# 13th EAST-WEST IMMUNOGENETICS CONFERENCE

## EWIC 2019 - BUILDING BRIDGES

# ABSTRACT BOOK

March 14-16, 2019
HOTEL PANORAMA
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University Hospital Center Zagreb, Croatia

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# 13th East-West Immunogenetics Conference

## Under the auspices of

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Hotel **"PANORAMA"**
Trg Kresimira Cosica 9, 10000 Zagreb, Croatia

# FACULTY LECTURES

# CROATIAN MODEL OF ORGAN DONATION AND TRANSPLANTATION

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Institute of Transplantation and Biomedicine, Ministry of Health, Zagreb, Croatia

## Introduction

There are several key elements common to all worldwide successful organ donation and transplantation systems. Croatia has successfully exercised its implementation in a manner adapted to the local health care system's reality. Namely, despite well-developed transplantation and immunogenetic segment (1971-1998), Croatian transplantation program, two decades ago had been greatly challenged by organ shortage. The Croatian Ministry of Health had launched a set of reforms carried out in a 10-year stepwise approach (2001-2011). Those reforms have resulted in a 10-fold increase in deceased organ transplantation rates. In 2015 Croatia reached 40 donors per million populations for the first time ever, thus ranked 1st in the world in deceased organ donation. Croatia is nowadays among the few worldwide countries with the highest capacity in terms of the provision of organ donation and transplantation services.

## Ethical principles

Croatian transplantation program is grounded on principles of altruistic donation, solidarity and equity. The altruistic act of donation has been nourished for over three decades as a highly appreciated gesture of loving care for others. Over time organ donation has become widely embraced by the whole Croatian society as a valuable contribution to the community.

## Organisation based on Coordinators Network

Key donation person(s) selected among the most skilled and experienced senior intensivists have been appointed in each hospital to provide a clinical leadership and intensive care expertise in deceased organ donation pathway. That was of the utmost importance in raising overall confidence and positive attitude towards deceased organ donation amongst critical care professionals.

## End of life care and Deceased Organ Donation Pathway

Deceased Organ Donation Pathway reveals several critical steps that should be timely and optimally addressed along the end of life care of the patient with a devastating brain injury; early identification of a patient who meets prognostic criteria for development of brain death; brain death determination; timely transition from a patient-oriented therapy to an organ-protective therapy - all should be facilitated under shared responsibility of critical care professionals and key donation person. Such operating protocols have been successfully implemented in all hospitals to ensure that organ donation is systematically imbedded into end-of-life practice, countrywide.

## Monitoring hospital performance

A specific set of quality indicators for the assessment of hospitals' performance in deceased organ donation program is specified within the national quality system for transplantation program, and is the subject of the regular audits performed by the health inspection.

## Optimal organ allocation

Croatia joined Eurotransplant in 2007. Eurotransplant membership has provided fine-tuned allocation system with a balanced exchange of organs between eight Eurotransplant member countries. Thus, since 2007 highly sophisticated computerised search for the best "organ match" has ensured optimal management of donated organs, evidence-based and fully transparent allocation process.

## Transplantation and immunogenetics

Transplantation surgical and immunogenetic areas have been additionally advanced and positively influenced due to increased organs availability, Eurotransplant membership and multidisciplinary approach.

## Conclusion

The Croatian organ donation and transplantation program is comprehensively grounded on the highest professional and ethical standards that both positively reflect Croatian health-care standards and social values. Enhanced governing and clinical leadership provided in conjunction with stepwise implemented organizational measures, have effectively contributed to Croatian transplant program approaching self-sufficiency.

## HISTORY OF HLA

**Francesca Poli**

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Since the detection of MAC, the first alloantigen present on human leucocytes which has become the first human leucocyte antigen (HLA-A2), we have witnessed the development of the HLA field which has moved from histocompatibility to what will be remembered as one of the major fields in medicine of the second half of the 20th century and beyond.

The history of HLA can be considered as an unprecedented story of international scientific collaboration.

This is the history of the pioneers in the field and the direct result of intense cooperation through the International Histocompatibility Workshops (IHWSs). All IHWSs have been instrumental for solving the complexity of HLA and the development of transplantation medicine. Just to mention few IHWSs achievements: the importance of HLA compatibility in clinical renal and in bone marrow transplantation, studies of HLA associated diseases, antibodies in chronic rejection, collection of reliable typing reagents, sera and generation of HLA databases.

Several pioneers won the Nobel Prize: Snell, Dausset, Benacerraf, Zinkernagel and Doherty, but many others would have deserved this prestigious recognition.

Thanks to the efforts of the pioneers and to the scientific collaboration, we have seen in parallel the development of transplantation medicine.

## CLINICAL DECISION MAKING IN HIGHLY SENSITIZED PATIENTS

**Mladen Knotek**

University of Zagreb Medical School, University Hospital Merkur, Zagreb, Croatia

Sensitization to HLA antigens occurs in approximately 30% of kidney transplant (KT) candidates. Preexisting donor-specific HLA antibodies (DSA) are associated with a risk for acute and chronic antibody-mediated rejection (AMR), with potentially inferior graft function and survival. DSA-associated factors, related to rejection risk include the highest MFI of the immunodominant antibody, MFI sum of all DSAs, DSA ability to bind complement, presence of class I and class II DSA, Non-HLA antibodies, such as angiotensin II type 1 receptor antibodies may be also pathogenic, leading to vasoconstriction, inflammation and fibrosis.

Antigen specificity detected by Luminex is used to minimize risk of DSA+ KT. DSA+ transplantation is avoided by precluding kidney offers with unacceptable antigens, by inclusion of a recipient in the Eurotransplant acceptable mismatch (AM) programme, or by kidney paired donation. If DSA+ KT cannot be avoided (e.g. in case of a single living donor, or in very broadly sensitized patients with antibody strength bellow threshold for AM inclusion), desensitization can be undertaken, using either high-dose IVIg+ rituximab, or plasma exchange + rituximab with low-dose IVIg, with acceptable results. Newer protocols with tocilizumab, or IDES (imlifidase) have been developed, as well.

Adequate immunosuppression is important in highly sensitized patients. Usually, antithymocyte globulin and/or rituximab induction, in combination with maintenance tacrolimus, mycophenolate mofetil and corticosteroids is used.

To summarize, KT in highly sensitized patients can result in good outcomes, following careful recipient-donor selection and close patient follow up.

# CTS AND RECENT DATA ON POST-TRANSPLANT MONITORING

**Caner Susal**

Antibody Laboratory, Collaborative Transplant Study, Institute of Immunology, Heidelberg University Hospital, Heidelberg, Germany

The majority of published studies on the impact of post-transplant donor-specific antibodies (DSA) on graft outcome are based on small numbers of transplants with graft loss. We took a different approach and investigated in the Collaborative Transplant Study (CTS) Serum Project a series of 64 patients with failed kidney transplants on whom a post-transplant serum obtained before graft failure was available for a possible association of the post-transplant presence, de novo development, or persistence of pre-existing DSA with graft failure.

Recipients and donors were HLA typed for the loci HLA A, B, C, DRB1/3/4/5, DQA1, DQB1, and DPB1, which allowed precise definition of donor-specific antibodies (DSA). Single Antigen Bead (SAB) assay-detected DSA and non-DSA antibodies were compared between patients with graft loss and matched control patients who had functioning grafts.

At the 500 MFI cut-off, as many as 95% of patients with and 94% without graft loss showed evidence of SAB-detected HLA antibodies. The incidence of DSA in these patients was 44% and 36%, respectively (P=0.47). With higher MFI cut-offs the difference between the two patient groups became more pronounced. At MFI ≥5,000, the patients with graft loss had a higher incidence of SAB-detected post-transplant antibodies than patients without graft loss (total antibodies: 59% vs. 36%; P=0.013; DSA: 19% vs. 9%, P=0.20; non-DSA: 56% vs. 33%, P=0.013). For 51 patients with graft loss a pre-transplant serum was available in addition to the post-transplant serum, which allowed the analysis of de novo antibody production after transplantation. The incidence of weak de novo antibodies (DSA or non-DSA) at MFI ≥500 was higher in the graft loss group than in the non-rejector group (88% vs. 55%, p=0.003). Due to the low incidence of DSA, the difference was significant only for non-DSA (P=0.003). Similar results were obtained at higher cut-offs. Interestingly, when the C1q binding ability in sera of 21 rejectors and 15 non-rejectors with DSA was compared, 24% of rejectors had C1q-DSA, whereas we did not observe any C1q-DSA in patients who did not reject (P=0.06). None of the 11 non-rejectors with persistent DSA pre- as well as post-transplant showed C1q-positivity, whereas 7 (58%) of the 12 patients with graft loss and persistent DSA were positive in their post-transplant serum in the C1q assay (P=0.005).

Two additional local studies in pediatric patients with a serum at indication biopsy and in high-risk adult patients with serial serum samples confirmed the strong predictive value of C1q-DSA. An analysis of patients who lost their graft due to C1q-DSA revealed the presence of pre- as well as post-transplant CD30 positivity and phases of under-immunosuppression. Pre-transplant DSA influenced graft outcome only in the presence of high sCD30. Others reported an association of de novo DSA development with fluctuations in tacrolimus levels. CTS data indicate that, also in the late post-transplant phase, intra-patient variation of tacrolimus trough levels is a strong risk factor for subsequent graft loss.

Prevention of under-immunosuppression seems to be extremely important in dnDSA development and associated graft losses.

# IMPACT OF THIRD GENERATION HLA SEQUENCING ON HAEMATOPOIETIC STEM CELL TRANSPLANT OUTCOMES IN A UK COHORT

**Steven GE Marsh**

Anthony Nolan Research Institute, London, UK
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Neema P Mayor[1,2], James D Hayhurst[1], Thomas R Turner[1,2], Richard M Szydlo[1,3], Bronwen E Shaw[4], Will P Bultitude[1,2], Jex-Ray Sayno[1], Franco Tavarozzi[1], Katy Latham[1], Chloe Anthias[1,5], James Robinson[1,2], Henny Braund[1], Robert Danby[1,6], Julia Perry[7], Marie Wilson [1,7], Adrian J Bloor[8], I. Grant McQuaker[9], Stephen MacKinnon[10], David I Marks[11], Antonio Pagliuca[12], Michael N Potter[5], Victoria T Potter[12], Nigel H Russell[13], Kirsty J Thomson[10], J Alejandro Madrigal[1,2], Steven GE Marsh[1,2]

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13. Nottingham University Hospital, Nottingham, UK

The gold standard for matching recipients and unrelated donors (UD) for haematopoietic cell transplantation (HCT) is allelic level for HLA-A, -B, -C, -DRB1 and -DQB1 (10/10 matched); HLA-DPB1 matching is advised. HLA genes are hyperpolymorphic but variation outside of the antigen recognition domain (ARD) has not been well defined in HCT pairs and the impact on UD HCT outcome is unknown. Third Generation Sequencing by Single Molecule Real-Time (SMRT) DNA sequencing was used to re-type a cohort of 891 UD HCT pairs to determine the clinical benefit of HLA matching at ultra-high resolution (UHR). Patients were transplanted for malignant disease between 1996-2011. Matching was determined at full gene level for HLA class I and

at CDS level for HLA class II genes, including all exons that encode the expressed extracellular domains of the mature protein. 29.1% of pairs had their matching status redefined after UHR HLA typing, mostly due to non-coding differences in HLA class I alleles and changes in HLA-DPB1 typing. Overall survival (OS) was significantly improved in 12/12 UHR HLA matched pairs compared to those previously identified as 12/12 matches, but now known to be mismatched (5 yr 54.8% vs 30.1%; p=0.022) and when compared to pairs with any mismatch at UHR (5 yr 55.1% vs 40.5%, p=0.005). Significant differences in OS were observed between 12/12 matched, 10/10 DPB1 permissively mismatched and 10/10 non-permissively mismatched patients (HR 1.98, p=0.001), predominantly due to higher NRM (16.8% vs 29.9%; HR 1.83, p=0.071) and increased acute GvHD (OR 2.37, p=0.018). When CMV matching was also included in the model, the best OS probability was seen for 12/12 HLA matched, CMV matched pairs (62.5%) while the worst was seen in 10/10 HLA-DPB1 non-permissively mismatched, CMV mismatched pairs (17.5%; HR 3.25, p<0.001). This study shows that UHR HLA matching, achieved by including exons outside of the ARD, introns and untranslated regions, can significantly improve UD HCT.

## HLA ANTIBODIES IN HAEMATOPOIETIC STEM CELL TRANSPLANTATION

**Ann-Margaret Little**

Histocompatibility and Immunogenetics, Laboratory Medicine Building,
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The negative impact of donor specific HLA alloantibodies in solid organ transplantation is well known and understood within the Histocompatibility and Immunogenetics community. However, the influence of DSA in the outcome of haematopoietic stem cell transplantation is less well regarded. As donor choices have evolved from HLA matched siblings and extremely well matched unrelated donors to mismatched cord blood and haplo-identical related donors, we are now finding more patients with antibodies reactive against their donor mismatches. During this presentation I will discuss strategies for screening stem cell transplant patients for HLA antibodies i.e. how and when. I will also present an update on what we know about the clinical impact of such antibodies and how they should be treated.

# NOVEL METHODS TO DETECT HLA-SPECIFIC B CELL MEMORY

**Sebastiaan Heidt**

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Current pre-transplant immunological risk assessment focuses on detection of serum antibodies produced by plasma cells, but ignores the contribution of the memory B cell compartment to the alloimmune response. To overcome this limitation, we have developed several assays to detect and quantify HLA-specific memory B cells in the circulation of sensitized patients. These include ELISPOT assays for detection of B cell memory against specific HLA class I and II molecules, as well as a donor-specific ELISPOT assay, making use of lysates from donor cells as source of the complete donor HLA repertoire. While informative, ELISPOT assays are labour intensive and difficult to standardize. Therefore, we have developed a new technique which involves a polyclonal activation step of B cells in unseparated PBMC samples, an IgG isolation step, followed by regular luminex single antigen bead analysis. Benefit of this approach is the simplicity of the procedure, and the possibility to compare the specificity profile between serum and the memory compartment. We provided proof of principle in a pilot study on a cohort of kidney allograft recipients (n=20) transplanted in the presence of pre-transplant SAB assay-defined donor-specific HLA antibodies (DSA) but with negative complement-dependent cytotoxicity crossmatches. All patients had at least two allograft biopsies (indication and/or surveillance) within the first year post-transplant. Donor-specific memory B cell-derived HLA antibodies (DSA-M) were detected in 9/20 DSA positive patients (45%). Allograft recipients with concurrent DSA and DSA-M pre-transplant showed a higher incidence of (sub) clinical ABMR (p=0.032) and a higher extent (g>or=1+ptc>or=1) of microvascular inflammation (67% versus 9%, p=0.02). Persisting DSA post-transplant had more often pre-transplant DSA-M than non-persisting (50% vs 13%, p=0.04). In conclusion, our newly developed method is sensitive, easy to perform and applicable in a clinical setting. Pre-transplant analysis of DSA-M may serve as a novel tool to evaluate risk for ABMR in pre-transplant DSA positive patients.

# THE ROLE OF LABORATORY IN KIDNEY PAIRED EXCHANGE PROGRAMME

**Sabine Wenda**

Department for Blood Group Serology and Transfusion Medicine, Medical University Vienna, Vienna, Austria

Living donation plays an increasingly important role in solid organ transplantation. In case of donor specific antibodies, however, transplantations cannot be realised. One way of overcoming these limitations is to pool several recipients with their potential living donors and combine them to new pairs where the HLA antibodies are no longer donor specific. Such kidney paired donation (KPD) programmes have been implemented worldwide.

