P1
**HELIOS EXPRESSION AND FOXP3 TSDR METHYLATION OF IFNY+ AND IFNY− TREG FROM KIDNEY TRANSPLANT RECIPIENTS WITH GOOD LONG-TERM GRAFT FUNCTION**

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There is circumstantial evidence that IFNy + Treg might have clinical relevance in transplantation. IFNy + Treg express IFNy receptors and are induced by IFNy. In the present study we investigated kidney transplant recipients with good long-term stable graft function for the absolute cell counts of IFNy + Treg subsets and whether their expression of Foxp3 is stable or transient. Helios expression determined by eight-color-fluorescence flow cytometry and methylation status of the Foxp3 Treg specific demethylation region (TSDR) served as indicators for stability of Foxp3 expression. Methylation status was investigated in enriched IFNy + and IFNy- Treg preparations originating from peripheral blood using high resolution melt analysis. A total of 136 transplant recipients and 52 healthy controls were studied. Proportions of IFNy + Treg were similar in patients and healthy controls (0.05% and 0.04% of all CD4+ lymphocytes; p = n.s.). Patients also had similar absolute counts of IFNy producing Helios + and Helios- Treg (p = n.s.). Most of the IFNy + and IFNy- Treg in transplant recipients had a methylated Foxp3 TSDR, however, there was a sizeable proportion of IFNy + and IFNy- Treg with demethylated Foxp3 TSDR. Male and female patients showed more frequently methylated IFNy + and IFNy- Treg than controls. The data show increased levels of Treg subsets with stable as well as transient Foxp3 expression in patients with stable allograft acceptance compared to healthy controls.

P2
**ANGIOTENSIN II TYPE 1-RECEPTOR ANTIBODIES IN HIGHLY SENSITIZED RENAL TRANSPLANT CANDIDATES**

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Preformed before transplantation non-HLA antibodies (abs) are risk factors of acute rejection, appearance of de novo anti-HLA donor specific abs and poor graft survival with early graft loss. The aim of this study was to assess the incidence of angiotensin II type 1 receptor (AT1R) abs in highly sensitized renal transplant candidates (HSP, with PRAs > 70%) compared to patients awaiting renal transplantation without anti-HLA-abs (PRAs = 0%). Serum samples from 108 HSP (study group) and from 22 non-sensitized patients (control group-CG) were tested for AT1R-abs by ELISA (EIA-AT1R kit, One Lambda Inc). All sera were also tested for anti-HLA and anti-MICA antibody specificities by Single Antigen Bead Assay (Luminex, One Lambda Inc). Anti-AT1R-ab levels were classified as low <10 U/mL, at risk 10–17 U/mL and high > 17 U/mL. For anti-HLA and anti-MICA abs an MFI > 1000 was considered positive. Anti-AT1R-abs were detected in 41 HSP (37.9%) vs. 2 patients (9.1%) from the CG (p = 0.0057, Fisher exact p). The anti-AT1R-abs were detected either in intermediate (n = 29, 26.8%) or high levels (n = 12, 11%) with mean ± SD values of 12.4 ± 1.9 U/ml and 25.4 ± 10.5 U/ml respectively. The anti-AT1R-ab levels in the two positive patients from the CG were close to cut-off with mean ± SD values 11.2 ± 1.5 U/mL. Sensitization in the study group was due to previous transplantation (n = 78), pregnancies (n = 25) or transfusions (n = 78). The presence of anti-AT1R-abs was associated with pregnancies and/or graft loss. All HSP
with transfusions history only had anti-AT1R < 10 U/ml. Sixteen HSP had anti-MICA-abs (14.81%) vs. none from the CG (p = 0.04, Fisher exact p). The presence of anti-MICA-abs was significantly correlated with anti-AT1R-abs (p = 0.01, Fisher exact p). Highly sensitized renal transplant candidates have additional donor HLA risk factors before renal transplantation. Detection of anti-AT1R-abs helps assessment of pre-transplant immunological status of patients and force clinicians to establish therapeutic protocols in order to improve graft outcome.

P3

COMPARISON OF TWO LUMINEX-BASED SINGLE ANTIGEN ASSAYS FOR THE DETECTION OF ANTI-HLA ANTIBODIES AND THEIR COMPLEMENT VARIANT (C3D AND C1Q)

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Luminex based single antigen assays are widely used methods for defining anti-HLA specificities and their complement binding activity. The aim of the study was to compare two single antigen bead (SAB) assays in defining anti-HLA class I and II specificities and their complement binding capacity in association with Mean Fluorescence Intensity (MFI). We tested 30 pre-transplant sera from 30 different patients (2016) with PRA > 60%. All sera were analyzed by SAB for the presence of HLA class I and II antibodies with Immucor (IM) and One Lambda (OL). Positivity was defined according to manufacturer’s instructions. Analysis was followed by C3d and C1q assays. 769 class I and 612 class II specificities were totally assigned. The total concordance between IM and OL for class I and class II specificities was 65% and 48% respectively, with mean class I MFI values for IM and OL 9724 and 8946, respectively and mean class II MFI for IM and OL 8326 and 5524, respectively. 11% of class I specificities were detected only by IM with mean MFI 6317 and 23% only by OL with mean MFI 6409. 17% of class II specificities were detected only by IM with mean MFI 5524 and 35% only by OL with mean MFI 9009. Class I specificities were totally 44% C3d positive (mean MFI 16618) and 44.1% C1q positive (mean MFI 17134) and class II specificities were 66.7% C3d positive (mean MFI 19962) and 34.6% C1q positive (mean MFI 17938). From 489 class I specificities detected by both methods, 193 (39.5%) were both C3d and C1q positive, whereas 37 (7.6%) were only C3d positive and 43 (8.8%) only C1q positive. From 277 class II specificities detected by both methods, 104 (37.5%) were both C3d and C1q positive, whereas 82 (29.6%) were only C3d positive and 12 (4.3%) only C1q positive. OL detected more specificities in both class I and II than IM. Concordance was better in higher MFIs. Class II concordance was unexpectedly low compared to our previous results maybe due to lot differences. Higher rates of class II C3d positivity compared to C1q were observed totally and especially in concordant specificities.

P4

FREQUENCY AND PREDICTORS OF SUCCESSFUL STEROID WITHDRAWAL GUIDED BY SURVEILLANCE BIOPSIES

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Steroid withdrawal following renal transplantation is controversial and has to balance the risk of rejection against the risk of steroid-related side effects. The aim of this retrospective study was to investigate the frequency and determinants of successful steroid withdrawal guided by surveillance biopsies. We investigated 156 ABO-compatible HLA-DSA negative renal transplants receiving basiliximab induction and maintenance immunosuppression with tacrolimus-mycofenolate-stereoids. The absence of rejection in surveillance biopsies at 3 or 6 months post-transplant initiated steroid withdrawal, which was monitored by subsequent indication and/or surveillance biopsies. The primary outcome was the frequency of successful (i.e. rejection-free) steroid withdrawal at one year post-transplant. In the whole population, successful steroid withdrawal was achieved in 73/156 patients (47%). Steroid withdrawal was initiated in 98/156 patients (63%) having a rejection-free 3- or 6-months surveillance biopsy and was successful in 73/98 patients (74%). Subsequent clinical rejection occurred in only 198 patient (1%), whereas 2498 patients (24%) experienced sub-clinical rejection. Steroid withdrawal was not initiated in 58/156 patients (37%) mainly due to current or prior severe (Banff equal or greater than IA) sub-clinical rejection (64%). Prediction of successful steroid withdrawal by pre-transplant or early post-transplant parameters was poor. By multivariable analysis, the only significant predictor was the absence of clinical rejection within the first 3 months post-transplant (odds ratio 2.84; 95% CI 1.07-8.52; p = 0.04). In conclusion, (sub)clinical rejection-free steroid withdrawal can be expected in about half of pre-transplant HLA-DSA negative patients. As successful steroid withdrawal cannot be well predicted by pre- and early post-transplant parameters, guidance by surveillance biopsies is advisable.
P5
DONOR-SPECIFICITY BUT NOT BROADNESS OF SENSITIZATION IS ASSOCIATED WITH ANTIBODY-MEDIATED REJECTION AND GRAFT LOSS IN RENAL ALLOGRAFT RECIPIENTS

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Panel-reactive antibodies are widely regarded as an important immunological risk factor for rejection and graft loss. The broadness of sensitization against HLA is most appropriately measured by the ‘calculated population-reactive antibodies’ (cPRA) value. In this study, we investigated whether cPRA represent an immunological risk in times of sensitive and accurate determination of pre-transplant DSA. 527 consecutive transplantations were divided into four groups: cPRA 0% (n = 250), cPRA 1-50% (n = 129), cPRA 51-100% (n = 43), and DSA (n = 105). Patients without DSA were considered as normal risk and received standard immunosuppression without T-cell depleting induction. Patients with DSA received an enhanced induction therapy and maintenance immunosuppression. Surveillance biopsies were performed at 3 and 6 months. Median follow-up was 5.7 years. Among the three cPRA groups there were no differences regarding the one-year incidence of ABMR (p = 0.16) and TCMR (p = 0.75). 5-year allograft survival was similar and around 87% (p = 0.28). eGFR at last follow-up was 50–53 ml/min (p = 0.45). By multivariable Cox proportional hazard analysis, the strongest independent predictor for ABMR and (death-censored) graft survival were pre-transplant DSA. cPRA were not predictive for ABMR, TCMR, and (death-censored) graft survival. We conclude that with current DSA assignment the broadness of sensitization measured by cPRA does not imply an immunological risk.

P6
EXPRESSION OF CXCR3 MONOCYTES INCREASES SIGNIFICANTLY IN THE GRAFT BLOOD COMPARED TO PERIPHERAL BLOOD IN PATIENTS WITH STABLE RENAL FUNCTION

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We have recently reported that some lymphocyte populations do not maintain the same proportion in graft blood as in peripheral blood, despite a stable function of the transplanted kidney. These results suggest that the comparative study between leukocyte cells from the graft blood and those obtained in peripheral blood could provide information about the inflammatory state of the transplanted organ. In this work we selected the population of monocytes expressing CXCR3 to test this hypothesis. The study was performed by flow cytometry during the third, sixth and twelfth months after transplantation in 69 patients who received an isolated kidney transplant and the same immunosuppressive regimen. The peripheral blood sample was obtained by venipuncture and the graft blood by fine needle aspiration. We found a significant decrease in CXCR3+ monocytes throughout the first year of transplantation in peripheral blood (15.8 ± 20.7 vs. 12.6 ± 12.4 vs. 6.3 ± 9.0, p = 0.001), whereas the percentage of CXCR3+ monocytes in the graft blood did not change over this period. This situation resulted in a significant percentage difference between the CXCR3+ monocytes of the graft blood and those of the peripheral blood during the sixth (16.19 ± 9.54 vs. 12.6 ± 12.4, p = 0.027) and twelfth months (14.10 ± 8.94 vs. 6.3 ± 9.0, p < 0.001). We can conclude, therefore, that the significant percentage increase of CXCR3+ monocytes in the graft blood with respect to the peripheral blood suggests the presence of inflammatory activity despite having stable renal function during the second half of the first year after transplant.

P7
A SUCCESSFUL DCD STRATEGY WITH MINIMAL COLD ISCHEMIC TIME – A SINGLE CENTER REVIEW

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Belfast is the sole renal transplant center in Northern Ireland (population 1.8 million) and the local H&I laboratory supports approximately 120 transplants annually. Donation after Circulatory Death (DCD) donor organs have been used in this center since 2013. We present the strategy employed to achieve this and the outcomes for the year 2015–16. Samples from 35 local potential DCD donors were received in the laboratory for HLA typing. Eighteen were subsequently ‘stood down’ and in two instances both kidneys were exported to mainland UK leaving 15 that were used locally. In 5 cases both kidneys were transplanted locally, culminating in 20 transplants. HLA typing was commenced on all 35 potential donors by Luminex rSSO. The CDC crossmatch procedure was amended in that peripheral blood, received at the time of sampling for typing, was used instead of waiting for retrieval of spleen cells. Crossmatching then commenced immediately as a recipient was chosen using
cells extracted from donor peripheral blood and the latest serum sample available from the potential recipient; some donors were stood down during this process. Virtual crossmatch was considered suitable for three recipients and CDC crossmatch for the 17 remaining prospective recipients. Allele mismatches varied from 2–7 (mean 5.7) on considering mismatches at HLA-A, −B, −C, −DRB1, −DRB3,4,5, −DQB1 and −DPB1 loci. Mean age of recipients and donors was 58.6 and 48.8 years respectively. The mean CIT was 8.4 hours (range 4.4 – 18.1). This is substantially better than that achieved in any other UK transplant centers. The routine use of cells extracted from peripheral blood instead of waiting for retrieval of the spleen for crossmatching, facilitated a significant reduction in cold ischemia time in this sub optimal group of donors. Frequently crossmatch was reported before withdrawal of life support thus allowing timely transplantation. Other centers may proceed to surgery in a similar manner by restricting DCD transplants to recipients suitable for virtual crossmatching only. The Belfast method allows equity of access to DCD organs to all on the waiting list.

P8

NEW HLA GENOTYPING TECHNOLOGIES OFFER THE OPPORTUNITY TO BETTER ASSESS THE DONOR-RECIPIENT HLA MISMATCH IN SOLID ORGAN TRANSPLANTATION

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HLA antigens are divided into 1st field groups based on shared serological patterns. Although this categorization was meant to allow matching algorithms that minimize allo-immunization after transplantation, it fails to do so. Low resolution typing and antigen categorization does not discriminate between alleles with different presentation and it excludes entire allele families based on cross-reactivity (CREG) and the assumption that they all share the same immunogenic risk. This matching strategy therefore does not correctly reflect the actual risk of allo-immunization and could benefit from fine-tuning. Epitope matching is currently suggested as an opportunity to improve donor-recipient matching. To do so we need in depth molecular characterization of the antigens. New techniques offer the opportunity to do this and even include new relevant loci such as DPB1 in a cost effective manner. Pacific Biosciences’ Single-Molecule Real Time (SMRT) sequencing technology offers read lengths >15 kb which enable direct haplotyping of long range PCR products derived from individual antigens. Reducing the need for fragmentation this permits direct phasing of variants instead of sophisticated error prone imputation from short reads. We are currently integrating this technique into an HLA genotyping workflow for organ transplant recipients and donors, allowing us to correlate high resolution single bead antibody with allelic resolution genotyping data in a retrospective analysis. We are already able to multiplex up to 31 samples for HLA class I in a single SMRTcell, resulting in high-quality data without any mistypes compared to the consensus. Our setup provides sufficient coverage to correct for the high individual molecule error rates with costs that are comparable to that of commercial SSO methods, making this approach feasible for implementation in organ transplant recipient, related donor and post-transplant deceased donor allelic resolution genotyping. A similar approach for HLA class II molecules (HLA- DRB1,3,4,5, DQB1, and DPB1) is in development. To allow future epitope discrimination in prospective donor typing we are currently evaluating the potential of real-time PCR kits such as the single antigen bead resolution kit (SABR, Linkage Biosciences), which discriminates between the epitopes present in current antibody identification kits.

P9

DETECTION OF HLA-DQ COMPATIBILITY OF RECIPIENT-DONOR COUPLES IN RENAL TRANSPLANTS FROM DECEASED DONOR AND INVESTIGATION OF DE NOVO ANTIBODIES AFTER TRANSPLANTATION

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Kidney transplantation is used as treatment for end-stage chronic kidney failure. Blood group and HLA tissue type compatibility, DSA presence, and PRA positivity between recipient and donor are important for the success of kidney transplants. In this study, a total of 25 recipient-deceased donor couples who applied to our laboratory in January 2014-September 2016 were included. HLA tissue typing of the couples (HLA-A, −B, −C, −DRB1, −DQA1, and −DQB1) were performed by a Luminex-SSO method. Pre- and post-transplant anti-HLA antibodies were investigated by a Luminex-PRA method and the association of the results with graft survival were assessed. The patients were divided into two groups according to their alloimmunizations. The patients with previous transplantation, blood transfusion, and pregnancy were in group 1 (20.8% (n = 5)), while the patients with blood transfusion and pregnancy were in group 2 (79.2% (n = 19)). One of the patients had no alloimmunization. One patient of the first group (20%) and two patients of the second group (10.5%) were pretransplant PRA positive. Post-transplant PRA of two patients
in the first group (40%) and second group (10.5%) were positive. When the results were evaluated according to graft survival, it was found that one patient in the first group lost the allograft due to de novo donor specific antibodies. In the second group, de novo antibody did not develop and graft failure was not observed. In conclusion, it was determined that transplantation was an important determinant for anti-HLA antibody production and post-transplant donor specific antibody development was important for graft failure.

P10

EARLY POST-TRANSPLANT MONITORING OF ANTI-HLA ANTIBODIES IN HEART TRANSPLANT RECIPIENTS FAILS TO DETECT PATIENTS AT RISK OF HUMORAL REJECTION

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There is growing interest in the monitoring of anti-HLA antibodies after heart transplantation as their presence has been linked with coronary allograft vasculopathy (CAV) and implicated in allograft injury, but there is no consensus in their follow-up timing. 93 consecutive patients that underwent heart transplantation between 2011 and 2015 at the Marques de Valdecilla University Hospital were included in the study. The presence of anti-HLA antibodies was assessed in pre-transplant sera and during the first year every three months after transplantation by using LABScreen® Mixed and Single Antigen assays (One Lambda, Canoga Park, CA, USA), according to the manufacturer’s protocol. Positive specificities were considered when two out of three criteria were fulfilled: raw mean fluorescence intensity (MFI) above 1500, baseline MFI above 1000 and above 25% of maximum MFI. Anti-HLA antibodies were found prior to transplant in 6.5% of all recipients (two against HLA class I; two against class II and two against both class I and class II antigens). 8.1% of the patients analyzed prior transplantation had anti-HLA antibodies at three months and 8.6% at 12 months. Two out of six de novo reactions were donor-specific (all against class II antigens) and both with rejection episode confirmed. 37.6% of patients had transplant rejection, being humoral rejection in 20% of the cases. C4d staining was positive in 71.4% of the biopsies. 74.3% of the patients who lost the graft did not present anti-HLA antibodies at any time. Therefore, anti-HLA antibody monitoring in heart transplant patients did not allow predicting graft rejection. Multi-center and prospective studies on heart transplant candidates should be addressed in order to assess the best plan of anti-HLA antibodies monitoring in heart transplant recipients.

P11

CCR4HIGHCD4+ CELL POPULATIONS IN KIDNEY GRAFT BLOOD AFTER STEROID WITHDRAWAL: A PROSPECTIVE, RANDOMIZED, CONTROLLED, PARALLEL GROUP STUDY. PRELIMINARY RESULTS

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Steroids represent a mainstay of immunosuppression after kidney transplant. The infiltration into the graft of active T cells following KT depends on the expression of chemokines and their interaction with their T-cell receptors. However, the natural history of the expression of these molecules in patients who undergo steroid withdrawal after transplant is unknown. In a controlled clinical trial (NCT02284464), a total of 176 KT patients with low immunological risk were recruited to randomly receive either conventional triple immunosuppression: steroids, TAC and MMF (Group A) versus steroid withdrawal at the 3 post-KT month (Group B). We compared the evolution of CCR4highCD4+ and CXCR3highCD4+ lymphocyte sub-populations in graft blood (GB) extracted by fine needle aspiration puncture determined by flow cytometry in patients after steroid withdrawal at the 3 month post-KT versus patients who continue to receive conventional triple immunosuppression. Measurements were made at 3 (baseline) and 6 months post-KT in GB and in peripheral blood (PB). So far, 68 patients have been randomized (34 in each group). There were no significant differences in the clinical and demographic characteristics between the groups at baseline. The first analysis (at 3 months) in those patients who had completed 6 months of follow-up (Group A: n = 13; Group B: n = 15) showed a significant increase in the CCR4highCD4+ subpopulations in GB versus PB in both groups. However, at six months a significant increase in GB versus PB was only seen in Group A. There were no significant differences in the CXCR3highCD4+ lymphocyte subpopulation at the third or sixth month between GB and PB in either group. These preliminary results could suggest a possible effect of prednisone that would favor the recruitment of CCR4highCD4+ cells into the renal graft.
Abstract

P12
A RAPID METHOD TO ISOLATE HIGHLY PURIFIED T OR B CELLS FROM BLOOD, LYMPH NODE OR SPLEEN SAMPLES FOR USE IN DONOR-RECIPIENT CROSSMATCH ANALYSIS
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The crossmatch assay is used as part of a pre-transplant immunologic risk assessment to determine the compatibility between donor-recipient pairs. Isolated T or B cells from the donor are mixed with recipient serum and the presence of donor-specific antibodies is detected through a complement-dependent killing assay (CDC-crossmatch) or by flow cytometry (flow crossmatch). Isolation of specific cell types can be time consuming, and multiple methods must often be validated in laboratories that receive a variety of sample types. We have developed methods (EasySep) to isolate T or B cells directly from whole blood (WB) in 25 minutes, or lymph node (LN) and spleen samples in 11 minutes, without RBC lysis, sedimentation or density gradient centrifugation. Unwanted cells, platelets and RBCs were immunomagnetically labelled and then placed into a magnet. Labelled unwanted cells were retained, while untouched T or B cells were poured or pipetted off. Isolation of T or B cells using this method was tested on WB, peripheral blood mononuclear cells (PBMC) (model system for LN, which typically have few RBC) as well as on a suspension of PBMC/WB and a B cell line (model system for spleen, which has a high B cell content). Purities following T cell isolation were 97% +/-6 (n = 10) from WB, 94% +/-6 (n = 11) from PBMC (mock LN), and 94% +/-6 (n = 12) from mock spleen (mean +/-SD). B cell purities were 97% +/-4 (n = 10) from WB, 94% +/-7 (n = 18) from PBMC, and 95% +/-9 (n = 6) from mock spleen. On average, 650,000 T cells and 70,000 B cells were recovered per mL of WB. Starting with 5 x10^7 cells, 10 million T cells and 2.8 million B cells were recovered from PBMCs, while 10 million T cells and 1.8 million B cells were recovered from mock spleen samples. Isolations can be automated using RoboSep. This new method enables the isolation of highly purified T or B cells from multiple sample sources using the same reagents, thus simplifying validation for a busy HLA laboratory.

P13
LONG-TERM OUTCOMES AND DISCARD RATE OF KIDNEYS BY DECADE OF EXTENDED CRITERIA DONOR AGE
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Extended criteria donors represent nowadays a main resource for kidney transplantation, and recovery criteria are becoming increasingly inclusive. However, the limits of this approach are not clear as the effects of extreme donor ages on long-term kidney transplantation outcomes is not known. To address these issues, we performed a retrospective study on extended criteria donor kidney transplantation. In total, 647 consecutive extended criteria donor kidney transplantations performed over 11 years (2003-2013) were included. Donor, recipient, and procedural variables were classified according to donor age decades (group A, 50-59 years old [n = 91]; group B, 60-69 years old [n = 264]; group C, 70-79 years old [n = 265]; and group D, 80 years old [n = 27]). Organs were allocated in single- or dual-kidney transplantation after a multi-step evaluation including clinical and histologic criteria. Long-term outcomes and main adverse events were analyzed among age groups and in either single- or dual-kidney transplantation. Kidney discard rate incidence and causes were evaluated. Median follow-up was 4.9 years (25th; 75th percentiles: 2.7; 7.6 years); patient and graft survival were comparable among age groups (5-year patient survival: group A, 87.8%; group B, 88.1%; group C, 88.0%; and group D, 90.1%; P = 0.77; graft survival: group A, 74.0%; group B, 74.2%; group C, 75.2%; and group D, 65.9%; p = 0.62) and between dual-kidney transplantation and single-kidney transplantation except for group D, with a better survival for dual-kidney transplantation (P = 0.04). No difference was found analyzing complication incidence or graft function over time. Kidney discard rate was similar in groups A, B, and C (15.4%, 17.7%, and 20.1%, respectively) and increased in group D (48.2%; odds ratio, 5.1 with A as the reference group; 95% confidence interval, 2.96 to 8.79). Discard rate and long-term outcomes are similar among extended criteria donor kidney transplantation from donors ages 50-79 years old. Conversely, discard rate was strikingly higher among kidneys from octogenarian donors, but appropriate selection provides comparable long-term outcomes, with better graft survival for dual-kidney transplantation.

P14
PRE-TRANSPLANT HLA ANTIBODY SCREENING BY SOLID PHASE ASSAYS: INCIDENCE OF ANTI-HLA IGM ANTIBODIES IN KIDNEY TRANSPLANT CANDIDATES
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Extended criteria donors represent nowadays a main resource for kidney transplantation, and recovery criteria are becoming increasingly inclusive. However, the limits of this approach are not clear as the effects of extreme donor ages on long-term kidney transplantation outcomes is not known. To address these issues, we performed a retrospective study on extended criteria donor kidney transplantation. In total, 647 consecutive extended criteria donor kidney transplantations performed over 11 years (2003-2013) were included. Donor, recipient, and procedural variables were classified according to donor age decades (group A, 50-59 years old [n = 91]; group B, 60-69 years old [n = 264]; group C, 70-79 years old [n = 265]; and group D, 80 years old [n = 27]). Organs were allocated in single- or dual-kidney transplantation after a multi-step evaluation including clinical and histologic criteria. Long-term outcomes and main adverse events were analyzed among age groups and in either single- or dual-kidney transplantation. Kidney discard rate incidence and causes were evaluated. Median follow-up was 4.9 years (25th; 75th percentiles: 2.7; 7.6 years); patient and graft survival were comparable among age groups (5-year patient survival: group A, 87.8%; group B, 88.1%; group C, 88.0%; and group D, 90.1%; P = 0.77; graft survival: group A, 74.0%; group B, 74.2%; group C, 75.2%; and group D, 65.9%; p = 0.62) and between dual-kidney transplantation and single-kidney transplantation except for group D, with a better survival for dual-kidney transplantation (P = 0.04). No difference was found analyzing complication incidence or graft function over time. Kidney discard rate was similar in groups A, B, and C (15.4%, 17.7%, and 20.1%, respectively) and increased in group D (48.2%; odds ratio, 5.1 with A as the reference group; 95% confidence interval, 2.96 to 8.79). Discard rate and long-term outcomes are similar among extended criteria donor kidney transplantation from donors ages 50-79 years old. Conversely, discard rate was strikingly higher among kidneys from octogenarian donors, but appropriate selection provides comparable long-term outcomes, with better graft survival for dual-kidney transplantation.
A positive lymphocytotoxic crossmatch represents an absolute contraindication to kidney transplantation. This paradigm is widely accepted for IgG antibodies but few data on the clinical relevance of IgM antibodies are available and so their role is still controversial. Some studies report that they can be protective for transplant, while other suggest that IgM antibodies are harmful for the graft. Pre-transplant antibody screening by CDC technique does not allow us to discriminate IgG and IgM isotypes. Instead, serum treatment with DTT can indirectly highlight this because it is able to inactivate the IgM pentamer. Moreover, in both cases it is not possible to know if these antibodies are specific for HLA molecules. The new solid-phase techniques permit us to investigate anti-HLA antibodies and to discriminate their isotype. Since September 2016, 189 kidney transplant candidates on the waiting list at the Lazio Regional Transplant Center, were analyzed to evaluate the incidence of IgM antibodies. The antibody characterization was performed using FlowPRA Screening Test to detected either anti-HLA IgM alone, otherwise unknown, or in combination with IgG antibodies and by Luminex Single Antigen Beads to identify antibody specificity. The incidence of anti-HLA IgM antibodies was 10% (19/189). Only IgM antibodies were detected in ten (53%) patients, both IgM/IgG antibodies were presented in nine (47%) patients. IgM positive-group showed in 6 cases only anti-HLA class I antibodies (2000 ≥ MFI ≤ 7000) and 4 cases only class II antibodies (4000 ≥ MFI ≤ 12000). The antibody characterization of IgM/IgG positive-group evidenced in 3 patients the same IgM and IgG specificity, while 6 patients showed additional IgM specificity respect to evidenced IgG antibody specificity. In conclusion, our study suggests widening the antibody screening to anti-HLA IgM antibodies in transplant candidates. The strength and specificity of detected IgM antibodies highlighted the importance of their accurate characterization to understand the clinical significance and to improve graft survival.

P15
FEASIBILITY OF EPLET-BASED MATCHING FOR ALLOCATION OF DECEASED DONOR RENAL TRANSPLANTS
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Retrospective studies have shown a poor correlation between the number of broad and eplet mismatches at the same HLA locus. If eplet mismatches are to be incorporated in the deceased donor allocation pathway, using high resolution HLA typing to calculate the number of eplet mismatches is not practical in the short time-frame available. We aimed to determine whether low / intermediate resolution HLA typing could be used to accurately calculate eplet mismatches. 264 patients who underwent renal transplant between 2003 and 2007 were included. Prospective serological HLA typing and retrospective 4-digit Sanger sequencing was performed for donor / recipient pairs. Two-digit molecular typing was derived from the 4-digit sequencing results. The number of eplet mismatches was calculated using HLAMatchmaker for 2-digit typing and compared with the 4-digit typing derived eplet mismatches. Correlation and agreement of HLA-A, -B, -C, -DR and -DQ mismatches between the 2- and 4-digit results were analyzed. There was close correlation between the number of eplet mismatches calculated using serological and four-digit molecular typing methods at HLA-A, -B and -DQ loci with coefficients above 0.95 (Spearman correlation). In contrast, there was less correlation at HLA-C and -DQ loci with coefficients of 0.87 and 0.80, respectively. The correlation coefficients between the number of eplet mismatches calculated using 2-digit and 4-digit molecular typing at all loci were above 0.98 (p < 0.001). Consistency and absolute agreement in the number of eplet mismatches was similar using 2-digit and 4-digit molecular typing across class I and II loci. In contrast, consistency and absolute agreement was generally lower for serological typing, particularly at HLA-C (consistency: 0.875 and absolute agreement: 0.875) and HLA-DQ loci (consistency: 0.801 and absolute agreement: 0.792). There is good correlation and agreement between 2- and 4-digit typing for total eplet mismatches at all loci. These results suggest that 2-digit molecular HLA typing may be sufficient for the allocation of donor kidneys by eplet-based matching in deceased donor allocation pathways. Further studies evaluating the correlation and agreement in a broader ethnically diverse population group are required.

P16
COMPARISON OF ANTI-HLA ANTIBODY DETECTION METHODS IN CADAVERIC TRANSPLANT CANDIDATES IN TURKEY: A SINGLE CENTER EXPERIENCE
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Chronic kidney failure can result from diabetes, obesity and hypertension which are the most abundant diseases worldwide. The definitive treatment of chronic kidney failure is transplantation. However, organ rejections can occur due to the presence of anti-HLA antibodies after transplantation. Therefore detection of anti-HLA antibodies is important to prevent hyperacute or acute rejections. In this study crossmatch tests were performed before transplantation to 416 cadaveric transplant candidates who were admitted to Tepecik Training and Education Hospital Tissue Typing Laboratory between 2014 and 2016.
The lymphocyte cells were obtained from 113 cadaveric donors. 31.7% of DSA and 35.3% of Flow XM results of CI antibodies and 33.3% of DSA and 47.6 Flow XM results of CII antibodies were positive while determined as negative by CDCXM. 68.3% of CI antibodies were detected as positive by both CDC and DSA and 64.7% were positive by both CDCXM and FCXM. 66.7% and 52.4 % of CII antibodies were detected as positive by DSA and FCXM, respectively while determined as positive by CDCXM as well. It has been known that CDCXM is a gold standard to detect the complement dependent anti-HLA antibodies. However the sensitivity of CDCXM is lower than bead based methods. Thus, at least two different crossmatch tests should be performed before cadaveric transplantation.

Results of PRA CL1 and SAB CL1 of these patients were statistically significant (p <0.05). FCXM-B results of patients with PRA CL2 positive were statistically significant (p <0.05). Statistically significant results were obtained when the results of PRA CL2 and SAB CL2 of these patients were compared (p <0.05). Anti-HLA antibodies that can not be detected by serologic methods can be detected by flow cytometric methods and SAB test is more sensitive test for identification of these antibodies than PRA test. We found that the SAB test was a more reliable method for detecting DSA in renal transplant recipients than other antibody detection tests, according to the statistical values of our results.

Our aim was to analyze the frequency and rate of production of anti-HLA antibodies (both donor and non-donor specific) in a multi-centric prospective cohort from 9 renal transplant units in Spain. The cohort included 742 patients. Recruitment was conducted from 2009 to 2012 and all patients gave informed consent. Serum samples were collected at transplantation and at 3, 12 and 24 months after transplantation. Presence of anti-HLA antibodies was screened by Luminex LabScreen (One Lambda Inc). If positive, specificities were detected with Luminex Single Antigen bead arrays and monitored from first positive serum. Positivity was defined for those sera that fulfilled the following conditions: MFI (mean fluorescence intensity) >25% of positive control of the assay and MFI >1500. Donor HLA typing was performed by PCR-SSP (One Lambda Inc). 600 of the 742 patients (80.8%) included in the study did not present anti-HLA antibodies pre-transplantation. 39 of the 742 patients (5.26%) had donor-specific anti-HLA antibodies, whereas the 103 remaining (13.88%) had donor-specific anti-HLA antibodies (DSA).
Abstract

HLA incompatibility is associated with post-transplant adverse consequences in renal transplantation. In this study, the effects of HLA incompatibility on 10 year graft function and survival in renal transplantation were assessed. Outcome analysis was performed in 109 diseased (67) or living (42) donor renal transplantations during 2005–2007. Patients were grouped according to the level of HLA-A, -B and -DR mismatching into two groups, group A with 0–3 incompatibilities (n = 74) and group B with 4–6 incompatibilities (n = 35). Serum creatinine levels (mg/dl), 24 hour urine protein levels (mg/24 h) in 1st, 5th and 10th year post-transplantation were measured along with graft survival, time on waiting list, cold ischemia time, donor and recipient age, donor type (diseased or living) and acute rejection episodes. HLA antibody reaction frequency (% PRA) was also measured. Groups A and B were compared in association with the previous parameters. Statistical analysis was performed through SPSS using t-test, chi-square and Fisher’s exact test at a level of p < 0.05. The total graft survival was 85.3%, 81.7% and 80.7% in 1st, 5th, 10th year, respectively. Graft survival was higher in group A in 1st, 5th and 10th year post-transplantation (98.1%, 92.6%, 88.9%, respectively) versus group B (66.7%, 61.9%, 61.9%) (p < 0.001, 0.001, 0.007 respectively). 24 hour urine protein in 5th and 10th year was 100% and 93.2%, respectively. As for B lymphocytes, the sensitivity and specificity of the virtual XM were 66.7% and 100% and 93.2%, respectively. The purpose of our study was to evaluate the contribution of the use of the Luminex Single Antigen kit (LSA) for the detection of anti-HLA antibodies as compared to the serological cross-match (XM). We aimed to study the sensitivity and specificity of this technology and evaluate the possibility of predicting the evolution of the graft in post-transplantation. During the period from January 2011 to December 2015 we achieved 182 XM by complement dependent cytotoxicity (CDC). These XM were performed as part of the preparation for a renal transplant (RT) or post-transplant follow-up. Each serum was studied by the Labscreen Mixed 12 (LSM12) screening kit, and then, in case of positivity, the specificity and the raw of the anti-HLA antibodies were defined by the LSA1 and / or LSA2 kits. We realized a virtual XM by the EpViX software for each positive serum in LSA1 and / or 2. The cut-off of the virtual cross-match was 2000. A negative virtual auto-XM was a necessary condition for the validation of the analysis. We studied the sensitivity and specificity of the virtual XM with short-term graft success. For 182 donor / recipient pairs, the sensitivity and specificity of the virtual XM compared to the XM results by CDC were 100% and 92.1%, respectively. With T lymphocytes, the sensitivity and specificity of the virtual XM were 100% and 93.2%, respectively. As for B lymphocytes, the sensitivity and specificity of the virtual XM were 66.7% and 96.6%, respectively. Indeed, two cases of humoral rejection (positive with virtual XM) could be prevented. Our results confirm those of the literature. The use of the LSA kits and the realization of a virtual XM by the EpViX software makes it possible to reduce the number of acute rejections.
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P22
VIRTUAL CROSSMATCHES IMPACT ON KIDNEY DISCARD RATE
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A virtual crossmatch (VXM) is used to ascertain the presence of HLA antibodies in a transplant recipient’s serum and to determine, virtually, the flow cytometric crossmatch (FXM) results with an available donor. VXM testing is routinely used to aid in the selection of import donor kidneys for highly sensitized patients. A VXM can be a valuable tool in helping to reduce cold ischemia time and donor organ discard. This study evaluates the impact of the VXM on the discard rate of kidneys for a single Organ Procurement Organization (OPO). A calibration curve was developed for VXM by comparing the mean fluorescent intensity (MFI) values for the donor specific antibodies to the corresponding median channel shift (MCS) values. A linear regression was performed and the slope was calculated. The slope was entered into an algorithm for estimating the MCS for a given MFI value, therefore predicting a FXM result. A logistic regression model was utilized to compare the number of VXM tests performed between December 2014 and July 2016 to the monthly percent of discarded kidneys. A statistically significant relationship was observed between the possibility of a kidney being discarded and the number of virtual cross matches performed (p = 0.0347). We see that for each unit increase in the number of virtual cross matches leads to a 0.04 decrease in the log-odds of a kidney being discarded. The increase in the use of VXM is one of the factors responsible for a significant reduction in the discard rate of kidneys observed at our OPO.

P23
PROPER INTERPRETATION OF DQB1 ANTIBODIES COULD MINIMIZE THE RISK TO OVERLOOK RELEVANT DSA
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Detection and interpretation of DQB1 HLA antibodies has to be in the right manner especially in the presence of Donor Specific Antibody (DSA) and a mismatch in DQA1 between recipient and donor. A sensitive and specific method such as solid phase analysis can be useful with proper interpretation. In this review, due to presence of two or more DQB1 beads for the same antigen in all test kits offered in the market, interpretation of positive HLA antibody for DQB1 will be discussed considering strength and Mean Floresent Intensity (MFI). HLA typing was performed by One Lambda Sequence specific oligonucleotide probes and sequence specific primers (SSOP/SSP). DSA measurement was performed by a solid phase antibody assay using HLA class II single antigen beads with positive HLA Flow XM. HLA DSA for DQB1 was reviewed carefully and accurately for 5 pairs waiting for allogeneic renal transplant. More than one bead for the same DQB1 DSA has to be checked for the presence of combined antibody against donor DQA1 mismatches. It was found that DQB1 DSA has to be assigned according to their DQA1 mismatches and it shows good correlation with positivity in B cell flow cytometer XM in all 5 cases. This accurate assignment and procedure might be recommended in the interpretation of DQB1 DSA. Accurate pre- and post-transplantation workup, including immunological risk assessment for DQB1 DSA, could influence the outcome of organ transplantation significantly.

P24
A NORMALIZATION FACTOR FOR AN INCREASED COMPARABILITY WITHIN ANTIBODY RESULTS, TESTED BY BEAD-BASED SINGLE ANTIGEN ASSAYS
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The Implementation of bead based techniques (Luminex) for anti-HLA antibody (Ab) detection has increased the complexity of Ab identification and specification. MFI values in Luminex Single Ag assays do not automatically mirror a predictable Ab concentration. The mean fluorescence intensity (MFI) of Abs is used for the definition of “unacceptable antigens”, even though there are considerable inter- and intra-HLA locus differences in the MFI readout of the Luminex assays. The aim of our study was to compare reaction patterns of Abs positive sera with respect to the various HLA class I and II Ab targets. MFI values of HLA specificities were measured and calculated in 278 anti HLA class I Ab positive and 281 anti HLA class II Ab positive sera from patients on the local waiting list in Erlangen, Germany using Luminex Single Ag assays. Anti HLA-C Abs reacted with a lower mean MFI level compared to anti-HLA-A and anti-HLA-B Abs. In contrast, anti HLA-DQ Abs showed higher MFI values than HLA-DR and HLA-DP Abs. In order to define a comparable MFI cutoff for all HLA Ab specificities, two normalization factors were calculated, based
on a “positive” assumed cut-off of 3000 MFI: one for anti HLA-C Ab MFI (MFI x 2.11) and one for HLA-DQ Ab (MFI x 2.64) MFI. Due to the various Ag loading densities on the surface of the microbeads, MFI values of different HLA specificities are not comparable. Furthermore, HLA inter-specific differences in Ab binding cannot be ruled out. In the interest of treating all Ab MFI values equally, independent of the various HLA targets, a unique Ab MFI cutoff within a single patient’s serum should be calculated using a normalization factor for - at least - HLA-C and HLA-DQ Abs. In order to perform a “virtual crossmatch”, the definition of unacceptable antigens, based on MFI value without taking into account the inter-HLA locus variability should be discussed intensively. A comprehensive concept for the evaluation of this normalization factor would appear to be sensible.

P25

DE NOVO DONOR-SPECIFIC HLA ANTIBODIES AFTER STEROID WITHDRAWAL IN KIDNEY TRANSPLANT RECIPIENTS: A PROSPECTIVE, RANDOMIZED, CONTROLLED, PARALLEL GROUP STUDY. PRELIMINARY RESULTS

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Steroids represent one of the mainstays of immunosuppression after kidney transplant (KT). Steroid withdrawal reduces metabolic and cardiovascular complications, but whether it increases the risk of acute rejection and the generation of donor-specific anti-HLA antibodies (DSA) is currently undetermined. In a controlled clinical trial (NCT02284464), a total of 176 KT patients with low immunological risk were recruited to randomly receive either conventional triple immunosuppression: steroids, TAC and MMF versus steroid withdrawal at the third post-KT month. We compared the incidence of de novo DSA, determined by Luminex Mixed and Luminex Single Antigen (One Lambda®), and its impact on graft histology in patients with steroid withdrawal at the 3 post-KT month (after a protocol biopsy) versus patients who continue to receive conventional triple immunosuppression. So far, 68 patients have been randomized (34 per group), with no significant differences in the clinical and demographic characteristics between the groups. The intermediate analysis in those patients who had completed one year of follow-up (n = 28) showed no significant differences in the formation of DSA (0% vs. 0%), nor was there rejection in those patients in whom prednisone was withdrawn after randomization. Patients with triple therapy showed a trend toward better renal function compared to those without steroids at the first post-KT year (1.29 ± 0.25 vs. 1.56 ± 0.42 mg/dL, P = 0.088). HbA1c levels were similar between both group at the first post-KT year (5.79 ± 0.59 vs. 5.68 ± 0.81%, P = 0.734). The preliminary results show that steroid withdrawal at the 3 month post-KT seems safe when assessing the appearance of rejection and formation of DSA compared to the patients who continued to receive conventional triple immunosuppression.

P26

INFLAMMATORY AND REGULATORY CYTOKINE CHANGES IN HIV-POSITIVE TO HIV-POSITIVE RENAL TRANSPLANT RECIPIENTS

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Since 2008, 42 renal transplants have been performed from HIV+ deceased donors to HIV+ recipients with renal failure due to HIV associated nephropathy. Despite transplantation across HLA mismatches, these transplants have been relatively rejection-free, safe and successful in a 3–5 year follow-up. Anti-thymocyte globulin (ATG), as a conditioning regimen given immediately post transplant for 5 days, is known to deplete the majority of T-cells, B-cells, macrophages, monocytes, and dendritic cells. Recipients also receive maintenance immunosuppression of prednisone, mycophenolate mofetil and tacrolimus. The impact of ATG and immunosuppression along with transplantation on host inflammatory and regulatory cytokines was assessed to gain insight into immune homeostatic mechanisms after ATG treatment. Despite the HLA mismatches, there were no rejection events, losses of graft function or deaths at 1 year PT. CD4 counts at transplant had a wide range (132 – 973 cells/ml) but did not change significantly over the first year PT. All recipients are adherent to their combined anti-retroviral therapy (cART) and all viral loads were undetectable throughout follow-up, indicating effective anti-retroviral treatment. In a sub group of 10 participants, the Luminex
200 system was used to measure the concentrations of 37 cytokines in plasma immediately pre-transplant and then at 1, 3, 6 and 12 weeks post-transplant. In all these recipients, when compared to baseline, IL-35 significantly decreased at 1, 3, 6 and 12-weeks post-transplant, whilst IL-10 increased significantly at 1-week post-transplant in 5 recipients. Hierarchical clustering showed a decrease over time in IL-35, IFN-g, IL-20, IL-28A, and IL-11 for all assayed participants. This analysis showed that both inflammatory and regulatory cytokines decline over the 12 weeks of follow-up, although IL-10 is transiently increased after ATG treatment, suggestive of induction of an immune regulatory environment. In this environment, the organ recipients tolerate their grafts and have stable HIV suppression in the presence of cART.

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THE RELEVANCE OF TUMOR NECROSIS FACTOR-ALPHA LEVELS IN KIDNEY TRANSPLANT RECIPIENTS

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Allograft rejection and Cytomegalovirus (CMV) viremia are major predictors of long-term graft survival in kidney transplant recipients. The role of tumor necrosis factor-alpha (TNF-alpha) has not been well defined in this complex entities before. The aim of this study was to determine the diagnostic value of serum and urine TNF-alpha levels in allograft rejection and CMV viremia. A total of 65 patients (61.5% male; mean age 36 ± 12 years) who underwent living kidney transplantation between 2013 and 2015 were included. Serum and urinary TNF-alpha levels were measured at post-transplant 1st, 7th day; 1st, 3rd and 6th month. Additionally serum creatinine, proteinuria, serum CMV DNA levels are monitored during post-transplant follow-up. The mean follow-up time was 26 ± 9 months. Standard enzyme-linked immuno-absorbant assay (ELISA) was used for detection of TNF-alpha levels. Nine patients (9/65 (13.8%)) had biopsy proven rejection during the follow-up period. Serum TNF-alpha levels were significantly higher in the allograft rejection group in 7th day and 1st month (11.5 ± 4.7 vs. 15.4 ± 5.8 p = 0.029, 11.1 ± 4.8 vs. 17.8 ± 10.9 p = 0.003, respectively). Elevated urine TNF-alpha levels were found in the allograft rejection group compared to non-rejection group at 1st and 7th day, 1st, 3rd and 6th month (10.2 ± 2.5 vs. 14.1 ± 6.8 p = 0.002, 9.8 ± 2.2 vs. 14.5 ± 2.7 p < 0.001, 8.0 ± 1.7 vs. 11.8 ± 2.4 p < 0.001, 7.7 ± 1.6 vs. 9.6 ± 1.7 p = 0.002, 7.4 ± 1.6 vs. 8.9 ± 0.9 p = 0.005, respectively). Both serum and urine TNF-alpha levels were significantly higher at 1st day (11.6 ± 4.7 vs. 19.6 ± 4.4 p = 0.002, 10.4 ± 2.7 vs. 15.6 ± 9.9 p = 0.004, respectively). Serum TNF-alpha levels were elevated at 7th day, and 3rd month in CMV viremia group (11.7 ± 4.9 vs. 16.9 ± 5.3 p = 0.042, 10.9 ± 4.3 vs. 23.3 ± 10.3 p < 0.001, respectively). Serum and urine TNF-alpha levels may be a possible predictor for allograft rejection. Besides that serum and urine TNF-alpha levels may be related to CMV viremia.

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ASSOCIATION OF EFFLUENT PARAMETERS, BMI, LIVER QUALITY AND LIVER TRANSPLANT OUTCOMES

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Predictors of postoperative complications are considerable parameters to save patients and organs after liver transplantation. The aim of our study is to evaluate whether effluent parameters prior to re-perfusion show any correlation with post-transplant outcomes such as mortality, early graft dysfunction (EAD), acute rejection and viral and bacterial infections in clinical liver transplant recipients. Pre-transplant concentrations of HMGB1, M65, M30, ALT, AST, GGT, and ALP were measured in available effluent samples of 15 adult recipients who died during the first year post-transplant and 38 age and gender matched liver transplant recipients who survived the first year post-transplant. Effluent concentration of ALP (p = 0.006), AST (p = 0.050), and Ca++ (p = 0.003) were higher in patients with one year post-transplant bacteremia than those without. ALP (p = 0.015) was higher in patients with EAD than those without. Multivariate analysis of effluent parameters showed that Ca++ > 0.30 mmol/l (p = 0.012: OR = 7.12, CI 1.56-32.58) and ALP ≥ 27 IU/I (p = 0.033: OR = 5.31, CI 1.14-27.74) are significant associated factors of 1 year post-transplant bacteremia. They also showed that ALP ≥ 27 IU/I (p = 0.020: OR = 5.56, CI 1.32-23.46) is a significant associated factor of EAD. Donors with fatty liver >20% had significantly higher HMGB1 (140.87 vs. 42.38 pg/ml: p = 0.0001). HMGB1 > 54 pg/ml (p = 0.008: OR = 6.05, CI 1.59-23.00) is a significant associated factors of BMI and fatty liver (p = 0.005: OR = 11.68, CI 2.10-64.01). Effluent parameters are indicators of liver quality and predict outcome of liver transplantation. High effluent Ca++ and ALP are risk factors.
of post-transplant bacteremia, high ALP is a risk factor of EAD and HMGB1 an indicator of donor liver quality.

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FORBIDDEN HLA ANTIGENS IN KIDNEY RE-TRANSPLANTATION – A SINGLE CENTER STUDY
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One of the allocation strategies intended to reduce the incidence of antibody-mediated rejection (AMR) is the definition of unacceptable, or “forbidden” HLA antigens before transplantation, however, the approaches for definition of these antigens applied by different centers (even in the same country) considerably vary. In our center, forbidden HLA antigens were defined as mismatched antigens from previous transplantation(s) against which patients have HLA antibodies detectable by Luminex. 144 patients were included in our study and were divided into two cohorts: historical cohort including 90 patients who were transplanted without taking into consideration forbidden antigens and 54 patients who were transplanted after applying the forbidden HLA antigens approach (test cohort). After the implementation of this allocation policy, there was a significant decrease in the proportion of re-transplanted patients compared with the total number of organ transplants (21% vs. 13%; p = 0.0008). As far as the incidence of AMR during the first year after transplantation, 30 patients in the historical cohort experienced AMR and 22 in the test cohort (33% vs. 41% p = 0.3772). The incidence of T cell mediated rejection was also similar in the two patient groups 20 (22%) vs. 6 (11%) p = 0.1185. On the contrary, in case AMR was diagnosed in the first month after transplantation, graft failure was lower in the test cohort in comparison with the historical cohort p = 0.048. The introduction of forbidden HLA antigens in re-transplanted patients in our center did not positively influence the incidence of AMR, however resulted into a significant decrease in the number of re-transplantations and into accumulation of these high-risk patients on the waiting list.

