Pathology and clinico-pathological correlations

394 SUCCESSFUL DIAGNOSIS OF LYMPHOMA FROM CORE BIOPSIES

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Background: Current BCSH guidelines recommend excision biopsy as the method of choice for diagnosis of lymphoma unless the node or mass is within an inaccessible area. Many case series have shown that diagnostic yield from core biopsies is extremely variable.

Method: We audited 2 patient groups with a final diagnosis of lymphoma, who underwent core biopsy by experienced operators using US or CT guidance at our institution. The first group, 55 new patients at a single centre neck lump clinic and the second, 52 new patients from a single interventional radiologist's biopsy list. The nature of the final diagnostic biopsy and, where known, the size of the core was documented. Reporting was performed by a single specialist lymphoma pathologist.

Results: Of the 55 patients in group 1, 26 had Hodgkin lymphoma (HL) and 29 non-Hodgkin lymphoma (NHL). Seventy one percent (39/55) of initial core biopsies were diagnostic. Of these 15/39 had a final diagnosis of HL (8-NS, 5-MC, 2-other) and 24/39 had NHL (11-DLBCL, 8-FL, 2-CLL/SLL, 1-LPL, 1-MCL, 1-MZL). Within the non-diagnostic group; 11/16 had a final diagnosis of HL (7-NS, 2-MC, 1-NLP and 1-LR) and 5/16 had NHL (2-FL, 1-MZL, 1-DLBCL, 1-PTCL); 14/16 required excision biopsy for final diagnosis. The median size of cores (16G needle) was 5mm in non diagnostic group and 17mm in the diagnostic group.

Of the 52 patients in group 2, 10 had HL and 42 NHL. Eighty five percent (44/52) of initial core biopsies were diagnostic. Biopsy sites included 12 extranodal, (5-lung, 1-small bowel, 1-thyroid, 1-liver, 2-soft tissue, 1-muscle, 1-parotid) and 40 nodal (14-cervical, 22- deep abdominal, 3-mediastinal, 1-axillary). Within the diagnostic group 6/44 had HL (4-NS, 2-MC) and 38 had NHL (20-DLBCL, 9-FL, 3-MZL, 1-PTCL, 1-ALCL, 1-LPL, 2-PTLD, 1-LG NHL). Within the non-diagnostic group 4/8 had a final diagnosis of HL (3-NS and 1-MC) and 4 had NHL (2-FL, 1-DLBCL, 1-PTCL). All 8 with non-diagnostic cores were re-biopsied: 5 excisional and 3 repeat core biopsies. The median size of cores was 13mm in the non-diagnostic group and 23mm in the diagnostic group. There was no morbidity incurred from any biopsy performed.

Conclusion: Core biopsy, especially of deep tissue masses, is a reasonable, safe approach to diagnosis of lymphoma and can avoid the need for anaesthetic and laparotomy. Operator expertise and adequate sample size, however, are essential to obtain a high diagnostic yield as in our series (83/107, 78% of cases). Diagnostic failures mainly arise in with node fibrosis in NSHL and when core specimens are small.

395 ARE THERE ANY SIGNIFICANT VARIATIONS IN THE CLINICAL OR HISTOLOGICAL PRESENTATION OF LYMPHOID PATHOLOGIES OVER THE COURSE OF TIME?

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Introduction: Little data is available concerning variations in the clinical characteristics of lymphoid pathologies at presentation. We decided to investigate whether any variations in these aspects had occurred in our environment during the last few years.

Materials and Methods: The GOTEL group database is an archive of all new lymphoma cases, regardless of their histological subtype, diagnosed in the hospitals within the group. An analysis was made of all the records between 1st January 1999 and 1st January 2010.

The number of hospitals submitting data has changed over the course of time, however data has been provided by 26 hospitals from 16 Spanish provinces.

Results: A total of 3,651 cases of lymphoma were recorded within this period. Grouped by clinical features, 42.8% (1,561 patients) presented low-grade lymphomas, 30.4% (1,110 patients) intermediate-grade lymphomas, 15.2% (556 patients) Hodgkin's lymphomas, there were 208 patients with T lymphoma (5.7%), 111 patients with high-grade lymphomas (3%), and 105 patients (2.9%) with lymphomas that were difficult to classify.

