTHROID CHANGES IN BONE MARROW TREPHINES IN HAIRY CELL LEUKAEMIA

PW Johnston, C Roberts, RJ Thomson, SMTW Maung. *Dept of Pathology, Aberdeen Royal Infirmary, Foresterhill, Aberdeen, UK*

Infiltration of the marrow in hairy cell leukaemia (HCL) can be associated with erythroid changes that attract comment but little attention. This study set out to look for disordered erythroid growth and hyperplasia in HCL. We examined retrospectively pre-treatment bone marrow trephine biopsies from 12 patients with HCL. We compared them with biopsies from age and gender matched controls. Biopsies were stained with H&E, Giemsa and reticulin, glycophorin A and C and CD20 (n=8 for immunocytochemistry and quantification). Clinical and laboratory data were reviewed. Sections were quantified using a systematic random point counting method to produce relative volumes (RV) of tissue components. Group data were expressed as medians and compared using a Mann Whitney-U test (significance was $p < 0.02$ to take account of repeated tests).

HCL patients were anaemic at diagnosis (haemaglobin (Hb) 97 g/l, control 129 g/l; p=0.0072). The cellularity of HCL trephines was increased (RV cells 61%, control 33%; $p<0.02$). Red cell dyshaemopoiesis was present in all assessed HCL trephines and absent from controls. For glycophorin A the control RV was 3.7% and for glycophorin C 6.1%. Five of the 8 HCL trephines showed an RV of glycophorin A staining greater than the control group and 2 of these also showed increased RV of glycophorin C positive cells. CD 20 staining supported the presence of substantial numbers of hairy cells in the HCL group. Reticulin staining was increased in the HCL group.

This preliminary study indicates that all our cases of HCL show anaemia and dyshaemopoiesis at diagnosis. Quantification of glycophorin staining suggests the majority of HCL trephines show erythroid hypoplasia and a smaller group exhibit hyperplasia. In conclusion, these observations may relate to a genuine increase in erythroid volume in HCL. Alternatively, the findings may indicate abnormal aggregation of erythroid cells in HCL detected as increased relative volumes in these biopsies.

017 DISCORDANT BONE MARROW INVOLVEMENT IN DIFFUSE LARGE B-CELL LYMPHOMA: MOLECULAR ANALYSIS OF MICRODISSECTED BONE MARROW INFILTATES REVEALS A HETEROGENEOUS GROUP OF DISORDERS

M Kremer, M Spitzer, S Mandl-Weber, K Stecker, L Quintanilla-Martinez[1], F Fend. *Institute of Pathology, Technical University, Munich, Germany; [1]Institute of Pathology, GSF, Neuherberg, Germany*

Background: Discordant morphology between lymph node (LN) and bone marrow (BM) infiltrates occurs in up to 30% of patients with diffuse large B-cell lymphoma. Patients with small cell infiltrates in the BM show a prognosis similar to patients without BM involvement. Molecular data concerning the relationship between the two populations are sparse. The aim of this study was to determine whether the comparative analysis of immunglobulin gene (IgH) and bcl-2 rearrangements of LN biopsies and microdissected BM infiltrates could elucidate the clonal relationship of discordant lymphoma manifestations.

Design: Twenty-one cases of high grade B cell non-Hodgkin's lymphoma (B-NHL) with morphologically discordant BM infiltrates were analyzed. Immunhistochemistry was performed on paraffin sections using: CD3, CD10, CD20, BCL-2, BCL-6, p53 and MIB1. The presence of clonal IgH gene and bcl-2 rearrangements was investigated by PCR in LN and microdissected BM specimens. Adequate monoclonal and polyclonal control tissues were included.

Results: In 18 of 21 cases the morphology was suspicious of a malignant infiltrate (paratrabecular infiltrate, monomorphous B-cell infitrate), whereas in 3/21 cases the morphology was compatible with a benign lymphoid infiltrate. In 8 of the 18 cases (44%) with suspicious morphology, clonal identity of LN and BM infiltrate was confirmed by IgH and/or bcl-2 PCR. In two cases (11%) divergent clones proven by sequence analysis were identified. In 4/18 cases (22%), rearrangement analysis of the BM samples revealed a reproducible polyclonal pattern despite the suspicious morphology and the presence of a clonal band in the parallel LN biopsies. The remaining 4 cases were technically uninterpretable. In the three cases with morphologically benign lymphoid infiltrates, a polyclonal pattern was always identified in the BM.

Conclusions: Discordant BM infiltrates in patients with high grade B-NHL represent a heterogeneous group. Although they frequently are a manifestation of the primary nodal lymphoma, they may also represent a second, unrelated neoplasm. The reason(s) for the reproducible polyclonality of the BM infiltrates in the 4 cases with suspicious morphology remains to be clarified.

018 DYNAMICS OF CD34$^+$ PROGENITOR CELLS FOLLOWING ALLOGENEIC BONE MARROW TRANSPLANTATION IN PH^{1+}CML

*HM Kvasnicka, J Thiele, DW Beelen[1], UW Schaefer[1]. *Institute of Pathology, University of Cologne, Cologne, Germany; [1]Department of Bone Marrow Transplantation, University of Essen, Essen, Germany*

Background: Following bone marrow transplantation in Ph^{1+} chronic myeloid leukemia (CML) reconstitution of hematopoiesis confirmed by the normalization of blood parameters and cellularity includes a homing into an appropriate microenvironment with expansion of CD34$^+$ progenitor cells and their differentiation into mature elements.