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CASE REPORT: THE IMPORTANCE OF C3D ASSAYS IN KIDNEY TRANSPLANT
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Anti-body-mediated rejection (AMR) is a major cause of kidney graft loss. Here we tried to test whether the capacity of anti-HLA antibodies to bind complement components allows accurate risk stratification at the time of AMR diagnosis. We report a case of a young female (35 years old), who was admitted to Padua Hospital for a cadaveric kidney transplant. The Complement Dependent Cytotoxicity (CDC) Crossmatch was repeatedly negative. Anti-HLA antibodies pre-transplant were tested by using Luminex Technologies and no DSA were identified. The serum creatinine level was 4.1 mg/dL at 1 day after transplantation. Open biopsy was performed immediately. Histology revealed moderate peritubular capillaritis and mild glomerulitis without C4d immunoreactivity. Sera banked at the time of the renal biopsy were screened for the presence of donor-specific anti-HLA antibodies (DSAs) and our laboratory examination showed that the patient had donor-specific antibodies (DSAs) to DR52 and to DRB1*11. In parallel serum was tested for the presence of C3d-binding anti-HLA using a novel single-antigen flow bead assay in order to asses the ability of these antibodies (DSAs) to bind complement components. We found a positive correlation between the absence of C4d deposition and non C3d-fixing DSAs, and a better outcome of patient kidney transplant. The young patient was not treated with plasmapheresis, but we continued to monitoring her antibody status as per protocol. The presence of Donor-Specific anti-HLA Antibodies (DSA) was determined by testing Lifecodes Single Antigen and Lifecodes C3d (Immucor Transplant Diagnostic), detected by X-MAP Luminex Technology. This result was supported by recent studies showing that the presence of C4d deposition into the graft was not associated with higher risk of graft. In contrast, the presence of circulating C3d-binding DSA at the time of rejection was strongly associated with higher risk for kidney-graft failure.

P31
SIMULTANEOUS LIVER–KIDNEY TRANSPLANT FOLLOWED BY CHRONIC DUCTOPENIC REJECTION OF THE LIVER AND ANTIBODY-MEDIATED REJECTION OF THE KIDNEY IN THE CONTEXT OF DONOR+/RECIPIENT GSTT1 MISMATCH
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We describe the case of a 45-year-old male who suffered chronic renal disease secondary to IgA nephropathy and
alcoholic hepatic cirrhosis. He received a liver and kidney transplant from the same donor in 18-10-03. Patient’s HLA genotype was A*29/- B*44/45 DRB1*11/16 and shared B*44 with the donor. Initial immunosuppression was mycophenolate mofetil (MMF) and steroids with a delayed introduction of cyclosporine A. A liver biopsy performed 1 year after the transplant (11-10-04) confirmed chronic ductopenic rejection and he received a second liver from an A*23/29 B*44/- DRB1*07/- donor in 26-2-05. The kidney function was normal until 20-10-08 when humoral rejection was suspected; the biopsy showed C4d positivity and histological signs of antibody-mediated rejection. Ten previous serum samples were tested for donor-specific HLA antibodies (DSA) but none were detected. After graft failure, the patient returned to hemodialysis. On 24-7-13 he received a second kidney from an A*02/29 B*35/51 DRB1*01/11 donor. Despite the high variability of donors’ HLA alleles, DSAs have never been detected. Regarding non-HLA antigens, this patient showed GSTT1 mismatch with the three donors. He lacks any copy of the GSTT1 gene -therefore neither the liver nor the kidney produce this protein- and all the donors carry the wild type allele so that, the allografts will produce the antigen. Anti-GSTT1 antibodies at high titre were detected in at least 8 of the serum samples that were negative for HLA DSAs prior to kidney failure. We studied 2 kidney biopsies to define the cellular composition of the inflammatory infiltrates. Biopsy 1 had a strong presence of B (58%) and plasma cells (6%). The second sample was the nephrectomy and showed a completely different profile; B cells lowered to 7%, plasma cells increased to 15%, CD8 T cells augmented to 33% and macrophages were higher (35% vs. 26%). The results support the recognition of GSTT1 as foreign antigen and its potential role in rejection.

P32

EFFECT OF SENSITIZING EVENTS ON ANTI-HLA ANTIBODY DEVELOPMENT IN PATIENTS AWAITING KIDNEY TRANSPLANTATION

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The anti-HLA antibodies have been considered a risk factor for rejection in kidney transplantations. Therefore detection of the anti-HLA antibodies can affect the result of the transplantation and graft survival. In this study we aimed to obtain antibody profiles of the patients awaiting kidney transplantation. Serum samples were collected from 1656 patients who were admitted to the Tepecik Training and Research Hospital Tissue Typing Laboratory between 2012–2016. Samples were analyzed by panel reactive antibody screening method by using Luminex technology. 33.6% of the patients were analyzed as PRA positive. Anti-HLA antibody production rates against only class I and only class II HLA antigens were 26% and 25.5%, respectively. 48.5% of the PRA positive patients produced both CI and CII antibody. It was determined that transplantation had the strongest sensitization effect followed by pregnancy then transfusion [66%, 35%, 17%, respectively]. The strengths of the antibodies against HLA-DR antigen were the strongest in patients with previous transplantation. Prevalence of HLA CII antibodies were detected at a higher level in patients with previous pregnancy and transplantation than transfusion. Blood transfusion had a significant effect on production of HLA CI antibodies. Such difference could be partly explained by the different structure of the blood and solid organ and/or distinct biological activity of the antibodies that’s why by transplantation CII antibody levels increase while by blood transfusion CI antibody levels increase.

P33

ANALYSIS OF HLA-E POLYMORPHISMS IN PATIENTS SUBMITTED TO KIDNEY TRANSPLANTATION, PATIENTS WITH CHRONIC KIDNEY DISEASE AND HEALTHY CONTROLS

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Chronic kidney disease (CKD) is considered a public health problem worldwide. In Brazil, incidence and prevalence of kidney failure are increasing. Some patients are considered high risk if they have hypertension, diabetes, and cardiovascular diseases, among other conditions. The techniques involved in kidney transplantation have been developed over the years, to the point that currently they can be characterized as relatively common procedures, so technical failure rates are very low and the major barrier to the success of the procedure is the prevention and maintenance of graft rejection processes. For success of kidney transplantation HLA mismatches should be avoided, in order to improve graft survival and reduce the incidence of acute rejection. The human leukocyte antigen E is expressed in all tissues at low levels, differently from class Ia antigens. HLA-E is mainly involved in regulation of innate immunity by interaction with the CD94/NKG2 receptor highly expressed, but not exclusively, in NK cells. In this study, 24 kidney transplant patients (KTx), 48 patients with CKD and 50 healthy controls were analyzed for genetic variability of exon 3 of the HLA-E gene by SBT. Allele and genotype frequencies were compared between the three different groups by the global G test. Comparisons of allele frequencies among the three groups and two by two comparisons revealed no significant differences. In the comparisons of genotypic frequencies (HLA-
The humoral antibody-mediated rejection is responsible for a large part of transplant failure. An assessment of the humoral immune response gives the measurement of the HLA antibody load. By means of Luminex assay, DSA (donor-specific HLA antibodies) are measured. Sensitization to donor HLA antigens with the synthesis of DSA increases the risk of acute and chronic humoral rejection. Monitoring of DSA after transplantation is recommended by international guidelines. Data on prevalence and validated recommendations in pediatric patients with DSA after kidney transplant hardly exist. We retrospectively analyzed 105 HLA antibody class I and II determinations (regular controls and for creatinine increase >10% of the baseline) in a total of 24 children using Luminex (One Lambda) (10 ± 5 years, 8 girls). The observation period was 2006 to 2015, mean follow-up of 1985 days. Exclusion criteria were previous transplantations. Rejection occurred in 12/24. DSA were identified in 49 (80.0%) and 33 (55.0%) recipients, respectively. Patients with AMR had higher MFIsum (AUC = 0.718, NPV = 83.3%, PPV = 66.7%, ACC = 79.7%, p < 0.001), DSE-II-IgG > 1 (AUC = 0.705, NPV = 83.3%, PPV = 44.8%, ACC = 64.4%, p = 0.013), DSE-I + II-C1q > 1 (AUC = 0.798, NPV = 87.2%, PPV = 65.0%, ACC = 79.7%, p < 0.001), DSE-II-IgG > 1 (AUC = 0.705, NPV = 83.3%, PPV = 44.8%, ACC = 64.4%, p = 0.013), DSE-I + II-C1q > 1 (AUC = 0.718, NPV = 87.2%, PPV = 65.0%, ACC = 79.7%, p < 0.001), DSE-II-IgG > 1 (AUC = 0.705, NPV = 83.3%, PPV = 44.8%, ACC = 64.4%, p = 0.013), DSE-I + II-C1q > 1 (AUC = 0.718, NPV = 87.2%, PPV = 65.0%, ACC = 79.7%, p < 0.001), DSE-II-IgG > 1 (AUC = 0.705, NPV = 83.3%, PPV = 44.8%, ACC = 64.4%, p = 0.013), DSE-I + II-C1q > 1 (AUC = 0.718, NPV = 87.2%, PPV = 65.0%, ACC = 79.7%, p < 0.001), DSE-II-IgG > 1 (AUC = 0.705, NPV = 83.3%, PPV = 44.8%, ACC = 64.4%, p = 0.013), DSE-I + II-C1q > 1 (AUC = 0.718, NPV = 87.2%, PPV = 65.0%, ACC = 79.7%, p < 0.001), DSE-II-IgG > 1 (AUC = 0.705, NPV = 83.3%, PPV = 44.8%, ACC = 64.4%, p = 0.013), DSE-I + II-C1q > 1 (AUC = 0.718, NPV = 87.2%, PPV = 65.0%, ACC = 79.7%, p < 0.001), DSE-II-IgG > 1 (AUC = 0.705, NPV = 83.3%, PPV = 44.8%, ACC = 64.4%, p = 0.013), DSE-I + II-C1q > 1 (AUC = 0.718, NPV = 87.2%, PPV = 65.0%, ACC = 79.7%, p < 0.001), DSE-II-IgG > 1 (AUC = 0.705, NPV = 83.3%, PPV = 44.8%, ACC = 64.4%, p = 0.013), DSE-I + II-C1q > 1 (AUC = 0.718, NPV = 87.2%, PPV = 65.0%, ACC = 79.7%, p < 0.001), DSE-II-IgG > 1 (AUC = 0.705, NPV = 83.3%, PPV = 44.8%, ACC = 64.4%, p = 0.013), DSE-I + II-C1q > 1 (AUC = 0.718, NPV = 87.2%, PPV = 65.0%, ACC = 79.7%, p < 0.001) were significant predictors of AMR, while DSE-I-IgG, DSE-I-C1q and DSE-II-C1q alone were not. Also, MFIsum (OR = 12.63; p < 0.001), DSE-II-
IgG (OR = 4.06; p = 0.023), DSE-I + II-IgG (OR = 9.25; p = 0.002) and DSE-I + II-C1q (OR = 6.33; p = 0.018) were significant risk factors for AMR. Pre-transplant DSE IgG load for HLA class I and both class I and II, but also C1q for HLA class I and II, can be an additional reliable marker, alongside with MFIsum value, for AMR prediction in HLAi kidney transplantation.

P36
POLYMORPHISM OF HLA-A, −B, −DRB1 ALLELE GROUPS AMONG KIDNEY TRANSPLANT LIST PATIENTS IN CROATIA
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This research aims to study HLA-A, −B, and DRB1 allele group frequencies of 1075 patients listed for renal transplantation covering the period from 2010 to 2016. The underlying disease leading to their listing was not taken into consideration for the analysis. The patients coming from 35 different dialysis centers throughout Croatia were grouped as originating from four different geographical regions of Croatia (East-EC, N = 241; West-WC, N = 142; Central-CC, N = 93; and South-SC, N = 259) while the patients coming from Zagreb area (N = 348) were analyzed separately due to the unknown geographical origin of patients. All patients were typed by a PCR-SSP method as well as the control group which consisted of 1004 cadaveric donors from the same regions of Croatia. Seventeen HLA-A, 27 HLA-B, and 13 HLA-DRB1 allele groups were identified. The most frequent HLA genes in total group of patients were: HLA-A*02 (30.7%), A*01 (13.5%), A*03 (12.2%); HLA-B*35 (13.3%), B*51 (11.6%), B*18 (9.3%); HLA-DRB1*11 (18.7%), DRB1*13 (11.9%), and DRB1*01 (11.8%). Among all patients the highest frequency haplotypes were: HLA-A*01-B*08-DRB1*03 (4.3%), A*02-B*18-DRB1*11 (2.5%) and A*02-B*51-DRB1*11 (2.1%). Statistically significant differences were not observed in the HLA polymorphisms when comparing patients with controls except for DRB1*16 in the SC region (7.5% vs. 12.2%; P = 0.003). It was observed that some of the most frequent HLA genes show statistically significant difference in frequencies between regions. For example, A*01 was significantly lower in the CC region (8.6%) compared to the WC region (15.2%; P = 0.039) and the SC region (14.7%; P = 0.037). At the HLA-B locus, B*51 was detected with the lowest frequency (8.3%) in the WC in comparison to all other regions but significant P value reached only when compared to the SC (14.7%; P = 0.0124). Among DRB1 genes, the DRB1*01 gene in WC (15.2%) had the highest frequency among all regions but a significant difference was found compared to SC (10.0%; P = 0.036). In conclusion, the HLA-A, −B, and -DRB1 frequencies among patients correspond to those observed among cadaveric donors. The observed differences in frequencies between analyzed Croatian regions may reflect historical migrations and admixture of neighboring populations.

P37
IMMEDIATE SENSITIZATION: DETECTION OF ANTI-HLA ANTIBODIES AFTER PRECOCIOUS TRANSPLANTECTOMY BY VASCULAR THROMBOSIS
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Primary thrombosis of the graft is one of the main causes of early failure of the graft and leads to immediate transplantectomy. Our objective was to describe the relationship between early transplantectomy and the subsequent production of anti-HLA antibodies. We performed a retrospective study in a series of 22 kidney transplants to 21 patients who required early transplantectomy. We considered as variables to be analyzed the presence of specific HLA class I and/or II donor specific antibodies (DSA), % PRA class I and/or II, the time in which the antibodies were still detected after transplantectomy, and the evolution of patients. Anti-HLA antibodies were detected in 73% of patients, anti-class I in 68% and anti-class II in 50%. In patients with anti-HLA class I, DSA was detected in 93.7%. In 5 cases they were specific against a single antigen, while in 5 other cases the antibodies detected were specific against 2 specific donor antigens. The presence of class II DSA was detected in 100% of patients with anti-class II antibodies. In 3 cases anti-DR DSA together with anti-DQ were detected, in another 7 cases only anti-DR DSA, and anti-DQ DSA only in one case. The average value of the PRA in our patients after transplantectomy was 40 ± 41% in class I, and 34 ± 41% in class II. The duration of the antibodies was very variable after transplantectomy. We have observed that patients undergoing transplantectomy develop DSA antibodies both against class I and class II antigens in a high proportion. Such antibodies can be detected for a long time, although their presence does not prevent success of a new transplant. Patients who continued with immunosuppression after transplantectomy due to their disease (eg, liver transplantation) did not develop anti-HLA antibodies. Therefore, we should ask ourselves whether it would be advisable to maintain immunosuppression, at low doses, in those patients, and also if we should make special emphasis on attempting a new transplant early.
P38

PROZONE PHENOMENON IN SOLID PHASE ASSAY IN ORGAN TRANSPLANTATION

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Human Leukocyte Antigen (HLA) antibody identification solid phase assays play a key role in defining Donor Specific Antibody (DSA) which can determine the compatibility between the recipient and donor in crossmatch results. Luminex bead array technology is widely accepted and used in HLA antibody detection. However, HLA antibodies with high titre lead to activation of complement which and lead to removal of C1 complex on the bead. Subsequently, interference of HLA antibodies and the secondary IgG antibodies might occur. This may lead to false-negative or weak antibody results, which is known as the prozone phenomena. Technical methods, like treatment with EDTA, dithiothreitol (DTT), heat inactivation and dilution with saline can reduce this phenomenon. In our laboratory, HLA Typing is performed for all recipients and donors before renal transplantation using One Lambda Sequence specific oligonucleotide probes and sequence specific primers (SSOP/SSP). HLA Antibody identification, single antigen class I/II and C1q test, are performed by One Lambda. T and B cell IgG XM is performed by FACSCanto II flow cytometer, where the cut-off for positive XM is determined based on normal human studies. Native serum samples of 11 recipients were diluted 1:10 with normal saline when a discrepancy was found between DSA and HLA Flow XM (DSA < 2000 MFI and FXM > 300 MCS). All cases (n = 11) showed strong elevation of DSA after a 1:10 dilution, often >15000 MFI, which was correlated with strong positive HLA flow cross match results. Our results demonstrate the large incidence of the prozone effect in solid phase assays. It is therefore strongly recommended to treat the serum, for example by dilution, to reduce the prozone effect, particularly when there is no correlation between strong positive FXM and weak or negative DSA.

P39

IS THE C3D TEST USEFUL FOR PATIENTS AWAITING A RENAL TRANSPLANT? IS THIS TEST ABLE TO HELP US IN IDENTIFYING HARMFUL ANTIBODIES AMONG ALL ANTICLASS I ANTIBODIES DETECTED BY SINGLE ANTIGEN TESTS?

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The objectives of this study were to compare the C3d/HLA I single antigen assay with CDC in patients on the waiting list of renal transplantation, to confirm the apparent absence of prozone effect for sensitized patients with the classical Luminex test with Immucor products, and to confirm previous results shown in HLA class II in a post-transplant setting where C3d positivity was found only when MFI were > 9000. One serum sample from each of 24 patients awaiting a renal transplant was studied using single antigen with and without Cd3 and a CDC test on a home made panel of 45 cells. Group 1: five patients were polyspecific on CDC test. Group 2: ten patients had only a few cytotoxic antibodies and were also positive in Luminex with MFI higher than 6000. Group 3: nine patients negative in CDC, but all were positive with the Luminex assay (MFI > 6000), six out of nine had MFI >10000. Group 1: results confirm that polyspecific patients (their PRA is above 80%) are also found to be polyspecific in Luminex and C3d lumines assay. The number of positives is identical between both tests. No negative sera were found positive in C3d. Some MFI (11%) were higher with C3d than without.Group 2: the same antibodies were identified in classical Luminex with MFI >10000 for all of specificities except five of them (A1 A2 A11 or A25(2)). The C3d test is known to be more sensitive than the old CDC test. Group 3: 17 specificities with classical MFI were higher than 10000 in six patients. None of them were found in Cd3 test. Some of the specificities were found in the past in CDC, corresponding to an immunization (previous grafts or pregnancies). To conclude, both tests give concordant results in terms of specificities in CDC and C3D class I single test and are linked to a sensitization. The MFI can be higher in Cd3 (after dilution of the sera) but all specificities were determined. As in class II the best correlation is found between C3d positivity and MFI >9000 or CDC (but the threshold is 9000 for both). An interesting point could be to study the specificity with MFI > 10000 not found in Cd3 in order to allow it to graft patients.

P40

ASSOCIATION OF PERIPHERAL NK CELL COUNTS WITH HELIOS + IFNY- TREGS IN PATIENTS WITH GOOD LONG-TERM RENAL ALLOGRAFT FUNCTION

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Little is known about a possible interaction of NK cells with Tregs in long-term stable kidney transplant recipients. Absolute counts of lymphocyte and Treg subsets were studied in whole blood samples of 136 long-term stable renal transplant...
patients were 1946 ± 2201 days (153–10268 days) post-transplant and showed a serum creatinine level of 1.7 ± 0.7 mg/dl.

Renal transplant recipients investigated >1.5 years post-transplant showed higher total NK cells counts than recipients studied <1.5 years after transplantation (p = 0.006). High NK cell levels were associated with high glomerular filtration rate (p = 0.002) and low serum creatinine (p = 0.005). Interestingly, high NK cell levels were associated with high CD4 + CD25 + CD127-Foxp3+ Treg that co-express the phenotype Helios + IFNy- and appear to have stable Foxp3 expression and originate from the thymus. Furthermore, high total NK cell levels were associated with long-term good allograft function and the statistical association of these two lymphocyte subsets with each other suggests a direct or indirect (via DC) interaction of these cell sub-populations that contributes to good long-term allograft acceptance. Moreover, we speculate that regulatory NK cells are formed late post-transplant that are able to inhibit graft-reactive effector cells.

P41

HLA INCOMPATIBLE RENAL TRANSPLANTATION ACROSS BW4/BW6 ALLELES IN TWO PATIENTS

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HLA incompatible (HLAi) renal transplantation may be an option for highly sensitized patients with reportedly a superior survival compared to long term maintenance dialysis. Risk stratification for potential recipients in the United Kingdom is often performed as per British Society for Histocompatibility and Immunogenetics (BSHI) guidelines, which involves comprehensive evaluation by a combination of Complement dependent cytotoxicity crossmatch (CDCXM), Flowcytometry crossmatch (FCXM) and Luminex single antigen bead (SAB) assay, and correlation with sensitization history. Transplanting successfully across a broad specificity such as HLA-Bw4 or Bw6 may prove more difficult, because non-DSA reacting with Bw4 or Bw6 epitopes could have an additive effect and greater overall reactivity. The maintenance immunosuppression protocol comprised of standard triple drug regimen which includes prednisolone, Mycophenolate Mufti and Tacrolimus. In this paper the workup leading to successful outcome of two HLAi transplants is presented.

P42

EVALUATION OF SOLUBLE HUMAN LEUKOCYTE ANTIGEN-G LEVELS IN KIDNEY TRANSPLANTATION PATIENTS

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The expression of human leukocyte antigen (HLA)-G, a non-classical HLA class I molecule and its soluble forms (sHLA-G) are found to improve graft acceptance. However, there are previous data on the correlation of sHLA-G and graft rejection, as well as data on the correlation with viral infections (HCV) in kidney transplanted patients. We evaluated the expression of sHLA-G levels on both patients on a waiting list for kidney transplantation and on patients returned to the list after the first transplantation by comparing them with blood donors as a control group. In addition, we investigated the correlation between sHLA-G levels and HCV infections. Sera of 67 patients on the waiting list for kidney transplantation, n = 43 with anti-HCV and n = 24 without anti-HCV were analyzed. Among all these patients n = 39 were on thr waiting list for the first transplantation while n = 28 were patients were returned to list due to graft loss. The control group included n = 23 blood donors and n = 28 were patients were returned to list after the first transplantation by comparing them with blood donors as a control group. The levels of sHLA-G were analyzed by Enzyme-Linked Immunosorbent Assay (ELISA, BioVendor, Czech Republic). Preliminary data revealed that sHLA-G levels obtained from patient sera were higher compared to controls. In addition, we found that serum sHLA-G levels were significantly higher in patients returned to the waiting list with respect to patients at first transplantation (p = 0.004). No significant correlation was observed for HCV infection among groups. Interestingly, we found high levels of sHLA-G in transplanted patients compared to patients at first transplantation. Previous data seem to be consistent with the hypotetic protective function of HLA-G in the post transplantation period. Therefore, our findings might be the result of...
ischemia and re-perfusion injury suggesting a possible use of sHLA-G levels as a marker for measuring the state of kidney allograft acceptance.

**P43**

**THE CONTRIBUTION OF MICA-129 VAL/MET POLYMORPHISM ON GRAFT REJECTION AND CMV INFECTION IN SIMULTANEOUS PANCREAS AND KIDNEY TRANSPLANT PATIENTS – ONE-YEAR FOLLOW-UP**

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Simultaneous transplantation of pancreas and kidney (SPK) is an established therapy for diabetes type-I patients suffering from chronic renal failure. Emerging evidence suggest that other antigens besides HLA mismatches may play a role in immune cell activation, graft rejection and survival. The polymorphic MHC class I chain-related sequence A (MICA) gene encodes a membrane-bound protein that binds to the NKG2D receptor activating NK and T cells whereas soluble forms of MICA (sMICA) impair the effector functions of these cells. Importantly, a single nucleotide polymorphism (SNP) rs1051792 at position 454A/G of exon 3 causes a valine (val) to methionine (met) exchange at codon 129, which influences MICA expression patterns and binding affinity to NKG2D.

The clinical relevance of this dimorphism in solid transplantation is still not clear. Therefore we genotyped this MICA SNP in 50 SPK patients and donors and measured sMICA levels once during one-year follow-up in 18 patients. We evaluate the effect of val/met mismatch situation with regard to CMV infection and graft rejection in the one-year follow up. Distribution of allele (p = 0.261) and genotype (p = 0.411) frequencies of 129 val/met were similar between donors and recipients. We observed that the val-mismatched patients (n = 7) had a shorter CMV infection-free-survival than the remaining patients [p = 0.004; hazard ratio (HR) 7.36; 95% CI 1.47-36.9]. Similarly, a shorter kidney rejection-free survival (p = 0.012; HR 3.64, CI 1.24-10.6) were observed in val mismatched patients. Interestingly, the one-year follow-up sMICA levels could hardly be detected in val mismatched patients (N = 4, range: 0–45 pg/ml), whereas other patients revealed substantial amount of sMICA (N = 14, range: 269–1331 pg/ml; p < 0.001). Our study gives for the first time evidence that the functionally relevant mismatch situation of MICA at position 129 has an impact on kidney allograft recognition and CMV infection in the first year post SPK transplantation.

**P44**

**IMMUNE STATUS ASSAY – AN APPROACH TO EVALUATE IMMUNOSUPPRESSION IN KIDNEY TRANSPLANTED PATIENTS**

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A major challenge in transplantation medicine today is to develop an assay to detect patients with high risk of transplant rejection or infection due to inadequate level of immunosuppression. To measure drug concentrations of immune-modulating agents in vivo may not be sufficient to achieve an effective immune suppressive treatment as metabolism of these drugs can vary between patients. The aim here is to develop a flow cytometry based assay for immune monitoring of patients after transplantation. In initial experiments, heparinized whole blood cells from ten patients with various immunodeficiencies and ten healthy controls were collected and treated with polyclonal immune-stimulating agents such as pokeweed mitogen (PWM), Concanavalin A (ConA) and Staphylococcal Enterotoxin A and B (SEA/SEB) or medium alone for 1–3 days. The frequencies of CD3+/CD4+ T cells expressing the activation markers CD69, CD134, CD25, CD71, and HLA-DR were analyzed by flow cytometry (Immune Status Assay; ISA). The results were compared with the previously described 7-days reference FASCIA-assay. The stimulation index (SI) for each activation marker was calculated and compared with data obtained with FASCIA. Patients were divided into two groups, “negative” (n = 5) or “normal to high” (n = 5) according to FASCIA assay status. Preliminary results show significant differences between patients in these two groups for all studied activation markers using Con A as a stimulation agent for 2 or 3 days; CD134 (d2, p = 0.0457, d3, p = 0.0156), CD25 (p = 0.0133, p = 0.0064), CD69 (p = 0.0054, p = 0.0209), CD71 (p = <0.0001, p = 0.0071), HLA-DR (p = 0.0027, p = 0.0236). The data from ISA indicates that 2 days of immune stimulation with Con A is sufficient for discrimination between responsive and non-immune responsive patients. In vitro activation for 1 day or with PWM or SEA/SEB 2–3 days did not equally well discriminate between patients with low and normal immune activation status. A larger cohort of kidney patients, selected for living donor transplantation, will now be tested with ISA before and after transplantation. Taken together, we here demonstrate a novel assay (ISA) which shows excellent correlation with current reference assay evaluating immune stimulation. This assay requires little hands-on time and results are available within 2–3 days.
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CX3CL1 IS SIGNIFICANTLY UPREGULATED IN BIOPSIES FROM ACUTELY REJECTING KIDNEY TRANSPLANT RECIPIENTS

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The chemokine CX3CL1 can act as both chemoattractant and adhesion molecule and is expressed on the apical surface of tubular epithelium in human renal transplant biopsy specimens procured during acute rejection (Rej) but also by endothelial and mesangial cells. We studied CX3CL1 on transplant aspiration biopsies (AB) and its evolution post-Rej. First cadaver kidney transplant recipients were studied and divided into three groups: I, stable cases, where AB was done between 7 and 10 days post-transplantation, and which proved Rej-free for at least six months (n = 42), group II, Rej cases with AB done the first day of diagnosis (confirmed by a classical tru-cut biopsy) done concomitantly, and group III post successful treatment of Rej (n = 5) all coming from group II, done one week post treatment completion. The AB samples were cyto-centrifuged and the CX3CL1 immunostaining was done following APAAP methodology. Neither patient demographics or immunosuppressive treatments differed significantly. The results are expressed as absolute positive cells (abs), the ratio of positive cells for kidney cells (+/R) and ratio for mononuclear immune cells (+/LM). For group I, abs 10.1 ± 14.4, +/R 0.028 ± 0.046 and +/LM 0.038 ± 0.087; group II, abs 80.7 ± 76.6, +/R 0.22 ± 0.23, +/LM 0.39 ± 0.23; group III, abs 17.6 ± 18.5, +/R 0.024 ± 0.019, +/LM 0.16 ± 0.29. Group I was lower than II, abs (p = 0.0001), +/R (p = 0.0001), +/LM (p = 0.0001). Group II was higher than III, abs (p = 0.057), +/R (p = 0.006), and +/LM (p = 0.12). No differences were observed by comparing I versus III, respectively for abs, +/R and +/LM, p = 0.32, p = 0.42 and p = 0.43. The negative predictive value for acute rejection recurring to abs < 20 was 97.3% and positive predictive value was 53.8%. We confirm the significant association of CX3CL1 with acute rejection in human kidney transplants and we show for the first time its rapid normalization following successful treatment.

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HLA-G EXPRESSION VARIABILITY AS INDICATOR FOR RENAL TRANSPLANT OUTCOME

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HLA-G is a non-classical HLA molecule known to play a crucial role in allogeneic situations like pregnancy and allo-transplantation. Higher HLA-G expression is related with better transplant outcome. The study aimed to investigate the role of HLA-G in North Indian patients undergoing renal transplantation. A total of 39 recipient-donor pairs were enrolled in the study and results were compared with 50 healthy controls. Of the enrolled recipient-donor pairs, 11 recipients experienced graft rejection episodes. The HLA-G exon8-14 bp indel polymorphism was evaluated by PCR-SSP and sequencing. Serum soluble HLA-G levels (pre-transplant, day 15, 30, 60, and 90) were measured using ELISA. The distribution of HLA-G exon8-14 bp genotypes among the healthy controls was, ins/del-46%, del/del-24% and ins/ins-30%. Among the recipients, the genotype distribution was evaluated on segregation into rejection and non-rejection groups. A higher percentage of recipients in the non-rejection group carried the ins/ins genotype as compared to the rejection group (32.1% vs. 20% respectively). Distribution of the del/del genotype was similar in both groups. The median sHLA-G level was 31.7 U/ml in healthy controls. sHLA-G levels were higher in the individuals with ins/ins genotype in comparison to del/del or ins/del genotype. The sHLA-G levels were significantly higher in transplant recipients when compared with healthy controls. Among recipient cohort that experienced a rejection episode, sHLA-G levels were lower (median-105.4 U/ml) as compared to recipients maintaining well-functioning grafts (WFG) (median-260.4 U/ml). The sHLA-G levels were better maintained throughout the post-transplant period in the WFG group. In the rejection group, the sHLA-G levels improved only after anti-rejection therapy, however it remained lower than WFG. It is evident that HLA-G 3'UTR genotyping and serial monitoring of sHLA-G levels in serum/plasma may be useful tests for predicting any adverse event like rejection.

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IMPACT OF SENSITIZATION EVENTS ON HLA ALLOIMMUNIZATION IN KIDNEY TRANSPLANTATION CANDIDATES

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Alleantibodies against human leukocyte antigens (HLA) may develop after exposure to blood transfusions, pregnancy and/or previous transplantation. HLA antibodies represent an important immunological barrier to successful organ transplantation. The aim of our study was to assess the frequency of exposure
to different sensitizing events (SEs) and evaluate their effect on HLA alloimmunization in kidney transplantation candidates. We performed retrospective analysis of the HLA antibody screening results in 163 patients on kidney transplant waiting list in Clinical Hospital Center Rijeka tested from March 2012 until the end of December 2015. The screening of HLA antibodies has been performed periodically every three months, four times per year by CDC and Luminex assays. Information on earlier and recent SEs were obtained from transplantation candidates, their nephrologists and medical records. A total of 163 patients, 114 (69.94%) were exposed to one or more SEs. The most common SE were blood transfusions. At least one red blood cell unit was received by 92 patients (56.44%). Pregnancies were reported in 57 females (34.97%) and previously transplants were recorded in 24 recipients (14.72%). In 105 patients (64.42%), regardless of a history of SEs, HLA antibodies were not detected by CDC nor by Luminex techniques. HLA alloimmunization occurred in 48 patients (29.45%) exposed to SEs and in ten patients (6.13%) without history of SEs. In 21 patients (36.21%) they were detected by CDC and Luminex (CDC + LUM+) while in additional 37 candidates SEs. In 21 patients (36.21%) they were detected by CDC and Luminex (CDC + LUM+) while in additional 37 candidates (63.79%), including all sensitized patients without known SEs, HLA antibodies were detected by Luminex only (CDC-LUM+). Exposure of patients on the waiting list to SEs represents a risk factor to developing HLA antibodies. Blood transfusions are the most frequent and the most susceptible to our influence. They should be minimized or avoided in transplantation candidates whenever is possible.

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ACUTE ANTIBODY MEDIATE REJECTION DUE TO PRE-TRANSPLANT HLA-DP DONOR-SPECIFIC ANTIBODIES
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Here we report two patients who developed biopsy proven acute antibody mediated rejection due to pre-transplant HLA-DP donor specific antibodies. The first patient, a 40 year old woman, sensitized following two pregnancies, received transfusions vPRA 90%. The second patient, a 36 year old woman, sensitized following pregnancy; vPRA 6%. HLA antibody screens of serum samples was performed by Luminex using Lifecodes LSA Class I and Class II SAB kits (Immucor, USA). HLA class I and class II molecular typing including retrospective high resolution HLA-DPB1 typing, was performed by a PCR-SSP genotyping method (Olerup SSP®, Sweden). After a standard Eurotransplant allocation procedure both patients received a deceased donor kidney transplant immunologically selected according to the patients’ profile and negative T + B crossmatch by complement-dependent cytotoxicity (CDC), without and with DTT, at the time of transplant. Both patients were negative in Luminex screening for donor mismatched alleles at HLA-A, −B, −C, −DRB1 and -DQB1 loci. Both received standard induction therapy, consisting of interleukin-2 receptor antagonist basiliximab and steroids, continued with triple maintenance therapy with tacrolimus, mycophenolate mofetil and steroids. Both had delayed graft function and after the biopsy results were suspected for ABMR. As both patients were transplanted without HLA-A, B, C, DRB1, and DQB1 DSAs, in order to determine the cause of acute ABMR, we expanded the HLA typing of patients and their donors to DPB1 locus and re-analyzed the pre-transplant sera and sera samples drawn at the time of the ABMR diagnosis for the presence of HLA-DP DSAs. The analysis revealed that both patients had HLA-DP DSAs with an average MFI value of 5000. Furthermore, retrospective analysis demonstrated that donor-specific DP antibodies were present in all tested pre-transplant samples. Patients were treated with steroids 0.5 gram per day for three days and plasmapheresis. Both improved their kidney function in relatively short period of time. In both cases, there were no other donor-specific HLA alloantibodies, suggesting that the HLA-DP specific antibodies may be directly pathogenic. HLA-DP matching might become relevant for renal transplantation in patients with pre-transplant HLA-DP antibodies.

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CLINICAL SIGNIFICANCE OF COMPLEMENT-BINDING DONOR-SPECIFIC ANTIBODIES AFTER KIDNEY TRANSPLANTATION
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Recent studies have reported a worse clinical outcome after kidney transplantation in patients having complement-activating donor-specific antibodies (DSA) in comparison with non-complement-binding DSA. Besides the C1q technique, a newly available method detects the C3d-binding capacity of antibodies. The purpose of this study was to compare the two approaches for detection of complement-activating antibodies (C1q vs. C3d) and to correlate these results with findings in graft biopsies which were performed due to deterioration of graft function. 14 kidney recipients, who received kidney grafts between 2012 and 2016 and had de novo DSA detected by solid-phase assays after transplantation, were retested by the Luminex Single Antigen IgG and C3d assays. Patient age was 46.7 ± 7.1 years, 11 (79%) recipients were retransplanted and seven (50%) were sensitized pre-transplant. The median time of serum testing was 13 days post-transplant. The concordance rate between immunofluorescent detection of diffuse C4d deposits in peritubular capillaries and the positivity in the C3d
and C1q tests was 92.8% and 57% respectively. The agreement between the two vendors (OneLambda and Immucor) in detection of DSA on the Single Antigen level was 85.7% for HLA class I and 100% for class II. We conclude that the C3d binding assay reliably identifies de novo DSA with complement-activating capacity in kidney transplant recipients with elevated risk of graft loss.

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OPTIMIZATION OF DONOR

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The pre-transplant complement-dependent cytotoxicity (CDC) cross-match (Xm) assay is a gold standard method for organ rejection prediction. There are three possible tissue sources for lymphocyte separation: lymph nodes, spleen and peripheral blood. A long established procedure for cell purification is using nylon wool column T and B cells separation. This method is convenient and cost effective, but has been found to be impractical when the lymphocyte source is donor’s peripheral blood. In most of cases, results of CDC with nylon wool separated lymphocytes from peripheral blood are un-interpretable for cadaver sources due to high background non-specific cell mortality. However, the logistics of obtaining lymph nodes is more complicated and hence final result is usually much later than that of peripheral blood. The aim of this study was to determine the most effective way to obtain T and B lymphocytes from donor’s blood. Toward this end, we compared four methods of separation with regard to yield, purity and convenience: RosetteSep gradient negative selection (StemCell), MACS MicroBeads magnetic beads positive separation (Miltenyi Biotec), MagniSort magnetic beads negative selection (eBioscience) and MACSprep HLA magnetic beads negative separation (Miltenyi Biotec). We found a significant decrease in concentration of lymphocytes in blood of deceased organ donors compared to living donors (2.7 x10^6/mL vs. 0.57 x10^6/mL). Each separation method had its advantages and disadvantages. Overall in our hands, we found the MACSprep HLA kit advantageous especially in terms of yield, purity and convenience. In addition, we compared CDC Xm results with cells that were separated from blood versus cells that were derived from lymph node of the same donor. We found no difference in T cell Xm results; however the B cell CDC sensitivity was much higher with peripheral blood (50% positive vs. 15%). These results requisite additional consideration in the analysis of cross-matches.

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IMPACT OF C1Q BINDING TO HLA DONOR-SPECIFIC ANTIBODIES ON THE MORTALITY AND THE DEVELOPMENT OF CHRONIC REJECTION (CLAD) IN LUNG TRANSPLANTATION

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We investigated the impact of C1q binding to de novo HLA Donor Specific Antibodies (DSA) on the mortality and the occurrence of chronic rejection (CLAD) in 192 Lung transplantation (LTx) recipients from the Marseille Lung Transplant Center between December 2006 and December 2013 and with at least 30-day survival. Complement-binding antibodies were detected retrospectively for patients with de novo DSA at M1 and M3 after LTx, using the C1q Luminex assays. CD16 engagement were assessed within PBMC effector cells by flow cytometry analysis of the decrease of MFI CD16 at the surface of CD3-CD56 NK cell subsets exposed to allogeneic target cells in presence of DSA. During the study period, 45 LTx recipients developed CLAD (including 8 patients (17%) with RAS and 39 (83 %) with bronchiolitis obliterans) and median overall survival was 35 (+/- 30) months. DSA were detected in 31% of LTx at M1 and 18% at M3. The cumulative DSA MFI (cMFI) level of de novo DSA at M1 were not associated to persistence of DSA at M3 (p = 0.40). ROC curve analysis showed that DSA MFI threshold >17,000 was associated with death with 50% sensitivity and 85% specificity. Only DSA at M3 were associated with lower survival (p = 0.02) and CLAD occurrence (p = 0.01). C1q bound 52% and 33% of DSA at M1 and M3, respectively. Among ten C1q + DSA at M3, eight C1q + DSA were persistent and two were de novo DSA. The cMFI value of DSA at M1 > 10,500 were correlated with C1q binding with 89% sensitivity and 69% specificity. C1q DSA at M1 and M3 were not correlated with clinical occurrence. Interesting, the engagement of CD16 by LTx DSA were very low whatever the MFI intensity, the time after LTx and the clinical occurrence. Finally, these data show that biochemical and biophysical characteristics of LTx DSA are different to these of DSA in other organ transplantation. They should be confirmed by another larger LTx cohort.
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TUNISIAN EXPERIENCE IN HLA ANTIBODY MONITORING
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During the period of 2012–2015, we analyzed 903 sera belonging to 513 patients awaiting or had already received a kidney transplantation. Anti-HLA antibodies were screened by complement dependent cytotoxicity (CDC) and Luminex assays (Labscreen Mixed 12 (LSM12). Luminex single antigen assays (LSA1 and/or LSA2) were performed if anti-HLA screening was positive (NBG ratio > 5). Anti-HLA antibody screening was positive by CDC in 10 patients and by Luminex assays (LSM12) in 79 patients distributed on 55 non-grafted patients and 24 transplanted patients. Sixty one patients were positive for HLA class I of which 17 were positive for HLA-C; forty six patients were positive for class II. Anti-DP antibodies were never isolated. They have been associated with anti-DR or anti-DQ antibodies. Among our 79 patients, 24 were positive for at least one of the anti HLA-C or DP. Among the 24 transplanted patients, donor-specific antibodies (DSA) were detected in 11 patients after transplantation, while non-donor specific antibodies (DSA) were detected in 13 patients. Seven of the 11 patients with DSA lost their graft.

P53
AN HLA-B7-SPECIFIC ANTIBODY IN AN HLA-B*07 POSITIVE PATIENT EXPLAINED BY A NON-EXPRESSED ALLELE (HLA-B*07:181N)
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HLA specific antibodies are a major cause for acute and chronic rejection after solid organ transplantations. Accurate definition of HLA class I and class II antibodies and their consideration for organ transplantations result in better outcome and longer graft survival. Antibody identification by a bead array assay in a kidney patient revealed several HLA-specific antibodies including one directed against HLA-B*07. Low resolution typing of the patient indicated the presence of an HLA-B*07 allele. To rule out an HLA-specific autoantibody the HLA-typing of the patient was further refined by nucleotide sequencing on a next-generation sequencing platform and eventually showed an HLA-B*39:01:01:03 and HLA-B*07:181N genotype. Thereby an allospecific nature of the antibody was proven. The anti-HLA-B7 could be explained by an immunization during the first kidney transplantation in 1996 with an HLA-B*07 positive donor. For plausibility of antibodies, the presence of non-expressed alleles that usually are not ruled out during typing should be taken into consideration.

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INTERFERON-GAMMA +874 POLYMORPHISM IS NOT ASSOCIATED WITH CHRONIC GRAFT DISEASE: EVIDENCE FROM A META-ANALYSIS
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The success of renal transplantation and its clinical outcomes depends on many characteristics of both donors and recipients including ages, ethnicities, pre-formed antibodies, HLA mismatches and other immune system agents such as cytokines and their receptors. Interferon-gamma (IFNg) is a pro-inflammatory cytokine that promotes the development of the TH1 response, regulates the presentation of HLA antigens, and induces apoptosis. Several studies have indicated that IFNg levels have significant effects on susceptibility to various autoimmune diseases, infections, and allergies. It may also contribute to the severity of the rejection episode by stimulating neopterin by monocytes derived macrophages. Chronic Graft Disease (CGD) is a multifactorial process which likely includes a combination of immunological, apoptotic and inflammatory factors. The application of individualized immunosuppressive therapies will also depend on the identification of risk factors that can influence chronic disease. The purpose of this study was to clarify the involvement of IFNg-g +874 cytokine gene polymorphism and it possible association with CGD. Relevant published data were retrieved through Medline pertaining to kidney transplant outcome and IFNg-g polymorphisms. Odds ratios (OR) with 95% confidence intervals (CI) were used to assess the strength of the association. Z test was used to determine the significance of the pooled OR. Statistical heterogeneity was measured using the Q statistic. A total of 6 studies, including 394 CGD transplanted cases and 324 transplanted controls with stable graft function, were collected. For TT vs. TA or AA IFNg-g genotypes data were combined using the fixed-effects model (Q = 5.78; p = 0.33). For the total population, we did not find a statistically significant association of the TT genotype and CGD, when compared with the SGF group: effect summary OR = 1.02; 95% CI = 0.67-1.54; p = 0.92. In this meta-analysis we didn’t find statistical evidence for the association of IFNg-g genotypes and CGD. Further functional studies of IFNg-g gene polymorphism will help to understand the underlying mechanisms of this cytokine.
ASSOCIATIONS BETWEEN OBESITY-RELATED FTO rs9939609, PPARS GENE VARIANTS AND KIDNEY TRANSPLANTATION

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Obesity and lipid abnormalities have implications in various post-transplant pathological conditions, including infections, cardiovascular and metabolic diseases and chronic rejection. Some gene polymorphisms involved in metabolic processes, that could increase the medication-related post transplant risk, are poorly investigated in organ transplantation. FTO (fat mass and obesity-associated) gene variants affect obesity susceptibility in the general population; PPARs (peroxisome proliferator-activated receptors) are nuclear receptors both involved in lipid metabolism, insulin sensitization, inflammation and immune regulation. To evaluate if they could be additional risk factors for post transplant outcome, their associations with pre-post transplant clinical parameters were analyzed. Polymorphisms of FTO rs9939609, PPARA rs1800206 (Leu162Val), PPARG rs1801282 (Pro12Ala) and PPARD rs2016520 were detected using Taqman allelic discrimination methods. Variations of BMI, blood lipids, fasting plasma glucose and creatinine levels were examined in association with genotypes in 173 kidney transplant recipients. Results show that pre-transplant and post-transplant total cholesterol was increased in patients carrying the C allele of the PPARD rs2016520 polymorphism (pre-transplant: C/C + C/T, 190.18 ± 43.23 mg/dl, T/T, 164.62 ± 42.51 mg/dl, p = 0.001; 1 year after transplantation: C/C + C/T, 206.73 ± 47.42 mg/dl, T/T, 192.16 ± 35.10 mg/dl, p = 0.01). No other significant associations were found, although a trend to a slight increase of FTO A/A genotype frequency in obese patients in the first/second years after transplantation was present (1 year, obese, A/A = 34.8%, non obese 19.3%, p = ns). In conclusion, analyzing a possible impact of the examined gene variants on some of the main post transplant risk factors for cardiovascular and metabolic diseases, only slight effects of FTO rs9939609 on the increase of body mass/obesity were detected; an association of PPARD rs2016520 with hypercholesterolemia in the early post-transplant period gives the indication for deeper investigations in the view of possible personalized interventions.

FREQUENCY OF ANTI-HLA ANTIBODIES IN PRE- AND POST-KIDNEY TRANSPLANT PATIENTS IN THE REPUBLIC OF MACEDONIA

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Anti-HLA antibodies play crucial role in kidney graft survival, and should be routinely analyzed prior to kidney transplantation, but also monitored after the transplantation in order to predict and prevent graft failure. The anti-HLA antibodies could develop prior to transplantation due to different sensitization events, such as blood transfusions or pregnancies. We have analyzed in this study a total of 493 sera from kidney transplant patients at the Institute for Immunobiology and Human Genetics, at the Medical Faculty in Skopje, Republic of Macedonia. The samples were analyzed in a period of 4 years (2013 to 2016). One hundred and forty three samples were collected from patients before kidney transplantation, while the rest of 350 samples were post kidney transplantation. The presence of anti-HLA antibodies was determined using the LabScreen Kit with Luminex technology. We have found anti-HLA class I antibodies in 31.47% of the sera prior to transplantation, anti-HLA class 2 antibodies in 22.38% and anti-MICA antibodies in 10.49%. From the sera analyzed after kidney transplantation, 25.14% were found to be positive for anti-HLA class 1 antibodies, 19.14% for anti-HLA class 2 antibodies and 10% for anti-MICA antibodies. To assess the frequency of donor specific antibodies (DSA), we have analyzed total of 38 pairs for kidney transplantation. Only in 2 patients of the pairs analyzed were DSA detected. In 26 of the patients, DSA were not detected (in 4 of them non-DSA anti-HLA antibodies were detected). Sera from 10 patients were not analyzed for anti-HLA antibodies. In conclusion, we report relatively high percentage of sera positive for anti-HLA antibodies, while we have found a relatively low presence of DSA. This points out the importance of the anti-HLA screen and this fact should be taken into consideration when making policies for determination of anti-HLA antibodies and planning kidney transplantation.
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**HLA-DQB1*03:19: SHOULD WE REALLY CONSIDER DQ7 AS ITS SEROLOGICAL EQUIVALENT? A CASE REPORT**

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The HLA-DQB1*03:19 allele was differentiated from HLA-DQB1*03:01 in 2007 by DNA Sequence-Based Typing with a single T185I polymorphism in exon 3. However, high resolution typing does not resolve this ambiguity, as EFI standards require “the identification of HLA alleles that encode the same protein sequence within the antigen recognition site”, i.e. the same exon 2 sequence for class II molecules. Hence, despite the structural differences between these two molecules, both alleles have been suggested to have the same DQ7 serological equivalent. A case of an HLA-DQB1*03:19 in a male patient who has developed anti-DQ7 antibodies questions this assumption. After a kidney transplantation from a HLA-A1, 9, B8, 18, DR5, 6 serologically-typed donor, anti-HLA antibody screenings by Luminex were still negative just before the transplant failed. Numerous de novo antibodies were then identified by Luminex Single Antigen from the very first serum after graft failure. Among them, anti-DQ7 antibodies (not directed at the DQ alpha chain) were identified with a peak MFI at 19687. We retrospectively verified this observation by crossmatching this serum and two others by flow cytometry to a donor against which we identified no other HLA-A, −B, −C, −DRB1 or -DQB1 specific antibodies than the anti-DQ7 antibody by Luminex Single Antigen. All crossmatches were strongly positive (ratio 4.3-10.6 depending on the serum; cut-off = 1.7), confirming the presence of the anti-DQ7 antibodies. To solve the issue of the serological equivalent of HLA-DQB1*03:19, we propose to distinguish the donor from the recipient. For the donor, as long as no HLA-DQB1*03:19 bead will be available in either one of the two commercially available Luminex Single Antigen identification kits, we propose to keep considering DQ7 as the serological equivalent. By contrast, DQB1*03:19 recipients should be assigned a phenotypic blank to allow the selection of all potential DQ3 unacceptable antigens for organ allocation.

