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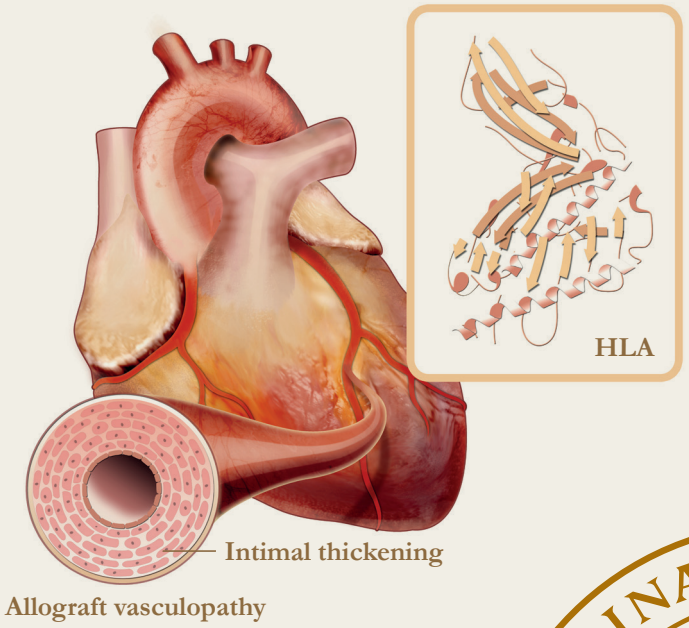

Immunological risk factors in heart transplantation

David Ansari, MD

LUND
UNIVERSITY

DOCTORAL DISSERTATION
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Title and subtitle Immunological risk factors in heart transplantation

Abstract

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Aim: The aim of the thesis was to increase our knowledge of the immunological factors that impact prognosis after heart transplantation, with special focus on HLA and immunotherapy.

Results/conclusion: (I) A systematic review showed that despite the considerable heterogeneity between studies, the short observation time, and older data, HLA matching improves graft survival in heart transplantation. In pooled analysis it was found that prospective HLA-DR matching is clinically feasible and should be considered as a major selection criterion. (II) Decreased long-term survival in heart transplantation was associated with HLA-A compatibility in HLA-B,DR incompatible grafts. This finding indicates that HLA mismatching vs HLA matching is associated with different long-term survival depending on the HLA-B and/or HLA-DR status of the patient. (III) In the International Society for Heart and Lung Registry experience, use of anti-thymocyte globulin rather than basiliximab as induction therapy appears to be associated with better long-term survival. (IV) In a group of pediatric heart transplant patients, the use of basiliximab for induction therapy was associated with an increased risk of mortality, when compared with those receiving anti-thymocyte globulin. (V) Increasing number of eplet mismatches is associated with worse survival in heart transplantation. The findings may have important clinical consequences for survival after heart transplantation.

Key words:human leukocyte antigen, HLA, heart transplantation, induction therapy, basiliximab, ATG, anti-thymocyte globulin, eplet, HLAmatchmaker, HLA-A, HLA-B, HLA-DR, ISHLT, UNOS, long-term survival , peadiatric

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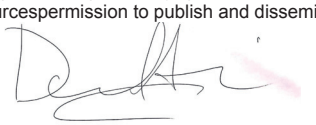
Signature 

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Innehållsförteckning

List of publications

The thesis is based on the following papers, which are referred to in the text by their Roman numerals. The papers are appended at the end of the thesis.

I. Ansari D, Bućin D, Nilsson J. Human leukocyte antigen matching in heart transplantation: systematic review and meta-analysis. Transplant International. 2014 Aug; 27(8): 793-804.

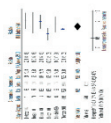
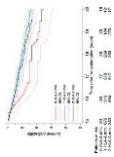
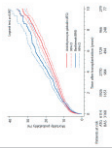
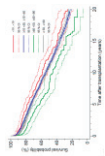
II. Ansari D, Bućin D, Höglund P, Ohlsson M, Andersson B, Nilsson J. Analysis of the influence of HLA-A matching relative to HLA-B and –DR matching on heart transplant outcomes. Transplantation Direct. 2015 Oct; 1(38).

III. Ansari D, Lund L, Stehlik J, Andersson B, Höglund P, Edwards L, Nilsson J. Induction with Anti-thymocyte globulin in heart transplantation is associated with better long-term survival compared to Basiliximab. The Journal of Heart and Lung Transplantation. 2015 Oct; 34(10): 1283-91.

IV. Ansari D, Höglund P, Andersson B, Nilsson J. Comparison of Basiliximab and Anti-thymocyte globulin as induction therapy in pediatric heart transplantation: A survival analysis. Journal of the American Heart Association. 2015: Dec 31; 5(1).

V. Ansari D, Ohlsson M, Bućin D, Höglund P, Andersson B, Nilsson J. Analysis of the influence of structurally based HLA mismatching on heart transplant outcomes. Submitted.

Thesis résumé

STUDY	QUESTION	METHODS	RESULTS & CONCLUSION
I 	What does the existing literature say about the role of HLA matching in heart transplantation?	Systematic review and meta-analysis.	HLA-matching in heart transplantation decrease the risk of acute rejection and graft failure but not overall patient survival.
II 	Is there an interaction between HLA-A and the other HLA loci, which impact long-term survival?	25,583 patients from the ISHLT registry were analyzed.	HLA-A mismatching vs HLA-A matching is associated with different long-term survival depending on the HLA-B and/or HLA-DR status of the patient.
III 	Is there a difference in long-term survival between ATG and Basiliximab in adult heart transplantation?	9,324 transplants were analyzed from the ISHLT registry. 3,180 received Basiliximab and 6,144 received ATG.	Induction with ATG in a population of adult heart transplantation is likely associated with lower all-cause mortality compared with induction with Basiliximab.
IV 	Is the more selective induction drug Basiliximab associated with a survival gain compared with ATG in a pediatric population?	2,275 patients were analyzed from the UNOS registry. 685 received Basiliximab and 1,590 ATG.	In pediatric heart transplant patients, the use of Basiliximab was associated with an increased risk of mortality, when compared with those receiving ATG.
V 	Can HLA matching with the use of HLAmatchmaker predict outcome in heart transplantation?	14,923 transplants with complete HLA class I and II typing were analyzed from UNOS registry.	75-100 % percentile cutoff in class I and class II eplet mismatches was associated with worse survival compared with 25-75 % percentile cutoff total eplet mismatches.

Abstract

Background: In the past three decades, there has been improvement in survival after heart transplantation due to advancements in postoperative intensive care and surgical technique, and more effective immunosuppressive strategies. However, graft failure still remains a major problem. Human Leukocyte Antigen (HLA) is the key molecule in the pathogenesis of graft rejection that ultimately can lead to graft failure. Therefore, increasing our knowledge about HLA and other factors that influence the immune system, such as immunotherapy, is crucial if the risk of graft failure is to be minimized.

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Populärvetenskaplig sammanfattning

Den vanligaste orsaken till död efter hjärttransplantationer är kardiovaskulära händelser och svikt av det transplanterade hjärtat (graftsvikt). Humant leukocyt antigen (HLA) är molekyler som finns på cellytan. Varje individ har en unik sammansättning av HLA molekyler. Det finns tre huvudtyper av HLA, HLA-A, HLA-B och HLA-C. Inom njurtransplantation har det visat sig att ju mer lika uppsättningen av HLA molekyler är mellan donatorn och mottagaren av organet desto bättre går det för den transplanterade njuren och följaktligen för patienten. Idag selekteras donatorer och mottagare vid en hjärttransplantation utifrån blodgruppering, ålder, kön och kroppsstorlek. Studier på HLAs betydelse vid hjärttransplantationer har varit svåra att genomföra. Detta beror på att begränsning i hur länge hjärtat kan vara syre innan det transplanteras, HLAs enorma variation och bristen på donatorer har gjort att välmatchade donatorer och mottagare är sällsynta inom hjärttransplantation. Eftersom graftsvikt fortfarande är ett stort hinder till en bra utgång efter hjärttransplantationer är det viktigt att öka vår förståelse kring de immunologiska faktorer som ligger bakom.

Observationsstudier från 90-talet fann att bättre matchning för HLA-A, B och C förbättrade graftets 3-års överlevnad. Flertalet studier har dock inte kunnat visa att bättre HLA matchning förbättrar prognosen. Studie på njurtransplantation har visat att i en grupp där donator och mottagare skiljer sig åt i HLA-B och –DR , går det bättre för de som har olika HLA-A hos donator och mottagare jämfört med gruppen där HLA-A är helt lika mellan donator och mottagare. Frågan är om samma sak kan visas på hjärttransplanterade patienter.

HLAmatchmaker är en datorbaserad algoritm som identifierar områden på HLA molekylens yta där antikroppar kan binda. Dessa områden som går under begreppet eplets, är således de kritiska områden på HLA molekylen som immunförsvaret tolkar som främmande från sitt eget. HLAmatchmaker räknar ut antalet eplets som är olika mellan mottagare och donator. Hittills har HLA matchning analyserats på serologisk nivå, d.v.s. bestämningen av HLA har utförts med hjälp av antikroppar på laboratorier. HLAmatchmaker som typar på molekylnivå har möjligheten att ge en mer exakt typning av HLA-matchning.

Immunosuppressiv behandling har möjliggjort hjärttransplantationer genom att minska risken för avstötning av det transplanterade hjärtat. Fortfarande är dock behandlingen suboptimal om man tittar på risken för avstötning på lång sikt och det saknas behandlingsprotokoll avseende den mest lämpliga behandlingen. Induktionsbehandling är en form av profylaktisk intensiv immunosuppressiv behandling som ges en kort period direkt efter en hjärttransplantation. Syftet är att minska risken för avstötning under det tidiga skedet efter en hjärttransplantation. De två vanligaste läkemedel som används som induktionsbehandling idag är

basiliximab och anti-thymocyt-globulin. Dessa två läkemedel skiljer sig vad gäller hur de påverkar immunförsvaret. Det finns få studier som jämfört dessa två behandlingar och ingen studie har jämfört de på ett tillfredsställande sätt vad gäller prognosen på sikt.

Delarbete I var en litteratur genomgång av den befintliga kunskapen av sambandet mellan HLA matchning och utfallet efter hjärttransplantationer. De flesta studier fann att bättre HLA matchning förbättrade graftets överlevnad samt minskade risken för angrepp av immunförsvaret, det som kallas för rejektion. Dock är sambandet mellan HLA matchning och den totala patientöverlevnaden inte lika tydligt. När vi slog ihop data från de olika studierna fann vi att framför allt HLA-DR har betydelse för graftets överlevnad.

I delarbete II använde vi oss av ISHLT registret som är ett världsomfattande register över hjärttransplantationer från 1980-talet fram till idag. Vi studerade sambandet mellan HLA-A matchning och långtidsutfall. Vi fann att HLA-A inkompatibla har en bättre prognos jämfört med HLA-A kompatibla transplantationer. i gruppen av patienter som är HLA-B, DR inkompatibla. Detta fynd ger stöd för begreppet tolerans, vilket innebär att immunsystemet kan acceptera främmande organ trots HLA molekyler som avviker från det egna.

I delarbete III använde vi oss av ISHLT registret och identifierade de patienter som hade fått induktionsbehandling med basiliximab respektive de som hade fått behandling med anti-thymocyt globulin. Långtidsöverlevnaden jämfördes mellan grupperna och vi fann att det gick bättre för de som fick anti-thymocyt globulin.

I delarbete IV, använde vi oss av UNOS registret som är ett register över hjärttransplantationer utförda i USA. Vi identifierade barn (ålder < 18 år) som hade fått induktionsbehandling med basiliximab respektive anti-thymocyt globulin. Vi fann att de barn som hade fått anti-thymocyt globulin överlevde längre än de som hade fått basiliximab.

I delarbete V använde vi oss av HLAmatchmaker för att räkna antalet eplets som var olika mellan donator och mottagare hos varje enskild patient. Vi använde oss av UNOS registret. Vi fann ett samband mellan antalet eplet mismatch och överlevnad. Mer uttalad mismatch ger ökad mortalitet, samtidigt som de med 18-31 eplet mismatch i class II, dvs 2:a kvintilen, hade bäst prognos. Detta illustrerar HLA matchningens komplexitet på strukturell nivå.

Abbreviations

ACR	Acute cellular rejection
AMR	Antibody-mediated rejection
Aza	Azathioprine
ATG	Anti-thymocyte globulin
BAS	Basiliximab
CAV	Cardiac allograft vasculopathy
CMV	Cytomegalovirus
CPH	Cox proportional hazard regression
CS	Corticosteroids
CsA	Cyclosporine
CYA	Cyclosporine
DSA	Donor-specific antibodies
ECMO	Extracorporeal membrane oxygenation
ELISA	Enzyme-linked immunosorbent assay
Foxp3	Transcription factor forkhead box P3
GF	Graft failure
HLA	Human leukocyte antigen
HR	Hazard ratio
ICU	Intensive care unit
ISHLT	International Society of Heart and Lung Transplantation
MAR	Missing at random
MCAR	Missing completely at random
MHC	Major Histocompatibility complex
MI	Multiple Imputation
MMF	Mycophenolate mofetil
MNAR	Missing not at random
PRA	Panel reactive antibody
PVR	Pulmonary vascular resistance
RAP	Rapamycin
SD	Standard deviation
SBT	Sequence-based typing
SSO	Sequence –based oligonucleotides
SSP	Sequence-specific primers
Ste	Corticosteroids
TAC	Tacrolimus
TCR	T-cell receptor
VAD	Ventricular assist device
UNOS	United Network for Organ Sharing

Chapter 1 Introduction

1.1. The history of heart transplantation

Carrel and Guthrie performed experimental heart transplantation in 1905 and were the first to report such experiments. They summarized the technique with the description: “anastomosing the cut ends of the jugular vein and the carotid artery to the aorta, the pulmonary artery, one of the vena cava and a pulmonary vein”. When this technique was successful the donor atria contracted almost immediately, whereas the ventricles started contracting approximately 1 hour later. Unfortunately, the experiment failed after 2 hours because of coagulation in the chambers of the donor heart.[1, 2] The next historical step in the history of heart transplantation came in 1933 when Mann and his co-workers transplanted the donor heart into the cervical region of the recipient. The benefit of this technique was that the coronary perfusion of the donor heart was increased.[3] Demikhov has been acclaimed the first to attempt transplantation of the donor heart into the thorax. In Demikhov´s experiments the donor heart was transplanted and the recipient heart left in place, i. e. heterotopic transplantation, but he also performed experiments where the recipients heart was removed, i.e. orthotopic transplantation [4]. Demikhov was very ambitious and came up with more than 24 different techniques for transplantation of donor hearts into the thorax. He experimented primarily on dogs, on which over 250 operations have been reported. His techniques involved most of the major vessels of the thoracic cavity. Unfortunately, the animals he experimented on did not survive more than a few days, because of technical problems. The works of Sen and his colleagues marked another milestone in the history of heart transplantation. Sen is known for developing a technique were the transplanted heart supported only the systemic circulation of the recipient. In one of his experiments the donor heart pumped for more than 48 hours. After the 48 hours the donor heart was removed and the circulation of the recipient again was taken over by the recipient heart. The donor heart in this experiment thus had functioned as a left ventricular assist device. [5]
As stated above, Demikhov was the first to report experiments on orthotopic heart transplantation. This was in 1951. What made the circumstances extra difficult was the fact that hypothermia and pump-oxygenator support were not yet invented. Demikhov´s technique involved end-to-side anastomosis between the corresponding thoracic aortae, superior and inferior venae cava and pulmonary

arteries. The two inferior pulmonary veins of the donor were joined together and connected to the recipient´s left atrial appendage. When these anastomoses were done, the ascending part of the recipient`s thoracic aorta and pulmonary artery were ligated and the recipient´s left atrium was indrawn at its border with the ventricle by means of a purse-string suture. The entire segment of the recipient´s heart thus excluded from the circulation was then excised. The result of this was that the animals survived more than 15 hours. Thus the notion of the donor heart managing the entire circulation of the recipient had become a reality and no longer a fiction.[4]

With the invention of artificial circulatory support, the interest of the possibility of successful heart transplantation in humans was again awakened. Hypothermia was used first by Neptune and his colleges and mechanical pump-oxygenator support first by Webb and Howard, as well as Goldberg and Berman. [6-8]

Cass and Brock refined the technique developed by Goldberg in 1959. They left both the atria intact in the recipient. In this technique the only vessels that are anastomosed are the atria, aorta and the pulmonary artery. With some improvements added to this technique by Barnard the technique is still used today in orthotopic heart transplantation.[9]

Lower and Shumway were successful in keeping dogs alive up to 21 days after heart transplantation. This was in 1960. [10] After the deaths of the dogs they studied the pathology report of the hearts and found that the myocardium was heavily infiltrated with cells of the immune system. The then proposed that a very important idea, i.e. that the donor hearts could have functioned longer if the immune system of the recipient had been suppressed. They could then prove their hypothesis by transplanting hearts that lived long-term. Furthermore, they made more contribution to the development of heart transplantation by showing that the heart could increase cardiac output when physiologic stress was increased, that 1 year after allotransplantation cardiac output of the heart reached normal levels, and found ingrowth of autonomic reinnervation in the transplanted heart.[11, 12]

Once the technical issues were solved there was another hurdle to overcome. The issue of the immune response had to be dealt with in order for long-term survival to be achieved. Methotrexate was used for the first time by Reemtsma and colleagues. With this drugs transplanted dogs survived up to 21 days compared with untreated dogs that survived only up to 10 days.[13, 14] In Stanford, a group of scientists used azathioprine and corticosteroids and improved the outcome even more. Furthermore, they used this drug combination to treat rejection episodes. [15]

Who became known as the father of heart transplantation was Barnard who on the 2nd -3rd December 1967 performed the first human to human heart transplantation at Groote Schuur Hospital in Cape Town. He transplanted the heart into a patient who was 57 years old and suffered from ischemic heart disease. [16] When the donated

heart, orthopically transplanted, functioned throughout the early post-operative period, the news of the first successful human to human heart transplantation was spread around the world. Azathioprine and corticosteroids were the drugs that were given to the patient of Barnard. The patients did not die from technical problems related to the transplantation procedure but from pneumonia 18 days after the operation. Another heart transplantation was carried out 1 month later in Cape Town. This patient lived an active and full life until 1,5 years after the transplantation.[17]

From then on heart transplantation became more and more common in hospitals around the world. Now post-operative complications remained as the biggest hurdles to surmount. These post-operative complications were infections and rejection. In first decades the outcome after heart transplantation kept improving as the patients were better selected, the post-operative care was improved, new and more efficient immunosuppressive drugs were introduced, and the infections were better prevented, treated and diagnosed. Percutaneous transvenous endomyocardial biopsy was introduced in 1973, and it became possible to diagnose and treat acute rejections, which improved prognosis even further.[18]

By late 1970s human to human heart transplantation was no longer something scientist experimented in the laboratories but an accepted treatment for end-stage heart failure. From then on more and more centers around the world began performing heart transplantation.

