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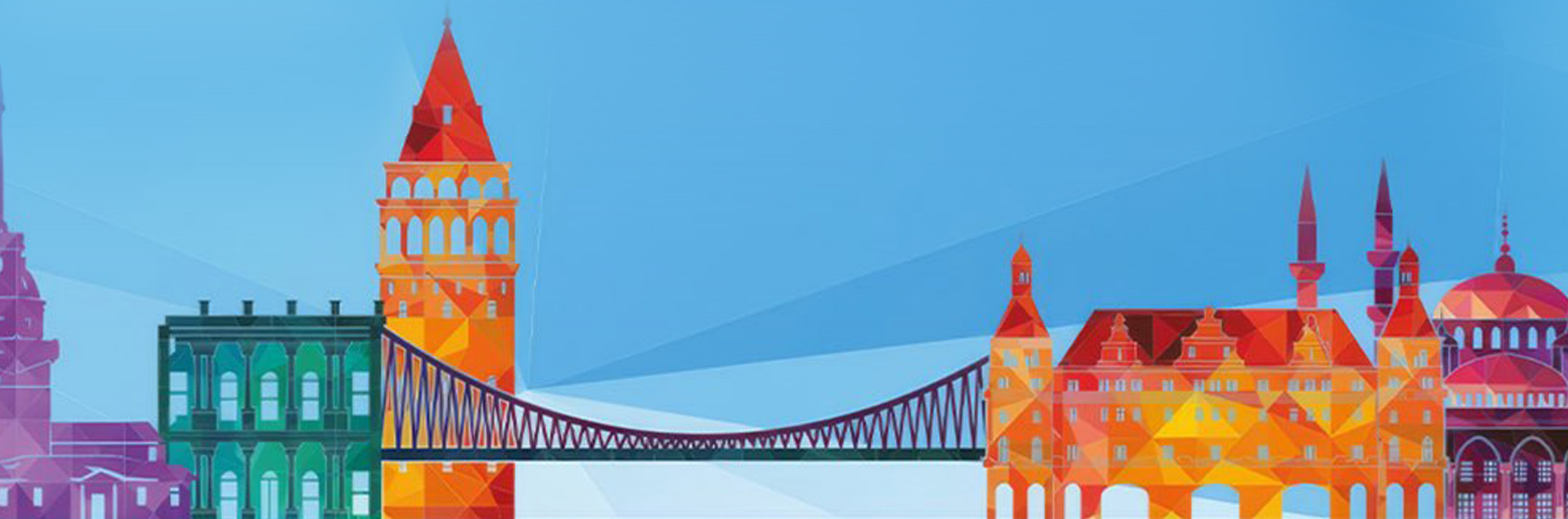

Oral Presentations

OP-01

The Use of Donor-Derived Cell Free DNA in Post-Kidney Transplant Monitoring

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Kidney transplantation is the optimal treatment for end stage kidney disease. However, rejection episodes remain a main cause of reduced graft survival and graft biopsy is the gold standard of the diagnosis.

Consequently, the need of a non-invasive and early detectable biomarker of rejection has recently led to the study of donor-derived cell free DNA (dd-cfDNA).

Thirty patients who underwent kidney transplantation (16 living and 14 deceased) from October 2023 until July 2024, were randomly selected for the dd-cfDNA quantification with the AlloSure test. Measurements of dd-cfDNA from patients' plasma took place 1 month, 3 months and 6 months post transplantation and in some cases before a "for cause" graft biopsy.

The preliminary results of dd-cfDNA measurements were combined with established laboratory markers used to diagnose rejection (serum creatinine, proteinuria, DSAs) and the tacrolimus level at the time of the measurement. Elevated values (equal or above the threshold of 0.5%) were noticed in 16/30 samples at 1 month after transplantation and correlated with T-cell-mediated rejection, acute cell-mediated rejection, mixed rejection and graft injury of other causes. Twenty-two patients of the sample underwent a second measurement at 3 months and 4 of them had a third measurement at 6 months. Also, in cases of "for cause" graft biopsy the results were associated with the dd-cfDNA value.

Dd-cfDNA is a new promising biomarker that contributes in the early detection of rejection. Therefore, threshold values and surveillance strategies in kidney transplant patients need to be established by further studies.

OP-02

Time-Dependent Change of $CD4^+$ and $CD8^+$ T-Cell Ratio in the Presence of Donor-Specific Antibody

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Abstract: In the context of cell, organ, and tissue transplants, the functionality of the graft tissue is a critical factor in determining survival outcomes. The cellular and secretory components of both the innate and adaptive immune systems initiate a rejection response against graft tissue that is recognized as foreign. $CD4^+$ T-cells have been shown to participate in the inflammatory response by secreting cytokines. In contrast, $CD8^+$ T-cells become effectors and kill target cells by exerting a cytolytic effect. B lymphocytes activate the complement system with donor-specific antibodies against human leukocyte antigen (HLA) structures of the donor that they recognize as foreign.

Introduction: Anti-donor HLA antibodies can be detected de novo after cell, tissue and organ transplantation and play an important role in long-term survival. *De novo* donor-specific antibodies (dnDSAs) are the cause of antibody-mediated allograft rejection (1,2). Rejection reactions, especially after heart transplantation, have been associated with death. In the context of antibody-mediated rejection, graft tissue damage is typically characterized by complement activation (3,4). This activation of the complement system subsequently leads to the migration of inflammatory cells, which plays a pivotal role in the rejection mechanism (5). $CD8^+$ T-cells have been observed to exert a cytolytic effect on target cells by secreting proteins such as perforin and granzyme. $CD4^+$ T-cells, on the other hand, have been shown to participate in the rejection response by secreting cytokines that promote inflammation (6). The aim of this study was to investigate the time-dependent changes in $CD4^+$ and $CD8^+$ T-cell ratios in the presence of donor-specific antibody. The aim of this study was to investigate the time-dependent changes in $CD4^+$ and $CD8^+$ T-cell ratios in the presence of donor-specific antibody.

Material and Methods: Lymphocytes were isolated from a healthy volunteer donor's peripheral blood sample. The presence of DSA was detected using LABScreen Single Antigen Assay (One Lambda). Mean fluorescence intensity (MFI) values greater than 1000 for DSAs against HLA-A, -B, -C, -DR and -DQ at four-digit levels were considered positive. The study protochol was planned as fallows; lymphocytes were divided into three major groups; first lymphocyte and DSA + A*24:02 (MFI: 10.173), DRB1*11:04 (MFI: 14.469), lymphocyte with autologous serum group and lymphocyte with medium groups. Each group incubated and cultured in CO_2 incubator at 37°C for 6 and 24 hours. Cell distributions from each group were evaluated by flow cytometry method using CD45, CD3, CD4, CD8 monoclonal antibodies. Each experimental setup was repeated 3 times and the results were evaluated as compatible with each other.

Results: As a result, it was clear that $CD45^+$, $CD3^+$, and $CD3^+$ $CD4^+$ cell ratios were increased in all of the three groups in a time-dependent manner and the increase was higher in the presence of DSA. For the $CD3^+$ $CD8^+$ cell group, a time-dependent decrease was observed and the decrease was not found to be correlated with the presence of DSA. Each experimental setups' results were aligned with each other (Figure 1).

Oral Presentations

Discussion: HLA epitopes and eplet mismatches are known to be associated with dnDSA production. Ashimine et al. (7) shows that T-cell epitope analysis might be more effective in predicting dnDSA production via memory response in potentially sensitized recipients, although both B- and T-cell epitope analyses are important in the primary immune response of unsensitized patients. Our study mimicked sensitized patients in the presence of both class-I and class-II DSA and study's results showed that total T lymphocyte ($CD45^+ CD3^+$) ratio and $CD3^+CD4^+$ T-cell ratio increased more with the presence of DSA compared to the control group. The result indicated that $CD4^+$ Th cells responded to DSA in a short time. On the contrary, a time-dependent decrease in $CD8^+$ T-cells was determined, but no DSA effect was detected. The cell group that responds to the presence of DSA in the first 24 hours has been identified as $CD4^+$ T-cells. Due to the cytokine effect secreted by $CD4^+$ T-cells, the immune system response starts in the early period.