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**ENDOTHELIAL PRECURSOR CELL CROSSMATCH USING TIE-2-ENRICHED SPLEEN CELLS**

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Non-HLA-antibodies against human endothelial progenitor cells (EPC) in pre-transplant recipient serum can have a deleterious influence on the graft. EPC enriched from peripheral blood have been commonly used for EPC crossmatching. In the present study, we describe crossmatches using EPC enriched from fresh or frozen-thawed spleen cell preparations, thereby widening the sample source for deceased-donor crossmatching and retrospective studies. EPC crossmatches were performed retrospectively using spleen cells and the flow cytometric XM-ONE crossmatch test kit. One of 29 (3%) sera of healthy controls contained IgG and IgM EPC antibodies, and three (10%) only IgM EPC antibodies. When sera of 11 random dialysis patients were studied, two patients (18%) showed IgG EPC antibodies, one (9%) additional IgM EPC and one (9%) only IgM EPC antibodies. When pre-transplant sera of 20 graft recipients with good long-term graft outcome (serum creatinine 1.0 ± 0.2 mg/dl measured 2463 ± 324 days post-transplant) were investigated using frozen-thawed and then separated Tie-2-enriched spleen cells of the original transplant donor, 2 patients (10%) had pre-transplant IgG EPC antibodies, and three (15%) additional IgM and one (5%) only IgM EPC antibodies. When pre-transplant sera of five patients with intra-operative graft loss were studied employing the original donor spleen cells, four (80%) patients had IgG EPC antibodies and two had, in addition, IgM EPC antibodies. Three of the four (75%) patients with graft loss and IgG EPC antibodies had, in addition, antibodies against angiotensin II type I receptor (AT1R), and one patient had, in addition, antibodies against endothelin ETA receptor (ETAR). Crossmatches with spleen cell-derived EPC using the XM-ONE assay are technically possible. Our very preliminary experience suggests clinical relevance.
DETECTION OF ANTI-HLA ANTIBODIES IN PRE-KIDNEY TRANSPLANTATION CANDIDATES IN THE KURDISTAN REGION OF IRAQ

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The presence of anti-HLA antibodies in the sera of patients waiting for kidney transplantation is a well-known risk factor for development of antibody-mediated rejection (AMR), which eventually might lead to graft loss. The Luminex based bead detection of anti-HLA antibodies has facilitated the task of determining the sensitization status of these patients. In this study, we aim to determine the presence or absence of anti-HLA antibodies in candidates of kidney transplantation in the Kurdistan region of Iraq. Also, to determine the correlation between the Luminex data and the CDC crossmatches that we routinely perform for such patients. From the period between September 2014 and December 2016 we tested 462 sera for the presence of anti-HLA antibodies using Immucor’s Deluxe LifeScreen, Class I and Class II ID (PRA), and LIFECODES LSA class I and II Single Antigens. Out of 462 sera, 170 (37%) were either sensitized for class I or class II anti-HLA antibodies or both. Of the sensitized sera, 30/170 (18%) had only class I anti-HLA antibodies, 61/170 (36%) had only class II anti-HLA antibodies, and 79/170 (47%) had both class I and class II anti-HLA antibodies. In the same period of time there were 16 positive CDC crossmatches between potential recipients and donors, of which 3 of them (19%) had only class I anti-HLA antibodies, 13 (81%) had both class I and class II anti-HLA antibodies and none had class II anti-HLA antibodies alone. The mean fluorescence intensity (MFI) values for the positive CDC crossmatch were all greater than 8000. This study is the first study to be done in the Kurdistan region of Iraq for the determination of the anti-HLA antibodies by using Luminex bead technology. Further studies in the region are required for better understanding the immunological patterns of the patients of the region.

PREDICTION OF FLOW CYTOMETRIC CROSSMATCH OUTCOME FROM BEAD ARRAY DATA

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In order to improve the virtual crossmatch for our center we studied the association of single antigen specific bead array results with the outcome of the flow cytometric crossmatch. Sera from 168 consecutive patients undergoing solid organ transplantation (kidney, liver, heart and lung) between May 2016 and November 2016 were drawn on the day of...
transplantation and analyzed in a solid phase screening assay and in a lymphocytotoxic test (CDC) for the presence of HLA antibodies. HLA antibody containing sera were further analyzed in a single antigen bead array test for the presence of donor specific antibodies (DSA). In addition, all sera were used for flow cytometric T and B cell crossmatches and CDC crossmatches. Regarding the bead array results, DSA could be detected in 12 out of the 168 patients, with MFI values between 1000 and 20000. The flow cytometric crossmatch was positive in eight patients when analyzing T cells and positive in 14 patients when analyzing B cells. Overall an MFI value of 6000 in the bead array test seems for our center to be the best cut-off value for the prediction of a positive flow cytometric crossmatch. We observed one discrepant false negative flow cytometric crossmatch and four false positive ones. These could be due to non HLA-specific alloantibodies and medication related artefacts. We conclude that there is a fairly good association between bead array tests and flow cytometric crossmatch. A larger number of parallel testing is however needed, before either test can be abandoned.

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ANTI-HLA ANTIBODIES IN LIVER TRANSPLANTATION: IS THERE ANY IMPORTANCE? A SINGLE CENTER EXPERIENCE

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Donor specific human leukocyte antigen (HLA) alloantibodies (DSA) are important among solid organ transplantations. The aim of the present study is to investigate if there is any importance of anti-HLA antibodies in liver transplantation and patient-graft survival. A total of 72 primary liver only transplant patients (2010–2016) were tested for anti-HLA antibodies with single antigen bead technology, before and after 1, 6, 12 months post-transplantation and thereafter annually. HLA typing was performed for all donor-recipient pairs. Pre-formed anti-HLA antibodies (PA) were detected in 32 patients (44.4%) who had 47.7% one-year survival rate vs. 77.8% in patients negative for PA (p = 0.030). Only 10% of these patients had pre-existing DSA class I or II with 60% one-year survival rate vs. 65.1% in negative patients (difference not statistically significant). 28 out of 72 patients (39.5%) developed de novo anti-HLA antibodies after transplantation and 15 of them (20.9%) had de novo DSA (five HLA class I and 13 class II). The difference in graft survival rate between de novo DSA positive (77.8%) and negative (86.2%) patients was not statistically important may be due to the small number of transplanted patients with de novo DSA. Patient and graft survival rate seemed to be affected by preformed anti-HLA antibodies but not by de novo DSA, although independent validation is needed. Long term outcome in patients with post-transplant DSA needs further study.

Haematopoietic Stem Cell Transplantation

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HIGH RESOLUTION ALLELE FREQUENCIES OF THE INVENTORY OF THE HELLENIC CORD BLOOD BANK (HCBB)

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Cord blood (CB), is an alternative source of hematopoietic stem cells for allogeneic stem cell transplantation. Although one of its advantages is the lesser degree of compatibility required between graft and recipient, there is increased demand for CB Units typed at high resolution. The Hellenic Cord Blood Bank is routinely using NGS-based typing for the CBUs of its inventory at 7 loci (HLA-A, -B, -C,-DRB1, -DQA1, -DQB1, -DPB1) permitting to obtain high resolution results, with minimal ambiguity, in one pass. The aim of this study was to estimate the distribution of HLA alleles and haplotype frequencies of the CB inventory. For this reason the allelic frequencies of 266 CBUs were obtained for 7 loci at the 2nd field. The most frequent alleles for HLA class I genes were: HLA-A*02:01 (23.9%), HLA-A*24:02 (17.1%); HLA-A*01:01 (10.1%); HLA-B*51:01 (13.8%); HLA-B*18:01 (10.7%); HLA-B*35:01 (9.4%); HLA-B*35:03 (4.5%); HLA-C*04:01 (19.5%); HLA-C*07:01 (13.1%). Regarding class II genes the most frequent alleles were HLA-DRB1*11:04 (19.3%), HLA-DRB1*16:01 (12.3%) and HLA-DRB1*11:01 (8.1%); for DQB1 HLA-DQB1*03:01 (33.6%), HLA-DQB1*05:02 (15.8%) and HLA-
DQB1*05:01 (10.7%); for DQA1 HLA-DQA1*05:05 (33.9%), HLA-DQA1*01:02 (18.1%) and HLA-DQA1*01:01 (8.5%); and for DPB1 HLA-DPB1*04:01 (37.4%), HLA-DPB1*04:02 (21.1%) and HLA-DPB1*02:01 (18.4%). The allele frequencies observed are in line with those previously reported for the Greek population. The most common haplotypes estimated by the Arlequin software were HLA-A*02:01 ~ B*18:01 ~ C*07:01 ~ DRB1*11:04 ~ DQB1*03:01 ~ DQA1*05:05 ~ DPB1*04:02 (2.05%) and HLA-A*02:01 ~ B*35:02 ~ C*04:01 ~ DRB1*11:04 ~ DQB1*03:01 ~ HLA-DQA1*05:05 ~ DPB1*04:01 (2.05%). In the future, an in depth and continuous analytical process of the CBUs haplotypes will ensure the dynamic evolution of the HCBB. Activities such as CB collection, planning or inventory renewal will be based on those results in order to achieve an optimal ethnic representation.

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EFFECT OF DONOR KIR GENOTYPE ON OUTCOMES AFTER HAPLOIDENTICAL STEM CELL TRANSPLANTATION
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In haploidentical stem cell transplantation (SCT), the selection of the “ideal donor” is not performed in a standardized way according to the KIR genotype expressed by potential donors. We retrospectively analyzed 30 patients and their donors submitted to haploidentical SCT at our center. All patients were prepared with a conditioning regimen including thiotepa-fludarabine with high doses of post-transplant cyclophosphamide (CyPT) and tacrolimus as prophylaxis of graft-versus-host disease (GvHD). HLA and KIR genotyping was performed by PCR-SSO using One Lambda commercial kits. The genotypic variables under study were: donor AA / Bx haplotype, donor B content (classified as neutral, better, best, according to Cooley et al., 2010), KIR inhibitor mismatches, presence of KIR2DS1 and KIR3DS1 in donor, mismatching in KIR ligands in the GVH direction, mismatching in KIR ligands in the HvG direction. We analyzed the impact of these KIR genotype variables on overall survival (OS), GvHD free survival, and event-free survival (EFS). Even with our small sample size, statistical significance was found when we analyzed the effect of the presence of mismatches on KIR ligands in GVH direction in relation to the diagnosis of chronic GvHD (54% vs. 100%, p = 0.004). Significance was also found when we compared the effect of the presence of AA or Bx haplotype of the donor with the diagnosis of chronic GVHD (50% vs. 86%, p = 0.033). We did not find statistical significance, in spite of the previous data in the literature that relate the existence of mismatches in KIR inhibitors or the presence of certain KIR genotypes (2DS1+, 3DS1+) with post-transplantation evolution regarding OS or EFS. Our results show that KIR genotyping can provide useful information to choose the donor for the haploidentical SCT without T-depletion with high doses of CyPT. Donors with a B haplotype that do not show incompatibilities of KIR ligands in the GvH direction could provide a lower risk of GvHD.

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IMPACT OF SINGLE NUCLEOTIDE POLYMORPHISMS IN THE MHC GAMMA BLOCK ON THE OUTCOME OF UNRELATED HEMATOPOIETIC STEM CELL TRANSPLANTATION
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The gold standard in unrelated hematopoietic stem cell transplantation (HSCT) is matching for HLA-A, −B, −C, −DRB1 and -DQB1 alleles. However, this is often not enough to prevent post-transplantation complications. MHC haplotype mismatches (MM) have been associated with higher risk of acute graft versus host disease (aGvHD). The gamma block (GB) is located in the central MHC region, between HLA-B/C and HLA-DRB/DQB blocks. It contains many inflammatory and immune regulatory genes. The single nucleotide polymorphisms (SNPs) within this block can be considered as markers for MHC haplotype matching. The aim of this research was to test whether the MM in the GB impact the outcome of HSCT. The study group included 30 patients diagnosed with acute myeloid or lymphoblastic leukemia (N = 18 and N = 12, respectively), transplanted with HLA 10/10 matched unrelated donor (MUD). The pairs were PCR-SSP typed using Gamma Type (GT) assay (Conexio Genomics, Australia) that detects 25 SNPs within the GB. Sixteen out of 30 (53.33%) pairs were GT matched (GT-M) while 14 out of 30 pairs (46.67%) were mismatched (GT-MM). The analysis showed no statistically significant difference in overall survival and relapse occurrence in our tested groups. There were also no significant differences in aGvHD incidence between GT-M and GT-MM patients. Although these preliminary results do not support the hypothesis that GT-MM is associated with higher risk of aGvHD and worse HSCT outcome, due to the small sample size, further investigation on a larger cohort is needed.
POLYMORPHISM AND EXPRESSION OF G-CSF RECEPTOR AND EFFECTIVENESS OF G-CSF-INDUCED MOBILIZATION IN THE RECIPIENTS OF AUTOLOGOUS PERIPHERAL BLOOD PROGENITOR CELLS

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The present study aimed to assess whether G-CSF receptor (CSF3R) expression contributes to G-CSF-mediated mobilization of CD34+ cells for transplantation purposes and could be affected by CSF3R polymorphisms as the rs3917924 wild type CC genotype was previously identified as a factor increasing mobilization efficiency. CSF3R expression on neutrophils and monocytes was investigated by flow cytometry in peripheral blood samples collected before and on the fifth day of G-CSF administration from 33 (F/M = 18/15) patients undergoing transplantation of autologous peripheral blood progenitor cells. G-CSF and SDF-1 (CXCL12) serum levels were assessed using a Luminex instrument. Additionally, genotyping for the CSF3R rs3917924, rs3918001, rs3918020, rs3918021 and rs146617729 polymorphisms was performed. Prior to introducing the mobilizing agent, the rs3917924 wild type CC homozygotes were characterized by higher frequency of CSF3R expressing monocytes compared with T allele bearers (mean of 28.266% vs. 13.371% of monocytes; t value of the t-Test for 2 independent means = 2.328; p = 0.027). The above relationship vanished by the fifth day of mobilization (t = 0.453; p = 0.654). Interestingly, at that time SDF-1 level (strongly correlated with the G-CSF level in the serum: Spearman’s Rho Correlation Coefficient R = 0.573; p < 0.0001) as well as changes in CSF3R expression on neutrophils were associated with percentage of CSF3R-positive monocytes (R = 0.461; P = 0.006 and R = 0.488; p = 0.004, respectively). In turn, expression of the G-CSF receptor on monocytes corresponded with mobilization yield, expressed by the number of CD34+ cells separated per kg of body weight (R = 0.393; p = 0.024). To conclude, efficacy of G-CSF induced mobilization is associated with CSF3R expression on monocytes and CSF3R rs3917924 polymorphism.

AN EVALUATION OF THE GAMMA-TYPE™ KIT

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GAMMA-TYPE™ (Connexio) is a PCR-SSP based kit which allows SNP profiling across the gamma block of the class III region within the MHC. This brief evaluation aimed to ascertain any added value in typing this region in a variety of clinical cases. 15 HSCT donor and recipient pairs were included in the study. Six recipients had second grafts from a different donor after a failed first transplant. Two further recipients had grafts from well-matched unrelated adult donors. Haplotypes were determined either by typing additional family members, or inferred by reference to frequency data. The level of HLA matching for unrelated donors was 9/10 (n = 3); 10/10 (n = 1); or 9/12 (n = 2); 10/12 (n = 2); 11/12 (n = 7); 12/12 (n = 3) including HLA-DPB1. Five unrelated donor pairs were also gamma block matched, and nine pairs were mismatched. The level of HLA matching did not predict identity within the gamma block: Mismatched pairs had HLA matching levels which ranged between 9/12-12/12, while gamma block matched pairs ranged between 9/12-11/12. All unrelated donor pairs with a 12/12 HLA match were mismatched at the gamma block. The population frequency of the HLA haplotype could not be used to predict the likelihood of additional matching within the gamma block in this limited study. Similarly, matching at the gamma block did not predict engraftment, or failure of the first transplant. One recipient had a 9/10 HLA-A mismatched sibling donor, in a family where haplotypes could not be identified by typing additional family members. Gamma block testing was used to assess whether this was most likely to be due to a crossover, or the presence of an additional haplotype in the family. The siblings were matched within the gamma block, indicating a probable cross-over event. This was extremely useful information, and in this case, the kit provided a rapid resolution. In summary, this is an interesting test, but its added value in matching unrelated HSCT donors requires further investigation.
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IMPLICATION OF KILLER-CELL IMMUNOGLOBULIN-LIKE RECEPTORS IN DONOR SELECTION FOR A MATCHED/HAPLOIDENTICAL STEM CELL TRANSPLANTATION
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Killer-cell immunoglobulin-like receptors (KIR) have an impact on transplant outcomes. KIR present on Natural Killer cells interact with HLA class I alleles and modulate the response of Natural Killer cells (increased/decreased cytotoxicity). The objective of the present study was to assess the impact of KIR HLA ligands, KIR gene haplotypes and donor characteristics like gender, age etc. on HSCT outcomes in haploidential related transplants. Thirty three patient-donor pairs undergoing T-cell replete HLA haplo-matched transplant at Tata Memorial Center, Mumbai were included in this study. HLA typing and KIR genotyping was carried out using PCR-SSP. The median age of patients undergoing haplotransplants was 25 years. In our cohort transplants with donor >30 years of age showed significantly lower chances of relapse (p = 0.040) and higher overall survival (p = 0.08). Also, transplants using male donors showed lower relapse (p = 0.873) and higher overall survival (p = 0.711). Transplants involving patient and donor of the male gender resulted in lower relapse (p = 0.173). Among patient-donor pairs having KIR ligand mismatches better overall survival and lower relapse rate were observed (p = 0.502 and p = 0.608 respectively). Patients carrying the C1C1 ligand for HLA-C epitope but did not carry the TI ligand for HLA BW4 epitope had significantly better overall survival (p < 0.0005). Transplants from KIR B/X haplotype donors resulted in lower relapse and better overall survival (p = 0.435, p = 0.852). Also, patients receiving transplants from donors with telomeric BX haplotype showed better overall survival as compared to transplants from donors with centromeric BX haplotype. This benefit was more pronounced when the patient carried the C1C1 KIR ligand (p = 0.140). We conclude that although HLA match is of primary importance, knowing the patient KIR ligands and donor KIR haplotype could help in selecting the best donor and minimize transplant related complications. The HLA-C and Bw4/Bw6 epitopes are important determinants of transplant outcomes whereas KIR genotypes of the donor seem to have no impact in haploidential/mismatched HSCT.

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IDENTIFICATION OF RARE HLA ALLELES IN POLISH POPULATION WITH THE USE OF NEXT GENERATION SEQUENCING
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The HLA (human leukocyte antigen) complex plays a significant role in matching for hematopoietic stem cell transplantation because allele-level HLA matching between donors and recipients reduces the likelihood of rejection and graft-versus-host disease (GvHD). The HLA genes are the most polymorphic in the human genome. Therefore, the changes in their sequence affect the specificity of antigen presentation and histocompatibility in transplantation. At present, sequence-based typing methods, in particular next-generation sequencing (NGS), allow the highest possible resolution. Most of the NGS methods are based on traditional PCR of the target regions followed by massive parallel sequencing of the amplicons. The advantage of NGS is a possibility to obtain the increased amount of sequencing reads per sample and locus. This allows for a highly confident allele-determination and verification of rare HLA alleles. The aim of our study was to determine all 11 HLA loci using TruSight HLA Panel (Illumina) and rare HLA alleles in Polish population. DNA samples isolated from peripheral blood were obtained from bone marrow donors. Typing of 11 HLA loci (HLA-A, −B, −C, −DRB1/3/4/5, −DQB1, −DPB1, −DPA1, −DQA1) was performed according to the HLA assay manual using the Illumina MiSeq platform. The raw sequencing data were assembled and analyzed by the Conexio software. Our data obtained by HLA NGS typing showed the presence of HLA alleles such as: DRB1*14:87, DRB1*11:69, DRB1*11:28:01, B*51:08:01, DQB1*03:12, A*11:37 and C*05:37. In addition, our study confirms that application of the NGS method gives more precise sequencing results and allows the identifications of rare HLA alleles by the high-quality typing compared to conventional techniques.

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CMV-IGG SEROSTATUS OF 290,438 GERMAN AND 31,776 POLISH DKMS DONORS
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The CMV (cytomegalovirus) infection is common in the general population and is usually benign in healthy individuals. However, in immunocompromised patients, CMV infection can lead to serious complications. The CMV-IGG serostatus of 290,438 German and 31,776 Polish DKMS donors is reported. The CMV-IGG serostatus was determined using an ELISA test. The serostatus was divided into three categories: CMV seronegative, CMV seropositive and CMV seroconverters. The results showed that the CMV-IGG serostatus distribution was significantly different between German and Polish donors. The CMV seroprevalence in German donors was higher compared to Polish donors. The CMV seroconversion rate was also higher in German donors. The results suggest that the CMV-IGG serostatus should be considered in the selection of donors for hematopoietic stem cell transplantation.
In the context of hematopoietic stem cell donations, CMV serostatus is becoming more and more important for donor selection. Consequently, DKMS started testing the CMV-IgG serostatus of all newly recruited donors in Germany and Poland by the beginning of 2014. For all donors recruited by collecting a blood sample the CMV-IgG was tested at “Institut für Immunologie und Genetik”, Kaiserslautern or “DKMS Life Science Lab”, Dresden. We report a total of n = 290,438 CMV-IgG serostatus for donors recruited in Germany and n = 31,776 for donors recruited in Poland. At the age of 18, 24% (n = 2334) of the donors tested positive in Germany, while 75% (n = 7355) tested negative. For 1% (n = 83) of the individuals the results were questionable. In contrast to that for donors at the age of 18 and recruited in Poland, a negative CMV-IgG serostatus was less common. Here, we report seropositive results for 62% (n = 1014) of the individuals and seronegative CMV-IgG results for 37% (n = 606). Questionable results have been obtained for 1% (n = 13) of the individuals. Analyzing the CMV-IgG serostatus of donors of different age groups recruited in Germany, we found that the share of seropositive individuals increases with age. For instance, in case of donors with ages between 18 and 25, a seropositive result is found for 28% (n = 18,374) of the donors while for the age group from 51 to 55 the share rises to 47% (n = 11,944). A comparable increase is observed for donors recruited in Poland. In the age group between 18 and 25 the seropositive share is at 63% (n = 4,236) while the share increases to 88% (n = 991) for the group between 18 and 25. The share rises to 47% (n = 11,944). A comparable increase is observed for donors recruited in Poland. At the age of 18 and recruited in Poland, a negative CMV-IgG serostatus was less common. Here, we report seropositive results for 62% (n = 1014) of the individuals and seronegative CMV-IgG results for 37% (n = 606). Questionable results have been obtained for 1% (n = 13) of the individuals. Analyzing the CMV-IgG serostatus of donors of different age groups recruited in Germany, we found that the share of seropositive individuals increases with age. For instance, in case of donors with ages between 18 and 25, a seropositive result is found for 28% (n = 18,374) of the donors while for the age group from 51 to 55 the share rises to 47% (n = 11,944). A comparable increase is observed for donors recruited in Poland. In the age group between 18 and 25 the seropositive share is at 63% (n = 4,236) while the share increases to 88% (n = 991) for the age group between 51 and 55. In summary, this report gives an impression on the proportion of CMV-IgG serostatus in different age groups but also different regions of Europe.

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INTERPLAY BETWEEN DONOR KIR2DS1 AND HLA-C IN THE OUTCOME AFTER HLA-IDENTICAL SIBLING HEMATOPOIETIC STEM CELL TRANSPLANTATION

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NK cells are critical for innate immunity and play a pivotal role in HSCT. NK function is controlled by an array of inhibitory and activating signals that are processed by receptors such as KIRs. Mainly inhibitory KIRs recognize the HLA class I ligand groups (HLA-C1, C2 and Bw4) and mediate tolerance to self but also alloreactivity during HSCT. The activating KIR2DS1 is well known to play a role in both NK cell activation and tolerance by means of HLA-C2 recognition. It has been described that activating donors’ KIR2DS1 affect outcome of HSCT in haploidentical settings and, recently, also in stem cell transplantation from matched unrelated donors. The functionally competent KIR2DS1-expressing NK cells from HLA-C1-positive donor could mediate the anti-leukemic effect in the recipient, mainly in patients with HLA-C2/C2. We evaluated the effect of activating KIR2DS1 in HSCT sibling pairs. We analyzed clinical data (in particular relapse and overall survival) of 53 patients (22 AML, ten ALL, 11 LMC, one NHL, four MDS, five myeloma) who received hematopoietic stem-cell transplantation from sibling donors matched for HLA-A,B,C, DR,DQ or with a single mismatch. We performed KIR and HLA genotyping with PCR-SSO/-SPP technique. Relapse was absent in 43 patients and overall mortality was absent in 38 patients. We found a reduced risk of relapse among patients whose donors were KIR2DS1-positive (62.8% vs. 60% p = 0.8). A similar scenario was evident considering overall survival: we noted a higher frequency of patients with KIR2DS1 positive donors lacking death event (68.4% vs. 46.7% p = 0.1). Allografts from KIR2DS1-positive donors with HLA-C1/C1 were associated with a decreased rate of mortality (28.5% vs. 34.6%). Even if without statistical significance we found, among patients with HLA-C2/C2 whose donors were KIR2DS1 positive, a higher rate of missing relapse (22.2% vs. 16.7% p = 0.9) and a better overall survival (23% vs. 14.8% p = 0.7). This is not a large cohort and it is necessary to increase our sample size. Nevertheless, it is possible that donor KIR2DS1 provides protection against relapse and reduces mortality, in an HLA-C-dependent manner, also in HSCT between identical siblings.

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IDENTIFICATION OF TWENTY-ONE NOVEL MICA ALLELES BY SEQUENCE-BASED TYPING

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The major histocompatibility complex class I-related chain A (MICA) proteins are encoded by a highly polymorphic gene on chromosome 6. To date, 106 MICA alleles are registered in the IPD-IMGT/HLA Database with most of the polymorphisms laying in exons 2–5. Whereas exons 2–4 mainly differ by SNPs, exon 5 sequences vary by a number of GCT repeats. 3262 patients and their unrelated donors from hematopoietic stem cell transplantations (HSCT), which were conducted between 2000 and 2011 in Germany, were MICA typed. Sequenced based typing was performed with an in-house kit using generic primers for exons 2–4 and group specific primers for exon 5. The group specific PCRs allowed for separation of specimens who are heterozygous for an indel at the 3'-end of intron 4 (7 T/T 8 T). For data analysis Sequence Pilot was used.
Sequence overlays caused by the presence of homozygous 7 T/7 T or 8 T/8 T SNPs were analyzed with an in-house tool using a string of 37 bases within exon 5 sequences. Out of 6524 typings, we found 21 (0.32%) novel MICA alleles. The exon 2–5 sequences of 16 novel alleles were accepted by GenBank and names were assigned by the WHO Nomenclature Committee for Factors of the HLA System. Five alleles are still in the process of submission. All detected polymorphisms were single nucleotide substitutions. Most of them occurred in exons 2–4 (5, 6, and 8 SNPs, respectively). Two SNPs were found in exon 5. Five SNPs resulted in silent mutations, 16 SNPs resulted in amino acid changes. Our results show that, even in a population of mainly Caucasian individuals, a significant fraction of novel MICA alleles occurs. MICA is clinically relevant in organ transplantation and in HSCT, is associated with autoimmune diseases and even with some tumors. For good reason, allelic variations of MICA genes are receiving increasing attention.

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UGT2B17 COPY NUMBER VARIATION AND GRAFT VERSUS HOST DISEASE AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN CZECH PATIENTS

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Despite complete HLA match at allelic level between the recipient and donor in allogeneic hematopoietic stem transplantation (aHSCT), substantial proportion of patients suffers from severe complications including graft versus host disease (GvHD). Development of GvHD in patients with HLA-identical donors may be attributed to minor histocompatibility antigens (mHA). UDP glucuronosyltransferase family 2 member B17 (UGT2B17) copy number variation (CNV) has previously been shown as mHA associated with acute GvHD after aHSCT, but this finding was not unequivocally replicated. The aim of the present study was, therefore, to provide further data on possible relationship between UGT2B17 CNV and aHSCT outcome. 235 patients who underwent aHSCT in four Czech centers and their stem cell donors were genotyped for UGT2B17 gene CNV by the quantitative-PCR TaqMan copy number assay. Distribution of UGT2B17 genotypes complied with Hardy-Weinberg equilibrium and was almost identical among the patients and donors. Concordant with the data from other Caucasian populations, the frequency of homozygous UGT2B17 deletion (d/d) genotype was 13% in the whole study group. 19 pairs (8.1%) mismatched for UGT2B17 in GvH direction (UGT2B17 gene carrying patients with d/d donors) were detected. Importantly, acute GvHD (grade II-IV) occurred in a similar frequency in UGT2B17 mismatched (33%) and matched (34%) patients. Nevertheless, UGT2B17 mismatched patients tended to develop life threatening grade IV acute GvHD more frequently (11% vs. 2% in matched patients, p = NS). In conclusion, the present study could not provide the data further supporting contribution of UGT2B17 CNV to the occurrence of acute GvHD after aHSCT. Whether the patients mismatched for UGT2B17 with their donors are in higher risk of the grade IV acute GvHD deserves further investigation in studies with higher statistical power.

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HLA-DPB1 TCE PERMISSIVITY AS A SECONDARY SELECTION CRITERIA IN UNRELATED STEM CELL TRANSPLANTATION (SCT) – 1 YEAR SINGLE CENTER EXPERIENCE

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With increasing number and typing quality of unrelated donors (UD) the probability of finding multiple perfectly matched UD for Caucasian patients is high. Current selection algorithm depends on matching for 5 genes, HLA-A,-B,-C,-DRB1, and -DQB1 (10/10 matched). Recent data has suggested that DPB1 compatibility is also important. In unrelated settings HLA-DPB1 disparity occurs in ~ 80% of pairs matched for 10/10 of the non-DS HLA loci due to low LD between HLA-DP and the other HLA class II loci. An approach to defining ‘non-permissive’, poorly tolerated HLA-DPB1 mismatches on the basis of alloreactive T-cell epitope (TCE) groups was recently introduced and shown to be of clinical relevance. We summarize a 1-year pilot experience with the policy of secondary selection according the TCE in 26 patients for whom 90 UD (median 4, range 2–6 UD) where six HLA loci were typed and TCE reactivity established. The primary selection consists of standard 5-loci matching, i.e. no additional UD was typed in order to find DPB1 matched/permissive UD. A DPB1 matched donor was found for 6 (23%) patients whereas out of 20 patients with DPB1 mismatched UD, a permissive UD was identified for 14 (54%) patients. Only for six (23%) patients were non-permissive DP-MM UD the only ones available. The proportions of DPB1 matched, DPB1-MM-permissive and DPB1-MM-non-permissive donors were 8, 50 and 32, respectively (9%, 56% and 36%). Overall 20 (77%) patients have DPB1-
matched/permissive DPB1-MM UDs and thus would have benefited from this selection. Our data confirm the feasibility of inclusion of HLA-DPB1 in UD selection algorithms without increasing the number of number of confirmatory typings for each patient.

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THE CHOICE OF SUITABLE POLYMORPHISMS FOR CELL CHIMERISM MONITORING OF PATIENTS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION
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Allogeneic hematopoietic stem cell transplantation (alloHSCT) is one of the most promising choice of therapy not only for leukemic patients but also for many non-malignant diseases. AlloHSCT leads to phenomenon called cell chimerism, coexistence of genetically different cells in one organism. The goal of cell chimerism monitoring is the early detection of relapse and disease recurrence. Sensitivity of the method and selection of suitable polymorphisms are crucial for correct determination of chimerism status. It is also important to take potential genetic changes into consideration (such as genome instability, loss of heterozygosity, cytogenetics of original disease), which are characteristic for different types of cancer. Among the most common cytogenetic abnormalities are changes at chromosome 5, 7 and 8 (associated with myelodysplastic syndrome or acute myeloid leukemia). In the case of mixed chimerism (MC, detection of recipient and donor genotype together) it is important to quantify portion of autologous hematopoiesis. If there are genetic changes in the recipient cells (for example deletion) it could half the ratio of donor-recipient blood cells and the patient could seem to be in better therapeutic condition, than they really are. The loss of heterozygosity can lead to complete chimerism detection (CC, detection of only donor genotype). Therefore MC can be overlooked. For monitoring of cell chimerism it is essential to precisely detect ratios of autologous hematopoiesis. To prevent incorrect detection, at least two informative markers are used and the association of the most common cytogenetic changes with each diagnosis are taken into account.

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RETROSPECTIVE ANALYSIS OF STORED UMBILICAL CORD BLOOD UNITS – ETHNICITY, QUALITY PARAMETERS AND HLA
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Precious Cells International Ltd (Precious Cells) is the first public/private hybrid bank established in the UK. Since May 2014, Precious Cells has been collecting altruistic umbilical cord blood (UCB) donations at six UK hospitals focusing its collections in ethnically diverse areas. To assess this approach, a retrospective analysis of ethnicity data for 457 UCB stem cell units was carried out. For 67% (n = 307) of units, both parents were of the same ethnic group; 32% were classified as white northern European, 27% as South Asian, 17% as Sub-Saharan African and 12% eastern European based on Precious Cells’ ethnic group classification. 33% of units were from parents of different ethnicities. Focusing on a subset of 96 units collected during 2015–2016, the mean weight of UCB units at collection was 141 g and the mean CD45+ cell count was 2.3 x10^9. Post-processing, the UCB unit mean CD45+ cell count was 1.13 x10^9, mean CD34+ cell count was 5.64 x10^6 and mean viability was 97.2%. Of these 96 units, 79 met the 6th edition NetCord-FACT requirements for these parameters. These units were ABO typed and HLA typed for HLA-A, -B, -C (exons 2 and 3) and HLA-DRB1/3/4/5, -DQB1 and -DPB1 (exon 2) by NGS. None of the units were homozygous. Using 2013 NMDP US donor registry haplotype reference data, the number of Precious Cells UCBs matching the top five haplotypes at HLA-A, -B, -C, -DRB1 and -DQB1 was determined for African American (AFA), Hispanic (HIS), Caucasianoid (CAU), Asian and Pacific Islander (API) groups. For two of the top three AFA haplotypes and for all three HIS and CAU haplotypes there was at least one UCB haplotype match. Two of the HIS and CAU haplotypes were common (HLA-A*01:01, -B*08:01, -C*07:01, -DRB1*03:01, -DQB1*02:01 and HLA-A*03:01, -B*07:02, -C*07:02, -DRB1*15:01, -DQB1*06:02). There were only HLA class I matches for two of three API haplotypes (HLA-A*33:03, -B*44*03, -C*07:01 and HLA-A*33:03, -B*53:01, -C*04:01) but no complete haplotype matches despite 24% of this cohort being defined as Asian. This may be due to the differences in the population making up the API ethnic group in the UK and the USA; further analysis of this cohort is required. However, Precious Cells’ UCB stem cells units are of high quality and carry common AFA, HIS and CAU haplotypes at five loci, potentially meeting the needs of these ethnic groups.
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HLA-DPB1 DISPARITIES IN 10/10 HLA-MATCHED UNRELATED HEMATOPOIETIC STEM CELL TRANSPLANTATION

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HLA-A, −B, −C, −DRB1 and -DQB1 matching improves the outcome of hematopoietic stem cell transplantation (HSCT), but the impact of HLA-DPB1 matching is controversial. The aim of our study was to evaluate the impact of T-cell epitope (TCE) HLA-DPB1 mismatches on outcome of patients who underwent 10/10 HLA matched unrelated HSCT. 51 patients treated with allogeneic HSCT in our transplant center from 10/10 HLA-matched unrelated donors were included in the study. High-resolution typing for HLA-A, −B, −C, −DRB1 and -DQB1 was performed by PCR-sequence-based typing (SBT) using Protrans S4 kits. High-resolution typing for HLA-DPB1 was done by PCR with sequence specific primers (SSP) using Olerup typing kits. HLA-DPB1 permissive/non-permissive mismatches were examined according to TCE grouping (http://www.ebi.ac.uk/ipd/imgt/hla/dpb.html). The diagnoses were: acute myelogenous leukemia (AML, n = 29), acute lymphoblastic leukemia (ALL, n = 17), chronic myelogenous leukemia (CML, n = 2), non-Hodgkin lymphoma (n = 2), chronic lymphocytic leukemia (n = 1). Early-stage disease included AML and ALL in first complete remission and CML in first chronic phase (n = 26). Advanced-stage disease included all other patients (n = 25). Overall survival (OS), event-free survival (EFS) and grade II–IV acute graft-versus-host disease (aGVHD) were the analyzed clinical end points. OS was defined as survival without lethal event from any cause, EFS was defined as survival in complete remission without lethal event from any cause. Grades of aGVHD were defined by Glucksberg scale. Proportional hazard Cox models were used for comparisons of time-to-event curves for OS, EFS and aGVHD. 10 donor-recipient pairs were HLA-DPB1 matched, 23 pairs had permissive HLA-DPB1 mismatches and 18 pairs had non-permissive mismatches. The factors significantly increasing risk of aGVHD were peripheral blood grafts, advanced-stage disease and male sex of recipients. No significant impact of HLA-DPB1 disparities on OS, EFS, aGVHD was observed.

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PRESENCE OF KIR2DS1 RECEPTOR IN DONORS OF ALLOGENEIC HEMATOPOIETIC TRANSPLANTATION DUE TO MYELOID MALIGNANCIES IMPROVES OVERALL SURVIVAL IN RECIPIENTS WITH HLA-C2 ANTIGENS

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By the recognition of HLA-C2 antigen of recipient cells, the activating killer immunoglobulin like receptor, KIR2DS1 on donor natural killer (NK) cell may lead to increased graft versus leukemia effect in patients treated by allogeneic hematopoietic transplantation (HSCT) influencing disease free (DFS) and overall survival (OS). The goal of the present study was to examine the effect of donor KIR status in conjunction with recipient HLA-C type on the outcome of HSCT. 249 consecutive adult patients, after first allogeneic HSCT (HLA-identical sibling n = 117 or unrelated donor n = 132) for a malignant myeloid condition, at a single center between 2007 and 2013, were retrospectively analyzed. Median follow-up was 36 months (range 6–92 months). Genotyping for the presence of KIR genes was performed by an allele specific multiplex PCR using archived DNA samples. Low resolution HLA-C typing was performed by sequence specific oligonucleotides as part of the routine work-up prior to HSCT. There was no difference in DFS or OS between patient subgroups stratified by the presence or absence of KIR2DS1 or HLA-C1/C2. In the patient subgroup with acute myeloid leukemia (AML) and with KIR2DS1 positive donors (n = 58), we found improved OS for patients carrying at least one copy of the HLA-C2 antigen (n = 36) compared to those homozygous for the HLA-C1 antigen (n = 22). OS at three years for HLA-C2 carriers was 71.7% compared to HLA-C1 homozygotes with 45.7% (p = 0.047). This association remained significant even with multivariate analyzes by Cox regression considering age, sex, the type of conditioning and type of donor as covariates, (hazard ratio = 0.422, 95% confidence interval: 0.179–0.995, p = 0.049). Our results indicate that the combination of donor KIR2DS1 and recipient HLA-C2 may be a favorable genetic constellation in allogeneic HSCT for AML with respect to overall survival.
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OPTIMIZING DONOR RECRUITMENT STRATEGIES: A COMPARISON BETWEEN THE HLA GENETIC COMPOSITION OF ITALIAN PATIENTS AND THE ITALIAN VOLUNTEER BONE MARROW DONORS ENROLLED INTO THE ITALIAN BONE MARROW DONOR REGISTRY DONOR CENTER RM01

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HLA incompatibilities represent a major barrier for allogeneic hematopoietic stem cell transplant. Therefore, the knowledge of immunogenetic profiles of enrolled marrow unrelated donors (MUDs) is very relevant for a successful search. A recent publication provides evidence that a 9–10/10 HLA MUD is available for about 75% of Italian patients, a slightly lower percentage respect to other populations. An explanation for such a high degree of genetic heterogeneity might be found in the history of Italy, where several different ancient populations settled down. The aim of the study was to analyze HLA-A, −B, −C and −DRB1 allele and phenotype frequencies in 1,926 Italian volunteer bone marrow donors enrolled into the Italian Bone Marrow Donor Registry Donor Center RM01, to obtain data about common, rare and very rare HLA alleles and phenotypes. Moreover, in order to plan a data-driven strategy for a future registry expansion, we compared the HLA profiles of the local enrolled MUDs with those of 315 Italian patients, coming from different Italian regions, which already carried out a MUD search. Among the 1,926 Italian MUDs we observed a total of 50 HLA-A, 96 -B, 45 -C and 53 -DRB1 alleles. The alleles showing a frequency >1% were 17/50 HLA-A, 28/96 -B, 18/45 -C, and 22/53 -DRB1, counting for more than 90% of the total cumulative frequencies for each locus, with A*02:01, B*18:01, C*04:01 and DRB1*07:01 reported as the most frequent alleles. Moreover we observed 1,699 HLA-A,-B and 1,895 HLA-A,-B,-DRB1 distinguishable phenotypes out of the total 1,926 MUDs, with a percentage of unique phenotypes of 88.2 and 98.4 % respectively, showing a good phenotypic variability of the registry. A comparison of allele frequencies between the group of 315 patients and 1,926 donors enrolled in the DC RM01 did not reveal significant differences, confirming that the enrolled donor population is near to represent a true reflection of the entire Italian population.

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HLA DPB1 MISMATCHING IN UNRELATED HEMATOPOIETIC CELL TRANSPLANTATION (MUD-HSCT) IN PEDIATRIC HEMATOLOGICAL DISEASES

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The allelic compatibility at HLA-A, −B, −C, −DRB1 and DQB1 loci (10/10) between donor and recipient plays an important role in the outcome of hematopoietic stem cell transplantation (HSCT). Unrelated donors with HLA compatibility 10/10 often have HLA DPB1 mismatches, due to the low LD between DPB1 and other HLA class II loci. Multi-center studies showed significant associations of DPB1 mismatches with the outcome of HSCTs. Classification of DPB1 mismatching, based on T-cell epitope groups, identify mismatches that might be tolerated (permissive) and those that could increase risks (non-permissive). The purpose of this study was to correlate DPB1 mismatches between donor (D) and recipient (R) with the non-engraftment, graft rejection and acute graft versus host disease (GvHD) in allogeneic HSCTs from unrelated donors. We studied two groups of children from the Stem Cell Transplant Unit in “Aghia Sophia” Children’s Hospital, 10/10 matched but with at least one DPB1 mismatch. Group A consisted of sixteen patients with hemoglobinopathies (10/16 boys, 13 thalassemia major, 3 sickle cell disease), where the non-engraftment/rejection was significantly associated with non-permissive DPB1 mismatches in the HvG direction. All but two patients are alive while 4 continue to receive transfusions. Group B included twenty five patients with hematological disorders (17/25 boys, 11 ALL, 10 AML and 4 MDS). Non-permissive DPB1 mismatches were observed in twelve (12/25) D-R pairs of which six (6/12) had GvHD III-IV. Four patients (4/25) are not alive (3/4 with DPB1 non-permissive mismatches), while twenty one patients (21/25) are alive. In this group our results do not show significant associations of DPB1 mismatches with the development of severe GvHD. In hemoglobinopathies the avoidance of DPB1 mismatches in HvG direction is favorable for the outcome of MUD-HSCT, confirming DPB1 T-Cell Epitope Algorithm as a useful tool for donor selection.
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NGS FOR HLA TYPING OF NEW, RARE OR PROBLEMATIC ALLELES: NEW OPPORTUNITY FOR THE IMMUNOGENETICS LABORATORY

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In the present study we describe the introduction of NGS technology for high resolution HLA typing in our laboratory. In order to contribute to the validation of the system we typed all the new alleles that we previously discovered in our laboratory using SBT and/or cloning methods. Moreover we confirmed the HLA typing of rare alleles found in individuals belonging to populations not very well known from an immunogenetic point of view, besides null, questionable expression and homozgyous alleles. Finally we also typed alleles characterized by intronic polymorphisms. Fifteen DNA samples were analyzed in a workflow using NType, Ion Torrent S5 platform and TypeStream software (Thermo Fisher - One Lambda, Canoga Park, CA, USA) for the loci HLA-A, -B, -C, -DRB1, -DRB3,4,5, -DQB1 and -DPB1, for a total of 193 alleles. Data showed a near-perfect concordance with results previously obtained at 2 fields for all the 193 alleles studied. Allele HLA-C*02:106, recently added to the nomenclature was identified as C*02:02:02:NEW. It is worthy to note that the characterization of HLA-C*02:106 was performed using a DNA cloning method. Similarly, DRB3*01:16, different from DRB3*01:01:02:02 for a single amino acid substitution in exon 3, was identified as DRB3*01:01:02:NEW, despite the simultaneous presence of DRB3*02:02:01:02. For heterozygous DNA samples, most were resolved, except in 2% of the cases, belonging however to “P” group. In particular, the presence of DRB1*09:01:02:09:21 was probably due to the challenge of investigating exon 1 for DRB1. All other ambiguities found at the DPB1 locus, as follows: DPB1*02:01:02,*04:02:01:01/DPB1*105:01,*416:01; DPB1*04:01:01:01,*04:02:01:02/DPB1*105:01,*126:01 and DPB1*13:01:01/*07:01. Moreover, our investigation showed that 24 alleles out of the 193 analyzed were characterized by polymorphisms located in intronic regions, lack full-length genomic reference sequences and have not been reported in the IPD-IMGT/HLA Database.

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FIVE-LOCUS HIGH RESOLUTION HLA HAPLOTYPE FREQUENCIES IN THE BONE MARROW DONORS REGISTRY OF PAVLOV FIRST SAINT-PETERSBURG STATE MEDICAL UNIVERSITY, RUSSIAN FEDERATION

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In December 2016 the Database of the Bone Marrow Donors Registry of Pavlov First Saint-Petersburg State Medical University included information about HLA phenotypes of 13000 volunteer donors. High resolution HLA typing by mononucleic Sanger-sequencing was carried out for 1000 donors. Data analysis was performed by Arlequin program version 3.5. The expectation–maximization (EM) algorithm was used for the determination of five-locus haplotype frequencies. In the studied group of donors the most common alleles were: HLA-A*02:01 – 23.9%, HLA-A*03:01 – 13.9%, HLA-A*01:01 – 10.9%, A*24:02 – 10.2%, HLA-A*11:01 – 4.6%, HLA-A*25:01 – 4.2%, HLA-B*07:02 – 11.6%, HLA-B*08:01 – 6.7%, HLA-B*18:01 – 5.1%, HLA-B*13:02 – 4.9%, HLA-B*35:01 – 4.9%, HLA-B*44:02 – 4.7%, HLA-C*07:02 – 13.1%, HLA-C*07:01 – 10.7%, HLA-C*04:01 – 9.3%, HLA-C*06:02 – 9.0%, HLA-C*12:03 – 8.3%, HLA-C*02:02 – 5.5%, HLA-DRB1*07:01 – 13.2%, DRB1*15:01 – 11.5%, HLA-DRB1*01:01 – 10.2%, HLA-DRB1*03:01 – 8.2%, HLA- DRB1*13:01 – 5.2%, DRB1*16:01 – 4.4%, HLA-DQB1*03:01 – 14.6%, HLA-DQB1*02:01 – 13.9%, HLA-DQB1*05:01 – 11.5%, HLA-DQB1*06:02 – 10.7%, HLA- DQB1*03:02 – 6.2%, HLA-DQB1*06:03 – 5.8%. The most frequent five-locus high resolution HLA haplotypes were: A*01:01-B*08:01-C*07:01- DRB1*03:01-DQB1*02:01 (3.4%); A*03:01-B*07:02-C*07:02- DRB1*15:01-DQB1*06:02 (3.3%); A*03:01-B*35:01-C*04:01-DRB1*01:01-DQB1*05:01 (1.8%); A*02:01-B*07:02-C*07:02-DRB1*15:01-DQB1*06:02 (1.3%); A*25:01-B*18:01-C*12:03-DRB1*15:01-DQB1*06:02 (1.0%). This study reports the most frequent HLA alleles and five-locus high resolution haplotypes in the donors of Bone Marrow Donors Registry of Pavlov First Saint-Petersburg State Medical University.
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IMPROVING GENOTYPING CAPABILITIES IN A CLOSE FAMILY COHORT BY EXPANSION OF A QPCR MARKER SET FOR CHIMERISM MONITORING

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Quantitative PCR not only allows quick and easy monitoring of chimeric mixtures after transplantation, but it can also accurately detect the presence of genetic material as low as 0.05%. The detection of adverse transplant events is thereby highly improved in comparison to STR analysis. Further development of chimerism monitoring by qPCR can be necessary in terms of the genotyping capabilities, especially for family members of the first and second degree due to their similarity in genetic make-up. This can be realized by increasing the number of available markers. We have now expanded our KMRtype®/KMRtrack® marker set to a total of 39 markers. This set is a combination of Celera (AlleleSeqR) and GenDx developed assays. The expanded marker set was tested by screening DNA samples of a close family cohort consisting of 4 grandparents, 2 parents and 12 children (Coriell Institute for Medical Research). The genotyping capabilities of the marker set expansion was assessed by determining the family member combinations that were distinguishable and resulted in informative markers. The KMRtype protocol was applied for genotyping, using a multiplexed qPCR system encompassing 3 markers in a single reaction (GenDx). All experiments were performed using the ViiA7 qPCR system (Thermo Fisher). When testing the close family cohort with the expanded chimerism marker set, each possible combination resulted in at least 1 informative marker. The majority of combinations (96%) resulted in more than 2 informative markers. As compared to the previous set, the expanded set reduced the number of combinations having 1 informative marker by 59% (from 29 to 12). It can be concluded that the genotyping capabilities of KMRtype/KMRtrack significantly improved with the addition of chimerism monitoring markers.