In Austria first experiences with KPD were gained in 2011 with the IT support of the Australian KPD programme. In 2015 a cooperation with the Czech KPD programme was started. This 'trans-border' cooperation brought the expected advantage of a larger recipient-donor pool; however, some challenges for both the laboratory and clinicians appeared, primarily of logistic nature.

Quarterly, a virtual crossmatch is calculated in Prague for all donor recipient combinations. Basis are the HLA typings, antibody identifications and the clinical history. All recipients and prospective donors in our centre were HLA typed at low and high resolution level with SSO and NGS for HLA-A, B, C, DRB1, DRB345, DQA1, DQB1 and DPB1. For HLA antibody identification CDC screening and Luminex Single Antigen analysis for class I and class II is performed for recipients and unacceptable antigens are defined. Results are reported to Praha. Pairs with a negative virtual crossmatch are included in the formation of chains. Potential two-way and three-way chains were reported. In case of successful chains final analyses start. These include a CDC crossmatch, performed with total lymphocytes and B-lymphocytes and a FACS crossmatch, carried out with T- and B-cells. Luminex Single Antigen analysis is repeated from fresh serum and additional high resolution typing of the external donor is performed. Each centre is responsible for its own recipient and its requirements. The more precise the HLA typing and HLA antibody definition is before chain calculation, the more concordant is the initial virtual crossmatch with the final crossmatch. Thus, logistic challenges can be reduced to a minimum.

## KIDNEY TRANSPLANTATION IN HUNGARY

**Aniko Szilvasi**

Transplantation Immunogenetics Laboratory, Hungarian National Blood Transfusion Service, Budapest, Hungary

The Hungarian kidney transplantation program started in 1973. Since then, 8612 kidney transplantations were carried out, 625 (7.3%) of which were living donations. Four kidney transplant centers operate in Hungary, located in the biggest academic cities: Budapest, Debrecen, Pécs and Szeged. Between 1973 and 2018, 4826 (56%), 1098 (13%), 1064 (12%) and 1624 (19%) kidney transplantations were performed by the transplant centers, in each city respectively. All transplant centers had been served by a local immunogenetic laboratory operating in the same city before Hungary joined Eurotransplant (ET). Budapest laboratory obtained EFI accreditation in 2013 and all immunogenetic testings were centralized to the Budapest laboratory, where we serve all solid organ and hematopoietic stem cell transplant centers as the only Hungarian HLA laboratory.

Between 2014 and 2018 (5 years of full ET membership), 1708 kidneys > were transplanted to Hungarian recipients, 207 (12.1% of all transplants) from living donors and 1501 from deceased donors, from which 370 (24.7% of deceased donors) originated in a foreign ET country. Comparing the 5-year period before Hungary joined Eurotransplant (2008-2012) with the 5-year period of Eurotransplant full membership (2014-2018) the average HLA mismatch number (MM) of kidney transplants using deceased donors decreased from 3.27 (n=1181) to 2.88 (n=1497). Transplantations with 0-3 MM increased; and the highest increase were observed in the ratio of 0 MM transplantations (from 1.95% to 5.28%); while transplants with 4-6 MM decreased. At the end of 2018, there were 862 active Hungarian kidney patients on the waitlist, 772 (89.6%) of them are not sensitized, 87 (10.1%) patients are sensitized and 3 (0.3%) are highly sensitized.

## MORE THAN 25 YEARS OF »SLOVENIA DONOR«

**Blanka Vidan Jeras**

Tissue Typing Center, Blood Transfusion Centre of Slovenia, Ljubljana, Slovenia

In the former common state, the modern method of autologous hematopoetic stem cell transplantation (HSCT) was initiated in 1983 in Zagreb which was associated with the launch of the nuclear power plant in the neighbourhood. Since it turned out that one transplant center is not enough in 1989 first autologous HSCT was also performed in Ljubljana. Slovenian patients not appropriate for autologous HSCT and lacking related donor were sent for HSCT from unrelated donor abroad, especially to UK. A precondition for establishing national unrelated transplantation program was Slovenian unrelated donor registry. HLA types of the first 100 potential donors travelled on a floppy disk to the BMDW on January 1992, making Slovenia Donor visible in the world. The registry experienced first unexpected big growth wave initiated through media by a patient. HLA types of donors mirror development of typing techniques in time and reveal extensive HLA-A-B-DR haplotype diversity where only 7 haplotypes with frequency more than 1% were observed. Italian and Hungarian minorities that live next to the border with countries of origin keep some HLA types significantly different from other Slovenian regions. While Italian donors were chosen for our patients and vice versa it was not the case with Hungarians. However, 42% of Slovenian donors donate for Slovenian patients. Although HSCTs from haploidentical donors are on their way, indications for unrelated HSCT are still growing as well as the number of transplantations performed yearly, where acute myeloid leukemia (AML) is the most commonly treated disease. Numerous students registered in the Slovenia Donor at the time of its 25$^{th}$ anniversary through the campaign "Put yourself on the list" initiated by the Slovenian Association of Patients with Lymphoma and Leukemia. As a consequence, a group of donors age 18-25 become 4-times bigger in one year. Our approach had to be changed crucially to be heard by generation Y.

# SELECTED IMMUNOLOGICAL PREDICTORS OF GVHD AND GVL IN ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION

**Frantisek Mrazek**

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Allogeneic haematopoietic stem cell transplantation (aHSCT) is used as a curative treatment in severe haematological malignancies, primary immunodeficiencies and hereditary metabolic disorders. Although clear improvement of the aHSCT approaches has been achieved in the last decade, substantial proportion of patients still suffer from immune-mediated complications including graft versus host disease (GvHD). On the other hand, in malignant diseases immune alloreactivity represented by graft versus leukaemia/lymphoma (GvL) effect mediated by similar donor-derived T-cell/NK-cell reaction may prevent relapses. At present, our real possibilities to predict and differentiate both damaging GvHD and beneficial GvL are limited. Nevertheless, apart from the well established immunogenetic factors associated with GvHD, several "intelligent" approaches have recently been introduced into the transplantation protocols, such as a model of HLA-DPB1 permissive mismatches or a preference for donors with highest KIR-B score among those equivalent according to HLA match for the patients allografted for acute myelogenous leukaemia. Furthermore, the important mediators and cells of the immune response are for long term investigated in the studies focused on the identification of systemic and local biomarkers of the aHSCT outcome. Recently, a prognostic score for acute GvHD prediction based on circulating molecules TNFR1, ST2, and REG3α was proposed and evaluated in a multicenter study. Another paper revealed tight association of decreased number of the regulatory T-lymphocytes (CD4+CD25hiFOXP3+) with the higher occurrence and severity of GvHD. Very recently, promising reports from animal models revealed new molecular and cellular targets for separation of GvHD and GvL (PD-L1, myeloid-derived suppressor cells, Notch signalling, Ikaros). These results bring us closer to the development of effective clinical strategies to enhance GvL while minimizing GvHD following aHSCT, and therefore to the haematopoietic stem cell therapy with higher overall benefit for the patients.

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## Haploidentical stem cell transplantation – single centre experience

**Milena Vrana,** Sara Nazarova, Hana Cechova

HLA department, Institute of Hematology and Blood Transfusion, Prague, Czech Republic

Allogeneic hematopoietic stem cell transplantation (HSCT) is one modality used in the treatment of hematologic malignancies. HLA match makes up the fundamental criterion for the success of allo-HSCT for many years. New approaches developed in last decades allow transplantation across HLA mismatch barrier from haploidentical family donors. This provides an opportunity for nearly all patients to benefit from HSCT when HLA-matched donor is not available.

Specific immunosupression is concerned on prevention of GvHD and HvG effects without loss of desirable graft versus leukemia and graft versus infection effects. Currently, the post-transplant administration of cyclophosphamide is the golden standard in haplo-HSCT.

There are some specificities in selection of haploidentical family donors. No benefit of better match in non-inherited haplotype is known, some studies declare lower rate of relaps and better disease-free survival associated with greater HLA disparities. The other important factor is a negative crossmatch; the presence of donor specific antibodies on crossmatch is associated with important increase of graft failure.

Haploidentical transplantation program started in our institute in 2014. Analyzed cohort included 56 patients transplanted till the end of 2017. Donors were mostly children (66%), haploidentical siblings (20%) and parents (14%). The most often diagnosis in transplanted patients was AML.

Crossmatch was crucial in selection of haploidentical donors. Presence of donor specific antibodies was contraindication for the transplantation. The degree of disparities in non-inherited haplotype was not taken into account. Other selection criteria (ABO, donor age, sex, CMV status, atc.) were identical to HLA-match donor search.

Myeloablative and non-myeloablative regiments similar to other HSCT were used. The difference was administration of fludarabin in days -5 till -1 and post-transplant administration of cyclophosphamide in days +3 and +5 in dose 50 mg/ 1 kg of patient body weight.

Post-transplant monitoring was based on standard tests of stem cell chimaerism. Additionally, specific HLA markers were searched. Relapse was detected in 25% of the cohort, no HLA-loss relaps has been detected so far.

Overall survival of the cohort was about 50%; these data are adequate to the results of HLA-match transplantations in the similar group of patients. The most often cause of death was relaps of the disease.

These preliminary data verified the suitability of haploidentical-HSCT as an alternative for our patients with no HLA-match donor.

Supported by the Ministry of Health, Czech Republic - conceptual development of research organization (00023736, UHKT).

## HLA POLYMORPHISM – WHAT CAN WE LEARN ABOUT THE EVOLUTION OF POPULATIONS

**Alicia Sanchez-Mazas**

Department of Genetics and Evolution – Anthropology Unit, University of Geneva, Geneva, Switzerland

The HLA diversity that we observe today in human populations is the result of a long and complex evolutionary history during which anatomically modern humans migrated across all continents from a single origin in Africa about 200'000 years ago. During their journey, human populations underwent major demographic changes and had to adapt biologically to very distinct environments. Among many other selective pressures, they were exposed to different kinds of pathogens to which they were more or less susceptible depending on their HLA genetic profiles. By analyzing the molecular diversity of HLA genes in present-day populations, it is possible to detect signatures of both demographic and adaptation events that occurred in their history. This presentation will show several examples of what we can learn in this context.

# DISTRIBUTION OF HLA ALLELES AND HAPLOTYPES ACROSS CROATIA –SIMILARITIES AND DIFFERENCES

**Zorana Grubic**

Tissue Typing Centre, Clinical Department of Transfusion Medicine and Transplantation Biology, University Hospital Centre Zagreb, Zagreb, Croatia

In this study we present differences in HLA allele and haplotype frequencies across Croatian's regional populations since the information on HLA allele and haplotype frequencies in Croatians published in a few previous publications was not considering regional diversities.

The study included HLA data of 4792 volunteer unrelated donors from Croatian Bone Marrow Donor Registry (CBMDR) collected in ten different recruitment cities from five Croatian regions (Slavonia-Osijek, Slavonski Brod; North Croatia-Varazdin, Cakovec; Central Croatia-Sisak, Karlovac; Istria and Primorje-Rijeka, Pula; Dalmatia-Zadar, Split).

The group size ranged from 243 individuals up to 1071 individuals and was in line with that of the city population as a whole. The capital, Zagreb, was excluded from regional distribution analysis. Regional distribution analysis was performed for ten most frequent HLA-A, B, C, and DRB1 alleles and four-locus haplotypes observed previously in the general population study.

HLA-A*02:01 was the most frequent HLA-A allele, with frequencies ranging from 24.4% to 31.9%, while depending on the recruitment city, HLA-A*01:01, A*03:01, A*11:01, and A*24:02 are second, third, fourth or fifth most frequent alleles, respectively. At HLA-B locus, in 8 out of 10 cities the most frequent allele was HLA-B*51:01, while HLA-B alleles reaching a frequency of 5% in at least one city were: HLA-B*07:02, B*08:01, B*15:01, B*18:01, B*35:01, B*35:03, and B*38:01. HLA-C*07:01 or C*04:01 alleles were the most frequent HLA-C allele, followed by C*02:02, C*06:02, C*07:02, and C*12:03 depending on the city. The distribution at HLA-DRB1 locus showed the highest differences, in 4 out of 10 cities the most present was DRB1*03:01 allele, DRB1*07:01 and DRB1*16:01 were most present in two instances, each, while DRB1*15:01 and DRB1*11:01 were most present in one city each.

A total of 31 alleles and seven haplotypes exhibited statistically significant differences between the city with highest observed frequency on one side and the city with the lowest observed frequency on the other side. These differences remained the same when the analysis was performed at the regional level. The peculiarity was found for North Croatia, revealing statistically significant diverging frequencies for two alleles (C*12:03 and DRB1*16:01) and two haplotypes (A*02:01~B*44:02g~C*07:04~DRB1*16:01 and A*02:01~B*27:02~C*02:02~DRB1*16:01) in comparison to all other four regions. Data presented depict allele and haplotype frequencies that appear to vary significantly across Croatia and consequently may be very useful for optimizing donor recruitment strategies in CBMDR.

## DISTRIBUTION OF HLA HAPLOTYPES IN RUSSIAN POPULATION FROM DIFFERENT REGIONS

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The aim of our study: to evaluate immunogenetic characteristics among Russian population from different regions of European part of Russia: from the North-west region (St. Petersburg), from the center of the East European Plain (Nizhniy Novgorod), from the Middle Volga (Samara), from the South-west region (Rostov-upon-Don) and from the central part of Eurasia (Pervouralsk). The study was performed in the 2016-2017 in the Russian Center of Tissue Typing (EFI accredited) in the Russian Research Institute of Hematology and Transfusiology. HLA typing (A*, B*, DRB1* low res.) was performed in 3161 persons included into the Registry of potential bone marrow donors (from St. Petersburg 1955 donors, from Nizhny Novgorod – 430, from Samara – 472, from Rostov-upon-Don – 174 and from Pervouralsk – 130). According to the data presented in the donor questionnaire, all the examined individuals self-determined as Russian.

The most frequent groups of alleles HLA-A* in all these regions are: *02, *03, *01, *24. The most frequent groups of alleles HLA-B* in St. Petersburg and Nizhniy Novgorod are: *07, *35, *44, *08, whereas in Samara – HLA-B *07, *35, *44, *18, in Rostov-upon-Don – HLA-B *44, *07, *18, *51 and in Pervouralsk – HLA-B *35, *07, *44, *15. The most frequent groups of alleles HLA-DRB1*in St. Petersburg are: *15, *07, *13, *11, in Nizhniy Novgorod and Samara – HLA-DRB1*01, 15, 13, 07, in Rostov-upon-Don – HLA-DRB1*11, *13, *07, *04, in Pervouralsk – HLA-DRB1*13, *01, *15, *04. The list of the first most frequent five HLA-A*-B*-DRB1*haplotypes of St. Petersburg, Nizhny Novgorod, Samara, and Rostov-upon-Don included A*01-B*08-DRB1*03, A*03-B*07-DRB1*15, A*02-B*13-DRB1*07, A*02-B*07-DRB1*15, A*03-B*35-DRB1*01 with different frequencies. Whereas in the Pervouralsk the most often determined haplotypes are HLA-A*02-B*07-DRB1*15. A*02-B*35-DRB1*01, A*03-B*07-DRB1*03, A*01-B*08-DRB1*03.

The distribution of HLA-A*-B*-DRB1* haplotypes in Russian population from the center of the East European Plain and the Middle Volga is similar, whereas in the North-west and South-west region and especially the central part of Eurasia has its own characteristics.