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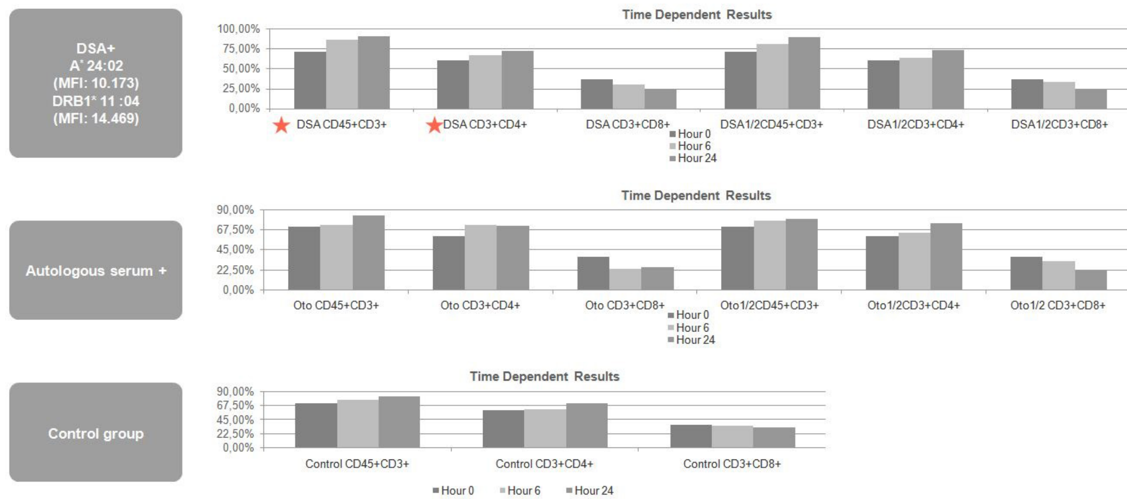

Figure 1. $CD4^+$ and $CD8^+$ cell ratios depending on time and presence of DSA.
DSA: Donor specific antibody

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OP-03

De Novo Donor Specific Antibodies: The Impact on Graft Survival and Rejection in Renal Transplantation

Tatjana Dukic, Jelena Dmitrovic, Barbara Jovanovic, Aleksandra Milojevic, Zorana Andric

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The presence of *de novo* donor specific antibodies (dnDSA), has been associated with poor transplant outcomes, including high incidence of antibody-mediated rejection, graft dysfunction, and inferior graft survival. Moreover, dnDSA can appear before graft loss, suggesting that dnDSA may represent a mechanism of repetitive injury and a potential prognostic biomarker (1-3). The development of dnDSAs after kidney transplant were reported in 13-30% of previously non-sensitized patients (4-6). DnDSA are predominantly directed to donor human leukocyte antigen (HLA) class II mismatches and usually occur during the first year of kidney transplant, but they can appear any time, even several years later (7). This study was based on the identification of donor-specific anti-HLA antibodies, their complex characteristics including antibody classes, HLA specificity, strength, immunoglobulin G subclass, and complement binding capacity in long-term post-transplant follow-up of patients, as well as their impact on graft survival.

This retrospective study included forty-one pediatric patients aged <18 years, who received first renal transplants at the University Children's Hospital, Belgrade, Serbia, between January 2008 and December 2018. All patients were non-sensitized and had been transplanted from ABO compatible donor with negative complement-dependent cytotoxicity (CDC) crossmatch. Nineteen patients underwent transplantation from a living donor and 22 from a deceased donor. Serum samples were collected at 0, 3, 6, 12 months and yearly thereafter, or at the time of biopsy when clinically indicated (graft dysfunction or suspicion of rejection). Sera were screened for HLA-specific antibodies using CDC (One Lmbda LCT 60 and in house panel) and solid-phase Luminex antibody-detection beads (Luminex, LMX, Immucor). Selected HLA-specific antibody-positive samples were analyzed using Luminex single-antigen class I and class II antibody-detection beads (lifecodes LSA1, LSA2). Specificities with mean fluorescence intensity values (MFI) >2000 were considered positive. If donor-specific antibodies were absent pretransplant, as determined by solid phase assays and became detectable post-transplant they were classified as dnDSA. All patients continue to be prospectively tested for dnDSA according to the serum collection schedule outlined above to detect new dnDSA or to assess the persistence of existing dnDSA.

During a mean follow-up time of 6.1±4.5 years, 20/41 (48%) patients developed dnDSA. Although the group of living donor kidney transplant correlated with 0-3 HLA mismatches (MM) ($p<0.01$), no statistically significant difference was proven in the presence of dnDSA in relation to the type of donor, living or deceased ($p=0.176$). By CDC method positive result for HLA antibody class I was obtained in five patients. In two cases, DSAs were confirmed, while in three others, detected antibodies were non-DSA.

By Luminex method, dnDSA class I were detected in two patients ($p<0.0001$), 12 patients had dnDSA class II only ($p<0.0001$) and both class I and II observed in 6 patients. The majority of dnDSAs were class II antibodies and HLA-DQ were among the most frequent specificity, with a statistically higher prevalence ($p<0.0001$).

Seven patients rejected the graft and were back on hemodialysis. In two cases, rejection occurred in the group of living donor recipients, in 5 cases it was in the group of deceased donor recipients. Time from the first detection of dnDSA to the graft failure was 2.7-3.9 years. Of the patients with dnDSA, all graft losses occurred in patients with MFI in the moderate, strong or very strong range with none occurring in those with weak DSA.

The 5-year graft survival was better in the group of living donor recipients. However, over a ten-year period, the log rank test did not show a significant difference in survival length between the groups (Figure 1).

The 10-year graft survival for patients with dnDSA was lower than that of the no dnDSA group (52% vs. 95%, $p<0.05$) (Figure 2). Patients with HLA-DQ DSA had lower 10-year graft survival compared to the group of patients without HLA-DQ DSA (41.3% vs. 91%, $p<0.05$) (Figure 3). The 10 - year graft survival was significantly worse when DSA were combined with non-DSA compared with DSA alone, non-DSA alone or without DSA ($p<0.05$) (Figure 4).

This study showed correlation of graft survival in relation to the type of transplantation, DSA, class of antibodies, strenghts and specificities. Patients with DSA and antibody-mediated rejection (ABMR) had lower graft survival than those without DSA. The proportion of dnDSA was high, with the majority against HLA-DQ. The detection of dnDSA prompted early diagnosis and treatment of ABMR. Long-term transplant outcomes may benefit from routine DSA monitoring. The challenge is to develop a cost-effective DSA monitoring algorithm. However, currently, there is no standard or consensus follow-up protocol for dnDSA after transplantation.

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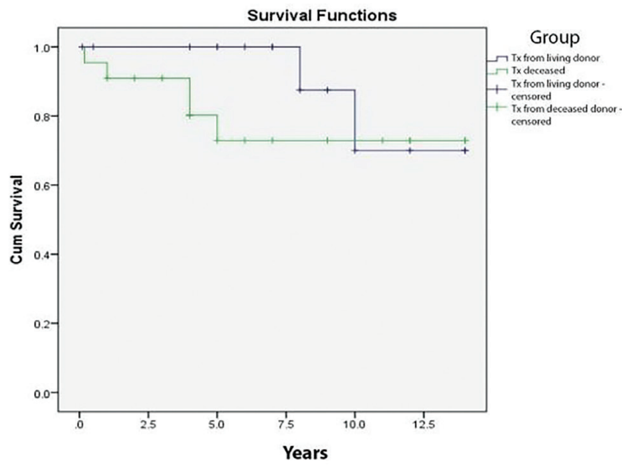

Figure 1. Graft survival related to type of donor (L/D).

