Pathology and clinico-pathological correlations

203 OCCURRENCE OF HODGKIN LYMPHOMA AND NON-HODGKIN LYMPHOMA IN THE SAME PATIENT. CLINICOPATHOLOGICAL FEATURES AND PROGNOSIS IN A SERIES OF 76 CASES

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Introduction: The possibility that non-Hodgkin lymphoma (NHL) may be diagnosed in patients with Hodgkin lymphoma (HL) is reported as single cases or in small series but its biological significance and clinical correlates are still discussed.

Patients and Methods: The database of HL diagnosed at three Italian Institutions have been analysed and 76 cases of HL and NHL occurring in the same patient have been retrospectively collected. Median age at HL diagnosis was 51. Male/female ratio was 41/35.

Results: In 37 cases HL was diagnosed first, in 17 simultaneously (9 composite) and in 22 after NHL diagnosis. The mean interval between the two diagnoses was significantly longer when HL occurred first (130 vs 77 months; P=.03). Histology of NHL included diffuse large B cell (DLBCL) in 36, T-cell in 10, follicular (FL) in 10, lymphocytic/CLL (SLL) in 9, marginal in 5. DLBCL was very rarely diagnosed before HL (P<.0001), whereas SLL and FL rarely followed HL (P=.014) and T-cell lymphoma and HL were never diagnosed simultaneously (P=0.028). Nodular lymphocyte predominance HL (NLPHL) occurred in 13 cases (17%). Nodular sclerosis was diagnosed in 59% of classical HL. RS cells were CD30+ in 97% and CD15+ in 64% of classical HL evaluable cases. CD20+ was present in 100% of NLPHL, 0% of HL associated with a T-cell lymphoma and in 48% of the remaining evaluable cases. Clinical presentation of HL was more aggressive, with 46% of patients in advanced stage (III-IV). However complete response rate (CRR) in HL was 81% and the overall response rate (ORR) 90% in 69 evaluable cases. Relapse occurred in 16 cases (21%). There were no differences in presentation, response and outcome according to the timing of HL diagnosis. Nineteen patients died. The most frequent cause of death was NHL followed by HL. With a median follow-up of 9,3 years, the overall 10-year survival rate was 74.5%+/-7%. It was 80%+/-9% when HL was diagnosed before and 66%+/-12% when HL was diagnosed after NHL; it was 79%+/-13% in simultaneous cases.

Conclusions: The occurrence of HL and NHL in the same patient is not rare. HL patients developing also NHL are older, have more advanced disease, but their response to treatment and overall prognosis seem not worse than in patients with HL as single lymphoma. Some distinctive clinicopathological associations as well as the higher frequency of CD20+ on RS cells deserve further studies.

204 IMMUNOHISTOCHEMICAL EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR A AND ITS RECEPTOR KDR IN CLASSICAL HODGKIN LYMPHOMA

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New vessel growth is a highly complex process involving a number of angiogenic molecules. The vascular endothelial growth factor (VEGF) and its receptor KDR play a key role in physiological angiogenesis and in controlling tumor growth. Both molecules have been demonstrated in Hodgkin/Reed-Sternberg (HRS) cells in cases of classical Hodgkin Lymphoma (CHL). The aim of the present study was to assess the immunohistochemical expression of these proteins, primarily in HRS cells, but also in their microenvironment. Moreover, we wanted to correlate their expression to a number of pre-therapeutic clinico-pathological features along with outcome-related parameters in a large cohort of previously untreated CHL patients. Using tissue microarrays, the HRS cell expression of VEGF and KDR was quantified in pre-therapeutic, paraffin embedded tumor tissue samples of 246 CHL patients. The expression of VEGF and KDR was assessed using a semi-quantitative approach. Clinical data were obtained from clinical records. HRS cells were found to stain positively for VEGF and KDR in the majority of the cases (69% and 98%, respectively). VEGF was also expressed by non-neoplastic cells in the tumor microenvironment e.g. tumor-infiltrating macrophages, endothelial cells, fibroblasts, plasma cells and lymphocytes. Expression of VEGF and KDR did not have prognostic influence in this cohort, but interestingly VEGF positivity correlated with the presence of EBV in the neoplastic cells (p<0.001). The present study is the first to demonstrate the co-expression of VEGF and KDR and the association between VEGF and EBV positivity in a large cohort of CHL patients. Further studies are needed in order to establish the biological and prognostic significance of these findings along with the therapeutic target potential of VEGF in CHL.

