''Focus on...'' session: immunodeficiency and lymphoma

049 GENOMIC COPY NUMBER ABERRATIONS (CNA) AFFECTING THE OUTCOME OF IMMUNODEFICIENCY-RELATED DIFFUSE LARGE B-CELL LYMPHOMAS (ID-DLBCL)

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Introduction: Both DLBCL originated in HIV-positive subjects (HIV-DLBCL) and post-transplant DLBCL (PT-DLBCL) differ in biology and genetics from DLBCL in immunocompetent individuals (IC-DLBCL) in terms of biology and genetics, and remain poorly understood. Aim: to evaluate the prognostic impact of the genomic CNAs in ID-DLBCL.

Patients and Methods: Minimal common regions (MCR) were estimated across a large dataset of 533 genomic profiles obtained with Affymetrix Human Mapping 250k Nsp I arrays from mature B-cell lymphoid neoplasms, including 166 IC-, 28 HIV-and 44 PT-DLBCL. MCR occurring in at least 3 patients (pts) with available follow-up data underwent univariate analysis (36/44 PT-and 19/28 HIV-, and 149/166 IC-DLBCL). A rituximab-containing regimen was used in 4/36 PT-, 1/19 HIV-, and 148/149 IC-DLBCL; 10/19 HIV+ pts had received HAART therapy, three before DLBCL diagnosis and 7 concomitantly with chemotherapy.

Results: 7 MCRs showed a statistical impact on OS in PT-DLBCL [+1q21.2-q42.3, +1q25.2, +1q44, +1q, del(15q12), +18p11.21-q23, del(18q23)] and 4 in HIV-DLBCL [del(18q23), +2p23.1, +2p16.3, del(3p14.2)]. Gains at 1q and 18q were associated with a poorer OS only in PT-DLBCL (P 0.008 & 0.001, respectively). 4/5 1q+cases were kidney recipients (P 0.06), but the latter condition was not, by itself, associated with worse OS. +1q or +18q identified a group with very poor outcome (P<0.0001). Del(3p14.2), including FHIT spanning the fragile site FRA3B, and gains at 2p were associated with a poor OS only in HIV-NHL (P 0.036 & 0.049, respectively). These lesions were independent of EBV status. Del(3p14.2) or +2p23.1 identified a group of HIV-DLBCL with a poor outcome (P 0.0072). Losses at 18q23, which includes CTDP1, were associated with poorer OS in both HIV- (0.006) and PT-DLBCL (P<0.0001), but not in IC-DLBCL. Del(15q12), ATP10A, was associated with a poor outcome in both PT- (P 0.044) and IC- DLBCL (P 0.037), while this lesion was not detected in HIV-DLBCL.

Conclusions: Genomic regions affecting outcome differ between ID- and IC-DLBCL and between HIV- and PT-DLBCL.

050 METHYLOME PROFILING OF POST-TRANSPLANT DIFFUSE LARGE B-CELL LYMPHOMAS (PT-DLBCL)

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Background: PT-DLBCL show clinical and biological features that distinguish them from DLBCL arising in immunocompetent individuals (IC-DLBCL), but genetic alterations underlying these differences remain poorly understood.

Aim: To evaluate the possible role of methylome aberrations in PT-DLBCL pathogenesis.

Material and methods: We compared the genome-wide promoter methylation status in 24 PT-DLBCL versus 10 de novo non-GCB IC-DLBCL using the Illumina Infinium Human Methylation27 arrays. Six normal peripheral CD19+ B-cells were also included as control group. Methylation beta values ranged from 0 (="not methylated") to 1 (="fully methylated''). Differentially methylated probes were identified by a t-test with multiple test correction (q<0.10, delta beta value >0.20).

Results: The methylome of PT-DLBCL and IC-DLBCL was highly enriched of PRC2/EED-EZH2 H3K27 trimethylating complex genes, whereas no specific biological pathway was identified (GSEA and DAVID functional annotation tools). Comparison of the methylation profile of PT-DLBCL and IC-DLBCL identified 78 CpG sites with a higher, and 231 probes a lower methylation in PT-compared with IC-DLBCL. Of the probes with higher methylation in PT-DLBCL, 27 were significantly more methylated in PT-DLBCL than in normal B-cells. These included the promoter region of SNX9 (sorting nexin 9), TIRAP (Toll-interleukin 1 receptor domain-containing adaptor protein isoform a), DUSP8 (dual specificity phosphatase 8), LHX3 (LIM homeobox protein 3 isoform a) and ATBF1 (AT-binding transcription factor 1). Methylation occurring at non-CGI CpG sites is considered to play a role in tissue-identity and is regulated differently from CGI-methylation. Analysis of non-CGI CpG sites allowed the identification of 66 probes with a higher, and 491 probes a lower methylation in PT- compared to IC-DLBCL. Also, 1,843 probes were preferentially unmethylated in PT-DLBCL versus normal B cells, whereas only 83 probes were more methylated in PT-DLBC than in normal B-cells, in accordance with the general hypomethylation of non-CGI CpG sites in tumor.