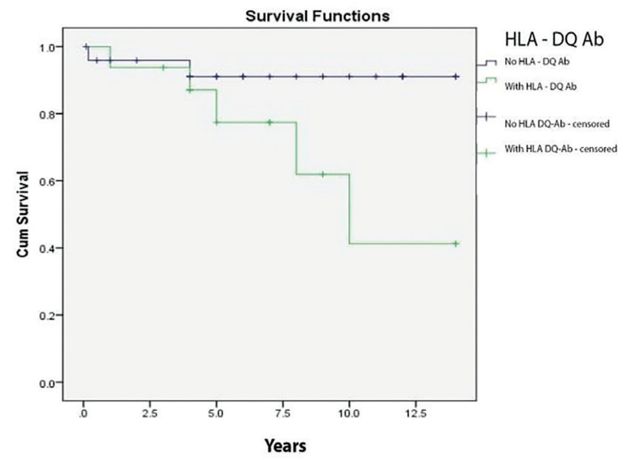

Figure 3. Graft survival in patients with and without HLA-DQ DSA.
HLA: Human leukocyte antigen, DSA: Donor specific antibodies

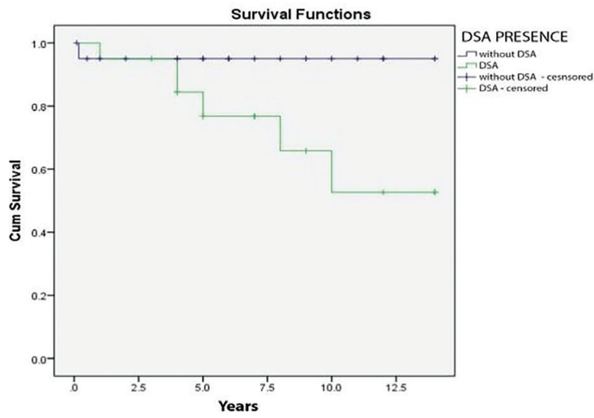

Figure 2. Graft survival in patients with and without DSA.
DSA: Donor specific antibodies

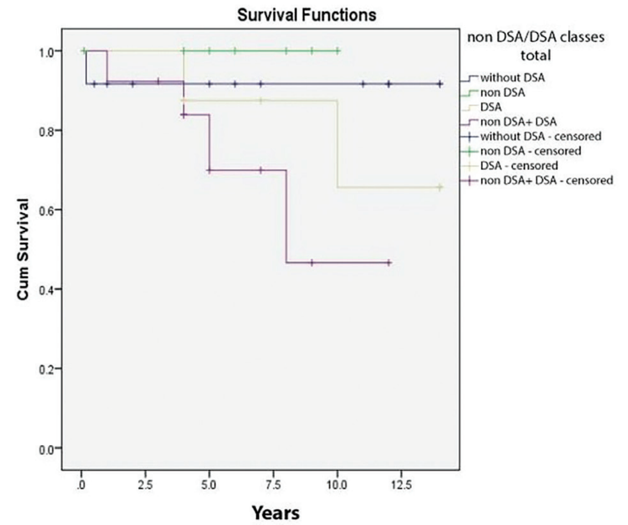

Figure 4. Graft survival in patients with DSA and non-DSA.
DSA: Donor specific antibodies

Oral Presentations

OP-04

Effect of Golgi Stress on CXCR5+ HLA- G+ Expression in T Follicular Helper Cells

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Objectives: The aim of study was to investigate the effect of Golgi stress induced in follicular B-cells and steroid treatment on human leukocyte antigen (HLA)- G expression in CXCR5- expressing T follicular helper (Tfh) cells.

Materials and Methods: T- and B-cells isolated by negative selection from spleen samples of 3 different volunteers were used in the study. Golgi stress was induced by treatment of B-cells with brefeldin A (BFA) and steroids were administered by T:B co-culture. For the effect of BFA and steroids, the time-dependent (0/6/12 hours) change in CXCR5+ HLA- G+ expression in Tfh cells was evaluated by flowcytometry method.

Results: Golgi stress induced in follicular B-cells significantly increased CXCR5+ HLA- G+ expression on Tfh cell surface in a time-dependent manner. HLA- G expression on the CXCR5+ Tfh cell surface was found to increase in a time-dependent manner with steroid treatment. In the presence of BFA, HLA- G expression on the CXCR5+ Tfh cell surface was found to increase in a time-dependent manner with steroid effect.

Conclusion: HLA- G is a non-classical class-I major histocompatibility complex molecule that plays a role in modulating the immune system. Expression of the HLA- G molecule on the CXCR5+ Tfh cell surface, which shows the chemotactic effect of T follicular helper cells, is increased. The increased expression of HLA-G on the surface of CXCR5+ Tfh cells indicates that the immunomodulatory effects of these cells increase depending on the relationship of these cells with B-cells under the effect of BFA and time.

OP-05

Using Machine Learning to Examine Pre-Transplant Factors Influencing *De Novo* HLA-Specific Antibody Development Post-Kidney Transplant

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Objective: The development of *de novo* donor-specific antibodies (dnDSA) against human leukocyte antigens (HLA) is linked to premature graft failure in kidney transplantation. However, rates and influencing factors of *de novo* DSA formation vary widely in existing literature. This study aims to identify pre-transplant factors affecting *de novo* HLA-specific antibody development post-transplantation using machine learning models.

Materials and Methods: Data from 460 kidney transplant recipients at a single center between 2009-2014 was analysed. Pre-transplant variables were collected, and post-transplant sera were screened for HLA antibodies. Positive samples underwent further investigation with single antigen bead testing. Machine learning models, including CART, Random Forest, XGBoost, and CatBoost, were trained on pre-transplant data to predict dnDSA formation. Models were evaluated with and without SMOTE oversampling, using F1 scores for performance and SHAP for feature importance.

Results: In the full cohort analysis, XGBoost models performed the best, achieving the highest F1 scores (0.54-0.59 without SMOTE; 0.72-0.79 with SMOTE). The strongest predictors were pre-transplant HLA antibodies, number of previous kidney transplants, cold ischemia time (CIT), recipient age, and female gender. Pre-existing HLA antibodies and past transplants increase the risk of dnDSA development. Notably, extreme CIT durations and older age (over 65) link to a lower predicted probability of dnDSA. In the unsensitised cohort, models had poor predictive power.

Conclusion: Machine learning models can identify pre-transplant risk factors for *de novo* DSA development in kidney transplantation. Monitoring and stratifying patients based on these factors may guide preventive immunological strategies and recipient selection, potentially improving long-term allograft outcomes.

Oral Presentations

OP-06

Is There a Relationship Between BK Virus and Human Leukocyte Antigen Subtype in Kidney Transplant Patients: A Retrospective Study

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Objectives: Renal transplantation is still recognised as the best treatment for patients with end-stage renal disease as it improves survival and quality of life (1). Human leukocyte antigen (HLA) compatibility plays a key role in the success of kidney transplantation (2). HLA consists of a 3.6 million base pair genomic region (6p21) located on the short arm of chromosome 6. In fact the HLA region is the most variable region of our DNA. HLA molecules are cell surface glycoproteins whose primary function is to present endogenous and exogenous antigens to T lymphocytes for recognition and response. Some HLA subtypes have been associated with various autoimmune, neurological, rheumatological and viral infections. HLA is also involved in the development of immune response against viral infections (3-5). The main target of the recipient immune system in renal transplantation is the HLA molecules on the surface of donor cells. Graft survival is better in transplants from full HLA-matched siblings than in transplants from less compatible living or cadaveric donors (5,6).

On the other hand, modern immunosuppressant therapies allow to increase graft survival in all cases. However, aggressive immunosuppressive therapies can cause increased opportunistic infections such as BK virus (BKV) infections (7). As with some viral infections, BKV has been associated with various HLA subtypes (8-11). In this study, the relationship between HLA subtype and BKV infections occurring due to immunosuppression in kidney transplant recipients was evaluated.

Materials and Methods: Our study was planned retrospectively and HLA A, B, C, DRB1, DQB1 and DQA1 tissue typing were studied before transplantation in the study group. DNA isolation was performed from whole blood samples taken from patients. Tissue typing tests were performed by sequence-specific oligonucleotides and/or sequence-specific priming method based on polymerase chain reaction (PCR). After transplantation, patients were tested for BKV. DNA was isolated from urine and/or plasma samples of patients. Isolated samples were quantitatively evulated for BKV by the real-time PCR method.

In our study, the data of 71 patients who underwent kidney transplantation between November 2018 and November 2024 were retrospectively analyzed. Statistical analyses were performed using the SPSS software version 24. The variables were investigated using visual and analytical methods to determine whether or not they are normally distributed. Since the HLA sub-groups numbers were not normally distributed; nonparametric tests were conducted to compare these parameters, as well as to compare the nominal variables. The Mann-Whitney U test used to compare HLA sub-groups and BKV condition between the groups. A p-value of less than 0.05 was considered to show a statistically significant result.

Results: Retrospectively analyzed 23 of 71 patients were found to be BKV positive. While HLA-A, HLA-B and HLA-DRB1 subgroups were analyzed in all patients, HLA-C and HLA-DQB1 subgroup analyses were performed in 17 BKV positive patients and 36 BKV negative patients. HLA-DQA1 subgroup analysis was performed in 13 BKV positive patients and 32 BKV negative patients. In our analysis, HLA-B*55 (p=0.003), HLA-C*03 (p=0.016), HLA-DRB1*13 (p=0,006) alleles were found to be significantly higher in the BKV positive group, while no statistically significant difference was found between the BKV positive and negative groups in HLA-A, HLA-DQB1 and HLA-DQA1 alleles. On the other hand, no statistically significant relationship was found between the BKV positive and negative groups in gender, donor type, and age (Table 1).

