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Oral presentations

BSHI O-01 ALLOANTIBODIES STIMULATED BY PREGNANCY, TRANSFUSION OR TRANSPLANTATION SHOW SIGNIFICANT QUANTITATIVE AND QUALITATIVE DIFFERENCES

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Introduction: A natural stimulus for HLA antibodies is pregnancy but less naturally transfusion and transplantation are also potent stimulators. Little is known about differences in the nature of the antibody response to these routes of exposure. Antibody level and isotype are likely to be variable characteristics of these responses suggesting different clinical significance associated with immunisation route. Such characterisation of HLA specific antibodies may improve pretransplant risk-assessment and provide further insight into humoral transplant rejection.
Methods: 106 separate HLA antibody specificities from 38 individual patients' sera were associated with potential sensitising event. Sensitisation due to pregnancy or graft loss was assigned if specificity corresponded to partner/donor HLA mismatches and consistent timing of antibody appearance. HLA specific IgG subclasses were identified using Labscreen Singles beads. Data are presented as presence of each isotype, isotype profile and bead MFI value.
Results: IgG1 was the dominant subclass for all sensitisation routes. While IgG2 was the second most prevalent in transfusion and transplant sensitisation (48% & 68%, respectively), pregnancy displayed high incidence of IgG3 (68% vs 40% from transfusion). The IgG1 only profile dominates antibody response to transfusion, while for pregnancy and failed transplant development of all subclasses is dominant. IgG4 is of low frequency in the transfusion sensitised group (26%) compared with pregnancy and transfusion combined (47%, p=0.046). For all subclasses lowest MFI values were seen with transfusion stimulated antibodies. Highest levels were seen in transplant stimulated antibodies for all subclasses except IgG3 where pregnancy gave highest MFI values.
Discussion: This is the first description that mode of sensitisation influences IgG heavy chain class switching and determines the relative level of each HLA specific subclass. These differences are relevant to subsequent transplantation because of the funtional differences of IgG subclasses.

BSHI O-02 CLASS I HLA-SPECIFIC ANTIBODY QUANTIFICATION

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Introduction: Recent advances in anti-HLA antibody detection methodologies has provided the clinical laboratory with a valuable tool to rapidly and accurately monitor the changes in antibody levels through the course of a kidney transplant. This is of particular importance in HLA antibody incompatible transplantation (HLAiTx) where daily monitoring of antibody status is often neccessary and clinical intervention can be heavily influenced by these results. However antibody data is given only as units of detectable fluorescence and no insight into the serum concentration of HLA specific antibody can be inferred. We describe a simple method for estimating serum concentration of HLA specific antibody using standard curves derived from human monoclonal HLA specific antibody.
Methods: HLA epitope specific monoclonal antibodies of IgG isoform were quantified and 'spiked' into HLA antibody negative AB serum and standard curves for single antigen bead binding were constructed in the dynamic range of 0.1-200μg/ml. Patient sera with the same single epitope reactivity as determined by inhibition analysis were then analysed using the concentration curve as reference.
Results: One transplant waiting list patient demonstrated a 142T epitope specific antibody (HLA-A2,28) at a consistent concentration of between 30-40μg/ml between three-monthly samples. The second patient underwent HLAiTX at our centre and had a pre-treatment serum antibody concentration of 12.8μg/ml which rose to a peak of 185μg/ml at day 17 post-transplant during a period of antibody mediated rejection.
Discussion: This pilot study provides a valuable insight into the concentrations at which anti-HLA antibody is found in the general circulation and the dynamic ranges of antibody concentration that can be observed during the early post-transplant period in HLAiTx. Knowledge of circulating HLA-specific antibody levels is of fundamental importance if designing more specific antibody reduction strategies.

BSHI O-03 DIFFERENTIAL EXPRESSON FOLLOWING A HOMOZYGOUS GENE DELETION AND INCREASED SEVERITY OF ACUTE GRAFT V HOST DISEASE

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Introduction: Haemopoietic stem cell transplantation (HSCT) is a central treatment modality in the management of haematological malignancies. Acute Graft vs. host disease (aGvHD) is a major cause of early morbidity post-HSCT, with donor T-cell responses towards recipient minor histocompatibility antigens (mHAs) being a contributing factor. One mHA encoded by the UDP glycosyltransferase 2 family polypeptide B17 (UGT2B17) gene has shown to be immunogenic because of differential expression in donor and recipient cells. This study was designed to assess the effects of a homozygous gene deletion of UGT2B17 on the occurrence and severity of acute aGvHD post-HSCT from a HLA-matched related donor.
Method: 115 patients who had received a HSCT from a related donor were recruited into the study. Genomic DNA was isolated from donor and recipient whole blood using a EZ1 workstation. Donor and recipient HLA types were determined using One Lambda LABType® reverse sequence specific oligonucleotide (rSSO) multiplex technology. The UGT2B17 gene deletion was determined using sequence specific primers, using human growth hormone (HGH) specific primers as a positive control. For all analyses, P values of $\leq$0.05 were considered statistically significant.
Results: There was no significant association between the homozygous deletion of UGT2B17 and occurrence of aGvHD (P=0.674). However, a significant increase in aGvHD severity (grades II - IV) was seen in UGT2B17 recipients expressing the protein with UGT2B17 deficient donors (P=0.036).
Discussion: We observed a significant association between UGT2B17 expressing recipients and UGT2B17 deficient donors with the severity of aGvHD. UGT2B17 encodes a cell surface protein that is highly expressed in the liver, intestines and skin; all of which are major targets of GvHD. This study provides evidence that genomic variations merit further investigation as potential mechanisms of GvHD.

BSHI O-04 SINGLE ANTIGEN BEAD (SAB)(NORMAL AND CLEAN BEADS) MFI THRESHOLD FOR DONOR SPECIFIC ANTIGEN (DSA), COMPARED TO FLOW AND CYTOTOXIC CROSSMATCH RESULT

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Aim: In this laboratory SAB are used at a positive threshold of 1000 MFI to determine unacceptable antigens. Concerned at the number of unacceptable antigens that the patients accumulate, we attempted to derive a more logical threshold by comparing the MFI against donor specific antigens (DSAMFI) with the result of the crossmatch in patients who are being considered for live renal transplantation.
Methods: The highest DSAMFI was taken for each patient.We further examined those patients who had negative crossmatch with positive DSAMFI using clean beads and beads from another vendor.
Results: Results of all crossmatches and DSAMFI with normal beads are shown below. Of the 12 patients crossmatch neg/DSAMFI pos, five had previous transplants and seven were females. Testing by clean beads showed little alteration in DSAMFI. In only one case did the clean bead go below our current threshold of 1000; DSAMFI (HLA-B8) reduced from 3000 to negative. Testing 10/12 crossmatch neg/DSAMFI pos with second vendor products showed that 7 were negative and the other 3 had DSAMFI of 1593,2190,2351. Eight of crossmatch neg/DSAMFI pos patients have been transplanted. One failed immediately (thrombosis,DSAMFI 9756),while the other 7 (DSAMFI 2016-7983) have good graft function 6-16 months out. MFI versus crossmatch result MFI+ve/xmatch+ve (n=9) MFI 4889 to 21339 MFI+ve/xmatch-ve (n=12) MFI 1042 to 18881 MFI -ve/xmatch+ve (n=0) MFI -ve/xmatch-ve (n=95)
Conclusions: We now believe that the MFI threshold considered as positive can be safely increased. This will be considered for each specific patient. We will continue to monitor crossmatch result with DSAMFI to obtain more results,

but currently our limited dataset indicates that an equivalent MFI to crossmatch could be as much as 4,000 for flow and 8,000 for CDC