A total of 6.3% of the diagnoses (231 patients) were performed prior to 1999, 29.5% between 2000 and 2001, 25.7% between 2002 and 2003, 19.7% between 2004 and 2005, 11.2 % between 2006 and 2007, and 200 entries from 2008 to the close of the study period, corresponding to 1.5% of the complete database. The median age at diagnosis was 60 years (range 7-105 years), by percentiles: 25 correspond to 44 years, 50 to 60 years and 75 to 71 years. The distribution by gender was 53.1% male, and 46.9% female.

An analysis was made of all the clinical variables collected, comparing their behaviour during the different diagnostic periods. Neither the periods, gender, ECOG, stage, LDH, â2 microglobulin, Hodgkin's or non Hodgkin's type neoplasia, B Lymphoma versus Hodgkin's, NK or T, nodal or extra-nodal origin, median age at diagnosis nor histological type by region of origin, showed any statistically significant differences in their distribution over the course of time.

Conclusion: In our experience, there are no significant variations in the clinical presentation or histological type in lymphomas diagnosed over the course of time in Spain.

396 WITHDRAWN

397 HIGHER LEVEL OF SERUM ANTI-TRIM68 AUTOANTIBODY AS AN INDICATOR OF AGGRESSIVE OR ADVANCED LYMPHOMA

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Background: TRIM68 (Tripartite motif-containing protein 68) is a member of ring finger proteins, and E3 ligase for ubiquitination. It is also known as ss56, whose autoantibody was detected in patients with AIDS, SLE, and Sögren syndrome. Recently, incidence of DLBCL is increasing in the AIDS patients after developing HAAT therapy. To clarify relationship between development of lymphoma and anti-TRIM68 autoantibody, we examined if anti-TRIM68 autoantibody is detectable in lymphoma patients without AIDS.

Patients and Methods: From September 2008 to June 2010, 254 patients with hematological malignancies and others visited and were treated at the Cancer Institute Hospital. All serum from the patients were separated and stored at -20°C after informed consent. All the histopathology samples were reviewed according to the WHO classification by expert hematopathologists. Recombinant His-tagged TRIM68 protein was made with cell-free system, ProtemistRDT, and adsorbed to 96-well plates. Serum were diluted at 1:100 with PBS (-), and put into the 96-well plates. After washing, goat anti-human (H-L) antibody was added, and the absorbance was determined at 450nm.

Results: Serum was collected from 254 patients (DLBCL 104 cases; FL 57 cases; MALT 9 cases; MCL 8 cases; BL 4 cases; CLL/SLL 2 cases; B-LBL 1 case; SMCL 2 cases; HL 15 cases; AITL 4 cases; MM 21 cases; ALCL 3 cases; NK/T 3 cases; PTCL 10 cases; ATLL 3 cases; AML 6 cases; CML 1 case; and other cancers 3 cases). Mean absorbance at 450nm (OD450) in DLBCL, MCL, B-LBL, CLL/SLL, SMCL, AITL, ALCL, NK/T, and ATLL was higher than PTCL, BL, FL, HL, MALT, MM, AML and other cancers as bellow: DLBCL 0.45; FL 0.32; MALT 0.16; MCL 0.46; BL 0.28; CLL/SLL 1.10; B-LBL 1.06; SMCL 0.87; HL 0.34; AITL 0.52; MM 0.21; ALCL 0.45; NK/T 0.82; PTCL 0.30; ATLL 0.52; AML 0.17; CML 0.31; and other cancers 0.33. Although there was no difference in mean OD450 among stages of DLBC, stage III/IV cases with more than 0.4 and less than 0.4 of OD450 were 45.5% and 32.1%, respectively. Stage IV patients with FL showed higher OD450 than Stage I-III. WBC count, Hb, Plt, LDH, sIL-2R, β2MG, and CRP were not correlated with OD450.

Conclusions: Serum anti-TRIM68 autoantibody in lymphoid malignancies, especially aggressive lymphoma, was detected higher than other hematological malignancies and cancers. Serum anti-TRIM68 autoantibody may be as an indicator of aggressive or advanced lymphoma.

