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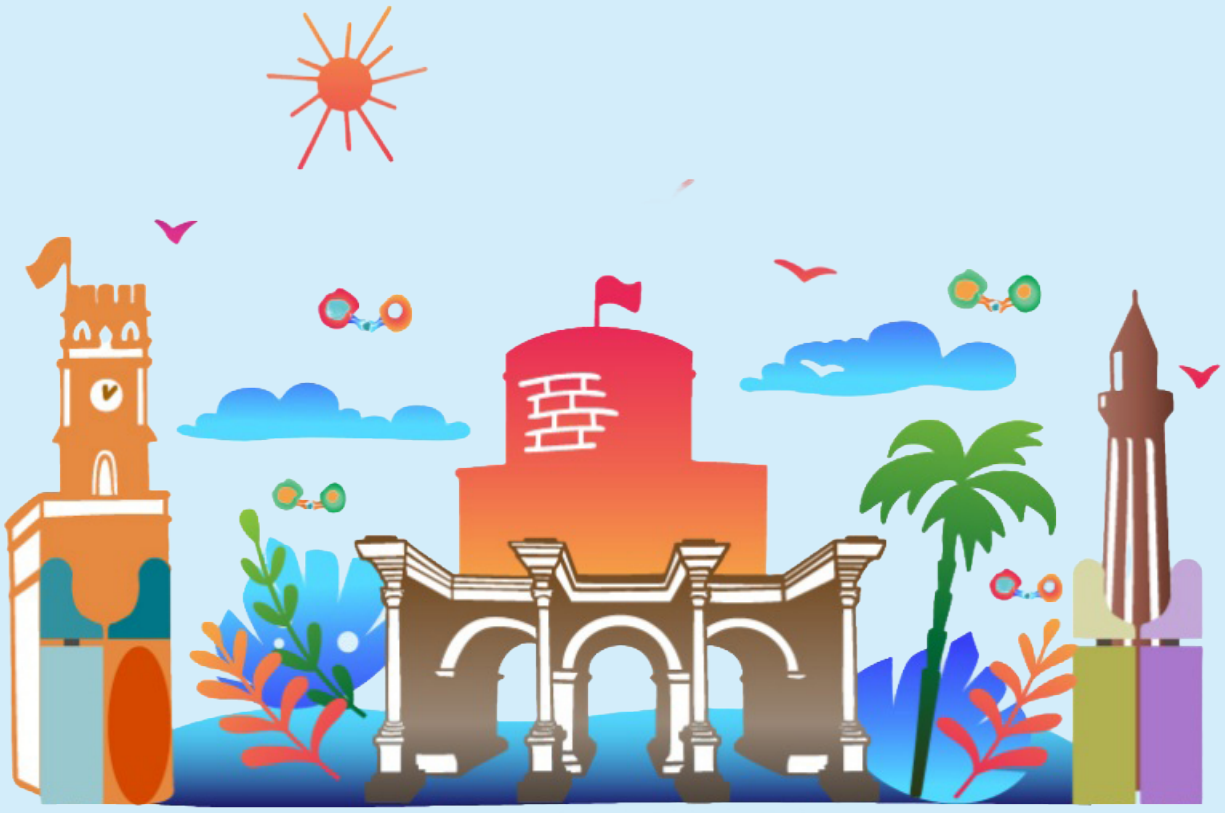

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Multi-Layered Immuno-Epigenetic Regulation of the YY1–SIRT7–H3K18Ac–PD-1/PD-L1 Axis in Renal Allograft Survival: Preliminary Findings

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Abstract

Objective: This study aimed to characterize the immuno-epigenetic role of the YY1–SIRT7–H3K18Ac–PD-L1 axis in renal allograft survival and to identify molecular biomarkers associated with rejection.

Materials and Methods: A total of 65 participants were enrolled: 20 patients with biopsy-confirmed allograft rejection, 25 with stable graft function, and 20 healthy controls. Blood samples were collected into EDTA-containing tubes and stored at –80°C. Total RNA was isolated and complementary DNA (cDNA) synthesized for gene expression analysis by real-time polymerase chain reaction (RT-PCR), using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and U6 small nuclear RNA (snRNA) as reference genes. Serum concentrations of SIRT7, H3K18Ac, and oxidative stress markers — superoxide dismutase (SOD) and glutathione (GSH) — were quantified by enzyme-linked immunosorbent assay (ELISA). Statistical analyses were performed using SPSS v23.0 ($p < 0.05$).

Results: Messenger RNA levels of STAT3, CD274 (PD-L1), H3F3A, SIRT7, and YY1 were significantly elevated in the rejection group, whereas PDCD1 (PD-1) was higher in the survival group ($p<0.05$). Long non-coding RNAs NEAT1, MALAT1, and GAS5 were upregulated in rejection ($p<0.05$). Significant alterations were detected in miR-21, miR-34a, miR-125, and miR-155 ($p<0.01$). SOD and GSH were decreased, while malondialdehyde (MDA) and catalase were elevated ($p<0.05$). NEAT1 and MALAT1 were predominantly upregulated in humoral rejection, whereas GAS5 was reduced.

Conclusion: The YY1–SIRT7 epigenetic axis, in conjunction with PD-L1 signaling, appears to be implicated in immune evasion and inflammatory amplification following renal transplantation, supporting the potential utility of these molecules as immuno-epigenetic biomarkers of allograft rejection.

Keywords: kidney transplantation; allograft rejection; epigenetics; PD-L1; non-coding RNA; biomarkers

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Introduction

Kidney transplantation remains the most effective therapeutic strategy for extending survival in patients with end-stage renal disease. Despite considerable advances in immunosuppressive therapy, acute and chronic rejection continue to be the principal barriers to long-term allograft survival. Emerging evidence suggests that epigenetic regulators — including Yin Yang 1 (YY1), Sirtuin 7 (SIRT7), and H3K18 acetylation (H3K18Ac) — may exert significant influence over immune responses, inflammatory signaling, and cellular metabolism (1,2). Nevertheless, the mechanistic interplay of these regulators in the context of solid organ transplantation has yet to be fully elucidated.

The present study aimed to characterize the role of the YY1–SIRT7–H3K18Ac–Programmed Death-Ligand 1 (PD-L1) axis at the immuno-epigenetic level in renal allograft survival, and to contribute to the identification of molecular biomarkers of rejection.

Materials and Methods

This study was approved by the Clinical Research Ethics Committee of Atatürk University Faculty of Medicine (Decision No: B.30.2.ATA.0.01.00/590; Date: 05.05.2025). Written informed consent was obtained from all participants. A total of 65 individuals were enrolled: 20 patients with biopsy-confirmed allograft rejection, 25 patients with stable graft function, and 20 healthy controls. Blood samples were collected from the rejection group prior to or during the early phase of biopsy-confirmed rejection, and from the remaining groups during routine follow-up visits. Samples were collected into ethylenediaminetetraacetic acid (EDTA)-containing tubes, centrifuged at 2,000 × g for 10 minutes, and stored at –80°C until analysis.

Total RNA was isolated using the NucleoGene RNA Extraction Kit, followed by cDNA synthesis. Gene expression was quantified by RT-PCR. GAPDH and U6 snRNA served as reference genes, and relative expression levels were calculated using the ΔCt method. Serum concentrations of SIRT7, H3K18Ac, and oxidative stress markers (SOD and GSH) were measured using commercially available ELISA kits. Statistical analyses were performed using SPSS version 23.0; a p-value of <0.05 was considered statistically significant.

Results and Discussion

At the messenger RNA (mRNA) level, STAT3, CD274 (PD-L1), H3F3A, SIRT7, and YY1 were significantly upregulated in the rejection group, whereas PDCD1 (PD-1) expression was higher in the survival group ($p < 0.05$; Table 1). Long non-coding RNAs (lncRNAs) — NEAT1, MALAT1, and GAS5 — were elevated in the rejection group ($p<0.05$; Table 2). MicroRNA (miRNA) expression profiling revealed significant alterations in hsa-miR-21, miR-34a, miR-125, and miR-155 ($p<0.01$; Table 3). Regarding oxidative stress, SOD and GSH levels were signifi-

Table 1. Comparison of mRNA expression levels of selected genes across rejection, survival, and control groups.