205 TOTAL NUMBER OF B CELLS, BUT NOT T CELLS AT DIAGNOSIS IN THE CLASSICAL HODGKIN LYMPHOMA MICROENVIRONMENT IS PREDICTIVE OF SURVIVAL

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Background: The non-malignant immune infiltrate comprises the bulk of pathological tissue in classical Hodgkin lymphoma (CHL). This microenvironment has the potential to induce both malignant cell suppression and support. We have previously confirmed the impact of regulatory T cell and macrophage infiltrate on outcome in classical Hodgkin lymphoma (CHL). A score combining the markers was a more powerful predictor of outcome than either alone. We have gone on to test the influence of overall T and B cell numbers to further characterize the interaction between tumor and microenvironment.

Methods: Formalin-fixed paraffin-embedded tissue was available for 122 patients: median age 30 (18-80), 65% male, 71% advanced stage, median follow up 16.5 (2-40) years. Triplicate cores were made avoiding fibrotic or a cellular portions, arrayed and stained for CD4, CD8, CD20 and CD21. Absolute numbers of CD4+, CD8+ and CD20+ cells were counted across a total of 50hpf equivalents using automated image analysis confirmed by expert histopathologists. Interfollicular CD20+ cells were counted by excluding areas with intact CD21+ follicular dendritic cell meshwork.

Results: High interfollicular CD20 expression is associated with superior OS (at 5 years 87% vs 70%, p=0.003) with a trend to benefit in disease specific survival (DSS: at 5 years 88% vs 74%, p=0.09). The OS benefit became more evident with long follow up, with OS at 10 years 84% vs 52% and at 20 years 76% vs 43%. No significant association of overall CD4 or CD8 cell infiltrate on prognosis was found.

Conclusions: Failing to confirm prognostic impact of total CD4 cells despite significance of FOXP3 expression strengthens the hypothesis that the nature of the T cell infiltrate, not merely total number confers the benefit. This will be explored by immunostaining for other T cell subsets. The association between CD20 and OS becoming more evident with longer follow up suggests a complex B cell interaction with the disease at the stage of initial therapy as well as in relapse, salvage and late effects which will be further tested by multivariate analysis.

206 THE PROGNOSTIC VALUE OF BIOLOGIC MARKERS IN CLASSICAL HODGKIN LYMPHOMA (CHL) PATIENTS

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Background: Many attempts have been made to identify CHL patients destined to have a poor response to therapy. So far, early-interim PET-scan (IP) after 2 ABVD courses represents the most effective predictor of the treatment outcome.

Materials and Methods: We analyzed the prognostic impact of a series of histological and immunohistochemical parameters on tissuemicroarrays (TMAs) from 209 CHLs and compared it to the IP predictive value. In particular, we tested 21 markers previously reported to have prognostic value in DNA and TMAs studies (STAT1, PCNA, SAP, TOP2A, RRM2, CDC2, MAD2L1, ALDH1A1, CD68, and BCL11a, CD20, EBER, BCL2, p53, PD1, FOXP3, CD68PGM1, CD163, TIA1, Granzyme B, Perforin). The molecules were assessed in both neoplastic (HRSC) and microenvironmental cell (MEC) components. All patients had been treated with standard ABVD ± Rx therapy.