398 FOLLICULAR LYMPHOMAS WITHOUT BCL2 REARRANGEMENT ARE HETEROGENOUS CONCERNING THE HISTOLOGICAL GRADING AND GENETIC FEATURES

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Background: Follicular lymphoma (FL) is genetically characterized by *BCL2*/18q21 chromosomal translocation with one the 3 immunoglobin chain loci. The resulting upregulation of this anti-apoptotic oncogene is considered as an important genetic event in FL lymphomagenesis although not sufficient. However, ~15 FL are reported with a *BCL2* germinal (*BCL2*-G) status, mainly in grade 3B with a diffuse pattern. Alternative genetic events have been identified in *BCL2*-G FL, such as *BCL6*, and 1p36 deletion. The latter is also detected in ~20% of FL with*BCL2* rearrangement and is linked with a worse prognosis. *TNFRSF14* has been recently identified as a candidate gene.

Material: We report a series of 9 *BCL2*-G typical FL further characterized for *BCL6*/3q27,*IGH*/14q32,*TNFRSF14*/1p36.33 and *mir34*/1p36.23 loci by FISH. The cases were selected after a morphological review which excluded 2 bordeline cases with marginal zone lymphoma and 3 cases of DLBCL suspected to be transformed FL.

Results: The median age was 61 [56-58] yr-old. Sex ratio (M/F): 5 /1. The main site was nodal in 6 cases (inguinal: 3) and extra-nodal in 3 cases (bladder, pancreas, duodenum). It is noteworthy that the 5 patients with available data had previous toxic exposition (4 professional exposures and 1 previously treated cancer). There was no prevalence for grade 3 (4 gr 1, 2 gr 3a, 2 gr 3b, 1 unknown). The FLIPI was relatively low when available (FLIPI 1, 2 and 3/5 in 4, 1 and 1 cases respectively). No obvious recurrent alteration was identified in the 3 cases with available karyotype. *IGH*locus was rearranged in 3 cases, including the only case with *BCL6* rearrangement (new partner loci in the 2 remaining cases). No deletion 1p36 was detected using BAC probes covering *TNFRSF14*and*mir34* loci.

Discussion: In this series, BCL2-G FL appear to be relatively heterogeneous concerning morphological aspect (grade) and genetic characteristics. Previous toxic exposure was seen in most of the patients with available data. Higher resolution genomic studies are under process to identify common oncogenic pathways.

399 QUANTIFICATION OF IL21 RECEPTOR IN FOLLICULAR LYMPHOMA USING MULTI-CHANNEL IMMUNOFLUORESCENCE SUGGESTS A POTENTIAL ASSOCIATION WITH POOR-OUTCOME DISEASE

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Background: Interleukin-21 (IL21), a recently discovered member of the common γ-chain family of cytokines, can enhance anti-tumor immunity and induce apoptosis in resting B-lymphocytes. Recombinant IL21 shows promise in cancer therapy. Since IL21 can induce apoptosis in follicular lymphoma (FL) cells mediated by the IL21 receptor (IL21R), differential expression of IL21R in FL biopsy samples may predict responses to IL21 therapy or define biologically- or clinically-important subsets of FL cases. Since FL samples are highly heterogeneous, and conventional bright field immunohistology is limited by a narrow dynamic range, we used quantitative immunofluorescence histology to evaluate the expression of IL21R selectively in CD20-positive B-cells in 114 FL biopsy samples. We then correlated these results with pathological and clinical features, including overall survival.

Design: Representative diagnostic, formalin-fixed, paraffin-embedded biopsy samples from subjects from whom clinical data were retrievable were represented in tissue microarrays (TMAs). TMA sections were stained simultaneously for CD20 (detected with Alexa-555) and IL21R (detected with Cy5), and AQUA software was used to determine Cy5 signal selectively within a mask defined by the presence of Alexa-555 signal. Receiver operating characteristic (ROC) curve analysis was used to dichotomize the cases into "high" versus "low" categories. Kaplan-Meier survival curves were analyzed by the log-rank test.

Results: In hyperplastic tonsil tissue IL21R showed preferential localization to the cell membrane and cytoplasm of CD20-positive cells within follicle centres. Among the FL samples, 45 and 69 cases were classified as "low" and "high", respectively, for IL21R expression. IL21R-high cases showed a trend towards reduced overall survival (p=0.078); this trend was more evident when cases with a component of diffuse large B-cell lymphoma in the biopsy sample were excluded from the analysis (p=0.061).