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EXTENDED MHC HAPLOTYPE DISPARITY LEVEL IS RELEVANT FOR THE PATIENTS SURVIVAL AND GRAFT VERSUS HOST DISEASE IN T-CELL REPLETE HSCT FROM UNRELATED DONOR

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Life-threatening risks in unrelated hematopoietic stem cell transplantation (HSCT) including graft versus host disease (GvHD) and mortality are associated with HLA disparity between donor and recipient. The increased risks might be dependent on disparity in not routinely tested multiple polymorphisms in the genetically dense MHC region, organized in two extended MHC haplotypes (Ehp). For modeling we considered that in HLA mismatched donor-recipient pairs increased frequencies of SNP disparities in MHC regions adjacent to mismatched HLA loci were discovered and in extremely strong linkage disequilibrium. Patients (N = 889) received T cell-replete HSCT from 2001 to 2012. We assessed the clinical role of Ehp disparity levels in donor-recipient pairs by the in silico detection of HLA allele phase mismatch using PHASE 2.1 algorithm. We compared cumulative incidences of acute (a)GvHD, chronic (c)GvHD, overall survival (OS) and non-relapse mortality (NRM) in patients given HSCT from unrelated donor with 1 or 2 Ehp mismatches. Co-variate adjustments were made in multivariate step-wise analysis with backward elimination. We found a highly significant increment of 100-day aGvHD (58% vs. 41%) and 5-year cGvHD (62% vs. 37%) with increasing Ehp mismatch level, even with the same level of 1 (out of 10) HLA mismatch. In adjusted multivariate models Ehp disparity level remained independent prognostic factor for aGvHD (p = 0.0000018, HR = 2.70, CI95% 1.75-4.14), cGvHD (p = 0.00000085, HR = 3.28, CI95% 2.04-5.25) and HLA mismatch level alone has been excluded from these models. Patients with double Ehp disparity had worse 5-year OS (45% vs. 56%, pa = 0.014, HR = 2.14, CI95% 1.13-4.05) and NRM (40% vs. 31%, p = 0.017, HR = 2.14, CI95% 1.75-4.14), cGvHD (p = 0.0000018, HR = 2.70, CI95% 1.70-8.97), as compared to patients with single Ehp disparity. We conclude that HLA-linked factors contribute to the high morbidity and mortality in recipients given HLA-mismatched unrelated HSCT.

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CLINICAL RELEVANCE OF DYNAMIC MONITORING OF HLA ANTIBODIES IN T-CELL REPLETE HAPLO-IDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Dynamic monitoring of anti-HLA antibodies detection before and after Hematopoietic Stem Cell Transplantation (HSCT) is not current and the data for the association between the dynamic changes of anti-HLA antibodies and HSCT outcomes are limited. The aim of this study was to determine the clinical relevance of the detection of anti-HLA in donors and in patients before and after transplantation by sensitive assays in Haplo-Identical (Haplo-ID) HSCT. We conducted a prospective study on 94 patients who received a Haplo-ID HSCT in Institut Paoli Calmettes, for malignant disease from related donor between August 2009 and August 2015. Pre-transplant sera and days(D) + 3, D + 8, D + 15 and D + 30 post-transplant sera of all patients and 57 donor-derived sera collected before transplantation (<30 days) were tested for anti-HLA class I and class II antibodies and for C1q binding using the LABScan™ 100 flow analyzer. Among the 94 patients, 15 out of 61 males (28%) and 12 out of 33 females (41%) were determined to be HLA-antibody positive. All recipient-derived HLA antibodies had a negative test for C1q binding. Among the 57 donors, 12 out of 33 males (36%) and 11 out of 24 females (46%) were determined to be HLA-antibody positive. Among them, 8 were RSA positive, 6 males (75%) and 2 females (25%). No donor-derived HLA antibodies and RSAs had a positive test for C1q binding. Among the 16 patients who had pre-transplant HLA allo-immunization, HLA detection remained for more than D + 30 in 7 patients (40%). One of these patients developed HLA antibody specificity similar to his donor. Besides, de novo HLA antibodies were detected in only two patients. Recipient- and donor-derived HLA antibodies and HLA antibody disappearance at D + 30 were correlated to occurrence of chronic GvHD in univariate analysis (p = 0.01, p = 0.11 and 0.05, respectively). In multivariate analysis by Cox proportional regression hazards modeling, the relative risk of cGVHD was 8 (CI 95%, 1.4-47) for HLA antibodies donors compared to non-HLA-allo-immunized donors. These results suggest that the production of de novo HLA antibodies and the transfer of donor-derived HLA antibodies after Haplo-ID HSCT are poor. Further studies are needed in a larger series of patients and donors to determine the relevance clinical of the monitoring of HLA antibodies after haplo-ID HSCT.

**P86**

**HIGH-RESOLUTION HLA-HAPLOTYPE FREQUENCIES OF 15,485 POTENTIAL HEMATOPOIETIC STEM CELL DONORS FROM IRAN**

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HLA haplotype frequencies play a pivotal role for search algorithms designed to identify matching unrelated donors for hematopoietic stem cell transplantation and are essential to estimate the probability of finding such a matching donor in a registry of a given size. We present HLA haplotype and allele frequencies derived from a data set of n = 15,484 potential hematopoietic stem cell donors enlisted with the Iranian Stem Cell Donor Program based in Tehran. All samples of potential volunteer donors were typed between August 2015 and December 2016 at high-resolution for HLA-A, -B, -C, -DRB1, and -DQB1 at DKMS Life Science Lab with a high-throughput amplicon-based next generation sequencing approach. Haplotype and allele frequencies were calculated by Hapl-o-Mat, our implementation of a maximum likelihood estimation via an EM algorithm. To account for sample size restrictions, haplotypes with frequencies smaller than 1/(2n) = 0.0000323 were disregarded. We observed A*11:01 g ~ B*52:01 g ~ C*12:02 g ~ DRB1*15:02 ~ DQB1*06:01 g to be the most frequent haplotype with an estimated frequency of 1.3%. A cumulative haplotype frequency of 25% and 50% is reached by the first 76 and 501 5-locus haplotypes respectively. The most common alleles per locus were found to be HLA-A*24:02 g (f = 13.1%), HLA-B*51:01 g (f = 10.1%), HLA-C*04:01 g (f = 18.2%), DRB1*11:04 g (f = 12.1%), and DQB1*03:01 g (f = 23.6%). Based on these frequencies, the chance of finding a matching 10/10 donor from Iran for a patient of the same ethnic group is 25% (50%; 75%) in a registry comprising n = 122,467 (n = 779,462; n = 4,581,415) donors.

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**EVALUATION OF HLA ALLELES BETWEEN MYELOID AND LYMPHOID ORIGIN OF THE LEUKEMIA**

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MHC has been the most frequently studied region of human genome for approximately 40 years, due to discovery of human leukocyte antigen (HLA) genes, which play important roles in disease susceptibility and clinical transplantations. HLA genes and proteins, which locate centrally in the immune system, play an important role in survival duration, population survival, infections, autoimmune diseases, cancer susceptibility and
To determine the phased haplotypes of individuals living in Switzerland, we analyzed HLA-A, −B, −C, −DRB1 and -DQB1 typings (to 2nd-field resolution) of 291 patients, potential candidates for an unrelated donor search, as well as the members of their families that were typed at HLA-A, −B and −DRB1. The comparison of frequencies estimated accounting or not for the phase information showed that among a total of 678 HLA-A ~ B ~ DRB1 haplotypes, only 39% were retrieved by both approaches and sometimes with marked frequency variation like for A*02:01 ~ B*08:01 ~ DRB1*03:01. The remaining 61% were either attributed a frequency of 0 by the algorithm although observed, or an estimated frequency above 0 although the haplotype was not observed. In fact, linkage disequilibrium analysis showed a lack of association globally between the 3 genes but also for many of the allele pairs. The HLA-A ~ B ~ DRB1 phased haplotypes in patients were compared to the HLA-A ~ C ~ B ~ DRB1 ~ DQB1 (2nd-field/group G) haplotypes estimated in 6114 volunteer donors from the Swiss registry. Accounting for linkage disequilibrium patterns between HLA-B and -C, on one hand, and between HLA-DRB1 and -DQB1, on the other hand, we could identify the most probable 5 loci haplotypes in Switzerland. In a further step, the HLA-A ~ B ~ DRB1 haplotypes estimated in Swiss donors were ranked by decreasing frequency order. The sum of ranks of the two haplotypes for each patient was used as a surrogate predictor of a successful search in the BMDW database. Logistic regression analysis showed a highly significant effect of the rank in the outcome of the search (p = 2.25e-14). Furthermore, a ROC curve analysis showed that the best trade-off between true and false positive rates was to use a cut off corresponding to a sum of ranks of 1000. This study confirms that using haplotype data requires much caution, especially when segregation is unknown, and provides very useful resources for the optimization and prediction of unrelated bone marrow donors in Switzerland and beyond.

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INCREASE IN THE NUMBER OF POTENTIAL UNRELATED BONE MARROW DONORS REGISTERED IN BONE MARROW DONOR WORLDWIDE AND NATIONAL REGISTRY INCREASES A CHANCE OF FINDING A BETTER DONOR AT HLA ALLELES AND EXTENDED MHC HAPLOTYPES

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Patients with malignant hematological disorders transplanted from a donor with a double mismatch at extended major histocompatibility complex (MHC) haplotypes (Ehp) are at highest risk of severe GvHD and mortality. During the last 15 years, the number of potential unrelated bone marrow donors registered in the Bone Marrow Donor Worldwide (BMDW) increased from 8 million (at the end of 2002) to more than 29 million (in 2016). In parallel, the number of Polish potential bone marrow donors increased from 2000 in 2002 to 1 million in 2016. Such an increase in the number of registered unrelated bone marrow donors promises a greater chance for finding a compatible donor matched for HLA alleles and at Ehp level. We analyzed retrospectively a group of 58 national patients for whom a donor with 2 mismatches at Ehp including 1, 2 or 3 HLA mismatches at HLA-A, −B, −C, −DRB1 and -DQB1 (9/10, 8/10 or 7/10) was
selected and accepted for hematopoietic stem cell transplantation (HSCT) in the years 2002–2011. In November 2016 we re-examined and analyzed the BMDW donor match results for the same group of 58 patients at risk. The median time from HSCT to re-analysis was 11.3 years (range 4.5 – 15.5). For the 53 recipients (91.4%) at least one donor with a lower level of Ehp mismatch and equal or higher degree of HLA matching was found. For 38 (66%) patients a fully HLA matched donor was found (HLA 10/10, Ehp 2/2), mostly in the Polish registry (69%). For 20 (34%) patients for whom the best donors found were HLA mismatched donors, 19 (95%) have a donor with one mismatched Ehp and only one (5%) with two mismatched Ehp. Overall, out of the 58 patients transplanted in the previous decade from sub-optimal risky donors, better donors could be found for 57 (98%) of the patients transplanted in the previous decade from sub-optimal risky donors, better donors could be found for 57 (98%) with current resources. The analysis shows that the development of the world’s donor database BMDW and national registries increases the chance of selection the optimal unrelated HSCT donor for the patients at highest risk of morbidity and mortality.

P90

GVHD-DIRECTED HLA-DPB1 MISMATCHES INCREASE INCIDENCE OF DEVELOPMENT OF AGVHD AFTER UNRELATED DONOR HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Allogeneic hematopoietic stem cell transplantation (alloHSCT) is now an established curative therapy for hematologic malignancies and other hematologic or immunological disorders. Human leukocyte antigen (HLA) molecules play an important role for immunoreactivity in alloHSCT. Despite HLA locus matching at the allele level, significant acute graft-versus-host disease (aGvHD) incidence remains a major barrier to successful alloHSCT. It is well defined that matching for HLA-A, –B, –C, –DRB1, –DQB1 is crucial, whereas typing for additional genes such as HLA-DPB1 is still controversial. To date, 67 consecutive adult patients undergoing their first alloHSCT at Vilnius University Hospital Santariskiu Klinikos were enrolled into the prospective study from June 1, 2014. Fifty-two unrelated donor (UD) - patient pairs (77.6 %) were 10/10 matched at HLA-A, –B, –C, –DRB1, –DQB1 loci and the remaining 15 pairs (22.4 %) were 9/10 matched. We found that 7 (10.4 %), 29 (43.3 %) and 31 (46.3 %) UD-patient pairs had no, permissive or non-permissive HLA-DPB1 mismatches (according to the DPB1 T-Cell Epitope Algorithms, IMGT/HLA), respectively. In a group of 10/10 matched pairs, only seven (13.5 %) pairs were identical for DPB1 alleles (12/12), 22 (42.3 %) pairs were found to be non-permissively mismatched and 23 (44.2 %) pairs had permissive HLA-DPB1 mismatches. In a logistic regression model, non-permissive DPB1 mismatches were found to be a significant predictor of development of aGvHD (grades II - IV) (p = 0.006). Further statistical analysis of DPB1 non-permissive mismatch subgroups revealed that only mismatches in graft versus host (GvH) direction were found to be responsible for increased occurrence of aGvHD (p = 0.004), whereas the predictive value of non-permissive mismatches in host versus graft (HvG) direction did not reach statistical significance (p = 0.13). In conclusion, our results suggest avoiding GvH directed HLA-DPB1 mismatches in UD selection procedure to minimize aGvHD incidence.

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THE IMPACT OF MICA POLYMORPHISMS ON OUTCOME OF UNRELATED HEMATOPOIETIC STEM CELL TRANSPLANTATION

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MICA (MHC class I polypeptide-related sequence A) is a non-classical HLA-molecule activating NK-cells, γδ-T-cells and
NKT-cells via the NKG2D receptor. MICA incompatibilities have been associated with an increased GvHD incidence and the MICA-129 (met/val) dimorphism has been shown to influence NKG2D signaling in unrelated hematopoietic stem cell transplantation (uHSCT). We analyzed the effect of MICA-Allele and MICA-129 matching on the outcome of uHSCT. We sequenced 3262 patients and their respective donors. All patients and donors were high-resolution HLA-typed and matched for 10/10 (n = 2107), 9/10 (n = 946) or 8/10 (n = 209) HLA-alleles. Within each HLA match group, cases matched and mismatched for MICA-Alleles/MICA-129 were analyzed for the endpoints of overall survival (OS), disease free survival (DFS), non-relapse mortality (NRM), relapse-incidence (RI) and GvHD. Mismatches at the MICA locus as well as MICA-129 increased with the number of HLA mismatches (MICA mismatched 10/10: 7.8%, n = 164; 9/10: 23.2%, n = 219; 8/10: 39.2%, n = 82; MICA-129 mismatched: 10/10 3.3%, n = 69; 9/10: 11.5%, n = 109; 8/10: 16.3%, n = 34). Adverse OS was observed in the 10/10 and 8/10 match group if MICA-129 was mismatched (10/10: HR 1.64, CI 1.18-2.30, p = 0.003, 8/10: HR 1.72, CI 1.06-2.80, p = 0.027). MICA-129 mismatches correlated with a significantly worse outcome for DFS in the 10/10 and 8/10 HLA match group (10/10 HR: 1.46, CI 1.07-1.98, p = 0.016, 8/10: HR 1.76, CI 1.11-2.79, p = 0.016). Higher rates of aGvHD were seen in MICA-129 mismatched cases. Our results in this extended cohort indicate that MICA-129 matching is relevant in uHSCT. Prospective typing of patients and donors in unrelated donor search may identify mismatches for MICA-129 and compatible donor selection may improve outcome for this small but high-risk subgroup.

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MALE-SPECIFIC SURVIVAL ADVANTAGE OF HLA-DRB1*04 FOR LYMPHOID MALIGNANCIES FOLLOWING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Distinct human leukocyte antigen alleles (eg. HLA DRB1*04) were described to predispose to lymphoid malignancies. Interestingly, this risk was identified as male-gender specific in several studies. The associations of HLA alleles with the outcome of allogeneic hematopoietic stem cell transplantation (allo-HSCT) have already been reported. We aimed to investigate the effect of HLA-A, -B and -DRB1 allele groups on overall survival (OS) following allo-HSCT in patients with lymphoid malignancies. 186 adult patients with lymphoid malignancy, who were consecutively treated with first allo-HSCT between 2007 and 2013, were analyzed retrospectively. The median follow-up was 44 months. In the case of HLA-A or -B allele groups, carriers and non-carriers displayed no survival difference, but in the case of HLA-DRB1*04, carriers showed significantly better OS compared to non-carriers (24-month OS 66 ± 8% [n = 36] vs. 50 ± 4% [n = 150], p = 0.01). Multivariate analyzes also confirmed an independent association (p = 0.05). In separate subgroup analyzes, we observed that the survival advantage of HLA-DRB1*04 was restricted to males (n = 114, p = 0.01), but not to females (n = 72, p = 0.41). The donor gender also affected the outcome in our cohort (p = 0.04). In male recipients the best survival was achieved in HLA-DRB1*04 carriers who received graft from a female donor, followed by those HLA-DRB1*04 carriers with a male donor. No clear explanation was discovered, except for a tendency toward less CMV reactivation/disease among carriers (8% vs. 21%, p = 0.097). In summary, HLA-DRB1*04 male carriers with lymphoid malignancies had significantly better overall survival following allo-HSCT compared to non-carriers. Our findings support that HLA-DRB1*04 carrier-ship might result in male-specific consequences in the lymphoid malignancies.

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DISTRIBUTIONS OF HUMAN LEUKOCYTE ANTIGENS IN PATIENTS WITH HEMATOLOGICAL DISEASES IN KAZAKH POPULATION

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Oncohematology is one of the most dynamic areas of medicine, which has been actively developing in the last 10 years. Over the years, many new scientific facts have been discovered which largely changed the fundamental concepts in oncology. The results obtained from modern molecular biological methods helped scientists to explain the structural changes in the genome of the cells, which are responsible for initiation of a tumour and tumour growth. In this study, we investigated the frequency of HLA class I (HLA-A and -B) and class II (HLA-DRB1) genes in patients with oncohematological, residing in Kazakhstan. The study enrolled 3740 participants: 3621 healthy blood donors considered as a control group (of them HLA-A – 3621; B- 3607; Cw- 3582; DRB1-3576) and 633 patients with oncohematological diseases. HLA-
typing (HLA-A, −B, −C, −DRB1 and -DQB1) for both groups was performed by a molecular genetic method using a kit from Protrans (Protrans, Germany). The study done to investigate the distribution of the HLA antigens for each oncohematological disease. Based on AML patients’ results, there is an association between the presence of the genes such as HLA-A*31, B*37 and the development of AML. The HLA antigens including HLA-A*30, B*44, C*16, DRB1*07, *16 marked with the development of ALL, whereas the genes such as HLA-A*11, HLA-B*18, *41, *47 *73 considered as genetic markers for the development of CML. In addition, the genes such as HLA-B*35, HLA- DRB1*11,*15 displayed the link between these antigens and the development of MDS, whereas the existence of HLA-A*24, HLA-B*40, *54, HLA-DRB1*14 antigens among AA patients revealed the association between these antigens and the development of this pathology. In addition, this study found that the presence of some HLA antigens in the genome has a protective role against oncohematological pathologies. For example, for AML- HLA-A*02, B*27, C*02, DRB1*01, *04, DQB1*06 genes; for ALL- HLA-A*02, C*02, DQB1*06 genes; for CML- HLA-A*01, HLA-C*02, *06 genes; for MDS- HLA-A*02, HLA-C*07 genes; for AA- HLA-C*02, *05, *07, *12, HLA-DRB1*03, *04, *07 genes. The HLA typing results can be used to study different diseases associated with the HLA antigens.

Immunotherapy, Gene Therapy & Cellular Therapy

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QUALITY AND SECURITY OF BLOOD, CELLS AND TISSUES IN THE EU LEGISLATION: IS THE CHOICE OF THE LEGAL INSTRUMENT A CHALLENGE?
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Medicine is booming because of the development of science and more particularly of biology and biotechnology. Health products based on genes, cells and tissues are developed and are questioning the existing legal frameworks because of their innovative character, their origin, the therapeutic hopes and the unknown risks. The European Union has an important role to play in the management of scientific advances in order to ensure a high level of protection of public health in the EU. Due to the extraordinary advances in the field, Europe has to update and evaluate the legal acts already implemented. In this regard, the proposal from the Commission is to turn the Tissues and Cells Directives into a Regulation which will be mandatory in all EU member states. This change of legal instrument is motivated by the different approaches developed by the Member States when transposing the Directives leading, then, to gaps in the national legislations. To this end we will analyze the public consultation launched on 17th January 2017 by the European Commission as part of its better Regulation Package, and the comments provided about the relevant legislation. The aim is to assess the legislation on blood, cells and tissues, so as to understand the positive and negative aspects of the current regulation. It is also a good opportunity to look at the issues under discussion during this public consultation as well as the legal nature of the acts in order to meet the initial objective of this regulation: minimize the risks in operations on blood, cells and tissues. It is a timely occasion to take stock of the legal actuality in this field and to understand the new issues which will arise in the coming months for the use therapeutic of tissues and cells.

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ELIMINATION OF ANTI-HLA ALLOANTIBODY PRODUCING B CELLS THROUGH THE USE OF A CHIMERIC ANTIGEN RECEPTOR-LIKE HLA MOLECULE IN T CELLS
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T cell immunotherapy using Chimeric Antigen Receptors (CARs) to generate tumor antigen-specific effector T cells has achieved excellent results in anti-leukemic clinical trials, and in a broader appraisal, promising immunotherapeutic results. A major problem in solid organ transplantation is the presence in the recipient of donor specific antibodies, which preclude the success of the transplant due to the associated high risk of antibody-mediated rejection. We hypothesize that a CAR (chimeric antigen receptor)-like molecule with a particular HLA molecule as the CAR extracellular domain will engineer T cells to kill alloimmune B cells with anti-HLA antibodies as BcR, completely eliminating alloantibodies in a specific manner. The first step was to create this chimeric receptor with the HLA-A2 antigen as the CAR-like extracellular domain: extracellular domains of the HLA-A*02:01 molecule were amplified by PCR from cDNA of an A*02:01 positive donor. A CAR comprising the extracellular domains of HLA-A2 and 4-1BB /CD3ζ signalling domain was constructed and delivered by lentiviral transduction into human T cells. The cytotoxic capacity of these transduced T cells will be assessed by co-culturing them with EBV-transformed B cells which produce anti-HLA-A2 alloantibodies. In conclusion, specific T cells directed to...
kill anti-HLA alloantibody producing B cells can be generated by means of the use of an HLA CAR-like receptor. This technology could open new ways of treatment and prevention of antibody-mediated rejection in solid organ transplantation.

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KYNURENINE AS AN INDICATOR OF THE MODIFICATIONS IN DIFFERENTIATED HEPATOCYTES

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Tryptophan (Trp) is an amino acid required for protein synthesis and metabolic functions. About 95% of Trp is metabolized via the kynurenine (Kyn) pathway. The initial step is oxidation of Trp to N-formylkynurenine, which is converted to Kyn by one of two enzymes, Trp-2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO). Kyn pathway metabolites act as immune regulators. Our aim is to investigate Trp metabolism via the kynurenine pathway in Primary human hepatocytes (PHH) and tumoral HepaRG cells.

TDO and IDO activity in supernatants of cultured PHH and HepaRG cells was estimated by measuring the degradation of Trp and accumulation of Kyn in the culture media using Reversed Phase-High Performance Liquid Chromatography. IDO activity was inhibited by 1-Methyl-tryptophan (1-MT). However, TDO activity was inhibited either by addition of 680C91 or by addition of LM10. TDO or IDO expression was determined by Real-time PCR. Additionally the influence of IFN-γ (an IDO enhancer) on IDO and TDO activity was studied. PHH as well as HepaRG cells degrade Trp in culture media with increasing amounts of Trp. The highest level of Trp degradation was at 72 h as compared to 24 and 48 h. In contrast, PHH supernatants of HepaRG showed increased levels of Kyn. Compared to unstimulated cells stimulated PHH and HepaRG cells with IFN-γ x did not show any significant difference in Trp degradation and Kyn accumulation. Degradation of Trp in the supernatants of differentiated hepatocytes was inhibited by 680C91 as well as LM10 and not by 1-MT. As expected TDO but not IDO gene transcription was detected in HepaRG cells. After IFN-γ stimulation, expression of IDO was extremely up-regulated but this did not enhance Trp degradation. Hepatic cells express only TDO protein which positively correlated with increased L-Trp levels in cell culture media. Enzymatic activity of TDO in HepaRG cells was only able to degrade L- but not D-isofom of tryptophan. To conclude, TDO is the key player of Trp degradation in differentiated intact primary- and tumor hepatocytes. HepaRG accumulate high levels of Kyn which can be a mechanism of tumoral immune resistance. Inhibitors of TDO activity-680C91 or LM10- could play an important role in cancer immunotherapy.

Bioinformatics

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COMPUTATIONAL APPROACHES TO FACILITATE EPITOPE-BASED HLA MATCHING IN SOLID ORGAN TRANSPLANTATION

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Epitope-based HLA matching has emerged over the last few years as an improved method for HLA matching in solid organ transplantation. The epitope-based matching concept has been incorporated both in the PIRCHE-II and the HLAmatchmaker algorithm to find the most suitable donor for a recipient. For these algorithms, high-resolution HLA genotype data of both donor and recipient is required. Since high-resolution HLA genotype data is often not available, we developed a computational method which allows epitope-based HLA matching from serological split level HLA typing relying on HLA haplotype frequencies. To validate this method, we simulated a donor-recipient population for which PIRCHE-II and eplet values were calculated when using both high-resolution HLA genotype data and serological split level HLA typing. The majority of the serological split level HLA-determined ln(PIRCHE-II)/ln(eplet) values do not or only slightly deviate from the reference group of high-resolution HLA-determined ln(PIRCHE-II)/ln(eplet) values. This deviation was slightly increased when HLA-C or HLA-DQ was omitted from the input and was substantially decreased when using two-field resolution HLA genotype data of the recipient and serological split level HLA typing of the donor. Thus, our data suggest that our computational approach is a powerful tool to estimate PIRCHE-II/eplet values when high-resolution HLA genotype data is not available.

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FERRET: A USER-FRIENDLY TOOL TO EXTRACT DATA FROM THE 1000 GENOMES PROJECT

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426 Abstract
The 1000 Genomes (1KG) Project provides a near-comprehensive resource on human genetic variation in worldwide reference populations, including high-resolution HLA typing. We implemented a user-friendly Java tool, “Ferret”, to ease the access of the community to the large and complex 1KG genomic data files. Ferret’s unique advantages encompass: (1) multiple inputs accepted such as locus, gene(s) or SNP(s) of interest; (2) fast extraction of 1KG individual genotypedata for SNPs and indels; (3) allelic frequency computation for SNP, indel and CNV in 1KG populations and (4) options for the Exome Sequencing Project populations. By converting the NCBI annotations and the 1KG data into files that can be imported into popular pre-existing tools (e.g. PLINK and HaploView), Ferret hence offers a straightforward way, even for clinicians and biologists, to manipulate, explore, and merge 1KG data with the user’s dataset, as well as visualize linkage disequilibrium pattern, infer haplotypes, and design tagSNPs. This tool could therefore empower the immunogenetics community to leverage the 1KG data, especially in the prospect of granting HLA data access. Ferret is publicly available at: http://limousophie35.github.io/Ferret/.

**P99**

**HLA AND THE EMR: DEVELOPING HL7 FHIR TOOLS FOR EXCHANGING NGS-BASED HLA GENOTYPING**

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While principles and standards have recently been developed for exchanging NGS-based HLA genotyping data between typing labs, researchers, and donor registries (MIRING & HML), it is still challenging to interoperate with clinical EHR/EMR and other healthcare systems, especially when the data includes clinical genomics and sequencing information. New standards are emerging that use modern approaches for interoperability with health care systems. The most promising standard is Health Level 7 Fast Healthcare Interoperability Resources (HL7 FHIR). HL7 FHIR has developed great momentum in the vendor community, evidenced by the Argonaut Project (argonautproject.org), a private sector initiative including EHR/EMR vendor and health care organizations to advance industry adoption of modern open interoperability standards for sharing electronic health records, and is focused on developing FHIR-base API and Core Data Services. FHIR’s fundamental building block is a resource (e.g. Patient, Observation, etc). These resources account for expected use by 80% of the implementations. The other 20% is accomplished by profiling the resource by constraining existing data elements and introducing domain specific elements with extensions. Using existing resources and profiles (including those developed for clinical genomics) in HL7 FHIR Standard for Trial Use 3, we have developed a transaction bundle for exchanging HLA typing reports. The scenario includes specimen collection (Specimen), typing labs (Organization), registries (Organization), lab orders for NGS-based HLA typing (Diagnostic Request), and typing reports (Diagnostic Report) that consolidate the genotype reported, and supporting evidence for allele assignment (nested Observations and Sequences). Tools are being developed to support this HLA typing scenario. This includes standing up a FHIR compliant server based on the Java based HAPI FHIR Server (http://fhirtest.b12x.org/), clients and middleware for creating FHIR resources and profiles for HLA either directly or converting from HML, and FHIR compliant terminology resources for HLA nomenclature.

**P100**

**THE KIDNEY TRANSPLANTATION APPLICATION (KITAPP): A VISUALIZATION AND CONTEXTUALIZATION TOOL IN A KIDNEY GRAFT PATIENT COHORT**

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Around 10% of adults suffer from chronic kidney disease (CKD). Among those more than 550,000 patients with advanced CKD evolved to end-stage renal disease (ESRD) and become candidates for kidney transplantation. With around 200 kidney transplantsations performed annually, our institute has had the opportunity to record data on more than 1,500 patients followed since 2008 gathering over 200 clinical and immunological items at the day of the graft and each year after that. How can this amount of data be used in a personalized way for both patients and practitioners to inform the decision-making process with data? The KiTApp (Kidney transplantation application) is a prototypic software designed to put in population context, a given patient facilitating actionable comparisons to his/her peers in the reference cohort. To some extent, KiTApp is a generalized version of paper-based growth charts used in pediatrics. The goal here is to understand how the immunosuppressive treatment and its intensity could affect the course of a patient’s disease. By comparing with the user-
defined sub-cohort, the ultimate objective is to help the clinical
decision by trying to anticipate the effect of possible decisions
on the patient’s evolution. The development of the app
required a careful curation of the data. We present the two
types of algorithms developed: 1) a populational contextualization
where we compare a patient to the different treatment
available, and 2) a referential contextualization where we com-
pare a patient to defined extreme groups (designed with the
help of clinicians) such as acute graft reject, humoral reject,
cellular rejection or tolerance. KiTapp is presented as a web
app displaying dynamic graphical view of the patient-centered
comparisons. On a clinical point of view, this app ambitions to
help clinical decision by facilitating access to large amount of
data actionable manner. On a technological point of view, soft-
ware and algorithms developed here could be applicable to vari-
sous chronic medical conditions.

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CWD-VIEWER: A TOOL TO VISUALISE AND INSPECT EFI AND OTHER CWD CATALOGUES
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The knowledge of which alleles are Common and Well Docu-
mented (CWD) is of great relevance to the HLA practitioner in
diagnostics and research. The EFI catalogue published in
February 2017 extends previous CWD catalogues by increas-
ing the amount of data for Europe and by providing information
about the CWD status of alleles at regional geographic levels. It is therefore possible to determine whether a given
allele is CWD across all of Europe or only within specific
regions. To facilitate access to this EFI CWD catalogue, we
have built a web-based interface accessible from any connected
device such as computers, tablets or smartphones (http://hla-
net.eu/cwd). The design of the interface is tailored to simplify
queries to all the CWD catalogue information. Queries, which can include several alleles and loci simultaneously, are either
visual or text-based. The results are synoptic tables and maps
illustrating the regional status (Common, Well-Documented or
other) of each allele and enriched with additional relevant
information retrieved from or linked to other databases. In par-
cular, as the CWD information does not reflect the population
diversity from the point of view of population genetics, the
viewer provides links to maps and charts displaying the fre-
cquency distribution of CWD alleles across Europe when such
information is available. The catalogue viewer presented here
is implemented as a tool that depends both on a data format
used to describe the data sources and on a set of computer pro-
grams used to query the data and produce statistical summaries
and detailed analyses of the CWD data. By providing the full
technical description of these formats and the algorithms used,
it will also be possible to build equivalent viewers for other
specific CWD catalogues. Finally, we discuss conditions for
automatic updates of such catalogues.

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SNP-HLA REFERENCE CONSORTIUM: HLA AND SNP DATA SHARING FOR PROMOTING
HLA CENTRIC ANALYZES IN GENOMICS
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SNP based imputation of HLA alleles is now essential in the
analysis of large human genomics studies. Recently, the Hap-
totype Reference Consortium aggregated over 20 studies to cre-
ate a very large reference panel of human haplotypes where
~50 M genetic variants are observed from >31,500 sequenced
whole genomes (http://www.haplotype-reference-consortium.
org/). Genotype imputation improves in accuracy with increas-
ing numbers of sequenced samples. While extensive studies
using SNP genotypes grew extremely fast; much work is still
needed to increase capacities of studying HLA alleles and their
association with different diseases where SNP genotypes are
available. Indeed, it became much easier to impute the HLA
alleles from SNPs genotyped in the MHC region. To fill this
gap between genomic data availability and HLA alleles studied
we propose to create a validated data framework for HLA
imputation: the SNP-HLA Reference Consortium. The aim is
to facilitate the HLA imputation from SNP studies and therefor-e
improve the correlation between SNPs and HLA genotypes.
We already have access to large cohorts of over 4,000 ethnici-
diverse individuals with HLA types and SNPs in high-
resolution genotypes. We plan to curate both SNP and HLA
data to improve the resources available for training a software
performing SNP-Based HLA imputation. We plan to make
available to the community through IHIW websites large
panels of references suitable to easily impute the HLA with
SNPs genotyped from chip arrays. We will present this ambi-
tious project to the next HLA and Immunogenetics Workshop
in San Francisco next September. To develop this very ambi-
tious tool we encourage willing participants with large HLA
types + SNP data to join the project and contribute to, as seen
for SNPs within the genomic community, accuracy increases
when we merge data from different ancestry backgrounds.
HIGH RESOLUTION HAPLOTYPE INFERENCE FOR HLA GENES FROM FAMILY TRIOS

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Full resolution genotypes of Human Leukocyte Antigen (HLA) genes can be obtained with next generation sequencing technology (NGS). All 11 major loci can be typed for multiple samples in a single run. Compared to traditional methods of SSO, SSP and Sanger sequencing, NGS delivers more complete coverage of the genome and phased contig sequences to resolve ambiguities. Combining the genotype at 4-field resolution and family pedigree information, haplotypes for HLA genes can be constructed with high accuracy. Here we describe a method for building phased haplotypes from family trios. Approximately 1500 family trios were typed by this method and the genotype calls made by the software are manually reviewed based on all the quality metrics. The accuracy of genotyping results at 3- and 4-field resolution is assessed through segregation analysis. Concordance is computed by comparing allele calls of the child to those of the parents. At 3 field resolution, the validated genotyping accuracies for automatic calls are above 98.8% and 98.3% for class I and class II genes respectively. For reviewed calls, the validated accuracies are around 99.9% and 99.8% for class I and class II genes respectively. For family trios, haplotype phase can be determined at multiple loci by applying Mendelian constraints. The child within a trio must share one allele identical by descent at each locus with each of the parents. This information allows resolution of the haplotypes at strongly linked loci. The challenge of constructing haplotype phase is at loci where all three family members have the same heterozygous genotypes. In this case, coalescent based methods and Hidden Markov models are used to resolve ambiguous assignment. A database of the haplotype frequencies is constructed from the phased haplotypes based on family trios. The method to build the haplotype database with high resolution and accuracy allows applying linkage analysis in population and disease association studies.

COMMUNITY RESOURCES FOR AUTOMATED ANNOTATION OF HLA, KIR AND BEYOND

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New sequencing technologies have increased demand for tools and methods for annotating and analyzing sequence data. The extreme allelic and structural polymorphisms present in HLA and KIR renders general genetic variation nomenclatures, as well as those used within the immunogenetics field, only marginally useful for describing 1) consensus sequence with partial phasing, 2) incomplete gene sequence coverage and 3) novel variants, especially intronic variants. In preparation for the 17th IHIWS, we have introduced open source web services that perform automated analysis of NGS consensus sequences and deliver Gene Feature Enumeration (GFE) strings, a computable shorthand description of consensus sequences. This GFE service [http://gfe.b12x.org/] accepts (curated or pre-curated) consensus sequences, performs alignment and annotation and leverages a simpler system for persisting sequence data called “feature service” [http://feature.nmdp-bioinformatics.org/]. Feature Service has been developed to authoritatively assign a unique identifier to any sequence indexed by its locus (any gene in the list maintained by the Human Genome Organization (HUGO)) and feature (any term in list maintained Sequence Ontology (SO)). We have demonstrated the utility of these services through the analysis of sequences generated from over 500 K genotyping results from HLA, KIR, ABO and other blood group antigen gene families with a variety of levels of coverage and phasing. In situations where targeted sequencing is used (e.g. exons only) we have extended and applied the Genotype List format and GL Service (gl.nmdp.org) for representing and persisting information about phase and allelic ambiguity. Applied together, these tools become a new platform for accelerating the development NGS data analysis for population genetics (LD, HWE), disease association, peptide binding, expression and clinical histocompatibility.
INTERNAL STRUCTURE OF HLA GENES REVEALED BY NEXT-GENERATION SEQUENCING

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MicroRNAs regulate the expression of genes by binding to the 3'UTR region of these genes. Polymorphism in this region affects the regulatory mechanism of microRNAs. This is particularly the case with HLA-C. The region of the HLA-C gene publicly known and available at the IPD-IMGT/HLA Database does not include the binding site for microRNAs. Next generation sequencing (NGS) allows the analysis of expanded regions of the genome. The microRNA-binding region of the 3'UTR segment of HLA-C was studied using Mia Fora (Immucor) NGS methodology in a large sample set. Seven polymorphic variants were identified in the HLA-C 3'UTR region. Most HLA-C alleles present only one of these genetic variants each, but some of them (C*02:10, C*04:03:01 and C*12:02:02) show multiple variants each. This is an example of the expanded horizons opened by the NGS HLA typing technology that go beyond allele assignment. Other poorly studied regions of the HLA genome are included in this study showing how these regions reveal not only important functional properties of HLA molecules but also phylogenetic cues useful to determine the evolutionary relationship among alleles. These cues from poorly studied low-entropy segments of the HLA genome show the internal structure of HLA genes, which is essential to develop tools to analyze their DNA sequence. Sequence analysis software tools can be enhanced by taking advantage of the internal structure of HLA genes. Specialized knowledge of the HLA environment is one of the keys to the efficiency of these software tools.

NOVEL HLA-G WHOLE GENE AMPLIFICATION APPROACH REVEALS LINKAGE DISEQUILIBRIUM BETWEEN EXTENDED 3'UTR AND HLA-G AT THE ALLELIC RESOLUTION LEVEL

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Several studies have been conducted which assessed the influence of the polymorphic sites present in the regulatory regions of the HLA-G gene on its expression. With regard to the 3 prime untranslated region (3'UTR) of HLA-G, a number of different haplotypes have been defined, including the 14-bp indel region and additional SNPs covering cDNA region 2940–3509. Linkage disequilibrium between the 3'UTR haplotypes and high-resolution HLA-G alleles has been studied by several research groups that are interested in the relevance of HLA-G in clinical transplantation and in pregnancy outcome. However, no HLA-G alleles have yet been submitted to the most recent IPD-IMGT/HLA Database (3.27.0) to provide the fully phased whole HLA-G gene sequence with inclusion of the 3'UTR region. We have developed an HLA-G whole-gene NGS-based typing assay (NGSgo-AmpX HLA-G, GenDx) enabling full phasing over the entire HLA-G region, and allowing the identification of HLA-G alleles at the allelic resolution level including the extended 3'UTR. Here, we present HLA-G typing data obtained for 450 clinical samples, using the NGSgo workflow compatible with Illumina platforms. HLA-G typings were confirmed using the CE-marked HLA-G NGSgo-AmpX assay and NGSengine® software (GenDx). A high, but not absolute, linkage disequilibrium between IMGT-defined HLA-G alleles and the extended 3'UTR region was identified. Full associations using estimated genotype and haplotype frequencies were found between HLA-G*01:01:01:01 and UTR-01, between HLA-G*01:01:01:05 and UTR-04, and between HLA-G*01:04 and UTR-03. In addition, several novel HLA-G alleles were identified.

QUALITY CONTROL IN NEXT-GENERATION SEQUENCING HLA Typing

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The main problem in the implementation of a new truly innovative technology is the lack of proper standards for its validation. Typing results from next-generation sequencing (NGS) cannot be compared to results by current standard methods because these fall short in many ways. In so far as validation of a new technology must rely on external verification, and since there is no current established typing method of comparable coverage and resolution, only a biological system with its own internal logic can meet the demands for proper validation. Reproducibility...
studies testing the same samples repeatedly do not meet the requirements for analysing alleles in different genetic contexts. Sexual reproduction and family segregation studies are a proper validation. A set of samples of mother-father-child trios is the ideal set to validate NGS HLA typing. These trios present the same alleles in different diploid combinations. A set of 500 such trios was used with Mia Fora (Immucor) revealing the strengths and problems of the methodology and providing the necessary feed-back for tuning up the sequence analysis and allele assignment algorithms. NGS software must have the feed-back control mechanisms to use typing-error data to optimise its analytical tools. In addition, automated sequential control systems in NGS should ideally incorporate the logic of population genetics. One of the main problems was the reference sequence library. Since the IMGT allele sequence library was built in sub-optimal and error-prone conditions it is not surprising to find it unfit to meet the data analysis demands of NGS. These demands include: 1) A realistic catalogue of HLA alleles based on adequate documentation. 2) Allele frequency information in the main broad world populations. 3) Quantitative linkage disequilibrium data specific for each of the main world populations.

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FULL-LENGTH SEQUENCING OF A NOVEL MICA ALLELE VARIANT
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The MICA (MHC I chain related gene A) gene is located on chromosome 6 within the HLA region and encodes cell stress-inducible, highly polymorphic cell surface proteins that function as ligands of the activating NKG2D receptor on natural killer (NK) cells. Recent reports describe the importance of MICA matching in the context of hematopoietic stem cell transplantation and therefore prospective typing may facilitate a further improvement in selecting suitable donors. After development of a full-length MICA next generation sequencing (NGS) assay as an integrated module of our NGS HLA typing routine we found a so far undescribed MICA coding sequence variant. This presumably new allele is almost identical to the MICA*008:04 allele except the A to G nucleotide exchange at the position 1087 in exon 6 which leads to an amino acid change in codon 340 from Thr to Ala. Next generation sequencing was performed on a MiSeq instrument with 500 cycles PE V2 chemistry from Illumina. The initially generated MICA full-length amplicons (13 kb) were enzymatically fragmented and adapter ligated according to a standard NEB library preparation protocol. For verification reasons the generated fastq data were analyzed with two different sequence analysis tools provided by the software suppliers Omixon (HLA Twin) and GenDx (NGSengine). The subsequent validation of the NGS results was performed by Sanger sequencing all six exons of this sample in forward and reverse directions on a 3730XL DNA Analyzer (Applied Biosystems) followed by an Assign SBT v4.7.1 (Conexio Genomics) sequence analysis. Sanger sequencing confirmed the initially observed nucleotide substitution as novel and so far undescribed MICA sequence variant. Implementing full-length MICA genotyping into HLA routine typing workflows will definitely increase our knowledge about the variability of the MICA locus at reasonable costs without neglecting allelic variants that might be important for donor selection and transplantation outcome.

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COMPARISON OF THREE COMMERCIAL KITS (STR PP16, STR MENTYPE CHIMERA AND INDEL MENTYPE DIPSCREEN) USABLE FOR CHIMERISM DETERMINATION BY FRAGMENTS ANALYSIS
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New multiplex PCR kits (Biotype diagnostic) CE-marked for chimerism determination using fragments analysis, performed by capillary electrophoresis, are commercially available. They must be evaluated and compared to the techniques routinely used. Three multiplex PCR were compared: STR Promega PP16, and Biotype diagnostic STR Mentre Chimera and InDel Mentype DIPScreen kits using insertion/deletion polymorphism. PCRs were performed according to manufacturer’s instructions. Amplicons were de-natured by formamide and migrated in polyacrylamide gel simultaneously with a fluorescent ladder. Results generated by Biotype diagnostic reagents were analyzed with ChimerisMonitor 2.0. This dedicated software proposes informative loci for % recipient calculation, and uses areas under peaks for the selected loci. These 3 kits were preliminary compared with 3 donor/recipient couples with different ADN mixes at 25, 5 and 1% recipient. Our work showed that all kits permitted manipulation with the same practicability. In addition, the results obtained are similar between the 3 kits on the tested samples. The commercial kits by Biotype diagnostic seems to be as appropriate as PP16 Promega kits for chimerism determination. A more extended evaluation should be performed, in particular concerning the informativity of the loci included in the kits and the Z scores obtained on external proficiency testing samples.
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HOW CAN NGS TECHNOLOGY HELP YOU IN GENOTYPING OF "TRICKY SAMPLES"?

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Sequencing based typing (SBT) has become a gold standard for high resolution HLA genotyping. With an increasing number of known HLA alleles, the number of ambiguous results for high resolution typing. Holotype HLA and its associated software, HLA Twin, gives tissue typers a new tool for HLA genotyping on an allelic level with almost unambiguous results. Here we show, with several examples, the power of NGS for HLA typing by identifying a novel stop codon and a new splice site. By displaying "noisy reads" that were removed during the analysis of the two alleles in the "noise track" it was possible to detect a triploid sample and separate a contamination in the sample. Using the amino acid display track we could immediately identify if our novel allele creates aberrant protein. NGS techniques continue to evolve rapidly and the trends are clear that they will soon become a routine method in almost every tissue typing laboratory. Holotype HLA and HLA Twin facilitate this transition to a new era of high-resolution, ambiguity-free, accurate HLA genotyping.

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HLA-E GENOTYPING – THE SOONER THE BETTER

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The Human Leukocyte Antigen E belongs to the non-classical HLA class I molecules located within the MHC on chromosome 6p21 and is described to display only limited polymorphism. The HLA-E complex is the major ligand for the natural killer inhibitory receptor CD94/NKG2A and the activating receptor CD94/NKG2C. The complex can also present peptides originating from the cellular antigen processing pathway and therefore induce T-cell mediated immune responses. Nowadays there is growing evidence that HLA-E polymorphisms have a major impact on the immune system and most likely affect hematopoietic stem cell transplantation outcome. To evaluate HLA-E polymorphism and their possible impact on transplantation we designed a NGS based long range PCR assay covering the entire gene. Due to the lack of suitable reference samples we sequenced 95 randomly selected DNA samples to prove amplification of so far described HLA-E alleles. The generated NGS data were analyzed with the HLA Twin software (Omixon) and confirmed by Sanger sequencing using a freeware tool (BioEdit). During analysis we found several so far undescribed sequence variants mainly located in the non-coding sections. One sample revealed a nucleotide substitution from G to A at position 1644 in exon 4 responsible for an amino acid change in codon 196 from Asp to Arg. From this observation we conclude that the frequency of polymorphism is higher than so far estimated. Implementing this in-house designed, full-length HLA-E sequencing assay into our NGS HLA typing routine will probably lead to the detection of more variants increasing our knowledge about HLA-E, hopefully to the benefit of transplantation outcome.

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ABO BLOOD GROUP TYPING WITH OXFORD NANOPORE MINION SEQUENCING

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Typing of the ABO blood groups, which encodes the transferase that catalyzes the last step in the synthesis of the A, B, and H antigens, is a prerequisite for both the recipient and donor in solid organ transplantation, stem cell transplantation, and blood transfusion. Historically, blood group typing has typically been done with serology, but in a number of cases, such as registry typing with buccal swabs and samples from cord blood, only DNA is available. The blood group types A, B, and O show clear differences in the genetic sequence of the ABO gene, and therefore DNA sequencing offers a valuable alternative to serology in these cases. In this study we have used MinION Nanopore sequencing to obtain full length sequences of the ABO gene. Two long-range overlapping amplicons were prepared which span the complete 18 kb length of the ABO gene for a set of samples which represent the six possible ABO combinations (AA, BB, AO, BO, AB and OO). The amplicons were sequenced on the Oxford Nanopore MinION, and reads were aligned with known reference sequences obtained from the NCBI dbRBC database. DNA sequence data was sufficient to differentiate between the six possible ABO combinations based on 3 polymorphic positions. Although the current
analysis focuses on the polymorphism that defines the blood group types as a proof of principle, the acquired full-length sequences can provide information about the extensiveness of polymorphism present in the ABO alleles, and help complete the full-length ABO allele database. Overall, this study shows that Nanopore sequencing on the MinION represents a novel platform for full-length high-throughput sequencing of ABO genes, suitable for cases where only DNA is available.

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TOWARDS FULL-LENGTH SEQUENCING: COMPARISON OF DIFFERENT SEQUENCING METHODS

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Excluding null alleles and resolving ambiguities within G groups are mandatory in the laboratories serving unrelated HSCT programs. Since routine sequencing strategies have mainly focused on exons 2 and 3, for more than 90% of the HLA alleles valuable full-length sequence information is lacking. The 17th IHIWG programme includes working groups for full length HLA gene sequencing. Using the full-length hemizygous Sanger sequencing method (SSBT 17th workshop protocol) we extended the sequence of a new allele, B*27:30 found in a family of Slovenian origin. In 6 individuals within family we confirmed the A*02:01-B*27:05-DRB1*01:01 haplotype, which is common in the Slovenian population. Oxford Nanopore MiniION® sequencing platforms, a small and low-cost single-molecule sequencer, which offers the possibility of sequencing long DNA fragments, was used for retyping all samples as well as for an additional nine randomly chosen individuals previously typed by Sanger SBT (AlleleSEQR HLA-SBT Reagents, Abbott) and another NGS platform (Roche reagents with the Roche GS Junior system). We obtained complete HLA-A, -B and -C full length sequences of all analyzed samples with SSBT that provide reference data for NGS. Raw data produced by MinION were concordant with SSBT as well as with EPI standards requirements enabling allelic resolution typing result interpreted by adequate commercial software. In conclusion, we find SBT with Oxford Nanopore MiniION® device as very promising method to be used both in high throughput, but definitely also in medium to small size H&I laboratories.

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ISOLATION OF VERY PURE LYMPHOID (CD3+, CD19+ OR CD56+) AND MYELOID (CD15+ OR CD33/CD66b+) CELL SUBSETS IN AS LITTLE AS 15 MINUTES FOR USE IN LINEAGE-SPECIFIC CHIMERISM ANALYSIS

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Chimerism analysis is used to monitor the presence of donor leukocytes in a recipient following hematopoietic cell transplantation. Lineage-specific chimerism can increase sensitivity compared to analyzing the entire leukocyte population, however it requires the isolation of highly purified cell subsets, as even a few contaminating cells can compromise the integrity of the assay. We describe a method (EasySep) to rapidly isolate very pure lymphoid (CD3+, CD19+ or CD56+) and myeloid (CD15+ or CD33/CD66b+) cell subsets directly from whole blood (WB) or buffy coat (BC) in as little as 15 minutes. Briefly, blood or BC was diluted with an equal volume of a red blood cell lysis reagent and target desired cells were immunomagnetically labelled and then placed in a magnet. Target cells were retained in the magnet while unwanted cells were poured or pipetted off. Target cell purities obtained after separation were as follows (mean +/- SD): CD3+: 99% +/-1 (n = 6); CD19+: 98% +/-1 (n = 4); CD56+: 99% +/-1 (n = 4); CD15+: 98% +/-1 (n = 10); myeloid (CD33+ and CD66b+): 99% +/-2 (n = 18). Starting from 1 mL of WB, 900,000 CD3+ T cells, 120,000 CD19+ B cells, 2.6 million CD15+ cells or 3 million myeloid cells were obtained. Approximately 600,000 CD56+ cells were obtained from 1 mL of BC (average start number 1.4 x10^7 cells). To save time and increase laboratory throughput, EasySep immunomagnetic cell separation can be fully automated with the RoboSep instrument. The separation procedure is compatible with downstream DNA isolation methods. These new EasySep kits provide immunogenetics laboratories a fast and easy method to obtain highly purified cells for use in lineage-specific chimerism testing.