BSHI O-05 FLOW CYTOMETRY CROSSMATCHING USING PURIFIED LYMPHOCYTES

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Flow cytometry crossmatching (FCXM) requires donor samples with adequate T and B lymphocytes. Contaminating cells may bind HLA antibodies and remove secondary antibody from the assay leading to decreased test sensitivity. Our objective was to establish whether cell preparations could be improved using magnetic cell separation (RoboSep®, STEMCELL Technologies) and to validate their use in FCXM. Peripheral blood lymphocytes (PBL) were isolated using density gradient centrifugation. Some lymphocytes were set aside for FCXM and the remainder underwent RoboSep® Total Lymphocyte Enrichment (TLE). Resulting cells (RSPBL) were compared with standard PBL in FCXM tests using dilutions of the positive control to establish positive thresholds and were also tested with patient sera to compare assay sensitivity. FCXM were performed using splenic lymphocytes and RSPBL to validate the use of RSPBL in deceased donor crossmatching. RoboSep® TLE gave increased purity for the 15 samples tested. Mean values for PBL v RSPBL were: T-cell purity: 41.7% v 82.7% B-cell purity: 6.2% v 14.5% Total lymphocytes: 47.9% v 97.2% 5 FCXM comparing RSPBL with PBL containing a low percentage of T and B lymphocytes demonstrated increased assay sensitivity for RSPBL: mean T-cell positive control 46.6 and 76.4 times negative control values and mean B-cell positive control 8.0 and 15.2 times negative control values for standard and RSPBL respectively. Negative FCXM gave equivalent results indicating RSPBL did not give false positive results. Detection of HLA antibodies was improved with RSPBL and the interpretation of the FCXM clearer. 4 FCXM comparing RSPBL with SPL gave equivalent results. RSPBL were of increased purity and gave improved FCXM sensitivity compared to standard PBL preparations especially those with low T and B-cell purity. This could be of particular benefit for FCXM using deceased donor PBL prior to organ retrieval.

BSHI O-06 SPECIFIC REMOVAL OF ANTIBODIES TO HLA-DRB1 FROM PATIENT SERA

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Introduction: Strong humoral responses to allogeneic Human Leukocyte Antigens (HLA) preclude organ transplantation, promote hyperacute rejection, and contribute to chronic transplant rejection. Therapies exist to prevent B cells from secreting antibodies, and bulk antibody removal is also an option, but there is no means to specifically deplete antibodies that recognize an allogeneic HLA molecule while leaving humoral immunity largely intact.
Method: Here, we produce milligrams quantities of the soluble class II molecule HLA-DRB1*11:01 in mammalian cells, covalently couple purified DRB1*1101 to a solid support, and generate a column for the purpose of removing anti-DRB1*11:01 antibodies in patient sera.
Results: When patient sera recognizing multiple HLA were passed over the class II matrix, antibodies specific for DRB1*1101 were removed while antibodies for other class II passed through intact. Furthermore, the total amount of Ig in patient sera remained unchanged after passage through the matrix. The bound DRB1*1101 specific antibodies could then be recovered, further characterized, and tested as a DRB1*1101 specific reagent.
Discussion: These data demonstrate the coupling of native class II molecules to an affinity matrix, the select removal of antibodies from a patient sample, and the recovery of antibodies specific for a class II HLA molecule. Specific antibody removal may be plausible as a pre and/or post transplant therapeutic.

BSHI O-07 SPECIFIC REMOVAL OF HLA CLASS I DIRECTED ANTIBODIES FROM HUMAN SERA

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Introduction: The success of antibody-incompatible renal transplantation is heavily reliant upon achieving reduction of donor HLA-specific antibodies (DSA) to manageable levels at time of transplantation. Current methods of antibody reduction: plasma exchange, double filtration plasmapheresis, protein A immunoabsorption, or therapeutic agents such as IVIg or rituximab are non-specific and thus deplete overall humoral immunity. We demonstrate that by using soluble class I HLA molecules produced in mammalian cells it is possible to deplete human sera of anti-HLA specific reactivity.
Method: Soluble phase antibody inhibition was carried out using HLA protein at a concentration of 0.05μg per 1μl sera. HLA protein was also coupled to a solid support generating columns for HLA class I specific antibody removal. Soluble inhibition and column efficacy was evaluated using class I single antigen beads.
Results: Soluble HLA-B7 protein inhibited reactivity of antibody directed against the 163E+166E epitope expressed on HLA-B7, B13, B27, B42, B48, B55, B60, B61, B67, B73, B81 and A*66:02. Inhibition with shared epitope specificity HLA-B13 also showed effective inhibition. Soluble inhibition using HLA-A2 protein did not result in reduction in reactivity due to lack of the relevant epitope. HLA-A2, A24, B57, and Cw2 proteins were then coupled to separate sepharose columns at a concentration of 100μg protein per 200μl matrix, and all were able to deplete human sera of corresponding antibody reactivity in an epitope specific manner. Anti-HLA reactivity could be reduced by up to 80% in a single pass.
Discussion: These data demonstrate for the first time that HLA-specific antibody removal can be achieved using HLA proteins bound to affinity matrix. Specific anti-HLA antibody reduction may be a plausible strategy in antibody-incompatible transplantation both in the pre and post-transplant phase, increasing access to transplantation for highly sensitised patients.

BSHI O-08 VARIATION OF ANTIGEN DENSITY ON HLA SINGLE ANTIGEN BEADS – IMPLICATIONS FOR SENSITISED PATIENTS AWAITING KIDNEY TRANSPLANTATION

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Luminex has revolutionised the identification of HLA antibodies but the clinical relevance of luminex defined HLA antibodies on transplant outcome is not fully elucidated. The aim of this work was to investigate antigen density across luminex single antigen beads. If any difference was found, correction for this variation might improve the consistency of luminex data or help to better predict crossmatch reactivity. This study utilised the WR18 antibody (PE-conjugated anti-HLA-DP/DQ/DR, AbCAM, Cambridge, UK) to correct for HLA class II antigen density using LABScreen Single Antigen Class II kits. Results demonstrated even numbers of DP, DQ and DR beads distributed in each quartile and a 5-fold difference in antigen density across the kit. HLA Class I single antigen beads (LABScreen Single Antigen Class I – Combi) were tested with W6/32 (Cedarlane, Burlington, NC). Results demonstrated that Cw and Bw4 beads have less antigen represented than other bead specificities, with only 6% of Cw beads and 11% of Bw4 beads found in the top 25th percentile. There was also an 8-fold difference in antigen density across the class I kit. A correction value was determined for each bead relative to the 75th percentile bead. Patient samples were subsequently tested, normalised for background fluorescence and the correction factor applied. The cohort was small but permitted some interesting observations. In one case study the results for donor specific antibodies were reduced by a third and luminex data correlated with historic crossmatch results more closely after correction. Class I data also fitted better into Cross Reactive Groups (CREG) and a B37 bead, known to give false positive results was assigned negative after correction. In conclusion these data suggests that correction may provide better resolution of borderline luminex reactions.