Conclusion: Our results based on the largest survey to date indicate prevalent expression of IL21R in FL and suggest that relatively abundant expression is more prevalent among cases with relatively unfavorable clinical outcome. Further investigation is required to determine whether therapy with exogenous IL21 may have a particular role in FL cases that do relatively poorly on current therapeutic regimens.

400 THE HISTOLOGIC SUBTYPE OF LARGE B-CELL LYMPHOMA PROGRESSION IN SPLENIC MARGINAL ZONE LYMPHOMA PATIENTS INFLUENCES CLINICAL COURSE AND PROGNOSIS

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Background: Splenic marginal zone lymphoma (SMZL) is an indolent extranodal B cell lymphoma which transforms to high-grade lymphoma in 10% of cases. The histological progression is usually represented by a diffuse large B cell lymphoma, not otherwise specified (DLBCL-nos), but, more rarely, it shows features of T-cell/histiocyte-rich large B cell lymphoma (TCHRLBC).

Aim: to retrospectively compare epidemiological, clinical, prognostic features and outcome of SMZL patients progressing to DLBCL-nos or to TCHRLBC.

Patients and Methods: From January 1995 to December 2010, 79 patients with SMZL were consecutively seen at our Institute. Their median age was 66 years; 95% were in an advanced stage and 17% had B symptoms. Twelve patients (15.2%) progressed to high-grade lymphoma. Histologically, five of them were classified as TCHRLBC, seven as DLBCL-nos. Clinical characteristics and outcome data of both groups were compared.

Results: Median age at diagnosis was 60 years in the TCHRLBC cases, and 73 in the NOS cases (p=0.01). Male/female ratio was 1:4 and 1:1.3, respectively. All patients had Ann Arbor's III-IV stage. The NOS cases had more frequent B symptoms (43% vs 20%), high-risk IPI (66% vs 50%) and high-risk SMZL prognostic score (Arcaini, Blood, 2006) (57% vs 40%). HCV infection rate was similar (16% and 20%). Treatment prior to progression included splenectomy in all TCHRLBC cases and in 3/7 NOS cases. Two NOS patients received alkylating agents +/- rituximab and two were managed expectantly. Median time from diagnosis to transformation was significantly longer in TCHRLBC cases than in NOS cases (55 vs 18 months) (p = 0.031). Progression occurred often at extranodal sites (80% TCHRLBC and 56% NOS), mainly bone marrow or GI tract. Four NOS patients died of lymphoma one month after progression, before any treatment. The other patients received chemoimmunotherapy w/wo anthracyclines. Two patients with TCHRLBC died of lymphoma and one of infection. Currently five patients are alive, four in continuous CR (two in each group). Median survival after progression was 36 in TCHRLBC vs 1 month in NOS cases. With a median follow up of 87 months, 5-year overall survival from diagnosis in TCHRLBC was 80% vs 38% in NOS patients (p=0.19). The TCHRLBC cases had similar 5-year survival than SMZL patients without transformation (87%).

Conclusions: The TCHRLBC variant of SMZL progression represents a distinct clinico-pathological entity, developing in younger patients, later during the course of disease, with less severe SMZL at diagnosis and with a better prognosis.

401 – 402 – 403 – 404 – WITHDRAWN

405 BCL-6 EXPRESSION IS A FAVOURABLE PROGNOSTIC FACTOR OF PRIMARY GASTRIC DIFFUSE LARGE B-CELL LYMPHOMA IN TAIWAN

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Introduction/Background: The gastrointestinal tract is the most common site of primary extranodal non-Hodgkin lymphoma (NHL). Most primary gastrointestinal NHL occur in the stomach with mucosa-associated lymphoid tissue (MALT) lymphoma and diffuse large B-cell lymphoma (DLBCL) as the most frequent histological types. The prognosis of primary gastric DLBCL differs in various studies.

Materials and Methods: We retrospectively searched our lymphoma file from January 1994 to December 2008 for primary gastric DLBCL. Cases with a history or concurrent MALT lymphoma and those with high stage (III and IV) diseases were excluded. We performed immunohistochemistry for CD10, bcl-2, bcl-2, MUM1/IRF4 and Ki67, and classified the tumour phenotype according to the algorisms of Hans et al (*Blood* 2004) and Muris et al (*JPathol* 2006). Medical records were reviewed, and clinical variables, LDH and phenotypic markers were analysed using Cox proportional hazard regression model.