Conclusion: Since the number of patients included in the study was relatively small and HLA-C subgroup analysis was not performed in all patients, studies involving a larger patient population are needed to establish a link between HLA-B*55, HLA-C*03, HLA-DRB1*13 alleles and the risk of developing BKV disease.

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Table 1. Clinical characteristics of patients positive and negative for BKV

Parameter	BKV negative (n=48)	BKV positive (n=23)	P-value
Sex			0.113
Male (n=43)	32	11	
Female (n=28)	16	12	
Donor type			0.334
Living (n=51)	35	17	
Deceased (n=20)	13	6	
Age group			0.286
Pediatric (n=15)	10	5	
Adult (n=56)	38	18	

BKV: BK virus

Oral Presentations

OP-07

Does Human Leukocyte Antigens Heterozygosity Influence the Gut Microbiota?

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Introduction

Within the human major histocompatibility complex (MHC), *human leukocyte antigens (HLA)* genes encode proteins that play a critical role in immune system function. *HLA* genes are some of the most polymorphic genes in the human genome. An individual HLA allele can present T-cells with a restricted repertoire of peptide motifs from self or non-self proteins. Differences between HLA alleles are predominantly due to differences in the peptide binding domain encoded by exons 2 and 3 in HLA class I alleles and exon 2 in HLA class II alleles. This allelic variation and subsequent variation in the peptide binding repertoire can lead to differences in immune response between individuals with different HLA phenotypes (1).

The human body is colonized by thousands of different microbial species that are key to our survival (2). The human microbiota is the ecological community of symbiotic, commensal and pathogenic microorganisms living on the surfaces and in specific niches of organisms (gut, skin, mouth, etc.) (3). The composition of the microbiota differs in various parts of the gastrointestinal tract and is influenced by factors such as pH, host secretion and substrate availability. Host HLA genotype may have a direct impact on the regulation of the host immune system through the recognition of specific molecules of symbiont microorganisms (4). It is not yet known whether HLA molecules control the composition of the microbiome through immune-mediated elimination or by directly influencing bacterial colonization.

In this study, we build on long-standing work in the HLA field and discuss the impact of HLA heterozygosity and homozygosity in shaping and regulating microbiome composition (and vice versa).

Materials and Methods

Thirty-three healthy individuals were included in the study. After nucleic acid isolation from stool samples, the samples were sequenced using the next generation sequencing. The operational taxonomic unit tables were plotted by calculating alpha diversity with R Statistical Computer Language. Statistical analyses were performed using R Statistical Computer Language. Associated pathways were analyzed with the KEGG database.

Results

The mean age was 45.27±12.37 years (27-67). The gender distribution was male/female: 17/16. Most common allels were HLA-A*24:02, HLA-B*35:01, HLA-C*04:01, HLA-DQB1*03:01 HLA-DRB1*13:01. The alpha-diversities of the gut microbiomes of people that are homozygous or heterozygous for five different HLA types (HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQB1) were compared using a Wilcoxon rank sum test. Diversities were calculated on the genus level. The results of the analyses conducted among the individuals included in our study revealed that individuals with HLA allele heterozygosity had a higher microbiota diversity than individuals with HLA allele homozygosity.

In the microbiota analysis between HLA homozygous and heterozygous individuals, *Corobacteriales Incertae Sedis* (p=0.04), *Streptococcaceae* (p=0.02), undefined *Corlobacterialiales Incertae Sedis* genus (p=0.04), *Streptococcus* (p=0.02) were predominant and statistically significant, whereas in individuals without HLA homozygosity, *Lachnospirales* (p=0.04), *Provetellaceae* (p=0.03), *Lachnospiraceae* (p=0.04), *Provetalla* (p=0.02), undefined *Lachnospiraceae* genus (p=0.04) were predominant and statistically significant. In HLA-A group some of significantly different groups based on p-values are; *Actinobacteria, Negativicutes, Lachnospirales.* In HLA-B group some of significantly different groups based on p-values are; *Streptomyces* spp.*, Oscillibacter, Lachnospiraceae* UCG-003.

In HLA-C group some of significantly different groups based on p-values are; *Candidatus Soleaferrea, Bacteroides caccae, Lactobacillus delbrueckii.* In HLA-DBQ1 group some of significantly different groups based on p-values are; *Verrucomicrobiae, Clostridia* UCG-014*, Lachnospirales.* In HLA-DQB1 group some of significantly different groups based on p-values are; *Oscillibacter, Lachnospira.* KEGG pathways are depicted here in three hierarchical levels. Level 1 KEGG pathways are the most broad pathways, which encompass the level 2 and level 3 pathways. The most number of significantly different pathways were seen in HLA-A homozygote vs heterozygote samples.

Discussion

The gut associated microbiome is one of the most studied and related diseases. The role of the HLA complex in some microbiota-related disease development is postulated through the theory of molecular mimicry, among others.

Persons heterozygous at a particular HLA locus may defend against a greater diversity of pathogens compared to homozygous individuals, or the utility of particular alleles may be regulated by pathogen incidence.

Metabolite pathway-mediated up- or down-regulatory interactions of the microbiota with HLA alleles may alter the course of the disease or the immune response.

Keyword: HLA, microbiota, homozygous, heterozygous

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Oral Presentations

OP-08

Evalutain of Patients with Donor Specific Antibody Positivity Using the Single Antigen Method in a Single Center Study

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Objectives: Antibodies to human leukocyte antigens (HLA) may develop in the blood of recipients due to blood transfusions, rejection after transplantation, pregnancy and abortion. These antibodies are called panel reactive antibody (PRA) because they are generally from common HLA panels in the world. As a result of this tests, patients with antibodies against HLA antigen above 70 % are consireder as hyper sensitized. High sensitization in kidney transplantation patients emerges as a major problem for transplantation. To day, different methods are used to detect them (Flow cytometry, Luminex, ELISA etc.). In the Luminex method we use, multi antigen coated beads tests are applied to detect antibodies. If the test serum contains anti- HLA antibody it will bind to the appropriate HLA molecule.

Materials and Methods: In this study, HLA antibody levels from the same sera samples of a total of 89 patients, 54 women and 35 men, who were prepared for kidney transplantation at Başkent University İstanbul Hospital between 2017 and 2024, were determined by using life codes PRA class ½ identification test and life codes ½ identification tests by Luminex method measured.

Results: As a result of multiple antigen tests, more than 70% sensitization was detected against both class 1 and class 2 antigens of the patients. When the same samples were re-evaluated with the single antigen bead method, it was observed that the desensitization values were considerably reduced compared to the values in the multiple antigen method. However, we observed that the donor-specific HLA antibodies (DSA) mean fluorescence intensity values also decreased to negative or acceptable limits.

Conclusions: Graft damage from alloimmune injury and the reduced access of highly sensitized patients to transplantation are two of the major challenges facing the transplant community. The accurate determination of clinically relevant HLA antibodies and an appropriate interpretation of their impact are essential in addressing these issues. In the detection of HLA antibodies, a single perfect test providing the desirable accuracy, quantitation, sensitivity and specificity does not exist. Consequently, HLA laboratories must incorporate multiple laboratory tests and analyses with information about each individual to ensure that the correct information regarding immunological risk is provided for each patient. In conclusion, in the light of these findings, we believe that the single antigen coated bead method is very useful in determining the risk of DSA in highly sensitized patients.

OP-09

Neutrophils Get All Worked Up When They See the Kidney!

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Neutrophils the immune system’s “first responders” who have often been dismissed as simple-minded, fast-acting foot soldiers. Yet these cells can be a nightmare in kidney disease; they are hidden main characters with a surprising range of terrific effects. Recent research reveals that neutrophils don’t just swarm into injured tissues and vanish. They actively contribute to kidney injury and even rejection after transplantation, often through NETosis-a dramatic process where they cast out webs of DNA and proteins to trap pathogens, but in the process, they trigger intense local inflammation.

The reality is that neutrophils’ enthusiasm for “saving the day” can make things worse for the kidney, driving fibrosis and damage that challenges graft survival. These findings call for a shift in our perspective it is time to rethink neutrophils as critical targets for kidney disease diagnosis and treatment. By harnessing insights into neutrophil behavior, we may open new doors to therapies that preserve kidney function and extend graft life. So, let’s give these overlooked cells the attention they deserve, before they do more damage to our transplants.