Results: The median age was 32 yrs (4-80), the stage IV in 32 patients, III in 50, II in 118, and I in 9. The mean FU was 52.3 months (3-93.7). Histopathology review showed: NS1 in 93 cases, NS2 in 37, NS syncytial variant in 8, NS cellular phase in 9, NS-NOS in 10, MC in 29, LD in 3, LR in 1, and CHL-NOS in 16. IP was positive in 37 patients (18%), while treatment failure was recorded in 49 (23,4%). In univariate analysis, the factors related to OS were: NS2/NS syncytial/LD histology (p .0227); BCL2 on HRSC and IP (p .000). NS2/NS syncytial/LD histology, p53 on HRSC, PD1 on MC, stage and IP turned out to be significantly related to a worse PFS (p .0094, .000, .0135, .0018, .0194, .000 respectively). In multivariate analysis, including all parameters significant at univariate analysis, BCL2 and IP maintained their prognostic value on OS

(p .0121 and .000 respectively) and P53, stage and IP on PFS (p .003, .000, 0.000 respectively). Restricting analysis to IP negative cases, expression of BCL11a on HRSC and CD163 on MC significantly correlated to treatment failure, and were included with IP in a new predictive model; the latter demonstrated a higher capability, than IP alone, to predict the treatment outcome (test misclass error 12,98% vs 13,94%).

Conclusion: These preliminary findings demonstrate the potential of biomarkers, in combination with IP, to foresee outcome in CHL. Statistical analyses are ongoing, aimed at constructing a algorithm predicting poor response to therapy: the result of these studies will be presented at Meeting.

207 CLINICAL SIGNIFICANCE OF BIOMARKERS OF DIFFUSE LARGE B-CELL LYMPHOMA IN THE RITUXIMAB ERA

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Introduction: The prognostic factors of diffuse large B-cell lymphoma (DLBCL) in the rituximab era are different from previously reported prognostic factors for DLBCL. We previously reported that a high nm23-H1 level was associated with poor prognosis in patients with DLBCL. We compared the prognostic factors of DLBCL between patients who received chemotherapy alone and those who received rituximab combined with chemotherapy (R-chemotherapy).

Patients and Methods: The subjects were 200 DLBCL patients who underwent R-chemotherapy and 146 DLBCL patients who underwent chemotherapy alone in whom markers could be analyzed. We evaluated CD5, CD10, BCL2, BCL6, MUM1, and nm23-H1 expression by immunohistochemistry.

Results: There were no significant differences in clinical characteristics between the chemotherapy alone group and R-chemotherapy group. The relationships between immunohistological markers and outcome among the patients with DLBCL who received chemotherapy alone were studied. CD5, CD10, and BCL6 had no prognostic impact on 5-year overall survival (OS) and progression-free survival (PFS) rates. When the patients were divided into the GCB and non-GCB groups, the 5-year OS of the GCB (n=71) and non-GCB (n=75) groups was 82% and 52%, respectively (p=0.0003). The 5-year OS rate of the BCL2-positive and -negative groups was 59% and 78%, respectively (p=0.008). As for nm23-H1 expression in DLBCL among patients who received chemotherapy alone, the 5-year OS of the nm23-H1-positive and nm23-H1-negative groups was 36% and 92%, respectively (p=0.0001). Next, patients who received R-chemotherapy were examined. CD5, CD10, BCL2, BCL6, and MUM-1 had no prognostic impact on 5-year OS and PFS rates. When the patients were divided into the GCB (n=91) and non-GCB (n=107) groups, the 5-year OS of the GCB group was 76% and that of the non-GCB group was 73%, showing no significant difference. The 5-year PFS of the GCB and non-GCG groups was 71% and 70%, respectively, showing no significant difference. In the rituximab era, BCL2, MUM1 and non-GCB were not prognostic factors. As for nm23-H1 expression in DLBCL, the 5-year OS of the nm23-H1-positive and nm23-H1-negative groups was 65% and 97%, respectively (p=0.001).

Conclusions: Among patients with DLBCL who underwent R-chemotherapy, patients with nm23-H1 expression had a significantly poorer prognosis than patients without nm23-H1 expression. These results suggest an important role for nm23-H1 in malignant progression and a potential therapeutic target for DLBCL.