Gene	Rejection Group (Mean±SD / Median [IQR])	Survival Group (Mean±SD / Median [IQR])	Control Group (Mean±SD / Median [IQR])	Test Statistic	*p*
MTOR	28 (22.53–28.18)a	23.29 (20.29–25.48)a	19.32 (18–21.29)b	60.065	<0.001*
PDCD1	20.55 ± 1.97a	25.58 ± 1.18a	24.91 ± 0.7b	23.827	<0.001†
CD274 (PD-L1)	25.18 (22.7–28.1)a	24.33 (20.46–31.77)a	20.6 (19.85–24.33)b	48.506	<0.001*
STAT3	24.79 (22.85–33)a	21.26 (20.19–29.28)a	16.56 (14.52–20.45)b	61.685	<0.001*
H3F3A	29.22 (22.93–29.53)a	27.39 (19.34–32.29)a	14.76 (13.23–16.47)b	60.042	<0.001*
SIRT7	24.73 (22.8–36.82)a	21.49 (21.74–33.73)a	16.74 (17.82–25.39)b	56.369	<0.001*
YY1	25.85 (21.28–29.36)a	21.36 (21.36–28.34)a	16.56 (15.37–19.77)b	60.247	<0.001*

*Values expressed as Mean±SD or Median [IQR]. Superscripts (a,b) indicate statistically homogeneous subgroups by post-hoc analysis. *Kruskal–Wallis test; †One-way ANOVA. IQR: interquartile range; SD: standard deviation; mRNA: messenger RNA.*

cantly decreased, while malondialdehyde (MDA) and catalase were elevated ($p<0.05$). The YY1–SIRT7–H3K18Ac axis was predominantly active in the rejection group, reflecting an immuno-epigenetic regulatory network. Among lncRNAs, NEAT1 and MALAT1 were upregulated in humoral rejection, whereas GAS5 expression was reduced (Table 4). These findings are consistent with previous reports implicating epigenetic dysregulation in immune-mediated allograft injury (3,4,5).

Taken together, these results suggest that the YY1/SIRT7 epigenetic axis, in conjunction with PD-L1 signaling, may be implicated in immune evasion and the amplification of inflammatory activation following renal transplantation (6). These findings contribute to the identification of novel immuno-epigenetic biomarkers in allograft rejection. Further validation in a larger cohort with complementary multi-omics analyses is planned.

Table 2. Comparison of lncRNA expression levels across rejection, survival, and control groups.

Gene	Rejection Group (Mean±SD / Median [IQR])	Survival Group (Mean±SD / Median [IQR])	Control Group (Mean±SD / Median [IQR])	Test Statistic	*p*
GAPDH2	23.53 (21.21–27.01)a	23.33 (20.28–27.01)a	17.81 (15.28–20.92)b	57.262	<0.001
NEAT1	20.06 (18.37–20.76)a	20.7 (18.33–22.57)a	14.54 (13.23–18.51)b	60.221	<0.001
MALAT1	22.31 (20.16–27.28)a	22.28 (20.16–27.28)a	19.38 (18.27–23.64)b	54.589	<0.001
GAS5	19.86 (20.15–23.1)a	22.58 (20.39–25.65)a	15.87 (15.15–17.53)b	59.808	<0.001
PVT1	23.04 ± 1.37a	22.22 ± 1.37a	18.58 ± 0.93b	117.201	<0.001

Values expressed as Mean±SD or Median [IQR]. Superscripts (a,b) indicate statistically homogeneous subgroups. Kruskal–Wallis test applied throughout. lncRNA: long non-coding RNA.

Table 3. Comparison of miRNA expression levels across rejection, survival, and control groups.

Gene	Rejection Group (Mean±SD / Median [IQR])	Survival Group (Mean±SD / Median [IQR])	Control Group (Mean±SD / Median [IQR])	Test Statistic	*p*
U6 snRNA	34.03 (23.73–36.37)a	34.03 (22.73–36.37)a	19.7 (15.47–25.43)b	58.563	<0.001
hsa-miR-21	28.67 (25.92–31.42)a	28.22 (23.42–31.67)a	20.55 (14.25–35.57)b	33.225	<0.001
hsa-miR-34a	29.63 (24.34–32.98)a	27.63 (23.94–33.32)a	19.95 (13.56–35.13)b	46.715	<0.001
hsa-miR-125	33.67 (29.05–36.63)a	32.96 (25.68–36.05)a	19.76 (15.34–33.52)b	51.223	<0.001
hsa-miR-155	34.01 (31.39–35.48)a	32.88 (24.39–36.29)a	20.5 (13.25–33.79)b	54.482	<0.001

Values expressed as Median [IQR]. Superscripts (a,b) indicate statistically homogeneous subgroups. Kruskal–Wallis test applied throughout. miRNA: microRNA.

Table 4. Comparison of ceRNA expression profiles between acute cellular and acute humoral rejection subtypes.

Gene	Acute Cellular Rejection (Mean±SD / Median [IQR])	Acute Humoral Rejection (Mean±SD / Median [IQR])	*p*
PDCD1	20.39 ± 1.94	24.99 ± 1.89	0.025
CD274 (PD-L1)	26.03 ± 1.85	21.33 ± 1.18	0.048
STAT3	26.85 ± 3.45	19.38 ± 1.18	0.014
H3F3A	26.03 ± 2.16	24.75 ± 0.7	0.005
SIRT7	28.49 (22.8–36.82)	21.69 (21.22–30.23)	0.047
YY1	29.96 ± 2.48	25.55 ± 1.98	0.025
NEAT1	25.99 ± 0.82	19.74 ± 0.84	0.050
MALAT1	23.74 ± 2.4	21.92 ± 0.9	0.026
GAS5	18.58 ± 1.16	21.99 ± 0.92	0.046
PVT1	23.15 ± 1.87	22.97 ± 1.04	0.050
hsa-miR-21	29.14 ± 1.18	24.6 ± 1.71	0.047
hsa-miR-34a	31.91 ± 1.74	29.34 ± 2.55	0.025
hsa-miR-125	23.57 ± 1.5	33.69 ± 2.26	0.004
hsa-miR-155	37.82 ± 1.19	33.84 ± 1.24	0.028

Values expressed as Mean±SD or Median [IQR]. Mann–Whitney U or independent samples t-test applied as appropriate. ceRNA: competing endogenous RNA.

Conflict of Interest: The authors declare no conflict of interest. No external funding was received for this study.

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ORAL PRESENTATION Turkish Journal of Immunology, 14(Suppl 1):5

Temporal Evolution of HLA Distribution: A 25-Year Single-Center Immunogenetic Analysis

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Abstract

Objective: The HLA system exhibits significant population variability due to demographic history. As a migration crossroad, Turkiye has a complex genetic structure. This study analyzes 25 years of data from a single center in West-Central Anatolia to document the evolution of regional HLA distribution between low-migration (2001-2011) and high-migration (2011-2025) periods and to evaluate its implications for transplantation immunology.

Materials and Methods: In this retrospective study, HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 data from 6550 healthy donors were analyzed. The cohort was divided into two periods: 2001-2011 (n=2089) and 2011-2025 (n=4461). Genotyping was performed via PCR-SSO. Population genetics analyses, including Hardy-Weinberg Equilibrium (HWE) and Linkage Disequilibrium (LD), were conducted using PyPop software. Statistical significance of allele frequency differences was assessed using Fisher's Exact test.

Results: Statistically significant frequency changes were detected in 18 alleles ($p<0.05$). In the post-2011 period, Middle Eastern/Asian-origin alleles (e.g., B*52, DRB1*03) increased, while European-associated alleles (e.g., B*44, DQB1*05) decreased. The high-migration cohort showed significant deviation from HWE due to excess homozygosity, indicating a "Wahlund effect" and population stratification. Furthermore, LD between HLA-C and HLA-B weakened significantly, whereas the strong LD between DRB1 and DQB1 was preserved. Specific haplotypes like A*30~B*51~C*16~DRB1*13 disappeared, replaced by new ones such as A*01~B*08~C*07~DRB1*03.

Conclusion: Dynamic changes in the gene pool directly affect donor matching probabilities and transplantation strategies. These findings highlight the necessity of periodically updating transplantation registries and donor search algorithms to reflect the current genetic profiles of populations.