Results: We identified a total of 47 cases of primary gastric DLBCL including 24 males and 21 females with a median age of 65. Eighteen (38%) patients at stage I, and 29 at stage II. Ten (21%) patients underwent surgery, and 2 of these patients received chemotherapy. Thirty-two (68%) patients received combination chemotherapy including 9 in combination with mebthera. The overall 2- and 5- year survival rates were 36% and 28%, respectively. Univariate Cox proportional hazard regression model showed that bcl-6 protein expression ($p = 0.004$) and type 2 phenotype ($p = 0.050$) as defined by Muris JJ et al were associated with a statistically significant better prognosis. Bcl-6 expression remained prognostically significant by multivariate Cox proportional regression model ($p = 0.004$).

Conclusion: The outcome of primary gastric DLBCL was poor. Although this is a small retrospective study with heterogeneous treatment modalities, we identify bcl-6 expression as a favourable prognostic marker.

406 A NOVEL METHOD FOR DETECTION OF NON-MTC BREAKPOINTS IN MANTLE CELL LYMPHOMA PATIENTS

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Introduction/Background: The t(11;14)(q32;q23) translocation leading to cyclinD1 over-expression is typically found in Mantle Cell Lymphoma (MCL). The breakpoint

is located in one of the *IGHJ* genes on chromosome 14; on chromosome 11, the breakpoints cluster in the Major Translocation Cluster (MTC) (approx. 40 % of MCL) or are dispersed centromerically over a large region of *CCND1*. We have developed a novel multiplex long-range PCR (LR-PCR) that detects non-MTC translocations.

Patients and Methods: The multiplex LR-PCR uses 95 primers centromerically tiling the 400kb region on chromosome 11 of the *CCND1* gene. These primers are combined with IGHJ primer into the set of 10 PCR reactions. In case of yielding amplification product in at least one PCR reaction, the PCR is repeated using a set of IGHJ primers designed specifically for individual IGHJ segments. The shortest product is then directly sequenced. 17 lymphnode biopsies, 54 bone marrow aspirates, and 9 whole blood samples were analyzed in 70 patients. All these samples were negative in standard PCR targeting MTC.

Results: With multiplex LR-PCR, we were able to detect and characterize translocation breakpoint in 26 patients (37%). Of the negative samples, 15 had low DNA quality and failed to amplify the 8 kb control fragment. Of the positive samples, 11 were detected in lymphnode biopsies, 13 in bone marrow aspirates, and 2 in peripheral blood samples. The identified breakpoints are localized in a broad region on chromosome 11 from approx. 2 kb up to more than 300 kb centromerically from *CCND1* transcription start (however 24 breakpoints were positioned within 210 kb from *CCND1*). 14 breakpoints were placed telomerically towards MTC, 12 centromerically. We observed no significant positional clustering of the detected breakpoints.

Conclusions: We have developed a novel PCR based assay for non-MTC t(11;14)(q32;q23) genomic breakpoints detection and proved its suitability for clinical sample analysis. This method might be used for fast and reliable identification of specific tumor markers for MRD monitoring, however its usability is limited to fresh and snap-frozen samples and samples with sufficient tumor load (at least 1 cell in 1000).

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407 PERIPHERAL T-CELL LYMPHOMA WITH A REGULATORY T-CELL PHENOTYPE: A NEW ENTITY?

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Background: FOXP3 is a key regulatory gene required for the development and function of regulatory T-cells (T-reg). T-regs are a CD4+/CD25+ subpopulation of T-cells specialized in maintaining the balance between immune responses and self-tolerance. Rare cases of FOXP3-positive peripheral T-cell lymphoma (PTCL) have been described in the literature. PTCL, not otherwise specified (PTCL-NOS) is a heterogeneous entity; hence, subtypes could be identified based on specific biological and/or pathological characteristics. This study aimed to report the clinical and pathological characteristics of two patients with a pathological diagnosis of PTCL-NOS with a T-reg phenotype.