P115

EVALUATION OF A COMMERCIALY AVAILABLE HLA TYPING KIT FOR NGS

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The HLA typing laboratory of the Hellenic Cord Blood Bank is routinely using NGS-based typing for the CBUs of its
inventory at 7 loci (HLA-A, -B, -C, -DRB1, -DQA1, -DQB1, and -DPB1) on the MiSeq platform, using the Holotype kit and HLA Twin software for analysis (Omixon). The aim of this study was the evaluation of the TruSight HLA v2 Sequencing Panel (Illumina) and the assign 2.0 software on 24 samples, previously typed with our validated method. The samples were processed in two batches of 12 and typed for 11 loci (HLA-A, -B, -C, -DRB1, -DRB3,4,5, -DQA1, -DQB1, -DPB1, and -DPB1). The workflow has certain differences to our routine as most steps are bead-based for the TruSight kit and do not require the use of dedicated instruments as in the Holotype kit. The total time for the two approaches from genomic DNA preparation to loading the MiSeq are similar, as well as the hands-on time. The TruSight is low throughput, as it is only available at a 24 sample configuration and only 12 samples can be processed simultaneously. Holotype can accommodate up to 96 samples that can be processed simultaneously although for bigger number of samples automation would be desirable. Of the loci tested only one failed to yield a result (DPB1), while the results for the 7 loci tested by both methods were concordant with the exception of 4 loci where possible novelties were detected. The only ambiguities observed with TruSight were in DPB1 (due to the lack of phasing between exons 2 and 3) and in B*39:01:01:02 L (due to a polymorphism in an off-target sequence). On the other hand, this kit resolves the A*02:01:01/02:01:01:02 L ambiguity observed with the Holotype kits. The analysis software Assign 2.0 provided, gives the correct assignments but in the version tested quality indicators like fragment size, minimum depth of coverage were not easily obtainable. The Trusight HLA v2 is an easy to use kit that gives high quality typings and would be well suited for a low through-put HLA typing laboratory.

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IC-HLA CASSETTE: AN AUTOMATED AND INTEGRATED SOLUTION FOR NGS-BASED HLA-TYPING
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Based on the arm-PCR core technology and iCubate system, we have developed an easy-to-use product that allows researchers to generate sequencing-ready libraries for HLA typing automatically. The user only need load genomic DNA sample into the cassette, amplification and purification can be executed in an automated fashion by the instrument in 7 hours. The amplified library is directly ready for sequencing using the Illumina MiSeq platform. The iC-HLA cassette provides the end-user a DNA library for HLA-NGS typing in an automated, disposable and fully closed format, without complex manipulations. Compared to current HLA-NGS products, the iC-HLA cassette has some significant advantages: (1) Amplification is robust. The assay uses our patented multiplex amplification strategy, arm-PCR (amplicon rescued multiplex PCR, Patent No. 7,999,092), allowing simplified library preparation while maintaining true high end-point resolution. (2) The system is closed. Once the cassette is loaded with a sample and closed, all the amplification and purification procedures can be conducted within the cassette. Since amplicons will not be exposed to the laboratory environment, and the majority of PCR reagents are pre-installed at the manufacturing site, both the contamination source and the opportunity for contamination are under control. (3) The system is easy to use. The integrated, fully automated design minimizes requirements for specialized training and facilities for technicians who perform the test. (4) The procedure is fast. The entire NGS workflow from gDNA amplification to genotype assignment could be completed within 2 working days. With the advantages of saving time and simplifying the protocol, iC- HLA cassettes will allow laboratories to have more time and energy to analyze and interpret the results.

P117
COMPLETE HUMAN LEUKOCYTE ANTIGEN GENE SEQUENCE DETERMINATION COMBINING LONG RANGE POLYMERASE CHAIN REACTION AND NEXT GENERATION SEQUENCING
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Complete genomic sequence data for HLA class I and II alleles are still limited. It was our intention to develop a workflow based on long range PCR (LR-PCR) and next generation sequencing (NGS) on a MiSeq platform (Illumina) to provide the complete genomic sequence of different HLA alleles. Therefore, we designed different HLA locus and/or allele specific LR-PCRs. After amplicon generation by PCR and NGS data evaluation with two different HLA software tools (NGSengine, GenDx and Omixon Twin, Omixon) we ended up with allele-specific contigs based on phased sequence alignment according to the individual single nucleotide variants (SNVs.) pattern present. The final alignment of these contigs was performed with the BioEdit (ClustalW) and AliView (Muscle) software along with published genomic sequences.
Abstract

With our approach, applying LR-PCR and NGS including phased sequence analysis, we were able to determine the complete gene sequence of different HLA class I and class II alleles (from 5'UTR to 3'UTR), separately. We are sure, that in the near future it will become much easier to identify the complete gene sequences of all HLA alleles with the combination of these methods. Apart from compatibility testing this would be helpful especially for evolutionary and ancestry studies.

P118

WHOLE GENE SEQUENCE DETERMINATION OF A RARE HUMAN LEUKOCYTE ANTIGEN DRB1 ALLELE BY COMBINING LONG RANGE POLYMERASE CHAIN REACTION AND NEXT GENERATION SEQUENCING

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Some years ago, we found a new HLA-DRB1*13 allele in a German family. It was found in a female potential stem cell donor, in two of her three children and in her mother. This allele, later called DRB1*13:67, seemed to be a hybrid allele between DRB1*13 and DRB3, at least in exon 2. To clarify the degree of recombination inside this allele, we developed a workflow based on long range PCR (LR-PCR) and next generation sequencing (NGS) on a MiSeq platform (Illumina) to provide the complete genomic sequence of this allele. Therefore, we designed different HLA locus and/or allele specific LR-PCRs. After amplicon generation by PCR and NGS data evaluation with two different HLA software tools (Omixon Twin, Omixon and NGSengine, GenDx) we ended up with allele-specific contigs based on phased sequence alignment according to the individual single nucleotide variants (SNVs.) pattern present. The final alignment of these contigs was performed with the AliView (Muscle) and BioEdit (ClustalW) software along with published genomic sequences (IPD-IMGT/HLA Database). With our approach, applying LR-PCR and NGS including phased sequence analysis, we were able to determine the complete gene sequence of HLA-DRB1*13:67 (from 5'-UTR to 3'-UTR). The recombination between the DRB1*13 and the DRB3 allele, seems to be restricted to the first part of exon 2. Our approach, to combine these methods, was very successful to get the complete gene sequence of this rare HLA allele.

P119

EXON PHASING PERMITS IDENTIFICATION OF NEW ALLELES BY NGS NOT DETECTABLE BY SANGER SEQUENCE-BASED TYPING

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A complementary typing on a bone marrow donor was firstly performed by bi-allelic sequence based typing (SBT, ALLELE SEQR kits, CELERA). The ASSIGN software (version 3.6, CONEXIO and IPD-IMGT/HLA Database 3.23.0.1, 2016-01-19) proposed interpretable results with cis-trans ambiguities: A*02:01:01:06, *11:172 / A*02:01:02, *11:01:34. PCR SSP technique (Olerup HLA-A*11 kits) excluded the allele A*11:172, resulting in the following typing: A*02:01:02, *11:01:34. Because the presence of these alleles seemed unlikely, we verified this result by NGS with HOLOTYPE HLA kits (OMIXON), and found a discordant result: A*02: NEW, *11:01:01:01. This new A*02 allele was characterized by a substitution of C > T in position 228.2 compared to A*02:01:01, accompanied by a amino acid modification (threonine > isoleucine). This substitution is what distinguishes an A*11:172 from an A*11:01:01:01. During sequence interpretation, exon phasing permits us to link distant polymorphic positions present in different exons, which is not possible with most of the classical SBT reagents. This example illustrates the SBT limitations because of the cis/trans ambiguities. For this individual, these ambiguities led to a wrong result because if two alleles already described can be interpreted, the new allele is undetectable. This limitation has been eliminated with the use of NGS provided that the introns are characterized and permitted to distinguish both alleles. Thus, combinations of two rare alleles found in an individual by SBT should be verified by an alternative technique.

P120

AN EVALUATION OF PAKLx™ – A LUMINEX®-BASED ASSAY FOR THE DETECTION OF ANTI-HUMAN PLATELET ANTIBODIES

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The ‘monoclonal antibody immobilisation of platelet antigens assay’ (MAIPA) is considered the ‘gold standard’ for the identification of alloantibodies to human platelet antigens (HPA) in patients with alloimmune platelet disorders. However, it is technically demanding, requires typed reference platelet panels and has a long turnaround time. New technologies, i.e. the Luminex-based PAKLx assay (Immucor, Lifecodes) are now
available for the detection of IgG antibodies to HPA-1, 2, 3, 4, 5 epitopes and ‘broad’ platelet glycoproteins (GP). We tested 48 sera from cases of suspected FNAIT (n = 26), MPTR (n = 5), PTP (n = 1), heredity platelet function disorder (n = 1), non-specified immune thrombocytopenia (n = 12) and healthy controls (n = 3), by the MAIPA and PAKLx assays in parallel. Doubling dilution titrations were also performed, to determine the comparative sensitivity of PAKLx, using three NIBSC reference reagents (05/106 - anti-HPA-1a, 03/190 - HPA-3a and 99/666 - HPA-5b). The MAIPA assay identified HPA and platelet GP antibodies in 32 of the 48 (66.7%) sera: HPA-1a (n = 18), HPA-1b (n = 4), HPA-2b (n = 1), HPA-3a (n = 1), HPA-5b (n = 7), GPIIb/IIIa (n = 3). PAKLx detected all antibodies found by the MAIPA and a further three, not MAIPA detected, in the 32 sera, viz., two broad GP-reactive and one HPA-2b. This gave the PAKLx assay a sensitivity of 100% and specificity of 98.8%. Titration studies showed that the PAKLx assay was more sensitive for the detection of HPA-1a antibodies, while the MAIPA showed increased sensitivity for HPA-3a and HPA-5b antibodies. In conclusion, the PAKLx assay is fast and easy to perform. Its superior sensitivity to detect anti-HPA-1a, implicated in 70-80% of FNAIT cases in the Caucasian population, supports its routine use in initial laboratory investigations. However, due to its limitations of reduced sensitivity for HPA-3a and HPA-5b antibodies and its inability to detect HPA-15 antibodies, the PAKLx assay should not be used as a standalone method.

P121

NGS-BASED HLA TYPING: COMPARISON OF FOUR PROTOCOLS AND CORRESPONDING SOFTWARE

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High resolution HLA typing results without ambiguities have become increasingly difficult to obtain when applying SBT and PCR-SSP. NGS (next-generation sequencing) based HLA Typing has great potential for generating precise and unambiguous results for multiple samples in a single assay potentially without additional testing to resolve ambiguities. We compared four commercially available reagent kits including their corresponding software (GenDx/NGSgo-Ampx, Illumina/TruSight HLAv2, Immucor/MIAFORA 11 Flex and Omixon/HOLOTYPE). To evaluate the four protocols, we considered the amplification strategy, the target generation, the flexibility of the kits and their CE certification, as well as the need for additional equipment, the practicability of the workflow and the costs. Altogether the same 24 samples (21 clinical and additional equipment, the practicability of the workflow and their CE certification, as well as the need for required reactions. Among the new multiplex assays that were validated, the example presented in this study combines 3 separate reactions targeting HLA-B*15, HLA-DPB1*03 and HLA-DQB1*02 alleles into a single reaction without requiring any protocol changes. Software algorithms were updated to define these new multiplexed results while keeping the same analysis.

P122

MORE WITH LESS: MULTIPLEXED REAL-TIME PCR HLA TYPING USING SYBR® GREEN AND MULTI-PEAK TM ANALYSIS

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HLA typing plays a critical role in solid organ transplantation. The degree of HLA matching and especially the avoidance of immunogenic antigens in pre-sensitized patients is directly correlated with improved outcomes. As of October 2016, the IPD-IMGT/HLA Database has grown to contain over 15,600 alleles and will certainly continue to expand. Current technologies used to evaluate immune status will also need to expand and broaden their ability to detect allele specific differences that code for immunogenic antigens. Epitope mapping will likely play an important role in this effort and provide a framework to group alleles in a practical way. HLA typing will also need to evolve to split these differences with improved resolution. This study validates a new multiplexing HLA typing method that allows better resolution and flexibility to add additional resolution over time. The LinkSeq™ typing kit from Linkage Biosciences uses real-time PCR combined with melt curve analysis to define HLA specificity. An evolution of this approach consists of multiplexing several targets into a single reaction and identifying the products by way of distinct melt curve signatures, thereby drastically reducing the number of required reactions. Among the new multiplex assays that were validated, the example presented in this study combines 3 separate reactions targeting HLA-B*15, HLA-DPB1*03 and HLA-DQB1*02 alleles into a single reaction without requiring any protocol changes. Software algorithms were updated to define these new multiplexed results while keeping the same analysis.
workflow and thus a transparent modification to end-users. This study demonstrates that multiplexing several LinkSseq assays into the same reaction is a practical and reproducible approach and should be applicable across the full spectrum of reactions. In addition to cost and labor advantages, multiplexing represents a leap forward for high-throughput HLA applications where the need for increased resolution is growing.

P123

VALIDATION AND ROUTINE SETTING OF HLA TYPING BY NEXT GENERATION SEQUENCING USING THE HOLOTYPE HLA (OMIXON) KITS: A MULTICENTRIC EXPERIENCE

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This study presents the experience of eight French laboratories that utilized the HOLOTYPE HLA kits (OMIXON) for performing HLA typing. This technique necessitates one dedicated PCR per locus. Several kits exist for different numbers of samples (24–96) and loci to type (2, 5, 7 or 11). Two different PCR programs are necessary for class I and II. Amplicon quantification is recommended by the manufacturer. Appropriately diluted amplicons are pooled for each individual and then fragmented, enzymatically, repaired and tagged with indexes. Indexed fragments are pooled and purified with magnetic beads. A selection of the appropriate fragments is performed based on their size by preparative electrophoresis. Afterwards, the library is quantified by qPCR. After appropriate dilution, the library is sequenced on a MiSeq (ILLUMINA). The interpretation of the sequences obtained is done by the HLATwin software using systematically two algorithms. The reliability of the interpreted results is expressed by the calculation of a number of quality criteria. The validation of the kits was performed in all eight laboratories. The sensitivity/specificity was evaluated on 20 EPT samples. The results obtained with the previous methods used in the laboratories (SBT Celera, SSO reverse Lumexin One Lambda + PCRSSP OLERUP) were compared on routine samples, including particular samples (new and null alleles). Results revealed excellent concordance with consensual typings obtained in EPT samples, or with results obtained with the other techniques on routine samples. The 4–6 digit resolution is excellent and new alleles are detected. In addition, quantification of the amplicons is not necessary. The only discrepancies observed in the results obtained, were due to new alleles not detected with the previously used technique. They concerned polymorphisms not explored by the previous technique: positions not tested by SSO probes, or exons not routinely sequenced in SBT.

P124

LONGITUDINAL TRANSCRIPTOMIC PROFILING OF DE NOVO HEPATITIS B VACCINE RECIPIENTS

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System vaccinology trials study the influence of a vaccine on gene expression and the resulting characteristics of the immune response by measuring induced antibodies and activated T cells. Not all vaccines are equally effective and there exists a large variability between individual immune responses. In this study we used the hepatitis B vaccine (Engerix-B, GSK) which is in most cases highly immunogenic. However, still 5% of the population are non-responders after the first 3 doses. We recruited 35 individuals without any known previous exposure to the hepatitis B virus or the hepatitis B vaccine. They received the Engerix vaccination on days 0 and 30. For profiling the gene expression changes due to hepatitis B vaccination, stabilized RNA was extracted from blood samples collected on day 0 (before the administration of the first vaccine dose), day 3 and day 7 after dose 1. The RNA samples were prepared using the QuantSeq 3’ mRNA-Seq Library Prep (Lexogen GmbH), an alternative for total RNA-seq which copies once the 3’ end of each mRNA strand. The prepared libraries were equimolar pooled and sequenced on an Illumina NextSeq (v2 high output, 150 cycles). We created a bioinformatics pipeline optimized to deal with the 3’ mRNA-seq data and processed the resulting reads using DESeq2. When we grouped all data of the principal component analysis, we noted that the variability between individuals was larger than the variation between different time points per individual. Nevertheless, on the individual level we found significant differentially expressed genes (log fold change above 2) in different pathways. The results are consistent with the fact that subunit vaccines, like Engerix-B, tend to have less significant general
changes in gene expression than live attenuated vaccines. The next step is to combine the short time gene expression data with the ELISA antibody titres to profile the immune response in order to identify RNA signatures that distinguish responders from non-responders.

P125

HUMAN HLA CLASS II MONOCLONAL ANTIBODIES: CRUCIAL TOOLS TO DEFINE ANTIBODY EPITOPES ON HLA CLASS II MOLECULES

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Recent evidence suggests that HLA epitope matching represents an efficient approach to prevent induction of donor-specific antibodies (DSA) after transplantation. For proper epitope matching it is essential to define the most immunogenic epitopes for these DSAs. Human monoclonal antibodies (mAbs) directed against various HLA antigens have been instrumental in defining antibody epitopes on HLA class I molecules. For HLA class II antigens, however, the number of human mAbs unfortunately remains very limited, which explains why the number of antibody verified epitopes on HLA class II molecules is still relatively small. The generation of a larger panel of well-defined HLA class II specific mAbs is therefore required. We decided to set up a system to isolate and produce human mAbs recombinantly. This approach will allow the generation of a well-controlled panel of anti-HLA class II mAbs characterized at the molecular level. A proof of principle experiment was performed with the established human B cell hybridomas WIM8E5 and MUS4H4, specific for HLA-A11 and HLA-A24 respectively. Variable heavy (VH) chain and variable light (VL) chain PCR products of the mAbs were generated and cloned into pcDNA3.3 expression vectors containing the heavy chain constant domain for human IgG1 and either the kappa or lambda light chain constant domain. Subsequently, the mAbs were expressed in Expi293 cells by co-transfection of the VH and VL chain containing vectors. The recombinant human mAbs produced, were tested against Luminex single antigen beads and showed similar specificities to the original mAbs. These proof of principle data indicate that it is feasible to clone and produce recombinant human mAbs against HLA. We are currently developing a system to culture flow cytometry-sorted memory B cells that are specific for HLA class II molecules with the aim to generate novel human HLA class II mAbs. These recombinant reagents will then be used for the verification of HLA class II epitopes.

P126

IMPROVEMENT OF HLA RESOLUTION WITH NEW LABTYPE® SSO XR KITS

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PCR-SSO is the most commonly used assay for HLA-typing in solid organ transplantation. Our laboratory used the One-Lambda LabType® SSO HD (HD) kits until January 2016 and changed to the new LabType® SSO XR (XR) kits for HLA-A, -B and -DRB1 typing after purchasing a LABScan3DTM. The XR kits have an increased number of bead regions in comparison with HD kits (500 vs. 100), which allows the number of sequence specific oligonucleotides and covered exons to be enlarged. Also, the XR kits are claimed to improve typing resolution by a diminished number of allele ambiguities, including null alleles that need to be resolved. The aim of this study was to measure the improvement in typing provided by XR kits. We retrospectively selected patients who had their first HLA-typing determination performed with the HD kit from September 2015 to January 2016, and their second determination with the XR kit from January to August 2016. Results were analyzed against the IPD-IMGT/HLA Database v3.21.1 (2015–8). Forty-two patients were selected for HLA-A typing comparison, 50 for HLA-B and 41 for HLA-DRB1. For the 3 loci, the number of ambiguities generated by the two kits that concerned exons 1, 2, 3, 4 and 5 were compared. XR kits significantly decreased ambiguities for HLA-A (65% for those concerning exons 2 and 3, 95% exon 4 and none for exon 5) and -B (48%, 51%, 42%, 90%, 95% for exon 1, 2, 3, 4, 5, respectively), but just moderately for HLA-DRB1 locus (14% for those concerning exon 2). No ambiguities were resolved for other DRB1 exons as only exon 2 was amplified. The XR kits allowed elimination of 88%, 61% and 26% of potential null alleles for HLA-A, -B and -DRB1 loci, respectively. Therefore, the XR kit allows a significant improvement of HLA-typing resolution for HLA-A and -B loci in comparison with HD kits. In contrast, an improvement of XR HLA-DRB1 kit is required through at least studying exon 3. It could also include oligonucleotides that allow HLA-DRB3,4 and 5 typing.

P127

IMPLEMENTING ABO GENOTYPING INTO HLA SEQUENCING WORKFLOW

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Lambda LabType® SSO XR KITS

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Lambda LabType® SSO XR KITS

PCR-SSO is the most commonly used assay for HLA-typing in solid organ transplantation. Our laboratory used the One-Lambda LabType® SSO HD (HD) kits until January 2016 and changed to the new LabType® SSO XR (XR) kits for HLA-A, -B and -DRB1 typing after purchasing a LABScan3DTM. The XR kits have an increased number of bead regions in comparison with HD kits (500 vs. 100), which allows the number of sequence specific oligonucleotides and covered exons to be enlarged. Also, the XR kits are claimed to improve typing resolution by a diminished number of allele ambiguities, including null alleles that need to be resolved. The aim of this study was to measure the improvement in typing provided by XR kits. We retrospectively selected patients who had their first HLA-typing determination performed with the HD kit from September 2015 to January 2016, and their second determination with the XR kit from January to August 2016. Results were analyzed against the IPD-IMGT/HLA Database v3.21.1 (2015–8). Forty-two patients were selected for HLA-A typing comparison, 50 for HLA-B and 41 for HLA-DRB1. For the 3 loci, the number of ambiguities generated by the two kits that concerned exons 1, 2, 3, 4 and 5 were compared. XR kits significantly decreased ambiguities for HLA-A (65% for those concerning exons 2 and 3, 95% exon 4 and none for exon 5) and -B (48%, 51%, 42%, 90%, 95% for exon 1, 2, 3, 4, 5, respectively), but just moderately for HLA-DRB1 locus (14% for those concerning exon 2). No ambiguities were resolved for other DRB1 exons as only exon 2 was amplified. The XR kits allowed elimination of 88%, 61% and 26% of potential null alleles for HLA-A, -B and -DRB1 loci, respectively. Therefore, the XR kit allows a significant improvement of HLA-typing resolution for HLA-A and -B loci in comparison with HD kits. In contrast, an improvement of XR HLA-DRB1 kit is required through at least studying exon 3. It could also include oligonucleotides that allow HLA-DRB3,4 and 5 typing.
The ABO system is the most important blood group system in terms of transfusion medicine lately gaining more relevance in donor/recipient matching in the transplantation context. For decades, serological phenotyping has been the most common and cost efficient method to determine the ABO blood group phenotype. ABO genotyping is even nowadays restricted to serologically ambiguous results or cases with limited availability of red blood cells. The frequently observed discrepancies between serological and molecular genetic typing results can be ascribed to the minimalistic way of ABO genotyping usually based on a limited number of SNPs residing in exon 6 and 7. In order to minimize these inconsistencies we developed a NGS based method to sequence the entire length of the ABO gene. Starting with the design of a long range PCR assay we finally succeeded generating large fragments covering the complete ABO gene including the regulatory enhancer region located 3.7 kb upfront of exon 1. The overlapping amplicons in the size range from 8–12 kb were basically sequenced according to standard NGS protocols on a MiSeq platform (Illumina). Developing and integrating an ABO analysis tool (HLA Twin, Omixon) turned out to be the major hurdle mainly hampered by the lack of useful reference databases and scarce sequence information especially in the remaining exons beside 6 and 7 and the non-coding regions. To prove the routine functionality of the established long range PCR assay and ABO software module integration we processed 300 serologically pre typed samples. With the described workflow we are able to sequence the ABO gene in its full length delivering ABO genotypes with so far unrivaled resolution not at last to fill the gaps in the reference databases. The most striking advantage for a high throughput registry typing laboratory is the avoidance of a serological ABO typing workflow in parallel.

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NANOPORE SEQUENCING IS COMING OF AGE: A NEW ANALYSIS PLATFORM FOR HLA ALLELE ASSIGNMENT BASED ON FULL-LENGTH DE NOVO ASSEMBLY

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In the years past, MinION has often been dismissed as a useful platform for clinical diagnostics, citing high per-base error rates and lack of analysis tools as the main apprehensions. Recently, the technology has advanced rapidly, and we regularly observe reads with an average Phred score of Q = 20, corresponding to 99.0% per-base accuracy. Oxford Nanopore releases regular updates on the sequencing and basecalling platforms, and members of the MinION community continually develop improvements on analysis and assembly tools. De novo assembly of nanopore reads remains a challenge, but data analysis has reached a point where reads are useable in analysis of complicated polymorphic regions, such as HLA. Our goal is to develop a high-throughput, highly accurate open-source analysis tool for HLA allele calling based on initial de novo assembly, followed by alignment to sequences from the IPD-IMGT/HLA Database. This analysis pipeline uses both community and in-house software in a structured way to sort, assemble, and classify high-quality Nanopore reads for applications in research, and ultimately routine diagnostics. As a proof of concept, we sequenced the full-length classical class I HLA genes (A,B,C) of three individuals with potentially novel HLA types. The MinION reads were analyzed, filtered for quality and length, and assembled into consensus sequences for each of the three class I loci. The sequences were aligned to allele references available in the IPD-IMGT/HLA Database for comparison with known alleles. For automated submission of novel full-length HLA alleles to the EMBL-ENA Database, an in-house allele submission tool was generated, and is available for general public use. Overall, the developed analysis tool represents a promising method for analysis of MinION data and assignment of HLA alleles based on full-gene Nanopore sequences.

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WHAT’S NEW GENOTYPING KIR2DL5?

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Killer-cell immunoglobulin-like receptors (KIR) expressed on NK cells are specialized in recognition of MHC lacking cells especially tumor and virus infected cells. In the last few years there is growing evidence that distinct KIR donor haplotypes lead to a better outcome of stem cell transplantations. Therefore extending donor profiles with KIR genotypes is desirable in order to improve donor/recipient matching. The inhibitory KIR2DL5 is a key gene to differentiate between the so far classified KIR haplotypes A and B. Most actual KIR genotyping methods unravel the presence or absence of the respective gene disregarding the variations of the present alleles. To investigate the impact of higher genotype resolution we established a NGS based typing strategy. Designing a long range PCR allowing a full-length gene amplification was the first step to implement...
KIR2DL5 genotyping into our NGS typing routine based on the Illumina MiSeq platform. During the initial testing of this KIR2DL5 NGS assay on 48 randomly selected samples the data analysis (HLA Twin software, Omixon) revealed several new variations in non-coding regions as well as an unknown polymorphism in exon 5. The C to A substitution at position 2860 leads to an amino acid change in codon 143 from proline to threonine. Validation of this so far undescribed variant was performed Sanger sequencing of exon 5 on a 3730XL DNA Analyzer (Applied Biosystems) with subsequent analysis using GeneStudio Professional software. The Sanger sequencing results confirmed the observed nucleotide exchange of this novel KIR2DL5 sequence variant. This observation highlights the importance of full-length, sequenced based KIR typing methods to improve knowledge about the variability of the KIR genes.

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SECOND FIELD HLA TYPING BY NGS FOR CLINICAL SERVICES

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Although high-resolution HLA typing by Next Generation Sequencing (NGS) has entered the field several years ago, its feasibility for clinical services is still a matter of debate. Here we report on 2nd field HLA-A, -B, -DRB1, and -DPB1 typing by an in-house NGS typing system. 22 HLA-typed control B-lymphoblastoid cell lines (B-LCL) and 334 samples from patients and their unrelated, 9-10/10 HLA-matched stem cell donors were included. Typing was performed for exons 2 and 3 on an Illumina platform, according to Lange et al. (2013), with an average read-depth of 780 reads per base and bioinformatic evaluation by the neXtype software. The frequency of partial or complete drop-outs was 2%. Concordance with the reported typings for the B-LCL control cell lines was 100% for all 4 loci. NGS was able to assign unequivocal G-group or 2nd field typing in 1265/1336 (94.7%) alleles. Total turn-around time for 167 pairs without NGS-robotics was 7 days, with an average cost per sample for the 4 loci of <10€. These data demonstrate the feasibility, reliability and cost-efficiency of in-house NGS protocols for unambiguous 2nd field HLA typing for clinical services, although the use of robotics for library preparation is recommended to reduce error risks and turn-around times.

**P131**

PACIFIC AND EUROPEAN ANCESTRY OF AMERINDIANS: A HLA RELATEDNESS STUDY IN WIWA (ARSARIO) COLOMBIAN POPULATIONS

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The HLA profile of an isolated Amerindian group from North Colombia (Wiwa) was studied in order to draw conclusions about its preventive medicine, the genetic relationship with worldwide populations and native American peopling since this last issue is hotly debated. Peripheral blood was obtained from volunteer blood donors belonging to Wiwa (also named Arsario) ethnic group. HLA-A, -B, -C, -DRB1 and -DQB1 genes were analyzed by standard methods. Wiwa Amerindians relationships with others were calculated by using Arlequin, Dispan and Vista software computer packages. Extended HLA haplotypes have been studied for the first time in this population. Classical Amerindian haplotypes have been found and also new Wiwa (Arsario) Amerindian haplotypes. New haplotypes are A*68:01 - B*15:01 - C*03:03 - DRB1*14:02 - DQB1*03:02, A*11:01 - B*07:02 - C*07:02 - DRB1*15:03 - DQB1*06:02 and A*68:01 - B*15:01 - C*03:04 - DRB1*14:02 - DQB1*03:01. Conclusions have been reached after exhaustive comparisons of Wiwa with other Amerindians and worldwide populations by using genetic distances, neighbor joining trees, correspondence analysis and specific groups of alleles which are common and frequent in both Amerindians and Pacific Islanders. They are: 1) The Americas First Inhabitants have probably come through the Bering Strait and the Pacific (from Austronesia and Asia) and Atlantic (from Europe) routes. A bidirectional gene flow is not discarded. 2) the genetic HLA Amerindian profile is distinct from that of other world populations. 3) Amerindians geographical proximity groups’ relatedness is not concordant with HLA genetic
relatedness, neither with language. This may be explained by a substantial population decrease that occurred after Europeans invaded America in 1492 and carried new pathogens and epidemics. 4) Our results are also useful for Wiwa and other Amerindians future preventive medicine (HLA linked diseases), HLA pharmacogenomics and transplantation regional programs.

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THE DISTRIBUTION OF ALLELES AND HAPLOTYPES IN RUSSIAN AND TATARIAN POPULATIONS OF SAMARA REGION POPULATION OF RUSSIA

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In the present work we analyzed HLA-A, −B, −DRB1 alleles and haplotypes of unrelated bone marrow donors of the Samara bone marrow donor registry. HLA-typing was performed using SSO and SSP techniques with the aid of OneLambda typing kits at low resolution level. The allele and haplotype frequencies of the samples of Russian (n = 1177) and Tatarian (n = 85) populations of the Samara region were estimated using the EM algorithm and Arlequin v.3.5 population genetics software. We found a significant difference in distribution of A*02 (27.6% in Russian population vs. 16.5% in Tatarian population), B*35 (11.8% vs. 16.5%), B*49 (1.9% vs. 5.3%). There was no significant difference in the frequencies of DRB1 alleles. The most frequent 11 haplotypes (frequency >1%) of the Russian population were: A*01-B*08-DRB1*03 (4.25%), A*03-B*07-DRB1*15 (3.61%), A*03-B*35-DRB1*01 (1.95%), A*30-B*13-DRB1*07 (1.78%), A*02-B*07-DRB1*15 (1.70%), A*25-B*18-DRB1*15 (1.61%), A*02-B*13-DRB1*07 (1.53%), A*23-B*44-DRB1*07 (1.27%), A*02-B*15-DRB1*04 (1.19%), A*02-B*41-DRB1*13 (1.15%) and A*02-B*44-DRB1*16 (1.15%). The most frequent 9 haplotypes (frequency >1.5%) of Tatarian population were: A*03-B*35-DRB1*01 (6.47%), A*25-B*18-DRB1*15 (4.12%), A*01-B*08-DRB1*03 (2.94%), A*02-B*44-DRB1*04 (2.35%), A*03-B*07-DRB1*15 (2.35%), A*24-B*13-DRB1*07 (2.35%), A*03-B*07-DRB1*07 (1.77%), A*23-B*44-DRB1*16 (1.77%) and A*26-B*49-DRB1*15 (1.77%). The knowledge of alleles and haplotype frequencies of different populations can be used for population genetic purposes to establish the genetic relationship, disease association studies and developing the typing and search strategies in bone marrow donor registries.

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CONFIRMATION OF THE RARE ALLELE B*35:311 IN POTENTIAL STEM CELL DONOR FROM SICILY

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In the context of the cooperation existing between donor center ME01 and transplant center RC01 for resolution of any ambiguous results, during HLA typing of new donors to include in Italian Bone Marrow Donor Registry, the rare allele B*35:311 was identified in a Caucasian male, born in Sicily. It was reported for the first time by N. Cereb of the Histogenetics Laboratory in 2015 on the IPD-IMGT/HLA Database, in an individual of unknown ethnic origin with the genotype A*01,*24; B*18,*35; C*04,*12; DRB1*03, *11. Only exon 2 and 3 coding for the antigen recognition site were sequenced. HLA-B*35:311 was until now unconfirmed and the status in CWD Catalogue v2.0.0 was not defined. There are no references published in the literature. Genomic DNA of the donor was extracted from peripheral blood leukocytes using an automated system. HLA typing was performed by PCR-SSO (Mr. Spot BAG) and SSP (ONE LABMDA) methods. The B*35:311 allele was confirmed performing sequencing in forward and reverse direction of exons 2 and 3 (SBT ROSE). Within these exons, the most similar allele is B*35:02:01 that differs by B*35:311 in two positions: 45 and 46 of the exon 2 (ATG to ACG and GCG to GAG respectively), generating a protein with a different binding site. B*35:311 was submitted to AFND in February 2017. The genotype of the donor was: A*24:02, *68:02; B*35:311, *53:01; C*04:01 homozygous, DRB1*01:02,*11:04. A family study will be extended to parents and sister of the donor in order to separate parental haplotypes. Since HLA matching between donor and recipient is a key factor on the incidence of engraftment and GvHD, detection and communication of rare alleles is important to select donors with optimal characteristics.

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HLA-DRB3 POLYMORPHISM IN DRB1*03 HAPLOTYPES

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The DRB3 gene which encodes a HLA-DR52 molecule is present on HLA-DRB1*03, *11, *12, *13 and *14 haplotypes. HLA-DRB1*03 allele is in strong LD with B*08 and
A*01 ~ B*08 ~ DRB1*03 is the most frequent haplotype in the vast majority of the European populations. In this study we present the results of the HLA-DRB3 polymorphism in unrelated individuals selected on the basis of HLA-B and -DRB1 typing. All 104 studied subjects were positive for DRB1*03 and the second allele at DRB1 locus were from non-DRB3 haplotypes so the DRB1*03 ~ DRB3 haplotype could be assigned unequivocally. According to the HLA-B typing, the subjects were defined as HLA-B*08 positive or B*08 negative. HLA-A,-B,-DRB1 and -DRB3 typing was performed by PCR-SSP (Luminex) at low resolution or by PCR-SSP at high resolution. In the entire group, only two out of 130 known DRB3 alleles were observed: DRB3*01:01:02 (63.5%) and DRB3*02:02:01:01 (36.5%). In the group of B*08:01 positive subjects DRB3*01:01:02 allele predominated with a frequency of 92.0% (46/50), which was highly significant in comparison with the frequency of DRB3*02:02:01:01 allele (8%; P < 0.0001). In contrast, in the group of HLA-B*08:01 negative subjects DRB3*02:02:01:01 allele was found more frequently (63.0%) than DRB3*01:01:02 (37.0%) and the difference was also statistically significant (P = 0.012). Further analysis in this group revealed that B*44:02:01G is more frequently found in DRB1*03 ~ DRB3*01:01:02 haplotype. Analysis of the association with HLA-A alleles in any of the studied groups, did not show statistically significant differences. These results will be useful in further population and disease association studies as well as implemented in DSA monitoring.

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DISTRIBUTION OF HLA-C ALLELES AND HLA-C1, –C2 LIGAND GROUPS FOR KIR FREQUENCIES IN MOSCOW STEM CELL DONOR REGISTRY

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Killer cell immunoglobulin-like receptors (KIR) through interaction with their ligands - major histocompatibility complex antigens (HLA) play a key role in regulating activity of natural killer (NK) cells. HLA-ligands for two-domain KIR include alleles of HLA-C locus. Large number of studies have focused on the association of polymorphism of KIR genes with the development of disease, its severity and outcome. At the same time the distribution of HLA-ligands for KIR received much less attention, especially it concerns the Russian population. The aim of this work was to analyze the frequency of allelic variants of HLA-C locus, and the distribution of HLA-C1 and -C2 groups as main ligands for KIR, among 1137 potential bone marrow donors from the registry of National Research Center for Hematology, Moscow. Donors were recruited between December 2014 and October 2016 years. 1137 donors who identified themselves as the Russians by ethnicity were enrolled in the study. High-resolution typing of HLA-C was performed by PCR-SSO on Luminex platform using Lifecodes HLA-C eRES SSO Typing Kit (Immunucor, USA). The most common variants of locus HLA-C in bone marrow donors group were HLA-C*07:02 (13.14% of the total sample), *04:01 and *07:01 (accounted for 12.7% and 12.48%, respectively), *12:03 (12.22%), *06:02 (11.21%). The rarest allelic variants, met in our study only once, were HLA-C *01:07, *04:03, *04:04, *15:06, pointing to their low frequency (0.04%). In general, the distribution of HLA-C alleles in our study, despite some of its peculiarities, corresponds to that of other European populations, in particular the Germans, Austrians and Poles. HLA-ligands for KIR in our group distributed in the predominance of HLA-C1 allele group (60%) and prevalence of the C1/C2 heterozygotes (49.9%) over C1/C1 (35.3%) and C2/C2 (14.7%) homozygotes. The prevalence of heterozygotes over homozygotes is also typical for European populations. The obtained data are important in evaluating the genetically determined functional activity of NK by their interaction with HLA-ligands. These results should be considered in the study of the role of KIR and HLA-ligands in predisposition to diseases, and problems related to the balance between the immune system and human reproduction.

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THE DISTRIBUTION OF HLA AND HPA ALLELES AND HAPLOTYPES IN HEALTHY PLASMA AND PLATELET DONORS FROM THE NORTH-WESTERN REGION OF RUSSIA

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The current study was performed with the aim of establishing the distribution of HLA and HPA alleles and haplotypes in regular plasma and platelet donors originated from the North-Western region of Russia. The study included 226 healthy donors (64 female and 162 male) in the age of 19 to 62 (mean age – 37 years). The typing of HLA (loci A, B, DRB1) and HPA genes was performed by PCR-SSO method with the commercially available kits. For the statistical analysis of the results the software Arlequin (v.3.5.1.2) was used. The study has shown that the most frequent HLA alleles were HLA-A*02, HLA-B*07 and HLA-DRB1*15 at 26.99%, 14.16% and 15.27% respectively. Two most common HLA haplotypes in our studied group were HLA-A*01-B*08-DRB1*03 and HLA-A*01-B*07-DRB1*15, which were found in 3.75% and 3.52% of donors respectively. The frequencies
of HPA alleles were as the following: HPA-A – 80.01%, HPA-B – 19.91%, HPA-2a – 90.27%, HPA-2b – 9.73%, HPA-3a – 55.97%, HPA-3b – 44.03%, HPA-4a – 99.56%, HPA-4b – 0.44%, HPA-5a – 88.94%, HPA-5b – 11.06%, HPA-6a – 99.56%, HPA-6b – 0.44%, HPA-15-a – 51.77%, HPA-15-b – 48.23%. Twenty five different haplotypes of HPA were evaluated in the studied group. Among them, the most common haplotypes were: HPA-1a-2a-3a-4a-5a-6a-15a and HPA-1a-2a-3a-4a-5a-6a-15b, which were found in 19.32% and 18.96% of cases respectively. From the current study we can conclude, that the distribution of HLA alleles and haplotypes in our healthy donors correspond to the frequency of respective HLA alleles and haplotypes in European population. The distribution of HPA alleles and haplotypes is in accord with the frequency of respective alleles and haplotypes in the population of the North-Western region of Russia.

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HLA GENES IN CHIMILA AMERINDIANS (COLOMBIA), THE PEOPLING OF AMERICA AND RELATEDNESS TO EUROPEANS AND PACIFIC ISLANDERS

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Our aim is to study the HLA-A, -B, -C, -DRB1 and -DQB1 gene frequencies in the Chimila Amerindian (Colombia) ethnic group. Results are compared with other World populations in order to obtain information about Chimila and Amerindian Health promotion, Amerindian origins and American peopling. Written consent was obtained from Chimila subjects to be included in this study. Peripheral blood was drawn and HLA DNA genotyping was carried out by standard methods. Analyses of Chimila relatedness with other Amerindians and worldwide populations was performed with a standard neighbor joining dendrogram and correspondence analyzes methodology. The Chimila HLA gene profile was related to that of other Amerindians groups. New complete HLA extended haplotypes were obtained. Some of them are described for the first time. Also, specific genealogical comparisons were done between Chimila Amerindians and Pacific Islanders by using specific HLA alleles. Our conclusions are: 1) These new data and HLA extended haplotypes are useful for present and future Chimila preventive medicine (HLA linked diseases), HLA pharmacogenomics and transplantation regional programs. 2) Classical accepted origin of America peopling should be revised: Pacific (Asian and Austronesian) and Atlantic (European) populations gene exchange may have occurred before 1492 AD. This is confirmed by our present studies using HLA autosomal genetic markers, 3) the genetic HLA Amerindian profile is separated from that of other world populations, 4) Genetics by itself may not be sufficient to ascertain populations origins and migrations: other cultural, linguistic and anthropological data must also be taken into account.

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THE CHARACTERISTICS OF THE NEW HLA-A*32 AND HLA-A*32:95 ALLELES, WHICH WAS IDENTIFIED IN THE DONOR OF THE NATIONAL REGISTER OF STEM CELLS OF THE REPUBLIC OF KAZAKHSTAN

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Since 2011, the immunological typing laboratory of the Scientific-Production Center of Transfusiology has conducted HLA typing of donor blood stem cells from the National Register of blood. During HLA typing of a potential donor, a new allelic variant of HLA-A was detected. Typing of HLA-A,-B,-C, –DRB1 and -DQB1 were carried out using a sequence based typing (SBT) method by Protrans S4 technology (Protrans, Hockenheim, Germany). During the PCR stage, haplotypes were divided and eventually determined using heterozygous sequencing. Nucleotide sequencing was obtained using BigDye v1.1 terminator, reagent (Applied Biosystems, Foster City, CA) and read on a genetic analyzer 3730XL (Applied Biosystems). Sequencing was carried out in the forward and reverse directions of exons 2, 3 and 4 for the loci HLA-A, -B and -C; exon 2 for DRB1 and exons 2 and 3 for DQB1. The Sequence Pilot Program (JSI medical Systems, Germany, version 3.25.1.) analyzed the results. The results of HLA-typing were as follows: HLA-A*02:06, *32:95, B*27:02, *35:01, C*02:02, *03:03, DRB1*09:01, *12:01, and DQB1* 03:01, 03:03. The new allele is similar to the already existing HLA-A*32:01:01 allele but differs in exon 2 at position 28. Adenine (A) was observed at this position, while previously only cytosine (C) has been described in this position. This replacement results in a change of codon 10 from ACA to AAA, altering the amino acid from lysine to threonine (T → K). In order to prove that the gene was inherited, the analysis of donor’s parents was performed. The allele was confirmed in father’s blood sample. The new allele variant is registered on GenBank and the IPD-IMGT/HLA Database and received an official name HLA-A*32:95 from the Nomenclature Committee of the
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EVALUATION OF ROUTINE DISEASE ASSOCIATION HLA-DRB1 TYPING FOR RHEUMATIC DISEASES

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HLA testing is commonly performed for selected patient populations for supporting the diagnosis of certain autoimmune diseases. The aim of this study was to investigate the distribution of HLA-DRB1 genes in 628 patients that were referred to our laboratory for HLA typing for rheumatic disease association testing (ICD-10, M00-M99) in a one year period. Individuals from the Croatian Bone Marrow Donor Registry (CBMDR) were used as controls. HLA-DRB1 typing was performed by a PCR-SSO method. Statistically significant differences were not observed between the entire group of patients and controls. Furthermore, according to the diagnosis, patients were grouped in 6 groups large enough to be included in a subsequent analysis, as follows: Inflammatory Polyarthropathy (IPA; N = 239), Rheumatoid Arthritis RF+ (RA/RF+; N = 70), RA/RF-(N = 83), Psoriatic Arthritis (PsA, N = 112), Ankylosing Spondylitis (AS, N = 61) and Spondyloarthritis (SpA; N = 63). The comparison of HLA-DRB1 allele frequency distribution between each group of patients and controls, revealed differences only in PsA patients and RA/RF+ patients. In PsA patients we confirmed the previously established association of DRB1*07 (P = 0.0106; OR = 0.74), but also the increase of DRB1*14 gene (P = 0.0003; OR = 1.16), while DRB1*11 showed lower frequency in comparison to the controls (10.7% vs. 16.7%; P = 0.0230; OR = 0.24). RA/RF+ patients showed significantly increased frequency of the shared epitope (SE) alleles (DRB1*01:01, *01:02, *04:01, *04:04, *04:05, *10:01) when compared with controls (26.4% vs. 17.1%; P = 0.005; OR = 1.74), but surprisingly also a statistically significant increased frequency of DRB1*16 (17.1% vs. 10.4%, P = 0.0155; OR = 1.77). These results reinforce the importance of evaluating disease susceptibility alleles in our population and suggest that additional analysis are required before DRB1 genotyping is incorporated into clinical diagnostic algorithms.

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FIRST REPORT ON ALLELIC DISTRIBUTION OF HLA LOCI OBTAINED BY SEQUENCE-SPECIFIC OLIGONUCLEOTIDE TYPING FOR POPULATION OF BONE MARROW REGISTRY DONORS IN BOSNIA AND HERZEGOVINA

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We tested a population of 140 samples (median age of 31) from the bone marrow donors’ registry in Bosnia and Herzegovina, which is about to be established at University Clinical Hospital Mostar, and is currently in process of obtaining donor samples. This study is the first in our country which obtained HLA population data of presumed healthy population by molecular HLA typing methods, namely polymerase chain reaction / sequence specific oligonucleotide (PCR-SSO). The aim of this study was to assess allele frequencies of 5 HLA loci at low resolution in the relatively heterogeneous population of Bosnia Herzegovina. Three most frequently detected alleles were: HLA-A*02 (32%), -A*01 (16%) and -A*24 (14%); -B*35 (13%), -B*51 (12%) and -B*18 (10%); -C*07 (31%), -C*12 (16%) and -C*06 (10%); -DRB1*15 (15%), -DRB1*11 (14%) and -DRB1*04 (12%); -DQB1*03 (30%), -DQB1*05 (25%) and -DQB1*06 (25%), per HLA loci, respectively. Distribution of the first 3 alleles per locus is highly comparable with published results of research from neighbouring populations. Our initial data show general similarities with frequencies of HLA alleles observed throughout the region. This study is setting a basis for future HLA studies as a national reference report. It will have its use within transplantation, disease association and anthropological studies, as well as more extended HLA population research on national and international level.

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HLA-DMB IN AMERINDIANS: SPECIFIC LINKAGE OF DMB*01:03:01/DRB1 ALLELES

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HLA-DMB proteins are important for intracellular trafficking to allow other major histocompatibility complex (MHC) molecules to present peptides to lymphocytes. In addition, HLA-
DMB alleles have been found to be linked to diseases in some ethnic groups and HLA-DMB molecules may be important in explaining HLA disease association. We aim to detect HLA-DMB allele profiles in Amerindians for the first time and compare them to other populations. This will establish a basis to study HLA-DMB linkage to disease in Amerindians. A group of 168 voluntary Amerindians have been typed for HLA-DMB alleles. They have been characterized both by genetic and genealogical basis. Cloning and automated HLA-DMB DNA (exons 2, 3 and 4) sequencing have been performed for allele assignment. HLA-DMB\*01:01:01 and HLA-DMB\*01:03:01 show the highest frequencies. These have been compared to other worldwide populations. HLA-DMB\*01:03:01 is tightly associated with specific HLA-DRB1 alleles in Amerindians. In conclusion, the specific Amerindian HLA-DMB allele frequencies and their linkage disequilibrium with other MHC alleles may be crucial to determine HLA-DMB World wide variation, evolution and specific linkage to disease in Amerindians and other populations.

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**ALLELE AND HAPLOTYPE FREQUENCIES OF HLA-A, −B, −C AND −DRB1 IN A MACEDONIAN POPULATION BASED ON A FAMILY STUDY**

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The HLA typing for patients in need of hematopoietic stem cell transplantation and members of their families is routinely performed at the Institute for Immunobiology and Human Genetics in Skopje, Republic of Macedonia, in order to precisely define HLA haplotypes and thus enhance the odds of finding a suitable related or unrelated HLA compatible donor. The aim of this study was to determine the allele and haplotype frequency of HLA-A, −B, −C and −DRB1 in the Macedonian population. The later results are obtained at a 4-locus level for the first time. HLA-A, −B, −C and −DRB1 alleles were identified in 78 unrelated individuals (parents of patients referred to our Institute for HLA typing in the period of 01/01/2012 to 31/12/2013) using Reverse Line Strip (RLS) and Sequence Specific Oligonucleotides (SSO) techniques. Allele and haplotype frequencies were analyzed using the software package Arlequin 3.5. A total of 13 different HLA-A alleles were detected, and the most frequent were: HLA-A*02 (28.2%), HLA-A*24 and HLA-A*01 (12.2%), and HLA-A*03 (10.9%). Twenty two different HLA-B alleles were found, with the most frequent being HLA-B*35 (17.3%), HLA-B*18 (15.4%) and HLA-B*51 (14.7%). Among 13 different HLA-C alleles were detected, with the most frequently found being: HLA-C*07 (26.9%), HLA-C*04 (19.9%) and HLA-C*12 (12.8%). The most frequent HLA-DRB1 alleles were: HLA-DRB1*11 (23.0%), HLA-DRB1*16 (14.1%), HLA-DRB1*13 (12.8%) and HLA-DRB1*03 (10.2%). A total of 12 HLA-DRB1 alleles were identified. The most frequent 4 locus haplotype was HLA-A*01-B*08-C*07-DRB1*03 (2.5%), followed by HLA-A*24-B*35-C*04-DRB1*11, A*33-B*14-C*08-DRB1*01, A*02-B*08-C*07-DRB1*03, A*02-B*35-C*04-DRB1*11 and A*26-B*08-C*07-DRB1*03 with frequency of 1.9%. We will soon enlarge our study population, and the data we report could be used for establishing a control group from our region in anthropology, transplantation and disease association studies.