208 DISRUPTION OF TP53 AND MIRN34A/B/C PREDICTS POOR PROGNOSIS IN DIFFUSE LARGE B-CELL LYMPHOMA

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In chronic lymphocytic leukemia it was recently suggested that miR34a expression could be used as a surrogate marker for TP53 disruption, and thus be an indicator of poor prognosis. In a complex circuit, p53 promotes transcription of the genes in the miR34 family (MIRN34A, and MIRN34B/C), which will then act as mediators of p53 signaling. All three miR34's are predicted to prohibit cell cycling at the G1/S transition checkpoint by targeting the mRNAs of several proto-oncogenes that counteract the p16 and ARF/p53 pathways including N- and C-MYC, CDK6, CDK4 and CCND1 (CyclinD1). Here, we have studied the combined status of TP53 mutations and MIRN34A and MIRN34B/C promoter hypermethylation in a total of 90 newly diagnosed DLBCL cases by using a panel of PCR based methods. Alterations of one or more of these genes were detected in 82 cases (91%). TP53 was mutated in 15 (17%), MIRN34A was methylated in 26 Cases (27%) and MIRN34B/C was methylated in 72 (80%) cases. All cases with methylation of MIRN34A also carried methylation of MIRN34B/C. Six cases carried combined TP53 mutation and MIRN34A/B/C methylation. The Kaplan-Meier estimate of overall survival for cases with methylation of MIRN34B/C did not differ from cases with unmethylated MIRN34B/C (P=0,81). Both mutation of TP53 and methylation of MIRN34A were borderline significant (P=0.077 and P=0.094, respectivly). Since TP53 mutation and MIRN34 methylation has been suggested to be mutually exclusive, we combined cases with either TP53 mutation and/or MIRN34A methylation. This group showed a poorer survival than cases with no alterations (median survival 5,6 vs 9,8 years, P=0,07). However, the 6 cases with disruption of both TP53 and MIRN34A/B/C methylation seemed to do even worse (median survival 1,4 years, P= 0.01). Our results suggest that the compound status of TP53 and MIRN34A/B/C may be a major determinant of outcome in DLBCL, and that the ''double-hit'' cases may be the most aggressive tumors with no response to current treatment strategies.

209 DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS PRESENT IMPAIRED NK CELL PHENOTYPE AT DIAGNOSIS

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Introduction: Diffuse Large B-Cell Lymphoma (DLBCL) is the most common subtype of aggressive non-Hodgkin's lymphoma. The treatment is based on a combination of chemotherapy and anti-CD20 antibody acting via complement and antibody dependent cytotoxicity (ADCC) and programmed cell death. Natural Killer (NK) cells are innate immune effector cells divided in two $CD56^{bright}$ and $CD56^{dim}$ sub-populations. They are both primarily involved in immunosurveillance to eliminate transformed cells. $CD56^{bright}$ NK cells are more efficient to produce inflammatory cytokines whereas $CD56^{dim}$ cells are more capable of either natural cytotoxicity or ADCC. The aim of our project was to examine the phenotype of circulating NK cells in DLBCL patients at diagnosis.

Patients and Methods: Frozen PBMC samples from age matched DLBCL patients at diagnosis (n=39) and healthy controls (HC, n=16) have been collected. Phenotypes of NK populations have been characterized by flow-cytometry.

Results: The balance between the $CD56^{bright}$ and $CD56^{dim}$ subpopulations was not modified in patients as compared to HC. However, the levels of expression of some NK receptors were disturbed on one or both subpopulations. $CD56^{bright}$ cells were moderately modified with a down-regulation of the activating NK receptors NKp46 and DNAM-1. By contrast, the $CD56^{dim}$ subset showed an impaired expression of DNAM-1 but an over-expression of the inhibitory C-type lectin heterodimer CD94/NKG2A.

We also analyzed the expression of molecules involved in antibody mediated immunotherapy: the CD16 (FcγRIII) receptor because of its role in the ADCC, and CD137, a potential and controversial new target to modulate the immune response against lymphoma. Our results showed that both molecules were down-regulated on the $CD56^{dim}$ subset as compared to HC.