Conclusions: Promoter regions affected by aberrant methylation differ between PT- and IC-DLBCL, suggesting that specific genes are epigenetically silenced in PT-DLBCL and potentially involved in their pathogenesis.

051 EBV-POSITIVE AND EBV-NEGATIVE POST-TRANSPLANTATION LYMPHOPROLIFERATIVE DISORDERS: TWO DIFFERENT DISORDERS?

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Introduction: Patients receiving immunosuppressive therapy after organ-transplantation are at risk to develop a Post-Transplantation Lymphoproliferative disorder (PTLD), in most cases of B-cell type and indistinguishable from diffuse large B-cell lymphoma (DLBCL). Epstein Barr Virus (EBV) has been linked to the pathogenesis in most, but not all cases and gene expression profiling (GEP) studies comparing EBV+ and EBV- PTLD show discordant results.

Materials and Methods: To study the pathogenesis of both EBV+ and EBV- PTLDs, we reviewed in 145 biopsy proven PTLDs the clinical and morphological characteristics. From this series, we selected 70 cases for molecular genetic analysis (Affymetrix Cytogenetics Whole-Genome 2.7M arrays and HU133A Plus 2.0 GeneChips). In addition 15 DLBCL control cases, were studied.

Results: on the first 30 PTLDs show that:

(i) in an unsupervised principal component analysis the EBV+ PTLD segregate into two groups at either side of the EBV- PTLD, the latter clustering with the DLBCL cases.

(ii) The two EBV+ PTLD groups show a different morphology and IHC profile.

(iii) One EBV+ group displays a GEP signature of germinal centre derived B-cells, while the other EBV+ group displays a signature of activated B-cells and shows upregulation of genes indicative for an innate immune response to viral infection, a tolerant immune response and upregulation of PDGFRA.

(iv) Comparison of the EBV- PTLD group with DLBCL identifies a gene set, that also separates the EBV+ from DLBCL.

(v) Cytogenetic data reveal complex karyotypes including translocations involving CMYC and BCL2.

Conclusions: The identification of two morphologically and genetically different EBV+ PTLD groups explains discordancies in previously published GEP results. The identification of an EBV-unrelated gene set differentially expressed between DLBCL and PTLD, indicates the existence of a non-EBV related pathogenetic mechanism of PTLD. We hope our integrated genomic profiling method will give insights in the identification of involved pathways.

052 THE ACCURACY OF PET IN THE DETECTION OF POSTTRANSPLANT LYMPHOPROLIFERATIVE DISORDER (PTLD)

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Introduction: The often difficult diagnosis and the urgent nature of PTLD frequently leads to treatment initiation when PTLD is suspected, even before biopsy confirms the diagnosis. We investigated the accuracy of PET in 165 patients with suspected PTLD.

Material and Methods: We retrospectively reviewed all patients in our center between 2003 and 2009 who had a PET-scan for the indication PTLD. After elimination of all patients in which the original PET-images were lacking or no clear diagnosis could be established, 176 PET-scans in 157 patients were eligible for evaluation (11 and 4

patients had two and three episodes of suspected PTLD respectively). In 50 cases, the patient had already a bioptic proof of PTLD before PET-scanning and PET was merely performed for *staging* of the disease. In the remaining 126 PET-scans, PET was performed to *differentiate between PTLD and other diseases*. Clinical symptoms and EBV increase were recorded (see also D. Dierickx, related abstract). PET-scans were blindly scored on a four-point scale (negative, probably no PTLD, preference for PTLD, definitely positive for PTLD). The first two scores were grouped as negative in the final analysis, preference for PTLD was considered positive. Results were compared with biopsy as the gold standard.

Results: Of the 176 cases, 65 turned out not to be associated with PTLD but with other diseases (mainly infections). In 95 patients, biopsy proof was available. The other 16 patients were diagnosed as PTLD based on clinical grounds. 93 of the 176 PET-scans, (53%) were scored as positive for lymphoma by the nuclear physician, and 83 (47%) were considered negative. We found a sensitivity of PET of 86%, specificity of 93%, positive predictive value (PPV) of 95% and negative predictive value (NPV) of 83%. In a subanalysis of the 126 scans performed for differential diagnosis PTLD versus other diseases, sensitivity, specificity, PPV and NPV were 86%, 94%, 92% and 89% respectively. FDG-uptake in PTLD was generally high with a mean and maximal standardized uptake value (SUV) of 9.0 [range 2.0-18.6] and 17.4 [range 2.6-26.4]. PTLD had often an atypical presentation on PET (diffuse lung involvement, gastro-intestinal lesions,. . .) and differentiation with other (mainly inflammatory) diseases is not always evident.

Conclusions: From these data, we can conclude that PET is highly sensitive for the detection of lesions of PTLD, and that PET has an excellent ability to differentiate PTLD from other diseases.