Patients/Methods: A retrospective immunohistological and morphometric study was performed on bone marrow trephine (BM) biopsies derived from 113 patients with Ph^{1+}-CML before and at standardized intervals following allogeneic transplantation (BMT) with full unmanipulated BM specimens. We investigated the numbers of CD34$^+$ progenitor cells and determined their dynamics during the posttransplant period. Moreover, we tried to correlate their number with corresponding changes in the amount of nucleated erythroid precursors and megakaryocytes including prokaryoblasts and megakaryoblasts and the fiber content.

Results: Monitoring the quantity of these precursors after BMT revealed a very rapid recovery in comparison to a control group. However, a more detailed evaluation showed that at day 22 ±6 a higher number of progenitor cells was significantly associated with an earlier independence for platelet transfusion and also with a more pronounced growth of erythro- and megakaryopoiesis including precursor cells. Furthermore, a slight increase in the density of the fibrous matrix (reticulin fibers) was present in these patients with a more favorable engraftment. The latter feature sheds some light on the complex pathomechanisms of homing and differentiation of progenitors. In confirmation with in-vitro findings this phenomenon is dependent on proper anchoring sites to the fibrous bone marrow stroma. Finally, size of full BM graft exerted a distinctive influence on the number of CD34$^+$ precursors in the early posttransplant period.

Conclusion: The present study has validated a number of BM features by focusing on the CD34$^+$ progenitor cells and associated hematopoietic reconstitution including reticulin fibers and precursor cells of the erythroid and megakaryocyte lineage, which are not readily evaluable by FACS analysis.

019 HETEROGENEOUS METHYLATION PATTERNS OF THE P15INK4B GENE IN CHRONIC MYELOMONOCYTIC LEUKAEMIA (CMML)

*U Lehmann, M Tessema, F Länger, H Kreipe. *Institute of Pathology, Medizinische Hochschule Hannover, D 30625 Hannover, Germany*

Chronic myelomonocytic leukaemia (CMML) is a stem cell disorder of the bone marrow with a high incidence of blast transformation, which belongs to the category of myelodysplastic/myeloproliferative diseases according to the new WHO classification.

For the myelodysplastic syndrome (MDS) an aberrant methylation of the tumour suppressor gene p15INK4B has been shown. Concerning CMML there are so far only very limited data available regarding the epigenetic inactivation of this important cell cycle regulator.

Different methylation specific PCR (MSP) and genomic sequencing protocols were employed for the analysis of the promoter methylation of p15INK4B to establish a reliable methylation assay. The analysis of genomic DNA extracted from formalin-fixed paraffin-embedded bone marrow trephines from a series of CMML patients (n = 25) enabled a direct comparison with the morphological features of the respective biopsies and the clinical data.

It turned out that hypermethylation of the CpG-island in the p15INK4B gene is a frequent event in CMML (88%). However the methylation patterns in different patient samples are quite heterogeneous.

The functional consequences of the hypermethylation of the p15INK4B gene concerning the gene expression were elucidated by measuring mRNA transcript levels using quantitative real-time PCR technology.

The analysis of aberrant methylation was performed to determine its discriminatory value regarding CMML and other hyperproliferative bone marrow changes as well as its predictive power regarding the occurrence of blast crisis. The methylation incidences and patterns

were studied in comparison to normal bone marrow biopsies and trephines displaying reactive changes as well as to chronic myelogenous leukemia (CML) samples.

020 BONE MARROW MORPHOLOGY IN CML PATIENTS DURING TREATMENT WITH STI571 (GLIVEC'): EVIDENCE OF COMPLETE MORPHOLOGICAL REMISSION AND CORRELATION TO HEMATOLOGIC AND CYTOGENETIC RESPONSE

*A Lugli, M Ebnöther[1], A Tichelli[1], A Gratwohl[1], A Zimpfer, S Cogliatti[2], M Linn[3], S Dirnhofer. *Departments of Pathology and [1]Hematology, University of Basel, Switzerland; [2]Department of Pathology, Kantonsspital St Gallen, Switzerland; [3]Novartis Pharma Schweiz AG, Bern, Switzerland*

Background: The recently developed STI571 (Glivec', Novartis) has been shown to be an effective treatment for chronic myelogenous leukemia (CML), but there is little information about bone marrow morphology under STI571 treatment.

Purpose: The aim of our study was to analyze morphologically and cytogenetically bone marrow biopsies and aspirates of 31 CML patients before and during treatment (week 13 and 25) with STI571.

Results: At week 13, normocellularity or hypocellularity was observed in 92% of patients in chronic phase (group 1) and in 64% of patients in accelerated phase/blast crisis (group 2). The M:E-ratio and the megakaryocyte number was markedly reduced in both groups (78% and 57%, respectively). "CML-megakaryocytes" could still be found in 39% of all patients. Cytomorphologically, pseudo-Gaucher cells were found in 11% in both groups. In group 1, cytogenetic response occurred in 7 patients (2 complete (CR), 3 major (MR), 1 minor (mR) and 1 minimal response (minR)) and in group 2 in 4 patients (3 CR and 1 minR). At week 25, 88% of group 2 patients showed a normalization of cellularity. The M:E-ratio was now reduced in 86% and the megakaryocyte number in 57% of all patients. The presence of "CML-megakaryocytes" increased in both groups. Pseudo-Gaucher cells were observed in 17% in group 2 and in no patients in group 1. Cytogenetic response occurred in 7 patients in group 1 (4 CR, 1 MR, 1mR and 1 minR) and in 2 patients in group 2 (2 CR).