## TRALI AND LEUKOCYTE ANTIBODIES

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Transfusion related acute lung injury (TRALI) even after the introduction of mitigation strategies in most countries is one of the most common elicitors of severe transfusion-related morbidities.

Immune TRALI is a non-cardiogenic lung edema presenting within 6 hours after blood transfusion, characterized by hypoxemia and new bilateral interstitial infiltrates on frontal chest radiograph. Antibodies to human neutrophil antigens (HNA)-1, 2, 3, 4 and 5 as well as HLA class I and class II in the plasma of multiparous donors are the main elicitors. Transfused HNA or HLA class I antibodies induce neutrophil aggregation, priming and interaction with the lung endothelium. Reactive oxygen species and soluble mediators released by the activated neutrophils cause capillary leakage and interstitial lung edema. Especially antibodies with HNA-3a specificity induce severe and fatal TRALI because they do not only bind and activate the neutrophils but also endothelia of the lung capillaries. Contrary, HLA class II antibodies do not bind to neutrophils but act via prompting monocytes to release cytokines that in turn activate the neutrophils and can induce severe TRALI cases. Since the introduction of a male plasma only strategy in many countries the incidence of TRALI was significantly reduced. However, transfusion of platelet units usually is not included in this strategy because the loss of products would be too high. Instead, many blood transfusion services test their female platelet donors with a history of pregnancy for the presence of the respective antibodies.

HNA antibody testing includes the classical granulocyte agglutination test (GAT), especially for HNA-3a, and -3b antibodies, indirect granulocyte immunofluorescence (GIFT) and the monoclonal antibody-specific immobilization of granulocyte antigen (MAIGA) test. Fluorescent bead assays (Luminex) are available, too, but actually cannot reliably detect all HNA-3 antibodies. Testing for HLA antibodies includes ELISA methods, flow cytometry, and the classical CDC. Luminex assays are possible, too, but the sensitivity is higher than required. HLA and HNA typing usually is performed by PCR methods, except for HNA, that to date only can be determined by means of serology.

Mitigation strategies significantly reduced the risk of TRALI but specialized laboratory diagnostics are still required.

# THE ROLE OF HLA TYPING IN RHEUMATIC DISEASES

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Over 100 diseases are associated with classical HLA class I and II genes. When addressing the relevance of HLA and disease association studies, we should not forget that ethnic differences, definition of the patient group, the advancements of typing techniques, statistical methods and finally, linkage disequilibrium, might all influence the application of research results to clinical practice. The aim of this presentation is to review the role of HLA typing in the management of some rheumatological diseases, namely Rheumatoid Arthritis (RA), Spondyloarthritis (SpA) and Juvenile Idiopathic Arthritis (JIA).

RA is the most common inflammatory arthritis which primarily targets synovial joints resulting in significant pain, functional limitations and mortality. The most relevant biomarkers used in clinical management of RA are Rheumatoid Factors (RF) and antibodies to citrullinated peptides (ACPA); however, neither of the tests is sufficiently specific to establish the diagnosis of RA and prognosis varies widely with seropositive and seronegative patients. HLA-DR4 has been known to be associated with RA Caucasian patients for more than 4 decades (Stastny 1976), and it is widely accepted that the HLA-DRB1 gene is the major genetic susceptibility locus for RA. Two alleles, HLA-DRB1*04:01 and DRB1*04:04, mostly explain the originally observed serological association. Moreover, some HLA-DRB1*01 and DRB1*10 alleles have also been found to be associated with RA in patients who are negative for DRB1*04. In the late '80s, it was found that the associated alleles shared a region of highly similar amino acid (a.a.) sequence leading to speculate that this portion of the HLA-DRB1 molecule (a.a. 70 to 74), also called shared epitope (SE), controls susceptibility to disease (Gregersen 1987). In non-Caucasian populations with a low frequency of HLA-DRB1*04, the associated alleles still contain the SE. More recently, it was shown that a small number of a.a. sites within HLA-DRB1 protein (positions 11 and 13 which are tightly linked, 71 and 74) are highly associated with RA. Individuals of Caucasian origin can be classified into 16 different risk categories solely based on their two field HLA-DRB1 type or on the a.a. carried at positions 11/13, 71, and 74 of HLA-DRB1 (Raychaudhuri 2012). The highest risk categories correspond to SE positive individuals, while the lower risk categories are defined by protective HLA alleles (for example, HLADRB1*13:01). HLA typing is not useful for diagnosis or screening of RA; although certain HLA alleles are strongly associated with severe RA, these alleles are common in the normal population. Routine HLA typing and SE status cannot be recommended for estimating prognosis, since RF testing and ACPA testing are widely available and the most useful markers for predicting which patients with early inflammatory arthritis will develop erosions. Once the diagnosis of RA is established, genotyping for SE alleles may help predict which patients are at highest risk of severe and erosive disease, and thus which are candidates for early and aggressive intervention.

Spondyloarthritis (SpA) are a family of rheumatic disorders which share some clinical features: inflammation of axial joints (especially the sacroiliac joints), asymmetric oligoarthritis (especially of the lower extremities), dactylitis (sausage digits), enthesitis (inflammation at sites of ligamentous or tendon attachment to bone) and a strong association with HLA-B*27. There are no laboratory findings that are absolutely specific for SpA. Most notable are HLA-B*27 and elevated acute phase reactants. In most ethnic groups, more than 90 percent of patients with ankylosing spondylitis (AS) and 50 to 70 percent of patients with other forms of SpA are positive for HLA-B27. In AS Caucasian patients, HLA-B*27:05 confers greater risk whereas, in Asian patients, it is B*27:04. HLA-B*27:06 and B*27:09 are not associated with AS. Approximately 20% of Psoriatic Arthritis are HLA-B*27 positive, and other associated HLA specificities are HLA-C*06:02, B*38, B*39 and B*08. HLA-B*27 testing is frequently obtained in patients suspected of SpA; however, the presence of HLA-B*27 by itself is not diagnostic of SpA, since a significant proportion of subjects in the general population are also positive. The diagnosis of axial SpA should be doubted if both HLA-B*27 and imaging (including magnetic resonance imaging) for sacroiliitis are negative. HLA-B*27 typing is a stronghold of algorithms for SpA diagnosis.

Juvenile Idiopathic Arthritis (JIA) is characterized by a variable pattern of articular involvement and systemic symptoms and thus has been classified in several subtypes. Genetic predisposition to JIA is mainly due to HLA class II molecules (HLA-DRB1, HLA-DPB1), although HLA class I molecules and non-HLA genes are also implicated. We carried out a meta-analysis designed to assess HLA genetic background of JIA patients, compared to healthy controls. Our analysis showed four main findings regarding HLA-DRB1 locus as a genetic factor of JIA: i) HLA-DRB1*08 is a strong factor predisposing to JIA, both for oligo-articular and poly-articular forms; ii) HLA-DRB1*01 and HLA-DRB1*04 may be involved in the genetic predisposition of RF+ forms of JIA; iii) HLA-DRB1*11 was confirmed to be predisposing to oligo-articular JIA; iv) HLA-DRB1*04 was confirmed to have a role in systemic JIA. Importantly, RF positivity seems to select the JIA clinical subset with the strongest immunogenetic similarities with adult RA.

In conclusion, it is evident that HLA plays a notable role in rheumatic disorders, even though its relevance for clinical management is not homogeneous in the different diseases. It is now fully recognized that early diagnosis of rheumatic diseases is pivotal in beginning treatment promptly, and in this view, in rheumatic conditions which lack the classical biomarkers or in the overlapping and complex cases, HLA typing may be of support for the optimal management of patients.

## HLA AND SARCOIDOSIS

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Sarcoidosis, a systemic granulomatous disease with immune features, has been a long term research subject of both medical and immunobiological investigators. Association of especially its pulmonary manifestation with HLA genetic variation was revealed already at serology, antigenic level (HLA-A1, HLA-B8). Subsequently DNA typing has revealed a range of HLA associations, importantly also with disease clinical course and some typical for particular ethnicities such as African Americans or East Asians [Ref. 1]. In this review, these developments will be summarised and expanded with current concepts of sarcoidosis immunogenetic research such as efforts to refine existing associations with help of NGS HLA allelic typing [2], investigations of possible immunopharmacogenetic component or developments of simplified tagging approaches for the most clinically meaningful associations exemplified by HLA-DRB1*03 for mild disease and HLA-DRB1*15 for progressive sarcoidosis [3]. Contribution of GWAS / WES studies will be mentioned as well [4].

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# SELECTED ABSTRACTS

# ORAL PRESENTATIONS

## PIRCHE EPITOPE MATCHING IN LIVER AND HEART TRANSPLANTATION – A SINGLE CENTER STUDY

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Studies from several centers have indicated that PIRCHE scores in kidney-transplant patients may correlate with the incidence of *de novo* production of donor specific antibodies (DSA). According to these data, recipients with high PIRCHE indexes have increased incidence of *de novo* DSA compared with patients with low PIRCHE scores. On the other hand, data on the predictive value of PIRCHE matching in patients who have undergone other organ transplantation (besides kidney) are scarce. The aim of our study was therefore to analyze the clinical relevance of PIRCHE epitope matching for DSA production and the incidence of acute rejection after liver and heart transplantation. A retrospective analysis of 103 liver and 53 heart transplant recipients was performed. HLA antibody presence and specificity, HLA mismatches with respective donors, rejection incidence and other parameters were analyzed. Our preliminary data showed that PIRCHE scores clearly correlate with the HLA mismatches between patients and their respective donors in all patient groups. A significant correlation was also observed between PIRCHE and the incidence of antibody-mediated rejection in heart transplant recipients (139.49 ± 63.78 vs. 97.43 ± 41.52, p =0.0328). No relationship was found between PIRCHE scores, the occurrence of rejection and the production of *de novo* DSA in liver transplant patients. Surprisingly, patients with acute cellular rejection after heart transplantation had lower PIRCHE indexes in comparison with patients free of rejection (91.89 ± 42.66 vs. 120.64 ± 46.50, p =0.0269). Our preliminary data suggest that taking into account PIRCHE score numbers may reduce the immunological risk after heart transplantation, however, the predictive value of PIRCHE matching concerning the production of *de novo* DSA and the incidence of rejection after liver transplantation would need further investigation.

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## CAN PREDICTED T-CELL/B-CELL EPITOPES AND MOST POSSIBLE SELECTIVE ANTIGEN RECOGNIZED BY HLA ANTIBODIES USING IMMUNOINFORMATIC METHODS HELP FOR BETTER DONOR MATCH?

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Aim: Epitopes bind to the antigen-specific membrane receptors on T-Cells/B-Cells and HLA antibodies of the immune system. We aimed to select the most possible selective antigen could be recognized by HLA antibodies which share same predicted T-Cell/B-Cell epitopes by using the binding specificity computed by bioinformatic methods.

Methods: Both B and T cells can react with epitopes, but specifications of epitopes are different for both reactions. B-Cell epitopes comprise the structural protrusion specifications for B-Cell receptor or antibody. But, T-Cell epitopes presented to the T-Cells processed by MHC I and II complex can be predicted by identifying the shortest peptides within an antigen which can stimulate either CD4 or CD8 T-Cells. T-Cell epitope prediction method uses the three basic steps as (i) the MHC binding prediction which calculates threshold values for peptides binding to specific MHC molecules using the binding energy and the binding strength calculation, (ii) the MHC processing prediction using the predicting proteasomal processing of peptides in the cell and (iii) the MHC immunogenicity prediction using the amino acid properties and their position within the peptide sequence. Predicting the most possible selective antigen calculates the score for higher affinity and selectivity together with the T-Cell and B-Cell epitope prediction methods. These immunoinformatic methods are combination of sequence motif, structure-based, motif matrix, quantitative affinity matrix, support vector machine, artificial neural network and quantitative structure-activity relationship for both T-Cell and B-Cell epitope prediction.

Results: We found that HLA-A*30:02 antigen is the most possible selective antigen for the patient's antibody against to the HLA-B*27:05 by using these immunoinformatic methods for prediction. Both HLA-B*27:05 and HLA-A*30:02 antigens share the predicted T-Cell/B-Cell epitopes having "KSSGGKGGSY" peptide sequence with the highest binding specificity according to the combination of artificial neural network method and stabilized matrix method.

Conclusion: The immunoinformatic epitope prediction methods can help by predicting T-Cell/B-Cell epitopes shared by antigens against to the HLA antibodies and by predicting the most possible selective antigens using the binding energy and the binding strength calculations for predicted epitopes for better donor match and increased survey time for solid organ transplantation.

# LONG-TERM OUTCOME OF KIDNEY TRANSPLANT REJECTION WITH MICROVASCULAR INJURY

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Aim: To investigate the long-term kidney allograft outcome in patients with microvascular injury and its association with donor-specific HLA antibodies (DSA) and non-HLA antibodies (Ab).

Methods: Retrospective analysis included 46 patients who had kidney transplantation, or simultaneous pancreas and kidney transplantation at Merkur hospital between 2007 and 2016, with microvascular injury (MVI) on protocol or indication biopsy. DSA were tested before transplantation and at the time of rejection, while anti-angiotensin II type 1 receptor (anti-AT1R) and anti-endothelin A receptor (anti-ETAR) Ab were determined at the time of rejection.

Results: The baseline patient characteristics are shown in Table 1. Median time from transplantation to rejection was 41 days (IQR 10-191). DSA and/or non-HLA Ab were identified in 36 out of 46 patients at the time of rejection. Among them 11 patients were positive only for de novo DSA, 12 patients were positive only for non-HLA Ab (8 AT1R-Ab positive, 4 positive both for AT1R-Ab and ETAR-Ab), while 13 patients had both DSA and non-HLA Ab (4 preexisting DSA, and 9 de novo DSA). The cumulative treatment for graft rejection included corticosteroids in 80.4%, anti-thymocyte globulin in 28.3%, plasmapheresis in 37%, intravenous immunoglobulins in 21.8%, bortezomib in 21.7% and rituximab in 4.3% of patients. Kidney function at the time of rejection was 28.9±19.7 ml/min/1.73m2, with subsequent improvement after multimodal rejection treatment. During the median follow up period of 3.4 years (IQR 2.6-6.1) death-censored graft survival was 77.2% and did not differ between patients either positive or negative for DSA and/or non-HLA Ab.

Conclusion: The results show that patients with microvascular injury either positive or negative for DSA and/or non-HLA Ab may have acceptable long-term kidney allograft survival when multimodal treatment of rejection is applied.