Interestingly, patients with early relapse or refractory to rituximab-CHOP combination presented a higher expression of CD137 whereas CD16 was not significantly associated to the clinical outcome.

Conclusions: This study demonstrates for the first time a disturbed phenotype of NK cell subpopulations in DLBCL patients at diagnosis. The impaired expression of CD16 and CD137 may play a role in the response to antibody-mediated immunotherapy strategies.

210 CYTOGENETIC FEATURES OF CD5-POSITIVE DIFFUSE LARGE B-CELL LYMPHOMA: UPDATED RESULTS FROM A MULTICENTER, RETROSPECTIVE STUDY WITH 76 PATIENTS

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Background: CD5-positive (CD5+) diffuse large B-cell lymphoma (DLBCL) is known as an immunohistochemical subgroup of DLBCL in the 2008 WHO classification. We reported cytogenetic features in 23 patients with CD5+ DLBCL previously (Gene Chromosomes Cancer 2005). With the revision of the WHO classification, we conducted a multicenter, retrospective study to investigate chromosomal changes in a larger number of patients with CD5+ DLBCL.

Materials and Methods: We collected 76 patients with CD5+ DLBCL diagnosed according to the 2008 WHO classification. No patients were included in the present study who were examined previously. Intravascular large B-cell lymphoma (IVLBCL), primary DLBCL of the central nervous system, evident secondary CD5+ DLBCL, and cyclin D1+ cases were excluded from the study population.

Results: Eleven patients (14%) had polyploid clones around 4n. The major chromosomal breakpoints were 19p13/q13 (36%). Although a substantial number of patients had 3q27 and 17p11 abnormalities, chromosomal aberrations affecting 8p21 or 11q13 were rarely observed, which were different from our previous results. A small number of cases having a translocation with 18q21 or 8q24 were intermingled in the current study population.

Conclusions: Our current study updates the specific cytogenetic features of CD5+ DLBCL based on the 2008 WHO classification. A decreased incidence of abnormalities with 8p21 may be caused by an elimination of IVLBCL. The high incidence of 19p13/q13 abnormalities may play a key role to elucidate molecular mechanisms underlying clinicopathologic features of CD5+ DLBCL.

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211 MARKERS OF INITIAL TREATMENT RESPONSE IN GCB VERSUS NON-GCB DIFFUSE LARGE B-CELL LYMPHOMA

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Introduction/Background: Diffuse Large B- Cell Lymphoma (DLBCL) is a heterogeneous disease that does not always respond to standard treatment, i.e. R-CHOP. Genetic profiling studies have shown that gene expression in DLBCL varies and that tumors can be subdivided into at least two biologic subgroups, GCB and non-GCB. Overall, GCB tumors have a better prognosis than non-GCB tumors. This study explores which genes are differentially expressed in patients who achieved complete remission (CR) versus in patients who did not (non-CR) for both GCB and non-GCB DLBCL.

Materials and Methods: 205 *de novo* DLBCLs were included in this study. RNA from all tumors was purified from formalin fixed, paraffin embedded diagnostic biopsies. Eight samples were excluded due to poor quality of RNA. All samples were categorized as GCB or non-GCB using the Hans algorithm. All patients received R-CHOP and their treatment responses were recorded as CR or non-CR (post-therapy status). The expression of 29 potentially prognostic genes and three normalizer genes (*GUSB, TBP, ABL*) were studied by qRT-PCR using Taqman Arrays®. Results were analyzed for differential gene expressions with dedicated qRT-PCR software (RealTime StatMiner®). A fold change >1.5 in gene expression was considered a "real" difference. P-values <0.05 were considered significant.