1.2. The Major Histocompatibility Complex

The host cells express Major histocompatibility complex (MHC) molecules on their cell surfaces anchored to the cell membranes. The MHC molecules interact with $CD4^+$ and $CD8^+$ molecules found on T-cells which enables these cells of the immune system to recognize host cell-associated antigens. T-cell receptors (TCRs) are specific for MHC molecules. In the maturation process of the $CD4^+$ and $CD8^+$ T-cells, their binding to the MHC molecules is a crucial step. This step in the maturation of the T-cells is important as it makes sure the T-cells only recognize MHC molecules that are associated with antigens. MHC molecules are polymorphic meaning that there are an immense variation among individuals. The term MHC restriction is defined by a specific T-cell that recognizes protein fragments on only a specific MHC molecule among all the existing ones.

It was in the 1940s that research on mice came up with the conclusion that there must be a gene region that could induce graft rejection.[19] The scientist gave this gene region the name major histocompatibility complex. The MHC complex consists of multiple genes that are interlinked. The MHC genes harbor the genetic code for the

MHC molecules, with which the T-cells interact. In humans the MHC molecules are named human leukocyte antigens (HLA).[20]

The genetic code of the MHC locus is divided into two major classes of genes, both of which are highly polymorphic. These two classes are named the class I and class II MHC genes. Class I genes have the genetic code for the MHC molecules that the $CD8^+$ T-cells interact with, whereas the class II genes have the genetic code for MHC molecules with which the $CD4^+$ T-cells are capable of binding to. Class I molecules are found on all cells that have a nucleus. Class II molecules are found on dendritic cells, B lymphocytes, macrophages and a few other cell types. Over 10,000 class I alleles and over 3000 class II alleles were known to mankind by the year 2015.[21] In contrast to many other genes both the HLA allele that are inherited from the mother and the father are expressed on the cell surface. The MHC locus in humans is part of chromosome 6´s short arm. The locus has a size of about 3500 kilobases. The class I MHC genes is further divided into the HLA-A, HLA-B and HLA-C genes. The class II genes are divided into the HLA-DP, HLA-DQ and HLA-DR genes.[22]

The molecular structure of the class II MHC molecule is a protein composition of α and β chains. The DP, DQ and DR loci each is divided into a A and a B gene section of which the A part encodes the α chains and the B part the β chains. In every human one can find two HLA-DP genes (called DPA1 and DPB1, encoding α or β chains), two HLA-DQα genes (DQA1, 2), one HLA-DQβ gene (DQB1), one HLA-DRα gene (DRA1) and one or two HLA-DRβ genes (DRB1 and DRB3, 4 and 5). HLA-DR α-chain is encoded by the HLA-DRA gene. HLA-DRB1 gene encodes HLA-DR β1-chain determining specificities DR1, DR2, DR3, DR4, DR5 etc. HLA-DRB3 encodes the HLA-DR β3-chain determining specificities DR52, Dw24, Dw25 and Dw26. HLA-DRB4 encodes HLA-DR β4-chain determining HLA-DR53. HLA-DQA1 encodes the HLA-DQ α-chain. HLA-DQB1 gene encodes HLA-DQ β-chain. HLA-DPA1 gene encodes the HLA-DP α-chain and HLA-DPB1 encodes the HLA-DP β–chain.[21]

1.2.1. Nomenclature of HLA alleles

Each HLA allele name is composed of up to four sets of two-digit number, each set of numbers separated by a colon. The allele name´s first two digits is the same as the HLA-type or serological antigen. Then follows the subtype number, and the numbers of this position were given to the allele in the order in which their DNA sequences have been determined. Alleles that differ in these two sets of numbers, not only must have different nucleotides but these nucleotides have to lead to a different amino acid sequence of the protein. The third set of numbers are so called silent substitutions, which means that the alleles differ in nucleotide sequence that doesn´t necessarily have to alter the amino-acid sequence of the protein. The fourth

sets of numbers define the alleles by the nucleotide sequence of the introns or 5^{I} or 3^{I} untranslated regions that flank the exons or introns.[21]

Example of an allele-name:

HLA-A* 02: 101: 01: 02

1.2.2. MHC molecules

Each MHC molecule is composed of an extracellular part which harbors the crucial peptide-binding cleft, an immunoglobulin-like domain, a part that spans the membrane and a cytoplasmic domain inside the cell.

The building blocks of the class I MHC molecule are the α chain, the genetic code of which is found in the MHC gene region and the β_2-microglobulin, which is encoded by a non-MHC gene region. Class II MHC molecules differ in this regard as both of the two polypeptide chains are genetically coded by MHC genes. The peptide-binding cleft is the polymorphic region of the MHC molecule. It is surrounded by and consists of highly variable amino-acid sequences. If one studies the molecular structure of this cleft one will find that the two walls of the cleft is composed of α helices and the floor of the cleft is composed of an eight-stranded β-pleated sheet. This configuration is created by the folding of the amino termini of the MHC molecule. One finds the highly variable regions in the floor and inner walls of the cleft. The cleft binds peptides that the T-cells can recognize. The MHC molecule consist of a part that is not polymorphic and has the shape of an immunoglobulin. This part is important as it is the docking site for the $CD4^{+}$ and $CD8^{+}$ of the T-cells. Approximately three quarters of the α-chain of the MHC class I molecule is found outside the cells. The part that is anchored to the cell membrane is relatively short and carboxy-terminal residues are found inside the cells (cytoplasm). The peptide-binding cleft cannot take up proteins of any size. Only proteins of 8-11 amino-acids are taken up. Any larger proteins must first be processed into smaller protein fragments and in an extended linear shape to fit into the cleft. The α-chain contains the α1 and α2 domains. These regions are the polymorphic regions in the peptide binding cleft. Thus it is the special amino acid sequence of the α1 and α2 domains that makes the MHC molecules unique and determines its unique characteristics. The α3 domain of the α-chain, on the other hand, has the shape of an immunoglobulin. All the class I MHC molecules have similar structure in the α3 domain. β_2-microglobulin is also an immunoglobulin-like protein and this too is similar among MHC-molecules. Interestingly, only when a protein fragment attaches to the cleft of the polymorphic region of the MHC molecule does the β_2-microglobulin and the α-chain become structurally stable. The cell expresses only stable class I MHC-molecules with a bound peptides, and when no peptides are bound, the MHC-molecules are degraded.[23] As stated before, class I α-chain alleles from both the father and mother are expressed. Therefore, every

individual can have up to 6 different MHC class I molecules, i.e. two HLA-A, two HLA-B and two HLA-C molecules.

One α-chain and one β-chain build up the class II molecules. Class II MHC molecules differ somewhat from class I MHC molecules in the structure of the peptide binding cleft. In the MHC class II molecules it can be found at the amino-terminal of α1 and β1 segments. The floor of the binding cleft is composed of four strands of the α1 segment and four strands of the β1-segment, and one of the walls from the α1 segment and the other wall from the β1-segment. The highly variable amino-acid regions are situated in and around the peptide-binding cleft. Like class I the class II molecules has immunoglobulin like parts, composed of α2 and β2 segments These regions does not differ among MHC molecules. The α-chain and the β-chain both have segments in the cell membrane and portions that resides inside the cells. Also true for class II molecules, only stable MHC molecules, i.e., those with bound proteins in the protein biding clefts are expressed on the cell surface. One DPA and one DPB gene is inherited from the mother and one DPA and one DPB gene is inherited from the father. The DPA has the genetic code for the α chain and DPB the genetic code for the β chains of the HLA-DP molecule. In the same way DQA and DQB are inherited and expressed from both the father and the mother, and encode HLA-DQ. Similarly, one DRA and one or two functional DRB are inherited and expressed. This means that every individual can have up to 6-8 class II MHC alleles. In usual circumstances MHC proteins are not paired between different loci, for example between HLA-DQα and HLA-DRβ, and genes from the mother or father are inherited as one unit. However, this is not always the case. As an example sometimes there exists an extra HLA-DRB loci that unite with HLA-DRA and there are cases where DQA from one chromosome unite with the DQB from another chromosome. This means that one individual may express on the cell surface more than eight MHC class II molecules.[22]

The allelic disparities of class II MHC molecules may have clinical implications. For example, the allelic variation may lead to different ability to bind antigenic peptides and therefore to stimulate specific helper T-cells. A hepatitis B virus vaccine would be ineffective in patients with MHC molecules that did not bind antigens of hepatitis B virus surface antigen.[24] As another example a patient with allergy might have MHC molecules that bind allergenic antigens, such as penicillin.[25]

1.3. Tolerance

When we speak of tolerance in organ transplantation, we mean that the transplanted organ functions well and when histology specimens are studied there is no sign of rejection. Fuchs and Matzinger showed that B-cells are capable of inducing virgin cytotoxic T-cell tolerance to the male-specific minor histocompatibility antigen H-Y. [26] They showed that female mice that were injected with male resting B-cells did not reject male skin grafts until about 100 days. On the other hand, those that were injected with dendritic cells could not sustain their grafts for long.[26] Unfortunately the results cannot be used in a clinical setting as there are multiple minor histocompatibility antigens that differ between donors and recipients even in the situation where the recipient and donor are HLA matched.[27]

Yet another way of inducing tolerance is by intravenous injection of allogeneic spleen cells and cyclophosphamide. This was shown in MHC-compatible strains.[28] It is believed that three major mechanisms are essential to cyclophosphamide-induced skin allograft tolerance. Cyclophosphamide treatment destroys donor-antigen stimulated T-cells in the periphery. Cyclophosphamide can also delete donor-reactive T-cells in the thymus. Lastly this drug can generate tolerogen-specific suppressor T-cells.[29]

By hematopoietic chimerism it is meant transfused donor blood cells or progeny of the cells that survive for extended periods in the recipient. Hematopoietic chimerism has been shown to induce tolerance to transplanted solid organs.[30] In one study it was shown that kidney and bone marrow transplantation, could increase the proportion of $CD4^+$, $CD25^+$, CD127 $FOXP3^+$ regulatory T-cells. These type of T-cells down-regulate the immune response against the donor up to 1 year. This dual transplantation incorporating hematopoietic cells also was thought to induce long-term tolerance by deletion or anergy mechanisms.[31]

Tolerance is a crucial part of the immune response in transplantation and in other responses to, for example, cancer, infection, or autoimmunity. Furthermore, the immune response comprises interactions between up- and down-regulative processes. As an illustration of a general principle, the activation of up-regulative response may induce and activate a down-regulative immune response as shown by interaction of CD28 and CTLA-4 antigens with CD80, CD86 ligands.[32, 33] In contrast to the 80s or 90s at the present time numerous of tolerance inducing genes/structures have been identified, for example non-classical HLA class I genes (HLA-G,F,E), where the tolerance induction of HLA-G genes were extensively studied in pregnancy and transplantation.[34, 35] Furthermore, some of the epitopes of HLA-A antigens have been found in association with decreased risk of delayed allograft function in renal transplantation.[36]

1.3.1. T-regulatory cells and CTLA-4

There is a selection in the thymus during the maturation of the immune cells so that T-cells that react to proteins that belong to the host are either killed or inactivated. Still this maturation process is not without errors. T-cells sometimes arise that are not eliminated, yet react with self-proteins. T regulatory cells have an important task to suppress the T-cells that have evaded the control-system in the thymus and been released into the periphery.[37] CTLA-4 is a T-cell costimulatory molecule that can suppress the T-cell response.[32] CTLA-4 has been shown to prevent autoimmunity and this has been well-studied.[38-40] But T-regulatory cells not only prevent autoimmunity. They have also been implicated in suppressing immunity against tumor cells, have a role in tolerance to the fetus during pregnancy and infectious agents.[41-43] Furthermore T-regulatory cells have also been shown to be involved in organ transplantation. When $CD25^{+}CD4^{+}$ T-regulatory cells were removed from normal mice they rejected skin grafts quicker.[44] The transcription factor Foxp3 is a key component of the regulatory system of the T regulatory cells.[45]

1.4. Rejection and graft failure

1.4.1. Graft dysfunction

Primary graft dysfunction does not have a discernable cause whereas in secondary graft dysfunction we have identified a cause, such as hyperacute rejection, pulmonary hypertension or known surgical complications. To make the diagnosis of primary graft dysfunction it is required that it is made within 24 hours after completion of the heart transplantation.[46]

1.4.2. Cardiac allograft vasculopathy

One of the main causes of death in the long run after heart transplantation is cardiac allograft vasculopathy (CAV).[47] In order to make a diagnosis of CAV one has to look at the coronary angiography and assess cardiac allograft function. [48] In an assessment of the time of first appearance of CAV and clinical events, it was found that early ($\leq$ 2 years) post-transplantation detection of CAV had more rapid progression to ischemic events than late (> 2 years) detection of CAV.[49] When one studies the pathology samples of coronary arteries with CAV one finds involvement of both the intramyocardial and epicardial coronary arteries. Uniquely to CAV the coronary obstructions are diffuse and concentric.[50] It is not unusual to also find perivascular fibrosis and signs of myocardial ischemia. The cells that dominate the picture in autopsy specimens from patients with CAV are cytotoxic T-cells, macrophages, and a proliferation of smooth muscle cells in the intima. [51] Some have

hypothesized that endothelial cell injury is the central event in the development of CAV.[51, 52] Both immunological and non-immunological factors, such as dyslipidemia, cytomegalovirus infection and brain death are believed to be important in the pathogenesis of CAV.[53-56] The humoral and the cellular immune response are both crucial in the development of CAV.[57, 58]Studies have found an increased level of antibodies to cardiac self-antigens myosin and vimentin, as well as an increased frequency of IL-17 secreting CD4+ T-cells against myosin and vimentin[59], in patients with CAV, indicating that they may be involved the pathogenesis of CAV. Also donor specific antibodies to mismatched HLA are significantly associated with the development of antibodies to self-antigens[59].

1.4.3. Antibody-mediated rejection

Antibody-mediated rejection (AMR) is caused by recipient antibodies directed against HLA antigens on the donor endothelial cells.[60] This leads to the complement system become activated and complement and immunoglobulins are then accumulated within the microvasculature of the allograft, leading to an inflammatory process and in the end graft dysfunction.[61] It has been proposed that the complement split products C4d and C3d be used as diagnostic markers for AMR. Not only HLA antibodies but also non-HLA antibodies have been shown to matter in the development of AMR.[59] AMR can develop both early and late. If the recipient is sensitized to donor antigens it occurs as early as 0-7 days after transplantation. It can also arise within the first month after transplantation due to development of de-novo donor-specific antibodies (DSA) or preexisting DSA. AMR can also occur months to years after transplantation.[60] If one detects DSA a diagnosis of AMR is supported but is not required to detect DSA in order to make the diagnosis.