Keywords: HLA Antigens, emigration and immigration, biological evolution, linkage disequilibrium, genetics population

OP-01

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ORAL PRESENTATION Turkish Journal of Immunology, 14(Suppl 1):6

Identification of Rare HLA Alleles with Non-Coding Region Variants by NGS and Their in Silico Evaluation at the Eplet Level

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Abstract

Objective: Genetic variation within the HLA region plays a pivotal role in transplantation compatibility and the shaping of immune responses. Next-generation sequencing (NGS) technology enables the detection of non-coding region variants that cannot be identified by conventional methods, thereby allowing a more comprehensive assessment of HLA allelic diversity. However, the structural differences of these rare variants at the eplet level and their potential immunogenic effects on KIR-ligand epitopes, namely Bw4/Bw6 and C1/C2 groups, have not yet been fully elucidated.

Materials and Methods: In this study, rare HLA alleles harboring non-coding region variants were identified among 15,350 HLA alleles characterized by NGS at the Tissue Typing Laboratory of Istanbul Faculty of Medicine. These alleles were validated and named through comparison with reference sequences in the IPD-IMGT/HLA database. The identified alleles were as follows: A02:01:01:164, A02:01:01:118, A02:06:01:15, A23:39, A24:02:01:02L, A24:314, A31:234, A02:01:01L, B44:02:01:35, B44:02:01:02S, B51:01:01:17 (in two individuals), B51:01:01:69, B51:11N, B58:01:01:10, C17:38, C02:02:02:47, C12:323, C15:171, DRB113:157, DRB114:23, DRB111:310, DRB401:03:01:02N, and DPB1*1321:01. Eplet characterization of these alleles was performed at the amino acid level by comparison with their respective reference alleles. In addition, Bw4/Bw6 epitopes of HLA-B alleles and C1/C2 epitope characteristics of HLA-C alleles were evaluated.

Results: For the majority of alleles, the observed differences were confined to non-coding regions and did not involve amino acid changes within the antigen-binding domain. Consequently, their eplet profiles and Bw4/Bw6–C1/C2 epitope characteristics were found to be similar to those of the corresponding reference alleles. However, certain alleles—namely C17:38, A23:39, A24:314*, A31:234*, C12:323*, C15:171*, and DRB1*13:157*—were predicted to harbor potential amino acid substitutions within the antigen-binding groove, which could theoretically give rise to novel eplet combinations. Alleles categorized as L, N, and S were also considered to be of potential clinical relevance due to their altered expression patterns.

Conclusion: Rare HLA alleles with non-coding region variants identified by NGS generally exhibit eplet and KIR-ligand (Bw4/Bw6, C1/C2) profiles similar to those of their reference alleles; however, certain variants may still possess immunogenic differences. These findings indicate that the combined evaluation of high-resolution HLA typing results at both the eplet and KIR-ligand levels may provide more precise predictive insights in transplantation immunology.

Keywords: Non-coding, NGS, HLA alleles, eplet analysis

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ORAL PRESENTATION Turkish Journal of Immunology, 14(Suppl 1):7–8

The Impact of HLA Eplet Mismatching on GVHD, Relapse, and Survival Following Hematopoietic Stem Cell Transplantation: A Multidimensional Clinical and Statistical Evaluation

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Abstract

Objective: Graft-versus-host disease (GVHD) remains one of the leading causes of morbidity and mortality following allogeneic hematopoietic stem cell transplantation (allo-HSCT). Even when conventional HLA matching is achieved, epitope-level discrepancies may still trigger alloimmune responses. Eplet mismatching represents a novel and more sensitive parameter for evaluating such immune incompatibilities and has gained increasing importance in predicting transplant outcomes. In this study, the associations between donor- and recipient-directed Class I and Class II eplet mismatches and the development of acute/chronic GVHD, relapse, and overall survival (OS) were investigated.

Materials and Methods: A total of 56 allo-HSCT recipients followed at the Department of Hematology, Istanbul Faculty of Medicine, were included in this study. According to HLA matching, 18 patients received 5/10 matched, 7 received 6/10 matched, 1 received 7/10 matched, 2 received 8/10 matched, and 28 received 9/10 matched transplants. High-resolution HLA typing was performed using next-generation sequencing (NGS) for all patients. Donor–recipient eplet mismatches at HLA-A, -B, -C, -DRB1, and -DQB1 loci were calculated using the HLAMatchmaker algorithm. Clinical endpoints included GVHD, relapse, and overall survival. Statistical analyses were performed using multivariate Kruskal–Wallis and Dunn–Bonferroni post hoc tests, Cox proportional hazards regression, and logistic regression models.

Results: Donor-directed Class II eplet mismatching was significantly higher in patients who developed chronic GVHD ($p<0.05$), with the most prominent organ involvement observed in the gastrointestinal tract. Furthermore, Class II eplet mismatch showed an independent positive association with relapse development (OR>1, $p<0.05$). Kaplan–Meier survival analysis demonstrated that a high Class II eplet burden was significantly associated with reduced overall survival ($p<0.05$). In multivariate Cox regression analysis, chronic GVHD and Class II eplet mismatching were identified as independent predictors of mortality (HR>1, $p<0.05$). When relapse was added to the model, the risk of mortality increased markedly (HR≫1), while the effect of Class II eplet mismatching diminished, indicating that Class II mismatching affects mortality indirectly through relapse and/or chronic GVHD. In contrast, Class I eplet mismatching showed no significant effect on GVHD, relapse, or overall survival.

Conclusion: Our findings demonstrate that Class II eplet mismatching adversely affects survival indirectly by

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increasing the risk of chronic GVHD and relapse. In contrast, the impact of Class I eplet mismatching appears to be limited. These results are consistent with previous reports indicating that HLA-DR/DQ eplet disparities enhance alloreactive T-cell activation and increase the risk of chronic GVHD and relapse.

In conclusion, eplet-level matching provides a more sensitive and predictive approach for evaluating transplant outcomes compared with conventional antigen-based HLA matching. Incorporating eplet mismatch analysis into donor selection strategies may improve risk stratification and post-transplant prognosis.

Keywords: Eplet analysis, HLA, GVHD, HSCT

ORAL PRESENTATION Turkish Journal of Immunology, 14(Suppl 1):9

The Relationship Between NK Cell Function and Non-KIR Receptor Levels with GVHD in Patients Undergoing Allogeneic Stem Cell Transplantation

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Abstract

Objective: Hematopoietic stem cell transplantation (HSCT) is a curative treatment for hematological malignancies; however, graft-versus-host disease (GVHD) remains a major complication. While donor T cells are central to GVHD pathogenesis, natural killer (NK) cells, via activating and inhibitory receptors, may also influence disease course. This study evaluated post-transplant changes in non-KIR receptor expression on NK cells and their association with GVHD.

Materials and Methods: Twenty patients (M:3, F:13) who underwent allogeneic HSCT at Hacettepe University (2021–2024) were included. The mean age was 50, with a median follow-up of 26.1 months. Eleven patients received fully matched and nine haploidentical transplants; GVHD developed in 10 patients. Peripheral blood samples were collected at day 0 and on days 30, 100, and 180. Patients were followed for at least 6 months. Mononuclear cells were isolated, and non-KIR receptor expression in NK subsets was analyzed by flow cytometry. NK cells from days 0 and 30 were co-cultured with the K562 cell line to assess CD107a, perforin, and granzyme expression.

Results: Eight AML, six ALL, four MDS, and two lymphoma patients were included. NKG2A and NKG2D decreased significantly (p=0.0001; p=0.0049). NKG2C declined until day 30, then increased. NKp30, NKp44, and NKp46 decreased. ILT-2 and ILT-4 also showed significant reductions (p=0.004; p=0.0001). Cytotoxicity analysis showed increased granzyme and decreased perforin and CD107a, with only perforin reduction significant (p=0.025). Increased NKp46 and NKp30 expression was associated with reduced GVHD risk (AUC: 0.819; 95% CI: 0.576–1.000).

Conclusion: Allogeneic HSCT is associated with reduced NK cell non-KIR receptor and perforin expression. Although no significant relationship was found between GVHD development and receptor profile, changes in expression levels suggest that NK cells are affected by chronic inflammation. Higher NKp46 and NKp30 expression may indicate reduced GVHD risk, supporting their potential as biomarkers and the need for larger studies.