**Table 1.** Baseline characteristics

| | |
|---|---|
| **Patient characteristics** | |
| Age (years) | 47 (36-57) |
| Male/Female | 63%/37% |
| Time on dialysis (days) | 1650±1938 |
| Prior transplantation | 10.9% |
| Kideny transplantation/SPKT | 91%/9% |
| **Donor characteristics** | |
| Age (years) | 50 (46-58) |
| Male/Female | 43%/57% |
| Deceased donor | 84.8% |
| **Immunological characteristics** | |
| Sensitized patients | 47.8% |
| Peak PRA | 32.3±38.2 |
| HLA Mismatch<br>• A locus<br>• B locus<br>• DR locus | <br>1.2±0.5<br>1.3±0.7<br>1.0±0.6 |
| **Immunosupression** | |
| Induction agent:<br>Anti-IL-2 ab<br>ATG<br>Rituximab | <br>84.8%<br>8.7%<br>6.5% |
| Maintenance:<br>• TAC/MMF±prednison<br>• CyA/MMF±prednison<br>• Others | <br>91.3%<br>8.3%<br>0.4% |

## INFLUENCE OF INFREQUENT HLA ALLELES AS PREDICTIVE VALUE TO FIND A MATCHED UNRELATED HEMATOPOIETIC STEM CELL DONOR - EXPERIENCE OF THE SERBIAN BONE MARROW DONOR REGISTRY

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The probability to find HLA matched unrelated hematopoietic stem cell donor (MUD) within a relatively short time is useful information to make decision on the transplantation strategy for patients without sibling donor. The aim of this study was to analyze influence of infrequent HLA alleles as predictive value to find MUD. This study included 244 patients for whom the searching was performed through the Serbian BMDR. Five loci high resolution typing by PCR-SSP (Olerup) were performed for all patients and their donors. Twenty-four HLA-A, 50 HLA-B, 25 HLA-C, 35 HLA-DRB1 and 17 HLA-DQB1 different alleles were identified and 48 of them were observed less than three times. The most infrequent alleles were present in locus HLA-B (18 alleles), then in loci HLA-DRB1 (11 alleles), HLA-A (8 alleles), HLA-C (7 alleles) and HLA-DQB1 (4 alleles). A 10/10 matched donor was found for 131 (60.09%) out of 218 patients for whom the search of donor was finalized. The presence of at least one infrequent allele was observed in 11.45% (15/131) of patients with 10/10 matched donor which was significant difference regarding to 37.93% (33/87) of patients without a fully matched donor ($p<0.05$). The most patients had infrequent allele in HLA-B (16.09% of patients without a fully matched donor and 2.29% of patients with a 10/10 matched donor) and HLA-DRB1 (8.04% and 3.05%). There were 7 patients with one and 1 with two infrequent alleles in the group of patients with a 10/10 matched donor, opposite to 22 patients with one and 3 patients with two infrequent alleles in the group of patients without a 10/10 matched donor. The study confirmed that the presence of low frequent alleles influence probability of finding suitable donor and detailed analyses of HLA allele and haplotype polymorphisms would help to predict in advance which patient's HLA will result in an unsuccessful search.

## CROATIAN HSCT PROGRAM – HLA TYPING OVERVIEW

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Tissue Typing Centre (TTC) at University Hospital Centre Zagreb is the single Croatian tissue typing laboratory providing services for allogeneic HSCT program in Croatia. Aim of this study is to present this work in the period 2010-2018.

The number of patients referred to TTC for HLA typing has been increasing progressively in the past decade, and has reached the average number of 200 patients and 350 family members in the last three years. If a matched donor is not found (72% of cases), search for an HLA matched unrelated donor is initiated. In the period from 2010-2018, TTC provided search services for >500 Croatian patients, and >3,500 patients from other countries, reaching more than 500 preliminary searches performed yearly for the last three years. A successful search with a 10/10 donor resulted in 65% of Croatian patients, while for the patients for whom a 9/10 matched donor was identified, the most frequently disparate locus was HLA-A (38%), followed by HLA-C and -DRB1 (22% and 19%, respectively). Over the past several years, number of performed haploidentical HSCTs has increased and surpassed the number of related HSCTs done in Croatia in 2018. In those cases, in addition to first and verification HLA typing of the patient and respective donor, cross-match as well as screening test results were provided by TTC. Finally, by the end of 2018, Croatian Bone Marrow Donors Registry (CBMDR) enlisted more than 53,000 HCS donors. The majority of CBMDR donors (99.3%) are typed by TTC for HLA-A, -B and -DRB1 at low/intermediate resolution. In addition, 24% of CBMDR donors are typed for HLA-C locus, and 0.6% for HLA-DQB1 locus. PCR-SSP method and SBT method are used for high resolution typing which is done only upon request for a national/foreign patient. Total number of HLA typing performed upon request (2010-2018) was 147 and 1,558 (low and high resolution, respectively). In conclusion, the activities performed by TTC relating to HSCT program in Croatia have shown a steady rise over the past decade and reflect the value of a tissue typing laboratory as a key part of a successful HSCT program.

## HLA GENES AND HAPLOTYPES IN THE RUSSIANS AND THE BURYATS OF TRANSBAIKAL TERRITORY

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The population of Transbaikal Territory (Zabaykal'skiy kray) of the Russian Federation is more than a million people. Until now distribution features of HLA genes of this population have not been studied. Our study included potential unrelated hematopoietic stem cell donors of Transbaikal Territory, who according to their self-assessment were the Russians (n=296) and the Buryats (n=150). HLA-typing was performed using Luminex 200 with Lifecodes HLA SSO typing kits (Immucor). Olerup SSP kits (CareDx) were used for resolving ambiguities. HLA-A*, -B*, -DRB1*, -DQB1* loci were typed at low resolution, HLA-C* locus at high. Statistical analysis was done using ARLEQUIN 3.5 software. Haplotype frequencies were estimated by expectation–maximization algorithm. Nei's standard genetic distances between the investigated populations and other 42 populations (available at http://www.allelefrequencies.net) were calculated using the PHYLIP 3.695 program. Among the Russians we found 15 allele groups for HLA-A, the most frequent: A*02 (28.7%), A*03(15.5%) and A*24 (11.7%). HLA-B had 15 allele groups, the most frequent: B*07 (13.5%), B*44 (10.8%) and B*35 (9.8%). HLA-C had 24 alleles groups, the most frequent: C*07:02 (14,7%), C*07:01 (13.2%), C*04:01 (12,8%). HLA-DRB1 had 13 alleles groups; the most frequent: DRB1*07 (13%), DRB1*13 (12.8%), DRB1*15 (12.7%), DRB1*11 (12.3%) and DRB1*01 (12.1%). HLA-DQB1*03 was 36%. 345 different HLA- A*-B* -C*-DRB1*-DQB1*-haplotypes were estimated, A*01-B*08-C*07:01-DRB1*03-DQB1*02 was the most frequent (6.1%) followed by A*03-B*07-C*07:02-DRB1*15-DQB1*06 (4.9%) and A*03-B*35-C*04:01- DRB1*01-DQB1*05 (4.9%). Among Buryats we found 14 allele groups for HLA-A, the most frequent: A*02 (24.7%), A*24 (23.0%) and A*01 (10%). 25 allele groups were found for HLA-B, the most frequent: B*40 (18.3%), B*44 (9.3%) and B*15 (8.7%). HLA-C had 24 alleles groups, the most frequent: C*03:04 (18.7%), C*06:02 (11.7%), C*07:02 (10.3%). HLA-DRB1 had 13 alleles groups; the most frequent: DRB1*04 (15.3%), DRB1*07 (14.7%) and DRB1*12 (10.7%). HLA-DQB1*03 was 43%. 206 different HLA-A*-B*-C*-DRB1*-DQB1*-haplotypes were estimated, A*23-B*44-C*04:01-DRB1*07:01-DQB1*02 was the most frequent (4.7%) followed by A*02-B*15-C*03:03-DRB1*04:01-DQB1*03 (2.7%) and A*01-B*37-C*06:02- DRB1*10:01-DQB1*05 (2.3%). By HLA genetic distances the Transbaikalian Russians are very close to the Russians of Moscow, Chelyabinsk and Novosibirsk, the Transbaikalian Buryats are close to the Irkutsk Buryats and the Khalkha Mongolians.

## SHARED EPITOPE AND POLYMORPHISM OF MICA AND NKG2D ENCODING GENES IN GREEK AND POLISH PATIENTS WITH RHEUMATOID ARTHRITIS

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Aim: Our previous studied showed that polymorphisms within genes coding for MICA (MHC class I-chain related protein) and its NKG2D receptor may affect the outcome of biological treatment in Polish patients with rheumatoid arthritis (RA). The present study aimed to analyse and compare the genetic variability of Greek and Polish patients with respect to the presence of the HLA-DRB1 Shared Epitope (SE), and MICA and NKG2D variants.

Methods: Selected single nucleotide polymorphisms (SNPs) in genes coding for MICA and NKG2D were assessed in 61 Greek and 100 Polish RA patients. Genotyping for *MICA* rs1051792 (Val/Met; G>A), *KLRK1* rs1154831 (A>C) and *KLRC4-KLRK1* rs1049174 (C>G) was performed using a LightSNiP assay (TIB MOLBIOL). The *KLRC4-KLRK1* rs2255336 was studied employing TaqMan SNP genotyping assay (ThermoFisher Scientific). HLA-DRB1 alleles were genotyped by PCR-SSOP, PCR-SSP or SBT while anti-CCP antibodies were detected by ELISA. Genotyping results were correlated with clinical parameters.

Results: 62.3% of Greek and 69.3% of Polish RA patients were characterized by the presence of the HLA-DRB1 Shared Epitope (SE), mostly represented by DRB1*01:01, *04:05 and *10:01 among Greek and DRB1*01:01 and *04:01 among Polish patients, respectively. No significant differences were observed in the SE presence among Greek patients with various *MICA* rs1051792 alleles or genotypes. The Polish *A* allele carriers were more likely to possess one of the HLA-DRB1 SE alleles (p=0.004), especially DRB1*01:01 and/or DRB1:01*02 (p=0.008). As for the NKG2D polymorphisms, *KLRC4-KLRK1* rs1049174 *G* variant was more frequently observed in the Greek than in Polish population (p<0.001). In the Greek population, the *MICA GG* homozygous patients more frequently presented with anti-CCP antibodies and RF as compared to those carrying the *A* variant (p=0.038 and p=0.028, respectively). Moreover, the *KLRK1* rs1049174 *G* variant and rs1154831 *CC* homozygotes were characterized with lower disease activity score (p=0.032 and p=0.015, respectively). These relationships were not observed in Polish patients.

Conclusion: These results showed that genetic associations of RA may be different in the Greek than in the Polish population, *MICA* and *KLRK1* polymorphisms might be associated with clinical parameters of RA in Greek patients.

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## THE INCIDENCE OF HLA-DP SPECIFIC DSA IS DEPENDENT ON HLA-DP EXPRESSION LEVELS

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Haematopoietic cell and solid organs transplantation is a treatment modality for otherwise incurable diseases. Immunisation with cells carrying mismatched HLA molecules, e.g. after transplantation, can lead to the production of antibodies. If the recipient possesses antibodies that are specific for mismatched HLA antigens of the donor, an unfavourable outcome of the transplantation becomes more likely. Interestingly the expression of HLA-DP molecules is associated with a SNP in the 3'UTR: While the A variant of rs9277534 predicts low, the G variant predicts high expression. We asked, whether these expression patterns would skew the occurrence of HLA-DP antibody production in patients having undergone kidney transplantation. One hundred three patients and their donors were HLA-DPB1 typed by NGS. HLA specific antibodies were detected by beads array assay. Chi-square tests were performed to explore whether the occurrence of HLA-DP specific antibodies was associated with the presence of rs9277534 variants in donors. In 86 of the transplantations, a DP mismatch occurred. In this cohort DSA were detected in 20 cases. Donors were grouped according to the presence of (one or two) high expressed HLA-DPB1 alleles. Thirty-one donors showed no high expressed allele, 28 were DSA negative, three DSA positive. Fifty-five donors possessed high expressed alleles, 38 were DSA negative, 17 DSA positive. Fisher's exact test showed a p value of 0.03. Thus the incidence of DSA in the group with high expressing DP molecules appears to be statistically significant increased. A larger number of observations will be needed to confirm these findings.

## PLAUSIBLE' HLA SPECIFIC ANTIBODIES CAN BE TRIGGERED BY IMMUNISATION WITH DIFFERENT HLA ANTIGENS

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In solid organ transplantation, antibodies of the recipient, specific for HLA antigens of the donor, are suspected to play a major role in antibody-mediated rejection and graft survival.

For the definition of the clinical relevance of HLA specific antibodies, mainly the quantity of the antibodies is taken into consideration. In addition, the 'plausibility' of antibodies, i.e. whether they can be traced back to immunisations during previous pregnancies, transfusions or transplantations with mismatched donors, influences the interpretation of those antibodies, who are then regarded as more clinically relevant.

When performing antibody identification after transplantation we observed that 'plausible' antibodies can become triggered, during immunisation with an HLA different graft. We report this phenomenon on two patients. Both were women and had children. One was aged 42, when she received a heart (her son being 8 years old then). She had not detectable HLA antibodies pre-transplant. On day 6 post TX she showed a positivity in the Luminex-SA-assay, indicating an HLA-B12 specific antibody. MFI levels increased to 7000 and a retrospective flow crossmatch with an HLA-B12 positive target cell became positive. Those antibodies were surprisingly not donor specific – but specific for the paternal HLA antigen of her child. The other patient, aged 65 when she received a lung, was mother of two sons then at the age of 39 and 43 years. She had no detectable antibodies pre-transplant, however, developed one month after the transplantation an HLA-DQ5 donor-specific antibody plus an HLA-B57 antibody, specific for the paternal HLA antigen of her two sons.

In conclusion, HLA specific antibodies against children's HLA antigens can be triggered after transplantation. The donor must not necessarily carry the same antigens. These observations could be explained by epitope sharing and could help identifying immunological relevant epitopes.

## DETECTION OF IgA HLA ANTIBODIES USING A BEAD-BASED LUMINEX SCREENING ASSAY

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Due to the clinical relevance of IgG isotype HLA antibodies for transplant outcome, a routine procedure before the transplantation is screening serum samples for the presence of these antibodies using luminex (LMX) bead-based assay. However, other isotypes of HLA antibodies, such as IgA may also contribute to the alloimmune response. Due to the lack of a reliable screening assay, the role of IgA HLA antibodies in the transplantation setting still remains elusive. Therefore, we modified the commonly used IgG-LMX for IgA HLA antibody detection by replacing the standard IgG secondary antibody with a polyclonal IgA-specific secondary antibody. The modified IgA-LMX assay was optimized and validated using IgG, IgA1 and IgA2 isotype variants of the HLA-specific recombinant human monoclonal antibody (mAb) MUS4H4 (recognizing HLA-Bw4/A24/A32/A25). Reactivity pattern of the bead groups for IgA1 and IgA2 isotype mAbs in IgA-LMX were identical to those of IgG isotype with comparable reaction strength to IgG-LMX, while no cross-reactivity with IgG antibodies was observed. Next, we used this novel assay to screen serum samples from individuals without any HLA exposure (n=18), kidney waitlist patients with a history of transplantation (n=84) and women with a history of pregnancy (n=92). None of the samples from alloantigen non-exposed individuals showed IgA HLA antibodies. IgA HLA antibodies were detected in 5.7% (n=10) of alloantigen exposed individuals (n=176), 90% of them being against HLA class II. All IgA positive serum samples also contained IgG HLA antibodies. The majority of IgA positive samples were from waitlist patients with a history of previous transplantation (n=8) and 2 from pregnancy immunized women. Our results suggest that the current modified IgA-LMX can serve as a cost-effective assay to screen for IgA HLA antibodies before utilizing expensive single antigen bead kits. In that way, this novel IgA-LMX assay may facilitate the research on the relevance of HLA IgA antibodies for transplantation outcome.

## COMPARISON OF VIRTUAL CROSSMATCH AND PHYSICAL CROSSMATCH IN CADAVERIC KIDNEY TRANSPLANTATION – SINGLE CENTER EXPERIENCE

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Aim: Virtual crossmatching (VXM) refers to the identification of donor-specific antibodies (DSAs) in recipients on the knowledge of the donor's human leucocyte antigen (HLA) type, providing reasonable prediction of the physical crossmatch (PXM) result. The aim of this study was to correlate VXM and PXM results in a five-years period (2014-2018) performed by Tissue Typing center Zagreb in a cadaveric donor kidney transplantation program.