BSHI O-09 HIGH RESOLUTION ANALYSIS OF RENAL ALLOGRAFT REJECTION – HLA CLASS II MISMATCH AND ANTIBODIES

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Aim: To analyse in detail the HLA Class II antibody associated with allograft rejection in 94 renal transplant patients.
Method: The HLA mismatch between donor and recipient was assessed using nucleotide sequencing to define the precise HLA-DRB1*,-DRB3*,-DRB4*,-DRB5*,-DQB1* and -DPB1* alleles at the amino-acid level. HLA-DQA1* and -DPA1* were similarly defined using high resolution PCR-SSP. The HLA specific antibodies at the time of graft loss were analysed using single antigen beads and correlated with the HLA Class II epitope mismatch assessed as eplets by the HLA Matchmaker programme (1)
Results: Thirteen of the donor/recipient pairs had no HLA Class II mismatch at high resolution and produced no Class II antibody. 36 produced no HLA Class II specific antibody despite being mismatched. There was a lower mean num-

ber of HLA Class II mismatches in the 36 antibody non-responders (4.7) when compared to the 45 antibody responders (6.1) and this was evenly spread between the loci. We analysed the 45 antibody responder pairs for HLA mismatch, eplet mismatch and the Class II anti-donor specific antibody as described by consistent eplets. There were similar numbers of HLA mismatches in the antibody responders at each of the Class II loci apart from DPA1 and DRB3/4/5 which had fewer mismatches. There were significantly more eplets per mismatch for DQB1 when compared to DRB1 (p=0.002) and DRB3/4/5 (p=0.003). This emphasis towards DQB1 was supported by analysing the eplets consistent with the antibody where DQB1 produced significantly more eplet response than the other loci (p=0.0005).
Conclusion: In this cohort DQB1 appears to be the dominant anti-donor antibody in the serum of renal allograft recipient's immediately post graft loss. The implications of the differences between the Class II loci in antibody generation will be discussed. 1 Hum.Immunol.2007,68:12-2

BSHI O-10 THE POSITION OF THE NATURAL KILLER CELLS ACTIVATING KIR GENES PROTECT FROM REACTIVATION OF CYTOMEGALOVIRUS INFECTION AFTER KIDNEY TRANSPLANTATION

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Cytomegalovirus (CMV) infection is a common complication after organ transplantation. Immunosuppression inhibit efficiently T cells activation but have a modest effect on natural killer cells which can play a critical role to prevent or cure infection. Previous studies have demonstrated that natural killer cells activating killer-cell immunoglobulin-like receptors (KIR) may reduce the rate of CMV infection. KIR genes can be divided into haplotype A (containing a fixed set of inhibitory receptors) and haplotype B (carrying additional activating KIR genes). The KIR locus is divided into a centromeric and a telomeric portion, both of which may carry A or B haplotype motifs. We studied a cohort of 339 kidney transplant recipients to elucidate which KIR genes protect from CMV infection. CMV infection occurred in 139 patients (41%). Possession of telomeric (hazard ratio 0.64, 95% confidence interval 0.44-0.94, p=0.02) but not centromeric (HR 0.86, 95% CI 0.60-1.23, p=0.41) B motifs was associated with statistically significant protection from CMV infection. Due to linkage disequilibrium, we were not able to identify a single protective gene within the telomeric B complex (which may contain the KIR2DS1, KIR3DS1, KIR2DL5A and KIR2DS5 genes). Presence of known or putative ligands to activating KIR did not significantly modify the influence of telomeric B group genes. The natural killer cells activating KIR genes play a critical role to protect against CMV reactivation after solid organ transplantation.

BSHI O-11 EFINING SERUM CYTOTOXICITY: A COMPLEX MIX?

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Introduction: Of the IgG subclasses, IgG3 has the greater complement fixing activity, followed by IgG1, then IgG2, with IgG4 having the least. A serum may therefore have the property of being cytotoxic by virtue of either its isotype composition or primarily because of the total amount of IgG, or a combination of both factors. We investigated this issue by comparing relative IgG subclass levels and C1q binding ability of HLA specific antibody in crossmatch positive sera with those in crossmatch negative sera.
Methods: 51 known antibody incompatible potential transplants were crossmatched using donor peripheral blood lymphocytes with rabbit serum as a source of complement, DTT and not AHG: 35 were CDC negative, 16 positive. Donor specific IgG was confirmed in all cases by single antigen bead assay. IgG subclasses were measured using a subclass-specific secondary antibody and standard luminex methodology. Antibody strength was taken as raw MFI. Classical complement activation was measured using C1qScreen (OneLambda).
Results: For all subclasses crossmatch cytotoxicity was associated with significantly higher MFI levels; IgG1 $p>0.00003$; IgG2 $p>0.0005$; IgG3 $p>0.003$; IgG4 $p>0.02$. The C1qscreen assay showed excellent correlation with CDC results. In addition in those cases that were predominantly IgG3 positive no C1q binding was observed. Three cases had high levels (>10,000MFI) donor specific IgG1 but no C1q binding supporting a CDC negative crossmatch result. In addition a further three cases were CDC negative in the presence of high levels of IgG2 and IgG1, possibly indicating an inhibitory effect of IgG2 upon complement activation.
Discussion: Although associated with higher levels of IgG1, cytotoxic capacity of a serum appears to be dependant upon other as yet undefined properties of immunoglobulin.

BSHI O-12 NTERASSAY VARIABILITY OF ANTI-HLA ANTIBODY MEASUREMENT USING LUMINEX SINGLE ANTIGEN BEADS (SAB)

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Measurement of anti-HLA antibodies using Luminex SAB has been widely adopted, however there is no consensus about cut-off for clinically relevant cut-offs. There is little published data about the performance characteristics of these assays.
Objectives: To define the interassay variability of antibody levels in patient serum.
Methods: Retrospective review of internal quality control data from SAB assays performed in our laboratory over a one year period. A serum-pool prepared from multiple samples from a single, highly-sensitised patient served as an internal quality control (IQC), and was included in all runs of One Lambda SAB. The Tepnel positive control was similarly evaluated. Levy-Jennings plots were maintained for 3 beads giving high, low and borderline values. 128 runs were analysed for the one lambda SAB, while 23 runs of Tepnel SAB were included.
Results: The strongly positive IQC value gave a mean MFI of 19,185 (range 13,587 to 23,023, CV 8.5%). The low-positive bead gave a mean MFI of 1154 (range 714-2090, CV 16.9%). The borderline value gave a mean MFI of 647 (range 401-1235, CV 20%). Levy-Jennings plots showed significantly decreased lab temperature on MFIs for all beads rose significantly when the laboratory temperature dropped. Interassay CV was lower for the Tepnel assay with weak values, but greater with strongly positive values. The strongly positive bead showed a mean adjusted-value 3 of 47 (range 32-113, CV 28.4%). The medium bead showed a mean adjusted-value 3 of 10.9 (range 8.6-14.6, CV 13.1%), while the low IQC showed an adjusted-value 3 of 6.49 (range 5.4 -7.5, CV 9.4%).
Conclusion: Luminex SAB assays show significant interassay variability, within a single laboratory. Manufacturers should improve the quality of asays. Despite apparent simplicity, SAB assays should be interpreted by experienced H & I personnel.

BSHI O-13 SURFACE PLASMON RESONANCE (SPR) TO DEFINE THE AFFINITY/AVIDITY OF THE ANTI-ABO RESPONSE

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Introduction: Measurement of ABO-specific antibody levels by haemaglutination (HA) is notoriously unreproducible despite this being the standard method for about 100 years. This is a significant problem in organ transplantation because of the rapid growth in ABO incompatible (ABOi) procedures. Previously, we have demonstrated an ABO antibody detection and quantitation assay using the XPR36TM SPR platform (Biorad). This study analyses the binding characteristics of the anti-ABO response and provides a robust mathematical model to assess the kinetic properties of this interaction.
Methods: In order to determine reaction constants (such as binding affinity) mathematical models describing the reaction between free-flowing analyte and surface immobilised ligand (antigen) are applied. The three typical models are considered: the Langmuir model, in which the reaction is considered as between two well-mixed substrates; a modified Langmuir model that incorporates the effects of transport; and the Effective Rate Constant (ERC) approximation, derived from consideration of the fluid dynamics of the analyte in the flow and the receptor layer, and the subsequent binding. The model parameters (reaction constants etc) are estimated using the data from the SPR platform.
Results: It is shown that heterogeneity in analyte binding characteristics results in models in which there is not a unique correspondence between parameters and response, so that care must be taken in determining binding affinities. In particular, unless the concentrations of all unknown binding species are known the Langmuir model cannot be applied to uniquely determine binding affinity. Parameter estimates (including affinities) for the transport and ERC models are comparable.
Discussion: Importantly, this robustly modelled multiplex assay will allow a detailed characterisation of the physical changes in antibody quantity, affinity and avidity associated with accomodation and antibody modulation during the response to incompatible grafts.