1.4.4. Acute cellular rejection

A very typical sign of acute cellular rejection (ACR) is the massive inflammatory infiltration of lymphocytes in pathology specimens.[62] A collection of these cells cause damage to the myocardium. An international grading system for cardiac allograft biopsies was adopted by the International Society of Heart and Lung Transplantation (ISLHT). The categories of cellular rejection are Grade 0 R (no rejection), Grade 1 R (mild rejection), Grade 2 R (moderate rejection), and Grade 3 R (severe rejection) (Figure 1). [62] In one study it was found that the percentage of Th17, Th1 and FoxP3$^+$ CD4$^+$cells and their associated cytokines were increased in endomyocardial biopsies during acute allograft cellular rejection and were correlated with the grade of acute rejection.[63]

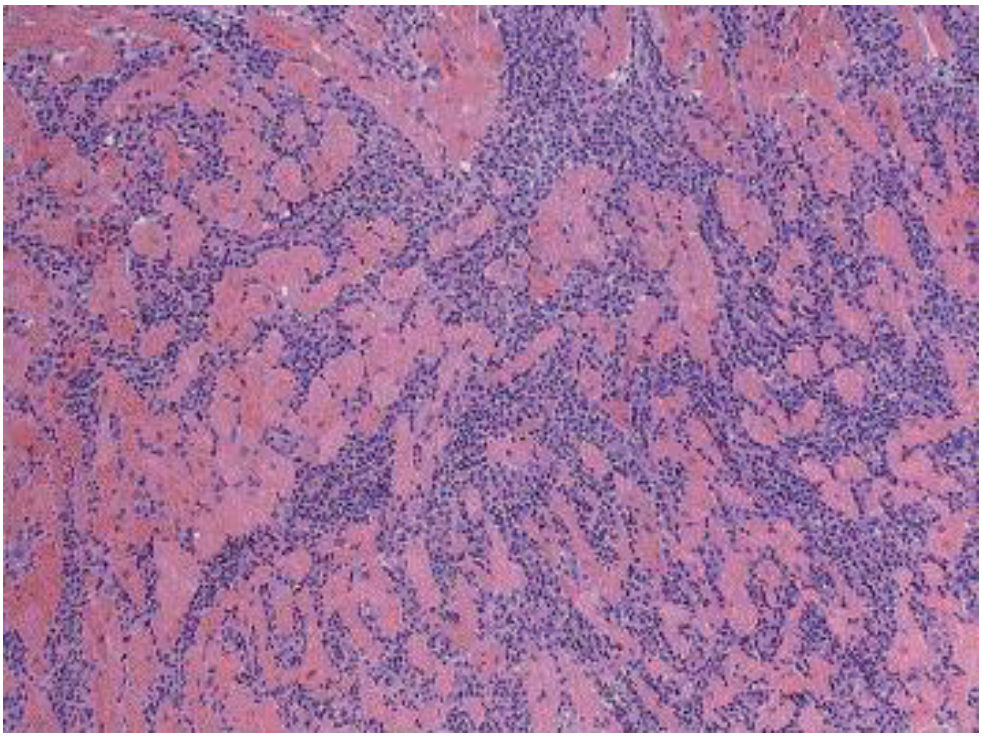


Figure 1.
Grade 3R: Diffuse damaging infiltrates with encroachment of myocytes and disruption of normal architecture. Reprinted from The Journal of Heart and Lung Transplantation. November 2005, Volume 24, Issue 11, 1710-1720. Susan Stewart et al. Revision of the 1990 Working Formulation for the Standardization of Nomenclature in the Diagnosis of Heart Rejection, with permission from Elsevier.

1.5. Panel reactive antibodies

With the use of panel reactive antibody assays allosensitization can be assessed in patients that are undergoing orthotopic heart transplantation. It has been shown that patients that are sensitized and who have a significant reduction in panel reactive antibody activity also have a decline in the incidence of graft failure compared with those without a panel reactive antibody activity reduction. [64] There are various typing methods for the detection of panel reactive antibodies.

1.5.1. Cytotoxic (Cell based) antibody screening

Lymphocytes are randomly selected from the population, thought to represent the donor. Between 20 and 40 cells are selected. These form the panel of cells. The basic idea is that the HLAs of these lymphocytes mirror the distribution of the HLAs that are found in the population from which the donor is derived. The percentage of "cell donors" in the panel that the recipient has antibodies to is then calculated and is thought to represent the percentage of donors in the population that the recipient would have a positive cross match to. The typing method is carried out by serum from the recipient mixed with "cell donor" lymphocytes. Complement and the vital dye is also added. If there are antibodies in the sera that bind to the cells, complement will be activated and the vital dye indicate that a reaction has occurred. If in a panel of 40 cells, 30 cells react, then the PRA (panel reactive antibody) would be reported as 75 %.

The PRA might change if the cells used in the lymphocyte panel are altered, even though amount or type of the antibodies of the recipient are the same. This is a limitation of the cytotoxic antibody screening method. Often the commercially panels that are used, do not with certainty represent the particular region that the donor come from. Racial differences in a particular region might lead to alteration in the HLA distribution. Another problem with this method are false positive results that occur because of non-HLA antibodies. Also false negative results occur. Sometimes when the titer of antibodies is low complement will not be activated. High titer of antibodies is required to activate the complement system.[65]

1.5.2. Solid phase antibody screening

Antibodies can cause damage-even when the PRA is normal. In solid phase antibody screening soluble or recombinant HLA are used instead of lymphocytes. HLA molecules are purified and applied to solid phase media. They bind only HLA antibody when recipient serum is added. After the initial step of recipient sera mixed with solid phase media containing recombinant HLA, antibodies to human IgG linked with enzymes are added. They will detect any HLA antibody in the serum that is bound to an antigen. Optical density reading or fluorescence are the technique used for the detection of the enzyme-linked antibodies that have reacted. Because one can choose the antigens placed on the beads, the assays can be specific for HLA antigens only, one can discriminate between class I and class II antibodies, and the precise antibody specificities may be determined.
Because of the high sensitivity of the solid phase antibody screening method low level antibodies, even below the level of clinical importance, can be detected. This can lead to potential donors be excluded and limit transplants available. Another problem is that today there are thousands HLA alleles identified, and apparently solid phase methods cannot contain every HLA allele. Fluorescence or optical

density are used to say that a result from solid phase assays is positive or negative. The results are continuous and controversy exists as to what thresholds should be considered positive. This has led to substantial variability between laboratories. [65]

1.6. Cross matching

In cross matching what is analysed is whether a recipient has antibodies to a particular single donor of interest.

1.6.1. Complement-dependent cytotoxicity crossmatch methods

Similarly to cytotoxic antibody screening this method is considered positive when T-cells or B-cells from the donor bind to donor specific antibodies, and killed after addition of complement. It was found that AHG improves this method by increasing sensitivity by requiring lower titers to be positive. With low titer antibodies false negative results may arise or false positive can result if non-HLA IgG antibodies are detected instead of antibodies directed to HLA.[65]

1.6.2. Flow cytometry crossmatch methods

In contrast to cytotoxic cross matching this method detects donor specific antibodies regardless of its ability to bind complement. It detects only the presence or absence of IgG DSA in the donor lymphocytes. Serum is added to donor lymphocytes and then fluorescein conjugated anti-IgG antibodies are added. These cells only remain bound if the DSA are initially bound to the donor lymphocytes. The thresholds of positivity can vary between laboratories. [65]

1.7. Induction therapy

One separates induction and maintenance immunosuppression in heart transplantation. Induction therapy is defined as a treatment given prophylactic and temporarily in the immediate post-operative period, whereas maintenance treatment is given lifelong to the patient. Below are listed those inductions immunosuppressive drugs have been used in heart transplantation[66]:

Rabbit antithymocyte globulin (rATG) – Thymoglobulin (Genzyme) or ATG Fresenius (Fresenius)

Horse antithymocyte globulin (hATG) – ATGAM (Pfizer)

IL-2 receptor antagonists – basiliximab (Simulect, Novartis) or daclizumab (Zenapax, Roche)

Anti-CD3 antibodies – Muromonab- CD3 (Orthoclone OKT3, Janssen –Cilag)

Anti-CD52 antibodies – Alemtuzumab (Campath , Genzyme and Lemtrada, Sanofi)

In the beginning there was a hope that induction therapy would make the immunosystem tolerant against the graft. However, induction therapy has not been able to achieve this dream. When the available evidence was gathered in the ISHLT guidelines for the care of heart transplants there was a lack of support of use of induction treatment in patients indiscriminately. However, the guideline recommends the use of ATG in patients at high risk for acute rejection and in patients at high risk of renal dysfunction when used with the intent to delay or avoid the use of the calcineurin inhibitors, cyclosporine and tacrolimus. Furthermore, in pediatric heart transplantation, the guidelines recommend routine use of induction therapy with a polyclonal preparation when complete corticosteroid avoidance is planned after heart transplantation.[67]

1.7.1. Basiliximab

Basiliximab is a chimeric (murine/human) monoclonal antibody (IgG_{1k}). It is produced by recombinant DNA technology. Basiliximab exerts its effects by binding to and blocking the interleukin-2 receptor α- chain, which is found on activated T-cells. Another name for the interleukin-2 receptor α- chain is CD25 antigen. Basiliximab is developed in the laboratory from mouse myeloma transformed cell lines. These cell lines are then genetically changed so that they express human heavy and light chain constant region genes and mouse heavy and light chain variable region genes that encode the RFT5 antibody that bind selectively to the interleukin-2 receptor α- chain. Basiliximab binds with such a strong affinity to the interleukin-2 receptor complex that the binding of interleukin-2 in inhibited. Interleukin-2 is crucial for the activation of lymphocytes. Studies done in vitro on human tissues have not shown that basiliximab binds other cells than lymphocytes. It is unknown for how long basiliximab exerts its effect on the immune system but we do know that when combined with corticosteroids and cyclosporine interleukin-2 receptor α- chain was saturated with basilixmab for 36±14 days, when added to a triple regimen with azathioprine, cyclosporine and corticosteroids 50±20 days and when added to a drug combination with cyclosporine, corticosteroids and mycophenolate mofetil 59±17 days. In contrast to ATG, investigation with flow cytometry have not shown that the number of circulating lymphocytes or cell phenotype changes with basiliximab use. When basiliximab was originally introduced it was indicated for use in renal transplantation as prophylaxis of acute

organ rejection in combination with corticosteroids and cyclosporine. Its indication did not expand to the field of other solid organ transplantations because its efficacy for the prophylaxis of acute rejection had not been demonstrated.[68]

1.7.2. ATG

Two commercially available forms of polyclonal anti-lymphocyte antibodies exist; production from horses (ATGAM) or from rabbits ((Thymoglobulin). ATGAM was developed by the Upjohn Company during the 1980s. This was the first commercially available ATG in Europe and USA. Thymoglobulin became available for commercial release in 1984 in Europe and in 1999 in USA. Later, ATG-Fresenius, which is a rabbit ATG, was introduced in Europe.[69] These drugs have been used in organ transplantation for years and are among the most potent immunosuppressive drugs known. They cause various effects on the immune system; a rapid and profound lymphocytopenia by complement-dependent cytolysis, cell-mediated antibody-dependent cytolysis, as well as opsonization and subsequent phagocytosis by macrophages.[70] The polyclonal antibodies are directed against many surface molecules on both T-cells and B-cells.[71] The fact that ATG is polyclonal explains its diverse effects on the immune system. ATG depletes T-cells in blood and peripheral lymphoid tissue through complement-dependent lysis, T-cell activation and apoptosis, modulation of key cell surface molecules that mediate leukocyte/endothelium interactions, induction of apoptosis in B-cell lineage, interference with dendritic cell functional properties, and induction of regulatory T and natural killer T-cells.[72] ATGAM is produced from horse serum immunized with human thymus lymphocytes. ATGAM contains primarily IgG. ATGAM does not usually cause severe lymphopenia. It was originally indicated for use as induction treatment and treatment of rejections in renal transplantation as well as treatment of severe aplastic anemia by the US. Food and Drug administration.[73] Similarly Thymoglobulin is gamma immune globulin (IgG) produced by immunization with human thymocytes but instead of horse sera, rabbit sera is used. Thymoglobulin includes antibodies that exert an effect on several different molecules on T-cells, including HLA. By binding to these molecules, thymoglobulin can inhibit the proliferative responses to several mitogens. Thymoglobulin was indicated for the treatment of renal transplant acute rejection.[74] Thymoglobulin has been shown to deplete a variety of immune cells, but the primary mechanism of action is on T-cells.

1.7.3. Effect of ATG on regulatory T-cells

In 2006 it was shown by Lopez et al that ATG could cause a rapid and sustained expansion of $CD4^+CD25^+$ T-cells when cultured with human peripheral blood lymphocytes. Alemtuzumab or the interleukin-2 receptor antagonists did not have

this effect. ATG had the ability to convert $CD4^+CD25^-$ T-cells into $CD4^+CD25^+$ T-cells.[75] The authors showed that ATG could expand T regulatory cells ex vivo, mainly by inducing $CD4^+CD25^+Foxp3^+$ T-cells. When T-cells were cultured with Thymoglobulin the expression of GITR, CTLA-4 and Foxp3 was enhanced and this efficiently suppressed a direct alloimmune response of the original responder lymphocytes. What characterizes T regulatory cells are the expression of the interleukin 2 receptor α-chain, CD25, and the transcription factor forkhead box P3 (Foxp3).[76] $CD4^+CD25^+$ T regulatory cells have the ability to maintain and induce self-tolerance and tolerance toward autoantigens and alloantigens.[77] From these results the authors drew the conclusion that ATG exerts its effects both by depleting lymphocytes and by a continuous regulatory T cell activity. The question also was raised that maybe it would be possible to expand T regulatory cells ex vivo for the benefit of transplantation and autoimmunity.

1.8. Databases

1.8.1. The ISHLT database

The International Society for Heart and Lung Transplantation (ISHLT) was originally created as a non-profit, multidisciplinary, professional organization dedicated to improving the care of patients with advanced heart or lung disease through transplantation.[78] ISHLT started in 1981. The organization started as a small group consisting of 15 cardiologists and cardiac surgeons but today it has expanded to include more than 3000 members from over 45 countries representing over 15 different professional disciplines. The ISHLT International Registry for Heart and Lung Transplantation is a database that gathers information on thoracic organ transplantations that are carried out worldwide. The requirement to participate is that the countries perform a minimum number of transplantations. The public become aware of the results of the database through their website in the form of data reports quarterly and annually by data slides, that can be downloaded. Scientists that are members can use the data for research purposes. The Registry registers survival data, risk factor data, outcome data, demographic data, status at transplantation, indication for transplantation and follow-up data. Every year ISHLT publishes a report in the Journal of Heart and Lung Transplantation. There they present the analysis and interpretation of their data. ISHLT collects data in three ways; manually via web-based data entry system by individual centers, electronic download of data from individual centers and via sharing of data with regional/national Organ Procurement Organizations and Organ Exchange Organizations. 45 centers send in their data manually using the web-based data entry system and the Registry have data sharing agreement with the following organ

transplant organizations; United Network for Organ Sharing (UNOS) (USA), Eurotransplant (Austria, Belgium, Germany, Luxemburg, The Netherlands, Slovenia), Organizacion Nacional de Transplantes (Spain), Registro Espanol de Transplante Cardiaco (Spain), UK Transplant (United Kingdom and Ireland), Scandia Transplant (Sweden, Norway, Denmark, Finland), Australia and New Zealand Cardiothoracic Organ Transplant Registry, Agence de la biomedicine (France) and British Columbia Transplant Agency.[78]

1.8.2. UNOS database

UNOS, United network for Organ Sharing, is a database over all organ transplantations performed in USA. UNOS was started on March 21, 1984, as an independent non-profit organization. Data entry by all US transplant centers has been mandatory since the passage of the National Transplantation Act of 1984.[79]

1.9. Missing data

One issue that any scientist performing analysis in medical and epidemiological research must be aware of is that almost all data include some missing values. Missing data handled incorrectly may lead to bias and erroneous mean regression coefficients, confidence intervals and significance tests. Multiple imputation is a statistical technique to handle missing values. It has become popular because of its generality and recently software has been developed that makes it easier to use this technique.[80, 81] The basic concept in multiple imputation is that the missing data are replaced by probable values that are based on estimates of the distribution of the known data. Some random values are incorporated in the estimates in order to account for the uncertainty of the data. Multiple rounds of estimates for the missing values are calculated but in a final step, these individual data sets are combined into an overall estimate.[82] Missing values are divided into three types depending on the correlation with known or unknown data. When the probability of the data being missing is not dependent on the known or unknown data missing values are called missing completely at random (MCAR). When the probability of the data being missing is not dependent on the unknown values but dependent on the known values the missing data is named missing at random (MAR). In a third category missing data can be missing not at random (MNAR) which means that the probability of the data being missing is dependent on both the known and unknown data. The different groups of missing data can be exemplified by the following example; missing values in blood pressure are MAR if the probability of older patients having their blood pressures measured is higher but MNAR if the patients that have higher blood pressure, in addition to older patients, more often have their blood pressure

measured. One advantage of multiple imputation is that it can be used both when the missing data are MAR and MNAR.[83] Multiple imputation by chained equations is a special form of multiple imputation. It is especially suitable when dealing with large datasets when many of the variables have missing values. Another advantage of multiple imputation by chained equation is its ability to handle different variable types, for example continuous, binary, unordered categorical and ordered categorical, as different variable type can be imputed by different imputation models.[84]

1.10. HLAmatchmaker

HLAMatchmaker (www.hlamatchmaker.net) is a computer algorithm that determines HLA compatibility at so called epitope level. Each HLA antigen is considered as a string of amino acid configurations as key elements of epitopes that can elicit specific alloantibodies. It is the stereochemical modeling of protein antigen-antibody complexes and the critical amino acid residues that dominate in antigen-antibody binding that HLAmatchmaker uses to determine the number of eplet mismatches [85]. The computer algorithm of HLAMatchmaker compares the amino acid sequences that are crucial for antibody binding between donor and recipient alleles to identify and quantify differences. Not all amino-acids of HLA are considered but only those that are polymorphic and at or near the molecule´s surface accessible to antibody binding. The program finds special patches of polymorphic amino acids that are exposed on the antigen surface, consisting of amino-acids that are continuous or discontinuous in a linear sequence but are brought close to each other on the tertiary structure[86]. HLAMatchmaker uses low resolution, 2-digit alleles, and a subjects race to assign the most likely high-resolution 4 –digit alleles for each subject.