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**HLA ALLELES AND HAPLOTYPES IN CANARY ISLANDS (GRAN CANARIA): MIXTURE OF POPULATIONS OF DIFFERENT ORIGIN**

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The peopling of the Canary Islands has been much debated. Data on mitochondrial DNA and the Y chromosome have suggested an original contribution of Berber origin with a later contribution of Spanish genetic background coming from the invaders. Some data on HLA class II alleles from the Tenerife population was published postulating a Berber and Atlantic/Iberian origin but this study focused on the Y chromosome, without complete HLA class I and class II haplotypes. In order to study the different contributions of the genetic background of Canary Islands, the HLA class I and class II allele frequency, haplotype frequency and phylogenetic dendrogram were determined from 215 unrelated individuals who had at least three previous generations of ancestors from the Canary Islands. Results showed a first contribution coming from North African Mediterranean population (probably of Berber origin); a second from Iberian-North Africans (directly from North African or from Iberian Peninsula); a third was composed of Mediterranean populations, European Mediterraneans and Western Europeans, carried by Spaniards (invasions); a fourth from Atlantic/Iberian populations and finally directly from North European avoiding the Iberian Peninsula. These data conclude that the current genetic background of the Canary Islands inhabitants has been generated over the years by admixture of several waves of invader DNA with an original Berber background.
HLA MAY BE A USEFUL TOOL TO TRACK DOWN ETHNICITY AND GEOGRAPHICAL ORIGIN OF INDIVIDUALS: PRELIMINARY DATA FROM 46 FAMILIES OF VENEZUELA

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DATA FROM 46 FAMILIES OF VENEZUELA ORIGIN OF INDIVIDUALS: PRELIMINARY

HLA MAY BE A USEFUL TOOL TO TRACK DOWN ETHNICITY AND GEOGRAPHICAL ORIGIN OF INDIVIDUALS: PRELIMINARY DATA FROM 46 FAMILIES OF VENEZUELA

The patterns of HLA genetic variation worldwide show significant information about human geographic expansion, demographic history and cultural diversification. Here we investigate the contribution of HLA alleles and haplotypes in a scenario where also uniparental markers (mitochondrial DNA, mtDNA, and Y-chromosome, MSY) are considered. We analyzed the offspring DNA samples of 46 families from Venezuela, testing 32 and 20 unrelated individuals for mt-DNA and for MSY, respectively. 25 were assigned to Native American (A2 n = 4, A2am n = 2, A2k1a n = 1, B2 n = 1, B2b n = 1, B2d n = 4, B2e n = 1, B2j n = 2, C1b n = 3, C1c n = 1, C1d n = 1, C1d1 n = 1, D1f1 n = 1, D1f2 n = 1, D4h3a n = 1) and 7 to European and African mtDNA haplogroups (H n = 5, L2a1a2 n = 1 and T2b n = 1). For MSY, one belonged to the Native American haplogroup Q, while 10, 6 and 3 were assigned to T2b n = 1). For MSY, one belonged to the Native American haplogroup Q, while 10, 6 and 3 were assigned to T2b and R1b, E and J non-native haplogroups, respectively. Allele-level typing was available for HLA-A, -B, -C, -DRB1, -DQA1, -DQB1 and -DPB1, including the definition of maternal/paternal inherited haplotypes (MIH/PIH). We examined HLA alleles and haplotypes of our sample compared to the Sierra de Perija Yucpa and Bari. We found several alleles frequent in Venezuelan Amerindians, such as A*02:13 (n = 2), A*02:04 (n = 3) and B*35:43 (n = 4). We also found characteristic DBP1 alleles, such as *04:02 (n = 15) and *14:01 (n = 7), native DQA1*03:01-DB1*03:02 haplotype in 12 samples (60.6% in Venezuelan Amerindians), and native DQA1*04:01-DQB1*04:02 haplotype (12.8% in Venezuelan Amerindians) associated to DRB1*08 in 7 samples. Interestingly, A*02:04-B*35:43-DRB1*04:07 in the PIH was associated to non-native MSY haplogroup R1b. Moreover, the sample assigned to non-native mt-DNA haplogroup H showed native DRB1*04:03-DQA1*03:01-DQB1*03:02 (11.7% in Yucpa) in the MIH, while the sample belonging to MSY haplogroup Q showed a native haplotype in the PIH. HLA polymorphism could be complementary to uniparental markers, especially when parental inherited haplotypes are defined, providing a useful tool for anthropological studies.

HLA-A, -B, -C TYPING BY NEXT GENERATION SEQUENCING IN A SAMPLE OF TURKISH POPULATION

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HLA typing was performed on 767 unrelated subjects as per the EFI-accredited Alp Şen Tissue Typing and Genetic Research Laboratory in Istanbul University, Istanbul Faculty of Medicine using Illumina’s MiSeq Sequencing System. The subjects were genotyped for the volunteer bone marrow donor registry. HLA-A, −B, −C typing on next-generation sequencing data was achieved using Omixon Holotype HLA(TM) assay and Omixon HLA Twin(TM) software. Population genetic analyses were done on Arlequin v.3.5.1.3. Genotype frequencies at all three classical HLA class I loci were in Hardy-Weinberg equilibrium (P > 0.25). Also, when assessed by the inbreeding coefficient (FIS), observed and expected heterozygosity did not differ at any loci (P > 0.40). Ewens-Watterson tests of selective neutrality did not indicate any statistically significant selection (P = 0.99). The numbers of alleles detected in each locus were 55, 104, 51 for HLA-A, −B, −C loci, respectively. The most common three alleles (and their frequencies) at each locus were A*02:01:01 (0.201), A*24:02:01 (0.145), A*01:01:01 (0.111); B*51:01:01 (0.114), B*35:01:01 (0.081), B*18:01:01 (0.060); and C*04:01:01 (0.175), C*12:03:01 (0.114), C*07:01:01 (0.100). The most common B-C haplotypes (and their frequency / D’ as LD measure) were: B*35:01:01 - C*04:01:01 (f = 0.072; D’ = 0.864), B*49:01:01 - C*07:01:01 (f = 0.043; D’ = 0.967), B*38:01:01 - C*12:03:01 (f = 0.040; D’ = 0.965). These B-C haplotypes most frequently had the following HLA-A alleles as part of three-locus haplotypes: A*11:01:01, A*23:01:01, and A*26:01:01, respectively. However, the most common three-locus haplotype was none of these, but A*03:01:01 - B*07:02:01 - C*07:02:01 (f = 0.018). Of the 11 three-locus haplotypes with more than 0.01 frequency, three were the B-C haplotype B*35:01:01 - C*04:01:01 with different alleles at HLA-A: A*02:13 - B*07:02:01 - C*07:01:01 (f = 0.018). These B-C haplotypes most frequently had the following HLA-A alleles as part of three-locus haplotypes: A*11:01:01, A*23:01:01, and A*26:01:01, respectively. However, the most common three-locus haplotype was none of these, but A*03:01:01 - B*07:02:01 - C*07:02:01 (f = 0.018). Of the 11 three-locus haplotypes with more than 0.01 frequency, three were the B-C haplotype B*35:01:01 - C*04:01:01 with different alleles at HLA-A: A*11:01:01 - A*24:02:01 - A*03:01:01, suggesting the presence of a recombinational hot-spot between HLA-A and −C on this particular B-C haplotype. The addition of these high-resolution HLA class I types in a sample of Turkish population should fill a gap in global databases.
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COMMON AND RARE HLA-ALLELE GROUPS AND HLA-HAPLOTYPES IN THE MOSCOW RUSSIAN DONORS OF HEMATOPOIETIC STEM CELLS

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The aim of our study was to analyze frequencies of HLA-A, −B, −C, −DRB1, and -DQB1 allele groups haplotypes in a representative group of Moscow Russian donors of hematopoietic stem cells. The study included 1507 unrelated donors who according to their self-assessments were the Russians by ethnicity. The donors were recruited and HLA-typed in the registry of National Research Center for Hematology (Moscow). The HLA-A, −B, −C, −DRB1, −DQB1 typing (low or intermediate resolution) was performed using Luminex 200 system (Luminex, TX, USA) with Lifecodes HLA SSO typing kits (Immucor, CT, USA). Statistical analysis was performed using the ARLQUIN version 3.5 software [http://cmpg.unibe.ch/software/arlequin35]. We calculated maximum-likelihood estimates for HLA-A,-B,-C,-DRB1 and -DQB1 allele group frequencies. Five-locus haplotype frequencies were estimated by expectation–maximization (EM) algorithm. Hardy–Weinberg exact tests were performed for each of the HLA loci. There were no deviations from HWE in all investigated HLA loci. We found 16 allele groups with frequencies >0.001 in HLA-A, 29 allele groups with frequencies >0.001 in HLA-B, 13 allele groups with frequencies >0.001 in HLA-C, 13 allele groups in HLA-DRB1 and five allele groups in DQB1, all defined allele groups of HLA class II had frequencies >0.001. 1284 different five-locus HLA-haplotypes were calculated. 201 of HLA-haplotypes had frequency >0.001. Seven of HLA-haplotypes had frequency >0.01: A*01-B*08-C*07-DR*03-DQB*02 (0.0415), A*03-B*07-C*07-DR*15-DQB*06 (0.0265), A*03-B*35-C*04-DR*01-DQB*05 (0.0262), A*25-B*18-C*12-DR*15-DQB*06 (0.0176), A*02-B*13-C*06-DR*07-DQB*02 (0.0163), A*02-B*18-C*07-DR*11-DQB*03 (0.0153), A*02-B*07-C*07-DR*15-DQB*06 (0.0139). Distribution of HLA-allele groups and HLA-A-B-C-DRB1-DQB1 haplotypes in the Russian population of Moscow is similar to their distribution in the Russians from other regions of our state.

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DIFFERENTIAL HLA-C EXPRESSION IN CAUCASIANS: USEFUL DATA FROM DIRECT HLA-PROTEIN MEASUREMENTS IN 188 HEALTHY GERMAN DONORS

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Differential HLA-C expression, although already investigated in African Americans (Apps et al. Science 2013), has not been accordingly researched in Caucasians. Furthermore, the possibility that HLA-C expression heterogeneity could reflect an overall HLA class I expression regulation has not been yet experimentally addressed. This work aims at elucidating both issues through actual protein expression data retrieved from healthy German blood donors. 188 buffy-coats provided from the Ulm Blood Donor Service were used for leucocyte collection in order to determine by flow cytometry the protein expression levels of HLA-C and HLA class I molecules on lymphocytes as previously described. To this end a DT-9 and an anti-HLA-ABC Antibody (Ab) were used. Median Intensity Fluorescence (MFI) coefficients were calculated for each C allotype through implementation of a linear regression model, as previously described. According to our findings HLA-C*03, C*07 and C*08 (MFI: 408, 450, 481) were ranked as low-expressed C antigens in contrast to C*12, C*14 and C*01, which were notably higher expressed (MFI: 1261, 1280, 1322). In addition, our data on overall HLA class I expression levels indicated no direct correlation between HLA-C and HLA class I expression on the cell surface. First, the divergence range of expression levels for HLA class I molecules was markedly narrower (MFIs: 450–758 vs. 408–1322) and secondly, low expressed C alleles were found linked with high HLA class I expression levels (e.g. C*08 758 for C1-1 vs. 481 for C). Our HLA-C expression results match significantly those of Apps et al., at least for low and high -expressed HLA-C antigens, despite the different ethnicity of subjects included in the studies. Moreover, of high interest is our finding regarding the lack of association between HLA-C and HLA class I expression, which in turn points to an HLA-C rather than an HLA class I specific expression-regulating factor accounting for this marked variability in HLA-C expression levels. Further research is required before final conclusions can be drawn.
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TRI-ALLELIC PATTERN AT THE TPOX LOcus: BACK TO THE LITERATURE ABOUT ONE CASE

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Pre-graft short tandem repeat profile determination for a patient from Mayotte displayed a peculiar pattern, using PP16 Promega kits, suggesting a contamination. A tri-allelic pattern could be observed for the TPOX locus (8, 10, and 11 AATG repeat motifs). Three of four parental HLA haplotypes were from African origin, the fourth was from Malaysian origin. The 4 patient’s grandparents were from Madagascar and had Swahili as a native language, a typical Bantu idiom. Those tri allelic patterns for TPOX are not contaminants and are described, despite their rarity, present in 2% in the black sub-Saharan populations. In 90%, this third allele (10) is due to an insertion in the X chromosome. It would have occurred in the Bantu tribes before the beginning of their geographical expansion from the Cameroon-Nigeria region to south east Africa then Madagascar and the Comorian islands. The detection of this profile in America would be in line with the migration of African populations in the direction to America linked to the slave trade. In Brazil, where diverse populations were largely mixed, this pattern is also observed in individuals with Caucasian phenotypes. Indigenous populations from Madagascar are from Polynesian origin (in particular Indonesian) and speak an Austronesian language despite strong African immigration. Parental HLA haplotypes and grand-parents native language, indicate that they are of African origin, despite a possible race mixing with the indigenous population. This ethnic origin, probably explains the observed tri-allelic pattern in this patient. This genetic particularity must be known in the chimerism post graft follow up for these patients.

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ASSOCIATION OF THE CYP21A2 GENE P. V282L MUTATION WITH HLA ALLELES AND HAPLOTYPES IN THE CROATIAN POPULATION

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The CYP21A2 mutations that are in linkage disequilibrium with particular HLA-A, −B, −DRB1 alleles/haplotypes, cause deficiency of the 21-hydroxylase enzyme (21-OHD) and account for the majority of congenital adrenal hyperplasia (CAH) cases. The aim of this study was to investigate those associations linked to the non-classical (NC) form of CAH among Croatians. The study included parents of patients with the NC form of CAH, positive for the p.V282L mutation (n = 55) and cadaveric donor samples (n = 231). All subjects were HLA-A, −B, and −DRB1 typed and tested for the presence of the p.V282L mutation. Among parents of patients, 92.73% of subjects were positive for the B*14:02 allele and almost half of them carried the HLA-A*33:01-B*14:02-DRB1*01:02 haplotype. Among cadaveric samples 77 out of 96 subjects positive for the B*14:02 allele had the p.V282L mutation. Among them, 37 were positive for the HLA-A*33:01-B*14:02-DRB1*01:02 haplotype, 23 had the HLA-A*33:01-B*14:02-DRB1*03:01 haplotype, eight had the B*14:02-DRB1*01:02 combination and five were carrying the HLA-A*68:02-B*14:02-DRB1*13:03 haplotype. Four of these subjects were positive only for B*14:02 allele. HLA-B*14:02 was the only single allele which association with the p.V282L mutation reached statistically significant P value (RR = 12.00; P = 0.0024). Haplotypes B*14:02-DRB1*01:02 (P < 0.001) and HLA-A*68:02-B*14:02-DRB1*13:03 (P < 0.001) as well as HLA-A*33:01-B*14:02-DRB1*01:02 and HLA-A*33:01-B*14:02-DRB1*03:01 showed high relative risks (RR = 45.00, RR = 41.63 and RR = 36.96, respectively). Our data support the previously documented association of the HLA-A*33:01-B*14:02-DRB1*01:02 haplotype with the p.V282L mutation, but also point out a high frequency of the p.V282L mutation among Croatians with HLA-A*33:01-B*14:02-DRB1*03:01 and HLA-A*68:02-B*14:02-DRB1*13:03 haplotypes.

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THE FIRST POPULATION OF THE CANARY ISLANDS IS NOT ONLY NORTH AFRICAN: PRESENCE OF EUROPEAN ATLANTIC GENES AND ANCIENT IBERIAN LANGUAGE SCRIPTS

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Canary Islands (Spain) First Inhabitants (“Guanches”) where postulated to come only from North Africa. Our studies show that not only North Africans but also Iberian/Atlantic Europeans (and possibly others) must have been first Canarians. Debate whether North Africans or Iberians were the first “Guanches” is artificial since Iberian Peninsula-North African gene flow in ancient times was abundant and Iberians share a great part of genetic profile with North Africans. New genetic (HLA) and linguistic data shown in the present paper, is
supported by diverse early anthropological and “Guanches” mummies characters which confirm existence of at least two “Guanches” types and a correct interpretation of R1b Y chromosome high frequency in Atlantic Europe (Ireland, British Isles, North Spain, Basque Coast and Portugal), and also, is present in Canary Islands (10%). In the present paper HLA genes and presence of abundant old Iberian language scripts (which show an easy translation proposal by using Basque) in Fuerteventura and also in Lanzarote and the El Hierro Islands suggest that a present day dogma of a hypothetically North African single origin should be changed. Both Atlantic/Europeans and North Africans define origin of Canary Islands first inhabitants.

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**IDENTIFICATION OF GENOMIC FULL-LENGTH SEQUENCE OF HLA-E IN CHINESE INDIVIDUALS AND TWO NOVEL HLA-E ALLELES**

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HLA-E is one of non-classical HLA class I genes. At present, the study of polymorphism analysis in China is mainly aimed at the variation in exon 3 of HLA-E, which determines HLA-E*01:01 or E*01:03. However, study of identification of the full-length HLA-E and its novel alleles is rarely reported. We aimed to establish a method of identification of HLA-E genomic full-length sequences, and identify novel alleles in healthy blood donors in Shenzhen, China. We extracted DNA from peripheral blood of individuals and designed amplification and sequencing primers in conserved regions according to the sequences of HLA-E published on the IPD-IMGT/HLA Database. We used a high-fidelity polymerase to amplify genomic full-length of HLA-E followed by sequencing, assembling, confirming and typing. During this research, we successfully established a method for amplifying genomic full-length sequence and sequence-based typing. Two novel HLA-E alleles were detected and named by the WHO HLA Nomenclature committee as HLA-E*01:01:01:06 and HLA-E*01:01:01:07. Compared with the closer allele HLA-E*01:01:01:01, HLA-E*01:01:01:06 has one nucleotide change at position -26G > T in the 5’-promoter region, and HLA-E*01:01:01:07 has one nucleotide change at position 3345 T > C in the 3’-UTR. HLA-E*01:01:01:06 appears to be a common allele among Chinese. The polymorphism data of genomic full-length HLA-E in Chinese individuals need to be further investigated.

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**CHARACTERISATION AND FUNCTIONAL IMPLICATIONS OF THE TWO NEW HLA-G ALLELES FOUND IN AMERINDIAN (HLA-G*01:08:02) AND CARIBBEAN (HLA-G*01:20) POPULATIONS**

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HLA-G polymorphism has been found to be relatively low in all world populations. In the present paper two new HLA-G alleles are described in ancient American natives. A new HLA-G allele (HLA-G*01:08:02) from an Ecuadorian Amerindian individual (male) showed four codon changes with respect to HLA-G*01:01:01. Silent changes in the alpha-1 domain (residue 57, Pro, CCG > CCA) and alpha-2 domain (residue 93, His, CAC > CAT and residue 100, Gly, GGC > GGT) and one non-synonymous change in the alpha-3 domain (residue 219 changed from Arg to Trp). This alpha-3 change may dramatically alter HLA-G interactions with beta-2 microglobulin, CD8, ILT-2 and ILT-4 ligands present in subsets of T, B, NK, monocytes, macrophages and dendritic cells. Another HLA-G new molecule (HLA-G*01:20) was found in a woman from Hispaniola Island, Dominican Republic (Sto Domingo): it presented a silent change in the alpha-2 domain residue 107, Gly, GGA > GGT and non-silent change at residue 178, Met > Thr (with respect to HLA-G*01:01:01) which is close to class I molecule/clonotypic T cell receptor interaction sites. Functional implications of these findings are discussed.

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**THE DIVERSITY IN THE ASSOCIATIONS BETWEEN THE HLA-DRB4*01:03:01:02 N ALLELE AND HLA-DRB1 ALLELES**

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It is well known that the DRB1 gene is expressed for each HLA haplotype and depending on the DRB1 alleles present, additional DRB genes can be expressed. In the haplotypes with DRB1*04, DRB1*07, and DRB1*09 alleles the second active DRB gene is DRB4. This gene has now 57 known alleles and among them five are non-expressed: DRB4*01:03:01:02 N, DRB4*01:16 N, DRB4*01:38 N, DRB4*02:01 N and
DRB4*03:01 N. Here we describe HLA-DRB1 ~ DRB4 haplotypes in a group of 242 cadaveric samples positive for one of the DR53 alleles in Croatia. One hundred and eleven samples were DRB1*07:01 positive, 126 were DRB1*04 positive, while 5 samples were DRB1*09:01 positive. HLA-A, −B, −C, −DRB1 and -DQB1 typing was performed by PCR-SSP low resolution method, while HLA-DRB4 and HLA-DRB1*04 typing was performed by PCR-SSP at allelic level. Three different DRB4 alleles were observed among DRB1*04 samples; DRB4*01:02, DRB4*01:03 and DRB4*01:03:01:02 N with frequencies of 2.38%, 91.27%, and 6.35%, respectively. Of the eight times DRB4*01:03:01:02 N was observed among DRB1*04 samples, the associated allele was DRB1*04:02 (7/26; 26.92%). This is statistically significant in comparison to DRB1*04:01 samples (P = 0.0019) and DRB1*04:04 samples (P = 0.0113), while comparison with other subgroups of DRB1*04 samples did not reach statistical significance. Among six different DRB4 alleles in the group of DRB1*07:01 ~ DQB1*02:02 haplotypes, the most frequent were DRB4*01:03 (49.09%) and DRB4*01:01:01:01 (41.18%) while alleles DRB4*01:08, DRB4*03:01 N, DRB4*01:05 and DRB4*01:02 were detected once or twice. The DRB1*07:01 ~ DQB1*03:03 haplotype, in 98.21% of cases, carried the DRB4*01:01:01:01 (7/26; 26.92%). This study supports necessity of DRB4 sub-typing to correctly identify 98.21% of cases, carried the DRB4*01:03:01:02 N allele. This could help in searching donor for hematological patients and provide useful information for extended haplotype profiles of the Serbian population. A high number of unique haplotypes argue in favor of increasing the number of donors in Serbian registry.

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MAJOR HISTOCOMpatibility COMPLEX-DMB allelic diversity in old and new world non-human Primates: Intra-species Pattern of evolution
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New MHC-DMB complete cDNA sequences have been obtained in individuals belonging to the following primate species/families: Hylobates lar, Papio hamadryas, Macaca mulatta, Macaca fusciculata, Cercocebus aethiops and Saginus oedipus. Exonic allelism has been recorded all along the DM molecule domains and analyzes of the critical residues in the conformation of the MHC-DR peptide-binding site were done; it was found that an evolutionary pressure over the putative peptide-binding region of the DMB molecule favors synonymous changes. These results are in contrast with those found in the MHC class I and class II genes, where nonsynonymous DNA base substitutions are favored. The immunoreceptor inhibition motif Tyr230-X231-X232-Leu233 (ITIM) is invariably present in all extant studied primates since 40 million years ago. It confirms the important function for this molecule, directing DR molecules towards the endosomal/lysosomal HLA class II compartment and sending inhibitory signals to
cells in order to stop synthesis of unnecessary MHC-DR molecules. Some *Macaca* individual’s DMB molecules (appearing on Earth more than ten million years ago) do bear both short (without ITIM) and long cytoplasmic tails (with ITIM), similarly to what has been found in human individuals. These differences may have important functional implications. Obtained MHC-DMB allele phylogenetic trees suggest an intraspecies evolution, since alleles of the same species cluster together, as it occurs in other MHC related genes (Bf, C4d, HLA class I and class II). Other MHC class I and class II molecules’ phylogenetic trees show a trans-species pattern of evolution. Also, these trees show that gorilla and human are genetically closer than human and chimpanzee (like C4d phylogenetic trees).

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IDENTIFICATION OF TWO DISTINCT HAPLOTYPE STRUCTURES ELUCIDATED BY POLYMORPHISM AND GENE EXPRESSION ANALYZES IN DOG LEUKOCYTE ANTIGEN CLASS I AND II GENES: DLA-88, DLA-12, DLA-64 AND DLA-DRB1

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The current information on the polymorphism variation and haplotype structure of the domestic dog leukocyte antigen (DLA) genes is limited in comparison to other experimental animals. In this paper, to better elucidate the degree and types of polymorphisms and genetic differences. For DLA-88, DLA-12, DLA-64 and DLA-DRB1, we genotyped four families of 38 beagles and another 404 unrelated dogs representing 49 breeds by RT-PCR based Sanger sequencing. We identified 76 alleles for DLA-88, 21 for DLA-12, seven for DLA-64 and 47 for DLA-DRB1, of which 44, 20, seven and six were newly described, respectively. Phylogenetic analysis supported that the DLA-88, DLA-12 and DLA-64 alleles were independently generated after the original divergence of the DLA-79 alleles. Our genotyping data suggested that there were two distinct two-gene DLA-class I (DLA-88a and DLA-12/88b) haplotype structures, tentatively named DLA-88a - DLA-12 and DLA-88a - DLA-88b. Of them the haplotype frequency of DLA-88a - DLA-88b occupied 31.9% of the unrelated dogs. Quantitative real-time PCR analysis showed that the gene expression levels of DLA-88b and DLA-88a were the same, and that the gene expression level of DLA-12 was significantly lower. In addition, haplotype frequency estimations revealed 143 different DLA haplotypes (88a-12/88b-64-DRB1) overall, and 37 different DLA haplotypes in homozygous dogs for 29 breeds and mongrels. Further studies and large scale genetic screening of dogs would help to better identify and define various DLA haplotypes. This DLA polymorphism information and genetic differences among the dog breeds could be used as a standard internal control of the MHC genetic background for the benefit of biomedical research into regeneration medicine using the most common DLA haplotypes as models for human MHC related diseases.

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MICA ASSOCIATIONS WITH ORAL SQUAMOUS CELL CARCINOMA

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Major histocompatibility complex class I related chain A (MICA) is a ligand of the Natural killer group 2, member D (NKG2D) receptor. Recent studies have shown that MICA is up regulated in tumors from epithelial origin, playing a key role in immunological surveillance and different alleles are associated with diseases related to NK activity. The aim of our study was to analyze associations of MICA polymorphism with oral squamous cell carcinoma (OSSC). Twenty seven patients with histologically proven OSSC were included in the study. The majority of patients had G2-G3 tumors according to Anneroth’s classification. The control group included healthy subjects from the Bulgarian population. MICA genotyping was performed by a PCR-SSO kit (LABType SSO MICA, One-Lambda) and PCR-SBT. Our results showed statistically significant protective association for MICA*12:01 allele (Pc < 0.05, OR-0.07), encoding a full length protein. Interestingly this allele had a higher frequency in the healthy Bulgarian population compared to other European populations. With the highest frequency in patients with OSSC was observed MICA*08:01 allele, encoding truncated protein. However the difference with the control group was with a borderline significance (Pc = 0.053). Although our data are preliminary considering the small number of patients analyzed, the associations observed support the model that alleles encoding truncated, ectopic and soluble MICA molecules play an important role in
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**CR1 POLYMORPHISMS, SERUM LEVELS OF CR1 PROTEIN AND mRNA EXPRESSION: AN ANTI-INFLAMMATORY ROLE IN PEMPHIGUS FOLIACEUS**

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Pemphigus foliaceus (PF) is an autoimmune disease endemic in Brazil, characterized by epidermal blisters and autoantibodies against desmoglein-1. The recognition of the autoantigen activates the complement system, which collaborates with the acantholytic process. Complement receptor 1 (CR1) regulates the complement system by destabilizing C3 and C5 convertases and preventing tissue injury. In order to evaluate if there is an association between common CR1 single nucleotide polymorphisms (SNPs) and the susceptibility to PF, we developed multiplex sequence-specific PCR assays and simultaneously genotyped nine SNPs including rs2274567 (His1208Arg) in exon 22, rs3737002 (Thr1408Met) in exon 26, and rs17047660 (Lys1590Glu) in exon 29, in 282 PF patients and 63 controls. We identified 13 haplotypes. Genotype distribution was in Hardy and Weinberg (HW) equilibrium in both groups. The GTATCTACA and GCATCTACA haplotypes may have a protective effect against development of the disease (P = 0.03, OR = 0.62 and P = 0.02, OR = 0.23, respectively) while GCACCTACG haplotype may increase susceptibility (P = 0.0001, OR = 4.47).

Among patients under treatment, those with localized lesions had higher sCR1 serum levels than those prior to treatment (P = 0.02). Among patients under treatment, those with localized lesions had higher sCR1 serum levels than those having generalized disease (P = 0.0004). Carriers of the GCATCTACA haplotype allele presented lower CR1 mRNA expression levels (p = 0.02). The results lead us to suggest that CR1 haplotypes may modulate gene expression and susceptibility to PF. Furthermore, corticoid treatment seems to increase sCR1 serum level, and higher sCR1 levels may play a protective role in individuals with PF.

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**24 AUTOSOMAL STR MICROSONATELLITES PROVIDE NEW INSIGHTS INTO THE MEXICAN MESTIZO POPULATION FROM MEXICO CITY AND THE HIGHLANDS**

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STRs are versatile and informative genetic markers that have facilitated a great understanding of the genetic diversity present in natural populations, being a common tool in forensics, paternity and anthropological studies. Data collection is conducted based on many populations and allele frequency (AF) must be established in making an identification or any study. Mexican Mestizos are of mixed descent. Their ancestry is the result of admixture between the indigenous people of Asian ancestry that inhabited the country, different Europeans, and to a lesser extent, Africans. The aim of the study was to describe the population genetics in Mexico City and Highlands, by using the PowerPlex21 & GenePrint24(Promega) & Global filer & Identifiler (Thermo Fisher Sci.) systems. We investigated 24 autosomal STRs in genomic DNA extracted from blood samples of 721 healthy Mexican Mestizos (57.4% males; 42.6% females). The analysis was performed with a 3500 Genetic Analyzer (Thermo Fisher Sci.). The genotyping process was done with the Gene Mapper ID-X v1.4 software. We compared the frequency with other studies in Mexicans from different regions. Heterozygosity (HE), Linkage disequilibrium (LD); Hardy-Weinberg equilibrium (HWE), Power of Discrimination (PD), Match Probability (MP) and Power of Exclusion (PE) were estimated with the PowerStat software. A total of 339 alleles at the 24 STR loci were found with their corresponding AF, ranging 0.069-50.704. These STRs are highly polymorphic being the most diverse FGA, D18S51, Penta-E, D21S11, D6S1043 and SE33 (30–18 alleles). Penta E showed the highest PD(0.985) and SE33, the highest PE (0.947). Concordantly, these STR loci showed the highest heterozygosity together with D6S1043; one allele was not in HWE at vWA, D8S1179, Penta D, D12S391, D19S433, D16S539; two alleles at TH01, Penta E, D21S11; 3 at D18S51, FGA, CSF1P0, since they were rare alleles. LD displayed no association between paired loci. Our data are concordant with those shown in Monterrey (North Mexico) but show different AF from them & those found in other Mexico regions. The most frequent alleles >20% were at; D21S11; D6S1043; vWA;TH01;CSF1P0; D16S539; D8S1179; D3S1358; D19S433, D2S391; D7S820. STR loci provide highly informative polymorphic data for population genetics, paternity testing and forensic identification.
PRELIMINARY STUDY REGARDING THE ASSOCIATION BETWEEN TUMOR NECROSIS FACTOR ALPHA GENE POLYMORPHISMS AND CHILDHOOD IDIOPATHIC NEPHROTIC SYNDROME IN ROMANIAN PEDIATRIC PATIENTS

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Childhood idiopathic nephrotic syndrome (INS) is one of the most common glomerular diseases, characterized by heavy proteinuria, hypoalbuminemia, dyslipidemia and generalized edema. Patients with resistance to steroid treatment have a high risk of progressing to chronic kidney disease, which is considered a public health problem worldwide. New evidence suggests that several cytokines, including tumor necrosis factor alpha (TNF-alpha) may play an important role in the pathogenesis of this disease. Our objective was to analyze two single nucleotide polymorphisms (SNPs) of TNF-alpha gene in 70 patients with INS and 159 healthy controls. The two SNPs (rs1799724/-857C/T and rs1800629/-308G/A) were genotyped by TaqMan Genotyping Assays C_11918223_10 and C_7514879_10 (Real-time PCR System, Applied Biosystems, USA). Association tests were performed with the software PLINK v 1.07 and p values <0.05 were considered significant. Controls and patients were in Hardy-Weinberg equilibrium for the investigated SNPs. Minor alleles frequencies were 11.6% in INS patients versus 13.2% in controls for 857*T allele and 14.3% in INS versus 18.3% in controls for 308*A allele. Although the minor alleles were more frequent in controls than in patients, the difference was not statistically significant (p = 0.28, OR 0.74 and p = 0.63, OR 0.86). An analysis regarding the distribution of the two TNF-alpha polymorphisms was conducted in steroid sensitive INS patients (80%) versus steroid resistant patients (20%). We found a low frequency of 857*T allele in steroid resistant patients (8.3%) compared to steroid sensitive patients (15.3%) and controls (13.2%), but not statistically significant (p > 0.05). We conclude that neither -857C/T, nor -308G/A polymorphisms of TNF-alpha gene are associated with the susceptibility and the response to steroid treatment of INS in our population. Given the small sample size used, future studies are necessary to clarify the results observed in the present study.

COMPLEMENT RECEPTOR 1 POLYMORPHISM IS ASSOCIATED WITH LEPROSY IN BRAZIL

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Leprosy is a chronic infectious disease caused by the obligate intracellular pathogens Mycobacterium leprae and M. lepromatosis, which invade macrophages and Schwann cells. Complement receptor 1 (CR1) binds to C3b/C4b fragments and to mannose binding lectin (MBL) deposited on opsonized bacteria, facilitating bacterial entrance into phagocytes. Here, we developed a multiplex PCR sequence-specific assay and genotyped nine single nucleotide polymorphisms (SNPs) in 213 leprosy patients and 297 controls: rs6656401 (intron 4), rs3849266 (intron 21), rs2274567 (exon 22), rs3737002 (exon 26), rs11118131 (intron 26), rs11118167 (intron 28), rs17047660 (exon 29), rs4844610 (intron 37) and rs12034383 (intron 37). We measured the mRNA and soluble CR1 levels in a subset of up to 80 samples. We identified 18 haplotypes, whose frequencies differed between ethnic groups (p < 0.000001). Afro-Brazilians with A alleles from polymorphism rs6656401 and rs4844610 presented almost four times increased susceptibility to leprosy (OR = 3.89, p = 0.003). Euro-Brazilians with the intronic rs3849266T presented higher susceptibility to leprosy (OR = 1.63, p = 0.028). Carriers of the rs11118167C presented higher CR1 mRNA expression in comparison to T/T homozygotes (p = 0.036). Euro-Brazilians with the intrinsic rs3849266T presented higher susceptibility to leprosy (OR = 1.63, p = 0.028). Carriers of the rs11118167C presented higher CR1 mRNA expression in comparison to T/T homozygotes (p = 0.036). Euro-Brazilians with the variant rs12034383A exhibited higher sCR1 levels compared to G/G homozygotes (p = 0.0175). A negative correlation between sCR1 and MBL levels was also observed (r = −0.52; p = 0.007). The results lead us to suggest that CR1 polymorphisms modulate gene expression and sCR1 levels, as well as susceptibility to leprosy, with the different effects in distinct ethnic groups.

THE GENETIC PROFILE OF KIR RECEPTORS IN SERBIAN POPULATION

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Abstract

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Killer cell immunoglobulin-like receptors (KIRs) are expressed on natural killer (NK) cells and on some types of T-cells. KIR genes encode inhibitory and activating receptors. Polymorphism of KIR genes is present in variation of alleles and haplotype variation in gene content. Distribution of KIR genes varies between individuals and across populations. The aim of this study was to analyze KIR gene and genotype frequencies in a sample of 97 randomly selected, healthy, unrelated individuals from different parts of Serbia. KIR genotyping was performed by PCR SSO (One Lambda) and PCR SSP (Innotrain, Olerup). The observed frequencies of KIR genes and genotypes were determined by direct counting. KIR genotypes were assigned according to the Allele Frequencies KIR database. Framework genes KIR3DL3, KIR3DP1, KIR2DL4, KIR3DL2 were found in all individuals of study sample and pseudogene KIR2DP1 almost in all individuals (95.8%). The frequency of inhibitory KIR genes was higher (77.7%) than frequency of activating (39.2%) except KIR2DS4 gene which was found with frequency of 94.8%. KIR2DS5 gene was present with smallest frequency of 27.8%. The frequency of other activating KIR genes are: KIR2DS2 (56%), KIR2DS3 and KIR2DS1 (37.1%), KIR3DS1 (38.1%). Inhibitory KIR genes were present in all individuals, KIR2DL1 (96.9%), KIR3DL1 (93.8%), KIR2DL3 (86.6%) and with lower frequency KIR2DL2 and KIR2DL5 (55.6%). 28 different genotypes were observed. Among AA genotypes (28.8%), the most frequent was genotype ID1 (27.8%) and other was genotype ID203 (1.03%). Among BX genotypes (71.1%), the most frequent was genotype ID5 (12.4%) followed by ID2 (8.2%), ID4 (6.2%), ID3 (5.2%), ID6 (4.1%). Sixteen KIR genotypes were observed only once each. This data about the distribution of KIR genes and genotypes will be useful as a reference for further analysis of KIR genes diversity and diseases association study in Serbian population.

CD59 POLYMORPHISMS MARK DIFFERENTIAL EXPRESSION LEVELS AND ARE ASSOCIATED WITH INCREASED SUSCEPTIBILITY TO PEMPHIGUS FOLIACEUS IN FEMALES

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Pemphigus foliaceus (PF) is a bullous autoimmune disease characterized by acantholysis and autoantibodies against desmosomal antigens (particularly desmoglein 1), accompanied by complement system (CS) activation and painful epidermal blisters similar to burn injuries. CD59 is an important regulator of the complement cascade final step, in addition to mediating signal transductions and T lymphocytes activation. CD59 has different transcripts due alternative splicing, suggesting the presence of regulatory sites in their non-coding regions. However, there are no reports regarding the CD59 non-coding polymorphisms and their effect on autoimmune diseases. We haplotyped six CD59 non-coding polymorphisms with a possible effect in alternative splicing and gene expression: rs861256, rs831625, rs831629, rs704701, rs1047581 and rs704697, in 157 patients and 215 controls by PCR-SSP. Differential gene expression was evaluated in 82 subjects by qPCR. Genotype distribution was in Hardy-Weinberg equilibrium. The variant rs861256-G was associated with increased CD59 mRNA expression levels (P = 0.01) and PF susceptibility in women (OR = 4.11, P = 0.0001). We observed that female patients were more prone to developing generalized lesions (OR = 4.3, P = 0.009) and to not experience disease remission (OR = 3.7, P = 0.045). Genetic associations were also observed for rs831625-G (OR = 3.1, P = 0.007) and rs704697-A (OR = 3.4, P = 0.006) in Euro-Brazilian women, and for rs704701-C (OR = 2.33, P = 0.037) in Afro-
Brazilians. These alleles constitute GGCCAA haplotype, which also increases PF susceptibility (OR = 4.9, P = 0.045) and marks higher mRNA expression (P = 0.0025). In conclusion, higher CD59 transcriptional levels seem to be related to PF susceptibility, probably due to transcriptional changes or to the CD59 role in T cell signal transduction and in cytokine release. These results should be replicated in independent cohorts and our hypothesis further investigated in functional studies.

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**GENETIC CHARACTERIZATION OF IMMUNOGLOBULIN GAMMA HEAVY CHAIN (IGHG) GENE SEGMENTS IN BRAZILIAN URBAN AND AMERINDIAN POPULATIONS SUGGESTS EVIDENCE OF NATURAL SELECTION**

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The immunoglobulin gamma heavy chain (IGHG) gene segments encode the constant regions of the four immunoglobulins G (IgG) heavy chains, which are responsible for the effector functions of the mature molecules. Polymorphism of IGHG has been associated with susceptibility to cancer, autoimmunity and infection. However, presently knowledge of the diversity of IGHG is mostly restricted to that assessed by serological methods, because it has not been systematically sequenced in worldwide populations. Moreover, genetic polymorphism of immunoglobulins is not well covered in genome-wide studies and genomic databases. We characterized the genetic diversity of three IGHG gene segments (IGHG1, IGHG2 and IGHG3) by sequence-based typing in 348 individuals from several Brazilian populations: Japanese-descendant (n = 41), Euro-descendant (n = 41) and in five isolated Amerindian populations, Guarani Kaiowa (n = 51), Guarani Ñandeva (n = 51), Guarani Mbyá (n = 47), Kaingang from Rio das Cobras (n = 48) and Kaingang from Ivaí (n = 49). A total of 25 new alleles have been found, of which 10 have been confirmed by cloning and sequencing. The most frequent alleles in Japanese-descendants were IGHG1*02 (61%), IGHG2*03 (53%) and IGHG3*14 (49%); in Euro-descendants, IGHG1*03 (72%), IGHG2*02 (47%) and IGHG3*11 (71%), and, in Amerindians, IGHG1*02 (45 to 77%), IGHG2*03 (74 to 96%) and IGHG3*14 (84 to 99%). Neutrality tests were performed for each gene segment and population. Tajima’s D values showed evidence of purifying selection (−1.9; p < 0.001), and Fay and Wu’s H statistic suggested that IGHG polymorphism is possibly driven by positive selection in nearby regions (−3.8; p < 0.01). Further studies are needed to verify which evolutionary pressures shape the genetic diversity of these gene segments. However, description of such unique isolated populations is already providing information for disease association studies and insights in immunoglobulin population genetics and evolution.

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**SIGNIFICANT ASSOCIATIONS BETWEEN LONG NON-CODING RNA POLYMORPHISMS AND PEMPHIGUS FOLIACEUS SUSCEPTIBILITY**

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Pemphigus foliaceus (PF) is an epidermal bullous autoimmune disease, characterized by presence of antibodies against desmoglein 1, a molecule important for cell adhesion. Genetic and environmental factors contribute to this complex disease, which is endemic in certain regions of Brazil. Long non-coding RNAs (lncRNAs) are transcripts more than 200 nucleotides in length and lack coding potential. Most lncRNAs are involved in gene expression regulation by interacting with DNA, other RNAs and proteins. lncRNA dysregulation and polymorphism have emerged as important co-players in the onset or progression of complex diseases, being reported in associations with differential risk to cancer autoimmune and infection. We aimed to investigate whether SNPs (single-nucleotide polymorphisms) located within lncRNAs genes could be associated with differential susceptibility to pemphigus foliaceus. For this purpose we intersected data from lncRNASNP database with genotype data (Illumina Beadchip Human Core Exome-24) from 235 patients and 6,681 controls. After standard quality control, we found 2,080 SNPs mapped to lncRNAs in our data. We performed an association study applying a logistic regression using principal components as covariants to correct for possible population structure bias. We found 3 SNPs associated with pemphigus foliaceus with P < 0.001. The three associated SNPs are located in regions close to protein coding genes. The strongest association was found on chromosome 2, SNP rs1542604, mapped on the lncRNA AC133785.2. Suggestive associations (P < 0.01) were seen for other 25 SNPs. We showed, for the first time, that SNPs located at lncRNAs could be associated with pemphigus susceptibility. Moreover, our results suggested that lncRNAs may be among the factors that influence pemphigus pathogenesis. We expect that lncRNAs quantification and analysis of the functional impact of their polymorphism will help to clarify the biological significance of these associations.
FUNCTIONAL ANALYSIS OF ALLELIC TYPED DONOR NATURAL KILLER CELL RECEPTORS KIR2DL1 AND KIR3DL1 FOR IMPROVED OUTCOME IN STEM CELL TRANSPLANTATION

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Genomic studies on KIR-HLA ligand interactions reveal activity differences based on allelic polymorphisms of NK KIR receptors, leading to differences in functional activity. However, these variations in affinity are not completely understood. In our study, allelic typing was performed by sequence analysis of KIR2DL1 and KIR3DL1 in a modified version after Hou et al. (2007) and Belle et al. (2008) including 21 healthy donors. NK cell activity was assessed by the CD107-degranulation assay against L721.221 transfected cell lines expressing HLA-C group 1, −C group 2, HLA-Bw4 ligands or HLA negative L721.221 cells. KIR exon sequences were analyzed by GeneSearch software using the IPD-KIR database to assign allelic typing. KIR3DL1 positive donors were grouped into ‘low’ and ‘high’ affinity allele groups. KIR2DL1 sequence analysis included a total of 57 polymorphisms. Position 245 in exon 7 was selected to separate donors into two groups based on an arginine/cytosine-exchange (R245/C245). Flow cytometry of KIR3DL1 low and high affinity confirmed the low/high expression pattern of alleles. NK cells missing their specific ligand on the target cells in the C245-group (N = 5) showed general higher activity levels compared to donors of the R245-group (N = 7) in CD107-assay. Nine analyzed donors were heterozygous for both (R/C245). One new polymorphism was found showing the triplet GGC, coding for the amino acid glycine. To correlate our findings with clinical outcome data from the Children’s Transplantation Center Frankfurt, 60 additional family stem cell donors were sequenced for the KIR2DL1 245-polymorphism and stratified for KIR2DL1 arginine (R) (245R) *001, *002, *00301-03, *005, *008, *009, *010 RR or R+ grafts with less mortality and better progress free survival (PFS). Determining allelic polymorphism in combination with functional analysis will lead to better medical prediction of the NK effect and could fill the gap between theory and praxis of NK cell alloreactivity.

KIR3DL1, BUT NOT ITS LIGANDS, CONFER LOW RISK FOR THE DEVELOPMENT OF ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) IN MEXICAN MESTIZO PATIENTS

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ALL accounts for >30% of all malignancies diagnosed before the age of 15 years. The disease is multifactorial, and an important role for inherited genetic variation has been suggested for its development. A spectrum of HLA associations were reported and polymorphisms at the killer cell immunoglobulin-like receptors (KIR) influence ALL development as well. KIR genes present on NK cells, regulate their functions through interactions with their HLA ligands, and are also documented to be involved in the destruction of leukemic cells. We addressed this issue by performing a case–control study in Mexican Mestizo patients with ALL, looking for the involvement of KIR3DL1 and its Bw4, A23, A24 & A32 ligands with the development of ALL. DNA was extracted from peripheral blood with the Maxwell16 instrument from 137 patients (90% were children) and 356 healthy controls. KIR3DL1 was detected using a PCR-SSP technique. HLA typing was done using a Luminex PCR-SSOP method. Frequencies of KIR3DL1 and KIR-HLA ligands were compared between patients and controls, using the Chi2 test. A low frequency of 3DL1 in patients vs. controls (80.3% vs. 88.5%; p = 0.018; OR = 0.530) was shown; however, no difference was observed for Bw4 or for A23, A24 & A32 (p = 0.24) or for 3DL1 with any of its ligands (p = 0.34). We previously reported protection associated with KIR2DS4 (p = 0.002), KIR2DL2 (p = 0.001), KIR2DS2 (p = 0.0001) and KIR2DS3 (p = 0.002) but not KIR3DL1, having typed then, only 66 patients. However, when increasing the number to 137, KIR3DL1 was clearly shown to be a protective gene. We thus conclude, that 3DL1 confers a low risk for ALL development in Mexicans. These data agree with those reported in Chinese populations, but differ from data found in Canadian whites and Indians and in admixed Hispanics from California (which include different races). Activating KIR2DS1-C2, 2DS2-C1 and 3DS1-Bw4 were risk genes in Indians only. Ethnicity differences clearly influence the genetic distribution of KIR genes and their ligands, therefore, probably their associations too. Undoubtedly, KIR3DL1 plays an important role in the expression and clearance of ALL in Mexicans. Moreover,
these results are consistent across different ALL phenotypes, as shown in white Canadians. The importance of typing a greater sample size of patients is evident.

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EXPRESSED KIR GENE FREQUENCIES IN NORMAL POLISH POPULATION AND LEUKEMIA PATIENTS
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Killer-cell immunoglobulin-like receptor (KIR) molecules play a key role in cancer immunosurveillance. We performed KIR genotyping in a normal Polish population (N = 330) and in acute myeloid leukemia (AML, N = 191) and acute lymphoblastic leukemia patients (ALL, N = 114). In the normal population some framework genes (KIR2DL4, 3DL2, 3DL3, 3DP1) were common and remaining genes occurred with certain gene frequency pattern (2DL1-0.809, 2DL2-0.308, 2DL3-0.670, 2DL5-0.292, 2DP1-0.809, 2DS1-0.233, 2DS2-0.324, 2DS3-0.166, 2DS4-0.754, 2DS5-0.158, 3DL1-0.773, 3DS1-0.218). It is considered that normal variants of some genes are functional and non-expressed variants with deletions are not (2DL4 norm-0.478 and del-0.517, 2DS4 norm-0.181 and del-0.584, 3DP1 norm-0.782 and variant-0.165, 2DL5A expressed-0.211 and 2DL5B null-0.240). The association study revealed a strong protective role of normally expressed KIR2DL4 in AML (OR = 0.43, p = 0.000045, 95% CI 0.29-0.64) and ALL (OR = 0.46, p = 0.0013, 95% CI 0.29-0.74) but not in it’s deletion variant. In a ALL similar protective role has been revealed for expressed variants of KIR2DS4 (OR = 0.41, p = 0.0018, 95% CI 0.23-0.72). These associations withstand the Bonferroni corrections for 15 KIR gene comparisons. Of note, the only so far reported ligand of KIR2DL4 is the non-classical HLA class I molecule HLA-G, leading to the inhibition of the cytolytic NK cell function. The protective role of functional KIR2DL4 can be related to licensing capacity of inhibitory KIR upon engagement to HLA-G or another unknown ligand.