Material: Patients were the ones from Zagreb center (CZATT patients) and patients from other ET centers (ET patients). CZATT included, both, non-sensitized (PRA 0-6%) and sensitized (PRA>6%) patients, while ET consisted solely of sensitized patients. PXM was performed by complement dependent cytotoxicity method (CDC) without the addition of dithiothreitol (DTT) and with the addition of DTT, whenever indicated.

Results: On the basis of a negative vXM, PXMs were performed in kidney allocation procedure from 621 cadaveric donors. In total 3803 PXMs were performed, 3244 of them for CZA patients (85%) and 559 for ET patients (15%). Altogether, positive CM was obtained in 10% of tested sera samples. In the group of non-sensitized CZA patients (N=2939), positive PXM was obtained for 77 sera samples (2.6%), among which 34 were positive for patients that had always been historically PRA< 6%. In the group of CZA sensitized patients, 306 PXMs were performed, revealing positive results in 72 cases (23.5%). In the group of ET sensitized patients, 558 PXMs were performed, revealing positive results in 217 cases (39%). When the results were analyzed separately per ET center origin, frequencies of positive PXMs were in range 21%-58%. Analysis taking into account number of tested patients showed that PXM was performed for 1013 patients, 773 non-sensitized and 499 sensitized patients. Positive PXM was obtained in 20,4% of all patients, specifically in 8,8% of non-sensitized and in 37,5% of sensitized patients. Furthermore, 60 patients had positive PXM with more than one cadaveric donor (12 at the most). The analysis performed for CZA patients revealed that the possible reasons for PXMs positivity were mostly due to the presence of IgM antibodies and non-HLA antibodies, but also in some cases due to unreported transfusions.

Conclusion: Overall, the VXM correlated with PXM in 97,4% of all non-sensitized patient proving high sensitivity in predicting donor-recipient immunologic compatibility in this group of patients.

# HLA-C AND KIR HLA-LIGANDS ASSOCIATION WITH THE LIKELIHOOD OF BK VIRUS NEPHROPATHY IN KIDNEY TRANSPLANT PATIENTS

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Aim: BK virus-associated nephropathy (BKVAN) is a complication of kidney transplantation (KITX) which results in allograft loss in some 50% of cases. NK cells play an important role in BK virus reactivation through the interaction of killer cell immunoglobulin-like receptors (KIR) on virus-infected cells. Our aim was to explore genetic predisposition to BKV infection and BKVAN on NK cell level comparing the frequency of HLA genes and KIR HLA-ligands in patients with biopsy-proven BKVAN and BK virus-free patients.

Methods: We performed a case-control study of 23 first cadaveric kidney allograft recipients and their donors with biopsy-proven BKVAN (cases) and 46 first cadaveric kidney allograft recipients and their donors with persistently negative BK viruria and viremia after KITX (controls). Patients were matched for all relevant clinical parameters and withouth any statistically significant difference in the distribution of HLA-A, -B, -C, -DRB1 and –DQB1 alleles between cases and controls. Minimum follow-up time for controls was 560 days post KITX (median 1138; IQR 893-1440).

Results: KIR HLA-ligand phenotypes were determined retrospectively and compared among cases and controls. HLA-C*07 positive donors were less frequent in cases compared to controls (35% vs. 74%, p=0.004), while C1/C2 donors were more frequent in cases (52% vs. 35%). KIR HLA-ligand analysis showed that C1/C1 donor-C1/C2 recipient ligand combination was more common in BKVAN patients (30% vs. 15%), while donor C1/C2-recipient C1/C1 combination was more common in controls (17% vs. 4%). After adjustment for potential confounders, only donor HLA-C*07 positivity was significantly associated with lower odds for BKVAN (OR 0.09 [0.02, 0.45]), while no association was found with recipient HLA-C*07 status, donor or recipient age, total HLA-MM, immunosuppresion type or mTORi treatment.

Conclusion: The given data demonstrate that BKVAN is more common in kidney transplants with HLA-C*07 negative donors, this being a ligand for inhibitory receptors KIR2DL2 and KIR2DL3. A difference in donor-recipient ligand combination pairs between cases and controls as well as a difference in distribution of KIR HLA-ligands point to possible role of KIR genes in BKVAN occurence.

## EBV INFECTIONS ARE STRONGLY DEPENDENT ON ACTIVATING AND INHIBITORY KIR-HLA PAIRS AFTER T CELL REPLATE UNRELATED HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Aim: Viral infections are main cause of increased morbidity and mortality among recipients in hematopoietic stem cell transplantation (HSCT). NK cells fight virally infected cells provided their directional activation of cytotoxicity. In this study, we analyzed the role of receptor-ligand pairs that include inhibitory or activating KIR with their HLA class I ligands in the course of viral infections.

Methods: The study cohort included N=230 HSCT recipients with malignant and non-malignant diseases, in which the occurrence of viral infections were monitored with median follow-up of 1 year. HLA class I ligands of Bw4, C1 and C2 groups were paired with inhibitory or activating KIRs forming cognate KIR:HLA pairs.

Results: In our cohort we confirmed higher transplant related mortality (48% vs. 29%; p=0.013) and overall mortality (57% vs. 40%; p=0.028) in patients with than without Infections. In inhibitory KIR:HLA (iKIR:HLA) pairing model we found significantly higher frequency of EBV infection in patients whose donor lost a pair of iKIR:HLA in recipient's HLA environment as compared with patients with unchanged number of iKIR:HLA pairs post transplant (40% vs. 9%; OR=6.67; p=0.0057; 95%CI 1.74-25.62). The difference for CMV infections was of borderline significance (60% vs. 30%; OR=3.58; p=0.054; 95%CI 0.98-13.10). In the activating KIR:HLA (aKIR:HLA) pairing model, we found significantly higher incidence of EBV infections in recipients with at least one aKIR:HLA cognate pair post transplant as compared to recipients without aKIR:HLA pairs (15% vs. 5%, OR=3.58; p=0.023; 95%CI 1.19-10.73). Unlike EBV infection, in aKIR:HLA model the frequency difference of CMV infections was insignificant (34% vs. 27%; OR=1.39; p=0.28; 95%CI 0.77-2.52). Bacterial and fungal infections were not significantly modified by KIR:HLA pairs both in activating and inhibitory KIR pairing models.

Conclusions: These results suggest that i) Both, iKIR:HLA and aKIR:HLA cognate pairs are involved in NK cell-mediated immunosurveillance upon viral infections, ii) Opposite impact on infections suggest that iKIR:HLA-based education arms NK cells for better elimination of EBV and aKIR:HLA-based education disarms the NK cells, iii) It is possible to reduce Infectious viral complications in HSCT by selecting donors with appropriate KIR:HLA constellation.

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## Results of external proficiency testing „DETECTION OF HLA ALLELES ASSOCIATED WITH DISEASES“ in 2018

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EPT program “DETECTION OF HLA ALLELES ASSOCIATED WITH DISEASES“ is organized by HLA Department of the Institute of Hematology and Blood Transfusion in Prague since 2010. This program is offered annually started in March in three variants:

- Alleles of DQ loci associated with celiac disease (CD):
- B*27 (association with Morbus Bechterev and other rheumatoid autoimmune diseases):
- DQB1*06:02 (association with narcolepsy)

Samples are distributed in two parts for variant CD and all in one for variant B*27 and DQB1*06:02, distribution of samples is realized in April (and September for part 2 of variant CD). Results are evaluated in terms of correct determination of predisposing alleles/allelic groups and the clinical interpretation. Reports are issued in July (and December), final report is released at the end of the year.

### 2018 results:

29 laboratories participated in the regular part of PT (25 from CR, 2 from SR and 2 from Austria). In the second part of PT 2018 (for the variant „Alleles associated with CD“) participated 9 laboratories, some of them without attendance to the part 1.

## 2018 result overview:

| Variant | Category | Efficiency | Laboratories / Total | Successful |
|---|---|---|---|---|
| CD part I | Genotype | 100 % | 23 / 28 | yes |
| | | 90 – 99 % | 2 / 28 | |
| | | ≤ 89 % | 3 / 28 | no |
| | Serological equivalent | 100 % | 27 /28 | yes |
| | | Not evaluated | 1 / 28 | Not evaluated |
| | Interpretation | 100 % | 24 /28 | yes |
| | | 90 – 99 % | 3 / 28 | |
| | | ≤ 89 % | 1 /27 | no |
| | All together 3 laboratories did not pass at least one criteria. | | | |
| CD part II | Genotype | 100 % | 3/9 | |
| | | 90 – 99 % | 6/9 | |
| | Serological equivalent | 100 % | 8/9 | |
| | | Not evaluated | 1/9 | |
| | Interpretation | 100 % | 7/9 | |
| | | 90 – 99 % | 2/9 | |
| | All laboratories passed the criteria. | | | |
| B*27 | All | 100 % | 15/ 15 | yes |
| Narcolepsy | All | 100 % | 4 / 4 | yes |

All HLA laboratories willing to participate in our EPT are welcome. Please contact the guarantor Milena Vrana (milena.vrana@uhkt.cz) or our assistant Barbora Kinska (barbora.kinska@uhkt.cz).

# RESULTS OF EXTERNAL PROFICIENCY TESTING FOR CELL CHIMERISM ANALYSIS IN 2018

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Aim: Determination of cell chimerism is an important marker for the success of allogeneic hematopoietic stem cell transplantation. It allows to monitor engraftment dynamics, survival, multiplication of donor cells and early detection of autologous hematopoiesis, indicating the risk of relapse and graft rejection. External proficiency testing (EPT) for cell chimerism Analysis has been organized in the Institute of Hematology and Blood Transfusion since 2005. This year's EPT was divided into optional and compulsory part. The optional part was consisted of the identification of reference alleles based on the examination of recipient and donor DNA samples and the compulsory part was comprised of the sample quantification with the different recipient/donor ratio and their interpretation. The EPT was offered in two variants – basic (5samples) and extended (10 samples).

Methods: Each participant received DNA samples of a recipient and a donor and 5 (10) quantification samples by selected variant. DNAs were isolated from buffy coat fraction of peripheral blood and mixed in different ratios for the preparation of quantification samples. All samples were analysed before sending to participants using a combination of methods – analysis of sequence-based polymorphisms of short tandem repeats and short insertion and deletions. All participants analysed samples by methods commonly used in their laboratories.

Results: A total of 13 laboratories participated - 3 domestic and 10 foreign, 4 in the basic variant, 9 in the extended variant. The informativity examination (optional part) was sent by 7 participants. The results of cell chimerism were compared with the expected values and statistically evaluated by Z-score. The results were divided into five categories: Excellent, Good, Acceptable, Critical and Under the detection limit of the laboratory. Last category means that the participant's sensitivity was lower than the expected value. Overall, 64% of all results were Excellent, 11% Good, 6% Acceptable, 8% Critical and 11% Under the detection limit of the laboratory. Each laboratory also received a graphical comparison of all participants.

Conclusion: All 13 laboratories received the certificate. The next round of EPT for chimerism testing will be organized in the first half of 2019.

Supported by MH CZ – DRO (00023736, UHKT).

# SELECTED ABSTRACTS

# POSTER PRESENTATIONS

## HLA-A~B~DRB1~DQA1~DQB1 HAPLOTYPE ASSOCIATION WITH CELIAC DISEASE IN THE ALBANIAN PEDIATRIC PATIENTS FROM KOSOVO

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Aim: Genetic predisposition to celiac disease (CD) is strongly associated with the presence of HLA alleles in the individual genotype encoding HLA-DQ2 and/or HLA-DQ8 heterodimers. The aim of this study was to analyze the five-loci HLA haplotypes distribution as well as the prevalence of CD predisposing HLA-DQ genotypes in 60 Albanian pediatric CD patients from Kosovo and 124 non-CD children from Kosovo.

Methods: All CD patients and controls were typed for HLA-A, -B, -DRB1, -DQA1 and -DQB1 applying the standard polymerase chain reaction-sequence specific oligonucleotide probing (PCR-SSOP) method in combination with polymerase chain reaction - sequence specific primers (PCR-SSP) high-resolution protocol.

Results: The HLA genes observed with statistically significant higher frequency among CD patients were HLA-A*01, -B*08, -B*50, -DRB1*03, -DRB1*07, as well as -DQA1*02:01, -DQA1*05:01, -DQB1*02:01 and -DQB1*02:02 alleles. Three different 5-loci HLA haplotypes were found with statistically significant higher frequency among patients:
HLA-A*01~B*08~DRB1*03~DQA1*05:01~DQB1*02:01,
HLA-A*02~B*50~DRB1*07~DQA1*02:01~DQB1*02:02 and
HLA-A*68~B*44~DRB1*07~DQA1*02:01~DQB1*02:02.

A total of 95.0% CD patients were positive for risk heterodimers HLA-DQ2 or HLA-DQ8, while at the same time it was the case in 43.3% of controls. HLA-DQ2 heterodimer in *cis* position was present in 40.01% of patients and additional 6.66% of patients were positive for HLA-DQ2 heterodimer in *trans* position, both statistical significantly different in comparison with controls ($p<0.0001$ and $p=0.0088$, retrospectively). On the other side, non risk DQ5.1 and DQ6.1 heterodimers were more frequent in controls ($p=0.0017$ and $p=0.252$, retrospectively). Among the single dose risk genotypes, the most prevalent one was HLA-DQ2.5/DQX (58.3%), while among double dose risk genotypes the most prevalent one was HLA-DQ2.5/DQ2.2 (20.0%), both showing statistically significant difference compared with controls ($p<0.0001$ and $p=0.0005$, retrospectively). The frequencies of single dose risk genotypes DQ2.2/DQX and DQ8/DQX among CD patients compared to control group was without statistical significance.

Conclusion: The given data demonstrate similarity, but also differences in distribution of HLA haplotype among Albanian CD patients from Kosovo in comparison to other European and non-European populations as well as the important role of HLA-DQ2 and HLA-DQ8 heterodimers in the development of CD.

## HLA-DPB1 GENE IN RUSSIANS FROM CHELYABINSK REGION

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Aim: The purpose of the work was to establish the distribution of the HLA-DPB1 alleles in the population of Russians living in the Chelyabinsk region (South Ural of Russia). HLA-DPB1 frequencies were determined in 100 unrelated Russian, living in Chelyabinsk region. All subjects were normal, healthy unrelated blood donors, of between 18 and 55 years of age. Their ethnic origin was determined by a comprehensive questionnaire.

Method: Typing was performed by NGS (using the primer system «HLA-Expert», DNA-technology, Moscow). For population genetics analysis Arlequin v3.1 software was used.

Results: DPB1*04:01 prevails in the studied population with frequency (gf=0.034). Most frequent HLA-DPB1 alleles were: DPB1*04:02, DPB1*03:01, DPB1*02:01 and DPB1*17:01 (gf = 0.253; 0.246; 0.253; 0.012 respectively). Rare alleles (gf<0.1) were DPB1*01:01, DPB1*05:01, DPB1*06:01, DPB1*09:01, DPB1*10:01, DPB1*105:01, DPB1*11:01, DPB1*124:01, DPB1*13:01, DPB1*14:01, DPB1*15:01, DPB1*150:01, DPB1*16:01, DPB1*23:01.

Conclusion: Among the most frequent alleles and of Russian Chelyabinsk region the large fraction of them is typical for Europeans populations. HLA-DPB1 data may be included in national transplant standards.