Case Description: The first case is a 48-year-old female patient without evidence of immunodeficiency (HIV and HTLV-1 serology were negative). She presented with a lesion in the cavum, which was biopsied and showed tumoral cells that were positive for FOXP3, CD3, CD4 and CD25. Reed Stemberg-like cells were seen and were positive for EBV LMP-1. She had stage IIA PTCL-NOS and had a PIT score of 1 (increased LDH), and received six cycles of CHOP followed by involved field radiotherapy, achieving a complete remission. Two years later, the patient relapsed and received 6 cycles of ICE and 6 cycles of GDP without response. She died 36 months after her diagnosis from a duodenal perforation caused by her lymphoma. The second case is a 65-year-old man without immunodeficiency (serology for HIV and HTLV-I were negative) who presented with generalized lymphadenopathy and B symptoms. An excisional lymph node biopsy showed tumoral cells that were positive for FOXP3, CD3, CD4 and CD25. He had a stage III PTCL-NOS with a PIT score of 2 (age >60 years and elevated LDH). He received 6 cycles of CHOP and achieved a complete response. Seven months later, he relapsed and received 6 cycles of ICE without response followed by 4 cycles of pixantrone with a short-lived partial response. Patient died 36 months after his initial diagnosis.

Conclusion: Here we present 2 cases of a FOXP3-positive PTCL-NOS. Both cases also showed positive co-expression of CD4 and CD25. This pattern is consistent with a T-reg phenotype. Both patients had a very poor clinical course with relapsed and refractory disease. Further research is needed to identify similar cases of PTCL with a T-reg phenotype, which may represent a distinct clinicopathological entity.

408 UTILITY OF FLOW CYTOMETRY USING T-CELL RECEPTOR Vβ INTERROGATION IN THE CHARACTERISATION OF T-CELL LYMPHOMAS

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Introduction: Flow cytometric (FC) assessment of T-cell receptor Vβ chains (TCRVβ) is a well described method for assessing T-cell populations in patients with suspected T-cell lymphomas. Most studies report the correlation of TCRVβ analysis with other techniques. We describe its practical contribution to lymphoma diagnosis in a small series of patients. We aimed to establish where this study fits into the diagnostic pathway, in particular whether it prompts other tests to confirm the disease or merely confirms blood or marrow involvement in patients with an established diagnosis.

Methods: We retrospectively analysed clinical and laboratory data of sequential patients with a clonal T-cell population by TCRVβ analysis (IOTest® Beta Mark, Beckman Coulter). We then analysed the outcome and follow-up studies for patients with this finding.

Results: There were 13 patients with a median age of 69 (range 27 - 89) and female to male ratio of 1.6:1. One patient already had a confirmed diagnosis of mycosis fungoides (MF). Of the remaining 12, 10 were subsequently diagnosed with lymphoma- adult T-cell leukaemia/lymphoma (ATLL) (1 case), anaplastic large cell lymphoma (1), MF/Sezary syndrome (1), T-cell prolymphocytic leukaemia (4) and large granular lymphocytic leukaemia (3). Two patients have not had a diagnosis of lymphoma made.

Patients were referred by Haematologists (7), and Dermatologists (2). Clinical presentation varied: lymphocytosis (7 cases), skin rashes (2), lymphadenopathy (1), anaemia (1) and B symptoms (1). The median haemoglobin at presentation was 13 g/dL (range 8.6 - 15.1), platelets: 198 x 10^9/L (range 62 - 376) and lymphocyte count: 5.26 x 10^9/L (range 0.5 - 83). Blood films were examined in all cases.

Five patients also had gene rearrangement studies by PCR which were concordant in 100% of cases. Imaging was available for 7 patients- 2 had disseminated lymphadenopathy (ATLL and MF).

Four patients who underwent chemotherapy had a repeat assessment of TCRVβ by FC and were negative following treatment.

Conclusions: TCRVβ interrogation by FC was often the first study to suggest a T-cell disorder, especially in patients with peripheral blood lymphocytosis, indicating further studies are required to establish a diagnosis. We argue that this is a useful addition to the range of tests available for the diagnosis of T-cell disorders. In our small series, we noted resolution of the TCR clone with treatment, as recently described. The use of TCRVβ interrogation for minimal residual disease detection may be of value in patients undergoing treatment.