Chapter 2 Aim of the thesis

The general aim of the thesis was to expand our knowledge about the immunological risk factors in heart transplantation, with special reference to human leukocyte antigen and immunotherapy.

The specific aims were:

I. to evaluate the efficacy of HLA matching in heart transplantation by performing a systematic review and meta-analysis of the available evidence**.**

II. to investigate possible associations between HLA-A matching in relation to HLA- B, DR matching and long-term survival after heart transplantation.

III. we hypothesized that the different mechanisms of action of ATG and Basiliximab may result in different effects on long-term mortality after heart transplantation. We also aimed to compare Basiliximab with ATG with regard to graft failure, cardiovascular, infection and malignancy-related death.

IV. to determine whether any difference could be observed between Basiliximab and ATG, with respect to long-term mortality, in a population of pediatric cardiac transplant recipients.

V. to examine the association between long-term survival and donor-recipient mismatching based on HLA structure.

Chapter 3 Material and Methods

3.1 Study selection and population

Study I

We performed a systematic literature search by using PubMed (inception to January 25, 2013), Embase, and the Cochrane Library. 'heart transplantation' and 'HLA' were used as search terms. We followed the specific guidelines outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [87]. We also screened all the reference lists in the articles selected for any further articles not identified in the initial search. We considered only original articles in English.

Study selection

The titles and abstracts of all studies identified by the initial search were reviewed and irrelevant studies were excluded. For articles that might be of interest to our study we obtained the full text. These full-text articles were reviewed to see if they met the inclusion criteria of our study. Data on publication year, sample size, study design, patient characteristics, type of intervention, HLA data, follow-up, and outcomes were extracted from each article.

Inclusion criteria

We included articles that reported on HLA matching and outcome in adult heart transplantation.

Exclusion criteria

Publications reporting pediatric studies were excluded. Studies on HLA antibodies and studies on HLA without matching were excluded. Irrelevant topics and studies on organ transplantation other than heart were excluded. Articles with no original data, such as reviews and technical descriptions, were also disregarded. Conference abstracts were excluded. Duplicate reports were removed.

Study II

Data from heart donors and the corresponding recipients transplanted between January 1, 1988 and June 31, 2011, were collected from the ISHLT registry (n =

93,507). Pediatric cases (recipients younger than 18 years, n = 13,136); recipients with panel-reactive antibodies (PRA) >=10% (class I or class II) (n = 4,483), history of previous cardiac surgery including mechanical circulatory support, or previous transplantation (n = 10,129); recipients who died intra-operatively (n = 726); and those with missing value on recipient or donor HLA-A, duration of follow-up, or cause of death not reported (n = 39,450) were excluded. The final study population comprised 25,583 patients with at least one day of follow-up duration. The latest annual follow-up was on October 9, 2011.

Study III

The source of data was the ISHLT registry. Data from adult heart recipients transplanted between January 1, 2000 (BAS was introduced in 1999) and June 31, 2011, were analyzed (*n* = 42,474). Pediatric cases (recipients younger than 18 years, *n* = 5,182), recipients who did not receive ATG (equine anti-thymocyte globulin [Atgam], rabbit anti-thymocyte globulin [Thymoglobulin/Fresenius-ATG] or Nashville rabbit antithymocyte globulin/Nashville rabbit antithymocyte serum [NRATG/NRATS]) or BAS (*n* = 26,998), or no information of cause of death or vital status (*n* = 1,012) were excluded. The final study population comprised 9,282 patients, with at least one day of follow-up duration, corresponding to 9,324 transplants. The latest annual follow-up was on October 9, 2011. The duration of follow-up was limited to 10 years.

Study IV

De-identified patient data from UNOS research database were extracted. We identified all recipients of orthotopic cardiac transplants patients under the age of 18 years of age, transplanted between January 3, 2001 and September 30, 2013. The latest follow-up was on December 5, 2013. We chose to include patients transplanted after 2000 because BAS was approved by the U.S. Food and Drug Administration in 1998.[88] Using these criteria resulted in 7,341 transplant recipients for analysis. The study population was limited only to those patients receiving induction therapy with either BAS (Simulect) or ATG (equine anti-thymocyte globulin; Atgam, rabbit anti-thymocyte globulin; Thymoglobulin). Those with missing values in BAS or ATG treatment were excluded. The final cohort consisted of 2,311 patients.

Study V

We conducted a retrospective cohort analysis of the United Network for Organ Sharing (UNOS) database. We included subjects undergoing primary heart transplantation in the United States between October 1, 1987 and September 30, 2013 (n = 56,436). Those with missing values on total class I or class II eplet mismatches (n = 6,404) and pediatric patients (age <18 years, n = 6,010) were

excluded. We also excluded those with missing values in recipient class I antigen or in recipient class II antigen (n = 29,099). 14,923 transplants were included in the final analysis. Transplants were divided into three groups based on the percentile cut-offs of the distribution of the total number eplet mismatches in class I and/or in class II. The latest annual follow-up was on December 5th 2013.

3.2. Outcome measures

In study I, outcome measures were graft rejection, graft failure, patient survival, and CAV.

In study II and V, the primary endpoint was all-cause mortality. In study II, secondary endpoints were mortality attributable to graft failure (primary failure, rejection: hyperacute, acute or chronic, technical, graft infection, recurrent disease, non-specific), cardiovascular causes (myocardial infarction, cardiac arrest, arterial embolism, ventricular failure, coronary artery disease, atherosclerosis, rhythm disorder, carditis, aortic aneurysm, cardiogenic shock, other), infection (bacterial septicemia, bacterial pneumonia, bacterial -other, viral cytomegalovirus, hepatitis, viral septicemia, viral – other, fungal, protozoal, mixed) or malignancy (metastatic, primary, post-transplant lymphoproliferative disorder, lymphoma, skin, other) as defined by the ISHLT Registry.

In study III, and IV the outcomes were cumulative all-cause mortality (censoring those who died from trauma or unknown cause of death and those alive at last follow-up) and graft failure related death, cardiovascular related death, infection related death and malignancy related death (same definitions as above).

3.3. Statistics

In study I, meta-analysis was performed on studies that shared a common outcome and were similar with regard to follow-up, HLA analyzed, number of mismatches, and statistical analysis. Due to low power meta-analysis was not done if fewer than three studies. The methodological quality of the studies was assessed by the Newcastle-Ottawa Scale, which was designed for assessment of the quality of non-randomized studies in meta-analyses. It scores potential sources of bias and variation in cohort studies regarding selection, comparability, and outcome. We assessed publication bias with funnel plots. When asymmetry was found in the funnel plot this indicated that the results were subject to reporting publication bias whereas symmetrical funnel plot indicated a lack of bias.

In studies II-V, statistical analyses were performed using the Stata MP statistical package version 13.1 (2013) (StataCorp LP, College Station, TX). Unpaired Mann-Whitney U-tests or t-tests were used to compare continuous variables, and $\chi 2$ or Fisher's exact tests were used to compare categorical variables among groups. Log-rank test was used to compare the Kaplan-Meier survival curves. In study IV, statistical differences between the mortality curves were also assessed using cloglog test (at fixed point in time)[89]. Independent predictors of cumulative mortality were identified using Cox proportional hazard regression (CPH). In study II, any variable from the univariable test (simple CPH) with a *p*-value <0.25 was selected as a candidate for stepwise backward selection Cox regression analysis, resulting in a main effect model. In studies III-V, in the iterative process of variable selection using forward, backwards and stepwise selection, covariates were removed from the model if they were non-significant and not a confounder, as described by Hosmer-Lemeshow[90], resulting in the primary main effect model with no interaction terms. In study V, the following characteristics were considered to be potential confounders in examining the association between the number of HLA eplet mismatches and overall mortality: recipient donor age and sex; donor/recipient weight ratio; ischemia time; era of transplant; proportion of with peak panel reactive antibody (PRA) > 10 %; recipient and donor race; pre-transplant diagnosis; pre-transplant dialysis; pre-transplant extracorporeal membrane oxygenation (ECMO); pre-transplant ventilator and donor cause of death. In studies II and V, we further split episodes into two episodes at implied time points. Each resulting covariate record contained the follow-up on one subject through one time band [91]. In study IV, we fitted a Cox regression model in which we accounted for the effect of time-varying covariates, by specifying that the time-dependent covariates be interacted with the logarithmic function of analysis time.[92] Interactions between induction therapy or HLA group and clinical relevant risk variables were estimated by Cox regression analysis including covariates from the main model. The results were displayed in a forest plot. Hazard ratios (HRs) were presented with 95% confidence intervals. All tests were two-sided and p-values of <0.05 were deemed significant.

To minimize potential bias arising from missing data, multiple imputation (MI) was performed using the chained-equations imputation technique as described by White et al. [82]. In studies II and III, the imputation method was predictive mean matching for continuous variables, logistic regression for binary variables, and ordered logistic for ordinal variables. In studies IV and V, the imputation method was predictive mean matching. The number of iterations for each chain was 10 and the number of imputed data sets was 10.

In study V each donor-recipient pair with genotyped HLA-A, HLA-B, HLA-C (class I) and HLA-DRB1, HLA- DRB3/DRB4/DRB5, HLA-DQB1 and HLA-DQA1 (class II) were entered into the HLAMatchmaker algorithm. We used HLAMatchmaker ABC and DRDQDP matching software (version 3.0) to

calculate the number of eplet-derived epitope mismatches that were present in each donor-recipient pair.

Chapter 4 Results

Study I - Human Leukocyte Antigen Matching in Heart Transplantation: Systematic Review and Meta-analysis

In the first step of the literature search, we extracted 1,035 studies from PubMed, 2,688 from Embase, and 21 from the Cochrane Library. Searching for articles from the references did not yield any article of interest. This search resulted in fifty-seven studies that were included in the final analysis [88, 93-147]. Twelve articles were multicenter studies [88, 136-146], whereas forty-five of the studies were single-center [93-135, 147]. Mean follow-up was 3.4 years and 13 studies reported follow-up of 5 years or more. Tables 1 and 2 summarizes details of the study designs and outcomes of these articles.

Table 1.

Characteristics of the multicenter studies included

Reference	Year	*n*	HLA locus analysis*	Resolution	Follow -up (y)	Immunotherapy	Registry	Stat.	Outcome
Hosenpud et al. [136]	1996	10,752	A, B, DR, A+B+DR	Serology/DNA	3	-	UNOS	M	Progressive reduction in mortality risk at 3 years for greater matching, primary benefit at HLA-A and DR loci.
Jarcho et al. [137]	1994	1,190	A+B+DR, A, B, DR	Serology/DNA	2.5	-	CTRD	M	Number of HLA mm correlates with time to first rejection (not in blacks); HLA-DR associated with cumulative rejection frequency; no correlation with graft failure; HLA mm correlates with rejection-related death at 2 years.
Mascaretti et al. [138]	1997	661	A+B+DRB1, DRB1, A+B	DNA	3	A.L.G	NITp	M	No significant correlation with 1- and 3-year patient survival.
Opelz et al. [139]	1989	2,000	A+B, DR, B+DR	Serology/DNA	1	CsA, Aza, Ste	CTS	M	Significant correlation between HLA-B, -DR and graft survival at 1 year.
Opelz et al. [141]	1994	8,331	A+B+DR, A, B, DR, A+B	Serology/DNA	3	CsA, Aza, Ste	CTS	M	3-year graft survival correlates significantly with HLA mm.
Opelz et al. [140]	1997	103	A+B+DR, B+DR	Serology/DNA	3	-	CTS	M	No significant correlation with graft survival in black recipients.
Park et al. [142]	1997	336	A+B, DR	Serology	4.4	CsA, Aza, Ste		U	Modestly improved 10-year patient survival for HLA-A, -B compatibility (Caucasians), though not significant ($P = 0.06$).
Poli et al. [143]	1992	168	A+B+DR, DR	DNA	1	CsA, Aza, Ste, A.L.G	NITp	U	No significant correlation with patient survival.
Poli et al. [88]	1995	358	A+B, DRB1	DNA	2	CsA	NITp	M	No significant correlation with graft survival.
Thompson et al. [144]	1998	1,927	DR, A+B+DR	-	3	-	SEOPF		Clear effect of HLA-DR matching on 1- and 3-year graft survival.
Thompson et al. [145]	2000	14,535	A, B, DR, A+B, A+B+DR	Serology/DNA	3	CsA, Aza, Ste	UNOS	M	3-year graft survival superior for HLA-A, -B, and -DR matching. When analyzed separately, 1- and 3-year graft survival directly related to the number of HLA-DR mm.
Valeri et al. [146]	1990	92	DR, B+DR	Serology	3	CsA.		U	HLA-B and -DR matching have a positive effect on 1- and 3-year patient survival.

* HLA A, B, DR: each locus was analyzed separately, 0–2 mm. HLA A+B: A and B loci were analyzed together, 0–4 mm. HLA B+DR: B and DR loci were analyzed together, 0–4 mm. HLA A+B+DR: all loci were analyzed together, 0–6 mm.

HLA: human leukocyte antigen. UNOS: United Network for Organ Sharing, all centers in USA. CTRD: Cardiac Transplant Research Database, 27 centers in USA.

NITp: Northern Italy Transplant program, 5 centers. Collaborative Transplant Study, centers worldwide. SEOPF: Southeastern Organ Procurement Foundation. M: multivariable (Cox proportional hazards regression). U: univariable. mm: mismatch. . CsA: cyclosporine. Aza: azathioprine. A.L.G: antilymphocyte globulin. Ste: Steroids.

Table 2.
Single-center studies.

Reference	Year	n	HLA locus analysis*	Resolution	Immunotherapy	Follow-up (y)	Statistical model	Outcome
Almenar et al. [93]	2005	243	A+B+DR	Serology	CsA, MMF/Aza, Ste, Tac(s)	4.7	Univariable	Associated with PS (inverse relationship). No effect on GR and GS.
Arbustini et al. [94]	1997	429	A, B, DR, A+B, A+B+DR	Serology	CsA, Aza, Ste	3.8	Multivariable (Poisson)	HLA-B associated with CAV.
Aziz et al. [95]	1998	249	A, B, DR	Serology/ DNA	CsA, Aza, Ste	-	Univariable	HLA-DR associated with GR. No effect on CAV.
Baan et al. [96]	1991	118	A, B, DR, B+DR	-	-	0.5	Univariable	HLA-B and -DR associated with GR.
Botha et al. [97]	1969	5	-	-	Aza, Ste, A.L.G	-	None	No outcome analysis.
Brunner La-Rocha et al. [98]	1996	161	A+B+DR	-	CsA, Aza, Ste	3	Multivariable (Logistic regression)	Associated with GR.
Carrier et al. [99]	1990	20	A+B	-	CsA, Aza, Ste. ATG	0.1	Univariable	No effect on GR.
Cocanougher et al. [100]	1993	160	A+B+DR, B+DR	Serology	CsA/OKT3, Aza, Ste	-	Univariable	Associated with CAV. HLA-A, -B, and -DR associated with PS.
Cochrane et al. [101]	1992	55	A,B, DR	-	CsA, Aza, Ste	0.5	Multivariable (Cox)	HLA-DR associated with GR.
Costanzo-Nordin et al. [102]	1993	195	A, B, DR	Serology	CsA, Aza, Ste	3	Univariable	HLA-DR associated with GR. No effect on PS.
De Mattos et al. [103]	1994	132	DR	Serology	CsA/OKT3, Aza, Ste	7	Univariable	HLA-DR associated with GR and GS. No effect on CAV.
DiSesa et al. [104]	1990	51	A+B, A+B+DR	-	CsA, Aza, Ste	2.8	Univariable	HLA-A or -B associated with GR.
DiSesa et al. [105]	1994	31	A+B, A, B, DR	Serology	-	-	Univariable	HLA-A+B associated with GR.
Fieguth et al. [106]	1991	61	A, B, DR, B+DR	-	CsA, Aza, Ste	2.8	Univariable	HLA-B+DR or B associated with GR. No effect on CAV.
Foerster et al. [107]	1988	51	A, B, C, DR	Serology	CsA, Aza, Ste	1.1	Multivariable (Cox)	HLA-DR associated with GR and GS.
Foerster et al. [108]	1991	100	A, B, DR	Serology	CsA, Aza, Ste	2.2	Multivariable (Cox)	HLA-DR associated with GS. No effect on PS.
Foerster et al. [109]	1992	100	A, B, DR	-	CsA, Aza, Ste	5	Multivariable (Poisson)	HLA-B+DR associated with GR.
Frist et al. [110]	1987	164	A+B, A, B	Serology	CsA, Aza, Ste	5	Univariable	HLA-A+B or -A associated with PS. No effect on GR.