BSHI O-14 TRANSPLANTING THE "VIRTUALLY" UNTRANSPLANTABLE HEART PATIENT – A CASE STUDY

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The ability to accurately detect HLA antibodies in cardiothoracic transplant patients provides a robust basis for 'virtual' crossmatching, and in our centre has coincided with a reduction in acute rejection episodes. However, there are some patients with multiple HLA antibody specificities that are difficult to transplant, particularly when antibodies are reactive by complement dependent cytotoxicity (CDC). This problem is acute in children who only receive a limited number of donor offers. We report on a case of successful transplantation in a highly sensitised three year old patient treated with antibody reduction therapy, with antibody levels monitored in the laboratory both pre and post transplant. Sensitisation to HLA was likely due to transfusions given perioperatively during a Rastelli procedure to repair a congenital defect, along with a bovine conduit for reconstruction of the right ventricular outflow tract. Initial Luminex® antibody screening samples were reactive with all beads in identification tests with single antigen beads showing reactivity against all but self antigens, and testing by CDC was positive against all cells tested. The patient condition deteriorated and mechanical support was initiated with an Excor® Berlin heart fitted as a bridge to transplant. A desensitisation program was initiated in an attempt to reduce antibody levels, consisting of cycles of Bortezomib, Rituximab and IvIG with daily Mycophenolate mofetil and Prednisolone. Antibody levels were monitored weekly using samples diluted to 1/50 to demonstrate the effectiveness of treatment. The patient was transplanted with an ABO and HLA 122 mismatched heart, receiving triple volume plasma exchange on bypass. Pre transplant CDC and flow crossmatches were positive, post-transplant they were negative. Post-transplantation antibodies increased and a further course of Bortezomib was used. Cardiac function is good and the patient remains well four months post-transplant.

BSHI O-15 ANTI-C3D FIXATION ASSAY: A TOOL FOR PREDICTING THE RELEVANCE OF LUMINEX DETECTABLE HLA ANTIBODIES IN RENAL TRANSPLANTATION

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Complement fixing ability of HLA antibodies correlates with the development of acute antibody mediated rejection and decreased graft survival in renal allograft recipients. However, the clinical relevance of low-level Luminex detectable antibodies is unclear. We employed an anti-C3d fixation luminex-based assay to investigate post transplant HLA antibody production in two case studies. Patient 1: became highly sensitized post-transplant with a range of DSA (donor-specific antibodies) and non-DSA, despite no further sensitizing events. This coincided with reduction in cyclosporin A (CSA) in the period between December 2007 and March 2008. Post-transplant serum samples were tested using the anti-C3d fixation assay. The antibody profile could be attributed to sensitization via epitope cross-reactivity. Production of complement fixing antibody correlated with increasing reactivation of immune status, (via immunosuppression withdrawal) and consequently graft damage. These data suggests developmental differences and the ability of complement fixing to spread via a pattern of epitope spreading. Patient 2: had luminex defined HLA antibodies prior to receiving a HLA-A, B DR matched transplant in June 2007. The graft failed due to pyelonephritis and the patient was re-registered on the transplant waiting list in November 2009. Results from the anti-C3d assay showed the complement fixing ability of HLA antibodies detected post-transplant changed. Positivity in the anti-C3d assay was detected in the patient prior to graft rejection. Results suggests the change in antibody profile may be associated with the change in graft function and provides evidence for humoral rejection as a mechanism of graft damage. These data suggest the presence of C3d supports the hypothesis that antibody and complement deposition are involved in the pathogenesis of graft failure and poor prognosis. Therefore, this assay may be of benefit in post-transplant monitoring.

BSHI O-16 FREQUENCY OF DPB1 MISMATCHES IN HLA "MATCHED" SIBLINGS

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Typing of recipients and patients in haemopoietic stem cell transplantation (HSCT) for HLA-DPB1 is performed routinely in some but not all HLA laboratories. It is known that siblings matched for A, B, C, DRB1 and DQB1 can be mismatched for DPB1 and that recombination can occur between DQB1 and DPB1. Recent interest in the role of DPB1 mismatches in graft-versus-host disease and graft-versus-leukaemia reactions indicates that such mismatches may influence transplant outcome. The frequency of DPB1 mismatches was investigated in 265 patient-otherwise HLA matched sibling pairs, involving 220 patients. Tested took place in our laboratory for HSCT over a 10 year period (January 2001 – December 2010). HLA-DPB1 typing was performed by PCR-SSP. In this large group of sibling pairs matched for A, B, C, DRB1 and DQB1, 11 of 265 (4.15%) were mismatched for DPB1. The frequency of DPB1 mismatches in HLA 'matched' siblings suggests that the absence of DPB1 typing could compromise the accuracy of studies of transplant outcome in sibling HSCT. In addition, DPB1 type could be considered in the choice of donor should more than one potential sibling donor be available.

BSHI O-17 PILOT EVALUATION OF C1Q SCREEN IN EVALUATION OF PATIENTS FOR RENAL TRANSPLATATION

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Anti-HLA analysis by luminex single antigen bead is well-established in patient evaluation pre-transplantation, despite concerns about oversensitivity, and lack of concensus concerning the clinically-relevant cut-off. A modified technique detecting only complement-fixing antibodies is commercially available. This assay is not CE-marked and labelled for research use only. Users are advised to "use a known positive and negative reference serum to determine assay cut-off". The commercial assay appears to differ in configuration to that published in some papers. Aim: Pilot evaluation to determine if this novel assay differentiated donor specific antibodies (DSA) in patients who developed who developed antibody mediated rejection (AMR) post renal transplant from those who did not.

Methods: 4 patients who developed AMR in the first 2 months post-transplant were compared with 13 patients, chosen because of positivity in IgG SAB assays who did not develop significant rejection post transplant. Standard IgG SAB assays were also performed.

Results: 3/4 patients had donor-specific SAB positivity detectable on the day of transplant. All were negative for DSA in the C1q assay (positivity defined either by absolute or relative MFI). All of the patients with an uneventful post transplant course had IgG detectable in SAB assays (4 detectable in one assay only, and 9 positive in both SAB assays). 4 patients in the no-AMR group had donor specific C1q detected. Of the 4 patients with donor-specific C1q antibodies 2 have had prolonged graft function (9yrs & 12 yrs), while 2 grafts failed at 30 months and 4 years.

Conclusion: In this pilot evaluation, the commercially-available C1q assay does not detect DSA in patients who subsequently develop AMR, even when these are detectable by IgG SAB. The presence of donor-specific C1q antibodies may be compatible with long-term graft survival.

BSHI O-18 TRANSPLANT NEPHRECTOMY: WHO GETS IT AND DOES IT MAKE A DIFFERENCE?