Conclusions: STI571 induces a complete morphological remission in the majority of CML patients. The only morphologic evidence for biological persistence of the disease are "CML-megakaryocytes" and/or pseudo-Gaucher cells. Only 17% of patients show no morphological response.

21 A NEW ANTI-HEMOGLOBIN F (HBF) ANTIBODY AGAINST SYNTHETIC PEPTIDES FOR THE DETECTION OF F-CELL PRECURSORS (F-BLASTS) IN BONE MARROW AND ITS APPLICATION FOR MDS DIAGNOSIS

*M Ito. *Department of Pathology, Nagoya University Hospital, Nagoya 466–8560, Japan*

Aim: We generated a new polyclonal antibody specific to hemoglobin F (HbF) and examined bone marrow materials to evaluate the usefulness of HbF immunostaining for myelodysplastic syndrome (MDS) diagnosis. We investigated an incidence of F-blasts of MDS cases and relationship between F-blast/F-cell (Fb/Fc) ratio and apoptosis.

Materials/Methods: Formalin fixed, paraffin embedded bone marrow clot materials were used. HbF polyclonal antibody was generated by immunization of a rabbit with synthetic peptides of human HbF. Specificity and reactivity were confirmed by ELISA and immunohistochemistry. Anti-CD34, single-stranded DNA (ssDNA), HbF, HbA and glycophorin C were immunostained by using streptavidin biotin method.

Results: ELISA confirmed that the antibody showed strong immunoreactivity to HbF without reaction to HbA. There were positive reactions to fetal erythroblasts and to erythroblasts from patients with MDS and erythroleukemia but no reactions to normal adult erythroblasts. Marked increase of F-blasts in bone marrow was identified in 116 of 137 MDS patients, and F-cells were elevated in 54 cases. Among glycophorin C+ erythroblasts, mean proportions of F-blasts in MDS were significantly higher than for non-MDS patients with stress erythropoiesis, although there were no significant differences in number of F-cells. HbF expression was relatively predominant in immature erythroblasts or dysplastic erythroid precursors. The apoptotic rate was significantly higher in the patients with Fb/Fc ratio > 5.0 than with Fb/Fc ratio <1.0.

Conclusion: F-blasts are incapable of maturation to functional end-stage F-cells presumably owing to apoptosis. The measurement of F-blasts in bone marrow is needed for the exact evaluation of fetal-type erythropoiesis in MDS. This new antibody will be a useful tool for studying HbF synthesis and detecting F-blasts.

022 VLA AND SELECTIN ANTIGEN EXPRESSION IN ACUTE MYELOGENOUS LEUKEMIA

D Praprotnik, P Kotylo, C Orschell-Traycoff, A Sarker, R Hromas, A Orazi. *Indiana University School of Medicine/Clarian Health Partners, Indianapolis, IN, USA*

Background: The leukocyte adhesion glycoproteins are important regulators of cellular interactions with the extracellular matrix (endothelium, bone marrow stroma) and cell signaling. The clinical significance and prognostic importance of adhesion receptor antigen expression in leukemic blasts is unknown at this time.

Design: VLA (CD49d and CD49e) and selectin (CD62L) antigen expression on blasts were evaluated using multiparameter flow cytometry in 5 adults and 3 children with acute myelogenous leukemia (AML), including M0, M1, M2, M4, M5 and mixed lineage leukemia (MLL). We correlated these results with clinicopathologic data, including peripheral blood white cell count (WBC), numbers of circulating blasts, cytogenetics, extramedullary disease, remission status and clinical outcome.

Results: The results are summarized in the table. The expression of CD62L was low in 7/8 cases of AML and high in the case of one individual with AML-M1 who died 2 months after diagnosis. The expression of CD49d was variable and did not show any correlation with the peripheral blood white cell count and the number of circulating blasts. A patient with AML-M0 with the lowest CD49d expression died 14 months after diagnosis. Three other patients with higher CD49d achieved complete remission, two of whom are alive without disease.

CD49e expression was variable and showed no correlation with peripheral white counts, remission status, or circulating blast number.

Conclusion: This pilot study of AML showed variable expression of CD62L, CD49d and CD49e antigens on leukemic blasts. Limited follow-up precludes definitive correlation with clinical outcome at this time. Long-term studies and functional studies of adhesion molecules may elucidate a possible role for these antigens in the biologic behavior of leukemic cells.

Abstract 22 Summary of clinicopathological data and expression of CD49d, CD49e, and CD62L

AML FAB type	WBC (×10⁹/l)	Circulating blasts (%)	Extramedullary disease	Complete remission	CD62L expression (%)	CD49d expression (%)	CD49e expression (%)
M0	90	8	No	No	1	1	18
M1	21	92	No	N/A	1	31	81
M1	175	96	No	No	61	94	69
M2	1	0	No	No	1	72	75
M4	9	41	No	Yes	1	78	55
M4	88	20	Yes	N/A	1	86	65
M5	74	10	Yes	Yes	1	88	86
MLL	220	64	No	Partial	6	88	93

AML, acute myelogenous leukemia; N/A, not available; WBC, white blood cell count.