Balkan EPT Meeting

15-16 November 2024

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Balkan EPT Meeting 2024
Poster Presentations

Poster Presentations

PP-01

Anti-HNA-3a Antibodies: A Cause of Unexpected Positive Flow Crossmatches in Kidney Transplantation

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Objectives: Crossmatch-(XM) testing is the established method for donor-specific anti-human leukocyte antigen (HLA) donor specific antibodies (DSA) detection prior to kidney transplantation. However, a positive T/B-flow-XM-(T/B-FXM) can occur in the absence of HLA-DSA, highlighting the role of other histocompatibility systems. Recent, guidelines have included human-neutrophil-antigens antibodies 3-a (anti-HNA-3a) testing for cases with unexplained positive XM. The aim of this study was to investigate the frequency of anti-HNA-3a in kidney transplant candidates-(KTC) and to present three T/B-FXM (+) cases due to circulating anti-HNA-3a.

Materials and Methods: Anti-HNA-3a were measured in 509 KTC using the LabScreen Multi-kit (One-Lambda). A positive result was determined by a normalized background value-(NBG)≥10 and MFI>1000. HNA typing was performed using PCR-SSP.

Results: A total of 8 patients (1.57%), 7 of whom were women, exhibited positive anti-HNA-3a with an average MFI: 6512 and NBG: 54. All women had a history of pregnancy and/or blood transfusions. During the study period, three anti-HNA-3a positive patients were selected for XM with deceased donors (DD), according to the DD-allocation rank.

Patient 1: 62-year-old woman, cPRA: 63%,MFI-anti-HNA-3a: 13682, XM with 3-DDs.

Patient 2: 40-year-old woman, cPRA: 0%, MFI-anti-HNA-3a: 15420, XM with 5-DDs.

Patient 3: 49-year-old woman, cPRA: 0%, MFI-anti-HNA-3a: 4245, XM with 1-DD.

All candidates exhibited unexplained T/B-FXM (+) in the absence of HLA-DSA or autoantibodies, resulting in their exclusion from transplantation. HNA typing revealed that the three patients were homozygous for HNA-3b and all donors expressed HNA-3a, confirming that the positive T/B-FXM was caused by the anti-HNA-3a.

Conclusion: Anti-HNA-3a can lead to positive FXM in allo-sensitized HNA-3b homozygous patients, given that most donors express HNA-3a. Although the clinical significance of anti-HNA-3a has not been fully elucidated, their screening is important for immunological risk assessment and timely therapeutic intervention.

PP-02

High-Resolution Characterization of KIR Genes Polymorphism in Healthy Individuals From the Bulgarian Population

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Objectives: Killer-cell immunoglobulin-like receptor *(KIR)* gene content has been widely studied in health and disease. In recent years KIR allele-level diversity has been described across human populations and the results of these studies highlight the population-specific nature of KIR diversity. Considering this, in the present study we aimed to analyze for the first time the allele polymorphism of nine *KIR* genes defined by a high-resolution method in the Bulgarian population.

Materials and Methods: In the present *KIR* gene polymorphism was investigated in 155 healthy, unrelated individuals from the Bulgarian population by applying next-generation-sequencing (NGS). Nine *KIR* genes (KIR2DL1,KIR2DL2,KIR2DL3,KIR2DL4,KIR3DL1,KIR3DS1,KIR3DL2,KIR3DL3, and KIR2DS2) were typed by NGS methods using the commercially available kits of GenDx, NGSgo, and then analyzed by NGSengine software (v. 2.29.0).

Results: In Bulgarians, the allele frequency distribution of *KIR* genes was found to be comparable to that observed in other European populations. The highest degree of polymorphism was observed for the *KIR3DL3* gene with 23 observed common alleles. On the contrary, the *KIR3DS1* gene was found to have the lowest degree of polymorphism with only two observed alleles in Bulgarians: KIR3DS1*01301 (31.6%) and KIR3DS1*049N (0.7%). The obtained results from the Ewens-Watterson test of neutrality suggest selection events that maintain the genetic variation in the population. Pairwise linkage disequilibrium (LD) for the 10 KIR loci was also estimated where some instances of strong LD among specific sets of KIR alleles were observed.

Conclusion: This is the first study investigating KIR allelic polymorphism at high resolution in a Southeast European population. This data will contribute to a better understanding of the genetic heterogeneity of this region and can be also may be applicable in clinical practice.

Poster Presentations

PP-03

Evaluation of Nanopore DNA Sequencing Technology for Urgent HLA High-Resolution Typing of Cadaveric Donors

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Objective: The definition of human leukocyte antigen (HLA) epitopes necessitates high-resolution (HR) genotyping, often not attainable before the allocation of deceased donors. Nanopore DNA Sequencing technology (3rd generation) emerging as a promising avenue for rapid HLA HR typing.

Materials and Methods: This study evaluates nanopore sequencing for urgent HLA-HR typing of cadaveric donors. It introduces a rapid HLA typing method employing the Nanotype 24/11v2 Ruo assay (Omixon) using 11 loci (HLA-A,-B,-C,-DRB1,-DRB3/4/5,-DQA1,-DQB1,-DPA1,-DPB1) from 30 samples, paving the way for on-call deceased donor allocation. This assay employs multiplexed long-range polymerase chain reaction with library preparation within 90 minutes. Data, generated on a MinION sequencing device using a MinION flow cell type R9.4, undergoes high-accuracy base calling, followed by analysis in Nanotyper software, restricted only in exons.

Results: In comparing results with pre-typed data (AlloSeq Tx17 kit, CareDx) sequencing on MiSeq, the nanopore method yielded 100% concordance for 330 loci with the current next-generation-sequencing method, maintaining a minimum 2-field typing. The method also provided accurate data at all HLA loci in approximately 4 hours, without prolonging allocation time. The average read length was 3150 bp, with an average minimum coverage for key exons of 1630 for all HLA loci. Notably, key exon allelic imbalance for heterozygous samples at most HLA loci was over 0.6.

Conclusion: Conducting HR typing across all HLA loci for deceased organ donor allocation has notable clinical benefits: The implementation of nanopore HLA typing for deceased donors before transplantation, paired with antibody screening and identification, enhances virtual cross-match accuracy, especially for hypersensitized recipients, within a time-efficient framework.

PP-04

Eight Digit Human Leukocyte Antigen -A, -B, -C, -DRB1, -DQB1, Allele and Haplotype Frequencies with Next Generation Sequencing, Single-Center Experience

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Objectives: There is a lack of data on the 8-digit human leukocyte antigen (HLA) frequencies in the Turkish population, and there are no reports of haplotype analysis in this population. The aim of this study was to evaluate the 8-digit 5 loci (HLA-A, -B, -C, -DRB1, -DQB1 and -DPB1) allelic data of 364 individuals in our database.

Materials and Methods: The 8-digit allele were sequenced using next generation sequencing (NGS) methods, and the resulting data were analysed using MIA FORA NGS FLEX HLA genotyping software (version 3.0). The assessment of the complete HLA data set was conducted utilising PyPop software, version 1.0.2.

Results: A total of 54 HLA-A, 96 HLA-B, 62 HLA-C, 74 HLA-DRB1, and 45 HLA-DQB1 alleles were identified. The three most frequent allele for each loci were A*02:01:01:01 (0.15247), A*24:02:01:01 (0.14973), A*01:01:01:01 (0.125); B*51:01:01:01 (0.07143), B*49:01:01:01 (0.0522), B*35:01:01:02 (0.04396); C*12:03:01:01 (0.08791), C*07:01:01:01 (0.07692), C*04:01:01:06 (0.07555); DRB1*11:04:01:01 (0.09066), DRB1*03:01:01:01 (0.08516), DRB1*07:01:01:01 (0.06593); DQB1*03:01:01:02 (0.15659), DQB1*02:01:01:01 (0.08929), DQB1*03:02:01:01 (0.08379).

Haplotype calculation showed that A*01:01:0 1:01~B*08:01:0 1:01~DRB1*03:01 :01:01 (0.01374); A*01:0 1:01:01~B*08:01: 01:01~C*07:01:01:01~DRB1*03 :01:01:01 (0.01374) and A*01:0 1:01:01~B*08:01:01:01~C*07:01 :01:01~DRB1*03:01:01 :01~DQB1*02:01: 01:01 (0.01236) were the most frequent haplotypes in our population.

Conclusion: The present study identified the frequencies of 8-digit HLA-A, -B, -C, -DRB1, and -DQB1 allele and haplotype frequencies in a Turkish population using NGS. The newly acquired data can be employed in the mapping of HLA patterns in our country, thereby providing a foundation for the design of subsequent investigations.