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Introduction: The aim of this study was to identify the proportion of patients who undergo transplant nephrectomy following a return to dialysis and compare their clinical characteristics and outcomes to those in whom the transplant was left in-situ.

Methods: We identified all patients who underwent renal transplantation in our unit after 01/01/1985, whose renal allograft functioned for a minimum of three months and in whom allograft failure occurred after 01/01/1995. Baseline clinical, laboratory and demographic data were recorded. Occurrences of transplant nephrectomy and mortality were recorded. Comparison of nephrectomy and non-nephrectomy groups was made by univariate parametric and non-parametric testing.

Results: 285 patients experienced 301 episodes of renal transplant failure within these inclusion criteria. 154/285 (54.0%) of patients were male with a mean age at first transplant of 35.1yrs. 119/301 (39.5%) episodes of transplant failure were followed by transplant nephrectomy. 34/119 (28.6%) nephrectomies occurred within 4 weeks of transplant failure with 85/119 (71.4%) >4 weeks after failure. Discounting those patients with transplant nephrectomy within 4 weeks of failure, those who subsequently underwent nephrectomy were found to be younger at transplantation (40.0 yrs v 44.5 yrs, $p=0.01$), have shorter duration of allograft function (6.1 yrs v 9.1 yrs, $p<0.01$) and have higher rates of transplant rejection causing failure (83.3% v 58.2%, $p<0.01$). No significant difference in the rate of chronic allograft nephropathy (22.2% v 25.5%, $p=0.83$) or mortality (22.4% v 29.7%, $p=0.27$) was found.

Conclusion: Nearly 40% of patients with a failed renal transplant may undergo transplant nephrectomy. Whilst 30% of nephrectomies occur within 4 weeks of failure, 70% are undertaken later. Such patients are younger, have shorter duration of allograft function and experience more rejection. No difference survival was demonstrated.

Poster presentations

BSHI P-01 FLOW CROSSMATCH RESULTS IN COMBINATION WITH 2 DIFFERENT SINGLE ANTIGEN ASSAYS PROVIDE GREATER RESOLUTION IN PREDICTING RENAL GRAFT SURVIVAL

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Detection of HLA-specific antibodies pre-transplant helps prevent graft rejection, and aids risk assessment of donor-recipient pairs. Initial enthusiasm and widespread adoption of Luminex single antigen (SAB) assays has been tempered by conflicting reports of their clinical relevance.
Aims: To assess effects of Luminex-detected antibodies on long-term graft-survival, alone or in combination with a second Luminex assay and flow cytometry cross-matching.
Methods: Day of transplant sera from 204 sensitized renal transplant recipients were retrospectively analysed for the presence of donor-specific HLA antibodies (DSA) using HLA One Lambda Labscreen® (Canoga Park, CA) and Genprobe Lifecodes® (Stamford, CT) SAB.
Results: 122 patients (55%) had detectable DSA. When all antibodies were considered positive, there was no statistically significant difference in graft survival (>80% in both groups). This confirmed the suspicion that not all antibodies detected are clinically relevant, and that the non-significant subset of antibodies detected was masking clinically relevant antibodies. When only antibodies detectable by both methods are considered true positives, the result was highly significant ($p<0.001$). Survival rates in negative patients were >80% ten years post-transplant, and patients with single technique antibodies also had excellent graft survival after ten years (>80%). In contrast, graft survival in patients with antibodies confirmed by both Luminex assays was <80% approximately 2 years post-transplant, dropping to approximately 50% after ten years. When flow-cytometry crossmatch results were correlated with SAB results, patients with confirmed antibodies and a positive crossmatch had significantly worse outcomes than confirmed antibody positive/crossmatch negative patients (graft survival at 2 years 64% vs >90%; $p<0.002$).
Conclusions: SAB assays alone are inadequate to risk assess a donor-recipient pair. However, when used in combination together with flow-cytometry crossmatching, they facilitate detailed risk assessment.

BSHI P-02 PROVISION OF HLA SELECTED RED BLOOD CELLS IN RENAL TRANSPLANTATION

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Since the introduction of recombinant human erythropoietin, transfusion requirements of dialysis patients has significantly reduced. However in some circumstances transfusion of red blood cells (RBCs) is still required. In patients awaiting renal transplantation this carries a risk of causing sensitisation to HLA antigens. This is particularly unfortunate when a suitable living kidney donor has already been identified and antibodies specific for donor HLA antigens are produced. In the presented case a 50 year old female commenced transplant work-up September 2009. Antibodies to HLA-B7 and related antigens due to previous pregnancies were detected. Two units of RBCs were transfused December 2009. The patient's HLA sensitisation increased with multiple additional specificities including a donor specific HLA-Cw14 antibody. Antibodies to other donor mismatched HLA antigens (HLA-A1 and B51) were not detected. In July 2010 a further 2 units of RBCs were requested for transfusion. This time HLA selected RBCs were sourced to try and prevent further sensitisation to HLA-A1 or B51 which could preclude transplantation from the identified living donor. As NHSBT currently has no IT capability to search RBC stocks for HLA data a manual search method to support the request was devised. The patient also had antibodies to RBC antigens; therefore an initial search was performed against RBC criteria. This identified 86 suitable donors nationally. Donors were individually checked against BBMR records to identify HLA typed RBC donors. Of the 86 donors 12 had been typed and 4 units were identified as suitable since they did not contain any of the kidney donor's mismatched antigens. Antibody screening post transfusion confirmed that sensitisation to the potential donor's mismatched HLA antigens was avoided. This case demonstrates the potential value of providing HLA selected RBC in patients with identified living donors.

BSHI P-03 MFI VALUES DON'T TRAVEL: RESULTS OF AN INTER-CENTRE STUDY

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Preformed IgG donor HLA specific antibodies are detrimental to transplant outcome and are usually considered a contraindication to transplantation. One of the main functions of the histocompatibility laboratory is to identify unacceptable HLA antigens for patients awaiting transplant in order to prevent positive crossmatches, rejection and graft loss. Luminex bead-array technology is currently used in many laboratories for antibody definition. The high sensitivity of this assay means that low levels of antibody are detected that would be negative by other screening techniques and may not be detected at crossmatch. Listing such 'low level' antibodies as 'unacceptable' may preclude sensitised patients from potentially suitable organ donors. At present each histocompatibility laboratory determines its own "cut-off" value for antibody positivity using evidence based data. There has been a suggestion that this could lead to different laboratories reporting discordant results for the same sample and attempts to equate results between laboratories have been made by comparing median fluorescence intensity (MFI) values. We have compared the LABScreen single antigen results from 4 laboratories for 6 sera. HLA-Fusion analysis shows that although MFI values for these sera vary between different laboratories the antibody profiles are remarkably similar as are the defined specificities. For example, results for one sample showed positive control bead MFI values ranging from 18620 to 4299 and maximum specific MFI values ranging from 6104 to 2552 between laboratories however HLA-Fusion analysis gave the following positive specificities: Lab 1 HLA-A2; B7,27,49,55,60,61,81 Lab 2 HLA-A2,66; B7,27,49,55,60,61,81 Lab 3 HLA-A2,68; B7,27,49,55,60,61,81 Lab 4 HLA-A2,68; B7,27,55,60,61,73,81 Our results suggest that communication of MFI values between laboratories as a measure of antibody positivity is invalid but that antibody profiles generated from data in one centre can be transferred to and confidently used in another.