409 CYTOTOXIC MOLECULE (CM)-POSITIVE LYMPHOMA: CLINICOPATHOLOGIC COMPARISON AMONG CM-POSITIVE CLASSICAL HODGKIN LYMPHOMA, HODGKIN-LIKE ANAPLASTIC LARGE CELL LYMPHOMA AND NODAL PERIPHERAL T-CELL LYMPHOMA

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Background: Cytotoxic molecules (CM) are apoptosis-inducing molecules found in azurophilic cytoplasmic granules of T lymphocytes. Detection of CM expression (granzyme B, TIA1 and perforin) is essential to the diagnosis of some mature T-cell lymphomas. We recently highlighted the adverse prognostic significance of CM expression among patients with nodal peripheral T-cell lymphoma (PTCL-NOS) (*Am J Surg Pathol 2005*), Hodgkin's like anaplastic large cell lymphoma (HD-like ALCL) (*Histopathology 2007*) and classical Hodgkin lymphoma (CHL) (*J Clin Oncol 2006*).

Patients and Methods: To characterize CM+ CHL, we clinicopathologically profiled 32 patients with CM+ CHL in comparison with 439 with CM-negative (CM-) CHL. Further, we clinicopathologically compared these 32 patients with CM+ CHL, 21 with CM+ HD-like ALCL, and 55 with CM+ nodal PTCL-NOS.

Results: H-RS cells of CM+ CHL patients had the prototypic immunophenotype of CD15+ CD30+ fascin+, with positivity for Epstein-Barr virus in 38% of cases. All CM+ CHL tumor cells were negative for Pax5. No difference in clinical parameters was seen between CM+ and CM- CHL. Notably, survival curve for CM+ CHL was significantly inferior to that for CM- CHL ($P = .0003$). Nodal PTCL-NOS was characterized as follows: 63% of patients had a performance status greater than 1 ($P = .001$), 79% were at an advanced clinical stage ($P = .013$), and 77% had serum lactate dehydrogenase levels higher than normal ($P < .001$). Immunophenotypically, CM+ CHL and CM+ nodal PTCL-NOS were positive for CD3epsilon in 7% and 83% ($P < .001$), CD8 in 9% and 40% ($P = .007$), CD15 in 71% and 4% ($P < .001$) and CD30 in 97% and 51% ($P < .001$) of patients, respectively. Interestingly, like the other CM-positive T-cell lymphomas, CM+ CHL showed a poor prognosis. Survival curves of patients with CM+ disease somewhat overlapped in the two years after diagnosis.

Conclusion: The histological and immunophenotypic features of CM+ CHL were generally within the boundaries of the CHL category, but differed from typical CHL in its aggressive clinical behavior. The question of whether CM+ CHL should

be categorized as Hodgkin lymphoma or T-cell lymphoma requires further consideration, and novel therapeutic approaches should be explored.

410 PREDICTIVE VALUE OF TUMOUR BURDEN COMBINED WITH MOLECULAR, HISTOMORPHOLOGICAL AND CLINICAL PARAMETRES IN ADVANCED STAGE CLASSICAL HODGKIN LYMPHOMA

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Introduction: In advanced stage Hodgkin lymphoma (HL), treated with more aggressive therapies, data of prognostic value of bulky disease are less consistent than in early stage HL. Furthermore, in combination of macroscopic measurement of tumor mass with molecular, histomorphological and other clinical variables might increase the predictive value of tumor burden.

Material and Methods: In a cohort of 100 advanced stage cHL pts treated with ABVD (1997-2005) we analyzed the prognostic relevance of voluminous tumor mass and their correlation with molecular (Bcl-2, Survivin, Bax, NFkB, Ki-67 and active caspase 3 expression determined by immunohistochemistry), histomorphological (eosinophil tissue infiltration, morphologic atypia of HRS cells, clusters or sheets of the HRS cells, total involvement of the lymph node by the neoplastic and inflammatory cells, presence of coagulative necrosis, sclerotic bands within the lymph node) and other clinical variables (IPS, extranodal involvement, eleveated sedimentation rate >50mm/h and ≥3 involved sites) at diagnosis. The median follow up was 7 years. Their significance was tested according to response rate and overall survival (OS).

Results: Patients with mediastinal bulky disease had significantly decreased EFS ($p<0.05$) Shorter OS was associated with voluminous tumor burden ($p=0.001$). There was statistically significant correlation between bulky disease and high Bcl-2+ at threshold of 50% of labeled tumor cells ($p=0.02$), clusters or sheets of the HRS cells ($p=0.001$), morphologic atypia of HRS cells ($p=0.05$), sclerotic bands within the lymph node ($p=0.019$) and eleveated ESR>50mm/h ($p=0.042$). Additionally, there was positive correlation with tissue eosinophilia, but it was not statistically significant ($p=0.07$).