Keywords: Graft-versus-host disease (GVHD), Hematopoietic Stem Cell Transplantation (HSCT), non-KIR receptor, NK cell

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ORAL PRESENTATION Turkish Journal of Immunology, 14(Suppl 1):10

A Sensitive Alternative to Fragment Analysis–Based Methods in Chimerism Monitoring: Microchimerism Analysis Using Next-Generation Sequencing Technology

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Abstract

Objective: Chimerism monitoring following allogeneic hematopoietic stem cell transplantation (allo-HSCT) is of critical importance for evaluating graft success and the early detection of potential transplantation-related complications. Conventional methods such as short tandem repeat (STR) analysis and quantitative PCR (qPCR) are limited in terms of sensitivity. Next-generation sequencing (NGS) has emerged as a groundbreaking approach, offering unparalleled sensitivity and accuracy for the detection of donor and recipient cell populations. The aim of this study was to compare NGS-based chimerism analysis with the STR method and to assess its analytical sensitivity.

Materials and Methods: This study included 20 patients who underwent allogeneic bone marrow transplantation at the Department of Hematology, Istanbul Faculty of Medicine, along with 10 international external quality control samples. A total of 41 samples were analyzed at the Tissue Typing Laboratory of Istanbul Faculty of Medicine. DNA samples were obtained from peripheral blood, bone marrow, intra-abdominal ascitic fluid, and liver biopsy specimens. DNA isolation was performed using the Promega Maxwell RSC kit, after which all samples were analyzed using both STR and NGS methods. STR analysis was performed using the Qiagen IDPlex Plus kit with fragment analysis, while NGS-based analyses were carried out using the One Lambda Devyser™ Chimerism NGS Assay and the GenDx NGSTRack kit.

Results: Of the 41 samples analyzed by STR, 23 were reported as complete chimerism. However, NGS-based analysis revealed that 21 of these samples actually exhibited microchimerism or mixed chimerism. Additionally, quantitative differences in donor–recipient cell proportions were observed among samples identified as mixed chimeric.

Conclusion: NGS-based chimerism analysis demonstrates significantly higher sensitivity compared to conventional STR methods and enables the early detection of minimal residual disease. The presence of microchimerism in many samples previously classified as “complete chimerism” by STR indicates that NGS is a more reliable method for predicting early graft failure and relapse risk. The integration of NGS into routine clinical practice has the potential to initiate a new era in post-transplant patient management following hematopoietic stem cell transplantation.

Keywords: Chimerism, NGS, microchimerism, monitoring, HSCT

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ORAL PRESENTATION Turkish Journal of Immunology, 14(Suppl 1):11

Determination of Donor Specific Antibody Changes in Heart Transplant Patients by MagSort Method

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Abstract

Objective: One of the major challenges in achieving long-term success in heart transplantation is the presence of donor-specific antibodies (DSAs) against HLA antigens. The extent of mismatch can be associated with the likelihood of DSAs formation. These HLA-specific antibodies recognize clusters of accessible polymorphic amino acid residues, known as functional epitopes or eplets, shared by the target HLA molecules. Magnetic beads with a single HLA specificity (MagSort, One Lambda, USA) were developed to facilitate the isolation of HLA specific DSAs from complex serum samples and represent one of the most recent techniques. The aim of the study is to re-test serum samples in which the presence of DSAs was detected by LSA testing during pre- and post-transplant evaluation using MagSort, to determine the presence of the identified antibodies and to correlate these results with clinical outcomes.

Materials and Methods: A total of 6 serum samples collected at different time points from 4 heart transplant patients at Baskent University Ankara Hospital. LSA Class I–II tests (before and after MagSort treatment) (One Lambda, USA) were performed on serum samples from the patients at the Baskent University Adana Dr.Turgut Noyan Medical Center. The LSA Class I–II test was performed using the Luminex method.

This study was approved by Baskent University Institutional Review Board (Project no: KA25/171) and supported by Baskent University Research Fund.

Results: Immunology and pathology results are summarized in the Table 1.

Conclusion: The MagSort method, as a new technique, may support donor selection in the organ transplantation process by identifying antibodies defined as DSAs for recipients and/or determining changes in MFI levels. It may facilitate and enhance the accuracy of identifying shared eplets. It may enable clear identification of de novo DSAs after transplantation, thereby guiding the treatment plan.

Keywords: Donor specific antibody, heart transplantation, MagSort

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ORAL PRESENTATION Turkish Journal of Immunology, 14(Suppl 1):12

Early Monitoring of Soluble Immune Checkpoints in Kidney Transplantation and Their Potential Associations with Kidney Function

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Abstract

Objective: Kidney transplantation is the most effective treatment for end-stage renal disease. Soluble immune checkpoints may regulate immune responses and serve as biomarkers for monitoring and graft function prediction.

Materials and Methods: In this study, serum samples collected and stored from healthy controls (n = 15) and kidney transplant recipients (n = 30) before transplantation (day 0) and after transplantation (days 3 and 7) were analyzed for levels of sCD25, s4-1BB, sCD86, active TGF-β1, sCTLA-4, sPD-L1, sPD-1, sTIM-3, sLAG-3, sGalectin-9, sCD27, and sPD-L2 using a flow cytometry–based multiplex bead assay. Kidney function parameters (creatinine and GFR calculated by CKD-EPI) were retrospectively determined at different time points (day 0, day 3, day 7, week 2, month 1, and month 3). Data were analyzed using SPSS to evaluate the post-transplant dynamics of soluble immune checkpoints and their associations with kidney function parameters.

Results: In pre-transplant patients, levels of sCD25, sPD-L1, sTIM-3, sGalectin-9, sCD27, and sPD-L2 were significantly higher compared with healthy individuals. In the post-transplant period, statistically significant temporal changes were observed in the levels of sCD25, sPD-L1, sTIM-3, sGalectin-9, sCD27, sPD-L2, and sCD86. In contrast, no temporal changes were detected in the levels of s4-1BB, sLAG-3, sCTLA-4, active TGF-β1, or sPD-1, and these levels were comparable to those of healthy controls. Correlation analyses and subgroup analyses based on post-transplant GFR indicated that higher sLAG-3 and sCTLA-4 levels were associated with better kidney function, whereas higher sCD25 and sGalectin-9 levels were associated with poorer kidney function.

Conclusion: Soluble immune checkpoints are linked to early kidney function and may serve as biomarkers and therapeutic targets. Larger studies are needed to confirm their clinical value in improving transplant outcomes.

Keywords: Soluble immune checkpoints, kidney transplantation, transplantation immunology

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ORAL PRESENTATION Turkish Journal of Immunology, 14(Suppl 1):13

Distribution of Human Leukocyte Antigen Antibodies Among Renal Transplant Recipients: Single-Center Experience

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Abstract

Objective: Single antigen bead (SAB) tests on Luminex platform are widely used for donor-specific antibody (DSA) detection. This study aimed to evaluate the frequency of anti-HLA antibody profiles based on SAB test results.

Materials and Methods: A total of 317 SAB tests that were performed between January 2022 and September 2025 were included in the retrospective analysis. For patients under follow-up, only the most recent results were evaluated. Antibodies against HLA-A, HLA-B, HLA-DRB1, and HLA-DQB1 with a mean fluorescence intensity (MFI) ≥10,000 were analyzed.

Results: Among 145 SAB-positive patients with MFI ≥10,000, 63 (43.4%) were positive for Class I, 42 (29%) for Class II, and 40 (27.6%) for both Class I and Class II antibodies. A total of 1,440 Class I antibodies were detected (anti-HLA-A: 541, anti-HLA-B: 899). For Class II, a total of 740 antibodies were identified (anti-DQB1: 524 [78.8%], anti-DRB1: 216 [29.1%]). The most frequent antibodies were as follows: HLA-A: A2 (12.2%), A68 (9.2%), A24 (8.9); HLA-B: B57 (6.2%), B51 (5.0%), B27 (4.8); HLA-DRB1: DR4 (27.3%), DR9 (14.3%), DR15 (7.9); HLA-DQB1: DQ6 (25.6%), DQ7 (17.2%), DQ4 (16.9).

Conclusion: The distribution of detected antibodies was consistent with HLA allele frequencies reported in the Turkish population. Among Class II antibodies, the high prevalence of anti-DQB1 antibodies was particularly noteworthy. Accumulating evidence in the literature suggests that DQB1 antibodies may play a significant role in graft rejection. These findings indicate that the HLA-DQB1 locus should be included in pre-transplant compatibility assessment, especially for re-transplant candidates.