Hollander et al. [111]	2013	53	A+B+DR	-	CsA, MMF, Ste, Sir(s), Tac(s),	3	Univariable	No effect on GR.
Hornick et al. [112]	1997	534	A, B, DR, A+DR, A+B+DR	Serology/ DNA	CsA, Aza, Ste	3	Univariable	No effect on CAV.
Kaczmarek et al. [113]	2006	240	A+B+DR, A, B,DR	Serology/ DNA	CsA, Aza, MMF, Tac, Ste	5.9	Multivariable (Cox)	HLA-DR associated PS. No effect on CAV.
Keogh et al. [115]	1995	183	A, B, DR	Serology/ DNA	CsA, Aza, Ste	4	Univariable	HLA-A, -B, or -DR associated with GR.
Kerman et al. [116]	1994	448	A+B, DR	Serology	CsA, Aza, Ste	5	Univariable	HLA-A+B or -DR associated with GR. HLA-A+B associated with CAV (inverse relationship).
Khagani et al. [114]	1989	353	A, B, DR, DQ, DRW52/53	Serology	CsA, Aza.	2	Univariable	HLA-DR associated with GS.
Kirklin et al. [117]	1992	229	A+B+DR	Serology	CsA, Aza, Ste	10	Univariable	Associated with GR.
Laufer et al. [118]	1989	43	A, B, DR, B+DR, A+B+DR	Serology	CsA, Aza, Ste	0.5	Multivariable (logistic regression)	HLA-B+DR associated with GR.
Leivestad et al. [119]	1996	208	A, B, DR	Serology/ DNA	CsA, Aza, Ste	5	Multivariable (Cox)	HLA-DR associated with GR.
Ouwehand et al. [120]	1994	118	A, B, DR, B+DR	Serology	CsA, Ste	0.5	Univariable	HLA-B+DR associated with GR.
Pfeffer et al. [107]	1988	37	A, B, DR	Serology	CsA, Aza, Ste	0.3	Univariable	HLA-DR associated with GR.
Pollack et al. [121]	1990	113	A+B+DR	Serology	CsA/OKT3, Aza, Ste	5.5	Univariable	HLA-A+B+DR associated with GR. No effect on CAV and PS.
Radovancevic et al. [122]	1991	167	A+B+DR, A, B, DR	Serology	CsA, Aza, Ste	2.9	Univariable	HLA-A or total HLA mm associated with CAV (inverse relationship).
Raffoux et al. [123]	1987	266	A+B, A+B+DR, DR	Serology	CsA, Aza, Ste	2	Univariable	HLA-A+B or HLA-A+B+DR associated with GS.
Rementeria et al. [124]	1997	165	A+B+DR	-	-	0.5	Univariable	Associated with GR.
Shakin-Eshleman et al. [125]	1990	82	A, B, DR, A+B, A+DR, A+B+DR, B+DR, Bw4/6, Bw4/6+DR, DR52/53	Serology	CsA, Aza, Ste	1	Univariable	No effect on GR and PS.
Sheldon et al. [126]	1992	127	B+DR, DR	Serology/ DNA	-	5	Univariable	No effect on GR and PS.
Sheldon et al. [127]	1994	165	A, B, DR	Serology/ DNA	CsA, Aza, Ste	6	Univariable	HLA-B or -DR associated with GR.
Sheldon et al. [128]	1999	261	A, B, DR, A+B, A+B+DR	Serology/ DNA	CsA, Aza, Ste	8	Multivariable (Cox)	HLA-DR associated with GR. HLA-A+B or HLA-DR associated with GS.
Smith et al. [129]	1995	1,135	A, B, DR	Serology/ DNA	-	10	Univariable	HLA-DR associated with GR and GS.
Stempfle et al. [130]	1995	24	A+B+DR	Serology/ DNA	CsA, Aza, Ste	-	Univariable	Associated with CAV. No effect on GR.

Suberbielle et al. [131]	1994	202	A+B+DR, DR, A+B	Serology	CsA, Aza, Ste, ATG	1	Univariable	No effect on GR and GS.
Taylor et al. [132]	1997	477	A, A+B, A+B+DR	Serology/ DNA	CsA, Aza, Ste	5	Multivariable (Cox)	HLA-A associated with GS (inverse relationship).
Tenderich et al. [133]	2007	923	A, B, C, DQ	Serology	CsA, Aza, Tac, MMF, Ste	10	Multivariable (Cox)	No effect on PS.
Yacoub et al. [134]	1987	204	A, B, Bw4/6, DR, DQ, DRw52/w53, B+DR, B+DRw52/w53	Serology	CsA, Aza	2	Univariable	HLA-DR associated with GS.
Zerbe et al. [147]	1988	242	A, B, DR	Serology	-	-	Univariable	Associated with GR.
Zerbe et al. [135]	1991	413	A+B+DR+DQ	Serology	CsA, Aza, Ste	0.3	Multivariable (Cox)	Associated with GR.

* HLA A, B, DR: each locus was analyzed separately, 0–2 mm. HLA A+B: A and B loci were analyzed together, 0–4 mm. HLA B+DR: B and DR loci were analyzed together, 0–4 mm. HLA A+B+DR: all loci were analyzed together, 0–6 mm.

CAV: cardiac allograft vasculopathy. Cox: Cox proportional hazards regression. HLA: human leukocyte antigen. mm: mismatch. GR: graft rejection. GS: graft survival. PS: patient survival. CsA: cyclosporine. Aza: azathioprine. A.L.G: antilymphocyte globulin. Ste: Steroids. ATG: antithymocyte globulin.OKT3: orthoclone OKT3. Tac, tacrolimus. Sir: Sirolimus. s: small proportion of patients.

Graft rejection

Jarcho et al has published the only multicenter study with graft rejection as outcome. [137] This study included 1,190 patients from 27 institutions participating in the Cardiac Transplant Research Database. After multivariable adjustment, the number of HLA-A, -B, and -DR mismatches remained a significant and independent risk factor for time to first rejection (P=0.013), but not in black recipients. Up to two mismatches was associated with a 54 % freedom from rejection at 1 year, as opposed to 36 % for more than two mismatches (P=0.02). It was also found that the number of HLA-DR mismatches were associated with cumulative rejection frequency in the first year after transplantation (P=0.04). The majority of the single-center studies, (25/33) found that the degree of HLA mismatch was significantly associated with graft rejection.

Cardiac allograft vasculopathy

So far, there is no multicenter study published that have evaluated the relationship between HLA mismatch and CAV. Four out of eleven of the single-center studies, found that HLA mismatch has a significant effect on CAV. The degree of atherosclerosis or luminal narrowing to make a diagnosis of CAV was specified only in a small number of studies.

Graft survival

Three multicenter studies by Opelz and co-workers have been published that study a possible association between HLA matching and graft survival. The first report, on 2,000 patients, found a significant correlation between HLA-B, DR matching and graft survival at 1 year (88% for < 2 mismatches with HLA-B, DR vs. 78 % for $\geq$ 2 mismatches; P=0.05) [139]. The second study by Opelz et al, found that HLA compatibility was strongly correlated to three-year rate of graft survival. Graft survival decreased from 83% for the 128 patients with no mismatches or only one mismatch to 76% for the 439 patients with two mismatches, and to 71% for the 7,764 patients with three to six mismatches (P<0.001). This correlation remained significant after multivariable adjustment (P= 0.005) [141]. When mismatches at each HLA loci (A, B, and DR) was investigated separately the association with graft survival was not as clear, remaining significant only for HLA-DR. The third study was a study on black recipients only, and the 103 patients investigated did not show a significant effect of HLA mismatch on graft failure[140]. Thompson et al. [144] observed in their multivariable analysis of 1,927 cardiac transplants a clear effect of HLA-DR matching on 1- and 3-year graft survival. Thompson et al. [145], followed this up with a survey of 14,535 heart transplant recipients from the United Network of Organ Sharing Transplant Registry, and observed a beneficial effect of HLA-A, -B, and -DR compatibility on 3-year graft survival. Again mismatch in HLA-DR antigens was the most strongly correlated with 1- and 3-year graft survival. In

contrast, the multicenter study by Jarcho et al. [137] did not show any significant association despite the probability of rejection-related death or re-transplantation by 2 years being 0% with no, one, or two HLA mismatches and 5% with three to six mismatches (P=0.14). Poli et al performed a small multicenter study. [88] involving 358 heart transplant patients and could not find a relationship between HLA locus mismatch and graft survival either. Of the single-center studies, the majority (9/11) found a significant correlation between HLA mismatch and graft failure.

Patient survival

Hosenpud et al. performed a multicenter analysis of 10,752 heart transplants from the UNOS Registry, [136] and noted that mortality risk at 3 years was reduced as HLA compatibility increased (1 or 2 matches: RR=0.83; 3 matches: RR=0.67; 4–6 matches: RR=0.59; $P \leq 0.01$). The primary benefit was in HLA-A and -DR loci (RR=0.87 and 0.79, respectively; P<0.001). A small multicenter study by Valeri et al. [146] concluded that HLA-B and -DR matching had a positive effect on 1- and 3-year survival in the 92 patients analyzed. One-year survival for heart transplants that shared two or more HLA-B or -DR antigens was 100% as compared to 87.5% for heart transplants that shared one or no HLA-B or -DR antigens. At 3-years, the corresponding figures were 100 % and 50%, respectively. Mascaretti [138], Park [142], and Poli [143] , on the other hand, reported collectively on 1,165 heart transplant recipients in multicenter studies, without finding any significant correlation between HLA matching and patient survival. Of the single-center studies, only a minority (4/10) could report a significant effect of HLA mismatch on patient survival.

Effects of mismatch at the HLA-DR locus (0–1 vs. 2) on outcome, meta-analysis

Six of the studies had graft survival at 1 year as outcome (Fig. 2A). All trials except one (Sheldon[128]) were favorable for less mismatch, and pooled data showed that less mismatch increased graft survival significantly with RR of 1.09 (95% CI: 1.01–1.19; P=0.04). There was heterogeneity between study estimates (I^2=63%). To reduce heterogeneity, we restricted the meta-analysis to studies that included heart transplantations performed until 1991 (4 studies) (I^2=0%). In the pooled estimates RR increased and became more significant in favour of less mismatch (RR : 1.19 (95% CI: 1.09–1.30; P<0.0001). Four studies reported data on patient survival at 1 year (Fig. 2B). Fewer mismatches at the HLA-DR locus did not lead to a significant increase in patient survival (pooled RR=1.04; CI: 0.96–1.13; P=0.33). Heterogeneity was low (I^2=9%). Four studies were found that had graft rejection at 1 year as outcome. Matching at the HLA-DR locus decreased significantly the incidence of graft rejection, with a pooled RR of 0.81 (CI: 0.66–0.99; P=0.04) and with little heterogeneity (I^2=31%; P=0.22). No meaningful analysis of HLA-A or -B, or of HLA-A, -B, and -DR together could be made, as there were three or less

studies that shared a common outcome, follow-up, HLA antigen, and number of mismatches.

Publication bias

The funnel plots for graft survival, patient survival, and graft rejection showed adequate symmetry, suggesting minimal publication bias. However, the number of studies included was less than ten, making the funnel plots difficult to interpret.

Figure 2 Forest plots showing influence of matching (0–1 vs. 2 human leukocyte antigen-DR mismatches) on outcome. (a) 1-year graft survival; (b) 1-year patient survival; (c) incidence of graft rejection at 1 year. Data across studies were pooled using random-effects model. Risk ratios are shown with 95% confidence intervals.

Study II - Analysis of the influence of HLA-A matching relative to HLA-B & DR matching on heart transplant outcomes.

25,583 patients were analyzed in this study. Median follow-up time was 6.0 (range 0-23.6) years. The mean recipient and donor age was 51±11 and 33±12 years, respectively, and 20 % of the recipients and 31 % of the donors were women. Non-ischemic cardiomyopathy (48%) and ischemic cardiomyopathy (45%) were the most common diagnoses. After 10 years the overall patient survival rate was 56% and after 20 years 25%. A total of 10,233 patients (40%) died during follow-up. The majority of the patients died from major adverse cardiovascular event (n = 2,337), graft failure (n = 1,762), malignancy (n = 1,710), and infection (n = 1,598).

We divided the study population into two groups; patients with HLA-A compatible (no HLA-A mismatches) and HLA-A incompatible (1-2 HLA-A mismatches) grafts. The two groups differed significantly in diagnosis, use of amiodarone, use of inotropic support and medical condition at transplant. The proportion of patients with donor-recipient sex match was higher in the HLA-A compatible group. Other demographic data, blood group, blood group match, previous blood transfusion, comorbidity, hemodynamic and laboratory status were similar in the two groups.

There were differences between the groups regarding immunotherapy given at discharge from the hospital. HLA-A incompatible grafts were given Tacrolimus (TAC), mycophenolate mofetil (MMF), and steroids more frequently, whereas. the HLA-A compatible group were treated to a higher extent with cyclosporine (CYA) and azathioprine (AZA). Furthermore, basiliximab use was more common in the HLA-A incompatible group whereas steroids as a form of induction treatment more common in the HLA-A compatible group. During the follow-up differences in immunotherapy narrowed and at 15 years post-transplant the differences had been completely erased between the groups. At one year post-transplant a greater proportion of patients in the HLA-A incompatible group received steroids for the treatment of rejection. But when we looked at 5, 10 and 15 years post-transplant, there was no difference between the groups in the proportion of patients receiving steroids for rejection treatment.

We started by comparing HLA-A compatibility versus HLA-A incompatibility with regard to all-cause mortality for the entire cohort. One can see the result in Figure 3. We found no significant difference in survival between the groups over the entire follow-up period (P = 0,187, Log-rank test). However, the survival curves indicate that HLA-compatibility has a lower survival toward the end of the follow-up. Log rank test showed an almost significant survival difference (P = 0.064) during the later time interval (> 15 years post-transplant).

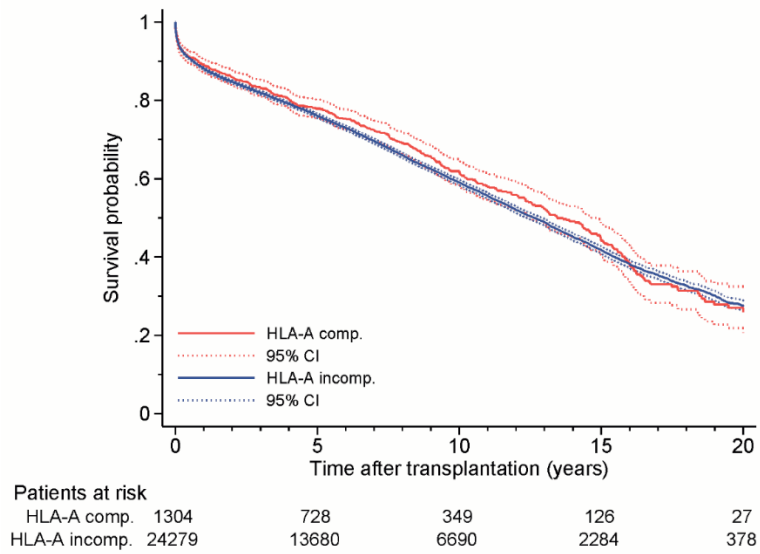


Figure 3.
Kaplan-Meier survival curves according to HLA-A compatible grafts versus HLA-A incompatible grafts in all patients for all-cause mortality (P = 0.187, Log-rank test). The red solid line shows the observed cumulative survival and red dotted lines show the 95% confidence interval (estimated with Kaplan- Meier survival function) in the HLA-A compatible cohort. The blue solid line shows survival and blue dotted lines show the 95% confidence interval (estimated with Kaplan- Meier survival function) for transplanted patients in the HLA-A incompatible cohort.

We next compared the two groups but this time in different subgroups. These subgroups were; recipients with HLA-B incompatible or compatible, HLA-DR incompatible or compatible and HLA-B and DR incompatible or compatible grafts. In the subgroups with incompatible grafts HLA-A compatibility had lower survival than HLA-incompatible grafts in the later time periods post-transplantation. The survival difference was smallest in HLA-B incompatible sub-group (P = 0.027, Log-rank test) but became more pronounced in HLA-DR incompatible grafts (P = 0.007, Log-rank test) and even more so in HLA-B and DR incompatible grafts (P = 0.002, Log-rank test) (Figures 4 A, C and E). This observation was not found in compatible HLA-B, DR or –B,-DR grafts (Figures 4 B, D and F.)

We next performed a multivariable analysis. This analysis showed that among those who survived to 15 years after transplant, those with HLA-A compatible grafts had higher mortality compared with those with HLA-A incompatible grafts in the subgroup of patients that had HLA-DR incompatible grafts (HR 1.59, 95% CI 1.11–2.28, P = 0.012, Cox proportional hazard test). This was also true for the subgroup of patients with HLA- B,DR incompatible grafts (HR 1.69, 95% CI 1.17–2.43, P = 0.005, Cox proportional hazard test) (Table 3A). Stratification of recipients by number of HLA-A mismatches further reinforced these results, demonstrating an association between fewer mismatches and higher mortality starting 15 years post-

transplant. Figure 5 shows this trend in HLA-B,DR incompatible grafts. These results were reflected in the adjusted HRs associated with having 2 HLA-A mismatches and 1 HLA-A mismatch, respectively, compared with recipients with 0 HLA-A mismatch.