053 ADAPTED MANAGEMENT OF EBV REACTIVATION AFTER SOLID ORGAN TRANSPLANTATION: AN EFFECTIVE PREVENTION OF POST TRANSPLANTATION LYMPHOPROLIFERATIVE DISORDERS (PTLD)? RESULTS OF THE LARGEST PROSPECTIVE STUDY ON 251 PATIENTS, AND COMPARISON WITH 820 TRANSPLANTED CONTROLS

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Background: EBV reactivation represents a major predictive factor for PTLD, especially during first years after transplantation, but there is no consensual attitude in this situation.

Aim: We conducted a monocentric prospective study on all new heart transplanted patients. EBV viral load (EVL) was systematically followed and confirmed reactivations were treated depending on viral load.

Methods: Heart transplanted patients who had at least one EVL between January 2004 and December 2008 were included. Immunosuppression consisted on anti-lymphocyte sera, ciclosporin, mycophenolate-mofetyl and prednisone. At least one EVL per month was analysed. If the EVL was more than 10.5 copies/ml, a CT scan or a PET-san was performed in order to detect any PTLD and patients were treated by diminution of the immunosupression (DIS). One injection of Rituximab (R) (375 mg/m^2) was used in case of failure and/or if EVL was over 10.6 copies/ml. Control cohort consisted in patients transplanted in the same unit since the year of the first local diagnosis of PTLD (Jan 1987) until the beginning of this study (Dec 2003), alive one month after the transplantation.

Results: A total of 251 adult patients were included. All but 6 were EBV positive before the graft. Reactivations were detected in 37 cases (15%) and treated by DIS only in 28 cases, DIS followed by R in 4 and directly by DIS and R in 5. All EBV negative patients developed a primoinfection in the first year, 2 with an EVL over 105, one presented non documented hepatic lesions which disappeared after DIS. All EBV reactivations were controlled. With a median follow-up of 3 years, only one PTLD has been diagnosed (in a patient lost to follow up and taken in charge in an other unit). Neither EBV reactivation nor R treatment increased graft rejection (respectively p=0.37 and p=0.50). Control cohort represents 820 patients with 24 (1,8/year) PTLD, of which 13 were early PTLD (all EBV positive). Median OS is not reached in the two cohorts, but OS is improved after 2004 (p<0,001). Incidence of PTLD decreased but not yet with a statistical significance (p=0.155).

Conclusion: EBV reactivation after organ transplantation can be managed efficiently and safely by diminution of immunosupression and/or rituximab. Larger studies are necessary to confirm the trend towards diminution of PTLD incidence.

054 PRIMARY CENTRAL NERVOUS SYSTEM (CNS) POST-TRANSPLANT LYMPHOPROLIFERATION (PTLD): AN INTERNATIONAL REPORT OF 52 CASES IN THE RITUXIMAB ERA

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Background: Several solid organ transplant (SOT) PTLD series have shown that CNS involvement is a poor prognostic factor (eg, Leblond et al, JCO 2001; Evens et al, JCO 2010). However, these reports contained small CNS numbers and most series were in the pre-rituximab era.

Methods: We performed a retrospective analysis of CNS PTLD patients (pts) diagnosed from 1998-2010. Cases with secondary CNS localization were excluded. Fisher's exact test was used to compare cases.

Results: We identified 52 CNS PTLD pts; the median age was 52 years (20-74). All pts presented with CNS-related symptoms and 51 pts had abnormal findings on MRI showing a parenchymal brain lesion(s). The median time from SOT to PTLD was 31 months (5-504); the most common SOT was kidney (69%). CNS characteristics included: CSF involvement 14%, >1 parenchymal brain lesion 48%, and ocular involvement 4%. Table 1 shows clinical comparisons of CNS vs non-CNS PTLD cases. As part of initial treatment (Tx), the majority of pts had reduction in immunosuppression (RI). Additional Tx consisted of high dose methotrexate-based Tx 54%, rituximab with radiation (RT) 15%, RT alone 12%, rituximab 10% alone, RI alone 7%, and cytarabine in 2%; rituximab was used as a part of first-line Tx in 41% of pts. Notably, only 4% of pts experienced organ rejection, which were of mild grade. With a median follow-up of 41 months (1-134), the 3-year progression-free and overall survival for all pts were 29% (95%CI 16%-42%) and 33% (95%CI, 20%-46%), respectively. No significant survival differences were noted among different Tx subsets.

Table 1. CNS vs Non-CNS PTLD.

Characteristics	CNS (n=52)	Non-CNS (n=102)	P
Late PTLD (> 1 year)	75%	64%	0.0552
Monomorphic	87%	66%	0.0098
EBV+ tumor	94%	52%	0.0001
PS 2-4	52%	31%	0.025
Elevated LDH	54%	31%	0.0191

Conclusion: These data represent the largest report of CNS PTLD to date. CNS PTLD appears to represent a distinct biologic and clinical entity within the PTLD family. Outcome of CNS PTLD remains overall poor. Continued study of this subset of pts is critically needed.