Keywords: Single antigen bead assay, HLA antibody, donor specific antibody

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ORAL PRESENTATION Turkish Journal of Immunology, 14(Suppl 1):14

Pyroptosis, Autophagy–Lysosomal Axis, and T-Cell Immune Regulation in Celiac Patients Lacking the DQ-Risk Genotype

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Abstract

Objective: Although celiac disease is predominantly associated with the HLA-DQ2.5 and/or DQ8 genotypes, it can also occur in individuals who do not carry these risk alleles. This suggests that mechanisms other than classical HLA-mediated pathogenesis may contribute to disease development. This study investigated the potential roles of selected single nucleotide polymorphisms (SNPs) associated with inflammasome–pyroptosis, autophagy-lysosomal system, T-cell activation, immune regulation, and cell migration in celiac patients without two risk DQ alleles.

Materials and Methods: A total of 89 celiac patients and 99 healthy controls were included in the study. DNA was isolated from peripheral blood samples, and HLA-DQ genotyping was performed to confirm inclusion criteria. Selected SNPs represented pathways including inflammasome/pyroptosis (IL18RAP rs917997, NLRP3 rs35829419), T-cell activation (TAGAP rs1738074), autophagy (FYCO1 rs2133660), and chemotaxis-cell migration (CCR3 rs6441961). SNP genotyping was performed using the Real-Time PCR method. Genotype and allele distributions were statistically analyzed and interpreted in the context of biological relevance.

Results: No significant difference was observed between groups in terms of age (p=0.324), whereas gender distribution differed significantly (p=0.0019). The T allele of IL18RAP (rs917997) and NLRP3 (rs35829419) was more frequent in the patient group. The TAGAP (rs1738074) TC variant and FYCO1 (rs2133660) CC variant were less frequent in patients. The CCR3 (rs6441961) TT genotype was found to be higher in patients. These findings suggest increased inflammasome activation, altered T-cell activation, impaired autophagic flux, and increased Th2/eosinophil-mediated chemotaxis in the patient group.

Conclusion: Our findings suggest that celiac disease development in patients without two risk DQ alleles cannot be explained solely by HLA-mediated mechanisms. Alterations in inflammasome activation, pyroptosis, autophagy-lysosomal pathway, and T-cell responses may represent an alternative immune axis. This study supports a distinct immune sub-phenotype in DQ-negative celiac disease and may contribute to future research on diagnostic biomarkers and targeted therapies.

Keywords: Celiac disease, inflammasome–pyroptosis, autophagy–lysosomal axis, t-cell immunoregulation

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ORAL PRESENTATION Turkish Journal of Immunology, 14(Suppl 1):15

The Importance of Antigen Presentation Mechanisms and HLA Expression in Cancer Stem Cells' Immune Evasion Strategies of Pancreatic Cancer

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Abstract

Objective: HLA molecules and antigen presentation pathways are critical for interactions between tumor cells and T cells. Defects in these mechanisms during carcinogenesis enable immune evasion. Cancer stem cells (CSCs) contribute to recurrence and treatment resistance due to their tumorigenic capacity and resistance to conventional therapies. Targeting CSCs and restoring HLA expression may improve outcomes. This study aimed to evaluate antigen presentation components and HLA expression in pancreatic cancer and CSCs.

Materials and Methods: In silico analyses assessed expression of 40 antigen presentation genes according to high and low levels of 10 CSC markers across two pancreatic cancer datasets. Survival analyses compared patients with mutated and unmutated antigen presentation genes. In vitro studies were performed using BxPC3 and CFPAC1 cell lines. CSC presence was determined using specific markers. Tumor spheres were generated, and CSC proportions, along with HLA-ABC and B2M expression, were analyzed in spheres and parental cells by flow cytometry. This study was supported by the Hacettepe University Scientific Research Projects Coordination Unit (No: 19477).

Results: Differential expression of antigen presentation molecules was observed across CSC marker groups. NLRC5 expression was significantly reduced in groups with high CSC marker expression, including PROM1 (CD133), SOX2, CD24, and CD44 ($p<0.05$). Survival was higher in patients without mutations in antigen presentation genes, although not statistically significant ($p=0.564$; $p=0.128$). In vitro findings showed increased CSC proportions in spheroids, while B2M and HLA-ABC expression was significantly decreased ($p<0.05$).

Conclusion: These findings indicate that impaired antigen presentation and reduced HLA expression contribute to immune evasion in pancreatic cancer and CSCs. Targeting key molecules in this pathway may improve treatment strategies and warrants further investigation.

Keywords: Antigen presentation mechanism, HLA expression, cancer stem cell, pancreatic cancer

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POSTER PRESENTATION Turkish Journal of Immunology, 14(Suppl 1):16–7

Evaluation of Methods and Results Used in Screening and Identification of Anti-HLA Antibodies: A Multicenter Study in Türkiye

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Abstract

Objective: Panel-reactive antibodies (PRA) or anti-human leucocyte antigens (anti-HLAs) are produced by the patient's immune system in response to donors during transplant procedures. Antibodies to HLA are known to develop following blood transfusion, pregnancy, and a history of transplantation. Donor-specific antibodies (DSA) arising in patient serum in response to donor-specific HLA can be detected using the single antigen bead (SAB) test. The objective of this multicentre retrospective study is to analyse the distribution of anti-HLA antibodies in Turkey, mean fluorescence intensity (MFI) values, and to assess the concordance of PRA tests across centres on a national scale.

Materials and Methods: The study will include adult patients and their donors who were on the living and cadaveric waiting lists and who underwent PRA screening/identification/SAB tests between 2013-2024. Data on patients' anti-HLA antibodies and demographics will be obtained from 12 tissue typing laboratories. Tests and MFI values will be recorded. The data will undergo rigorous testing for statistical significance using SPSS

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version 23.0, with a *p*-value <0.05.

Results: This study will reveal the distribution of anti-HLA antibodies across Turkey for the first time, and the effects of sensitization factors will be analysed. The correlation between Class I and II antibody positivity, MFI levels, and demographic data will be evaluated.

Discussion: The findings of this study will contribute to advancing laboratory consistency and establishing national test standardisation. It is hypothesized that the obtained data may also provide insights into updating the organ allocation system to include antibody testing. The comparison of PRA tests, SAB methods, and the MFI values will provide a new perspective on laboratory and clinical evaluation. This study aims to enhance the efficacy of patient-donor matching through the identification of shared criteria for pre-transplant immunological risk assessment within the national context.

Keywords: MFI, PRA screening/identification, SAB, transplantation

POSTER PRESENTATION Turkish Journal of Immunology, 14(Suppl 1):18

Demographic, Immunogenetic and Survival Analysis in Kidney Transplantation: A Single-Center Experience

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Abstract

Objective: To evaluate the impact of HLA mismatch level, donor type, donor relationship, sex matching, and age group on mortality among kidney transplant recipients in a single-center cohort.

Materials and Methods: A total of 365 kidney transplant cases were analyzed. Recipient and donor HLA-A, -B and -DRB1 loci were typed using sequence-specific oligonucleotide and sequence-specific primer methods. Donors were categorized as nuclear family members, other relatives, spouses, or cadavers. Total HLA mismatch (0–6) was calculated, and mortality outcomes were assessed. Statistical analyses were performed using JASP, with significance set at $p<0.05$.

Results: HLA mismatch increased progressively with decreasing donor relatedness ($\rho=0.87$). Sibling donors demonstrated the lowest mean mismatch (2.29), while spouse donors had the highest (4.33). Higher mismatch levels showed a strong correlation with mortality ($\rho=0.857$; $R^2=0.97$), with mortality reaching 10–25% at ≥4 mismatches. Mortality varied by donor type: 7.7% in nuclear family donors, 13.0% in other relatives or spouses, and 17.6% in cadaveric donors. Increased mortality in cadaveric transplantation appeared to reflect both immunologic and non-immunologic factors, including prolonged cold ischemia and higher rejection frequency.Sex matching influenced outcomes, with mortality rising from 9.4% in sex-matched to 13.4% in sex-mismatched pairs. Pediatric recipients had significantly lower mortality compared with adults (5.6% vs 11.6%; $p=0.048$). Presence of the DRB1*11 allele demonstrated a protective trend, with mortality of 7.4% in positive versus 12.1% in negative cases.

Conclusion: Survival after kidney transplantation is shaped by HLA compatibility as well as donor type, sex matching, age, donor relationship, and specific allelic characteristics. Optimizing both immunologic and non-immunologic risk factors—particularly in cadaveric transplantation—remains essential for improving long-term patient outcomes.