Results: Valid results were obtained from 198 tumor samples. One sample was excluded due to an undetermined treatment response. Within the GCB subset (n= 119), CR was associated with a relative 1.5 fold overexpression of *ACTN1* (P=0.01). *BCL2* was overexpressed (2 fold) in the non-CR tumors (P=0.03). Within the non-GCB subset (n= 78), CR was associated with a relative 2 fold overexpression of *LMO2* (P=0.03) and *PLAU* (1.8 fold, P<0.01) whereas *FOXP1* was overexpressed (1.5 fold) in the non-CR tumors (P=0.05).

Conclusion: Our findings of *LMO2*, *ACTN1* and *PLAU* overexpression being markers of good treatment response and overexpression of *BCL2* and *FOXP1* markers of a poor response, is in good accordance with previous studies, although the impact of *BCL2 has* been under debate. Further analysis of these genes' association with survival data are pending. The results presented here, however, emphasize that GCB and non-GCB tumors are biologically different and that genes associated with a good or bad prognosis need to be tested in DLBCL subtypes separately.

212 HOMOZYGOUS FCGR3A-158V ALLELES PREDISPOSE TO LATE ONSET NEUTROPENIA AFTER CHOP-R FOR DIFFUSE LARGE B-CELL LYMPHOMA.

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Introduction: The mechanism of action of rituximab is in part through antibody dependent cellular cytotoxicity and complement mediated lysis. Genetic polymorphisms of FCGR3A-158V/F and C1qA-276A/G respectively, may impact function. The high-affinity FCGR3A-158V allele (more common in Asian populations) correlates with the incidence of late-onset neutropenia (LON) after CD34+ enriched B-cell depleted autologous stem cell transplant. The influence of FCGR3A-V158F genotype on LON is unknown in Diffuse Large B-cell Lymphoma (DLBCL) treated with the 'gold-standard' combination chemo-immunotherapy regimen 'CHOP-R'. To date there is no data on C1qA polymorphisms.

Patients and Methods: 82 DLBCL patients treated at our institute with CHOP-R were typed for the FCGR3A and C1qA polymorphisms using allele-specific PCR. Cases investigated were chosen on the availability of tissue for PCR but were otherwise unselected. Maintenance rituximab was not administered. 100 healthy Caucasian controls were tested. Event free and overall survival (EFS and OS) and LON incidence was analysed for linkage to either polymorphism.

Results: The distribution of FCGR3A-158 (V/V 8%, V/F 46%, F/F 46%) patients versus (V/V 8%, V/F 48%, F/F 44%) controls and C1qA-276 (A/A 43%, A/G 51%, G/G 6%) patients versus C1qA-276 (A/A 40%, A/G 46%, G/G 14%) controls was equivalent (p=NS). Polymorphisms of FCGR3A or C1QA did not influence EFS or OS in our cohort. Notably, all patients possessing FCGR3A-158 V/V were long term survivors compared to 70% for F/F but this was not significant. There was a significantly increased risk with the FCGR3A but not the C1qA polymorphism of developing LON. Only 3% of patients possessing FCGR3A-158 F/F developed LON compared to 50% FCGR3A-158 V/V (p=0.003). Consistent with this, there was increased susceptibility in LON between FCGR3A-158 V/V and V/F (p=0.036) but not between the V/F and F/F patients.

Conclusion: In DLBCL treated with CHOP-R chemo-immunotherapy, C1qA polymorphisms do not predict outcome or LON. Larger studies are required to definitively determine the impact of FCGR3A on outcome. The high-affinity FCGR3A V/V polymorphism is associated with a highly increased susceptibility to the development of LON. Polymorphic analysis may be a predictive tool to identify those at high-risk of rituximab induced LON.

213 FAVORABLE OUTCOME IN YOUNG PATIENTS WITH NON-GERMINAL CENTRE DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) TREATED WITH R-ACVBP AS COMPARED TO R-CHOP. A GELA STUDY.