Poster Presentations

PP-05

"Immunogenetics Cerberus" or Donor Selection Problems for a Patient with Major Histocompatibility Complex Triple Haplotype After Acute Myeloid Leukemia Relapse

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Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is one of the most effective treatment for acute myeloid leukemia (AML) patients. At the same time, minimal residual disease and donor chimerism detection is critically important to monitor engraftment level as well as to predict early relapse development. Also, determination of HLA-LOH is vital for managing for immunological consequences of relapse.

The aim of this work was to establish the human leukocyte antigen (HLA)-loss of heterozygosity (LOH) presence or absence to understand the mechanism of immune evasion during relapse in a patient with AML for subsequent donor selection for second allo-HSCT.

Extraction of DNA from peripheral blood was performed using "Genomic DNA from Blood Extraction Kit" (Macherey-Nalel, Germany). For HLA-LOH determination we used SSP Typing Kits (One Lambda, USA) according to the manufacture's protocols.

Male 50 years old patient with AML was transplanted from haploidentical related donor (son). On day +106 the patient was diagnosed with main disease relapse. Post-transplant monitoring data demonstrated partial donor chimerism and hybrid blood group antigens expression (A- from donor and O+ from recipient), indicating incomplete engraftment. After that the patient's samples were directed to our department with suspicion of loss of heterozygosity in *HLA* genes. HLA typing revealed a tri-allelic combination of both donor and recipient alleles, which did not confirm HLA-LOH.

Taking into account obtained results, clinico-biological recommendations for the selection of the next donor and therapeutic treatment options of patients with similar immunogenetic changes are extremely complicated.

PP-06

Comparison of Higher Resolution of Sequence Spesific Oligonucleotide Probe vs. Next Generation Sequencing for HLA-A, B, C and DRB1 Typing

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The objective is to determine the accuracy and the level of resolution of higher resolution of sequence spesific oligonucleotide probe (SSO) vs. next generation sequencing (NGS).

Twenty-eight samples from bone marrow donors and 10 HR BEPT 2024 samples were included in the study. DNA samples were extracted using Maxwell RSC Whole Blood DNA Kit (Promega). Human leukocyte antigen (HLA) typing was done using LabType XR Class I A, B, C and Class II DRB1 Typing Test (One Lambda) and AllType FASTPlex NGS 11 Loci Flex Kit (OneLambda). All tests were done according to manufacturer's instructions using LabScan3D for SSO and iseq 100 (illumina) for NGS. Our study was funded by Ege University Office of Scientific Research Projects with project number 31968.

A total of 304 alleles from 38 samples were typed with both methods for HLA-A, B, C and DR loci. The results were concordant with NGS at low resolution except for two (0.65%) assingments for one specimen, which produced no result for HLA-C. High resolution typing results were concordant except for two (0.65%) ambiguities and two (0.65%) assignments among three samples. The HLA-A*23:01 NGS result was A*23:CJT (01/17) with SSO and HLA DRB1*14:54 with NGS was DRB1*14:BCAD (01/54). Low resolution HLA-B result could be obtained for one sample (B*27 B*35). All discordant results were tested in duplicate.

Among 304 alleles, 6 (1.97%) either produced no result or was discordant with NGS.

NGS should be used where available for highly sensitized kidney transplant candidates. Higher resolution SSO may be helpful when NGS isn't available and for deceased donors.

Poster Presentations

PP-07

Human Leukocyte Antigen Typing Results Among Patients with Probable Celiac Disease

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The objective is to determine the frequency of the presence of risk alleles for celiac disease (CD) among a cohort of patients whose samples were sent for differential analysis of inflammatory bowel disease.

Human leukocyte antigen (HLA)-*DQB1* and *DQA1* genes were typed with LabType SSO Class II DQA1/ DQB1 Typing Test (One Lambda) using LabScan3D. and Celiac Multiplex Real Time PCR Kit (SNP). HLA-DQB1*02/ HLA-DQA1*05 (DQ2.5), HLA-DQB1*03:02 (DQ8) and HLA-DQB1*02/ HLA-DQA1*02 (DQ2.2) were reported as risk alleles for CD.

Seventy-three patients were tested between January 2023 and September 2024. Fifty-two (71.2%) were women and 21 (28.7%) men with an average age of 58. Forty patients (54.8%) were positive and 33 (45.2%) negative for risk alleles. Among 40 patients a total of 45 risk alleles were detected; 22 DQ2.5 (48.9%), 13 DQ8 (28.9%) and 10 DQ2.2 (22.2%). Thirty-five patients had one allele; 19 (47.5%) DQ2.5, 10 (25%) DQ8 and 6 (15%) DQ2.2. Five patients were heterozygous with risk alleles: 2 (5%) DQ2.5/DQ2.2, 2 (5%) DQ2.2/ DQ8 and 1 (2.5%) DQ2.5/ DQ8. Both methods were concordant for risk allele typing except for one patient, where DQ8 was positive with reverse transcription-polymerase chain reaction (RT-PCR) but negative with next-generation-sequencing. RT-PCR could not detect DQ2.2, leading to six (8.21%) DQ2.2 positive patients being negative with RT-PCR.

DQ2.5 homozygosity, the highest risk for severe CD, was not detected. Five patients were heterozygous with risk alleles. Heterozygosity of risk alleles is considered as greater risk, compared with having only one heterodimer. United Kingdom National External Quality Assessment Service and British Society for Histocompatibility and Immunogenetics guideline recommends risk stratification to be included in patient reports.

PP-08

Investigation of Human Leukocyte Antigen and Killer Cell Immunoglobulin-Like Receptor Relationship in Acute Myeloid Leukaemia Patients

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Objectives: Acute myeloid leukemia (AML) is a complex disease and most common type of blood cancer with poor prognosis. AML is a malignancy of the stem cell precursors of the myeloid lineage (red blood cells, platelets and white blood cells other than B- and T-cells). Like other malignancies, it is caused by genetic variations leading to neoplastic changes and clonal proliferation (1). Natural killer (NK) cells, one of the cells of the innate immune system, originate from the bone marrow and constitute 10-20% of the entire lymphocyte population. NK cells, which are involved in the innate immune response, are important cells involved in the immune response against viral infections and tumour cells, especially leukaemia, lymphoma and metastatic tumour cells (2). NK cells are cytotoxic cells that regulate the activity of the immune system through the killer cell immunoglobulin-like receptor (KIR) they express. The activity of NK cells is regulated mainly through KIRs, which are composed of activator and inhibitory receptors and have great genomic diversity. The genes encoding KIRs are located on chromosome 19. Sixteen *KIR* genes have been characterised to date. Fourteen *KIR* genes encode receptors that trigger inhibition (3DL1-3, 2DL1-3, 2DL5) or activation (3DS1, 2DS1-5) or both (2DL4), while two pseudogenes (2DP1 and 3DP1) are not known to encode cell surface receptors. KIRs regulate the function of NK cells by binding with human leukocyte antigens (HLA) class I molecules on the surface of target cells (3-5). In recent years, there has been an increasing number of studies showing an association between AML and KIR. The aim of our study was to determine the frequency of KIR genotypes in patients diagnosed with AML.

Materials and Methods: The study included 22 patients (45.45% female and 54.55% male, with no gender or age difference) who were admitted to Başkent University Adana Dr. Turgut Noyan Research and Medical Centre, Department of Hematology (between 2017 and 2023), diagnosed with AML and died during treatment. HLA typing and KIR typing (Immucor, USA) tests were performed with Luminex method according to the manufacturer's protocol from the patient's DNA sample.

Results: In the study, haplotype A was identified in 6 of 22 patients, haplotype B was identified in 16 of 22 patients, 27.27% and 72.73% respectively. Two of the 6 haplotypes A were C1/C1 and 4 was C2/C1. C1/C1 was determined as 13.64%, C1/C2 was determined as 72.73% and C2/C2 was determined 13.64%. In all patients, KIR3DL3, KIR3DL2, KIR3DP1 and KIR2DL4 (framework genes) were detected. The inhibitory KIR2DL1 which recognize C2 group allele, was most frequently detected in the AML patient group. On the other hand, KIR2DS5 (36.36%) was detected with the lowest frequency. *KIR* gene frequency and profile in AML patients studied are given in Figures 1 and 2, respectively.

Discussion: Previous studies have investigated the relationship between KIR, HLA and AML, but the results have been found to vary according to the

ethnic groups studied. Notably, there is a distinct gene content profile among the several populations. Caucasians and Africans populations have both the A and B haplotypes. Northeast Asians predominantly display AA genotype. In contrast, B genotype (either AB or BB) carrier individuals were observed among the American natives, Australia, and India population (6). In our study, AML patients had both haplotype A and B.