Multivariate analysis revealed bulky disease as significant predictor for OS ($p=0.003$).

Conclusion: Advanced stage cHL pts with voluminous tumor mass, high Bcl-2+, cohesive clusters or sheets of the HRS cells and morphologic atypia of HRS are at a higher risk and could benefit from other, more intensive therapeutic approach.

411 CLINICAL RELEVANCE OF HISTOLOGICAL VARIABLES IN ADVANCED CLASSICAL HODGKIN LYMPHOMA

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Introduction: The clinical relevance of histomorphologic parameters in HL has been controversial. A subtle analysis of histology could lead to better identification of high risk patients.

Material and Methods: In a cohort of 100 advanced stage cHL (79 nodular sclerosis NS, 14 mixed cellularity, 4 lymphocyte rich, 3 lymphocyte depleted) treated with ABVD (1997-2005) we analyzed the prognostic relevance of pathomorphologic features such as tissue infiltration by eosinophils (>5% of all cells or clusters in at least 5 HPF), morphologic atypia of HRS cells (>25% of bizarre and highly anaplastic HRS cells with pleomorphic nuclear features and highly irregular nuclear outline), cohesive clusters or sheets of the HRS cells (sheets of >20% cohesive HRS cells), total involvement of the lymph node by the neoplastic and inflammatory cells (no focal residual secondary follicles), presence of coagulative necrosis (large areas of necrosis) at diagnosis. The median follow up was 7 years. Significance was tested according to the response rate and overall survival.

Results: Lower complete remission rate was associated with atypia of HRS cells ($p=.001$), total involvement of the lymph node ($p=.021$) and cohesive clusters or sheets of the HRS cells, ($p=.037$). Decreased OS had pts with tissue eosinophilia ($p=.013$), atypia of HRS cells ($p=.000$), total involvement of the lymph node ($p=.002$) and cohesive clusters or sheets of the HRS cells ($p=.004$).

Multivariate analysis revealed that tissue eosinophilia was significant predictor for OS ($p=.02$).

Conclusion: Advanced stage cHL pts with tissue eosinophilia are at a higher risk and could be eligible for more effective therapeutic approach.

412 PROGNOSTIC SIGNIFICANCE OF COMBINED MOLECULAR AND CLINICAL PARAMETRES IN ADVANCED CLASSICAL HODGKIN LYMPHOMA

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Introduction: Although the outcome of Hodgkin lymphoma (HL) has been significantly improved, identification of new prognostic factors might help in better stratification of patients (pts) towards more effective treatment.

Material and Methods: In a cohort of 100 advanced cHL pts treated with ABVD (1997-2005) we analyzed the prognostic relevance of Bcl-2, Survivin, Bax, NFkB, Ki-67 and active caspase 3 expression determined by immunohistochemistry, as well as IPS, bulky disease, extranodal involvement, elevated sedimentation rate (>50mm/h) and ≥3 involved sites at diagnosis. Immunohistochemistry was performed on formalin fixed, paraffin embedded lymph nodes tissue sections using an indirect immunoperoxidase method and a specific monoclonal antibodies. The percentage of neoplastic positive (+) cells was analyzed on 10 different high power microscopy fields (HPF, x400).

The median follow up was 7 years. Their significance was tested according to response rate and overall survival (OS).

Results: Lower complete remission rate was found in pts with IPS >2 ($p=0.0003$), extranodal involvement ($p=0.037$) and ≥3 involved sites ($p=0.028$). Shorter OS was detected in pts with high (>50% labeled tumor cells) Bcl-2+ ($p=0.006$), high Survivin+ ($p=0.047$), high Ki-67+ ($p=0.008$), IPS >2 ($p=0.0001$), extranodal involvement ($p=0.004$), bulky disease ($p=0.0002$) and ESR>50 ($p=0.0003$).

Multivariate analysis model combining clinical and molecular features revealed that high Bcl-2 + at threshold of 50% ($p=0.015$), IPS>2 ($p=0.0007$) and bulky disease ($p=0.001$) were significant independent prognostic variables for OS.

Conclusion: Advanced stage cHL pts with high Bcl-2+ (>50% of positive cells), IPS>2 and bulky disease are at a higher risk and could benefit from other, intensive therapeutic approach.