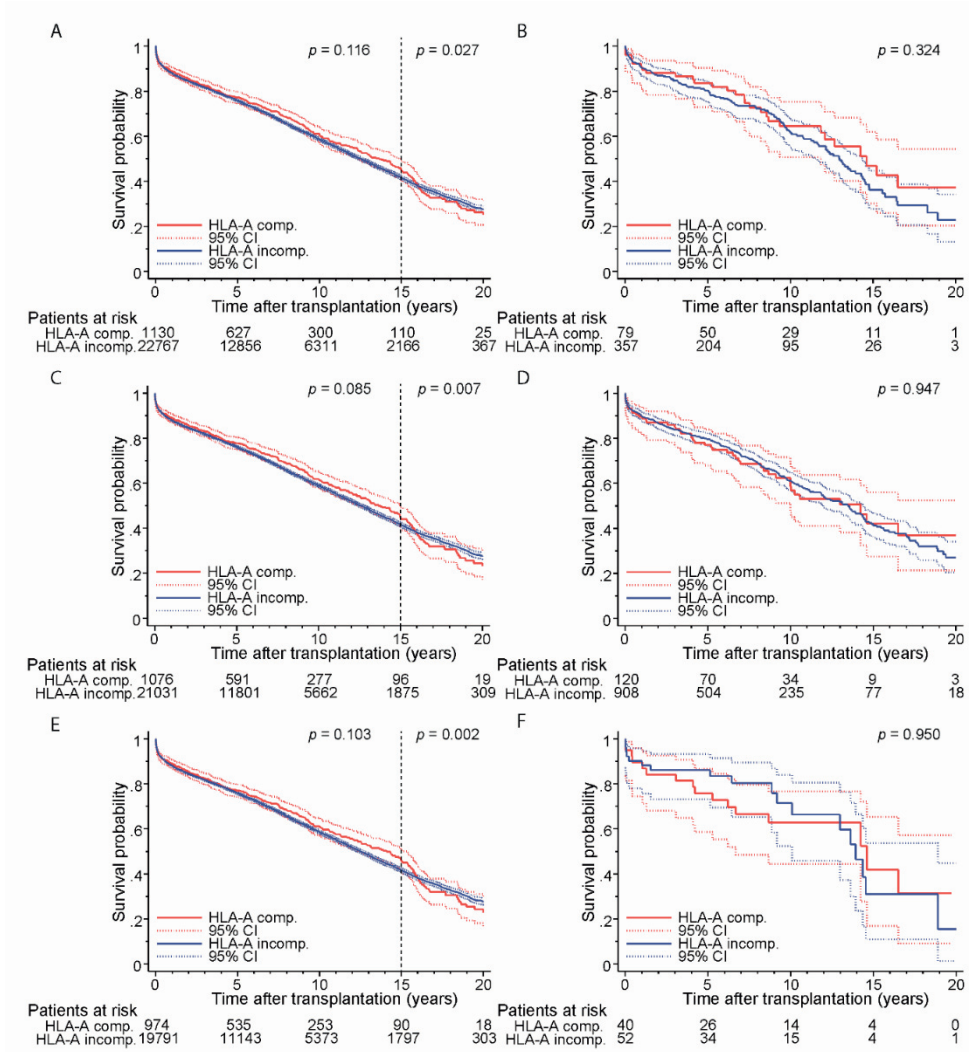


Figure 4 A-F.
Kaplan-Meier survival curves according to HLA-A compatible grafts versus HLA-A incompatible grafts for all cause-mortality in **A.** HLA-B incompatible transplants, **B.** HLA-B compatible transplants, **C.** HLA-DR incompatible transplants, **D.** HLA-DR compatible transplants, **E.** HLA-B & DR incompatible transplants and **F.** HLA-B and DR compatible transplants. The red solid line shows the observed cumulative survival and red dotted lines show the 95% confidence interval (estimated with Kaplan- Meier survival function) in the HLA-A compatible cohort. The blue solid line shows survival and blue dotted lines show the 95% confidence interval (estimated with Kaplan- Meier survival function) for transplanted patients in the HLA-A incompatible cohort. Statistical test: Log-rank test.

Table 3A.

Univariable and Multivariable Cox proportional hazards regression analysis in the later time interval (> 15 years post-transplant) affecting all-cause mortality for HLA-A compatbility vs incompatiblity in different HLA combinations.

			Univariable		Multivariable	
		n	Hazard ratio	P	Hazard ratio	P
HLA-A						
Incomp		24,279	1.00		1.00	
Comp		1,304	1.36 (0.98-1.90)	0.066	1.32 (0.95-1.84)	0.102
HLA-A	HLA-B					
Incomp	Incomp	22,767	1.00		1.00	
Comp	Incomp	1,130	1.46 (1.04-2.05)	0.028	1.41 (1.00-1.98)	0.052
HLA-A	HLA-DR					
Incomp	Incomp	21,031	1.00		1.00	
Comp	Incomp	1,076	1.64 (1.14-2.36)	0.007	1.59 (1.11-2.28)	0.012
HLA-A	HLA-B & -DR					
Incomp	Incomp	19,791	1.00		1.00	
Comp	Incomp	974	1.75 (1.22-2.51)	0.002	1.69 (1.17-2.43)	0.005

Values in parenthesis are 95 per cent confidence intervals. Incomp, incompatible; Comp, compatible. n, number of patients. Adjusted for transplant era, donor age (year), recipient work for income, recipient diabetes, recipient age (year), albumin level (g/L), donor hepatitis C virus status, recipient weight (kg), recipient infection within 2 weeks, recipient previous transfusion, recipient on ventilator, recipient obstructive pulmonary disease, donor sex, recipient hypertension, maintenance therapy; mycophenolate mofetil, maintenance therapy; corticosteroids, maintenance therapy; azathioprine, induction therapy; OKT3.

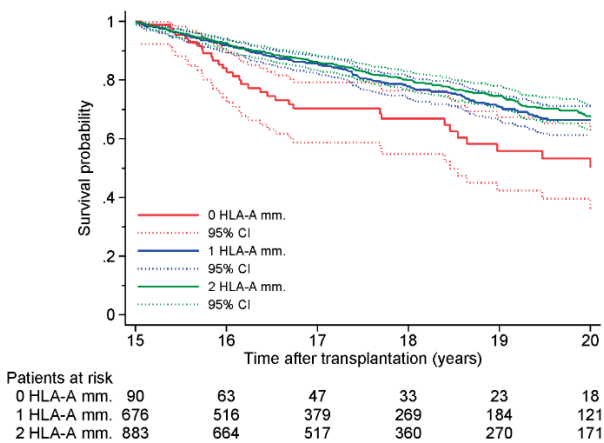


Figure 5.

Kaplan-Meier survival curves by number of HLA-A mismatches in the 15 – 20 years post-transplant time interval in HLA-B, DR incompatible grafts for all-cause mortality. The red solid line shows the observed cumulative survival and red dotted lines show the 95% confidence interval (estimated with Kaplan- Meier survival function) in the HLA-A cohort with zero mismatch. The blue solid line shows survival and blue dotted lines show the 95% confidence interval (estimated with Kaplan- Meier survival function) for transplanted patients in the HLA-A cohort with 1 mismatch. The green solid line shows survival and green dotted lines show the 95% confidence interval (estimated with Kaplan- Meier survival function) for transplanted patients in the HLA-A cohort with 2 mismatches. Statistical test: Log-rank test. mm; mismatches.

We performed the same uni- and multivariable analyzes for the secondary endpoints, i.e. cause of death. There was a trend for lower survival in the later post-transplant eras for HLA-compatibility for cardiovascular-, infection-, and malignancy-related deaths but not for graft failure-related deaths. As cardiovascular disease could be a manifestation of chronic rejection and infection and malignancy related to immunosuppressive agents given for chronic rejection, we studied the combined deaths caused by chronic rejection, cardiovascular disease, infection and malignancy. HLA-compatible grafts had lower survival in the later post-transplant time eras (P = 0.044, Log-rank test). Table 3B shows the results of the multivariable analysis for this outcome. Noteworthy, HR increased from 1.69 to 1.91 (95% CI 1.22 – 3.01, P = 0.005) in HLA-B, DR incompatible grafts. However, for the entire cohort the hazard ratio was not significant (P = 0.063). Thus in multivariable analysis the largest compromise in survival for HLA-A compatibility (vs HLA-incompatibility) was for chronic rejection (including cardiovascular-, infection- and malignancy-related deaths) in HLA-B and DR incompatible grafts, which is also shown in Figure 6.

Table 3B.

Univariable and Multivariable Cox proportional hazards regression analysis in the later time interval (> 15 years post-transplant) affecting mortality caused by chronic rejection, cardiovascular disease, infection or malignancy for HLA-A compatibility vs incompatiblity in different HLA combinations.

			Univariable		Multivariable	
		n	Hazard ratio	P	Hazard ratio	P
HLA-A						
Incomp		24,279	1.00		1.00	
Comp		1,304	1.52 (1.01-2.28)	0.046	1.48 (0.98-2.23)	0.063
HLA-A	HLA-B					
Incomp	Incomp	22,767	1.00		1.00	
Comp	Incomp	1,130	1.68 (1.11-2.55)	0.015	1.62 (1.07-2.47)	0.024
HLA-A	HLA-DR					
Incomp	Incomp	21,031	1.00		1.00	
Comp	Incomp	1,076	1.82 (1.16-2.84)	0.009	1.76 (1.13-2.77)	0.013
HLA-A	HLA-B & -DR					
Incomp	Incomp	19,791	1.00		1.00	
Comp	Incomp	974	1.98 (1.26-3.10)	0.003	1.91 (1.22-3.01)	0.005

Values in parenthesis are 95 per cent confidence intervals. Incomp, incompatible; Comp, compatible. n, number of patients. Adjusted for transplant era, donor age (year), recipient working for income, recipient age (year), albumin level (g/L), recipient weight (kg), donor hepatitis C virus status, recipient diabetes, recipient previous transfusion, recipient on ventilator, donor sex, recipient stroke, donor cytomegalovirus status, maintenance therapy; corticosteroids, maintenance therapy; cyclosporine, maintenance therapy; mycophenolate mofetil, induction therapy; anti-thymocyte globulin.

Similar analyses as for HLA-A match versus HLA-A mismatch was made for HLA-B match vs mismatch and HLA-DR match vs mismatch for the entire cohort and in different HLA subgroups. There was a trend toward lower survival seen in the survival curves for HLA-B compatibility vs HLA-B incompatibility in later post-transplant eras, but this could not be confirmed in uni or multivariable analyses.

Finally, we examined the effects of HLA-A matching on graft loss, defined as death or repeat transplantation (n = 575). The results remained essentially unchanged.

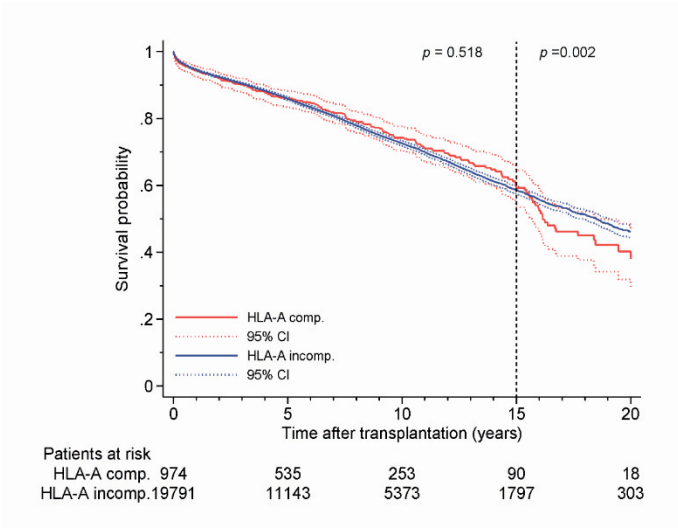


Figure 6.
Kaplan-Meier survival curves according to HLA-A compatible grafts versus HLA-A incompatible grafts in HLA B & DR incompatible transplants for mortality caused by chronic rejection, cardiovascular disease, infection or malignancy. The red solid line shows the observed cumulative survival and red dotted lines show the 95% confidence interval (estimated with Kaplan- Meier survival function) in the HLA-A compatible cohort. The blue solid line shows survival and blue dotted lines show the 95% confidence interval (estimated with Kaplan- Meier survival function) for transplanted patients in the HLA-A incompatible cohort. Statistical test: Log-rank test.

Study III - Induction with Anti-Thymocyte Globulin in Heart Transplantation is Associated with Better Long-term Survival compared to Basiliximab

This was a study on the 9,282 adult heart transplant patients (corresponding to 9,324 transplants) who received induction with either ATG (n = 6,144 transplants) or BAS (n = 3,180 transplants) between 2000 and June 2011. The median follow-up time was 3.0 (range 0-12) years. The mean age of the recipients was 52±12.2 years, and 23 % of the recipients were women.

Recipients that used BAS and ATG did not differ in recipient gender, recipient height, recipient blood group, donor ischemic time or mechanical ventilator support. Patients receiving BAS were slightly older than the those receiving ATG, but the age of the donors was higher in the ATG group. Panel reactive antibody (class 1 and class 2) was higher in the ATG group. More patients in the BAS group were in intensive care unit (ICU) pre-transplant. These patients also had non-ischemic cardiomyopathy to a lesser extent.

Patients in the ATG group received tacrolimus (TAC) and mycophenolate mofetil (MMF) less frequently but cyclosporine (CYA) or azathioprine (AZA) more frequently. Treatment for rejection during the early postoperative period (until discharge) using steroids was more common in the BAS group compared with the ATG group.

For the entire study group the overall 30-day mortality was 3 % and one-year mortality 9 %. A total of 2,257 (24 %) patients died during follow-up. Up to one year after transplantation patients treated with BAS had similar estimated survival compared with the ATG group (90 % versus 91 %, p= 0.858). This is illustrated in Figure 7. As also apparent from this survival curve up to five years and 10 years after transplantation, the use of BAS for induction was associated with poorer long-term survival compared to ATG (77 % versus 82 % at 5 years, p = 0.005, and 64% versus 67% at 10 years, p = 0.007, respectively). The increased mortality associated with BAS use remained significant after multivariable adjustment, incorporating 19 significant independent covariates, (HR, 1.22; 95 % CI, 1.09 - 1.37; p < 0.001), Table 4.

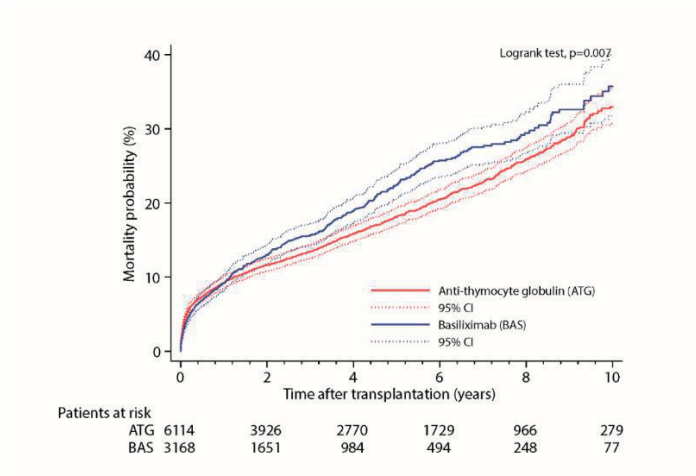


Figure 7.

Comparison of all-cause mortality probability between the Basiliximab (BAS) and Anti-Thymocyte Globulin (ATG) groups. p = 0.007 (Log-rank test).