In 2017, a study conducted in South China with 273 control groups and 253 AML patients to investigate the relationship between KIR and AML revealed that the frequency of 2DS4del was significantly higher in AML-M5 patients than in control patients, therefore 2DS4del may be susceptible to AML and genotype BX13 may have a protective effect on AML (7). In our study, 2DS4*all Ex.4 was found in 19 out of 22 patients, 2DS4*full length Ex.5 in 14 out of 22 patients and 2DS4*deletion Ex.5 in 17 out of 22 patients. *KIR* genes vary according to ethnicity and disease. In our study group, as expected, 100% of the genes were found to be part of the framework. Despite the limited size of the patient cohort, it was established that activator genes were less prevalent than inhibitors. This study was approved by Başkent University Instuitional Review Board and Ethics Committee (Project No: KA23/118) and supported by Başkent University Research Found.

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KIR genes	AML (N = 22) n (%)	M0 (N = 8) n (%)	M1 (N = 1) n (%)	M2 (N = 4) n (%)	M4 (N = 5) n (%)	M7 (N = 1) n (%)
3DL1	19 (86,36)	8 (100)	0 (0)	4 (100)	4 (80)	0 (0)
2DL1	21 (95,45)	8 (100)	1 (100)	4 (100)	5 (100)	1 (100)
2DL3	18 (81,82)	7 (87,5)	1 (100)	3 (75)	4 (80)	1 (100)
2DS4*all Ex.4	19 (86,36)	8 (100)	0 (0)	4 (100)	4 (80)	0 (0)
2DL2	15 (68,18)	5 (62,5)	0 (0)	4 (100)	2 (40)	1 (100)
2DL5	15 (68,18)	5 (62,5)	1 (100)	3 (75)	2 (40)	1 (100)
3DS1	9 (40,91)	4 (50,00)	1 (100)	0 (0)	1 (20)	1 (100)
2DS1	9 (40,91)	3 (37,5)	1 (100)	0 (0)	1 (20)	1 (100)
2DS2	16 (72,73)	5 (62,5)	1 (100)	4 (100)	2 (40)	1 (100)
2DS3	11 (50,00)	4 (50)	0 (0)	3 (75)	2 (40)	0 (0)
2DS5	8 (36,36)	3 (37,5)	1 (100)	0 (0)	1 (20)	1 (100)
2DL4	22 (100)	8 (100)	1 (100)	4 (100)	5 (100)	1 (100)
3DL2	22 (100)	8 (100)	1 (100)	4 (100)	5 (100)	1 (100)
3DL3	22 (100)	8 (100)	1 (100)	4 (100)	5 (100)	1 (100)
2DP1	21 (95.45)	8 (100)	1 (100)	4 (100)	5 (100)	1 (100)
3DP1	22 (100)	8 (100)	1 (100)	4 (100)	5 (100)	1 (100)

Figure 1. Frequencies of KIR gene in AML patients.

KIR: Killer cell immunoglobulin-like receptor, AML: Acute myeloid leukemia

Genotype ID	3DL1	2DL1	2DL3	2DS4 *all Ex.4	2DL2 *001	2DL5	3DS1	2DS1	2DS2	2DS3	2DS5	2DL4	3DL2	3DL3	2DP1	3DP1	AML (N = 22) n (%)	M0 (N = 8) n (%)	M1 (N = 1) n (%)	M2 (N = 4) n (%)	M4 (N = 5) n (%)	M7 (N = 1) n (%)
AA1																	6 (27,27)	3 (37,5)	0 (0)	0 (0)	3 (60)	0 (0)
BX106																	1 (4,55)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
BX13																	1 (4,55)	1 (12,5)	0 (0)	0 (0)	0 (0)	0 (0)
BX3																	1 (4,55)	1 (12,5)	0 (0)	0 (0)	0 (0)	0 (0)
BX4																	1 (4,55)	0 (0)	0 (0)	1 (25)	0 (0)	0 (0)
BX5																	2 (9,09)	0 (0)	0 (0)	2 (50)	0 (0)	0 (0)
BX6																	3 (13,64)	2 (25)	0 (0)	0 (0)	0 (0)	0 (0)
BX68																	1 (4,55)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)
BX7																	1 (4,55)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
BX70																	1 (4,55)	0 (0)	0 (0)	0 (0)	1 (20)	0 (0)
BX71																	3 (13,64)	1 (12,5)	0 (0)	1 (25)	1 (20)	0 (0)
BX88																	1 (4,55)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)

Figure 2. The profiles and distribution of KIR genotypes in AML patients

KIR: Killer cell immunoglobulin-like receptor, AML: Acute myeloid leukemia

Poster Presentations

PP-09

Correlation of Anti-HLA-DR51/52/53 Antibodies Positivity with Flow Cytometry Results

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Objectives: Interactions between recipient and donor immune cells occur in the allograft microvasculature. All three human leukocyte antigen (HLA) class II antigens, DR, DP and DQ, have been detected on renal epithelial cell with a markedly increased expression of HLA class II observed in renal allografts undergoing rejection (1). HLA-DR affects the rejection process, especially by playing a critical role in activating CD4+ T-cells. Some DRB1 locus alleles are inherited together with different DRB4 (DR53), DRB3 (DR52), DRB5 (DR51) loci (2). Other HLA-DRB molecules, which are encoded by loci different from HLA-DRB1 are weakly polymorphic (3). In our study, we aimed to investigate the effect of anti-HLA DR51/52/53 detected in PRA tests of kidney transplant candidates on Flow cytometry cross-matching (FCXM) positivity alone or in combination.

Materials and Methods: In our study, the results of 200 patients who underwent simultaneous PRA and FCXM tests in İstanbul University İstanbul Faculty of Medicine, Tissue Typing Laboratory between 2019-2023 were retrospectively analysed. PRA tests were performed with Luminex (Immucor) and cross match tests were performed with FCXM method.

Results: At least one of the antigens belonging to anti-HLA-DR51/52/53 subgroups was positive in 55.5% (n=111) of 200 class II PRA (+) patients included in the study. In the DR51 subgroup, DR15 (p=0.007) and DR16 (p=0.011) were associated with FCXM-B positivity both alone and in combination (p=0.006), while DR16 (p=0.017) was associated with FCXM-T positivity both alone and in combination with DR15 (p=0.019). The combination of DR13, DR14, DR17, DR18 in the DR52 subgroup was associated with FCXM-T positivity(p=0.027). The association of DR4 and DR9 in the DR53 subgroup was associated with FCXM-B positivity(p=0.003).

Conclusion: The results of our study suggest that typing of HLA-DR superfamily subgroups, which are critical for rejection risk, may be important in predicting FCXM positivity. Furthermore, although HLA-DR51, -DR52 and -DR53 antigens are significantly weaker expressed than the general DR antigens originating from the DRB1 gene, HLA-DR51, -DR52 and -DR53 antigens are known to always depend on DR antigens (4,5). Therefore, it is recommended that these antibodies should be taken into account during HLA-DR51/52/53 typing and donor-specific antibodies evaluation for all donors and recipients.

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PP-10

Distribution of HLA-DQA1 and HLA-DQB1 Alleles in Celiac Patients

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Objectives: Celiac disease is a chronic autoimmune enteropathy triggered by the ingestion of gluten, leading to small intestine damage and autoantibody formation. The disease is linked to human leukocyte antigens (HLA)-*DQ* genes that encode the HLA-DQ2 and DQ8 proteins.

Materials and Methods: In this study, 108 adult patients diagnosed with celiac disease and 100 healthy controls included. HLA-DQA1 and -DQB1 alleles were typing with SSO-PCR technique. Relative risks for different alleles were also evaluated.

Results: The allele frequencies between patients and healthy controls, DQA1*03:01 (p=0.011), DQB1*03:02 (p=0.017) are the alleles showing statistically increased in patients. In typical celiac patients DQA1*05:01 and DQB1*02:01 alleles were significantly higher and DQA1*05:05 allele was lower (respectively, p=0.003, p=0.006, p=0.027). There was no statistically significant difference in DQ2 heterozygous genotype frequency between patients and controls. The DQ2 homozygous genotype has a higher frequency in celiac patients, but this increase is not statistically significant. The DQ8 heterozygous genotype was found at a significantly higher frequency in celiac patients than in healthy controls (p=0.018). Gastrointestinal system related findings the DQB1*0501 allele showed a positive association with weight loss (p=0.049). The DQB1*0303 allele was strongly associated with reproductive system symptoms (p=0.041).