## THE RARE HLA ALLELES IN TURKISH POPULATION

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Introduction: HLA exons encode the critical amino acids which bind the foreign peptides. Particularly the structural positions of these exons initiate the gene conversion (GC) events, and result with the emergence of new alleles. These allelic variations mainly affect the structure and combination of the peptide binding groove, and thus modulate the peptide repertoire that is presented for the recognition of CD8 + or CD4 + T cells by the HLA class I, and class II proteins. 21.683 HLA alleles were currently defined in accordance with the International ImMunoGeneTics information system (IMGT) database. New polymorphisms are formed in HLA genes with point mutations, gene conversions, and part replacements, and the diversity continues to increase.

Aim: To identify the rare HLA alleles in the Turkish population.

Material-Method: DNA isolation of the peripheral blood of 5100 individuals, and HLA alleles were analysed using the Sanger-SBT (sequence-based typing) method, and HLA-A, B (exon1-5), HLA-C (exon1-7), HLA-DR (exon 2-3), and HLA-DQ (exon 2-3) between 2017 and 2019 in the present study. 27 alleles were described as rare in a total of 5 loci. The rare alleles were evaluated using the IMGT database (3.35.0, 2019-01-23). Each of the rarely detected alleles were confirmed using the NGS (Next Generation Sequencing) method.

Results and Discussion: 27 alleles were detected as HLA-A*01:155, 02:20, 02:66 (2), 02:90, 02:110, 02:343, 03:82, 24:28, 24:146, 24:276, 31:23, 33:26 (3), HLA-B*18:19, 35:193, 37:04, 40:303, 51:69 (2), 51:169; HLA-C*04:39, 06:40, 07:93, 12:149, 15:73; HLA-DRB1*11:12:02, 11:149, 13:14:02, and HLA-DQB1*03:27 in 5100 individuals whose sequence based typing was performed. Various new HLA alleles were discussed to have epidemiologically significant roles. New alleles are probably detected in low frequency in the population. In addition, however rare in the population, has significant contributions in defencing itself against the potential pathogens. We described the rarely detected alleles in the Turkish population by SBT, and NGS. The differentiation strength of some alleles was more limited with the Sanger sequencing, however alleles are definitely differentiated using the NGS. These studies provide data that the new generation sequencing technique will bring the new changes, and alleles to the HLA typing area.

## RARE AND UNIQUE HLA ALLELES DETECTED IN THE HLA DEPARTMENT

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Aim: HLA genes represent a highly polymorphic part of the human genome. In our HLA department we perform HLA typing for hematopoietic stem cell transplantation and for optimal donor selection. In the 2018 we started with a new project dealing with identification of rare and unique HLA alleles. Gathering information needed to describe rare and unique alleles found in our laboratory and their disclosure in an international IMGT/HLA database. Acceptance conditions of new alleles in the international database are confirmation by two different methods, complete HLA-ABDR genotypes, followed by publication.

Methods: Using next-generation sequencing (NGS) we have retrospectively analyzed a large cohort of 1479 samples for the presence of rare and newly detected HLA alleles. New alleles in our patients were confirmed by Sanger sequencing method.

Results: Using established NGS and Sanger sequencing, we detected 32 uncommon alleles seen in less than one patient in our settings, according Allele Frequency Net Database 12 from 32 are rare alleles. We detected 10 new alleles that have not been described in the IMGT/HLA database yet. Specifically we detected one new allele in HLA-A in 1 patient, two new alleles in HLA-B in 2 patients, two new alleles in HLA-C in 2 patients, one new allele in HLA-DRB1 in 1 patient, two new alleles in HLA-DQB1 in 2 patients and one new allele in HLA-DPB1 in 1 unrelated donor. In two patients, the presence of a new allele has been confirmed by a family study, which suggests their germ cell origin. In all patients, we continue to collect biological material from other family members for family study confirmation. Very interesting is somatic variant in one patient, which promotes a deleterious exchange of amino acids with *de novo* origin.

Conclusion: To date, we detected 10 new alleles and 32 uncommon alleles. In this time we are focusing on the exact specification of new alleles - on precise description, position of the nucleotide and amino acid substitution, the effect on the molecule's conformation and consequent influence on protein function.

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# HLA-DRB1 AND HLA-DQB1 IN MULTIPLE SCLEROSIS PATIENTS FROM EAST CROATIA

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Aim: Multiple sclerosis (MS) is a chronic disease of the central nervous system characterized by myelin destruction and resulting in progressive neurological dysfunction. The recent studies have shown multifactorial etiology of MS. They suggest that interaction between environmental influences and individual genetic predisposition modulates presentation and therapeutic responsiveness. Although the basis of genetic susceptibility to MS is complex, several alleles of the human leukocyte antigen (HLA) class II were identified as genetic component the most strongly linked to MS, particularly HLA-DRB1*15:01 allele. The aim of this study was to investigate the distribution of HLA-DRB1 and HLA-DQB1 alleles in a group of MS patients from East Croatia. Additional, to determine HLA-DRB1 and -DQB1 gene variants which contribute to MS risk in tested population.

Methods: This retrospective study included 46 unrelated individuals (33 female, 13 male), mean age 39 ±12 years, with a diagnosis of MS according to 2010 and 2017 revisions of McDonald criteria. Low resolution HLA-DRB1 and -DQB1 typing was performed using PCR-SSP (Olerup) and PCR-SSO (Immucor) techniques in the period of 2011-2019. The chi-square and Fisher´s test were performed to evaluate a correlation between HLA allele frequencies in MS patients and previously published results for general population of Croatia.

Results: The distribution of HLA-DRB1 and -DQB1 allele groups in our study cohort was as follows: DRB1*15 (15.64%,), DRB1*11 (11.96%), DRB1*03 (10.12%), DRB1*07 (10.12%), DRB1*01 (9.2%), DRB1*13 (8.28%), DRB1*16 (8.28%), DRB1*04 (7.36%), DRB1*08 (2.76%), DRB1*14 (0.92%), DQB1*06 (22.08%), DQB1*05 (19.32%), DQB1*02 (18.4%), DQB1*03(DQ7) (15.64%), DQB1*03(DQ8) (4.6%), DQB1*04 (2.76%), DQB1*03(DQ9) (1.84%). The most frequent haplotype observed was HLA-DRB1*15-DQB1*06 (HF 16.3%). The HLA-DRB1*15 showed statistically significant positive association with MS (OR 2.03, 95% CI 1.12-3.50, p=0.008).

Conclusion: The results of this study show that the genetic susceptibility with MS in the East Croatia region is linked to the HLA-DRB1*15 which is in concordance with results of the numerous studies in European populations.

## EVALUATION OF ANTI-HLA ANTIBODIES LEVEL IN POTENTIAL KIDNEY RECIPIENTS WITH APPLICATION OF CDC TEST AND LUMINEX X–MAP METHODS

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The work done by the scientific club of Department of Immunological Diagnostics, Pomeranian Medical University, Szczecin, Poland

Despite the huge progress that has been made in the selection criteria for donor-recipient transplantation and the use of immunosuppressive drugs, one of the reasons for the loss of the transplanted kidney is the process of its rejection. High immunized patients belong to the group of higher risk of rejection in the comparison with non-immunized recipients. They are particularly exposed on humoral acute rejection associated with presence of anti-HLA antibodies and complement activation. Therefore, it becomes important that the determination of the immune status, of the kidney recipient, which quite easily undergoes the alloimmunization process, both in the peri-and post-transplant period. However, despite the commonly used name for the assessment of immunization, which is referred to as PRA (Panel Reactive Antibodies), there are various diagnostic methods with significantly different sensitivity and specificity, which create difficulties in the interpretation of results. In Poland, the currently recommended method is the PRA-CDC serological method and, more recently, also the Solid Phase Assay, which involves the use of a fluorocytometer (Luminex), which additionally allows for the identification of specificity of anti-HLA antibodies.

Aim: Comparison of sensitivity of methods used for kidney recipients alloimmunization estimation – PRA – CDC and screening test for anti-HLA antibodies detection

Material and methods. The study involved 149 potential kidney recipients in whom the alloimmunization was assessed using two parallel methods: PRA-CDC and screening test for anti-HLA IgG antibodies performed with x-MAP Luminex method (One Lambda).

Results: In the group of 149 patients, the convergence of results obtained with both methods was obtained in 63 cases (55 positive and 8 negative). In 85 patients positive results were obtained in the anti-HLA antibodies screening test and negative in the PRA-CDC test. Only in one case a positive result was obtained in the PRA-CDC test and a negative result in the screening test.

Conclusion: It seems to be necessary using more sensitive methods for alloimmunization's estimation of potential kidney recipients, before transplantation based on the identification of lytic antibodies as well as monitoring the humoral response in patients after kidney transplantation.

## EFFECTS OF DIFFERENT VOLATILE ANESTHETICS ON IFN-γ AND TGF-β1 RELEASE IN LIVING RENAL TRANSPLANT RECIPIENTS

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Aim: Renal transplantation is the treatment of choice for patients with end-stage renal disease. Ischemia reperfusion injury (IRI) is one of the major causes of early renal dysfunction during the perioperative period. Ischemic hypoxic damage increases the local inflammation in the tissue by secreting pro-inflammatory cytokines which can lead to deterioration of graft functions. Studies have shown that pro-inflammatory cytokine IFN-γ–producing CD4+ T cells, NK cells, and GR-1+ neutrophils are important in the pathogenesis of kidney IRI. Anesthetic conditioning is a widely described strategy to attenuate IRI. It's plausible that volatile anesthetics cause the membrane externalization of phosphatidylserine to outer surface on renal tubuler cells leading to release of TGF- β1 resulting anti-inflammatory and anti-necrotic effects. We aimed to compare the effects of two different volatile anesthetic, desflurane and sevoflurane, on pro-inflammatory cytokine IFN-γ and anti-inflammatory cytokine TGF-β 1 release in living donor kidney transplant recipients.

Methods: Eighty donor /recipient couples were recruited in this prospective, randomized study. Anesthesia maintenance was provided by using desflurane for Group Des and sevoflurane for Group Sevo. Each patient's demographic characteristics, immunologic, clinical data and hemodynamic parameters were recorded. IFN-γ and TGF-β1 and serum creatinine (sCr) levels were studied from the samples drawn preoperatively and on the postoperative days 1, 7 and months 1, 3 after transplantation. Estimated glomerular filtration rates e(GFR) were calculated. The acute rejection attacks and the graft loss within 6 months of transplantation were recorded.

Results: There was no significant difference in demographic, immunologic and clinical data between groups ($p>0.05$). IFN-γ, TGF-β1 levels were similar preoperatively and on the postoperative days 1, 7 months 1, 3 ($p>0.05$). No significant difference was detected in sCr and eGFR between groups ($p>0.05$). There was no graft loss within 6 months after transplantation.

Conclusion: Both desflurane and sevoflurane have similar effects on IFN-γ and TGF-β1 release in transplant recipients and no difference was observed between the two groups in terms of kidney function, which is reflected in the clinic. We suggest that both agents have protective effects on IRI in the living donor kidney transplantation.

## THE IMPACT OF RECIPENT GENDER ON HLA ALLOIMMUNIZATION BEFORE KIDNEY TRANSPLANTATION

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Aim: Various immunizing events such as previous transplantation, transfusion, pregnancy can cause the development of anti-HLA antibodies that may have a deleterious effect on transplant outcome. The purpose of this study was to compare HLA immunization status between female and male kidney recipients before transplantation.

Methods: This retrospective analysis characterizes and compares the HLA antibody status between men and women before kidney transplantations that had been performed from 1985 to 2017 in Clinical Hospital Centre Rijeka, Croatia. Recipients with insufficient data or combined transplantations (kidney-pancreas) were not included in the study. The presence of HLA antibodies was detected by Complement-Dependent Cytotoxicity (CDC) assay.

Results: Among total of 825 kidney transplants, 285 (34.5%) recipents were women and 540 (65.5%) male. The median age of female recipients was 48, range 8-77 years. It was not different from the age of the male recipients; median 45, range 10-80 years. The first graft received 766 (92.9%) patients while 59 (7.1%) patients were retransplanted. The proportions of the retransplants were not different between male and female recipients. Anaemia was treated with blood transfusions in 561 (68.0%) transplant candidates among which the proportion of women was significantly larger (73.3% of all women recipients vs. 65.2% of all male patients; $\chi 2=5.32$, $P=0.02$). The history of pregnancy had 234 (82.1%) female patients. Pretransplant HLA antibodies were detected in 202 (24.5%) kidney recipients, more in female than male (32% vs. 20%; $\chi 2=11.27$, $P=0.0007$).

Conclusion: In our study, the gender of kidney transplant recipients had significant impact on the development of HLA antibodies. The proportion of immunized women were larger than man. Apart from the exposure to the previous pregnancy, women received more frequently blood transfusions in regards to male patients.

## OCCURENCE OF AUTO- AND ALLOANTIBODIES IN WOMEN WITH MISCARRIAGES

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Miscarriage is one of the major problems affecting women nowadays. Studies indicate that the statistics of abortions each year are increasing. Causes of abortions may be different from anatomical defects of genital tracts, trough infections of various etiologies as well as endocrine or immunological disorders.

Aim: Assessment of allo- and autoimmunity degree in patients with at least up to 2-fold miscarriage.

Material and methods: The study included 24 patients who were tested for:

- presence of anti-HLA antibodies with Luminex method (OneLambda)
- presence and specificity of autoantibodies with indirect immunofluorescence method (IFT) and ANA PROFIL 3 Euroline (Euroimmun)
- presence and level of antisperm antibodies (ASA) with indirect immunofluorescence method (Euroimmun).

The statistical analysis of the results was carried out based on the 2x2 test. The $p<0.05$ results were considered statistically significant.

Results: 9 patients were found to have both anti-HLA antibodies and autoantibodies (Scl70 and dsDNA - 1 patient; DFS70 – 2 patients; RNP/Sm - 1 patient; Ro-52 – 1 patient; dsDNA - 1 patient; Sm - 1 patient; unspecified autoantibodies - 2 patients); 12 patients were found to have only anti-HLA antibodies, and in 2 patients only presence of autoantibodies was found (one of them was found to have specific anti-ACA antibodies). In case of one patient the results of anti-HLA antibodies and autoantibodies were negative. In all examined patients, ASA antibodies were found in the low titer. The results of autoimmunization in relation to alloimmunization were statistically insignificant.

Conclusions: Immunological factors especially presence of ANA, ASA and anti-HLA antibodies from a clinical point of view may be the key cause of miscarriage. This applies not only to the process of autoimmunity, but also to alloimmunization. The presence or absence of specific anti-HLA antibodies in the woman's body may play an important role in keeping pregnancy. The routine diagnostics in this direction is important for making the correct diagnosis and making a decision about the inclusion of immunosuppressive treatment.

## IMMUNOLOGICAL LANDSCAPE OF HUMORAL IMMUNITY IN KIDNEY TRANSPLANT RECIPIENTS

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Aim: In searching for operational tolerance different biomarkers are taken into account. The important cells in the field are regulatory B cells (Bregs) that secrete IL-10, and CD5 positive B cells are one of them. It is possible that Bregs downregulates immune response to the transplanted organ that may result in low anti-HLA antibodies development and stable graft function. Thus, identification of B cell signature of humoral immunity may be beneficial for personalized risk stratification.

Methods: In this study, fifty-three low-risk kidney transplant recipients were recruited and followed-up to 24 months after transplantation for alloantibodies development and concomitant lymphocytes phenotype, as well as signs of organ rejection. Every three months anti-HLA antibodies, B lymphocytes phenotype, and cytokines were assessed. Th1/Th2 and BAFF serum levels were measured with a Luminex solid phase assay. Finally, graft survival was assessed.