BSHI P-04 A RETROSPECTIVE INVESTIGATION OF THE PREVALANCE OF "COMMON" NULL ALLELES IN A UK POPULATION

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In our laboratory we have recently made the transition to offering a fully molecular service for HLA typing. We currently perform high resolution typing of class II alleles for HSCT recipients and donors only. Therefore there is a potential risk of missing null alleles since our in-house PCR-SSP trays, as low/mid resolution typing, do not discriminate between expressed and non-expressed alleles. Guidelines from professional bodies, such as the NMDP, and the literature state that there are five "common" null alleles associated with certain haplotypes, these are A*24:09N, B*51:11N, C*04:09N, DRB4*01:03:01:02N, and DRB5*01:08N alleles. DRB4*01:03:01:02N was excluded in this study since this allele is frequently seen. The frequencies of these risk haplotypes were assessed over various time periods spanning 21-88 months in our local population. Retrospective DNA samples were tested using the HLA Ready Gene (PCR-SSP) kit (Innotrain) which definitively detects the above alleles. One B*51:11N allele and three C*04:09N alleles were identified. The A24:09N, and the DRB5*01:08N alleles were not identified in our population group. The identification of the B*51:11N, and the C*04:09N alleles highlights the importance of screening transplant patients for certain null alleles. The potential clinical impact of the identified null alleles has been reviewed. The B*51:11N allele was identified in a renal patient who has since been transplanted. Interestingly the patient was of Polish origin and the other documented B*51:11N allele that has been identified has also been found in an Eastern European patient. Further investigations are now underway in order to confirm the rare B*51:11N allele on the IMGT database. This study has contributed to the development of a laboratory strategy for the detection of these null alleles based on our knowledge of predicted frequencies.

BSHI P-05 THE EFFECT OF DONOR AND RECIPIENT CYTOKINE GENES POLYMORPHISM ON THE OCCURRENCE OF ACUTE REJECTION AFTER RENAL TRANSPLANTATION

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Acute allograft rejection remains an important cause of morbidity after kidney

transplantation. Accumulated data has indicated that the rejection process is regulated by a balance of a Th1/Th2 cytokine paradigm. In addition, the genetic makeup of the recipients and their donors play a significant role in the allograft outcome. These variations may be related to genetic polymorphisms within the regulatory regions of the cytokine genes. Accordingly, this study sought to explore the impact of the recipient and the donor cytokine gene polymorphisms and the outcome of renal transplantation. Assessment of SNPs distribution for TGF-b (codon 10 and 25), IL-10 (positions -1082, -819, -592), TNF-a (position -308), IL-6 (position-174), and IFN-g (position -874) using gnomic DNA from a cohort of 100 kidney transplant recipients and their matched living related donors in correlation with their graft outcome. Genotyping analysis was performed by allele-specific polymerase chain reaction (SSP-PCR). The majority of the donors demonstrated extended genotype profiles characterized by low TNF-α, high TGF-β, intermediate IL-10, high IL-6 and intermediate IFN-γ production. On the other hand, a significant difference (P value= 0.03) in IL-10 was detected in the classification of donors based on their recipients graft outcome with respect to cellular or antibody mediated rejection status, Further analysis showed that donor IL-10 genotype was also associated with severity of acute rejection. This study demonstrated that the donor IL-10 genotypes, among other factors, can be determinants in graft outcome after renal transplantation.

BSHI P-06 POSITIVE B CELL FLOW CROSSMATCHES ARE NOT ALWAYS CAUSED BY HLA ANTIBODIES – A TEN YEAR RETROSPECTIVE STUDY USING LUMINEX TESTING

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Historically it has been difficult to determine the cause of a B cell positive flow crossmatch (B+FXM). B+FXM interpretation is further confounded by difficulties in defining some class II antibodies, such as those directed against DQα and DP. The aim of this study was to use Luminex technology to re-examine samples from past B+FXM transplanted patients to see how many were caused by donor specific HLA antibodies (DSA). At transplant samples from 101 patients transplanted with a B+FXM from 1998-2009 were tested by Luminex using LabscreenTM mixed beads. Samples positive by screening were further tested using LabscreenTM single antigen beads (SAB). Forty two patients (41.5%) had no detectable HLA antibodies at the time of transplant. In 59 patients HLA antibodies were detected, of which 34 (33.6%) were DSA with an MFI $>$500. Seven of these had class I (6.9%) antibodies only, 21 had class II only (20.8%) and six (5.9%) had both. There were 13 class II DSA with MFI $>$5000 of which five were either DQα or DP. The remaining eight were DRB or DQB DSA and were detected in samples from B+FXM performed prior to 2003 when Luminex was introduced to the laboratory. No class I antibodies were found with an MFI $>$5000. In conclusion, 76.4% of B+FXM were not caused by HLA DSA. Luminex testing has increased the class II specificities we are able to detect and decreased the number of unexpected B+FXM caused by undetected DSA. The consequence is more confidence when transplanting across a B+FXM. This study also demonstrates that current and historical antibody screening strategies prevented patients with high levels of class I DSA from being transplanted.

BSHI P-07 EXTRACTING DNA FROM SERUM

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Regular screening of patient serum is essential for the identification of any HLA-specific antibodies that may affect their transplantation chances and treatment options. Since erroneous results could have fatal consequences, it is crucial that only data obtained using sera from the correct patient is considered clinically. There are occasions when samples may give unexpected screening results (eg an unexpected negative, or positive) and to this end it is beneficial to have a system in place whereby the identity of samples can be confirmed. The objective of this study was to identify a robust method to obtain sufficient DNA from serum that could subsequently be used to obtain an HLA type or STR profile to confirm patient identity. Following unsuccessful trials of a commercial kit, we investigated a modified version of our standard protocol for DNA extraction from peripheral blood and bone marrow samples using the Geno-M6/EZ1 robotic system. Using this protocol, DNA was extracted from 17 serum samples at a range of time-points after being drawn, varying from freshly isolated serum, to frozen samples stored for up to 18 years. The quantity and quality of DNA was assessed by NanoDrop® spectrophotometry, and identity with the patient was confirmed by LABType rSSO HLA typing (single locus or A,B,DR). HLA types were obtained from 15/17 samples which allowed adequate confirmation of identity. The two samples which failed to type had been freeze-thawed repeatedly, which is likely to have led to degradation of DNA. Further investigations are ongoing to improve DNA extraction from such samples. This study has identified a simple, reliable method for confirming the identity of serum samples using methodology and reagents already in routine use.

BSHI P-08 DEVELOPMENT OF GROUP SPECIFIC SEQUENCING BASED TYPING FOR EXON 3 OF THE HLA DQB1 GENE TO RESOLVE AMBIGUITIES

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To achieve single allele resolution for HLA DQB1 sequencing based typing (SBT), it has become increasingly necessary to include exon 3 sequence in the analysis. Because our laboratory already performs SBT for exon 2 of the HLA DQB1 gene using a group specific approach, a similar strategy was followed in developing SBT for exon 3.
Methods: Forward and reverse primer sequences for group specific amplification of exon 3 were selected from intron 2 and intron 3 sequence alignments assembled using the nucleotide database at NCBI (www.ncbi.nlm.nih.gov) and recently available from IMGT/HLA (www.ebi.ac.uk/imgt/hla). Universal sequencing primers were designed using the same alignments. Group specific amplification primers and sequencing primers are listed in Table 1. Amplification and sequencing steps were performed using standard protocols, samples were electrophoresed on an ABI 3130 Genetic Analyzer and results interpreted using ASSIGN software (Conexio Genomics, Perth, Australia).
Results: The strategy for SBT of HLA DQB1 exon 3 was validated using DNA extracted from samples distributed by UK NEQAS for Histocompatibility and Immunogenetics for EQA purposes. Gel electrophoresis showed clear group specificity for the amplification primer mixes. The PCR templates were subsequently used to sequence exon 3 of the corresponding HLA-DQB1 allele in both forward and reverse directions.
Conclusion: We have developed a group specific SBT protocol for exon 3 of the HLA DQB1 gene. This has enabled us to achieve single allele resolution for SBT at this locus.