023 COMPARATIVE STUDY OF HISTOPATHOLOGICAL FINDINGS IN CHRONIC EOSINOPHILIC LEUKEMIA AND HYPEREOSINOPHILIC SYNDROME: A REPORT OF THREE CASES

*G Petrusevska[1], L Cevreska[1], S Stankovic[1], R Jovanovic, B Georgievski[1], A Stojanovic[1]. *Institute of Pathology, Skopje; [1]Clinic for Hematology, Skopje*

Chronic eosinophilic leukemia (HES) is a rare myeloproliferative disease difficult to distinguish from hypereosinophilic syndrome (HES). In this comparative study, we present two cases of CEL and one case of HES. Clinical histories and histopathological analysis of paraffin embedded bone marrow tissue and buffy coat specimens were used for this report.

Paraffin sections were stained with H&E, Giemsa, PAS and reticulin, while semi-thin sections were stained with toluidine blue and PASM-basic fuchsin. Patients diagnosed as CEL were 55 and 26-years-of-age males, admitted at the clinic for hematology with symptoms of malaise and hepatosplenomegaly. Laboratory findings: leucocytosis (22.6×10^9/l and 56.6×10^9/l, respectively), with eosinophylic count of 39% and 46%, respectively. Bone marrow biopsies showed increased cellularity with obliteration of the fatty space. There was myeloid proliferation with increased number of eosinophilic myelocytes and promyelocytes, dominating the microscopic field. Erythropoesis and megakaryopoiesis were significantly depressed. Slightly increased peritrabecular reticulin fibrosis was detected. Cytogenetic abnormality was found [[t(1q–, 9p+)]] in the first patient. The third patient was 48-years-of-age old male with symptoms of pleural effusion, and bilateral hilar shadows with cardiomegaly, and hepatosplenomegaly. Leukocytosis (43×10^9/l) with 63% eosinophils was found in peripheral blood analysis. The bone marrow was hypercellular due to huge eosinophilic proliferation with signs of maturation (80%). Islands of erythropoiesis and solitary megakaryocytes, with no reticulin fibrosis were found. Cytogenetic examination showed no abnormalities. All patients were treated with Hydrea. An intriguing fact is that all patients appeared at the same time last year. Follow up for 6 months showed no clinical progression.

We conclude that morphological distinction between the two entities can be made by the presence of maturation of eosinophilic precursors in HES, and dysmaturation with increased immature eosinophilic precursors in CEL.

024 THE MEGAKARYOCYTIC PATTERN OF EXPRESSION OF *C-MPL* IN ESSENTIAL THROMBOCYTHEMIA CORRELATES WITH THE THROMBOTIC RISK

F Pierconti, L Teofili[1], N Maggiano, N Vianelli[2], S Ascani[2], S Pileri[2], G Leone[1], V De Stefano[1], LM Larocca. *Istituti di Anatomia Patologica, Università Cattolica del Sacro Cuore, Facoltà di Medicina e Chirurgia "Agostino Gemelli", Roma, Italy; [1]Istituti di Ematologia, Università Cattolica del Sacro Cuore, Facoltà di Medicina e Chirurgia "Agostino Gemelli", Roma, Italy; [2]Instituto di Ematologia "Seragnoli", Università of Bologna, Bologna, Italy*

Background: To investigate by immunohistochemistry the pattern of megakaryocyte expression of *c-mpl* among patients with essential thrombocythemia (ET) or with secondary thrombocytosis (ST).

Patients/Methods: We investigated by immunohistochemistry the expression of *c-mpl* in bone marrow megakaryocytes of 88 patients with ET, 6 patients ST, and 20 patients with lymphoma (as controls). Both the pattern of expression and the staining intensity were considered.

Results: We identified two patterns of expression of *c-mpl*: a uniform pattern and a heterogeneous one. The uniform pattern was found in all the controls, in all the cases with ST and in 28 patients with ET. In such subjects, the staining intensity was strong in the majority of megakaryocytes (>80%). In contrast, in 60 patients with ET the *c-mpl* expression was heterogeneous. Among them 18 (30%) suffered from thrombosis at diagnosis, at significant variance with the patients showing a uniform pattern (2 of 28, 7%; p = 0.026); in particular, the over-representation of thrombotic complications in respect to the last set of patients was found mainly among the individuals with a significant percentage (10–40%) of weakly stained and/or *c-mpl* negative megakaryocytes (heterogeneous-weak pattern) (13 of 30, 43% p = 0.002). Accordingly, the presence of such a pattern was associated with a 6.1-fold increase in risk for thrombosis in comparison with the patients with a *c-mpl* uniform pattern.

Conclusions: The presence of a heterogeneous *c-mpl* distribution pattern in bone marrow megakaryocytes could be a useful diagnostic criterion in the differential diagnosis of thrombocythosis. Furthermore, the detection of a significant percentage of weakly stained and/or *c-mpl* negative megakaryocytes can help to identify patients at higher thrombotic risk.