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KILLER-CELL IMMUNOGLOBULIN-LIKE RECEPTOR (KIR) POLYMORPHISM IN RETINOBLASTOMA: A CASE-CONTROL STUDY
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Retinoblastoma is the most common cancer of the eye in children. A higher disease burden is recorded in large populations, such as Asia and Africa. An association between KIR and retinoblastoma has not been yet been reported. The aim here was to investigate the frequency of KIR genes in patients with retinoblastoma and compare with controls. Children with retinoblastoma and healthy controls were enrolled. DNA was extracted from peripheral blood samples of cases and controls. KIR genotyping was performed by polymerase chain reaction using sequence-specific primer assay. Nineteen KIR genes were investigated. These included: inhibitory (2DL1, 2DL2, 2DL3, 2DL4, 2DL5A, 2DL5B), activating (2DS1, 2DS2, 2DS3, 2DS4*FUL, 2DS4*DEL, 2DS5, 3DL1, 3DL2, 3DL3, 3DS1) and pseudogenes (2DP1, 3DP1*FUL, 3DP1*DEL). The association was determined by chi-square or Fisher’s exact test. The significance was adjusted with Bonferroni correction; a p value lower than 0.0026 (0.05/19) was considered significant. The study was approved by the institutional ethics committee. Thirty two patients with retinoblastoma and 60 controls were enrolled. The mean age of patients was 71.2 ± 19.5 months (range: 5–96), with a M:F ratio of 1:1.1. Seven (22%) patients had bilateral retinoblastoma. Of the 19 KIR loci analyzed, a significant difference in cases as compared to controls was observed at 3DL1, 3DL2, 3DL3 and 3DS1 loci. The inhibitory KIR genes that were significantly less well represented in patients with retinoblastoma included, 3DL1 (p 0.0004), 3DL2 (p 0.0002) and 3DL3 (p 0.0023). In addition, there was a significant down regulation of activating KIR gene 3DS1 (p 0.001) in cases. There was a significant reduction in frequency of 3DL2 (p 0.0006) and 3DS1 (p 0.0007) in patients with unilateral retinoblastoma. No association was observed with bilateral disease. A protective effect of 3DL1, 3DL2, 3DL3 and 3DS1 KIR alleles was observed in patients with retinoblastoma. The study provides novel insights concerning distribution of KIR genes in patients with retinoblastoma and has implications for the development of innovative immunotherapies for this childhood cancer.

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ANALYSIS OF EDUCATED KIR 3DL1 IN A PATIENT WITH MULTIPLE MYELOMA
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Multiple Myeloma (MM) is a hematological malignancy characterized by accumulation in the bone marrow of plasma cells that secrete a monoclonal immunoglobulin (Ig). For over thirty years, the relationship between NK cells and MM has been described in clinical and scientific studies. The available data suggest that NK cells play a key role in the immune response. However, such role could be lost due to strategies of "immune escape" used by the myeloma cells. It is well known that NK cells are subject to an "educational" process that ensures the formation of a repertoire of functional but "self-tolerant" NK cells through the interaction of their cell surface receptors with the HLA molecules (ligands C1, C2, Bw4). These receptors provide the educational signal. The aim of the present study was to analyze the prognostic impact of KIR expression of NK cells in MM patients and in healthy controls, to assess the connection, if any, between KIR genes and MM. KIR genes and their HLA ligand were analyzed in 30 healthy subjects (controls) and 69 patients (MM) using the Genotyping SSP and SSO kit. Patients and controls were divided into two groups according to the presence of KIR genes, KIR Ligand, educated KIR genes and KIR haplotype AA/Bx. The gene frequencies were also compared using the Fisher’s t-test with GraphPad Prism software version 6.00. KIR 3DL1-edu was found to be significantly more prevalent among controls as compared to MM patients (76.7% vs. 43.59%, p < 0.0023; CI = 95%). For MM patients, the AA and Bx genotype frequencies were, respectively, 24.6% and 74.4% with an A:B ratio of 1:3.05. As for the healthy controls, the AA and Bx genotype frequencies were, respectively, 33% and 67% with an A:B ratio of 1:2. Our data suggest that the lower frequency of an educated receptor (3DL1edu) may be associated with a reduced cytotoxicity of NK cells against myeloma cells.

Reproduction, Autoimmunity, Infection & Cancer

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T-CELL INFILTRATION AND MACROPHAGE POLARIZATION CORRELATES WITH HLA CLASS I EXPRESSION IN NON-SMALL CELL LUNG CANCER

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The main objective of this work was to investigate whether tumor HLA class I (HLA-I) expression influences immune infiltration. Tumor tissues obtained from 40 patients diagnosed with lung carcinomas were analyzed for HLA-I expression and lymphocyte infiltration using immunohistochemistry and flow cytometry. 22 patients out of the 40 were completely negative for HLA-I expression or showed down-regulation of HLA-A, -B and -C molecules (55%). We quantified tumor infiltration with CD8+ T lymphocytes, and M1 and M2 macrophages. We found that HLA-I positive tumors have statistically significant increases in tumor T lymphocyte infiltration compared to HLA-I negative tumors. M1 and M2 infiltration was analyzed in order to determine if they are associated with tumor HLA-I expression. In addition, we observed that tumors with HLA-I positive expression have higher M1/M2 ratio than HLA-I negative tumors (also statistically significant). These data suggest that there is a phase during tumor development when anti-tumor responses mediated by CD8+ T-lymphocytes and pro-inflammatory M1 macrophages prevail. In contrast, noticeably reduced presence of T-CD8 lymphocytes and polarization of macrophages to M2 phenotype in HLA class I negative tumors may favor the immune escape. Additionally, using flow cytometry analysis of the tumor infiltrate we noted a positive correlation between the total percentage of lymphocytes and the proportion of Treg cells. In conclusion, this study revealed that HLA-I-positive and -negative tumors show striking differences in the composition of leukocyte infiltration and that greater tumor infiltration with lymphocytes correlates with increased presence of Treg cells, which is a feature of immunosuppressive tumor microenvironment.

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THE ABSENCE OF HLA CLASS I EXPRESSION IN NON-SMALL CELL LUNG CANCER CORRELATES WITH THE TUMOR TISSUE STRUCTURE AND THE PATTERN OF T CELL INFILTRATION

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We wanted to analyze whether tumor HLA class I (HLA-I) expression influences the pattern of the immune cell infiltration and stromal cell reaction in the tumor microenvironment. Tumor tissues obtained from 57 patients diagnosed with lung carcinomas were analyzed for HLA expression and leukocyte
infiltration. 28 patients out of the 57 were completely negative for HLA-I expression (49.1%) or showed a selective HLA-A locus down-regulation (three patients, 5.2%). In 26 out of 57 tumors (47.8%) we detected a positive HLA-I expression but with a percentage of HLA-I negative cells between 10 and 25%. The HLA-I negative phenotype was produced by a combination of HLA haplotype loss and a transcriptional down-regulation of beta 2-microglobulin (b2-m) and LMP2 and LMP7 antigen presentation machinery genes. The analysis and localization of different immune cell populations revealed the presence of two major and reproducible patterns. One pattern, which we designated “immune-permissive tumor microenvironment” (TME), was characterized by positive tumor HLA-I expression, intratumoral infiltration with cytotoxic T-CD8+ cells, M1-inflammatory type macrophages, and a diffuse pattern of FAP+ cancer-associated fibroblasts. In contrast, another pattern defined as “non-immune-permissive TME” was found in HLA-I negative tumors with strong stromal-matrix interaction, T-CD8+ cells surrounding tumor nests, a dense layer of FAP+ fibroblasts and M2/repair-type macrophages. In conclusion, this study revealed marked differences between HLA class I-positive and negative tumors related to tissue structure, the composition of leukocyte infiltration and stromal response in the tumor microenvironment.

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ASSOCIATIONS OF POLYMORPHISMS WITHIN TOLL-LIKE RECEPTORS AND NUCLEAR FACTOR KAPPA GENES WITH PROGRESSION OF RHEUMATOID ARTHRITIS
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Rheumatoid arthritis (RA) is one of the most common autoimmune diseases. The signal cascade mediated by Toll-Like Receptors (TLRs) and the nuclear factor kappa B (NF-xB) results in an immune response via rapidly increased production of cytokines, such as TNF-alpha, and chemokines, that may lead to inflammatory and joint destructive process. Genetic polymorphisms of TLR and NF-kB1 genes were investigated in 303 RA patients (237 females / 66 males) and in 162 healthy individuals. TLR2 (rs111200466, −196/-174 del/ins), TLR3 (rs3775391; Leu412Phe G > A), and NF-kB1 (rs28362491; −94ins/del ATTG) polymorphisms were assessed by real-time PCR (using LightSNiP assays) and capillary electrophoresis. Patients and controls did not differ with respect to genotype/allele distributions. More effective EULAR response to treatment with TNF-inhibitors was observed after 24 weeks as compared to 12 weeks of treatment (p = 0.001). Men had a higher activity of the disease (DAS28) before treatment compared to women (p = 0.05) and the response to treatment was better in female than in male patients after 24 weeks (p = 0.05).Remission rate was higher in women compared to men (p = 0.04).

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COMBINED EFFECT OF GLUTAMINE AT POSITION 70 OF HLA-DRB1 AND ALANINE AT POSITION 57 OF HLA-DQB1 IN TYPE 1 DIABETES: AN EPITOPE ANALYSIS
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The contribution of specific HLA Class II alleles in type 1 diabetes is determined by polymorphic amino acid epitopes that direct antigen binding. Therefore, along with conventional allele frequency analysis, epitope analysis can provide important insights into disease susceptibility. Within our highly genetically heterogeneous patient cohort we identified a subgroup that did not carry the DRB1*03:01-DQA1*05:01-DQB1*02:01 and DRB1*04:04:xx-DQA1*01:03-DQB1*03:02 risk haplotypes but a novel recombinant one, DRB1*04:XX-DQA1*03:01-DQB1*02:01 designated DR4-DQ2.3. Through epitope analysis we identified established susceptibility (DQB1 A57, DRB1 H13) and resistance (DQB1 D57) epitopes as well as other novel susceptibility epitopes DRB1 Q70, DQB1 L26 and resistance epitopes DRB1 D70, R70 and DQB1 Y47.
Prevalence of susceptibility epitopes was higher in patients and was not exclusively a result of linkage disequilibrium. Epitopes DRB1 Q70, DQB1 L26 and A57 and a 10 amino acid epitope of DQA1 were the most significant in discriminating risk alleles. An extended haplotype containing these epitopes was carried by 92% of our patient cohort. Sharing of susceptibility epitopes could also explain the absence of risk haplotypes in patients. Finally, many significant epitopes were non-pocket residues suggesting that critical immune functions exist spanning further from the binding pockets.

**P176**

**THE ROLE OF HLA-DRB1 ALLELES AND THE SHARED EPITOPE HYPOTHESIS IN THE SUSCEPTIBILITY TO SERONEGATIVE AND SEROPOSITIVE RHEUMATOID ARTHRITIS IN PATIENTS FROM NORTHERN SPAIN (CANTABRIA)**

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Rheumatoid arthritis (RA) is a complex, multifactorial disease in which HLA, mainly HLA-DRB1 has been shown to play an important role, but its exact contribution in the pathogenesis remains unclear. According to different populations, a lot of studies focused on finding the associated specific alleles have been performed. Alleles from the HLA-DRB1*01, *04, *10 and *14 have been found in association with RA. All these alleles share a RAA motif in the 72–74 position of the amino acid molecule which is known as the shared epitope hypothesis. In 2005, du Montcel ST et al proposed a new classification including the amino acids at positions 70 and 71 because of their role in modulating the susceptibility to RA. Since then, few studies taking into account this additional residues have been published. 331 seropositive and seronegative RA patients (presence / absence of anti citrullinated protein antibodies) and 252 age and sex-matched healthy controls were analyzed for the HLA-DRB1 allelic distribution. All of them, belong to a well conserved region from northern Spain (Cantabria). Our results showed that HLA-DRB1*01:01 (QRRRAA) and *04:01 (QKRAA) were significantly more frequent in RA patients and HLA-DRB1*07:01 (DRRQG) and *13 (DERAA / DKRAA) in healthy controls. Furthermore, when we compared seropositive and seronegative patients we found that HLA-DRB1*01:01, *04:01 and *04:08 were significant associated with seropositive RA whereas HLA-DRB1*03:01, *13:01 and *13:02 were associated with seronegative RA. Distribution of HLA-DRB1 alleles were also analyzed in men and women and we found that HLA-DRB1*13:01 and *15:01 were more frequent in men with RA. Finally, genotype distribution according the new shared epitope classification was studied. In conclusion, our findings regarding HLA-DRB1 allele distribution partially agree with previously reported data. Moreover, when we considered the presence or absence of anti citrullinated protein antibodies compared with HLA-DRB1 alleles distribution, we found different results than other published studies.

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**HLA-DRB1 AND HLA-DQB1 ANTIGENS IN BULGARIAN PATIENTS WITH PRIMARY AND SECONDARY ANTIPHOSPHOLIPID SYNDROME**

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Antiphospholipid syndrome (APS) is a disease with multifactorial and polygenic pathogenesis. Recently, genetic predisposition to APS has been subjected to wide discussion. The aim of this study is to determine the prevalence of HLA-DRB1 and -DQB1 loci in a Bulgarian population of healthy persons and patients with primary (PAPS) and secondary (SAPS) APS. We investigated the prevalence of DRB1 and DQB1 in 204 healthy volunteers and 127 patients tested for antiphospholipid antibodies (aPL), divided into five groups: I-29 patients with systemic lupus erythematosus (SLE) with SAPS, II-35 patients with PAPS, III-32 women with spontaneous abortions without aPL, IV- patients with different thrombosis without clinical and laboratory data for APS, and V-16 SLE patients without clinical and laboratory data for APS. HLA-DRB1 and DQB1 alleles were determined with a PCR-SSP/SSOP technique. Chi square, and Fischer’s exact test were used to evaluate differences between groups. Maximum-likelihood and descriptive statistic were also calculated. Patients from group I had more frequently DRB1*03 than the control group (p < 0.0001; O.R. 4.04), than group II (p = 0.025; O.R. 0.32) and those from group III (p = 0.02; O.R. 0.3); and more frequently DQB1*02 compared to control group (p = 0.002; O.R. 2.88) and patients from group II (p = 0.029; O.R. 0.35), but more rarely DRB1*11 compared to control group (p = 0.014; O.R. 0.29), patients from group II (p = 0.03; O.R. 3.82) and those from group III (p = 0.012; O.R. 4.5). Additionally, HLA-DQB1*03 was at significantly lower frequency in patients from group I (0.207) compared to control group (0.386; p = 0.01; O.R. 0.42), group II (0.382; p = 0.025) and group III (0.406; p = 0.028). There were no statistically significant differences between other groups. HLA-DRB1*03 and DQB1*02 antigens have a strong association with SLE patients with APS, whereas HLA-DRB1*11
and DQB1*03 seems to have a “protective” role. Patients with PAPS and those with spontaneous abortions have DRB1 and DQB1 alleles similar to a healthy population. Our results show the need for further investigations in order to identify the exact alleles associated with this complex autoimmune disorder with high socioeconomic status.

**P178**

ASSOCIATION OF HLA-DR4/HLA-DRB1*04 WITH VOGT-KOYANAGI-HARADA DISEASE

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Vogt-Koyanagi-Harada disease is characterized by chronic granulomatous panuveitis and diffuse bilateral etiology, whose pathogenesis has been related to immunological disorders affecting the melanocytes and involving mediated cytotoxicity by T lymphocytes and apoptosis. It develops towards the detachment of the exudative retina, often associated with immunological abnormalities. The incidence is estimated at 1 / 400,000 cases a year. The disease affects mainly female individuals and those with darker skin pigmentation from Asian, Hispanic, Middle Eastern and Native American populations. The disease can be divided into four clinical stages. The prodromal phase is characterized by a non-specific symptomatology. The next phase shows neurological symptoms, while the convalescent phase that occurs within three months of the onset of the disease is characterized by cutaneous signs such as poliosis of eyelashes, eyebrows and hair. Recurrent uveitis and ocular complications characterize the chronic recurrent stage. In one case, a 56 year-old woman was at the prodromal stage, without specific symptoms such as fever, headache, dizziness (vertigo) and nausea, then after a few days progressed to the ophthalmologic stage. The patient complained of blurred vision, orbital pain, and bilateral central scotoma. HLA class II typing identified DRB1*04:05 in this patient. Confirming the diagnosis, the patient was treatment with high-dose corticosteroids and after being under observation for 10 months, the patient has had no recurrences of disease.

**P179**

DISCOVERY OF HYBRID PEPTIDES IN AUTOIMMUNE DISEASE

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We recently discovered hybrid insulin peptides (HIPs) as a new class of antigens targeted by autoreactive T cells in Type 1 Diabetes (T1D). These peptides form in pancreatic beta cells as a result of the post-translational crosslinking of insulin fragments to other naturally occurring peptides that are present in the secretory granules of beta cells. At the junction between two peptide binding partners HIPs contain entirely new amino acid sequences, which provide plausible explanations on how immune self-tolerance may be broken in T1D. We are currently developing proteomic strategies to characterize the hybrid peptide landscape in beta cells, setting the stage for the identification of hybrid peptide antigens in other autoimmune diseases. The amino acid sequences of hybrid peptides are not represented in traditional protein databases used for mass spectrometric analyzes of biological samples. Consequently, we are working on the generation of new databases, which can be used for the mass spectrometric identification of hybrid peptide sequences. For this we are using biochemical strategies to identify potential hybrid peptide binding partners in beta cell extracts, which can be used to generate hybrid peptide databases. The identification of hybrid peptides in tissue targeted by autoimmune attacks may provide tools to predict, prevent or reverse various autoimmune diseases.

**P180**

AUTOIMMUNE CYTOPENIAS IN PRIMARY IMMUNODEFICIENCY DISEASES: SINGLE CENTER EXPERIENCE

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Primary immunodeficiency diseases (PIDs) are associated with hematologic complications such autoimmune hemolytic anemia (AIHA) and thrombocytopenia (ITP). The most common autoimmune cytopenia is ITP. Although ITP is observed in 7.6% of patients with PID, AIHA is seen at 4.8%. Also, we aimed to present the patients who had autoimmune cytopenias and PID. Fifty six PID patients who were followed at the Pediatric Immunology Department of Erciyes University Medical Faculty (analyzed genetically) were evaluated retrospectively. Autoimmune cytopenias such as ITP and AIHA were detected in 9 (16.07%) of the patients (combined immunodeficiency: 4 patients, common variable immunodeficiency: 2 patients, hyper-immunoglobulin E syndrome: 1 patient, X-linked lymphoproliferative: 1 patient, chronic granulomatous disease: 1 patient). ITP was detected in 80 of 9 patients and AIHA was also detected in 6 patients. In four patients (LRBA deficiency: 2 patients, hyper IgE syndrome: 1 patient and CGD: 1 patient), both ITP and AIHA were observed. Immunosuppressive therapy with steroid, cyclosporine, mycophenolate mofetil and intravenous immunoglobulin were given to all patients. Bone
marrow transplantation was performed to the four patients. However, five patients died because of immunodeficiency. There is a paradoxal situation between PID and autoimmunity. The reduction of central and peripheral tolerance is held responsible for autoimmunity in PID. As a conclusion, we wanted to point out autoimmune cytopenias in patients with PID and the requirement of multidisciplinary approach for treatment.

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**HLA-DRB1, KNOWN TO BE ASSOCIATED WITH DIFFERENT AUTOIMMUNE DISORDERS, IN PREGNANCIES COMPILCATED BY AUTOIMMUNE RHEUMATIC DISEASES**

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Rheumatic diseases are chronic systemic diseases often affecting young women during reproductive ages, so pregnancy is a major issue in their management. The fetus represents a natural allograft that is not normally rejected. While the maternal immune system preserves the ability to respond to foreign antigens, tolerance mechanisms are up-regulated to protect the fetus from immunologic attacks by the mother. This deep immunologic adaptation influences maternal rheumatic diseases in several ways. Associations between HLA molecules and systemic autoimmune diseases have been reported and vary among ethnic groups and diseases. This is a case–control study including 30 pregnancies in women with preexisting rheumatic diseases and 177 physiological pregnancies. The rheumatic diseases included: 22 undifferentiated connective tissue disease cases, five of primary anti-phospholipid syndrome, one systemic sclerosis, one Sjögren’s syndrome, and one fibromyalgia case. PCR-SSP/-SSO techniques were used to define HLA-DRB1 allelic polymorphisms. The frequency of the HLA-DRB1*01,*04,*10 rheumatoid arthritis shared epitope was similar in patients and controls (10% vs. 11%). The same was observed for DRB1*15, described as associated with Sjögren’s syndrome (3.3% vs. 4.5%). The allele frequency of HLA-DRB1*03, significantly associated with SLE, was slightly increased in patients with respect to controls (10% vs. 6.2% p = n.s. OR = 1.68). Only HLA-DRB1*07 showed a significantly higher frequency among patients with respect to controls (18.33% vs. 8.76% p = 0.023). Considering this study as a genetic counseling, it may be useful to predict the evolution of undifferentiated connective tissue diseases to overt rheumatoid arthritis. We found six rheumatic pregnant women carriers of the HLA-DRB1 shared epitope peculiar to rheumatoid arthritis and we informed the clinicians in order to properly follow them up. The high frequency of HLA-DRB1*03 and DRB1*07 in rheumatic patients might be indicative of a silent celiac disease and suggests a need to perform a search for anti-tissue transglutaminase antibodies.

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**RELATIONSHIP BETWEEN HLA-DQA1, HLA-DQB1 AND HLA-DRB1 ALLELES IN IMMUNOGLOBULIN A NEPHROPATHY**

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Immunoglobulin A nephropathy (IgAN) is the most prevalent primary glomerular disease worldwide and an important cause of end stage renal disease. Data obtained in recent years have shown the ethnic-specific association between certain human leukocyte antigen (HLA) alleles and IgAN susceptibility. This study was designed to explore the relationship between HLA-DQA1, HLA-DQB1 and HLA-DRB1 alleles and disease susceptibility and clinical manifestations of patients with IgAN in Turkish population. A PCR-SSO Luminex typing technique was used to detect HLA-DQA1, HLA-DQB1 and HLA-DRB1 alleles in 265 IgAN patients and 232 healthy subjects. Clinical data were collected from each patient at the time of renal biopsy. Twenty-one HLA-DQA1 alleles, twenty-seven HLA-DQB1 alleles, sixty-one HLA-DRB1 alleles were detected in IgAN patients and healthy subjects. A high frequency of DQA1*01:01 [odds ratio (OR) = 2.22, P = 0.0021], DQB1*05:07 (OR = 45.2, P = 0.0027) and low frequency of DQA1*01:02 (OR = 0.45, P = 0.006), *01:04 (OR = 0.05, P = 0.004), *03:03 (OR = 0.04, P = 0.002) and DRB1*16:01 (OR = 0.3, P = 0.018) were observed in IgAN patients compared with healthy controls. Further stratification analysis revealed that the frequency of DRB1*14:01/54 was higher in patients with urine protein ≥ 1.0 g/24 h than in patients with urine protein < 1.0 g/24 h. But this did not remain significant after application of the Bonferroni correction. ur study indicated that HLA-DQA1*01:01 and DQB1*05:07 alleles may be a potential predictor of high-risk IgAN susceptibility in Turkish populations.

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**ANALYSIS OF HLA-DRB1 AND TLR POLYMORPHISMS IN SPANISH Q FEVER PATIENTS**

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Q fever is a bacterial infection caused by *Coxiella burnetii* that can spread to humans by infected animals, mainly sheep, cattle and goats. Variations in human immune response genes are well recognized to influence the course of infection, particularly by obligate intracellular pathogens which have a similar cell dependency to that of *Coxiella burnetii*. The relation between immune response genes variations and disease outcomes may be clarified by the analysis of polymorphic variations in individual candidate genes deduced to be of direct importance in disease pathogenesis. The aim of this study was to determine the possible association of Q fever with 3 polymorphic immune genes (TLR2, TLR4 and HLA-DRB1) in our population. Low resolution HLA-DRB1 typing, TLR2 (Arg753Gln) and TLR4 (Asp299Gly, Thr399Ile) polymorphisms were genotyped in 38 patients with acute Q fever and 38 healthy controls (relatives and patients co-workers). In addition, HLA typing results were compared with a representative sample of 121 donors from the same geographical area (Gran Canaria, Spain). No differences were observed in the three TLR polymorphisms studied. However, when the results of HLA-DRB1 frequencies of the patients were compared with their control group, an increase in the frequency of HLA-DRB1*04 (26.68% vs. 14.67%; p = 0.049, pc = ns) and HLA-DRB1*16 (3.5% vs. 0%; p = 0.038, pc = ns), as well as a decrease in the frequency of HLA-DRB1*08 (1.32% vs. 6.67%; p = 0.044; pc = ns) was observed. Also, when HLA-DRB1 patients frequencies were compared to the general population, a significant association was observed between HLA-DRB1*04 alleles and the presence of Q fever infection (26.68% vs. 12.41%; pc = 0.014). In conclusion, HLA-DRB1*04 allele might be associated with an increase in susceptibility to the development of Q fever in our area.

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**EVALUATION OF SARCOIDOSIS GENETIC RISK BASED ON 18 SUSCEPTIBILITY MARKERS IN A WEST-SLAVONIC POPULATION: SUBANALYSIS IN CONTEXT OF LÖFGREN SYNDROME**

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Sarcoidosis is a multi-systemic inflammatory disease, affecting mostly lungs. The etiology is unknown and its disease course is variable. The sarcoidosis sub-phenotype Löfgren syndrome (LS) is characteristic by acute onset and better prognosis. The disease is suggested to be influenced by environmental factors and genetic susceptibility. Several genes involved in immune responses have been reported, suggesting pulmonary sarcoidosis as a polygenic disorder. In this study, we analyzed 18 SNPs among 564 sarcoidosis patients, its sub-phenotypes LS (n = 94) and non-LS (n = 414) in comparison to 301 healthy controls from West-Slavonic population (Czech Rep. and Poland). Genotyping was performed using MALDI-TOF mass spectrometry based MassARRAY iPLEX platform (Agena Bioscience, San Diego, CA). Association analysis using allelic model and odds ratios (OR) was estimated using Fischer’s exact test; all significance statements and p values are given after Bonferroni correction for multiple comparisons. Analysis of sarcoidosis cases revealed association of total 7 variants associated with sarcoidosis as a whole. Analysis for sub-phenotypes LS/non-LS showed four variants specifically associated with Löfgren syndrome: HLA-DQA rs2187668*T (OR = 3.14, p = 1.09x10-6); HLA-DRA rs3135394*G (5.23, 8.25 x10-13); TNFalpha rs1800629*A (2.66, 5.94x10-7); LRRC16A rs9295661*C (2.97, 4.29x10-4). One variant was specifically associated with non-LS: ANXA11 rs1049550*A (0.66, 2.71x10-4). Our results replicate the findings from large cohort studies performed mostly in western Europeans and extend them to the Polish population, which has not been investigated in this context. Characterization of possible predisposing gene variants for sarcoidosis, and its sub-phenotypes such as Löfgren syndrome could be helpful for diagnosis of acute onset of the disease.

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**THE INFLUENCE OF HLA-SHARED EPITOPE (SE) AND SMOKING IN CCP AUTOIMMUNITY IN GREEK PATIENTS WITH RHEUMATOID ARTHRITIS**

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Rheumatoid Arthritis (RA) is a complex, multifactorial autoimmune disease, whose etiopathogenesis involves genetic and
environmental factors. The aim of the study was the assessment of association of HLA-DRB1*-SE in the presence/absence of CCP autoimmunity in Greek patients with RA (smokers and non-smokers). Eighty-three (83) RA patients (41 smokers, 42 have never smoked) were typed for HLA-DRB1 by molecular techniques (PCR-SSOP and SSP). In 62 out of 83 (74.7%) anti-CCP abs were detected by ELISA. In RA patients in comparison to the controls, increased frequency of HLA-DRB1*01:01 (28.9% vs. 6.8%, OR = 4.4), *10:01 (16.9% vs. 2.4%, OR = 8.4), *04:01 (3.6% vs. 2%, OR = 1.8), *04:04 (7.2% vs. 1%, OR = 7.6) and *04:05 (15.7% vs. 3.7%, OR = 4.8), as well as decreased frequency of DRB1*04:02 (1.2% vs. 2%, OR = 0.6) and *04:03 (4.8% vs. 6.8%, OR = 0.7) was found. Among RA patients, 77.1% possessed 1SE vs. 18.9% of controls (OR = 14.4), whereas 10.8% possessed 2SE vs. 1% of controls (OR = 11.8). In CCP(+) RA patients in comparison to CCP(-), an increased frequency of HLA-DRB1*01:01 (27.4% vs. 14.3%, OR = 2.3) and *10:01 (21% vs. 4.8%, OR = 5.3) was observed. Furthermore, 88.7% of CCP(+) carried 1SE vs. 42.9% of CCP(+) patients (OR = 10.5). CCP(+) smokers patients in comparison to CCP(+) non-smokers were presented with an increased frequency of DRB1*01:01 (41.9% vs. 12.9%, OR = 4.9). Among the CCP(+) smokers, 96.8% possess 1SE vs. 80.6% of CCP(+) non-smokers (OR = 7.2), whereas 12.9% possess 2SE vs. 12.9% of CCP(+) non-smokers (OR = 1). In conclusion: a) an increased frequency of HLA-DRB1*01:01, *10:01, *04:05 alleles, as well as the protective role of *04:02, *04:03 alleles, in Greek patients with RA was confirmed, b) the presence of any SE, particularly *10:01 allele, strongly influences the production of anti-CCP abs and c) interaction between smoking and any SE, particularly DRB1*01:01, is associated with anti-CCP positive RA in Greek patients.

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HLA ANTIGENS AND AUTOANTIBODIES AS SCREENING TEST AMONG FAMILY MEMBERS OF CELIAC DISEASE PATIENTS

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Celiac Disease (CD) is a permanent intolerance to gluten, which in genetically susceptible individuals leads to bowel mucosal villous atrophy. It affects at least 1% of caucasoids, however is more common in certain high risk groups, such as family members (FMs) of CD patients. The aim of this study was the evaluation of serologic and genetic markers in investigation and assessment of familiar CD prevalence, in a Greek population. The study included 29 CD patients and 101 first-degree FMs not presenting with typical symptoms and signs of CD. HLA typing by high resolution PCR-SSP techniques for -DQA1/-DQB1 alleles and serological identification of anti-tissue transfugaminase (tTG) by ELISA and anti-endomysial (EMA) autoantibodies (AAbs) by IIF, were performed. At least one CD predisposing HLA allele was typed in 28/29 (96.5%) patients and 75/101 (74.3%) FMs. More specifically 86 (66.1%) were DQ2 positive, 12 (9.2%) DQ8 positive and 7 (5.3%) DQ2/8 double positive. HLA-DQA1*05:01/DQB1*02:01 haplotype was present in 70 (53.8%), *02:01/02:02 in 19 (14.6%), *03:01/03:02 in 17 (13.1%), *03:03/02:02 in 7 (5.4%) and *03:02/03:02 and *03:01/03:05 in 1 (0.8%) individuals. All patients and 21 FMs were positive in at least one of the above AAbs and all of them but one patient carried a high risk HLA allele, 41 - DQ2 and 8 -DQ8. However, 56 individuals (43.1%) without positive AAbs expressed a high risk HLA allele. The study revealed 21 new CD cases (4 parents, 10 offspring, 7 siblings) according to ESPGHAN diagnostic criteria, all possessing both genetic and serological CD markers. The prevalence of CD among first-degree relatives (21/101) appears higher (20.8%) than in general population. Most of these patients had an atypical form of the disease and would therefore be overlooked, leading in serious complications. Therefore, a more pro-active screening strategy with HLA genotyping as well as tTG and EMA AAbs serological testing, should be strongly recommended in FMs of CD patients.

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LACK OF ASSOCIATION OF PROGRAMMED DEATH-1 GENE POLYMORPHISMS rs2227981 AND rs41386349 WITH MULTIPLE MYELOMA

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Multiple myeloma (MM) is a B-cell malignancy characterized by the proliferation and an accumulation of isotype – switched immunoglobulin-producing monoclonal plasma cells in the bone marrow. A variety of T-cell abnormalities was reported in MM patients, such as marked reduction in the proportions of CD4 and CD8 cells expressing co-stimulatory molecules, signal transduction components, and Th1/Th2 imbalance. Therefore we focused our attention on one of the immune checkpoint molecules - Programmed Death 1 protein (PD-1). PD1 is a cell surface receptor belonging to the immunoglobulin super-family, which negatively regulates the immune response by limiting the activation and proliferation of T-cells. Expression of this transmembrane protein, its ligands and different genetic variations of PD-1 gene have been widely studied as a part of attempts to identify the pathogenesis of many diseases related to immune system. The aim of this study was to examine whether the PD-1 SNPs: rs2227981 C > T (PD-1.5) and rs41386349 C > T (p.7209), are genetic modifiers for risk of MM. Our case-
control study was conducted in a Polish population consisting of 192 cases with multiple myeloma and 271 healthy individuals for PD-1.5, and 182 cases and 246 controls for p.7209. Genotyping was performed by polymerase chain reaction and restriction enzyme digestion: PvuII for PD-1.5 and MluI for p.7209. We found no significant differences in distribution of genotypes between MM patients and controls for both studied SNPs (rs2227981 C > T; CC - 32.3% vs. 33.6%, CT - 51.6% vs. 49.1%, TT 16.1% vs. 14.4% and rs41386349 C > T; CC - 82.4% vs. 86.6%, CT - 17.6% vs. 13.0%, TT - 0% vs. 0.4%). The haplotype analysis showed the trend toward higher risk of disease for patient with TT haplotype (OR = 1.58; p = 0.1). Our results indicates that rs2227981 C > T (PD-1.5) and rs41386349 C > T (p.7209) are not associated with the risk of multiple myeloma in Polish population, while the results should be confirmed on larger group of patients.

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ASSOCIATION OF POLYGLANDULAR AUTOIMMUNITY WITH HLA: IT’S WORTHWHILE TO TAKE A CLOSER LOOK INTO DETAIL

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The association of glandular autoimmunity, e.g. type 1-diabetes (T1D) or autoimmune thyroid disease (AITD), with HLA class I and class II alleles is well documented. Data are more scarce concerning HLA class I alleles and polyglandular autoimmunity (PGA). HLA-B*08:01 has been associated with the development of PGA, but due to its high linkage to the susceptibility alleles DRB1*03:01, *04:01 and *04:04, the informative value is restricted. This study aimed to determine HLA class I allele association with PGA with a special impact on gender-related differences and discrimination for the involvement of AITD. HLA-A and HLA-B alleles of 143 PGA patients and 350 healthy controls were determined by PCR-SSO (Luminex, Immucor HLA-A and HLA-B alleles of 143 PGA patients and 350 healthy differences and discrimination for the involvement of AITD. This study aimed to determine HLA class I allele association. This demonstrates the important influence of e.g. hormones and metabolic parameters on the development of polyglandular autoimmunity.

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STEVENS-JOHNSON SYNDROME FROM ALLOPURINOL WITH HLA B*58

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To date there are studies on the association of HLA-B*58 with allopurinol-induced severe allergic dermatitis. It is known that this drug, mainly used for the treatment of hyperuricemia, can cause the appearance of severe allergic reactions such as SJS and Lyell’s syndrome in susceptible individuals. Typing of HLA-B can identify a predisposition to allergic reaction to allopurinol, avoiding the administration of this drug to patients with HLA-B*58. A 41 year old man in treatment for a month with allopurinol 300 mg/day for hyperuricemia, came to our observation for the appearance of a rash on the palms of hands, soles of feet and subsequent commitment of the trunk and face. Erythematous lesions were presented as a rounded shade of deep red, with normal color in the center. These lesions were followed by desquamation of the lips and erythema of the oral mucosa making it difficult for the patient to feed himself. The patient was admitted to intensive care, where he was subjected to treatment with intravenous methylprednisolone 40 mg and topical therapy with fluocortolone. On the 7th day the patient was discharged in good clinical condition. The HLA study of the HLA-B*58*01 allele was positive. In another case, a 52 year old hypertensive man with familial hypercholesterolemia, who had been subjected to routine checks, was discovered to have high levels of uric acid. The hyperuricemia was subjected to therapy with allopurinol 300 mg/day. On the 8th day he was hospitalized in E.R. for fever, erythema of the oral and conjunctival mucosa. Erythematous lesions on the skin, centered by a taut clear blister with negative Nikolsky’s sign gave the diagnosis of Stevens-Johnson Syndrome. The patient started intravenous and topical steroid therapy for the skin lesions. The HLA typing showed the presence of the HLA-B*58. HLA-B*58 is relatively rare in Caucasians. HLA typing is a useful tool for the protection of the patient’s health and is a useful for the prevention of medical risk.
THE KIR2DS1 RECEPTOR IS A MINOR FACTOR FOR SUSCEPTIBILITY TO PSORIASIS INDEPENDENT OF HLA-C*06:02: A STUDY OF THE SARDINIAN POPULATION

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Psoriasis is a multi-factorial disease known to have a strong genetic susceptibility component. The HLA-C*06:02 allele has been suggested to be the most likely candidate for a predisposing role in disease pathogenesis since it has been found strongly associated with the condition in most populations worldwide. However, association of this allele is still a matter of controversy. Moreover, there are as yet no functional data supporting its pathogenic role in psoriasis. In the study of psoriasis, killer-cell immunoglobulin-like receptors (KIRs), expressed by natural killer (NK) cells, have aroused considerable interest (KIRs) for their binding affinity with specific HLA-C molecules. Natural killer cells or natural killer-T (NKT) cells infiltrate psoriatic plaques and have been shown capable of causing the disease in immune-deficient murine models. In the present study, we compared the distribution of HLA-C and KIR genotypes (KIR2DS1, KIR2DS2, KIR2DL1, KIR2DL2 and KIR2DL3) between 340 Sardinian psoriatic patients and 200 healthy controls. Statistical analysis of the comparisons performed between the two groups revealed a significantly higher frequency of KIR2DS1 in the group of psoriatic patients (51% vs. 41%; P = 0.02). The KIR2DS1 locus was associated with psoriasis independent of the HLA-C*06:02 allele and did not seem to be influenced by clinical parameters such as: onset age, psoriasis area and severity index (PASI) or response to anti-tumor necrosis factor (TNF) drugs. These findings support the hypothesis that KIR2DS1 is a minor factor for susceptibility to psoriasis with a different mechanism of action compared to with respect to HLA-C*06:02.

ASSOCIATION OF THE MICA POLYMORPHISM WITH CLINICAL RESPONSE TO ANTI-TNF THERAPY IN RHEUMATOID ARTHRITIS PATIENTS

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Major histocompatibility complex (MHC) class I chain-related A (MICA) proteins are stress-induced molecules involved in transmission of activating signals through NKG2D receptor. The MICA proteins are abberantly expressed on synovial tissue in rheumatoid arthritis patients. A total of 280 RA patients receiving anti-TNF therapy were enrolled to the study. Genotyping for MICA rs1051792 was performed using a polymerase chain reaction (PCR) amplification employing LightSNiP assays. Clinical response was evaluated according to the European League Against Rheumatism (EULAR) criteria at 12th and 24th week after initiation of the therapy. The MICA rs1051792 was significantly associated with clinical response after 12 weeks of anti-TNF treatment among patients. Patients carrying the heterozogous MICA rs1051792 AG genotype achieved significantly better EULAR responses to anti-TNF agents than patients with other genotypes (p = 0.003). Moreover, inefficiency of anti-TNF therapy was more frequently observed after 12 weeks in patients with GG genotype than in patients bearing the A allele (p = 0.010). No association was observed between MICA rs1051792 polymorphism and predisposition to RA development. These results imply that the MICA rs1051792 polymorphism may affect clinical response to therapy with TNF inhibitors in patients with RA.

A STUDY ON THE CORRELATION OF HLA-E GENE POLYMORPHISM WITH LEUKEMIA

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We studied the correlation between the gene polymorphism of HLA-E and leukemia, with the aim of determining new
molecular markers for the mechanism of leukemia occurrence and development. Peripheral blood DNA were extracted from patients with leukemia (n = 59) and healthy individuals (n = 132). The full-length HLA-E gene was amplified by long-range high-fidelity PCR and exon 3 was sequenced to identify the genotype of HLA-E. The genotype and allele frequency were calculated respectively in the leukemia patients and healthy controls. In the leukemia group, we detected 35 homozygous cases (59.3%), of which 29 (49.2%) were homozygous for HLA-E*01:03, and only 6 cases (10.2%) were homozygous for HLA-E*01:01. In healthy individuals, we detected 62 homozygous cases from healthy donors (47.0%), 36 cases (27.3%) were homozygous for HLA-E*01:03, 26 cases (19.7%) were homozygous for HLA-E*01:01. The allele frequencies of HLA-E*01:03 in leukemia patients and healthy individuals were 69.5% vs. 53.7% (P = 0.004, OR = 2.0). The detection rate of HLA-E*01:03 homozygous in two groups were 49.2% vs. 27.3 (P = 0.003, OR = 2.6). We conclude that HLA-E*01:03 is a susceptibility allele for leukemia.

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CYTOKINE GENE POLYMORPHISMS AND CLINICAL LABORATORY PARAMETERS IN PATIENTS WITH MULTIPLE MYELOMA FROM THE NORTH-WEST REGION OF RUSSIA
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Polymorphic cytokine genes are involved in the pathogenesis of multiple myeloma (MM). We compared single nucleotide polymorphisms (SNP) of cytokine genes (IL-1α, IL-1β, IL-1Ra, IL-2, IL-4, IL-4Ra, IL-6, IL-10, IL-12, TNF-α, IFN-γ, TGF-β1) in 100 healthy unrelated Caucasoid blood donors with frequencies observed in 80 patients with MM (inhabitants of the same region). Genomic DNA was extracted from the peripheral blood; gene genotyping was performed by PCR-SSP, p-values less than 0.05 were considered statistically significant. The largest differences between two studied groups were found for IL-1 and IL-6. Frequencies of IL-1α –889 TT, IL-1β +3962 TT, IL-6 -174 GG, IL-6 nt565 GG in patients were higher than in donors (p < 0.05). Further we analyzed albumin and beta2-microglobulin levels in the patients with/without homozygous genotypes for IL-1 and IL-6. All patients were divided into four groups: 1st – with IL-1α –889 TT, IL-1β +3962 TT, IL-6 -174 GG, IL-6 nt565 GG in their genotypes; 2nd – with IL-1α –889 TT, IL-1β +3962 TT; 3rd – with IL-6 -174 GG, IL-6 nt565 GG and 4th – without homozygotes in IL-1 and IL-6. Frequency of albumin low levels (up to 3.5 g/dl) was significantly higher in the 1–3 groups compare to the 4th group. The lowest levels of albumin (3.3 g/dl) was detected in the 1st group, however, the highest (more than 3.5 g/dl) was detected in patients without TT and GG homozygotes in IL-1 and IL-6 genotypes (p <0.05). The highest level of beta2-microglobulin was detected in the 1st group (5.5 mg/l) but no significant differences were detected compared to the other groups of patients (p > 0.05). Low levels of beta2-microglobulin weren’t registered in the 1st and 2nd group. The average level of beta2-microglobulin (3.5-5.5 mg/l) was frequently observed in the 4th group (0.70) and least registered in the 3rd group (0.42; p <0.05); high levels of protein (more than 5.5 mg/l) most rarely seen in patients from the 4th group (0.26).

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BREAST CANCER: GENETIC RISK OR PROTECTION IN ITALIAN INDIVIDUALS
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Breast cancer (BC) is one of the most common and malignant diseases among women and hormonal and environmental factors could be involved in the presentation of BC. There is also evidence that genetic factors may favor or interfere with the occurrence of BC. The innate immune system with natural killer receptors (KIR) recognizing class I HLA molecules and tumor surveillance with HLA class II antigens presenting of tumor peptides to T lymphocytes are involved in anti-tumor immune response. The aim of this study was to investigate the association between the KIR genes and HLA-C alleles in Italian patients with BC and matched controls (Ctrs). In addition, we analyzed the potential relationship between HLA-DRB1 alleles and cancer. Our SSP results regarding KIR and HLA-C gene polymorphisms (43 BC patients and 39 Italian female controls) showed a higher incidence of KIR2DS1 gene in advanced carcinoma patients (stage III-IV) compared to stage I-II patients (66.7% vs. 28.0% p = 0.0156; 46.1% in Ctrs). In particular, the KIR2DS1/HLA-C2+ combination was predisposing to a more advanced cancer (III-IV 42.9% vs. I-II 6.3%, p = 0.0309). On the contrary, KIR2DS4ins alleles seemed to be protective towards more advanced stages (11.1% vs. 35.9% in Ctrs, p = 0.06). In addition, HLA-DRB1 sequencing of 114 breast cancer patients and 120 female donors revealed the association of DRB1*11:03 allele with an increased risk to develop BC (p = 0.025, OR = 3.5674) and a protective role of HLA-DRB1*16:01 towards the BC cancer onset (p = 0.019, OR = 0.2328). Our findings suggest a potential role of KIR activating receptors licensed by HLA-C ligands in BC tumoral progression and an effect of some MHC class II alleles in the
etopathogenesis, suggesting that in the development of breast cancer exists a disorder of immune regulation.

P195
IMMUNOGENETIC CORRELATES OF HIV IN THE NORTH INDIAN POPULATION

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Genomic architecture of specialized cohorts of viremic controllers, elite controllers, exposed uninfected individuals, and rapid and slow progressor individuals indicate population specific genetic correlations of HIV/AIDS vulnerability. We performed candidate gene based studies to explore the genetic predisposition to HIV infection in a North Indian population. Particularly, we analyzed the genes that influence i) HIV cell entry (chemokine co-receptors like CCR5, CCR2 and their ligands like CCL3L1), ii) viral replication (tripartite interaction motif 5 a (TRIM5α), apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like 3G (APOBEC), T cell/ transmembrane, immunoglobulin and mucin (TIM) family proteins, NFKB inhibitor like 1 (NFKBIL1) as well as iii) pro- and anti-inflammatory cytokines. Our major findings are (a) the well known protective variant CCR5 delta 32 is rare (<1%), and CCR5 promoter haplotypes were found associated with susceptibility and development of AIDS, (b) no association of CCR2 64I, CCL3L1 copy numbers and APOBEC3B deletion (29.5 kb) with HIV susceptibility and/or resistance, (c) among the observed seven TIM-1 exon-4 haplotypes, significantly higher CD4 counts were observed in D3-A + ve HIV patients, (d) TRIM5 α-exon 2 variant 43Tyr-allele and haplotypes carrying this allele were associated with susceptibility and development of AIDS, (b) no association of CCR2 64I, CCL3L1 copy numbers and APOBEC3B deletion (29.5 kb) with HIV susceptibility and/or resistance, (c) among the observed seven TIM-1 exon-4 haplotypes, significantly higher CD4 counts were observed in D3-A + ve HIV patients, (d) TRIM5 α-exon 2 variant 43Tyr-allele and haplotypes carrying this allele were associated with resistance to HIV infection, e) low expressing allele *01 of NFKB IL1 gene and APOBEC3(H) haplotypes carrying rs139292 (N15del) and rs139297 (G105R) were associated with HIV susceptibility and (f) significantly higher allelic frequencies of IL-1α -889 T and IL-4 -1098 T were observed in HIV patients, while IL-1α -889 CC, IL-4 -1098 GG and IL-6 nt565 AA genotypes were observed significantly lower as compared healthy uninfected controls. The study represents distribution of immunogenetic variants and their influence on HIV/AIDS outcome in a North Indian population.

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INHIBITORY LEUKOCYTE IMMUNOGLOBULIN-LIKE RECEPTORS LILRB1 2, 3 AND 4 ON MYELOMONOCYTIC CELLS AND LYMPHOCYTES OF PATIENTS WITH BEHÇET’S DISEASE

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Behçet’s disease (BD), with a strong genetic risk association to HLA-B*51, represents a chronic multi-system vasculitis, in which increased chemotaxis and hypersensitivity of neutrophils could play an important pathogenetic role. The aim of this study was to investigate expression of inhibitory leucocyte Ig-like receptors LILRB1,2,3,4 which could down-modulate innate and adaptive immune responses by binding to classical and non-classical HLA-class I (LILRB1, B2), matrix proteins (LILRB3) and still unknown ligands (LILRB4) in BD patients and healthy controls. Expression of LILRB1-4 were investigated on myelomonocytic cells in comparison to lymphocytes in 21 BD patients and 21 healthy controls by indirect immunofluorescence labeling with specific monoclonal antibodies and FACS after specific density gradient separation. For data, statistical analysis using Flow Jo 7.2 software and graphical display of the results was performed using GraphPad Prism v5.0. Our results showed that LILRB1 expression was significantly reduced in patients (mean of LILRB1+ granulocytes: 17.3% in patients vs. 25.7% in controls p = 0.0244) and mean LILRB1+ lymphocytes: 15.6% in patients vs. 27.4% in controls; p = 0.0013). BD patients were also found to have significantly reduced expression of LILRB3 particularly on granulocytes (mean of LILRB3+ granulocytes: 12.1% in patients vs. 25.9% in controls p < 0.0001), but also on monocytes (mean of LILRB3+ monocytes: 13.3% in patients vs. 21.7% in controls p = 0.0134) LILRB4 was also shown to be significantly reduced on granulocytes of BD patients (mean of LILRB4+ granulocytes: 16.8% in patients vs. 26.2% in controls p = 0.0123). LILRB2 showed only a slight, but not significant weaker expression on myelomonocytic cells in BD patients. The analysis indicates that down-regulation of the expression of LILRB1, 3, 4 particularly on myelomonocytic could be involved in reduced inhibitory activity of specific innate immune cells in BD and thus participate in its pathogenesis.
SHORT TANDEM REPEATS IN DISPUTED PARENTAGE ANALYSIS: A CURRENT APPROACH

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Short Tandem Repeats (STRs) and Humsan Leukocyte Antigens (HLA) constitute genetic systems widely used in disputed parentage analysis. The aim of this study was to evaluate the HLA polymorphism contribution in parentage cases investigation. A total of 489 DNA samples (147 trio/24 duo parentage cases) were analyzed. Genomic DNA was isolated from whole blood or buccal swabs. Analysis was performed using 16 STR loci typing by DNA sequencing and HLA typing (LR and HR) by PCR-SSP/SSOP. Power of Exclusion (PE), Random Man Not Excluded (RMNE), Combined Parentage Index (CPI), and Probability of Parentage (W) values were calculated using allele frequency of Caucasoid (STRs) and Greek (HLA) population databases. Out of 171 cases, 42 (30 trios/12 duos) were excluded by HLA & STRs and 128 (116 trios/12 duos) were not excluded by both approaches. In one case, where the two alleged fathers were relatives, there was exclusion by STRs, but not by HLA alleles. The low W rates (0.9870-0.9999) using HLA typing were increased up to 0.999999 using STRs. The CPI was ranged from 15.173:1 (duo) to 6.76x1013:1 (trio) with statistically significant difference between trios and duo, RMNE (from 1.46x10-4 to 8.83x10-12), while PE was estimated up to 0.999999 using STRs. Additionally, STRs mutations were observed in seven cases: SE33, D10S1248, vWA, D12S391(2), D2S1338, D21S11 and 1 Null allele (SE33), in equal number of parentage disputes cases (8/129, 6.20%), (7 trios/1 duo) without excluding relation by blood. In mutation cases (ratio of paternal versus maternal 7:1) the low W as determined by STRs typing (0.9972-0.9999) was increased up to 0.999999 using both systems. In conclusion, STR-genotyping is the current approach used in parentage or kinship cases. However, the combination of two systems (HLA & STRs) diminishes the possibility of false exclusion due to STRs mutations, and minimizes the risk of wrong inclusion due to the absence of mother’s genotype.