BSHI P-09 SBT FOR MICA – A PRACTICAL APPROACH

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MICA genes are located close to HLA-B and although the gene products have structural similarities to classical HLA Class I molecules they are not associated with beta 2 microglobulin nor do they bind peptide. MICA proteins are stress inducible and are expressed only on certain types of cells. Recently antibodies to MICA have been demonstrated in transplant patients and have been implicated in graft rejection.
Methods: We have established a sequencing based typing (SBT) protocol adapted from a previously published method for unambiguous MICA allele typing (Zhu, F. et al Tissue Antigens 2009; 73: 358-363). A peculiar problem with MICA typing is the heterogeneity of exon 5 which encodes the transmembrane (TM) region where at least 7 different single tandem repeats (STR's) have been identified (the number of GCT repeats determines A4, A5, A5.1, A6, A7, A9 & A10 patterns). The number of TM STR's has usually been based on the size of fragments as determined by Genescan fragment analysis. However, we have preferred to use the same SBT methodology throughout and sequence exons 2 to 6. Results were interpreted in the first instance using ASSIGN software (Conexio Genomics, Perth, Australia). For individuals who are heterozygous at the MICA locus, exon 5 was analysed separately using a simple algorithm to establish the TM combination.
Results: The strategy for SBT of MICA, including the algorithm for analysis of exon 5, was validated using DNA extracted from cell lines of known MICA type.
Conclusion: The SBT protocol for MICA may be used successfully to type patients and donors in the further investigation of MICA antibodies.

BSHI P-10 ANTIBODY REDUCTION: REMOVING BOTH HLA AND ABO ANTIBODIES IN A LIVING RELATED KIDNEY TRANSPLANT – A CASE STUDY

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Introduction: Current technologies for antibody removal and monitoring of antibody levels have allowed development of ABO and HLA incompatible transplantation protocols. Here we describe a case of a 52 year old female patient with end stage renal failure receiving an HLA and ABO incompatible living donor transplant. The patient was blood group O Rh positive and had been previously transplanted from a 101 HLA mismatched (MM) deceased donor. The living donor (husband) was a 212 HLA MM and ABO A1 Rh positive. Baseline ABO titres were anti-A1 IgM 16 and IgG 64. The patient had donor specific antibodies to HLA B62 (peak MFI 1000), DR7 and DR9 (historical samples).
Method: HLA and ABO antibodies were removed using four cycles of

Therasorb® adsorption. Antibody levels were monitored pre and post treatment using antibody identification/SAB Luminex® technology. A mean fluorescence intensity (MFI) <500 was defined as negative. ABO antibody titres were monitored by agglutination using Diamed® gel cards.
Results: Pre-transplant: The cytotoxic crossmatch was negative. The flow crossmatch was T cell positive, B cell negative before antibody reduction treatment. ABO antibody titres gradually reduced by one dilution per treatment. HLA antibody levels reduced to <500 MFI before transplantation. Post transplant: By day +30, ABO titres were anti-A1 IgM 2 and IgG 4, and the donor specific HLA B62 antibody was negative (MFI 94). The graft is still functioning 9 months post transplant.
Discussion: In this case the patient probably produced potential DSA through pregnancy, necessitating antibody reduction pre-transplant. This case demonstrates that antibody removal columns can reduce antibodies to both HLA and ABO in the same treatment and how close monitoring can guide treatment.

BSHI P-11 OUTCOME OF KIR PREVALENCE AND INCOMPATIBLITY IN KINDNEY TRANSPLANT RECIPIENTS VERSUS LIVING RELTED DONORS

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KIR and/or HLA mismatching in organ transplantation, may lead to NK cell activation. In return, the activation of NK cells depends on the balance between signals from activating and inhibitory receptors. The purpose of this study was to investigate the effect of KIR/HLA mismatch and KIR genes on graft outcome in a cohort of 100 Saudi kidney transplant recipients and their living related donors. HLA and KIR genotyping was performed by allele-specific polymerase chain reaction (SSP-PCR). The prediction of NK cell alloreactivity was done based on the "missing ligand" hypothesis. Allograft rejection was observed in 17 (17%) recipients. Similar to most published data, we observed the dominance of the two framework genes 3DL2 and 3DL3 which are present in 100% of all recipients and their related donors. Higher gene's frequencies (56%, 81%) of the inhibitory and activatory genes; 2DL5a and 2DS4 respectively, were found to be associated with donors with graft rejection, compared to 20% and 50% genes frequencies in donors with no rejections. In contrast, a lower frequency (18%) of activatory KIR gene 3DS1 is detected in donors with no rejection compared to those with rejection (50%). A predominance of AA1 genotype was observed in recipients and donors with stable graft followed by ABC5. In addition, we detected a higher HLA-C2 homozygous in patients with acute rejections compared to patients with stable graft. Moreover, the number of 2DL pos and 2DS2pos with no corresponding ligand were three times higher in controls than the rejected patients. In conclusion, KIR and HLA mismatching and gene frequencies were found to have significant impact on allograft outcome.

BSHI P-12 ANALYSIS OF THE HLA-A, -B, AND -DRB1 ALLELES IN NORTH-WESTERN ROMANIANS

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Purpose: To analyze the polymorphism of HLA-A, -B, and -DRB1 alleles in North-Western Romanians.
Materials and Methods: The 264 patients currently awaiting kidney transplants from our transplantation unit have been A, -B, -DR typed polymerase chain reaction (PCR) with the sequence-specific primer (SSP) method by low resolution Olerup SSP® kits. DNA was extracted from whole blood using innuPREP Blood DNA Mini Kit. The application of DNA based procedures has increased the accuracy of HLA typing and lead to the identification of serologically undetected alleles and of many subtypes of serological specificities
Results: Thirteen different HLA-A, 34 -B and 18 -DR alleles were observed among the patients. Fourth HLA-A alleles of a total of 460 had frequencies higher than 10% (A*02, A*01, A*24, A*03) and then are characteristic of Central European. Among 508 HLA-B alleles, B*35, B*18, B*51, B*44, B*08, B*07, B*27 were the most frequent alleles in the studied population. We have investigated the distribution of HLA class II alleles for the HLA-DRB1 loci. Among the 18 alleles at HLA-DR locus, the most prevalent five alleles included DRB1*11 (11%), DRB1*13 (7%), DRB1*16 (5%), DRB1*03 (5%), DRB1*01 (5%).
Conclusions: This study can be used to aid clinicians to know the HLA alleles frequencies of patients on the cadaveric kidney transplant waiting list and to establish probabilities of finding compatible donors.