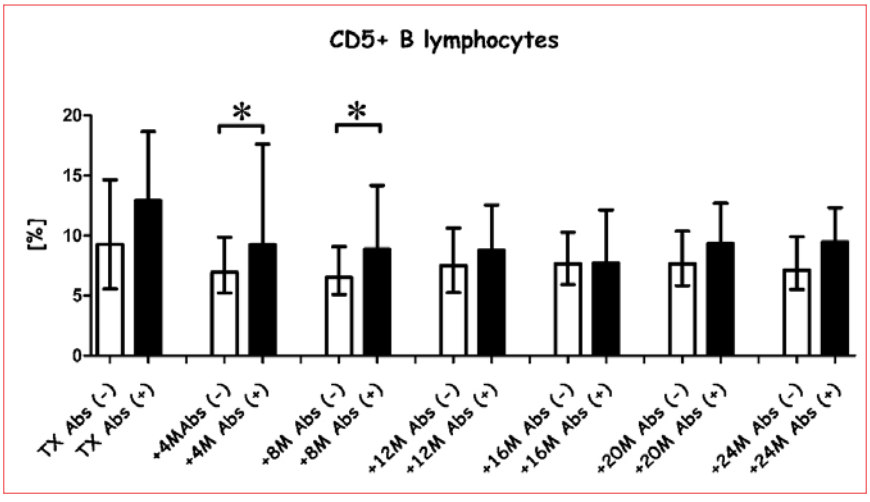


**Figure 1.** The levels of CD5+ B lymphocytes in two years follow-up
The percent of CD5+ B cells for (Abs–), indicated as open bars and for (Abs+), as black bars for KTX recipients up to 2 years after TX.

Results: The most important observation was that increased CD5+ B lymphocytes levels were found in kidney transplant recipients who do produce alloantibodies in the first year after transplantation (Figure 1). The entire landscape of tested parameters compared between alloantibodies positive and negative recipients can be found in Figure 2. Another important observation was that 24 months after KTX comparable levels of creatinine were found for both alloantibodies positive and negative allorecipients (Figure 2). Interestingly, alloantibodies development was correlated with memory B cells, Rs=0,96 (Spearman rank correlation).

Conclusion: Lymphocytes B phenotype monitoring after kidney transplantation is useful for alloantibodies development and may serve as an additional marker of humoral immunity activation. These could be also beneficial for individual risk stratification and tailored immunosuppression protocol development after kidney transplantation.

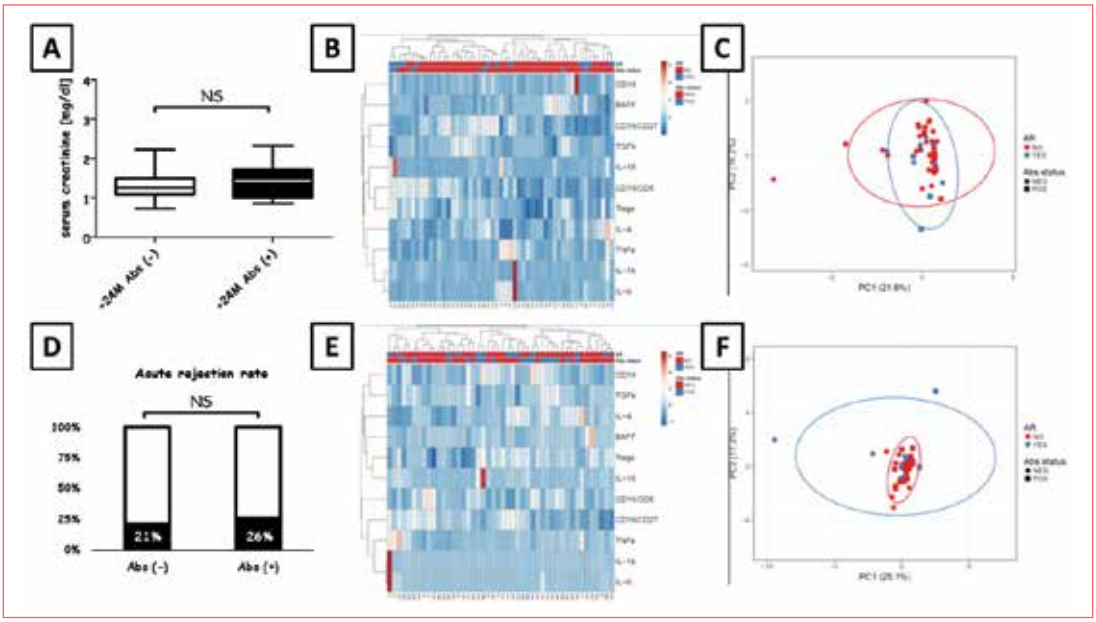


**Figure 2.** The two years transplant outcome

The collective differences between (Abs–) and (Abs+) KTX recipients 2 years after KTX.

## IMPACT OF HLA ANTIGEN DISTRIBUTION AMONG CADAVERIC DONORS ON KIDNEY GRAFT ALLOCATION

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Aim: Becoming a member of the Eurotransplant (ET) in 2007, Croatia adopted joined kidney allocation program based on the cross border exchange between eight ET country members. In this study we compared HLA-A, -B, -DR antigen distribution among cadaveric donors from Croatia (CRO donors) and donors from other Eurotransplant countries (ET donors) as well as we assessed mismatches (MM) within a group of patient who received kidney from CRO or ET donor in order to estimate the benefit of kidney exchange programme.

Methods: The study included 861 patients who received kidney graft from 707 cadaveric donors (CRO donors N=422, ET donors N=285). All patients and CRO donors were HLA typed by polymerase chain reaction - sequence specific primers (PCR-SSP), while HLA typing of ET donors was obtained from the ET allocation protocol. The number of MM was determined according to the Eurotransplant rules –at the broad level for HLA-A and -B antigens and at the split level for HLA-DR antigens.

Results: Statistically significant differences of HLA-A, -B and -DR antigen frequencies between CRO and ET donors were observed. HLA antigens significantly more present among ET donors were B7 (P=0.0048), B8 (P=0.0316) and B44 (P=0.0061) while B35 (P=0.0022), DR11 (P=0.0350) and DR16 (P=0.0001) were more frequently present in CRO donors. Consequently, patients positive for HLA-B7, B8, or B44 antigens more often received kidney from ET donors (P=0.0016, P=0.0198 and P=0.0312, respectively), while HLA-DR16 positive patients received kidney more frequently from CRO donors (P =0.0306). The ABDR MM 000 was more frequently present in the case of transplantation from ET donor (P=0.0005), while MM 222 was significantly more frequent when the donor was from Croatia (P=0.0277). The MM number analysis by single locus showed statistically significant difference between the two groups of donors for MM 0 on the HLA-B locus (CRO donors - 4.0%; ET donors 11.1%; p = 0.0001), whereas on HLA-A and -DRB1 loci statistically significant difference for any degree of MM was not observed.

Conclusion: HLA antigen distribution differences found between CRO and ET cadaveric donors is also the factor that influence kidney allocation.

## PRINCIPLES AND METHOD OF PAIRING ANALYSIS AT THE LEVEL OF FUNCTIONAL PAIRS OF INHIBITORY KIRS WITH COGNATE HLA IN HAPLOIDENTICAL HSCT

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Aim: NK cells of donor origin are crucial effectors in graft versus tumor (GvT) effect in hematopoietic stem cell transplantation (HSCT). NK cells trigger their tumor immunosurveillance via different receptors including killer cell immunoglobulin-like receptors (KIR). Direct comparisons indicated that inhibitory KIR (iKIR) pairing with cognate HLA class I molecules have independent, primary influence on GvT effect (but not missing HLA ligand or mismatched HLA class I alone). In haploidentical transplant setting the iKIR:HLA pairing process is much more complex than in other kinds of alllo-HSCT due to increased number of HLA mismatches. Here we present rationale and method of unambiguous iKIR:HLA pairing in haploidentical HSCT for prediction of transplant outcome in malignant patients.

Methods: In haplo-HSCT the iKIR:HLA pairing was designed using basic findings for HLA mismatched unrelated HSCT, including i) Effector NK cells post transplant are of donor origin, therefore the donor KIRs are taken for pairing, ii) iKIR:HLA pairs post transplant are composed of donor iKIRs and patient HLA ligands, iii) number and quality of iKIR: HLA pairs post transplant are compared with cognate pairs in healthy donor (pre transplant), iv) Number of cognate iKIR:HLA pairs is dependent on the number of donor KIRs (and not on the number of HLA molecules, whereas quality of HLA molecules pre and post transplant is considered).

Results: Three levels of result of pairing analysis are possible including 1) FAVORABLE, the acquisition of at least one HLA:KIR pair in recipient HLA environment (powered anti-cancer function of the transplant, reduced risk of relapse or progression), 2) ADVERSE, loss of at least one HLA:KIR pair (increased risk of relapse or progression), 3) No change in the number of HLA:KIR pairs (maintaining of the anti-cancer function of the transplant at the level of a healthy donor). An example result of lifetime iKIR:HLA pairing analysis in haplo-HSCT included: 3 cognate pairs in donor (Bw4-80I:KIR3DL1; C1:KIR2DL2; C1:KIR2DL3) and 4 cognate pairs post transplant (Bw4-80T:KIR3DL1; C2:KIR2DL1; C1:KIR2DL2; C1:KIR2DL3). Net result: FAVOURABLE (+1 pair post transplant).

Conclusion: This type of prediction of haplo-HSCT outcome can improve selection of better bone marrow donor for malignant patients.

## PRINCIPLES AND PRACTICES OF UNRELATED BONE MARROW DONOR SELECTION IN POLAND IN YEAR 2018

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Bone marrow donor searches are performed by individual search units (SU) and are supposed to be reimbursed by the Polish Ministry of Health (PMH). Each year, Poltransplant (its agency for transplant coordination, also Central Bone Marrow Donor Registry PL5) imposes strict rules and regulations regarding bone marrow donor searches as well as for HLA testing of recipients and donors. Payment is only granted when a search strictly adheres to the Poltransplant rules and any "violation" is punished by rejection of payment or reimbursement for Search Costs made by the SU. Below, we compare Poltransplant "national standards" to those recommended by EBMT (European Society for Blood and Marrow Transplantation), WMDA (World Marrow Donor Association) and EFI (European Federation for Immunogenetics) and discuss the differences.

# ASSESSMENT OF THE EFFICIENCY OF THE ACTIVITY OF THE BONE MARROW DONOR REGISTRY

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Aim: We evaluated the effectiveness of the Russian Bone Marrow Donor Registry (Kirov).

Materials and methods: Since 2009, the Kirov Registry has been systematically working with bone marrow donors (as of 31/12/2018, the total number of donors was 39,741, of which 46% - male, 53% - female; 43% - regular blood donors, 57% - volunteers from donor actions), since 2013 the Registry has its own Collection Center.

Results: As of 31/12/2018, donors of Kirov Registry carried out 135 donations of hematopoietic stem cells. Every year the number of donations during the activation of donors from the Registry is steadily increasing, including through requests for partially compatible donors (9/10). 94.8% donations were performed in Kirov Collection Center, 5.2% were carried out in other transplant centers. The use of the created and validated activation model with the employment of the Registry and the Collection Center currently provides the following activation times: the period for sending a sample for confirming HLA typing does not exceed 14 days; activation request sent by the transplant center 1.5-2 months prior to the transplantation is implemented in the specified period of time. Indicator of the efficiency of the Registry is the number of refusals to donation. During the period from 2013 to 31/12/2018, the Registry received 174 requests for the collection of cellular material: 78% were fulfilled; the remaining 22% were not completed due to a number of reasons. Classification of unfulfilled requests organized by reasons and donors' categories is presented in Table 1.

Table 1's data analysis showed that 54% of incomplete donations were unfulfilled due to the donor's refusal for personal reasons; 46% - due to unrelated to donor's reasons. It should be noted that there are 1.5 times more donation refusals in regular blood donors than among volunteers recruited at donor actions, and the number of withdrawals for medical reasons is the same in both categories.

Conclusion: The efficiency of the Registry is confirmed by the demand for donor resources, the timeliness of activation periods and a relatively low percentage of donation refusals

**Table 1.** Classification of unfulfilled requests organized by reasons and donors' categories

| Reasons for unrealized requests | Number of unrealized requests | Donor categories | |
|---|---|---|---|
| | | Regular blood donor (male/female) | Volunteer with donors action (male/female) |
| **Proper refusal to donate:** | **21** | **13 (6/7)** | **8 (2/6)** |
| Relatives against | 6 | 3 (1/2) | 3 (1/2) |
| Pregnancy planning | 4 | 2 (0/2) | 2 (0/2) |
| Without explanation | 3 | 3 (0/3) | - |
| Fear for their health | 3 | 1 (1/0) | 2 (0/2) |
| Refusal to re-donate | 1 | 1 (1/0) | - |
| Lack of remuneration | 3 | 3 (3/0) | - |
| Work, study | 1 | - | 1 (1/0) |
| **Reasons not related to donor position** | **18** | **10 (8/2)** | **8 (6/2)** |

## QUANTITATIVE PCR TECHNOLOGY IN CHIMERISM MONITORING AFTER HSCT - ONE CENTRE EXPERIENCE

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Aim: Chimerism status evaluation is included in routine patient follow up in order to assess hematopoietic stem cell transplantation (HSCT) outcome. Aim of the study was to retrospectively analyse results obtained by quantitative (qPCR) method in one-year period.

Methods: A total of 170 patients were referred to our Centre for chimerism monitoring, resulting in 800 performed tests. Patient's and respective donor's DNA were tested for 30 polymorphic markers (KMRtype, GenDx), patient's informative markers were identified and subsequently used for patient's post-transplantation sample analysis (KMRtrack, GenDx).

Results: The patient post-transplantation samples (N=800) could be divided into four groups according to the chimerism detection result: samples exhibiting full donor chimerism (FDC, 100% donor DNA detected, N=465), mixed chimerism (MC, >1% patient DNA detected, N=156), microchimerism (mMC, 0.1-1% patient DNA detected, N=174), and no chimerism detected (100% patient DNA, N=5).

Further analysis included patients with three or more post-transplantation samples tested (N=113) in order to consecutive monitor chimerism status in a time period. FDC status was detected for 86 patients, either from the beginning or after a period of decreasing MC, decreasing mMC or transient mMC. One patient rejected the transplant. Constant or increasing mMC was observed in 12 patients, while constant or increasing MC was seen in three patients. Finally, for a group of 11 patients, a transition from MC to mMC or vice versa was detected. Among them, disease relapse was confirmed for four patients who displayed increasing mMC that reached MC level. The qPCR chimerism monitoring method in these cases indicated an increase of patient's DNA at least one month earlier than it would have been detectable with the PCR-STR method.

Conclusions: The introduction of qPCR method into chimerism detection procedure enabled the discovery of mMC in 40% of patients in consecutive monitoring. This information has clinical value as it provides earlier warning of a possible disease relapse and indicates those patients for whom a more frequent monitoring should be considered.

## OUR EXPERIENCE WITH NGS HLA TYPING

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Aim: In the last few years, many donor registries worldwide adopt high resolution NGS typing of the donors as a standard strategy. The aim of this study was to evaluate the first results of NGS HLA typing in the Republic of Macedonia.

Material and Methods: We have implemented NGS technology in our lab at the end of 2018. DNA from 37 subjects (26 donors and 11 patients) was isolated using 2 automated methods for isolation (DuplicaPrep, EuroClone, Italy and Qiagen, Germany). HLA typing was performed using AllTypeTM NGS kits from OneLambda, USA on S5 Ion Torrent machine. The typing included the folowing loci: HLA-A, -B, -C, -DRB1, -DRB3,4,5, -DQA1, -DQB1, -DPA1 and –DPB1.