Keywords: Kidney transplantation, HLA mismatch, donor type, mortality, immunogenetics, DRB1*11

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Association of HLA-G Allele Distribution with Rejection in Heart Transplant Patients

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Abstract

Objective: HLA-G is a non-classical human leukocyte antigen (HLA) class-I molecule that regulates immune tolerance and plays a key role in transplantation immunology. Solid organ transplantation is a life-saving option for patients with end-stage organ failure. Despite advances in immunosuppressive therapy, allograft rejection remains a major complication. Identifying immunogenetic factors associated with rejection may improve post-transplant monitoring and long-term graft survival. The aim of the study was to investigate the relationship between HLA-G alleles and rejection in heart transplantation.

Materials and Methods: This study included 31 patients who underwent heart transplantation between 2007 and 2024 at Başkent University Ankara Hospital. Patients were tested Luminex Single Antigen (LSA) assay, and an MFI value ≥1000 for donor-specific antibodies (DSAs) was considered positive. Endomyocardial biopsies were evaluated for antibody-mediated rejection (AMR). Rejection was detected in 13 patients.

HLA-G typing was performed using Sanger-based sequencing by using SBTexcellerator HLA-G Core Kit (GenDx, Netherlands) at Baskent University Adana Dr.Turgut Noyan Medical Center. Genomic DNA was extracted from peripheral blood using the EZ1 DNA Blood Kit (QIAGEN, Germany).

This study was approved by Baskent University Institutional Review Board and Ethics Committee (Project no: KA25/36) and supported by Baskent University Research Fund.

Results: Results are given in Figure 1. According to study, HLA-G*01:01:01 is the most common allele and the highest genotype frequency in both the rejection and non-rejection groups belonged to G*01:01:01/*01:01:01, observed at rates of 30.77% versus 22.22%, respectively. HLA-G*01:01/*01:01 homozygosity was exclusively detected in the non-rejection group, with a frequency of 16.66%.

Conclusion: Although the number of patients in the study was limited, the HLA-G allele is thought to play a role in the rejection mechanism. The presence of homozygous HLA-G*01:01 was detected only in the non-rejection group and is thought to be effective in protecting against rejection.

Keywords: HLA-G, heart transplantation, rejection

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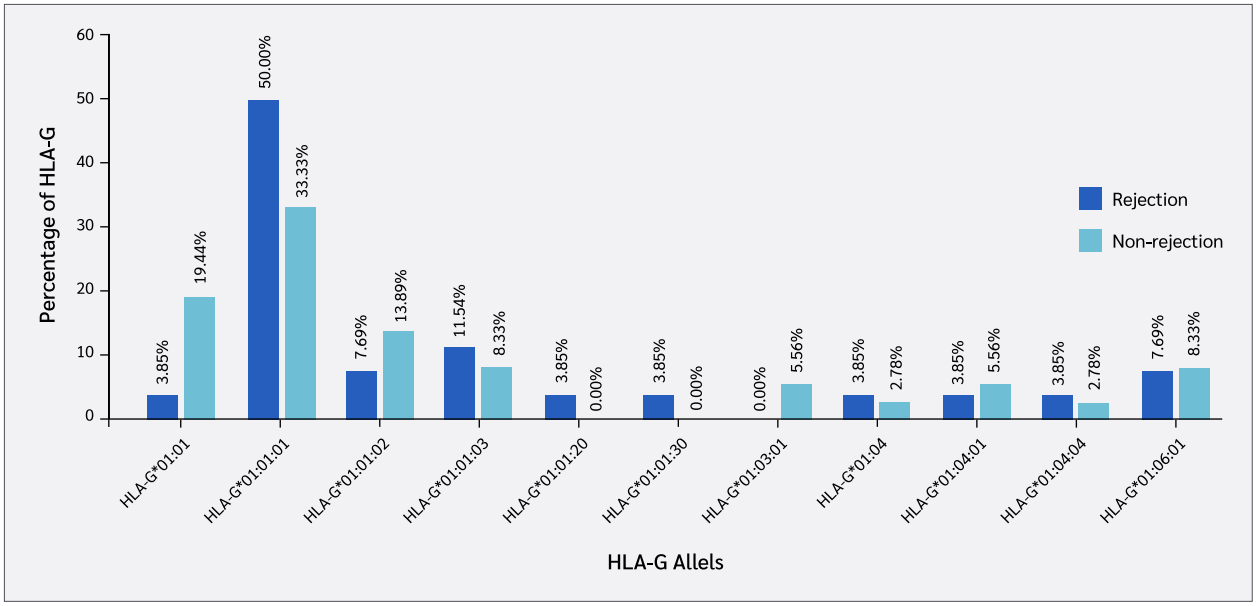

Figure 1. HLA-G allelic profile of heart transplant patients.

POSTER PRESENTATION Turkish Journal of Immunology, 14(Suppl 1):21

Verification of Flow Cytometry Crossmatch within the Framework of CLSI H62 and ICCS Q&S Module 25

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Abstract

Objective: Flow cytometry crossmatch (FCXM) is a highly sensitive assay for detecting donor-specific antibodies (DSA) in solid organ transplantation. International standards such as ASHI and EFI mandate the verification of performance characteristics before routine clinical use. This study aims to present a practical and guideline-compliant verification roadmap for tissue typing laboratories by re-analyzing routine data to minimize additional workload and kit costs.

Materials and Methods: In accordance with CLSI H62 and ICCS (Module 25) guidelines, three core performance characteristics were evaluated: accuracy, intra-assay precision, and cut-off verification. Pooled negative and positive control sera were utilized. For accuracy, routine FCXM results (2024–2025) were retrospectively compared with virtual crossmatch (VXM) results based on single antigen bead (SAB) assays (MFI >1000). Intra-assay precision was assessed using negative, weak positive, and strong positive controls on a flow cytometer. The internal 95% operational upper limit (OUL95) was calculated using the non-parametric nearest-rank p95 method from negative control distributions (T-cell n=214, B-cell n=211).

Results: FCXM demonstrated 100% specificity for both T and B cells and an overall accuracy of 91% for T cells and 85% for B cells. Sensitivity was 83% for T cells and 71% for B cells. Precision data showed coefficient of variation (%CV) values ranging from 0.7% to 13.5%, confirming suitable reproducibility for clinical use. The laboratory-specific OUL95 values for median IgG-FITC were determined as 190 for T cells and 426 for B cells. Laboratory-derived OUL95 values were fully compatible with routine cut-off criteria.

Conclusion: The FCXM method successfully met the verification requirements of CLSI H62 and ICCS guidelines. The study demonstrates that high specificity and robust intra-assay precision can be achieved with a minimal verification set, providing a reliable and cost-effective model for clinical laboratories.

Keywords: Donor-specific antibodies; flow cytometry; HLA; method verification; transplantation; virtual crossmatch

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HLA Allele Distribution in Patients with End-Stage Renal Disease: A Cross-Sectional Study

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Abstract

Objective: Our study was conducted to analyze the association between HLA polymorphism and end stage renal disease (ESRD) in patients and to determine the underlying mechanisms of the onset and progression of renal failure.

Materials and Methods: In our study we included 120 patients on the kidney transplant waiting list and 100 unrelated donors who underwent HLA typing using the polymerase chain reaction-sequence specific primer (SSP) method at the Tissue Typing Laboratory of Demiroğlu Bilim University Gayrettepe Florence Nightingale Hospital in between 2021-2023. The frequencies of HLA-A,-B,-DRB1 alleles were compared between ESRD patients and the control group, and the relationship between ESRD risk factors, HLA allele frequency (AF), haplotype frequency (HF) was investigated.