025 ISOLATED BONE MARROW INVOLVEMENT IN HUMAN IMMUNODEFICIENCY VIRUS-ASSOCIATED HODGKIN LYMPHOMA

M Ponzoni, L Fumagalli[1], G Rossi[2], M Freschi, A Re[2], MG Viganò[1], M Guidoboni[3], R Dolcetti[3], RW McKenna[4], *F Facchetti[5]. *Department of Pathology, Ospedale S. Raffaele, Italy; [1]Department of Infectious Diseases, Ospedale S. Raffaele; [2]Department of Hematology, Spedali Civili, University of Brescia, Italy; [3]Experimental Oncology 14, CRO, Aviano, Italy; [4]Department of Pathology, University of Texas Southwestern Medical Center, Dallas, USA; [5]Department of Pathology, Spedali Civili, University of Brescia, Italy*

Background: Human immunodeficiency virus-associated Hodgkin lymphoma (HIV-HL) frequently involves the bone marrow (BM) and is usually associated with lymph node HL but occasionally is the only apparent manifestation of disease. In the latter setting diagnosis can be problematic.

Purpose: Six HIV-HL cases with BM as the only site of disease at diagnosis are described.

Methods/Results: 22/42 HIV-HL patients (pts) had positive BM involvement at diagnosis. From this group 16 pts were found to have concomitant substantial histological, and/or clinical extramedullary HL. In the remaining 6 pts BM was the only site of disease at diagnosis. In all 6 cases, BM biopsy (BMB) revealed obvious HL in either nodules (4 cases) or diffuse infiltrates (2 cases). Reed-Sternberg cells or variants were easily identified in all cases, morphologically and immunophenotypically (CD3–, CD15+, CD20–, CD30+, CD45RB–, ALK1–, and Oct2–). EBV was demonstrated by in situ hybrizidation in 3 of 3 cases studied. Spared BM tissue constantly showed fibrosis. All pts were males presenting with fever and blood cytopenias, with a median age of 35 years and a median CD4+ lymphocyte count of 70 cells/mm^3. Total body CT scans and all other staging procedures were negative. All pts were treated with chemotherapy. Median survival was 4 months; longer survival was achieved in the pts who completed chemotherapy regimens. Three subjects died shortly without the full completion of chemotherapy, two from HL: two of them underwent autopsy and one of showed disseminated HIV-HL four months from diagnosis.

Conclusions: Isolated BM HIV-HL may be an underestimated condition in the HIV setting: in those pts with unexplained fever and blood cytopenia, BMB should be performed with the aim of assessing for HL, even in the absence of typical nodal and visceral HL. A rapid diagnosis of isolated BM HIV-HL could expedite therapy.

026 ENDOGLIN (CD105)-POSITIVE VESSELS ARE INCREASED IN CHRONIC IDIOPATHIC MYELOFIBROSIS

M Ponzoni, Il Shendrik, AJM Ferreri, G Pruneri, P Servida, F Bertolini, *A Orazi. *Departments of Pathology, Radiochemotherapy and Hematology, S. Raffaele H Scientific Institute; Departments of Pathology and Laboratory Medicine and Hematology, European Institute of Oncology, Milan, Italy; Departments of Pathology and Hematology, Indiana School of Medicine, Indianapolis, USA*

Background: Recent data indicate that microvessel density (MVD), a surrogate marker of angiogenesis, is increased in bone marrow biopsies (BMB) of chronic idiopathic myelofibrosis (CIMF) patients (pts). Endoglin (CD105) is preferentially expressed by recently-formed vessels. CD105 immunoreactivity has not been investigated so far in vessels of CIMF pts. Data from the literature suggest a potential anti-angiogenetic role of anti-CD105-targeted drugs.

Purpose: To assess the immunoreactivity (IR) and the prognostic role of CD105 MVD in CIMF pts.

Methods: Fifty-six paraffin-embedded BMB of CIMF pts and 21 normal controls (staging BMB of non-Hodgkin lymphomas) were collected at diagnosis and stained with reticulin and anti-CD34 (clone QBEnd/10, dil.1:400) and -CD105 (clone 4G11, dil. 1:100) monoclonal antibodies, respectively. For CD105 and CD34 MVD assessment, 49 and 55 cases of CIMF were evaluable: each full-length BMB was counted at 20× HPF and the obtained mean MVD value ±standard deviation was registered. Fibrosis (F) was graded and subdivided into <2 and >2. The presence of megakaryocytes (Mgk) immunoreactive for CD105 was also reported.

Results: The mean CD105 and CD34 MVD was significantly higher in CIMF pts than in controls: 30.5 ±7.8% and 37.2 ±24.4% vs 8.2 ±2.8% and 2.7 ±1.4%, respectively (t-test; p <0.00001). CD105+ve Mgk were more frequent in CIMF (73% in CIMF vs 14% in controls, c2 p<0.00001). CD105 MVD was correlated with G3–4 F (31.6±7.8% for G 3–4 F vs 25.7±7.5% G 1–2 F, p= 0.05). No correlation was observed between degree of F and CD34 MVD.

Conclusions: CD105 MVD is significantly increased in CIMF and is correlated to substantial marrow F. This may lead to the employment of anti-CD105-targeted drugs to more advanced stages of disease.