REPORT OF A FALSE NEGATIVE AMPLIFICATION SHOWN TO BE A NEW NULL ALLELE VARIANT

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This case study highlights the complementary strength of different molecular techniques used in the HLA field. Three different techniques and cooperation between three different labs allowed this novel null allele to be characterized. A complaint was received by Olerup SSP regarding a false negative reaction for A*01:04 N in one of Olerup SSP’s HLA typing kits. The customer tested the sample as A*01:04 N using Sanger sequencing. A new SSP primer targeting A*01:04 N was designed for confirmatory and investigational purposes. This primer failed to amplify the sample, but amplified another allele, A*24:11 N, identical to A*01:04 N at the target motif. Primer functionality was then confirmed using an A*01:04 N reference sample. As it still was not possible to achieve a positive amplification with the customer’s sample, it was sequenced again by another lab. The two sequencing attempts indicated a C insertion in the c.621-627 poly C region and an A629G substitution in line with an A*01:04 N / A*33:01:01:01 result. However, the SSP results from Olerup did not amplify the customer’s questionable sample but amplified other A*01:04 N samples and alleles with identical motifs. A new primer was designed to amplify a theoretical new allele with a C insertion at position c.627_628insC and a A629G substitution. This new primer amplified the customer’s sample but not A*01:04 N and identical A*24:11 N samples, supporting the theory of a new allele. This was confirmed by Pacific Biosciences’ SMRT Sequencing. The sample was shown to be A*01:01:01:01 and A*33:01:01:NEW, with a c.627_628insC insertion in codon 186, exon 4. This frameshift creates a premature stop codon 196, and it was determined to be a novel null allele variant. The novel A*33 null allele has been reported to EMBL with accession number LT707076.
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**HOLOTYPE HLA™ AUTOMATED ON TBG DX-A™ AND SEQUENCED USING MINISEQ**

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Next Generation Sequencing (NGS) is a powerful new method for HLA genotyping. Currently, the rate of NGS adoption by HLA laboratories is rapidly increasing driven by the accuracy, resolution, reproducibility and high-throughput nature of the technology. The elimination of human and random error is a desirable attribute of any diagnostic test and the implementation of a liquid handling system can significantly reduce both sources of error in an NGS workflow. Holotype HLA is a commercially available HLA genotyping product developed in a clinical lab with clinical routine in mind. With Holotype HLA one can easily scale up to 96 samples at 11 loci on a single MiSeq run. Due to its flexibility, it is compatible with multiple liquid handling systems, such as the TBG DX-A, which is a small footprint robot capable of reducing the hands-on time for technicians for both pre- and post-PCR steps in the Holotype workflow. Together, Holotype HLA and DX-A provides an ideal solution for labs who wish to fully automate their HLA genotyping workflow, reducing variation and human error, thus increasing the specificity, sensitivity and accuracy of the genotyping. The DX-A reduces the total hands-on time of the Holotype workflow to 40 minutes. An Illumina’s MiniSeq system was used to test a set of 192 samples. In this study, the PCR setup, amplicon quantitation and normalisation, and library preparation were automated on the DX-A for HLA-A, −B, −C (class I) and HLA-DRB1, −DQA1, −DQB1, DPB1 (class II) loci for each sample. In each setup, 24 samples were prepared on the robot, then four runs of 24 samples were collected together for each sequencing run on the MiniSeq. Two MiniSeq runs have been performed to sequence 96 samples for 7 loci per run. The MiniSeq results were compared to known genotyping calls previously determined from Sanger-based sequencing data. All MiniSeq data was analyzed using Omixon HLA Twin. Here we present the results of our validation of Holotype HLA automated on DX-A and sequenced on the MiniSeq system - an accurate, reproducible and high-throughput system for HLA typing in less than 48 hours from gDNA to genotype.

**P200**

**CALRETICULIN MUTATIONS IN BULGARIAN MPN PATIENTS – FREQUENCY AND FUNCTIONAL IMPLICATION**

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Somatic mutations in JAK2, MPL and CALR are recurrently identified in most cases of Philadelphia chromosome negative myeloproliferative neoplasms (MPNs). Reports on CALR mutations in south-eastern populations are relatively limited. We applied four molecular genetic methods for identification of CALR exon 9 mutations, including high resolution melt (HRM) analysis, Sanger sequencing, semi-conductor target genes sequencing and whole exome sequencing. A total of 78 patients with myeloid malignancies were included in the study. We identified 14 CALR exon 9 mutated cases out of 78 studied patients with myeloid malignancies. All mutated patients were diagnosed with MPN being either PMF (n = 7) or ET (n = 7). Nine cases had type 1 mutations and five cases had type 2 mutations. CALR exon 9, MPL exon 10 and JAK2 p. V617F were mutually exclusive. There were no statistically significant differences in the hematological parameters between the cases with CALR and JAK2 or MPL mutations. Notably, all four techniques were fully concordant in the detection of CALR mutations and in this study we demonstrated their utility. All mutations observed in exon 9 lead to a shift in the open reading frame and expression of peptides with a novel mutant C terminus. We applied further a straightforward bioinformatic approach (PONDR-FIT, RONN and peptide charge calculator) to address the mechanisms through which CALR mutations promote neoplastic transformation. We observed structural and electrochemical properties of mutated C termini of CALR probably leading to the loss of Ca2+ binding activity and therefore contributing significantly to the pathogenetic mechanisms of these mutations. However, as CALR is a protein with versatile cellular functions, this might be just one of several mechanisms. This will hopefully lead to innovative therapies for this subset of MPN patients.

**P201**

**HAPLOTYPE OF HLA-B*13:01/HLA-B*15:02 AND DAPSONE-INDUCED DRUG HYPERSENSITIVITY SYNDROME IN THAI**

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Dapsone is the 5th most common cause of the drug reaction of eosinophilia and systemic symptoms (DRESS) and the 20th most common cause of drug-induced Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) in Thailand. To investigate the association of HLA genotypes with dapsone-induced hypersensitivity syndrome (DHS) in Thai non-leprosy patients, we prospectively enrolled 35 subjects (13 DHS and 22 dapsone tolerant controls) from the Thai Severe Cutaneous Adverse Drug Reaction (THAI-SCAR) project. In addition, data for a general Thai population was obtained from 986 subjects undergoing HLA-B genotyping through the Laboratory for Pharmacogenomics, Somdech Phra Debaratana Medical Center (SDMC), Ramathibodi Hospital. The HLA-B genotypes were determined by the reverse polymerase chain reaction sequence-specific oligonucleotides probe (PCR-SSOs). The HLA-B*13:01 allele was significantly associated with DHS sufferers compared to tolerant controls with an odds ratio of (OR) 33.33 (95% CI 4.77-232.79, p-value = 0.0001) in patients and OR = 21.19 (95% CI 5.76-78.00, p-value = 0.0001) in the Thai population. In addition, HLA-B*15:02 was significantly associated with dapsone-induced SCARs when compared with dapsone-tolerant controls (OR = 13.13; 95% CI 1.32-130.43, p-value = 0.0185) and in the general Thai population (OR = 3.30; 95% CI 1.07 - 10.22, p-value = 0.0450). The haplotype of HLA-B*13:01/HLA-B*15:02 were only found in DHS (n = 3/13, 23.08 %). There were significantly associated with DHS by OR 73.65, p-value = 0.0001. This study demonstrated the association between HLA-B*13:01/HLA-B*15:02 and DHS in Thai non-leprosy patients. Moreover these results suggest this haplotype screening may be a useful genetic marker for prevention of DHS in Thai population.

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IDENTIFICATION OF A NOVEL HLA-DRB3 ALLELE
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We describe the identification of the new HLA-DRB3*02:61Q allele during routine confirmatory human leukocyte antigen (HLA) typing of a volunteer bone marrow donor. Initial typing of HLA-DRB3 locus by SSP (Olerup SSP AB, Sweden) showed an unusual reaction pattern detecting the rare alleles DRB3*02:37/51/52 by analysis in SCORE Standard Version (Helmberg, Austria) with IPD-IMGT/HLA Database Release 3.25.0. To verify this result sequence based typing of DRB3 exon 2 was performed using Protrans S1 HLA DRB3 kit (Protrans GmbH, Germany) following manufacturer’s protocol. Subsequent allele assignment with SBTengine (Genome Diagnostics B.V, Utrecht, Netherlands) identified the new allele. Compared to the common DRB3*02:02:01 allele, the new sequence results from a continuous deletion of 3 nucleotides in exon 2 between nucleotide positions 187 to 196. The nucleotide stretch from 188 to 195 of DRB3*02:02:01 represents an incomplete triplet repeat of AGG (AGG AGG AG), thus any deletion of a triplet within this section delivers an identical sequence. The consequence of this deleted triplet is the removal of one glutamic acid in the protein sequence. According to these facts expression of the allele on the cell surface is questionable. The sequence was submitted to GenBank (accession number BankIt1966791 Seq1 KY099331) and to the IPD-IMGT/HLA Database (accession number HWS10027219). The new allele was officially named DRB3*02:61Q by the World Health Organization (WHO) Nomenclature Committee in December 2016.

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FREQUENCY OF HLA-B*57:01 ALLELE IN HIV-INFECTED PATIENTS AND SCREENING FOR ABACAVIR THERAPY
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Abacavir (ABC) is an anti-retroviral drug recommended for Human Immunodeficiency Virus (HIV) infected individuals. During ABC therapy hypersensitivity reactions (HSR) can occur approximately 4-9% of the patients. The development of HSR to ABC is strongly associated with the presence of HLA-B*57:01. Its frequency varies in different ethnic populations at about 5% in caucasian populations, while other populations, especially Africans, have a lower allele frequencies. This study was designed to establish HLA-B*57:01 frequency in our population of HIV infected patients to establish the effectiveness of prospective HLA-B*57:01 screening. A total of 212 HIV-infected patients, from January 2013 to July 2016, were admitted to the prospective HLA-B*57:01 screening for the prevention of ABC-correlated HSR. 210 healthy blood donors were used as a control group. Patients mean age was 40 years (range 14–74); 67 females (32%) and 145 males (68%), 53% (113 patients) were of Italian origin. Control group mean age was 48, (range 21–73). 111 females (53%) and 99 males (47%). HLA-B low resolution genotyping was performed using SSO-PCR (HistoSpot, BAG) and/or SSP-PCR (One Lambda). In HLA-B*57 positive samples we searched for the presence of HLA-B*57:01 allele using high-resolution HR) technique, such as SSO-PCR (HistoSpot 4D, BAG) and/or SSP-PCR (Olerup). Among 212 HIV-infected patients, 15 (7%) were carriers of HLA-B*57 allele; 5 of Italian origin and 10 of
African origin. HR typing allowed us to identify HLA-B*57:01 allele among 5 Italian patients, but not in any African patients. HLA-B*57:01 allele frequency was 4.42% in Italian HIV infected patients and 5.24% in the control population. HLA-B*57:01 allele frequency in HIV-infected local patients was similar in Italian population and controls (about 5%), similar to caucasians. Since the clinical importance of HSR to ABC treatment in HIV infected patients and the frequency of HLA-B*57:01 carriers reported in this study, we suggest the preventive use of genetic screening in Italian population before ABC treatment.

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CYTOKINES AND SOLUBLE HUMAN LEUKOCYTE ANTIGEN G MARROW StromA LEVELS AND Survival in CHILDHOOD T-CELL ACUTE LYMPHOBLASTIC LEukemia

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Abstract

Leukemic cells can induce defective expression of soluble factors and can change marrow cytokine profile, leading to aberrant cell signaling, cell fixation and cell proliferation in bone marrow. T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive disorder which accounts for 15% of pediatric ALL. To evaluate the contribution of immunological factors on T-ALL survival, we measured Th1, Th2, Th17 cytokines and soluble human leukocyte antigen G (sHLA-G) levels in bone marrow from 32 Brazilian children at diagnosis (D0), after induction (D19) and after consolidation (D49) of the chemotherapy phase. Data analyses were performed using the Wilcoxon and Friedman tests. Correlations were evaluated by the Pearson coefficient and reciprocal influences of the analyzed factors were determined by multivariate analysis using Pearson linear regression. Survival analyses were performed by the Kaplan-Meier method using log-rank test. TNF, IL-10 and IL-6 marrow levels were increased at diagnosis compared to the D19 and D49. IL-10 marrow levels < 4.57 pg/mL at diagnosis were associated with increased survival rates. In this group, a positive correlation was observed between IL-2 and IL-17 levels. Increased survival rate was also associated with IFN-γ marrow levels < 1.17 pg/mL at consolidation phase. In this group, we observed a positive correlation between IL-4 and IL-2 as well IL-4 and IL-17 levels. In contrast, a worse survival rate was associated with IL-2, IL-4 and IL-10 levels imbalance. In addition, increased SHLA-G levels at diagnosis were associated with increased leukocyte count, a well-known factor for poor prognosis.

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A SIMPLE AND FAST METHOD FOR ENRICHMENT OF LYMPHOCYTE SUBSETS FOR COMPLEMENT-DEPENDENT CYTOTOXICITY ASSAYS

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Abstract

Serological cross-match analysis such as complement-dependent cytotoxicity (CDC) assay is routinely done before solid organ transplantation to detect donor-specific antibodies that may lead to graft rejection or dysfunction. This analysis is based on isolated donor lymphocyte subpopulations and recipient serum. Enrichment of cells for CDC can be exceedingly time consuming or result in co-enrichment of non-target or dead cells. We aimed to develop a fast and convenient method for enrichment of donor lymphocyte subsets directly from whole blood or spleen cell suspensions. Using MACSxpress® technology, untouched human leukocyte subsets can be isolated within only 20 minutes from up to 30 mL of anticoagulated whole blood. For the enrichment of B, T cells, or a combination of both cell types, anti-coagulated whole blood or cell suspensions obtained from rinsed donor spleens were incubated with the respective bead cocktails for 5 min at room temperature. Then the open tube was placed in the magnetic field of a MACSxpress Separator for 15 min. With the tube inside the magnetic field, the supernatant containing the enriched target cells was collected and transferred into a new tube. Magnetically labeled non-target cells as well as aggregated erythrocytes were retained in the tube. Purity and viability of magnetically enriched cell populations were determined by flow cytometric analysis and functionality shown in CDC assays. MACSxpress-enriched lymphocyte subsets (B/T/BT cells) from whole blood had average purities of 94/96/98 % with yields of 70/54/40 % (n = 17/19/21) respectively. Viability analysis of enriched fractions showed that the frequency of dead cells was below 5% for all samples. Cell enrichment from spleen single cell suspensions resulted in purities of 97/96/98 % and recoveries of 27/32/29 % (n = 5) (B/T/BT cells) respectively. Viability of the enriched fractions varied due to the age of the samples but was never below 90%. CDC analysis of positive and negative control sera with MACSxpress enriched cells showed no influence of fluorescent readout by the enrichment process. Using MACSxpress technology, lymphocytes for crossmatch analysis can be enriched within 20 minutes from whole blood or spleen cell suspensions without the need of expensive lab equipment or preparation of PBMC by density gradient centrifugation.
ASSOCIATIONS BETWEEN OMMEN SYNDROME AND CYSTINURIA: A CASE REPORT
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Ommen syndrome is one type of combined immunodeficiency, characterized with hepatosplenomegaly, lymphadenopathy, recurrent infections and has an autosomal recessive pattern of inheritance. T lymphocyte count can be normal in peripheral blood but their functions are impaired. B lymphocyte count is very low to none. Cystinuria is renal reabsorption defect of dibasic amino acids, inherited autosomal recessive. Because cysteine solubility is lower than other amino acids cysteine stones are formed in kidneys. In the literature, no association was found between Ommen Syndrome and cystine stones hence, our case is very interesting. A five month old girl applied to the Pediatric Immunology Department of Erciyes University Children Hospital with skin eruption. There was no family history for immune deficiency and no consanguineous marriage between mother and father. The patient had one sibling who is healthy. The patient was not treated with BCG or other live vaccines. In her physical examination, we observed exfoliative erythroderma and hepatomegaly. In laboratory examination, leukocyte count 6540/mm3, absolute neutrophil count 2270/mm3, absolute lymphocyte count 1560/mm3, absolute eosinophil count 581 mg/dl, IgG level 171 mg/dl, IgM level 24.5 mg/dl, IgE level 1270 mg/dl were found. T lymphocyte count 1092/mm3, B lymphocyte count 6/mm3, NK count 332/mm3 were found respectively. Blood sample of patient was sent to Erasmus for genetic analysis. The patient had no full-match family donor. Hence, haplotype of bone marrow transplantation from her father was planned. In preparation for bone marrow transplantation, bilateral kidney stones were showed in abdominal CT. Cystinuria was detected in urine and thought to be bilateral cystine stone. Percutaneous nephrolithotomy operation was performed, then the patient was given scholl solution. Stone analysis revealed to be cystine stone. Association between two different diseases inherited autosomal recessive is very interesting. This challenging incident may or may not be coincidental, but alteration of cysteine and tyrosine amino acids in known to occur in Ommen syndrome, perhaps suggesting a cause of the cystine stones.

Sarcoidosis is a multi-organ inflammatory disorder with heritability estimates up to 66%. Previous studies have shown the MHC region to be associated with sarcoidosis, suggesting a functional role for antigen presenting molecules and immune mediators in the disease pathogenesis. The aim of this study was to investigate genetic variance in five candidate genes: LTA, TNF, AGER, BTN2L, and HLA-DRA within the MHC Classes III and II with tag-SNP genotyping approach. We also addressed the question whether the associations were due to the strong linkage disequilibrium (LD) with HLA-DRB1. Here, we present results from a joint analysis of four study populations (Finnish, Swedish, Dutch and Czech). A total of 805 sarcoidosis patients and 870 controls were included in the study. Patients were further subdivided based on the disease activity and the presence of Löfgren’s syndrome. Seven SNPs were associated with non-Löfgren sarcoidosis (NL; the strongest association rs3177928, P = 0.012) and eight association rs3129843, P = 3.44E-12, OR = 3.4) when compared with controls. Five SNPs associated with sarcoidosis disease course (the strongest association with rs3177928, P = 0.003, OR = 1.9). The high LD between SNPs and HLA-DRB1 controlled were included in the study. When the SNPs and HLA-DRB1 alleles were analyzed together, independent association was observed for four SNPs in the HLA-DRA/BTN2L region: rs3135365 (NL; P = 0.015), rs3177928 (NL; P < 0.001), rs6937545 (LS; P = 0.012) and rs5007259 (disease activity; P = 0.002). These SNPs act as eQTL for HLA-DRB1 and/or HLA-DRB5. In conclusion, we found novel SNPs in the BTN2L and HLA-DRA regions associating with sarcoidosis. This multi-population study demonstrates that at least a part of these associations are HLA-DRB1 independent (e.g., not due to LD), and shared across ancestral origins. The variants that were independent of HLA-DRB1 associations, acted as eQTL for HLA-DRB1 and/or -DRB5, suggesting a role in regulating gene expression.
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THE DETECTION OF HLA-B*27:12 IN UK NEQAS FOR H&I’S HLA-B27 TESTING SCHEME

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HLA typing is performed as an aid to disease diagnosis and susceptibility, with the most common test being for ‘HLA-B27’ due to its strong association with the spondyloarthropathies. UK NEQAS for H&I’s Scheme 1B assesses participants’ ability to correctly determine B27/B*27:08/B*27 status as ‘positive’ or ‘negative’. The scheme’s performance assessment is based on 75% or more of participants’ being in agreement. In 2016, 10 selected blood samples were distributed to participants. Unusually, sample 1B09/16 did not reach the 75% consensus level and, therefore, was not assessed. Of the 115 laboratories that reported a result for 1B09/16, 72 (62.6%) reported the sample as ‘B27 positive’ (14 assigning HLA-B*27:12) and 43 (37.4%) as ‘B27 negative’. A further 7 laboratories reported the sample as ‘equivocal’. Sample 1B09/16 was subsequently confirmed, by SBT, as B*27:12. This allele is similar to B*27:08, as both allele products possess the SLRNLRG Bw6 motif at residues 77–83 rather than the usual HLA-B27’s Bw4 motif. The B*27:12 specificity generally gives poor variable positive, or negative reactions, with HLA-B27 alloantisera and monoclonal antibodies. 40 laboratories tested the sample by flow cytometry (n = 34) or cytotoxicity (n = 6) with 32 (80%) of these reporting sample 1B09/16 as ‘B27 negative’ and 5 (12.5%) as ‘equivocal’. Only 3/34 laboratories, using flow cytometry only, reported the sample as ‘B27 positive’. These findings clearly reflect the poor reactivity of the B*27:12 product with B27 antibodies. Disappointingly, of the 82 laboratories using a molecular method, 11 (13.4%) reported ‘B*27 negative’. This is the first time a Scheme 1B sample has been unassessed due to the 75% consensus level not being met. Importantly, this exercise has clearly shown that is essential to be aware of an HLA-B27 assay’s ability to detect different HLA-B*27 alleles/specifications, particularly when testing patients from diverse ethnic backgrounds.

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PRESENCE OF BW4 KIR LIGANDS AS A PREDICTIVE FACTOR OF A ‘DIFFICULT’ RESPONSE TO ETANERCEPT IN MODERATE-TO-SEVERE PSORIASIC PATIENTS

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Psoriasis is an immune-mediated dermatosis with a multifactorial etiology. In the last few years, the therapeutic options for moderate-to-severe psoriasis have been broadened by the introduction of biological drugs. There are some immunogenetic factors, such as the polymorphism of KIR receptors and of their natural ligands, which could influence both the clinical presentation and the response to therapy. The aim of this study was to determine the correlation between HLA-A and HLA-B KIR ligands (Bw4 Ile80 or Bw4 Thr80) and the response to biological therapy in patients with moderate-to-severe psoriasis. HLA-A and B polymorphisms have been determined through low resolution PCR-SSO (Luminex LabType) in 68 psoriatic patients (54 males) on biological therapies (Adalimumab, Etanercept, Infliximab and Ustekinumab) for at least 6 months. The patients have been analyzed as a whole and, subsequently, they were divided in two subgroups: patients that showed a good/excellent response after a single biological drug (responders, n = 39) and patients who needed two or more drugs to reach the same clinical result (non-responders, n = 29). At first instance, non-responders patients showed a higher number of at least one ligand HLA-A Bw4 Ile80. In particular, we report findings relative to 52 patients treated with Etanercept, 32 responders and 20 non-responders. HLA-A Bw4 Ile80 ligand resulted significantly over-represented among the non-responders to Etanercept (45% compared to the 18.75% in the responders, p = 0.04). The same trend was found for the HLA-B Bw4 Ile80 ligand (70% vs. 43.75%; p = 0.06). These results show that the presence of HLA-A Bw4 Ile80 ligands, which have a higher affinity for KIR3DL1, is correlated to a poor response to biological therapies, and in particular to Etanercept. Further research is needed to confirm preliminary results of the present study and of some others that have already shown how KIR genotypes and their ligands can influence the response to anti-TNF-alfa agents in other pathologies.

P210

UK NEQAS FOR H&I’S EDUCATIONAL SCHEME FOR COMBINED RENAL HLA TYPING, ANTIBODY DETECTION/SPECIFICATION AND CROSSMATCHING

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UK NEQAS for H&I’s schemes aim to reflect existing clinical practice and laboratories often interpret results from multiple assays to make clinical decisions. Consequently, an exercise was trialed comprising HLA typing, HLA antibody detection/specification and crossmatching together with their
prothrombin G20210A variant, methylenetetrahydrofolate reductase (MTHFR) and plasminogen activator inhibitor (PAI) mutations were investigated. Mean ages of patients were 151.90 ± 48.21 months old and none had parental consanguinity. Nine patients (90%) had family-history. The mean C4 level was 4.71 ± 1.62 mg/dl, mean value of C1-INH level was 50.10 ± 19.22 mg/L. Eight patients (80%) had recurrent attack (range: per 2 weeks-2 months). None of them received prophylactic treatment. One patient (10%) was heterozygous for a F V G1691A mutation, another one was also heterozygous for a protrombin G20210A mutation. The heterozygous MTHFR mutation were identified in seven patients (70%) and homozygous MTHFR mutation were found in two patients (20%). Furthermore, four patients (40%) were heterozygous and one patient (10%) was homozygous for a plasminogen activator inhibitor (PAI) mutation.

C1-INH, inhibits circulating kallikrein, activated FXI and F XII and tissue plasminogen activator. If C1-INH is deficient, dermal vascular thrombosis and systemic coagulation occur. A case of HAE who had heterozygous Factor V leiden mutation and purpura fulminans was reported in the literature. In our study, there was no clinical evidence supporting thrombosis. Our patient with a homozygous PAI mutation had an attack more frequently. As a conclusion: In HAE, it’s important to be known protrombotic risk factors to estimate both frequency of attack and risk of thrombosis.

P211
INHERITED PROTHROMBOTIC RISK FACTORS IN TURKISH CHILDREN WITH HEREDITARY ANJIOEDEMA. SINGLE CENTER EXPERIENCE
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Hereditary angioedema (HAE) is a rare, inherited autosomal dominant disease. If plasma C1 inhibitor (C1-INH) is deficient, complement, quinine-bradykinin, coagulation and fibrinolytic systems are unregulated and angioedema develops, leading to thrombosis. Treatment with C1-INH concentrate, danazol and anti-fibrinolytic drugs may also stimulate thrombosis. Therefore, protrombotic risk factors are important in patients with HAE. This study was planned to investigate protrombotic risk factors in patients with HAE. Ten patients who were followed up at the Department of Pediatric Immunology and Allergy of the Erciyes University were included to the study. The type and frequency of attacks, use of prophylaxy and family-history of HAE were questioned. Factor V G1691A, prothrombin G20210A variant, methylenetetrahydrofolate reductase (MTHFR) and plasminogen activator inhibitor (PAI) mutations were investigated. Mean ages of patients were 151.90 ± 48.21 months old and none had parental consanguinity. Nine patients (90%) had family-history. The mean C4 level was 4.71 ± 1.62 mg/dl, mean value of C1-INH level was 50.10 ± 19.22 mg/L. Eight patients (80%) had recurrent attack (range: per 2 weeks-2 months). None of them received prophylactic treatment. One patient (10%) was heterozygous for a F V G1691A mutation, another one was also heterozygous for a protrombin G20210A mutation. The heterozygous MTHFR mutation were identified in seven patients (70%) and homozygous MTHFR mutation were found in two patients (20%). Furthermore, four patients (40%) were heterozygous and one patient (10%) was homozygous for a plasminogen activator inhibitor (PAI) mutation.

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P212
NEW SCHEME FOR CROSSMATCHING IN THE BALKAN EXTERNAL PROFICIENCY TESTING PROGRAM
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The Balkan external proficiency testing (BEPT) program started in 2004 and during the recent years has evolved by adding new schemes to fulfill the demands of the accreditation process. This program provided more accessible participation in different EPT categories for the laboratories from EFI region 8 and helped their successful accreditation. Here we report the results from the new BEPT scheme run in 2016 on donor/recipient crossmatching (XM) by lymphocytotoxicity (CDC) and flow cytometry (FC). Two blood samples and ten sera were sent, giving a total of 20 cell/sera combinations. The scheme organization was logistically challenging: the two types of biological materials (cells and sera) required for crossmatching were distributed by two different BEPT providers. Twenty six laboratories (CDCXM – 12; FCXM – 4; CDCXM + FCXM – 10) from 7 countries participated in this EPT round. The result for each cell/serum
combination was determined by 75% consensus and assessed separately for PBL/T and B cells. Satisfactory performance was 85% of the total number of results in agreement with the consensus findings. The majority of participants achieved a satisfactory performance. Seven labs reported “unacceptable” results for CDCXM (one for PBL/T cells, five for B cells, and two for both T and B cells XM). There were three participants with unsatisfactory performance for T cells and only one – for B cells FCXM. Most of the incorrect assignments were due to reported positive for consensus negative combinations. It could be suggested that different methodological approaches, scores and cut-off values used by the participating laboratories may have contributed to discrepant results and/or non-assessed combinations. In summary, the overall results in this new BEPT scheme are encouraging but further improvement is needed.

P213
REPORT FROM THE HLA-CELIAC DISEASE WORKSHOP (STRATEGIES IN TYPING AND REPORTS) ORGANIZED BY THE SPANISH SOCIETY OF IMMUNOLOGY
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Different strategies are used in commercial kits to perform HLA typing in patients with celiac disease symptoms (complete HLA-DQ typing or only alleles associated with the disease). Also, different reports are used to communicate the typing results. The Spanish Society of Immunology sent a survey about the typing and report strategies to 35 HLA laboratories in Spain, and 21 were completed. Regarding the typing strategy, 20 laboratories report that besides DQB+ they also perform DQA1 typing, while 14 laboratories also perform DRB1 typing. Regarding the kind of reports, 20 laboratories report complete typing. Most of the laboratories do not report risk graded by haplotypes. Half of the laboratories add comments when the patient does not carry any risk haplotype. Regarding the different haplotypes, there is no consensus among the participating laboratories about the risks of DRB1*07-DQB1*02:02-DQA1*02:01 (homozygous or heterozygous), and DRB1*11/12-DQB1*03:01-DQA1*05:05 haplotypes. In a second phase, another survey was sent to a series of 14 clinicians to find out when HLA typing was requested, and their preferences about the results report. In these questionnaires we observed different strategies between paediatricians (requiring HLA in every patient with celiac disease suspicion) and gastroenterologists (only in doubtful patients or for confirmation). 78% of clinicians consider that HLA typing is useful to rule out the disease, while 45% consider that is also useful to confirm it. All of them expressed their wish to have information about the risk inside the report. Also, they reflect doubts about the typing reports, and its complex terminology and claim the importance of reporting the serological equivalence (DQ2-DQ8). In conclusion, there is no consensus among the HLA laboratories on the way they report the HLA results in Celiac Disease. The clinicians claim for an interpretation of the HLA results.

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TET2 GENE MUTATIONS IN CHILDREN SECONDARY ACUTE MYELOID LEUKEMIA
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Secondary acute myeloid leukemia (sAML) is a heterogeneous disease with different genetic abnormalities. sAML rarely develops in child patients. Ten-Eleven Translocation-2 (TET2), which belongs to a family of three genes, is a tumor suppressor gene that plays a role in DNA modification through the oxidation of methyl-cytosine. We presume that TET2 mutations occur in sAML patients and could be a cause of cells' malignant transformation. Amplicon-based next generation sequencing (NGS) of TET2 gene was performed in 10 patients with sAML. Obtained data was compared to the reference NC_000004.12 ref.seq with further analysis. In all samples known SNPs which occur in the normal population were
identified in heterozygote states. Mutations, leading to the replacement of amino acids in the protein or the formation of a stop codon with nucleotide substitution frequency of 13 – 21%, were detected in 3 patients (30%). Since the frequency of replacement is less than 21% and it is not common for normal population, these changes could take place in malignant clones.

In the first patient we identified a missense mutation p. Met1164 > Ile in exon 3 (Polyphen 0.997). In the second one a missense mutation p.Pro576 > Leu (rs201433011) in exon 3 - (Polyphen 0.548) was detected. The third patient had a mutation leading to a termination codon R1216Stop in Chr4:105243621 position. In addition, we found a 3 nucleotide deletion in the coding region resulting in a loss of the protein by one amino acid (CAC - histidine) on chromosome 4 (Chr4:105316098 position) in the last patient. Here we report, that TET2 gene mutations occur in 30% of sAML. Most of identified SNPs were found in exon 3. TET2 gene mutations monitoring can be included in laboratory diagnostics of sAML patients.

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A NOVEL HLA-DRB1*13 ALLELE, DRB1*13:192 WAS REVEALED BY SEQUENCE-BASED HLA TYPING OF A LEUKEMIA PATIENT
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In this paper we report the identification of a novel HLA-DRB1 allele, officially designated HLA-DRB1*13:192. In our laboratory, a leukemia patient and all relatives were typed for HLA-A, -B, -C, -DRB1 and -DQB1 at low resolution using LABType HD class I and II test kits (OneLambda, San Diego, CA, USA). The tests were run on a Lumines 200 FluoroAnalyzer (Tepnel, Manchester, UK) following the manufacturer’s protocol. In the DNA sample of the patient’s father (laboratory code 227977), a new allele was identified after LABType HD class II DRB1 Typing Test analysis using Fusion 2.0 software (OneLambda). The analysis suggested the typing result of HLA-DRB1*13:09 with a *15:05. Sequence based DRB1 typing using allele specific amplification primers disagreed with the SSO result and on further investigation the existence of a novel allele, DRB1*13:192, in combination with a *15:01 was determined. PCR amplification, amplification control, cycle sequencing PCR purification and cycle sequencing reaction were performed as described previously. Sequence analysis using uType Software (Life Technologies, Invitrogen, Darmstadt, Germany) and the web based typing tool of the NCBI revealed a nucleotide substitution at position 286 in the DRB1*13 allele sequence. The allele sequence described here, DRB1*13:192 is most closely related to DRB1*13:09. In DRB1*13:192, there is a C at nucleotide position 286 (codon 96) instead of an A. Isoleucine (ATC), present in DRB1*13:09, is replaced by serine (CTC) in the novel allele. The novel allele found in the father was not found in the leukemia patient. Our finding adds one more member to the HLA-DRB1*13 family.

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A METHOD FOR SELECTING MHC CLASS I-SPECIFIC ANTIBODIES IN RHESUS MACAQUES
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Humans and rhesus macaques share a common ancestor, which is reflected by high levels of similarity for the immune system. Due to this similarity macaques are widely used, for instance as pre-clinical model species in transplantation research and to evaluate human immunodeficiency virus type-1 (HIV-1) vaccine candidates. If untreated, most HIV-1 infected humans develop AIDS. However, some individuals can control the HIV-1 infection without treatment, and turn out to be elite controllers. This phenomenon is strongly associated with the presence of particular major histocompatibility complex (MHC) class I allotypes, such as HLA-B*27:05 and -B*57:01. Elite control is also observed in the rhesus macaque simian immunodeficiency virus (SIV) infection model, and likewise, particular MHC class I allotypes are associated with it, such as Mamu-B*003, -B*008, and -B*017. The pre-clinical transplantation and SIV control studies would benefit greatly from the availability of non-human primate MHC class I allele specific monoclonal antibodies (mAbs). With only one non-human primate MHC class I allotype specific mAb available so far, namely for Mamu-A*01, we tested a series of HLA class I-specific human mAbs for cross reactivity by flow cytometry with macaque MHC class I allotypes, using our large panel (n = 30) of single antigen expressing cell-lines based on K562, transfected with Mamu-A and -B allotype constructs (SAL). Two mAbs, OK4F9 and OK4F10, both recognizing an epitope that is shared by many HLA-A allotypes and is defined by an isoleucine (I) at amino acid position 142, turned out to cross react with Mamu-B*008:01 allotype expressing SAL. This allotype uniquely harbors the I421 substitution (shared epitope). This is the first description of human mAbs that can specifically recognize a Mamu-B allotype. In potential these mAbs are powerful tools for in vitro monitoring of the presence and inhibiting the cell surface expression of Mamu-B*008:01 and related responses.
DETECTION OF HLA AND HNA ANTIBODIES USING THE LABSCREEN MULTI ASSAY AS PART OF A TRALI RISK REDUCTION PROGRAMME

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Transfusion related acute lung injury (TRALI) is a rare condition with significant morbidity and potential mortality associated with the transfusion of plasma rich blood components containing HLA (class I and II) and HNA antibodies. Previously NHSBT, introduced a TRALI risk reduction programme to screen female apheresis donors using a Luminex screening assay for HLA antibodies (LSM12) and the granulocyte immunofluorescence test (GIFT) for HNA antibodies. Recently, a Luminex assay (LSM Multi) enabling simultaneous detection of HLA and HNA antibodies has been introduced. Preliminary studies confirmed that this kit was able to detect all HNA antibody specificities detectable by GIFT but it was necessary to increase, the individual HNA bead cut-offs to avoid false-positive reactions. In 416 samples from female apheresis donors, 240 were HLA/HNA antibody negative, 129 were HLA antibody positive (class I and/or class II) and HNA antibody negative, 37 samples were positive for HNA antibodies but later confirmed negative by GIFT. Nine samples had HNA specificities confirmed by GIFT as follows: HNA-1a (1), –1c (1), –2 (3), –3a (3), –3b (1) with a further sample containing an apparent anti-HNA-5b. The false positive HNA antibody rate was 8.9% and the confirmed HNA antibody positive rate was 2.2%. Most of the 37 false positive reactions were due to spurious HNA-3 antibodies (22). One sample was negative for HLA/HNA antibodies by the LSM Multi but found to contain HNA-3a antibodies using recombinant cell lines. There was no association between the fluorescence values in the LSM Multi and those obtained by GIFT. In conclusion, these results indicate that the LSM Multi-kit has potential to be used as a screening tool for TRALI risk reduction but care is needed when defining the positive cut-off values and the assay should be supported by other techniques to confirm potential HNA antibody specificities.

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UK NATIONAL EXTERNAL QUALITY ASSESSMENT SERVICE FOR HISTOCOMpatibility AND IMMUNOGENetics ‘EDUCATIONAL HLA TYPING SCHEME’ FINDINGS IN 2016

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UK NEQAS for H&I’s ‘Educational HLA Typing Scheme’ provides undisclosed blood / DNA samples, with interesting HLA specificities/alleles, to its ‘HLA Phenotyping’ and/or ‘DNA HLA Typing Scheme’ participants. This scheme is gratis and participation is at the discretion of each laboratory. Findings are not assessed but participants can compare their results with some 40 other laboratories. In 2016 four DNA extracts were provided with an HLA-A, HLA-B or HLA-C allele of interest. Here we present participants’ findings: HLA-A*01:01:65 [identical to A*01:01:01 except for position 576 (C > T), exon 3] - 27 out of 46 labs (58.7%) reported as A*01:01:65. There were 13 reports of A*01, 4 of A*01:01 and 1 of A*01:01:99/102/167. Disturbingly, there was 1 report of A*36. HLA-A*02:24:01 [identical to A*02:01:01:01 except for position 453 (A > C)], corresponds to the annealing site of a primer once commonly used in A*02-amplifying PCR-SSP mixtures - 30 out of 46 labs (65.2%) reported as either A*02:24:01(n = 17) or A*02:24 (n = 13). There were 16 reports of A*02. We distributed an example of A*02:24 in 2012 (Tissue Antigens 2013, 81, 359). Here, 17 out of 30 labs (56.7%) reported A*02:24 (n = 11) or A*02:24:01 (n = 6). 3 labs assigned A*02 allele groups of between 5 and 20 alleles, while 10 labs reported A*02 only. The detection of A*02:24 has improved since 2012, although not significantly so (p > 0.1). HLA-B*40:10:01 (differs from B*40:01:01 by 4 bases in exon 2) - 33 out of 45 labs (73.3%) reported either B*40:10 (n = 26), B*40:10:01 (n = 6), B*40:10:01:02 (n = 1), or B*40:10/10/22 N43 (n = 1). There were 10 reports of B*40, HLA-C*02:22 (differs from C*02:02:02 by 3 bases in exon 3) - 34 out of 44 labs (77.3%) reported as C*02:22. There were 10 reports of C*02. We distributed an example of C*02:22 in 2013 (Tissue Antigens 2015, 85, 366). Here, 22 out of 25 labs (88.0%) assigned C*02:22 and 3 reported C*02. The detection of C*02:22 has declined in the last 3 years, although not significantly so (p > 0.1).

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DETECTION OF HLA ALLELES ASSOCIATED WITH COELIAC DISEASE – THE OVERVIEW AND QUALITY OF TESTING THE CZECH REPUBLIC

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Association of specific HLA alleles with various diseases is a useful tool for differential diagnosis of these diseases. In the case of coeliac disease (CD), HLA genotyping is an integral part of the non-biopsy approach. The testing is done by the detection of specific associated alleles/allelic groups of DQ
loci: DQA1*02, *03, *05, DQB1*02,*03:02. Since 2010 our department organizes external proficiency testing (EPT) program "DETECTION OF HLA ALLELES ASSOCIATED WITH DISEASES" for the detection of CD predisposing alleles. As a part of the program we map the number of tests carried out for individual diagnoses as well as methods used. In the Czech Republic about 3500 HLA genotyping for the diagnosis of coeliac disease is annually performed in almost three dozen of the laboratories. Investigations are carried out in laboratories of molecular genetics and other specializations however only 1 in 4 of the labs performs HLA testing for other purposes and is better aware of its specificity. The correct and exact identification of the predisposing HLA genotypes is essential for its successful implementation. Although the number of successful participants is still increasing, the correct genotyping remains a problem for a significant number of them.

An important issue is the correct distinction between alleles such as DQB1*03:02 and DQB1*03:03. Examination of HLA is crucially important for the non-biopsy diagnosis of coeliac disease. The impact of the test can be further improved by its standardization from the viewpoint of indication as well as performing the test, including clear laboratory interpretation. Coordination of the form of reporting the results and their interpretation in the context of the EPT can contribute to improvement of the informative value of the tests and its clinical clarity.

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HLA-B AND DRB1 ASSOCIATIONS WITH NEVIRAPINE HYPERSENSITIVITY REACTIONS

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Nevirapine (NVP) is a non-nucleoside reverse transcriptase inhibitor widely used in HIV-1 therapy, especially in developing countries and pregnant women. However, its use has been greatly limited by a 6-10% incidence of adverse effects, with two different phenotypes: cutaneous or hepatic manifestations. Several studies have investigated associations between HLA alleles and the risk of NVP-induced hypersensitivity reactions (NVP-HSR). HLA-C*04, HLA-B*35 and HLA-DRB1*01 alleles have been associated with NVP-HSR, but results are inconsistent. To evaluate the possible association between HLA alleles and NVP-HSR in a Gran Canaria island (Spain) population, we have studied HLA-B and HLA-DRB1 frequencies in 161 HIV patients that were previously exposed to NVP. Hypersensitivity reactions were reported in 20 patients (10 with cutaneous and 10 with systemic manifestations). No association with HLA-B alleles was found in our cohort, and only a decreased frequency of HLA-B*15 allele was observed in patients with HSR, but without statistical significance (0% vs. 9.9%, p = ns). This allele has been recently associated with protection against HSR in Caucasian and Asian patients. Study of HLA class II alleles has revealed an increased frequency of HLA-DRB1*01 allele in patients with NVP-HSR (40% vs. 17.9%, p = 0.048, pc = ns). The reduced number of cases does not allow us to associate this allele with hepatic or cutaneous manifestations of HSR. Our results may indicate that some HLA alleles are associated with NVP-HSR as protective or susceptibility factors in our population, but a larger cohort of patients is required to confirm these preliminary observations.

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CLINICAL DECISION MAKING IN H&I LABORATORIES – RESULTS FROM THE UK NEQAS FOR H&I’S INTERPRETATIVE EDUCATIONAL SCHEME

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UK NEQAS for H&I has offered a free interpretive educational scheme since 2013. Three clinical scenarios are distributed yearly covering solid organ/HSC transplantation/platelet transfusion. Each case provides laboratory results and clinical information: they require affirmed clinical decisions/clinical advice. The findings for 2016 scenarios are:

1/2016 (50 labs): Female patient, previous liver transplant needs renal transplant. From provided Luminex SA results (MFIs 0–20251) and HLA types of 4 potential deceased donors, 90.9–98% of labs would not transplant based on a virtual crossmatch (VXM) with 3/4 donors. For donor 4, 50% would transplant this sensitized patient from a VXM due to the absence of HLA DSAs. When provided with CDCXM and FCXM results for 2 potential live donors, 84.0% assigned a contraindication/high risk category for live donor 1 and 42.0% a medium risk for donor 2. 2/2016 (45 labs): Adult AML patient with ‘challenging’ HLA type required HSCT. From provided unrelated donor search results, 95.0% of labs chose the same donor as one of their 3 selections, with 80.0% selecting it as their first choice. From cord blood search results, 75.6% would require a double, not single, cord blood transplant. 55.5% of labs selected the same 2 cord blood units (84.4% selected the same 1 unit). When a DPB1 DSAs was detected, 80.0% of labs specified an increased transplant risk. 3/2016 (23 labs): Post HSCT transplant patient with HLA and HPA-5b antibodies refractory to random donor platelets. From provided HLA class I Luminex SA results (MFIs 0–12679), 95.6% of labs chose the same donor as 1 of 3 selections from HLA and HPA typed donors. From platelet increment data,
after transfusion of several HLA and HPA compatible units, 87.0% (n = 20) of labs would perform additional tests; 17/20 performing ABO antibody titre tests. Scenarios are not formally assessed but allow clinical interpretation/advice to be compared between labs; there was good agreement on many aspects of this in 2016.

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HLA DISEASE ASSOCIATION SCREENING: DEVELOPMENT OF A MULTIPLEXED ASSAY AND SOFTWARE PACKAGE

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HLA genes are the most polymorphic genes in the human genome. The encoded HLA molecules play a pivotal role in generating a target dependent immune response. Several HLA molecules are even associated with specific disease or drug responses. For example; HLA-B27 is associated with Ankylosing Spondylitis, HLA-B*57:01 is associated with abacavir hypersensitivity, and the HLA-DQ2 and HLA-DQ8 haplotypes are associated with Celiac Disease. We have developed a single tube multiplexed amplification assay for fast and cost-effective genotyping of HLA-related diseases based on exon 2 and exon 3 of HLA-B, HLA-DQA1 and HLA-DQB1 genes (NGSgo-HLAlinkX). Amplicons were processed in our NGSgo workflow for Illumina, and the resulting library was sequenced on the Illumina MiSeq. Obtained sequencing data was analyzed in our newly developed software package LINKengine, which reports whether a sample is positive or negative for the specific HLA allele. Furthermore, LINKengine reports the reliability of its results. Here we present the first results using NGSgo-HLAlinkX in combination with LINKengine. The fast and robust amplification assay, in combination with the reliable analysis and clear typing reports, provides a fast and easy method to genotype disease-related HLA genes. As the workflow of NGSgo-HLAlinkX after amplification is identical to the workflow of other NGSgo amplified products, all NGSgo amplicons can be combined in a single sequencing experiment, enabling more efficient use of the capacity of the NGS sequencing equipment.

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ASSOCIATION OF HUMAN LEUKOCYTES ANTIGENS WITH DRUG HYPERSENSITIVITY REACTIONS IN PERSIANS

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Drug hypersensitivity is an immune-mediated adverse reaction to a certain drug. Immune responses are based on the pharmacological-interaction, which involves the direct drug binding to certain HLA molecules. The main cause of drug hypersensitivity is not clear but many of these diseases show familial and ethnic clustering that can be explained by genetic susceptibility. To investigate the association between HLA alleles and drug hypersensitivity reactions (DHR), HLA-A, -B and -DRB1 alleles were determined in 43 southwestern Persian patients with severe DHR by PCR-SSP method and the results were compared to 40 healthy individuals from the same geographical region. Among our patients, 17 (39.5%) were sensitive to beta-lactams, 9 (21%) to non-beta-lactam antimicrobial agents, 14 (32.5%) to anticonvulsants. Three patients (7%) showed DHR to NSAIDs, anesthetics or herbal medicine. HLA-A*02:01 was associated with beta-lactam sensitivity (P = 0.02) and A*26:01 was associated to susceptibility to non-beta-lactam antibiotics (P = 0.019) while unlike to healthy controls, A*31:01 was observed among the sensitive patients to anticonvulsants with a frequency of 14.3% (P = 0.0038). HLA-B*51:01 was observed to be associated with sensitivity to both beta-lactams (P = 0.002) and anticonvulsants (P = 0.0042). HLA-DRB1*04:01 and DRB1*11:01 were associated with sensitivity to non-beta-lactams (P = 0.019 and P = 0.02, respectively) and anticonvulsants (P = 0.0155 and P = 0.0032, respectively). Considering the complex interaction between gene(s) and the environment, simultaneous analysis of other relevant genes like TLRs, PRRs and DAMPs as well as environmental risk factors would be helpful to define the exact mechanism of DHR to a certain drug which in turn will help drug developers to produce safe derivatives and will also help clinicians to a better management of patients with DHR.

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THE ACCURACY OF HLA TYPING TO 2ND FIELD RESOLUTION IN UK NEQAS FOR H&I’S SAMPLES 2013–2016

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UK NEQAS for H&I’s Scheme 4A2 assesses participant’s ability to correctly determine HLA alleles to the 2nd field resolution. As a minimum requirement, participants must resolve all ambiguities resulting from polymorphisms within exon 2 and 3 for HLA class I loci, and exon 2 for class II loci. Ten blood samples are distributed per year and participants can report 2nd field results for any combination of HLA loci. Alleles reported by at least 75% of labs are taken as the consensus HLA type. Results from 2013–2016 were analyzed.
69 labs tested between 5–40 samples, resulting in 23,928 allele assignments to the 2nd field. The most commonly typed loci were HLA-DRB1 (n = 4,080) followed by DQB1 (4,033), HLA-A (3,547), HLA-B (3,682), HLA-C (3,539), DPB1 (2,438), DQA1 (1,299), DRB3/4/5 (1,125) and DPA1 (185). A total of 163/23,928 alleles were incorrect; thus the overall error rate was 0.68%. The highest error rate was for DRB3/4/5 (2.13%, n = 24), followed by HLA-C (1.10%, n = 39), DQB1 (0.69%, n = 28), DRB1 (0.66%, n = 27), DQA1 (0.62%, n = 8), DPB1 (0.57%, n = 14), HLA-A (0.34%, n = 12), HLA-B (0.30%, n = 11); with no errors for DPA1. 61/163 errors involved multiple errors (2–6 incorrect alleles) on the same sample. 58.0% (40/69) of labs reported an incorrect allele during the 4 years; 15 labs had 1 allele error, 25 had multiple incorrect alleles. Many errors (49.1%, n = 80) were due to reports not meeting the minimum typing requirements, i.e. reports of allele strings with alleles differing in exon 2 (class II) and exons 2 and 3 (class I). 59 (36.2%) errors were due to reports with the incorrect 2nd field HLA (e.g. B*07:01, not B*07:02). 14 errors (8.6%) were due to missed alleles and 10 errors (6.1%) were at the 1st field (e.g. C*06:02 not C*07:02).

It is important that laboratories are able to perform accurate HLA typing to the 2nd field level, especially in support of HSC transplantation. The low overall error rate is encouraging, however further work is required to eliminate errors that could impact on patient care.