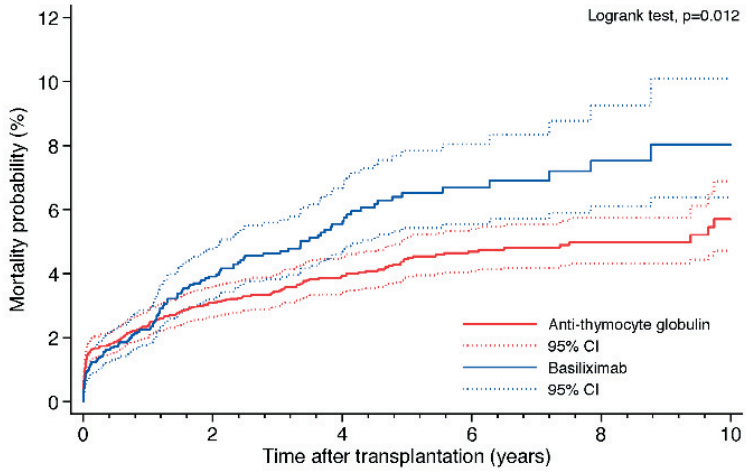
Table 4.
Cox multivariate logistic regression analysis

Variables	HR	95% CI	p-value
BAS versus ATG unadjusted	1.15	1.04 – 1.28	0.007
BAS versus ATG adjusted for age and gender	1.15	1.04 – 1.28	0.008
BAS versus ATG adjusted for 19 covariates*	**1.22**	**1.09 – 1.37**	**<0.001**

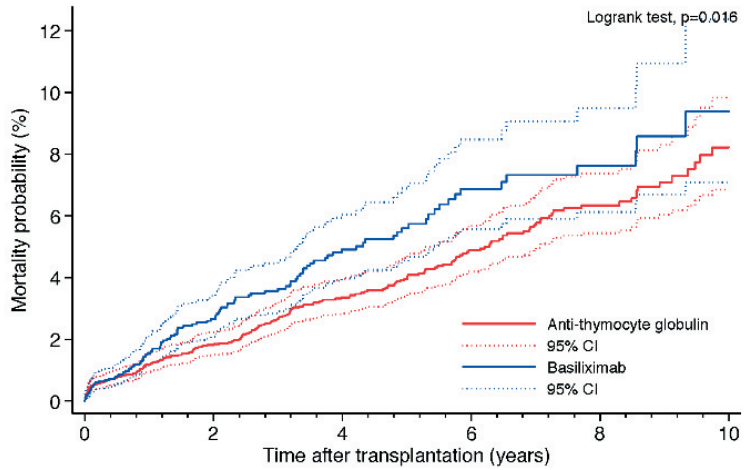
ATG, anti-thymocyte globulin; BAS, Basiliximab; HR, Hazard ratio; 95% CI, 95 per cent confidence interval.
*Adjusted for Diagnosis, Oxygen consumption at exercise, Hypertension, Creatinine at transplant, Medical condition at transplant, Pulmonary vascular resistance, Use of Mechanical ventilation, Use of Extra-Corporeal Membrane Oxygenation, Ventricular assist device type, Age (donor), Hepatitis C virus (donor), History of smoking (donor), Ischemic time (min), Induction drug- Daclizumab, Maintenance drugs – Cyclosporine A, Tacrolimus, Mycophenolate Mofetil, Azathioprine, and Corticosteroids.

We examined the cumulative incidence of death, based on specific causes of death in the BAS and ATG groups, censoring other causes of death. Figure 8a-d shows that patients in the BAS group had higher risk of death due to graft failure ($p = 0.012$), cardiovascular event ($p = 0.016$) and infection ($p = 0.037$), but not death due to malignancy ($p = 0.618$). Patients in the ATG group appeared to have higher risk of malignancy-related deaths in later time eras post-transplantation (Figure 8d). A nearly significant trend toward higher malignancy-related deaths in the ATG group was found after 7 years post-transplantation. ($p = 0.070$). These findings were further confirmed in a multivariable Cox regression model (adjusting for the same covariates as in the main Cox regression model) where BAS was associated with higher risk of death due to graft failure (HR 1.53; 95% CI, 1.21–1.94; $p < 0.001$), cardiovascular event (HR1.35; 95% CI, 1.05–1.73; $p = 0.018$) and infection (HR 1.34; 95% CI, 1.05–1.73; $p = 0.021$), but not death due to malignancy (HR 0.89; 95% CI, 0.60–1.31; $p = 0.547$).

Cause of death: Graft failure



Cause of death: Cardiovascular



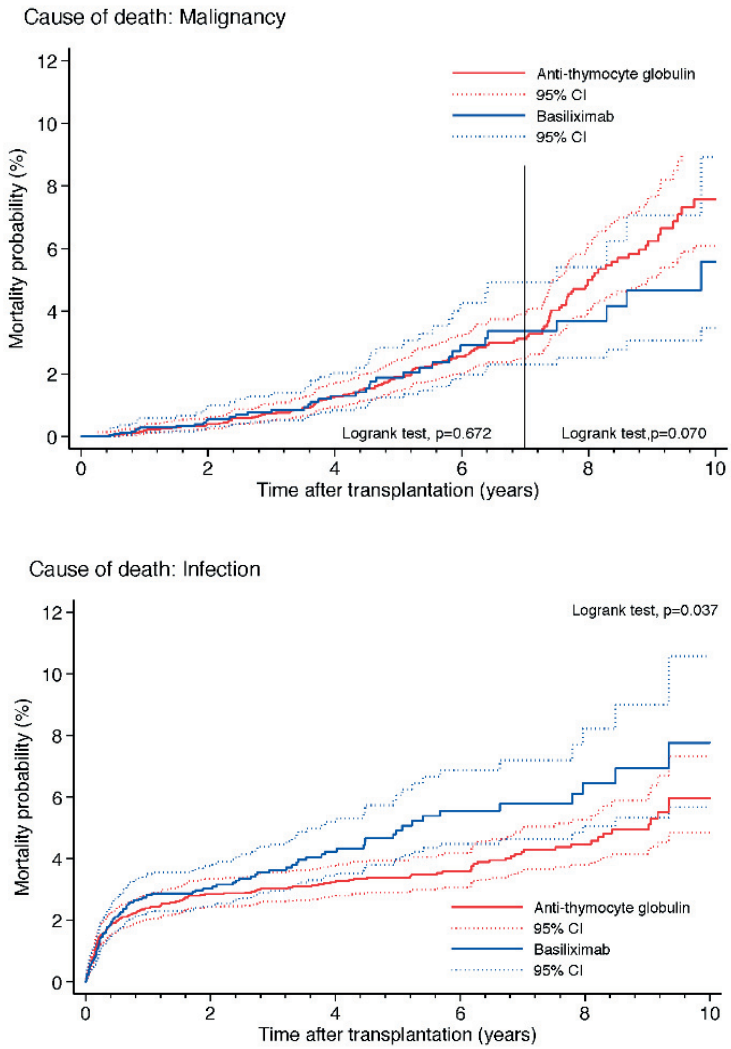
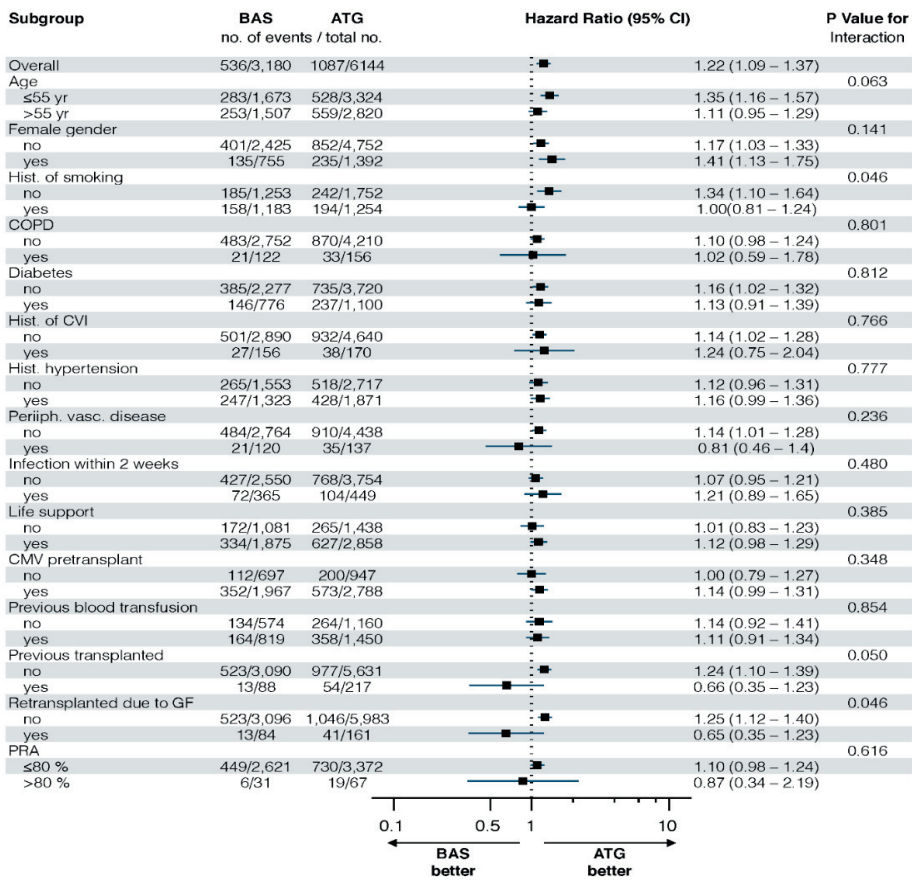


Figure 8.
Mortality probability curves by treatment group: **a** graft failure-related death, **b** cardiovascular-related death, **c** infection-related death, **d** malignancy-related death. **a** $p = 0.012$, **b** $p = 0.016$, **c** $p = 0.037$, **d** $p = 0.672$ (< 7 years post-transplant), $p = 0.070$ (> 7 years post-transplant). (Log-rank test). Graft failure; primary failure, rejection – hyperacute, acute or chronic, graft infection, recurrent disease or non-specific. Cardiovascular; myocardial infarction, cardiac arrest, arterial embolism, ventricular failure, coronary artery disease, atherosclerosis, rhythm disorder, carditis, aortic aneurysm, cardiogenic shock or other.

Subgroup analyses with interaction testing were performed to determine whether the increase in the HR for death (adjusting for the same covariates as in the main Cox regression model) after induction treatment with BAS was consistent across 20 important subgroups. No significant interactions were observed except for in four

subgroups. As shown in figure 9a, there was an interaction between the use of induction agent in patients who were re-transplanted due to cardiac graft failure (p = 0.046) and previously transplanted (previous kidney, liver, pancreas, pancreas islet cells, heart, lung, intestine and/or bone marrow transplant, p = 0.050), respectively, suggesting that ATG was superior in recipients without, but that ATG and BAS were no different in recipients with re-transplant due to graft failure or previous transplant. As shown in Figure 9b, there were interactions suggesting that ATG was considerably superior in the minority of patients who did not receive corticosteroids and that while ATG was superior regardless of MMF use, it was more superior in those who did not.



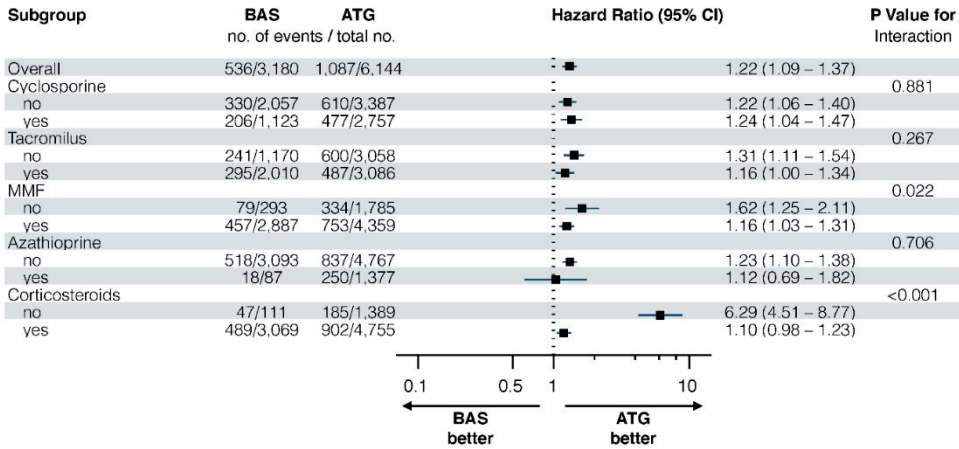


Figure 9a and b.
Subgroup Analyses of the Primary End Point of Death from Any Cause. Squares represent the adjusted hazard ratio (HR) for the treatment effect, Basiliximab (BAS) versus Anti-Thymocyte Globulin (ATG) for different subgroups. The lines represent the 95% confidence interval. The P value for interaction represents the likelihood of an interaction between the subgroup variable and the treatment effect. The overall effect included no interaction terms. The adjusted HR was calculated using the same covariate as presented in Table 4. COPD, chronic obstructive pulmonary disease. CVI, cerebrovascular insult. CMV, cytomegalovirus. PRA, panel-reactive antibodies. MMF, Mycophenolate Mofetil. Retransplantation due to GF, Retransplantation due to graft failure. Previous transplantation, previously kidney, liver, pancreas, pancreas islet cells, heart, lung, intestine or/and bone marrow transplantation.

Study IV – Comparison of Basiliximab and Anti-Thymocyte Globulin as Induction Therapy in Pediatric Heart Transplantation: a Survival Analysis.

Figure 10 shows use of the different induction drugs by transplantation year. We analyzed 2,311 pediatric heart transplants, corresponding to 2,275 patients. 699 transplants (685 patients) were given BAS and 1,612 transplants (1,590 patients) were given ATG. The median follow-up time was 2.7 (range 0-12) years. The mean age of the recipients was 6.9 ± 6.3 years and 48 % were female.

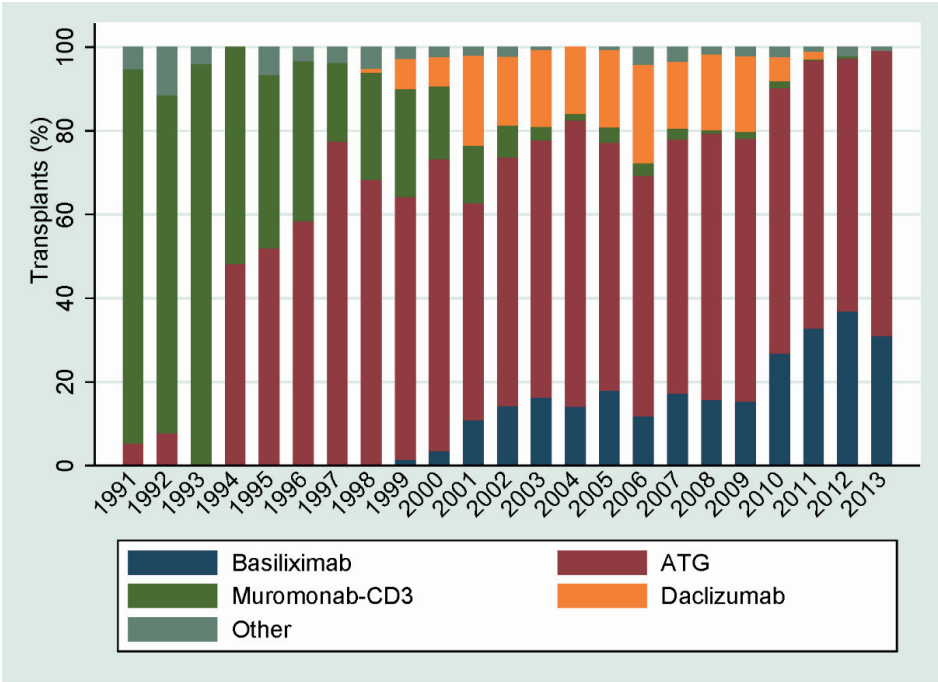


Figure 10.
Type of induction therapy for recipients with induction. Distribution is shown by year of transplantation, 1991-2013. ATG; equine anti-thymocyte globulin [Atgam], rabbit anti-thymocyte globulin [Thymoglobulin/Fresenius-ATG] or Nashville rabbit antithymocyte globulin/Nashville rabbit antithymocyte serum [NRATG/NRATS]. Other; cyclophosphamide (Cytoxan), methotrexate (Folex PFS, Mexate-AQ, Rheumatrex), alemtuzumab (Campath), rituximab (Rituxan).

Patients in the BAS group were older. Furthermore, recipient weight and height, recipient diagnosis and the proportion of patients in ECMO differed significantly between the groups. Panel reactive antibody class I and II was higher in the ATG group. The donors were older in the BAS group. The groups differed also with respect to donor weight and height, the proportion of donors with blood group A and O and in the proportion of donors with hypertension.

Fewer patients in the ATG group had been given tacrolimus (TAC), mycophenolate mofetil (MMF) but they were more likely to have received cyclosporine (CYA) or azathioprine (AZA).

For the entire study group the overall 30-day mortality was 3.8 % (95 % CI, 3.1% – 4.6%) and one-year mortality 10.5 % (95 % CI, 9.3% – 11.8%). A total of 493 (21 %) patients died during the follow-up. As illustrated in Figure 11, patients treated with BAS had similar estimated survival compared with the ATG group at 30 days and at one year after transplantation (97 % versus 96%; $P = 0.545$, and 90 % versus 89 %; $P = 0.727$, respectively). However, at 5 years and 10 years after

transplantation, the use of BAS was associated with poorer long term survival (68 % versus 76 % at 5 years; $P<0.001$, and 49 % versus 65 % at 10 years; $P<0.001$, respectively). The poorer mortality associated with BAS use remained after multivariable adjustment (Table 5). Patients treated with BAS (versus ATG use) had an increased mortality risk of 27 % (HR of 1.27; 95% CI, 1.02-1.57; $P<0.030$). The multivariable model incorporated 11 significant independent covariates and two time-varying covariates.