027 FLOW CYTOMETRY AS A DIAGNOSTIC AID IN MYELODYSPLASTIC SYNDROME

A Porwit, E Björklund, E Hellström-Lindberg[1], M de Santiago[2], A Orfao[2]. *Dept of Pathology, Karolinska Hospital, Stockholm, Sweden; [1]Dept of Hematology, Huddinge Hospital, Stockholm, Sweden; Dept of Cytometry, University of Salamanca, Salamanca, Spain*

The diagnosis of myelodysplastic syndrome (MDS) may be difficult, especially in cases with no clonal chromosomal changes. As a part of a study conducted within the Immunophenotyping Force of the European Working Group for Clinical Cell Analysis we have investigated the possibility of applying multiparameter flow cytometry to the diagnosis of MDS. A four-color immunostaining (CD13/CD11b/CD45/CD34) was systematically applied to bone marrow (BM) samples from 27 untreated patients with MDS and 18 BM samples from patients with anemia and/or thrombocytopenia due to other causes. The median age of the MDS patients was 75 years and 61 for the controls. According to the FAB classification MDS patients corresponded to: 12 RA, 2 RARS, 8 RAEB, 2 CMML and 3 RAEB-t.

From the analytical point of view it was possible to divide granulopoiesis into five distinct subsets of cells, from CD34+ stem cells, most immature CD13 high/CD11b low blasts, CD13 low/CD11b low precursors, CD13 low/CD11b high more differentiated precursors and CD13 high/CD11b high granulocytes. In the control group these five stages corresponded to 1.2%, 2.9% 14.7%, 36.2% and 44.2% of all granulopoiesis, respectively. The average values for MDS patients were 8% for CD34+ cells and 4.6%, 21.9%, 32.4% and 31.7%, for the other four stages of differentiation, respectively. Further analysis showed that the results in RA/RARS BM samples did not differ significantly from the control group. The results in RAEB, RAEB-t and one of two patients with CMML showed significant left-shift in differentiation with the CD34+ cells comprising 14.5% of granulopoiesis, and the four stages of differentiation corresponding to 7.5%, 20.9%, 28.3% and 26.1%, respectively. In several cases a clear block in differentiation was noticed.

We conclude that flow cytometry can be applied as a useful aid to diagnose aberrant differentiation patterns in granulopoiesis.

028 BONE MARROW HISTOLOGY IN CD4/CD56 POSITIVE NEOPLASMS

M Raphaël, Z Rudzki, S Lazzi, M Ffrench, T Petrella, Le Thi Thanh Mai, Cracovie, N Hurwitz. *Hôpital Avicenne, Université Paris 13, France; Departments of Pathology Universities of Krakow, Siena, Basel*

The rare CD4+/CD56+ maligancies are now better characterized by cytologic and immunophenotypic studies. Type 2 dendritic cell (DC2) or plasmacytoid dendritic cells have been proposed as a normal equivalent to these neoplasms.

Apart from the histological findings of the extramedullary presentatioin showing a diffuse infiltration by blastic cells, the histological description of the bone marrow biopsy infitration was not reported in the various series found in the literature.

In order to describe the histologic bone marrow pattern of the infiltration of the CD4+/CD56+ neoplasms we have analyzed bone marrow biopsies using conventional and immunohistochemical techniques and compared this with the cytology and immunophenotypic analysis by flow cytometry in 6 cases. For the immunophenotypic studies by flow cytometry the following antigens were tested : CD2, CD3, CD4, CD5, CD7, CD8, CD20, CD34, CD45, CD56, CD56, CD68, CD117, TdT, MPO. By immunohistochemistry, the expression of CD3, CD8, CD20, CD34, CD45, CD56, CD68, MPO and TiA1 was analyzed.

Bone marrow biopsy showed normocellular to hypercellular hematopoietic tissue. The normal hematopoietic tissue was still present in all cases except one. Some dysplastic features were observed as atypical megakaryocytes and hypogranular mature myeloid cells. The blastic infiltration was heterogeneous, ranging from mild infiltration within normal hematopoietic tissue and adipocytes to diffuse and massive infiltration especially at the relapse. Irregular nuclear outlines of blasts are a prominent feature. Some tumour cells are present in the sinuses. There is no or mild myelofibrosis. The results of immunophenotyping by flow cytometry are correlated with the detection of tumor cells by immunohistochemistry.

This analysis shows the spectrum of the histological infiltration in bone marrow in CD4+/CD56+ neoplasms and emphasizes the need of both techniques (flow cytometry and immunohistochemistry) to characterize the infiltration and to identify mild infiltration. Diagnostic problems in cases with primary extramedullary presentation will be briefly discussed.

029 BONE MARROW HISTOLOGY IN GAUCHER DISEASE TREATED WITH IMUGLUCERASE

*Z Rudzki, K Okon, M Machaczka[1], M Rucinska[1], B Papla, AB Skotnicki[1]. *Departments of Pathomorphology and [1]Hematology, Collegium Medicum, Jagiellonian University, Kraków, Poland*

Clinical aspects of response to enzyme replacement therapy in Gaucher disease are widely reported, whereas little is known about the alterations in bone marrow histology in the course of the enzymatic treatment.

We report the results of the bone marrow histological examination in five patients (2 M, 3 W, aged 32–55 yrs, 2 splenectomized) treated with recombinant beta-glucocerebrosidase (imiglucerase, 30U/kg/2 weeks) for 26 to 32 months.

In routinely processed trephines the relative volumes of basic histological components of bone marrow (Gaucher cell population, hematopoiesis, fat, and trabecular bone) were estimated using the standard point intercept method, and the unbiased mean volumes of Gaucher cells using the random sampled line intercept method.