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Microarray analysis has shown that patients with DLBCL expressing a gene expression profile of germinal centre B cells have a longer survival than those with an activated B-cell profile, when treated with R-CHOP. Hans algorithm using immunohistochemistry correlates well with gene expression data and has demonstrated in some studies clear survival differences between germinal-centre (GC) and non-germinal centre (n-GC) B-cell DLBCL groups treated with R-CHOP. We undertook an immunohistochemical study on tissue microarray (TMA) in a subgroup of patients aged 18 to 59 years with aaIPI 1 included in the GELA trial LNH 03-2B that compared R-ACVBP intensified immunochemotherapy and R-CHOP. This trial demonstrated an improvement of survival among R-ACVBP-treated patients. Our goal was to evaluate survival in the different arms of the trial according to Hans algorithm.

We analyzed by immunohistochemistry the expression of CD10, BCL6 and MUM1 and classified patients as GC or n-GC according to the Hans algorithm. Among the 380 patients enrolled in this study, 171 DLBCL cases were present on the TMA and available for Hans algorithm classification.

91 patients were classified as GC and 80 as n-GC. 86 patients were treated by R-ACVBP and 85 by R-CHOP. Progression-free survival (PFS) and Overall Survival (OS) were not different between the GC and n-GC profile among the whole population (*P*= .94, *P*= .65, respectively). There was no difference in PFS and OS between R-ACVBP and R-CHOP in GC patients (*P*= .61, *P*= .52, respectively). PFS and OS were significantly longer among n-GC patients treated by R-ACVBP compared to R-CHOP (*P*= .005, *P*= .002, respectively).

This study suggests that patients with non-GC immunophenotype may preferentially benefit from intensified immunochemotherapy as compared to R-CHOP.

214 CD10 AND ICOS EXPRESSION BY MULTIPARAMETRIC FLOW CYTOMETRY IN ANGIO-IMMUNOBLASTIC T-CELL LYMPHOMA

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Angioimmunoblastic T-cell lymphoma (AITL) is immunologically defined by the expression of CD10 and follicular helper T-cells (TF_H) markers such as CXCL13, PD-1 and ICOS. The frequent blood dissemination in AITL is in the most cases easily attested

by CD10 expression and may allow a rapid orientation to the diagnosis. However the CD10 expression by neoplastic T-cells may be absent justifying the search of other markers. Among a series of 20 AITL cases histologically proven, the expression of CD10, ICOS and PD-1 were studied in 15 lymph node (LN) and 13 peripheral blood (PB) specimens by multiparametric (8-colour) flow cytometry in addition to usual pan-T markers. In this series, all cases were CD4+ but one (CD4-/CD8-). In 10/20 cases, an atypical T-cell profile by the lack of surface CD3 (n=8) or the dim CD7 expression (n=3) was observed. A T-cell clone was identified by PCR in 18/19 availables cases (including PB and LN with and without abnormalT-cell profile). The CD10 expression was present in 14/15 (93%) LN and in 10/13 (77%) PB cases and ICOS in 13/15 (87%) LN and in 6/13 (47%) PB cases, whereas neither significant CD10 nor ICOS T-cells were identifeid in the control group (LN with reactive hyperplasia=10, PB of healthy donors=15). PD-1 expression was less informative as observed in AITL as in control cases. Furthermore, when the presence of neoplastic T-cells was demonstrated by atypical T-cell profile such as lack of surface CD3, this sensitive approach suggests that neoplastic T-cells could be composed of distinct immunological subsets: CD10+/ICOS+, CD10+/ICOS-, CD10-/ICOS+ and CD10-/ICOS-. Among the 8 pairs of LN/PB studied at the same time, 7/8 were CD10+ in both LN and PB and among these 7 cases, 6/7 (87.5%) were ICOS+ in LN, but only 2/7 (28%) were ICOS+ in PB. This multiparametric approach allowed us to confirm the frequent blood dissemination in AITL and to show that ICOS+ neoplastic T-cells whatever CD10+ or CD10- have a less tendency to disseminate in blood than ICOS- T-cells. Consequently, if ICOS expression, easily detected by flow, constitutes an useful additional feature for AITL diagnosis in LN, the study of a larger series in PB is necessray for precise evaluation of its value in the detection of circulating neoplastic T-cells.