Results: Similar alleles were observed at high frequencies in both the ESRD patient and control groups, with these alleles which are HLA-A*02 (0.239/0.245), HLA-B*35 (0.225/0.183), and HLA-DRB1*11 (0.230/0.216). The most common haplotypes in patients were A*24~B*35~DR*11 (0.0495), A*02~B*51~DR*11 (0.0485), A*03~B*44~DR*04 (0.0270), A*11~B*35~DR*01 (0.0225), A*02~B*35~DR*04 (0.0225), while in controls they were A*24~B*35~DR*11 (0.0231), A*23~B*49~DR*11 (0.0192), A*02~B*51~DR*13 (0.0179), A*03~B*18~DR*11 (0.0149), A*02~B*18~DR*04 (0.0142) in the controls. For the HLA-B locus, the risk factors were B*50 (*p*:0.017), B*51 (*p* <0.0001), B*52 (*p*:0.023), B*55 (*p*:0.0017), B*57 (*p*:0.0017) were identified as risk factors, while B*14 (*p*:0.016), B*15 (*p*:0.002) were identified as protective factors. Furthermore, in patients with a primary diagnosis of diabetic nephropathy, A*11 (25.0%), B*51 (31.3%), DRB1*11 (25%) were found at high frequencies. A*02 (27.3%), B*35 (36.4%), DRB1*04 (22.7%) were found at high frequencies in patients with a primary diagnosis of polycystic kidney disease.

Conclusion: This study has revealed the relationship between HLA polymorphism and susceptibility to ESRD in the patient population. In patients awaiting kidney transplantation, five susceptible alleles and four susceptible haplotypes were identified. These HLA alleles and haplotypes might serve as genetic markers for susceptibility to ESRD in our population.

Keywords: End stage renal disease, human leukocyte antigen, haplotype, alleles frequency

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POSTER PRESENTATION Turkish Journal of Immunology, 14(Suppl 1):23

A Comparison of STR-PCR and Digital PCR Methods in the Monitoring of Chimerism in a Patient Diagnosed with AML

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Abstract

Objective: STR-PCR is the gold standard for monitoring chimerism following haematopoietic stem cell transplantation. However, it fails to detect mixed and microchimerism due to a sensitivity limit of 1%, as well as technical issues such as stutter peaks and allelic imbalance. Despite these limitations, this study aims to compare STR-PCR with digital PCR (dPCR), which achieves 0.01% sensitivity using high-specificity markers.

Materials and Methods: This study examined chimerism following an allogeneic bone marrow transplant from a TÜRKKÖK donor in a patient diagnosed with acute myeloid leukaemia (AML), using STR-PCR and digital PCR methods. The patient's chimerism status at 1 and 2 months post-transplant was assessed using both methods with the same samples and under the same conditions. Subsequent follow-ups (on days 99 and 227) were performed using dPCR only.

Results: While STR-PCR yielded a 100% donor profile in the first month, dPCR revealed the presence of microchimerism with a donor-to-recipient ratio of 99.04% to 0.96%. In the second month, STR-PCR yielded a donor to recipient ratio of 61% to 39%, while dPCR revealed a ratio of 70.73% to 29.27%. On day 99, the dPCR analysis showed a donor-to-recipient ratio of 11.24% to 88.76% and on day 227, a ratio of 12.58% to 87.42% was observed, which was consistent with clinical relapse.

Discussion: This study demonstrates that STR-PCR is a reliable and informative method for monitoring HSCT chimerism. However, it cannot detect microchimerism at levels below 1%. Using the same samples, the dPCR-INDEL method detected microchimerism and, due to its quantitative accuracy, produced more reliable results than STR. Later analyses (Days 100 and 227) performed exclusively with dPCR captured the relapse process with a high recipient contribution.

Keywords: Acute Myeloid Leukemia (AML), chimerism analysis, dPCR, STR-PCR

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POSTER PRESENTATION Turkish Journal of Immunology, 14(Suppl 1):24–5

Effect of Steroids on $CD4^+ICOS^+$ Expression in Follicular T Helper Cells and $CD19^+HLA\text{-}DR$

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Abstract

Objective: T follicular helper (Tfh) and B follicular (Bf) cells play key roles in adaptive immunity, particularly in germinal centre formation and high-affinity antibody production. Inducible T cell co-stimulator (ICOS), expressed on activated Tfh cells, interacts with ICOS-L on B cells to support Tfh survival. HLA-DR, presents antigens to $CD4^+$ T cells, promoting their activation and differentiation. Steroids are widely used in the treatment of alloimmune responses. The aim of this study was to investigate the effects of steroids on Tfh and Bf cells.

Materials and Methods: Spleen samples were used, and lymphocytes were isolated by Ficoll density gradient centrifugation and isolated by negative selection (Stemcell, Germany). T–B cell co-culture, steroid treatment was applied. To evaluate the effects of steroids, time-dependent changes in $CD4^+ICOS^+$ expression on Tfh cells and $CD19^+HLA\text{-}DR^+$ expression on Bf cells were analyzed by flow cytometry (DxFLEX, Beckman Coulter).

This study was approved by Başkent University Institutional Review Board and Ethics Committee (Project no: KA22/308) and supported by Başkent University Research Fund.

Results: Expressions of ICOS on Tfh cells (Figure 1) and HLA-DR on Bf cells (Figure 2) are shown.

Conclusion: The interaction between Bf and Tfh cells is suppressed by the effect of steroids. The decrease in HLA-DR expression caused by steroids suggests that the antigen-presenting capacity of B cells is weakened, which may subsequently affect T-cell activation. The time-dependent increase in ICOS expression indicates that Tfh cells become activated; however, this activation is suppressed by the steroid effect. The reduction in ICOS expression caused by steroids is thought to inhibit the development of antibody-secreting plasma B cells. This situation indicates the regulatory role of steroids in immune responses and their potential to modulate the immune reaction by suppressing Tfh–B cell interactions in particular.

Keywords: B follicular cell, HLA-DR, ICOS, steroid, T follicular helper cell

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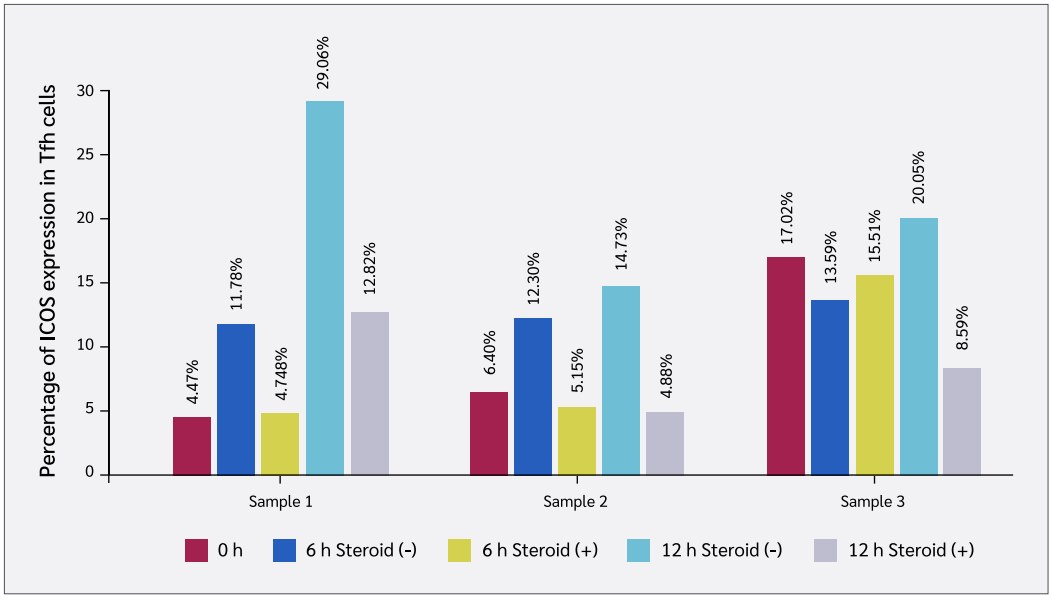

Figure 1. Effect of steroid treatment on ICOS expression in Tfh cells.