The comparison of pre-treatment and control biopsies revealed qualitatively similar alteration in all patients: reduction in the relative volume occupied by Gaucher macrophages (Wilcoxon matched-pair test, $p = 0.04$), increase in hematopoiesis ($p = 0.079$) and fat ($p = 0.04$), and surprisingly, a decrease in the relative volume of non-cortical bone ($p = 0.04$). Evolution to osteopenia was particularly spectacular in one patient (from 33.4% of the initial biopsy volume to 7.5% in the control biopsy, with histological picture involving extensive "button phenomenon"). This was paradoxically accompanied by disappearance of bone pain. The number and sizes of Gaucher cells decreased to various extents. Quantitatively the changes in morphometric parameters showed significant interindividual variability and no strict correlation with objective clinical values (platelets and hemoglobin).

The trephine bone marrow biopsy provides additional data in the assessment of enzyme replacement therapy. Accelerated osteopenia may be a hitherto overlooked side effect of enzyme replacement and needs further studies.

030 CD79a CAN BE USED TO STAIN MEGAKARYOCYTES IN BONE MARROW SECTIONS: COMPARISON WITH VON WILLEBRAND FACTOR STAINING

A Sarker, C An, A Orazi. *Indiana University School of Medicine, Indianapolis, IN, USA*

Background: Although mature, multilobated megakaryocytes (MKs) are easy to identify in routine bone marrow biopsy sections, identification of megakaryoblasts and atypical MKs requires the use of immunohistochemical techniques. Von Willebrand factor (vWF) is considered to be the most reliable immunohistochemical marker for identifying MKs in tissue sections (Thiele *et al* Virchows Arch 1987;54:89). Preliminary evidence has shown that CD79a can be used to identify MKs in normal bone marrows. However, no information is available on the specificity of this method as compared with the more conventional vWF staining.

Design: A total of 169 bone marrow biopsies were immunostained by CD79a (clone HM57; dilution 1:25, DAKO) and vWF (polyclonal, prediluted, DAKO). These included the following cases: 50 myelodysplastic syndromes (MDS); 4 chronic myelomonocytic leukemia (CMML); 44 chronic myelogenous leukemia (CML); 34 other chronic myeloproliferative disorders (CMPD) which included 11 agnogenic myeloid metaplasia/chronic idiopathic myelofibrosis, 13 essential thrombocythemia (ET), and 10 polycythemia vera (PV); 21 acute myelogenous leukemia (AML); 2 acute megakaryoblastic leukemia (AML-M7); 4 reactive marrow from patients with anemias; 10 normal controls. All MKs in a bone marrow biopsy section were counted and the number of MKs expressing CD79a and vWF was recorded and expressed as a percentage of the total number of bone marrow MKs.

Results: Except for AML-M7, in all cases MKs were reactive for both CD79a and vWF but the percentage of reactivity varied. The results are shown in Table 1. One of 2 AML-M7 cases showed expression in blasts of both CD79a and vWF with a percentage reactivity of

Abstract 30 Comparative immunohistochemical staining results in 169 cases

Antibody	MDS (n=50)	CMML (n=4)	CML (n=44)	CMPDs (n=34)	AML (n=21)	Reactive/normal marrows (n=14)
CD79a	97.1 ±5 (79–100)	82.5 ±5 (78–89)	92.8 ±10.8 (46–100)	93.1 ±15.4 (64–100)	95 ±8.2 (67–100)	95.9 ±6.9 (79–100)
vWF	97 ±5.6 (65–100)	81.9 ±12.2 (74–100)	94.8 ±8.1 (67–100)	91.7 ±8.2 (55–100)	89 ±13.5 (50–100)	90.9 ±10.4 (67–100)

Results are given as the mean (±SD) and ranges (minimum–maximum) percentage values of positive megakaryoblasts for all cases in each category. The results include two cases of AML-M7 of which one showed reactivity in megakaryoblasts with both CD79a and vWF.
AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; CMML, chronic myelomonocytic leukaemia; CMPD, chronic myeloproliferative disorder; MDS, myelodysplastic syndrome; vWF, vonWillebrand factor.

98.5% and 99.2%, respectively. Statistical analysis using Student's t test demonstrates no significant difference in immunostaining results between CD79a and vWF and also among the respective groups (see table).

Conclusion: Our results obtained on a large number of hematologic conditions show an overall comparable reactivity for vWF and CD79a in megakaryocytes. Therefore, CD79a can also be employed as a useful additional marker to identify normal and atypical MKs in routine bone marrow biopsy.

031 OVEREXPRESSION OF POLYCYTHEMIA RUBRA VERA RECEPTOR-1 (PRV-1) GENE IN ESSENTIAL THROMBOCYTEMIA

L Teofili, M Martini[1], M Luongo, A Di Mario, G Leone, V De Stefano, LM Larocca[1]. *Istituti di Ematologia and [1]Anatomia Patologica, Università Cattolica del Sacro Cuore, Rome, Italy*

Background: To examine the utility of polycythemia rubra vera receptor-1 (PRV-1)-specific reverse transcriptase polymerase chain reaction (RT-PCR) to discriminate essential thrombocytemia (ET) and polycytemia vera (PV) from secondary thrombocytosis (ST) or secondary erythrocytosis (SE).