Discussion: HLADQ2, HLADQ2.5 and DQ8 genotypes play important roles in determining the genetic susceptibility to celiac disease. The DQ2 heterozygous genotype does not play a significant role in predisposing and DQ8 homozygous carriers may be predisposed to celiac disease. These genetic findings may help to diagnose celiac disease earlier and more accurately and to develop personalized treatment approaches.

Keywords: Celiac disease, human leucocyte antigen, polymerase chain reaction sequence-specific oligonucleotide, HLA-DQA1/DQB1

Poster Presentations

PP-11

Investigation of Non-Classical MHC Class I Molecules in Mesenchymal Stem Cells Derived from Wharton's Jelly

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Objectives: Mesenchymal stem cells (MSCs) can be derived from various sources, including Wharton's jelly (WJ). WJ-derived MSCs stand out with their advantages of easy isolation, expandability, and notable immunomodulatory and anti-inflammatory effects. Recent studies have demonstrated that MSCs derived from WJ express high levels of non-classical MHC-class I molecule, human leukocyte antigens (HLA)-G. This study investigates the effects of interferon-gamma (IFN-γ) stimulation on HLA-G expression in WJ-derived MSCs after treatment with the demethylating agent 5-AZA. MSCs are multipotent somatic stem cells with the capacity to differentiate into various cell types (1,2). Among the mechanisms underlying the immunosuppressive properties of MSCs, the HLA-G molecule has been shown to play an important role (3). HLA-G gene expression significantly decreases in serial passages of MSC cultures (4). DNA methylation is one of the key epigenetic mechanisms controlling gene expression (5). Demethylation in the promoter region of a gene is associated with transcriptional activation (6). Based on this information, adding DNA demethylating agents to cell cultures may help maintain stable HLA-G expression across all passages for therapeutic applications.

The expression of MHC class I genes can also be induced by various cytokines. IFN-γ, a proinflammatory cytokine, is the most potent inducer of *MHC class I* gene expression (7). This study aims to elucidate the effects of HLA-G promoter region methylation and IFN-γ stimulation on *HLA-G* gene expression in MSCs derived from WJ.

Materials and Methods: WJ was isolated from umbilical cord tissue and cultured in an appropriate incubator. Cell characterization was performed using a flow cytometer. Following MSC characterization, 10 µM 5-AZA was applied to passage 1 (P1) cells for 72 hours. After this treatment, one group was stimulated with 10 ng/mL IFN-γ for 3 days after removing 5-AZA, while the other group was stimulated with IFN-γ without removing 5-AZA. Soluble HLA-G levels in cell culture supernatants from all groups were analyzed by ELISA, and the results were subjected to statistical analysis.

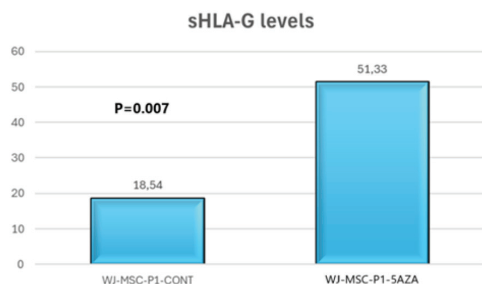

Figure 1. Intra-group comparisons of HLA.

HLA: Human leukocyte antigens

Results: HLA-G levels in P1 samples treated with 10 µM 5-AZA for 72 hours were significantly higher compared to control (p=0.007) (Figure 1). After removing 5-AZA and stimulating with IFN-γ, HLA-G levels increased but were not statistically significant (p=0.172) (Figure 2A). In contrast, when 5-AZA was not removed, HLA-G levels were significantly higher compared to the control group (p=0.026) (Figure 2B). HLA-G levels in the P1 group.

Discussion: One of the primary mediators in the immunomodulatory effects of MSCs is the HLA-G molecule (8). Increased HLA-G expression is correlated with the immunosuppressive effects of MSCs (9). This study demonstrates that demethylating agents like 5-AZA and cytokines like IFN-γ can enhance HLA-G expression. The combination of continuous 5-AZA presence with IFN-γ stimulation is more effective than removing 5-AZA before stimulation. Future research will contribute to a deeper understanding of these mechanisms.

Keywords: HLA-G, mesenchymal stem cell, Wharton's jelly

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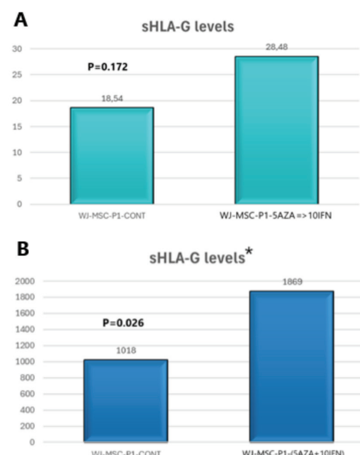

Figure 2. Intra-group comparisons of HLA-G levels in the P1 group.

**: A different ELISA kit was used for measurement, HLA: Human leukocyte antigens*

Poster Presentations

PP-12

What Happens? If Donor and Recipient Carrying PKHD1 and PKD Gene Variants in Renal Transplantation

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Objectives: In this case report, we present a patient who was diagnosed with autosomal dominant polycystic kidney disease (ADPKD) at the age of 1 year and given to our clinic as a 17-year-old renal transplant candidate. In living donor screening, human leukocyte antigens (HLA) 4/6 compatible with the mother was a carrier for polycystic kidney disease-1 (PKD1) and *polycystic kidney disease with/without hepatic disease (PKHD1)* gene variants.

Materials and Methods: HLA tissue typing was performed on the patient and the donor candidate's mother and father. 4/6 HLA compatibility was detected with the mother. Due to the patient's diagnosis of ADPKD, the family analyzed the DNA-next-generation-sequencing "Kapa Hypercap Hereditary" kit. After genetic testing, kidney transplantation was performed from the donor candidate's mother to her daughter. After the transplantation, the patient is under follow-up with stabilized routine follow-up.

Results: The mother was 37 years old and had no history related to ADPKD. However, the maternal genetic results revealed the PKD1 C.2695C>G likely pathogenic and PKHD1 c.5353T>C *VUS* gene variants. The PKHD1 c.525delT pathogenic variant and the maternal PKHD1 c.5353T>C and PKD1 c.2695C>G variants were analyzed. The patient received a kidney from the mother in August 2024. He returns once a month for routine monitoring.

Conclusion: It was determined that the PKHD1 c.525delT pathogenic variant with autosomal dominant inheritance was the underlying cause of the patient's etiopathogenesis. The absence of this variant in the mother of the donor candidate increased the transplant's success. The detection of the PKD1 c.2695C>G probably pathogenic and PKHD1 c.5353T>C VUS variants in heterozygous genotype in autosomal recessive inheritance led to the mother's evaluation as a carrier.

Keywords: PKD1, *PKHD1* gene, renal transplant

PP-13

Association of Severe Spinal Involvement with HLA Alleles in Patients with Radiographic Axial Spondyloarthritis

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There are limited studies showing the association between human leukocyte antigen (HLA) alleles and clinical severity in patients with axial spondyloarthritis (axSpA). In this study, we aimed to determine the HLA alleles that may be associated with disease severity and severe spinal involvement in radiographic axSpA (r-AxSpA) patients. Inclusion criteria for the study were patients with axSpA have a symptom duration of more than 10 years and their Bath Ankylosing Spondylitis Radiology Index, calculated by two rheumatologists. Patients were divided into two groups according to age at diagnosis by receiver operating characteristic analysis: ≤28 years and ≥29 years. HLA genotyping was performed using the sequence-specific oligonudeotide probe method for HLA alleles. Logistic regression analysis and decision tree models were used to assess the association between radiographic parameters and demographic and genetic characteristics. Overall, 100 patients were included. 72 (72%) of (r-AxSpA) patients were HLA-B*27 positive. Sixty-two (62%) of the patients were male, the mean (standard deviation) duration of symptoms was 21.1 (9) years. Syndesmophytes were found in 88.9% of HLA-A*02 homozygous male patients without extra-articular involvement. When the decision tree was evaluated, male sex, any extra-articular involvement, and HLA-A*02 homozygosity were associated with syndesmophyte. Hip involvement appears to be associated with HLA-A*02 homozygosity and early age at diagnosis in the decision tree. As a result, we found out that HLA-A*02 is related to syndesmophyte, but further studies are needed to support the frequency of HLA-A*02 homozygosity and its association with radiographic severity in r-AxSpA patients.