BSHI P-13 PARADOXICAL EFFECTS OF BRAIN DEATH AND ASSOCIATED TRAUMA ON RAT MESENTERIC MICROCIRCULATION: AN INTRAVITAL MICROSCOPIC STUDY

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To evaluate the role of brain death (BD) compared with BD-associated trauma on the development of the inflammatory response at the mesenteric microcirculation and its systemic effects. Male Wistar rats (250-300g) were anesthetized with isoflurane (2-5%) and intubated. A balloon catheter was placed into intracranial cavity and inflated to induce BD. Sham-operated rats (Sham) were trepanned only. Animals (7/group) were evaluated 30 and 180 minutes thereafter. The mesenteric microcirculation was analyzed by intravital microscopy, the expression of adhesion molecules by immunohistochemistry, and cytokines by ELISA. Total and differential cell counts were determined in blood samples. Proportion of perfused small vessels (<30 μm diameter) was reduced in BD-rats compared to Sham rats either at 30 minutes (BD 30.2±0.06% vs Sham 77.9±0.07%, $p<0.0001$) or 180 minutes (BD 27.9±0.07% vs Sham 74.3±0.06%, $p<0.0001$). Number of rollers were: BD30 94±4 cells/10 min vs Sham30 178±37 cells/10 min ($p<0.05$); BD180 87±8 cells/10 min vs Sham180 329±24 cells/10 min ($p<0.001$); adhered leukocytes: BD180 4.6±0.9 cells/100 μm vs Sham180 6.9±0.06 cells/100 μm ($p<0.05$); and migrated leukocytes: BD180 2.3±0.3 cells/5000μ^2 vs Sham180 1.3±0.3 cells/5000μ^2 ($p<0.01$). Expression of P-selectin did not differ among groups, and ICAM-1 increased in BD-rats at 180 minutes ($p<0.01$). BD-rats exhibited a reduction in the number of total white blood cell counts (BD 8,018±763 cells/mm^3 vs Sham 11,771±1,122 cells/mm^3, $p<0.05$) and neutrophils (BD 4,871±691 cells/mm^3 vs Sham 7,414±759 cells/mm^3, $p<0.05$) at 180 min. Serum concentrations of TNF-α, IL-1β, IL-6, IL-10, CINC-1 and CINC-2 were not different among groups. In conclusion, BD-associated trauma is responsible for most of the inflammatory events observed. Otherwise perfusion of mesenteric microvessels was promptly interrupted by BD itself. This was accompanied by a pronounced leucopoenia, and increased ICAM-1 expression and migrated leukocytes. Financial support: FAPESP.

BSHI P-14 AVOID OR ADMINISTER MMF IN RENAL TRANSPLANT RECEIPIENT WITH IDIOPATHIC (AUTOIMMUNE) THROMBOCYTOPENIA?

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Idiopathic thrombocytopenic purpura is an autoimmune disease characterized by thrombocytopenia with normal WBC count and hemoglobin concentration. A 27-year-old female with end-stage renal disease due to chronic glomerulonephritis underwent kidney transplantation from deceased donor on August 10th, 2004. Compatibility was 4 HLA mismatches (A-2, B-1, DR-1). She was immunosuppressed with tacrolimus and methylprednisolone, and was maintained thereafter on tacrolimus and prednisone. Primary immunosuppression did not include MMF due to idiopathic thrombocytopenia which was diagnosed in childhood (13y). The posttransplant follow-up included surgery due to hematoma. The two acute rejection episodes (not-biopsy proven AR because of thrombocytopenia) were treated with methylprednisolone (3x500 mg iv) with good clinical response. On December 2007, she underwent bone marrow biopsy, which was not characteristic, but anti-platelet antibodies were present (antigen: GPIIb/IIIa, GPIb/IX, GPIa/IIa). Platelet count was 2,000-4,000/m^3. SLE was excluded. High doses of prednisone and danazol were administered. She also underwent laparoscopic splenectomy without complications in October 2008. After splenectomy platelet count ranged from 4,000/m^3 to 16,000/m^3. On November 2010, increasing proteinuria (2.0 g/l) was found. Diagnostic kidney biopsy was not performed again due to thrombocytopenia. MMF 250mg twice a day (b.i.d.) was started when thrombocytopenia reached 4,000/m^3. MMF doses were gradually increased to 2×500 mg, which led to satisfactory response. Proteinuria has decreased to mild (less than 0.5 g/l) and platelet count has increased to 34,000 and 40,000/m^3. She has done well till now and maintained on prednisone, MMF (750+500 mg) and tacrolimus (blood level of 5-6 ng/ml) with a stable serum creatinine 126 μmol/l.
Conclusion: MMF originally used as an immunosuppressive agent in solid organ transplantation can also be useful in management of idiopathic (autoimmune) thrombocytopenia.

BSHI P-15 COMPLETE RESPONSE TO RITUXIMAB® IN LIVER-TRANSPLANT PATIENT WITH DIFFUSE LARGE CELL LYMPHOMA

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Objective: Effectiveness of Rituximab® in the treatment of post-transplant lymphoproliferative disorder (PTLD).
Methods: Prospective following of one patient diagnosed by diffuse large cell lymphoma nine months after a liver transplant and treated with mycophenolate mofetil, mTOR, and Rituximab®.
Summary: Transplant patients show a higher incidence of lymphoproliferative diseases (PTLD), being B-lymphocyte proliferation usually the responsible. The primary infection transmitted by an EBV donor is the main risk factor. It is known that Cyclosporine A (CsA) at normal or high doses or antiCD3 monoclonal antibodies (OKT3) increase the risk of PTLD development, lower incidence rate than that presented by patients treated with tacrolimus. Mycophenolate (MMF) is associated with a lower incidence in both adults and infants, obtaining lower rates than other immunosuppressants such as azatioprin (0.6% vs. 1.1%). Our experience shows a PTLD incidence in liver transplant patients of 0.45%. We show a 64-year-old patient undergone liver transplantation who nine months later suffered from a bowel obstruction episode and was diagnosed by large cell immunoblastic B lymphoma, located in mesogastrium and with affectation of retroperitoneum and several hepatic nodes and bilateral lung nodes. The induction immunosuppressive therapy was tacrolimus, mycophenolate mofetil and prednisone, and it was changed to mycophenolate mofetil (MMF), mTOR (sirolimus) and anti-CD20 monoclonal antibodies (Rituximab®). The patient developed sustained complete response.
Conclusions: As well as mTOR inhibitors (sirolimus) in renal transplant patients seems to be associated with complete remission of PTLD, Rituximab® is a effective therapy for obtainind a complete response in liver transplant patients with PTLD. Chemotherapy used as initial treatment or as treatment associated with immunosuppressive drugs reduction (either as CHOP or as anthracycline chemotherapy) must be administered as a second-line treatment in those patients who do not respond to Rituximab®.

BSHI P-16 THE USEFULNESS OF LIFECODES DONOR SPECIFIC ASSAY IN A SOLID ORGAN TRANSPLANTATION SETTING

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Crossmatching for deceased and living donor transplants can give ambiguous and misleading results using the flow cytometry platform. Auto reactive/non HLA antibodies can bind to the target cells giving a positive result which is not clinically significant in solid organ transplantation. In our centre approximately 35% of solid organ transplants are performed despite the presence of a "positive" flow crossmatch, sometimes leading to delays as additional testing is required such as an auto-crossmatch or single antigen assay to elucidate the results. The study aims to evaluate the Lifecodes Donor Specific Antibody (DSA) assay by comparing a set of actual donor/recipient results to the recipients' single antigen assay results and their flow cytometry crossmatch results. The study also looks at some patients transplanted with low level DSA The study contains 5 groups of patients (not all of whom were transplanted) 1- Patients with known DSA, 2– patients who gave positive crossmatches in the absence of DSA, 3– patients who have DSA that was only detected by one of the two single antigen assays on the market, 4– patients who have DSA confirmed by both single antigen assays and 5– DSA and crossmatch negative patients. Initial findings from the study indicate that low-level DSA do not give a positive crossmatch in the assay, similar results have been found for patients with single technique antibodies. We are looking at patients who have detectable antibodies without a history of classical sensitising events. The study includes titration of known DSA in the assay to try and determine an MFI range (cut off) that will result in a positive crossmatch. Preliminary conclusions so far indicate that the assay could well be a useful addition/alternative to the flow cytometry technique.