Patients/Methods: We analyzed the expression of PRV-1 in granulocytes isolated from 35 patients with ET, 12 patients with ST, 37 patients with PV, 10 patients with SE and from 20 normal individuals by PRV-1-specific RT-PCR. In female patients, PRV-1 expression was correlated with clonality analysis as assessed by the human androgen receptor (HUMARA) polymorphism assay.

Results: PRV-1 was not expressed in granulocytes isolated from normal individuals and from patients with ST and SE. On the contrary, all ET patients and 35/37 PV patients overexpressed PRV-1. All the cases with monoclonal hematopoiesis (17/21 with ET and 12/12 with PV) expressed PRV-1; yet PRV-1 overexpression extended to the cases of ET showing polyclonal hematopoiesis (4/20).

Conclusions: The overexpression of PRV-1 appears to be a useful tool to discriminate ET and PV from secondary thrombocytosis and erythrocytosis, so offering an innovative diagnostic approach based on the detection of positive diagnostic criteria instead of exclusion criteria.

032 PROGNOSTIC IMPACT OF MORPHOLOGICAL FEATURES IN PH1+ CHRONIC MYELOID LEUKEMIA

*J Thiele, HM Kvasnicka. *Institute of Pathology, University of Cologne, Cologne, Germany*

Background: Until now, in multivariate risk classifications for Ph[1+] chronic myeloid leukemia the putative prognostic property of bone marrow (BM) morphology has been generally neglected or even contested.

Patients/Methods: A multicenter clinico-pathological study was performed on CML patients to determine BM characteristics that exert a significant impact on survival under standard therapy regimens. Therefore 510 patients with chronic phase Ph[1+] CML and BM biopsies were entered into this trial. Treatment included interferon alpha-2b (IFN) and hydroxyurea (HU). Immunohistochemistry and morphometry were applied to identify BM cells and to quantify fiber density. Patients were separated into learning and validation samples and tree-structured survival (CART) analysis was performed to establish a prognostic decision tree.

Results: Application of the Sokal index and the recently proposed European score failed to distinguish three clearly defined risk groups. Both scores were able to establish a low risk profile with a significantly better prognosis in the IFN- and chemotherapy-treated cohorts. Regarding BM characteristics, a borderline increase in (reticulin) fiber content and a relevant reduction of erythropoiesis were important predictors for survival even in low risk classified patients according to both clinical scores. Considering optimal treatment strategies, patients with manifest myelofibrosis showed no significant difference in survival rates under IFN or HU therapy. Multivariate CART analysis of the validation sample (123 patients with HU therapy) revealed amount of erythroid precursors in the BM, myelofibrosis and splenomegaly as the most important prognostic features. Three risk profiles with significantly different survival patterns were established with median survival times ranging between 33 and 108 months. The new score was confirmed by application to the learning sample with IFN therapy.

Conclusion: Our data implicate that prognostic classification of Ph[1+] CML can be significantly improved by inclusion of morphological parameters which are acting independently of treatment modalities.

033 HEMATOGONES VERSUS NEOPLASTIC LYMPHOBLASTS: A DIAGNOSTIC PITFALL IN ASSESSING REMISSION AFTER CHEMOTHERAPY FOR ACUTE LYMPHOBLASTIC LEUKEMIA. SOLITARY CRITERIA ARE MISLEADING

*A Tzankov, P Mensah[1], L Verda[1], AB Skotnicki[1], Z Rudzki[1]. *Institute of Pathology at the University of Innsbruck, Austria; [1]Collegium Medicum, Jagiellonian University, Krakow, Poland*

Background: The term hematogones (HG) designates a population of benign B-lymphocyte progenitors in the bone marrow (BM), characterized by various positivity for CD10, CD34, TdT and CD19, thus resembling phenotypically B-lymphoblasts (LB). Expanded populations of HG, especially following treatment for ALL, have been well documented in pediatric patients, while little is known about their morphological appearance in adults. No solitary reliable criterion distinguishing HG from LB exists.

Purpose: By reporting our microarchitectural and immunohistochemical observations on two BM biopsies we aim to contribute to the determination of reproducible differentiating criteria between HG and LB.

Summarized description: First case: 17a, F; 5M after 2nd chemotherapy regimen for relapsed ALL—suspicious BM population of—compared to the LB (large, CD10, CD79a, CD34 and TdT+ cells)—smaller, variably CD10 and CD79a+, TdT+/–, as well as CD34– cells, forming huge clusters—hematogones. Second case: 69a, F; control BM biopsy 3M after 1st consolidation for ALL—40% infiltration with variably CD10, TdT and CD34+, CD99– small lymphoid cells, forming clusters; compared to the neoplastic LB (large, CD10, CD34, TdT and CD99+ cells), the population classified as hematogones.

Conclusions: The affinity to assemble clusters has been designated to be an inevitable hallmark of LB and thought of as a major differentiating criterion to HG. In the present cases, we observed and immunohistochemically proved the presence of hematogones building clusters with >5 cells in adult bone marrow specimens after ALL consolidation. In the second case the HG appeared CD99–, while the LB were CD99+. Concerning the normal maturation of BM B-cells, CD99 emerges after CD34, when the cells already become CD34–. It is tempting to speculate that the CD99/CD34 co-expression in LB could be a malignancy-associated phenotypical aberration. Further studies should verify the value of this phenotypic difference in distinguishing HG from LB.