Results: There was no significant difference in the quality of the results obtained from samples isolated with DuplicaPrep and Qiagen. In HLA-A, 71 of the alleles were with 4 fields result and 3 with 3 fields. In HLA-B, 36 were 4 fields results, 30 three fields, 1 two fileds, 3 one field, 2 alleles were ambigious and for 2 alleles there was no result. Forty nine of the alleles in HLA-C were with 4 fields, 18 with 3 fields and 7 with 2 fields. DRB1 results gave 4 fields in 43 alleles, 3 fields in 22 alleles, 1 field in 8 and 1 allele had ambigious result. In DRB3,4,5 loci, 35 of the alleles were with 4 fields, 33 with 3 fields and 4 had 1 field result. DQA1 locus had 53 four fields result, 18 three fields result, 2 two fields and 1 one field result, while in DQB1, 22 had 4 fields, 32 three fields, 7 with two fields and 1 field, 2 had ambigiuos and 4 no result. DPA1 gave 3 or 4 fields result for all allels, while DPB1 had the most allels with ambigious result, 21.

Conclusion: Of the total 664 analyzed alleles, 592 (89.15%) had result with 4 or 3 fields. High resolution typing for HLA is considered valubale tool in donor typing stratigies and will be used for donor typing in the Macedonian Bone Marrow Donor Registry in the forthcoming period.

## THE IMPORTANCE OF USING SENSITIVE METHODS FOR CELL CHIMERISM MONITORING

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Aim: Chimerism is defined as a status, when the cells from the genetical different organisms coexist in one body. This phenomenon arises after allogeneic hematopoietic stem cell transplantation (alloHSCT). The aim of cell chimerism monitoring is the early detection of relapse and disease recurrence. Sensitivity of the method and selection of suitable polymorphisms are crucial for correct determination of chimerism status.

Methods: A variety of methods is used for monitoring of cell chimerism. Methods, such as phenotypization of erythrocytes or cytogenetic methods are less used and could be time consuming with lower sensitivity (1-5%). The most frequently used methods are the molecular genetic techniques, especially the determination of Short Tandem Repeats length polymorphism with sensitivity 1% and the analysis of polymorphism type short insertion and deletion by quantitative real-time PCR (qPCR) with sensitivity range from 0.01 to 0.001%.

Result: QPCR allows to detect microchimerism (Mc, detection less than 1% of recipient genotype). The group of 207 patients transplanted since 2011 from HLA match related and unrelated donors was divided into the three categories according to cell chimerism status (complete chimerism – CC, Mc and mixed chimerism – MC). In comparison with overall three-year survival probability in category CC vs MC and CC vs Mc was discovered significant difference $p < 0.0001$, but in category Mc vs MC was insignificant ($p = 0.0620$). In the correlation of three-year relapse rates, it was found the significant difference between all categories: CC vs MC patients ($p<0.0001$), CC vs Mc patients ($p<0.0001$) and MC vs. Mc ($p = 0.0002$).

Conclusion: The high sensitivity methods for monitoring of cell chimerism significantly specify evaluation of patients after alloHSCT. In the case of MC or Mc detection, it is necessary to accurately quantify the proportion of recipient genotype and to monitor its dynamics. With an identification of rising trend, effective treatment needs to be initiated as soon as possible to prevent the relapse of the disease. Detection of mixed chimerism is a high risk factor and microchimerism is a potential risk factor in post-transplantation course.

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## FREQUENCY OF HLA-B*27, HLA-DRB1*01, HLA-DRB1*04, HLA-DRB1*10 ALLELES IN PATIENTS AT THE UNIVERSITY CLINICAL HOSPITAL MOSTAR – ONE YEAR STUDY

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Aim: Rheumatoid arthritis and ankylosing spondylitis have been strongly related to the expression of specific HLA alleles, the aim of this study was to determine the frequency of these alleles in the population of patients with diseases of the musculoskeletal system.

Methods: Study was conducted at the Department for Tissue Typing and Immunogenetics, University Clinical Hospital Mostar, during the period from January 1, 2018 to January 1, 2019. Data were collected and processed on the basis of medical documentation of the Clinic for Internal Medicine, from where the health cards of the patients referred to our department under the diagnosis of musculoskeletal diseases were taken. HLA typing was performed by PCR-SSO (Immucor GTI Diagnostics USA), using HLA LIFECODES kits. Results were evaluated using MATCHIT! DNA software (Immucor GTI Diagnostics, USA).

Results: During the year 2018 a total of 448 samples of peripheral blood samples were received. Most patients did not have a specific diagnosis but were guided under the M46.1 code, according to MKB10 classification (Sacroileitis, unspecified elsewhere). Allele HLA-B*27 was found in 61 (13.62%) patients, there was 53 patients with DRB1*01 (11.83%), and 58 had HLA-DRB1*04 (12.95%), only 3 had DRB1*10 (0.67%). 7 patients had both HLA-B*27 and DRB1*04 alleles (1.56%).

Conclusion: Obtained results cannot be compared with those from neighboring countries, and the reason is that from the total number of patients included in the study the exact number of patients with confirmed diagnosis of rheumatoid arthritis or ankylosing spondylitis was not known by a clinician who sought HLA typing, this was a limiting factor for the exact determination of the occurrence of the alleles associated with these diseases. The plan is to establish a better co-operation with doctors who refer patients to HLA typing, then continue to investigate the association of alleles with the aforementioned diseases and establish registries of patients suffering from diseases whose association with certain HLA alleles is determined. As our department is recently established, this study is above all educational and for the purpose of achieving quality data exchange, which contributes to the well-being of patients of the University Clinical Hospital Mostar.

# ASSOCIATION OF HLA-DRB1 ALLELES WITH ORAL LICHEN PLANUS (OLP) IN SOUTHERN CROATIA REGION

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Aim: Human leukocyte antigen (HLA) system is a highly polymorphic family of genes which has been linked with various immune and autoimmune diseases. Lichen planus is a chronic autoimmune, mucocutaneous disease that affects the oral mucosa as well as the skin, genital mucosa and other sites. There are several subtypes of LP based on location of clinical presentation of the disease. In this study we concentrated on oral lichen planus (OLP), one of the most common dermatological diseases manifested in oral cavity. The aetiology of OLP is unknown, however, it is considered that the pathogenesis of the disease is related to T-cell-mediated response. Since previous reports have shown a correlation between HLA-DRB1 alleles with subtypes of lichen planus in different populations, the aim of this study was to investigate the possible association of these alleles with OLP among patients from southern Croatia (SC) region.

Methods: The group of patients included 55 patients clinically diagnosed with oral lichen planus. Control group consisted of 99 healthy blood donors from SC. Genotyping for alleles of HLA-DRB1 loci was performed using sequence-specific oligonucleotide probing method on a Luminex platform using commercial kits (Immucor HLA SSO Typing kits). Allele frequencies in both group of patients and control group were calculated by direct counting and compared for significance by Fisher's exact test (statistical significance was considered at $p<0,05$).

Results: Comparisons between HLA-DRB1 allele frequencies in control and patient groups showed significant increase of HLA-DRB1*01 allele frequency (9,6 % vs 17,3%, $p=0,039$). No differences were found between other HLA alleles and the disease, although we noticed slight increase in HLA-DRB1*03 and DRB1*07 alleles, while the frequency of HLA-DRB1*11 was lower in group of patients.

Conclusion: In this study we detected significant increase in HLA-DRB1*01 allele frequency in a group of patients with OLP, which is in line with the previous results in Mexican, Italian and American populations. These results indicate a possible correlation between HLA-DRB1*01 allele and OLP and could be considered as a useful tool in differential diagnosis of OLP from other oral cavity diseases. Given the relatively small sample number of patients involved in this study further investigation with a larger sample number from different populations is needed.

## INVESTIGATION OF THE ROLE OF HLA-DR ALLELES AND MBL2 GENE VARIANT IN THE ETIOPATOGENESIS OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Infections play an important role in the natural history of ALL, and especially childhood ALL (c-ALL). Although no specific infectious agent has been identified, scientific evidence suggests that exposure to infections has some effect on c-ALL etiology. Epidemiological data have showed that infections may occur as a consequence of an abnormal interaction between the immune system and infectious agents. Mannose-binding lectin 2 (MBL2) is an acute phase protein that is involved in first-line immune defence. HLA-DR alleles are involved in presentation of peptides derived from extracellular proteins to T cells and subsequent activation of the immune response.

Aims: The aim of this study was to investigate both the functional variant of the MBL2 (rs:1800450-missense mutation) gene and HLA-DR alleles in patients and to evaluate the possible playing role in the ethiopathogenesis of this disease.

Methods: 100 healthy volunteers and 86 high risk pediatric ALL patients who were consecutively admitted to the Pediatric Hematology Unit of Istanbul Medical Faculty and Yeni Yuzyıl Medical Faculty where included in this study. Polymorphism for the MBL2 was determined by PCR-RFLP and HLA-DR alleles were identified by using next generation sequencing.

Results: A total of 86 patients were recruited (62 M/24 F). HLA-DRB1*04, DRB1*07 alleles were significantly more common in patients with c-ALL than controls (p0.05). In patient group, the association of MBL2 BB genotype and DR7 was found to be high and significant compared to those without DR7 (p=0.048; OR=3.933). At the same time, the association of MBL2 BB genotype and the presence of DR7/4 was found to be high and significant compared to those without DR7 (p=0.022; OR=9.125).

Conclusion: The haplotype association of MBL2 functional variant with non-expressed BB genotype and DR7 and/or DR7/DR4 were first shown in this study. The presence of DR7 and/or DR7/DR4 with the MBL2 BB genotype suggests that it could play a role in the ethiopathogenesis of the disease.

## HLA-B TYPING IN HIV POSITIVE PATIENTS IN REPUBLIC OF MACEDONIA: SINGLE CENTER EXPERIENCE

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Aim: Clinical care guidelines for treatment of HIV positive patients suggest HLA-B*57:01 screening in order to reduce the risk of hypersensitivity reactions from abacavir. The aim of this study was to determine the incidence of HLA-B*57:01 among HIV positive patients from Republic of Macedonia.

Material and Methods: In the period from 2016 to 2018 we have performed HLA-B typing for 46 HIV positive patients from the Republic of Macedonia. DNA was isolated using automated method (Duplica Prep, EuroClone, Italy). HLA-B typing was performed using SSO method with Luminex technology (OneLambda, USA). The patients positive for HLA-B*57 were additionally typed to 4 digits using SSP-B*57 high resolution kits (Olerup, Sweden).

Results: In a cohort of 46 HIV positive patients that were typed for HLA-B locus, only 2 (4.34%) were positive for HLA-B*57. These two patients were further typed to HLA-B*57:01 high resolution typing. The frequency of HLA-B*57 among Macedonian population is 1.39% (previously published data).

Conclusion: During the last three years since the introduction of the HLA-B*57:01 screen among HIV positive patients, a steady increase of the number of tested individuals is seen (6, 8 and 32, respectively). However, since around 50 new HIV positive patients are detected each year, the proportion of typed patients prior to treatment with abacavir is still low in the Republic of Macedonia and needs to be improved.

## SINGLE NUCLEOTIDE POLYMORPHISMS WITHIN THE TERT GENE – COMPARISON BETWEEN PATIENTS WITH VARIOUS MALIGNANCIES AND HEALTHY CONTROLS

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Aim: The human telomerase reverse transcriptase (TERT) gene encodes the telomerase catalytic subunit that is essential for maintenance of telomere length. Genetic variability within the TERT gene may affect telomere length and telomerase activity, thus contributing to various types of cancer. Therefore we aimed to compare the distribution of alleles and genotypes of selected single nucleotide polymorphisms (SNPs) within the TERT gene in patients with various cancers and in healthy individuals.

Methods: Four SNPs in the gene coding for TERT were assessed in 157 patients with non-small cell lung cancer (NSCLC), 74 with non-Hodgkin's lymphoma (NHL), 68 with chronic lymphocytic leukaemia (CLL), and 221 with multiple myeloma (MM) as well as in 238 blood donors serving as a control. The TERT polymorphic variants (rs2736100, rs2853690, rs33954691, rs35033501) were detected with the use of LightSNiP typing assay (TIB-Molbiol, Berlin, Germany), employing real-time polymerase chain reaction (PCR) amplifications with melting curve analyses.

Results: The greatest diversity between the studied groups was observed for the rs35033501 SNP. Only this SNP was found to be associated with the risk of cancer development. The presence of the T variant prevailed among CLL patients as compared to controls (OR=3.623, p=0.039). This allele was also more frequently detected among CLL patients than in those with MM (OR=4.996, p=0.007) or NSCLC (OR= 2.841, p=0.051). Moreover, patients with CLL differed from those with MM with respect to distribution of the rs33954691 alleles, with the T variant being more frequently present among CLL than MM patients (OR=2.678 p=0.010).

Conclusion: These results showed that genetic variability in patients with various types of cancer and controls may differ with respect to TERT SNPs. The rs35033501 SNP is characterised with the greatest diversity among studied groups and is associated with CLL risk.

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## HLA-A, -B, AND -DRB1 ALLELES AND HAPLOTYPES AND THE RISK OF ENDEMIC NEPHROPATHY IN CROATIANS

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Aim: Endemic nephropathy (EN) is classified as a tubulointerstitial progressive chronic disease that affects kidneys with a slow clinical course. Disease occurs in rural communities along the river flows of the Danube river basin in Croatia, Bosnia and Herzegovina, Serbia, Bulgaria and Romania. In Croatia, the endemic focal point includes fourteen villages and affects population of about 10 000 people located in the Brodsko-Posavska County on the Sava River bank, west of Slavonski Brod. Over the past period, the influence of hereditary and environmental factors on the appearance of EN (heavy metals, microelements, various viruses and bacteria, soil, drinking water, polycyclic hydrocarbons aromatic compounds) were investigated. Recently, next generation sequencing nominated three genes tightly connected to process of angiogenesis as candidate genes for predisposition to Balkan endemic nephropathy, one of them (KCNK5) being located on chromosome 6p21.2, in close proximity to the HLA region.

Methods: Prompted by this finding, we investigated HLA-A, -B, and -DRB1 alleles and haplotypes in the population of patients with EN (N=103) and matched healthy controls (N=190). All individuals were tested by PCR-SSO for low resolution typing and PCR-SSP to obtain a high-resolution typing.

Results: The results showed higher presence of HLA-DRB1*04:02 allele in EN (P=0.013, OR=3.006, 95% CI=1.28-7.07), in contrast to the lower frequency of HLA-A*01:01, B*57:01 and B*27:05 alleles (P=0.002, OR=0.373, 95% CI=0.19-0.72; P=0.032, OR=0.339, 95% CI=0.13-0.90 and P=0.006, OR=0.098, 95% CI=0.01-0.74, respectively). Moreover, when EN patient's HLA haplotypes were compared to controls, two haplotypes were present with higher frequency within EN patients group, HLA-A*02:01~B*27:02~DRB1*16:01 and HLA-A*26:01~B*38:01~DRB1*04:02 (P=0.002, OR=0 and P=0.054, OR=4.702, 95% CI=0.90-24.45, respectively) while HLA-A*02:01~B*57:01~DRB1*16:01 (P=0.031, OR=0) haplotype showed a significantly lower frequency. Two other haplotypes, HLA-A*02:01~B*27:05~DRB1*01:01 and HLA-A*01:01~B*57:01~DRB1*07:01, were also less frequent among EN patients, but this difference did not reach statistical significance.

Conclusion: The results point toward genetic susceptibility to EN indicating the necessity of further studies.

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