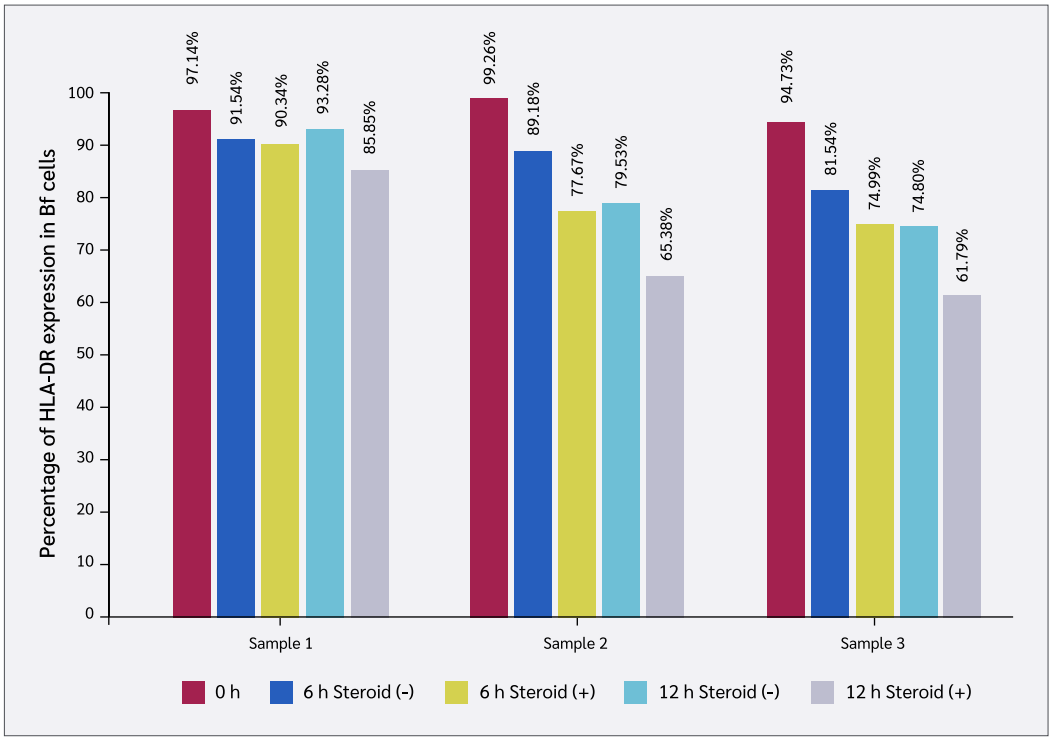

Figure 2. Effect of steroid treatment on HLA-DR expression in Bf cells.

POSTER PRESENTATION Turkish Journal of Immunology, 14(Suppl 1):26

Hidden Hierarchy of HLA Haplotypes: Resolution-Independent Directional Relationships via Asymmetric Linkage Disequilibrium

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Abstract

Objective: The Human Leukocyte Antigen (HLA) region is characterized by strong Linkage Disequilibrium (LD). However, traditional symmetric LD metrics fail to provide information on the direction of allelic relationships. This study aims to examine the effect of HLA typing resolution on directional relationships detected by Asymmetric Linkage Disequilibrium (ALD) and to investigate whether this hierarchy is a statistical artifact or a reflection of evolutionary architecture.

Materials and Methods: High-resolution (8-digit) HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 loci genotyping was performed using Next-Generation Sequencing (NGS) in 150 healthy and unrelated individuals of Central Anatolian origin. The 8-digit data were reduced to 2- and 4-digit levels to analyze three different resolution layers. Standard LD (*D'*) and directional relationships (ALD) between locus pairs were calculated using PyPop v1.2.1 software.

Results: Standard LD (*D'*) values increased significantly with higher resolution, showing the strongest signals in C: B (*D'*: ~0.94) and DRB1:DQB1 (*D'*: ~0.96) pairs. ALD analysis revealed that directional dominance was consistently preserved regardless of allele number or resolution. The predictive power of HLA-C for HLA-B was systematically higher than the reverse direction (C → B dominance) at all resolutions ($p<0.0001$). Similarly, HLA-DQB1 predicted HLA-DRB1 significantly better than the reverse (DQB1 → DRB1 dominance; $p<0.0001$). These hierarchies remained stable across 2-, 4-, and 8-digit levels.

Conclusion: ALD analysis is a powerful tool for revealing the hierarchical and directional structure of HLA haplotypes hidden by traditional symmetric metrics. The consistency of C → B and DQB1→DRB1 directional relationships, unaffected by typing resolution, suggests this hierarchy reflects the evolutionary history and functional integrity of the HLA region rather than statistical coincidence. These findings offer new perspectives for mapping genetic architecture and donor selection algorithms.

Keywords: HLA antigens, linkage disequilibrium, haplotypes, immunogenetics, ALD

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POSTER PRESENTATION Turkish Journal of Immunology, 14(Suppl 1):27

Necessity of a Turkish National HLA CWD Catalog: Inadequacy of Global Databases and a Proof-of-Concept Study

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Abstract

Objective: Common and Well-Documented (CWD) allele catalogs serve as global references for resolving ambiguous HLA typing. However, they may fail to represent the genetic diversity of specific populations like Türkiye. This proof-of-concept study aims to demonstrate the inadequacy of global catalogs in reflecting local diversity and to emphasize the critical need for a Turkish National CWD catalog based on Next-Generation Sequencing (NGS) data.

Materials and Methods: High-resolution (8-digit) HLA-A, -B, -C, -DRB1, and -DQB1 loci genotyping was performed using NGS in 142 healthy unrelated donors from the Eskişehir region. Allele frequencies were calculated using the Expectation-Maximization (EM) algorithm. The obtained genetic profile was compared with the Global CIWD 3.0.0 catalog (European and MENA datasets) and national catalogs of Germany, China, Russia, and Brazil.

Results: Although the Turkish population shares features with European and Middle Eastern populations, it exhibits a unique profile. Out of 228 distinct alleles identified at 8-digit resolution, 76 (33.3%) were not recorded in the Global CIWD 3.0.0 EU and MENA datasets. While representation was high at low resolution, it dropped dramatically at high resolution. Comparisons with other national catalogs revealed significant differences, confirming the necessity of local data. Additionally, 8-digit analysis showed that G-grouping masks dominant subtypes (e.g., A*02:01:01:01).

Conclusion: Global HLA catalogs are insufficient to represent the high-resolution genetic diversity of the Turkish population. Relying solely on global data may lead to overlooking optimal donors for Turkish patients. Establishing a dynamic National HLA CWD Catalog is a scientific and clinical necessity to improve donor matching accuracy, reduce ambiguous typing costs, and support disease association studies

Keywords: HLA Antigens, gene frequency, population groups, CIWD, catalog

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POSTER PRESENTATION Turkish Journal of Immunology, 14(Suppl 1):28

Impact of HLA Typing Resolution on Hardy-Weinberg Equilibrium: How 'Hidden Heterozygosity' Reveals Evolutionary Pressures?

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Abstract

Objective: The extreme polymorphism of the HLA system complicates Hardy-Weinberg Equilibrium (HWE) analyses. Low-resolution typing may create artificial homozygosity by grouping functionally distinct alleles. This study aims to determine how 2-, 4-, and 8-digit HLA typing resolutions systematically alter HWE results and to reveal mechanisms like "hidden heterozygosity".

Materials and Methods: High-resolution (8-digit) genotyping for HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 was performed using Next-Generation Sequencing (NGS) in 150 healthy, unrelated donors from Central Anatolia. The 8-digit data were reduced to 2- and 4-digit levels for comparison. HWE compliance was assessed using the Markov Chain Monte Carlo (MCMC) exact test via PyPop v1.2.1 software.

Results: Allele richness (***k***) increased systematically with resolution (e.g., HLA-B: 23 to 58 alleles), reducing observed homozygosity (e.g., HLA-C: 34 to 14 homozygotes). HWE compliance was highly resolution-sensitive. HLA-B consistently deviated from HWE at all resolutions ($p<0.05$). Conversely, HLA-C showed deviation only at 2-digit resolution ($p<0.0001$) but aligned with HWE at 4- and 8-digit levels, indicating a low-resolution artifact. Notably, HLA-DPB1 appeared compatible at lower resolutions but showed significant deviation only at the 8-digit level ($p<0.0001$), revealing hidden evolutionary pressure.

Conclusion: Low-resolution typing causes a "dilution effect," masking allelic diversity and leading to erroneous HWE assessments. The emergence of significant deviations in loci like DPB1 only at high resolution proves that evolutionary forces are obscured by lower-resolution methods. Therefore, 8-digit typing should be the gold standard for accurate population genetics and disease association studies.

Keywords: HLA antigens, gene frequency, heterozygote, genetics, population, NGS

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POSTER PRESENTATION Turkish Journal of Immunology, 14(Suppl 1):29

The Novel HLA Allele: HLA-C*12:05:03

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PP-11

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Abstract

The novel allele *HLA-C*12:05:03* differs from *HLA-C*12:05:01* by one nucleotide change G>T in exon 5 (GT-G>GTT).

The findings presented in this poster were previously reported as a novel allele and published in HLA: Immune Response Genetics.

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