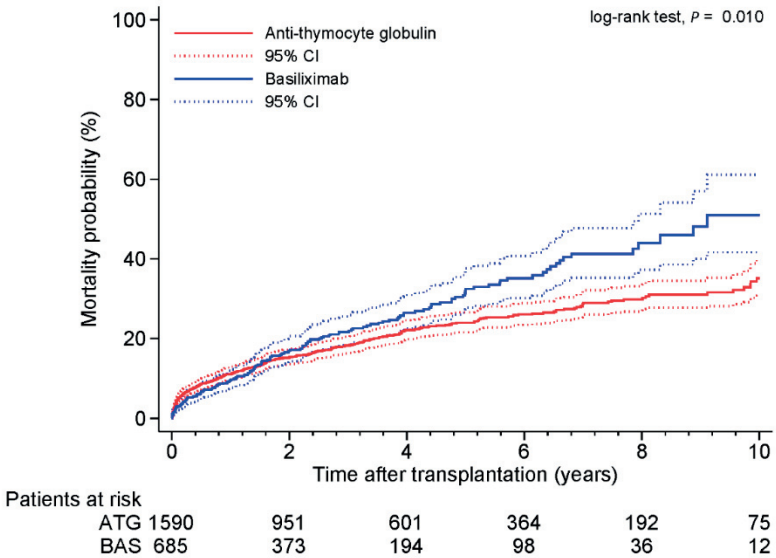


Figure 11.
Comparison of all-cause mortality probability between the basiliximab (BAS) and anti-thymocyte globulin (ATG) groups ($P = 0.010$, log-rank test).

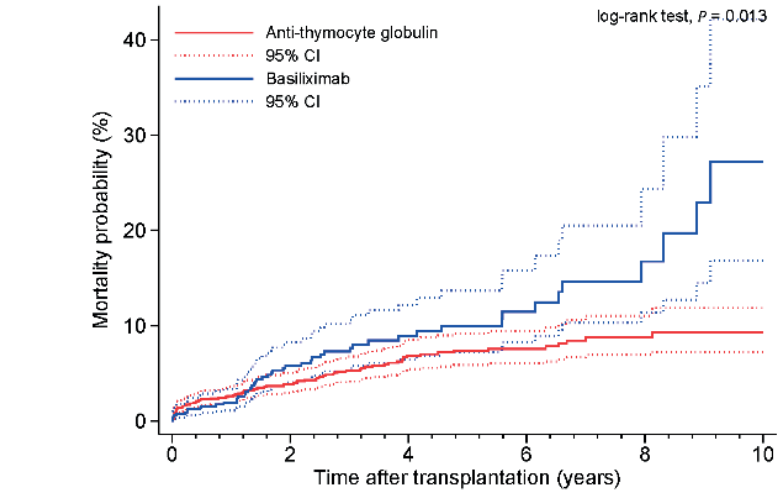
Table 5.
Cox multivariate logistic regression analysis (n=2,311)

Variables	HR	95% CI	*P Value*
BAS versus ATG unadjusted	1.27	1.06 – 1.55	0.011
BAS versus ATG adjusted for age and gender	1.23	1.01 – 1.49	0.035
BAS versus ATG adjusted for 11 covariates and time*	1.27	1.02 – 1.57	0.030

ATG, anti-thymocyte globulin; BAS, Basiliximab; HR, Hazard ratio; 95% CI, 95 per cent confidence interval. *Adjusted for previous cardiac surgery, pulmonary systolic artery pressure, infection within two weeks, recipient age, underlying diagnosis, panel reactive antibodies, recipient diabetes, recipient on dialysis, recipient on ventilator, maintenance drug tacrolimus and maintenance drug azathioprine. Time varying variables: recipient age and induction therapy.

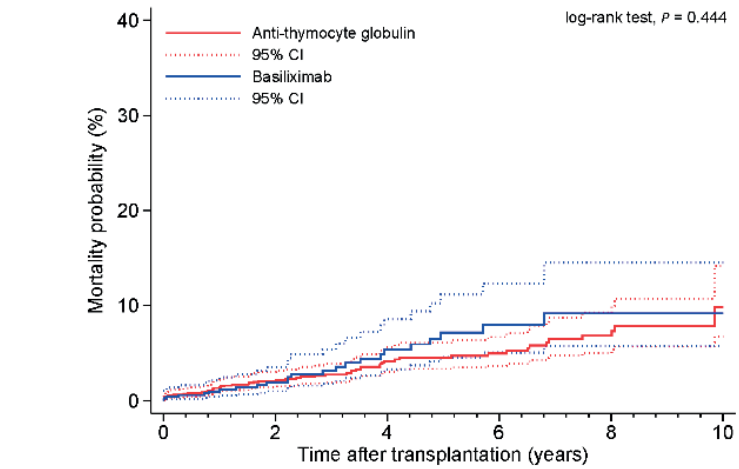
The BAS and ATG groups were compared to see if there were any differences with regard to the cause of death. Figure 12 a-d show the survival curves for the two groups for the outcomes graft failure-, cardiovascular-, infection-, and malignancy-related deaths, respectively. We observed that BAS use was associated with higher risk of death due to graft failure ($P = 0.013$), but not due to cardiovascular event ($P = 0.444$), infection ($P = 0.095$) or malignancy ($P = 0.392$).

Subgroup analyses with interaction testing were performed to determine whether the increase in the HR for death (adjusting for the same covariates as in the main Cox regression model) after induction treatment with BAS was consistent across 18 clinical important subgroups (Figure 13 a and b). No significant interactions were observed except for one subgroup. As shown in Figure 13b, patients treated with BAS who did not receive corticosteroids had more than double the risk for death compared with those who received corticosteroids. Furthermore, there was no interaction with any of the 11 UNOS geographic regions.



Patients at risk

ATG	1590	951	601	364	192	75
BAS	685	373	194	98	36	12



Patients at risk

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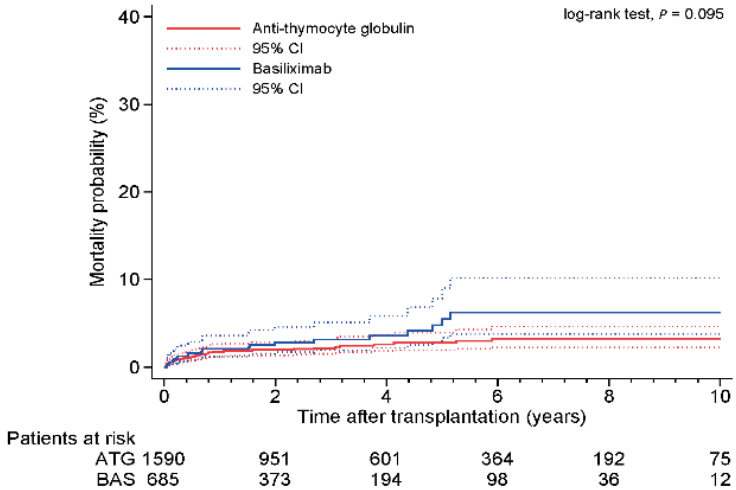


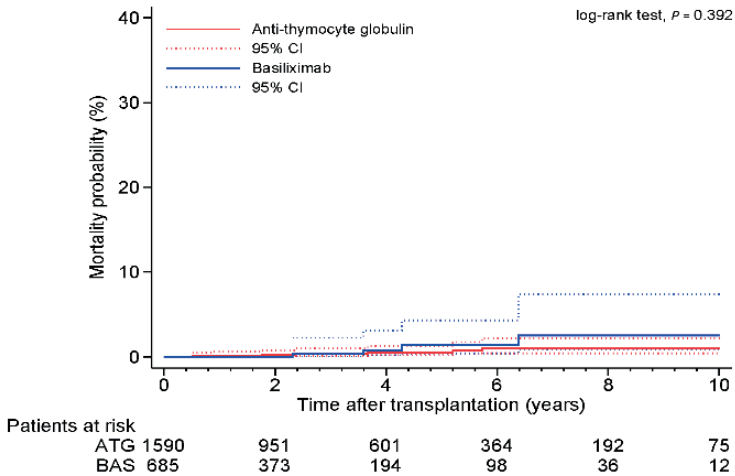
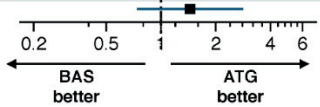


Figure 12.
Mortality probability curves by treatment group: **a** graft failure-related death (P = 0.013, log-rank test), **b** cardiovascular-related death (P = 0.444, log-rank test), **c** infection-related death (P = 0.095, log-rank test) and **d** malignancy-related death (P = 0.392, log-rank test).

Subgroup	BAS	ATG	Hazard Ratio (95% CI)	*P* Value for Interaction
	no. of events / total no.			
Overall	160/699	333/1,612	1.26 (1.02 – 1.56)	
Age				0.337
≤2 yr	45/201	154/728	1.54 (1.09 – 2.20)	
>2 yr	115/498	179/884	1.26 (0.97 – 1.63)	
Female gender				0.559
no	76/336	179/856	1.34 (1.00 – 1.79)	
yes	86/363	154/756	1.19 (0.89 – 1.59)	
Diabetes				0.400
no	133/634	323/1586	1.28 (1.03 – 1.60)	
yes	24/57	10/24	0.92(0.43 – 1.95)	
Infection within 2 weeks				0.064
no	106/524	242/1305	1.43 (1.12 – 1.82)	
yes	54/175	91/307	0.96 (0.66 – 1.38)	
Life support				0.643
no	89/330	112/527	1.29 (0.95 – 1.75)	
yes	71/369	221/1085	1.17 (0.88 – 1.56)	
CMV pretransplant				0.101
no	73/323	140/744	1.61(1.19 – 2.19)	
yes	59/274	133/608	1.13(0.81 – 1.57)	
Previous blood transfusion				0.180
no	21/47	32/141	2.08 (1.16 – 3.73)	
yes	53/235	191/831	1.33(0.95 – 1.85)	
Blood group match				0.969
no	32/159	66/348	1.28 (0.8 – 1.98)	
yes	128/540	267/1264	1.27 (1.00 – 1.60)	
Gender match				0.527
no	78/323	177/807	1.19 (0.89 – 1.58)	
yes	82/376	156/805	1.34(1.01 – 1.79)	
Previously transplanted				0.398
no	144/664	300/1501	1.25 (1.00 – 1.57)	
yes	15/34	32/109	1.66 (0.88 – 3.12)	
Retransplanted due to GF				0.905
no	152/673	304/1511	1.28 (1.02 – 1.60)	
yes	8/26	29/101	1.22 (0.55-2.69)	
PRA				0.413
≤80 %	116/543	238/1216	1.22 (0.96 – 1.56)	
>80 %	4/13	36/107	1.92 (0.68 – 5.50)	
VAD				0.774
no	135/575	266/1302	1.30 (1.03 – 1.64)	
yes	13/81	30/204	1.44 (0.74 – 2.83)	



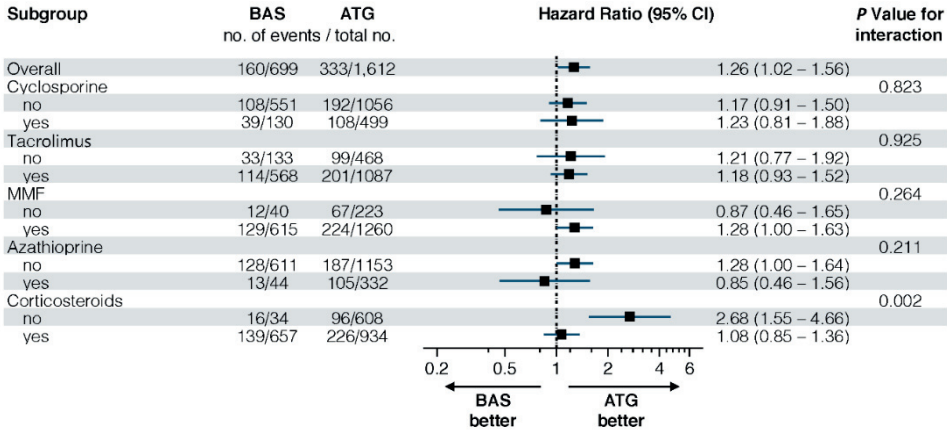


Figure 13a and b.
Subgroup analyses of the primary end point of death from any cause. Squares represent the adjusted hazard ratio (HR) for the treatment effect, basiliximab (BAS) versus anti-thymocyte globulin (ATG) for different subgroups. The lines represent the 95% confidence intervals. The *P* - value for interaction represents the likelihood of an interaction between the subgroup variable and the treatment effect. The overall effect included no interaction terms. The adjusted HR was calculated using the same covariate as presented in Table 5. CMV, cytomegalovirus; PRA, panel-reactive antibodies; VAD, ventricular assist device; GF, graft failure; previously transplanted, previously kidney, liver, pancreas, pancreas islet cells, heart, lung, intestine or/and bone marrow transplantation; MMF, mycophenolate mofetil.

Study V - Analysis of the influence of structurally based HLA mismatching on heart transplant outcomes

Study population

During the study period, 44,022 transplants underwent heart transplantation, corresponding to 43,186 recipients. Median follow-up time was 5.1 (range 0 - 25.9) years. The mean recipient and donor age was 51.7 ± 11.9 and 30.7 ± 12.2 years, respectively, and 23 % of the recipients and 29 % of the donors were women. The most common diagnoses were non-ischemic cardiomyopathy and ischemic cardiomyopathy. The overall patient survival rates were 53 % after 10 years and 20 % after 20 years. A total of 20,116 patients (46 %) died during follow-up. The main causes of death were major adverse cardiovascular event, graft failure, infection, and malignancy.

Mortality and number of mismatched

We found a non-random correlation between missing values in recipient HLA and the number of total eplet mismatches. Therefore, we excluded those with missing values in recipient class I antigen or in recipient class II antigen (n = 29,099). The

baseline characteristics of this subgroup (n = 14,923) were similar to those of the entire cohort (n = 44,022). There was no survival difference between patients with complete HLA typing and those with incomplete HLA typing.

The distribution of the number of the total class I and II donor-recipient eplet mismatches are shown in Figure 14 A and B. Transplants were divided into three groups based on the percentile cut-offs of the distribution: Low number eplet mismatch groups, defined as 0 – 25th percentile and corresponding to 0-13 class I eplet mismatches and 0-21 class II eplet mismatches, respectively; intermediate number eplet mismatch groups, defined as 25th – 75th percentile and corresponding to 14-24 class I eplet mismatches and 22-50 class II eplet mismatches, respectively; and high number eplet mismatch groups, defined as 75th – 100th percentile and corresponding to 25-45 class I eplet mismatches and 51-98 class II eplet mismatches, respectively, Table 6.

Table 6. Grouping of transplants into low, intermediate and high number mismatch groups according to the 0 – 25th, 25th – 75th and 75th – 100th percentile cut-offs for total number of eplet mismatches in class I and class II, respectively (n = 14,923)

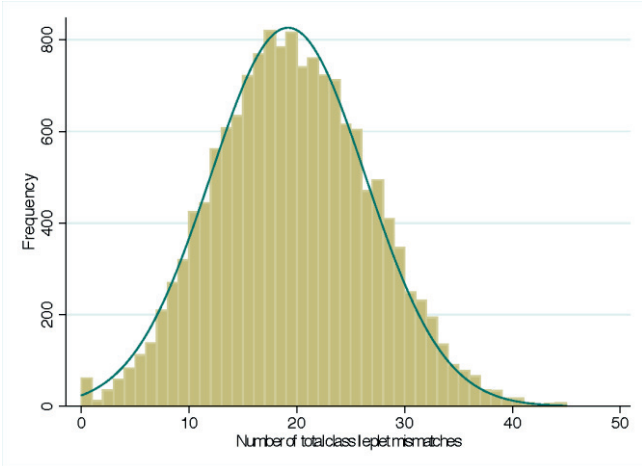
Group	Class I, number of mismatches	Class II, number of mismatches
Low no. mismatch, 0 - 25th percentile	0 - 13	0 - 21
Intermediate no. mismatch, 25th - 75th percentile	14 - 24	22 - 50
High no. mismatch, 75th – 100th percentile	25 - 45	51 - 98

Figure 15 shows the survival curves of the low, intermediate and high eplet mismatch groups for class I HLA loci. Univariable and multivariable analysis showed that there was a trend toward higher mortality for the high number mismatch group compared with the intermediate eplet mismatch group, Table 7A. As the survival curves indicate there might be a survival difference between high and low eplet mismatch groups starting 8 years post-transplant. We therefore performed interaction analysis that showed a significant higher mortality with higher degree of eplet mismatch between 8 and 15 years post-transplant in the multivariable analysis (HR: 1.17, 95 % CI 1.00 - 1.38; p = 0.049), but not before 8 years post-transplant (p = 0.475).

Table 7A. Cox multivariate logistic regression analysis for class I HLA loci (n = 14,811)

Variables	HR	95% CI	p-value
Unadjusted			
Low no. eplet mm (0 – 13)	1.01	0.95 – 1.07	0.797
Intermediate no. eplet mm (14 – 24)	1.00	Reference	
High no. eplet mm (25 – 45)	1.06	1.00 – 1.13	0.059
Adjusted for Age and Gender			
Low no. eplet mm (0 – 13)	1.00	0.94 – 1.07	0.989
Intermediate no. eplet mm (14 – 24)	1.00	Reference	
High no. eplet mm (25 – 45)	1.07	1.00 – 1.13	0.051
Adjusted for 14 covariates*			
Low no. eplet mm (0 – 13)	1.00	9.94 – 1.06	0.918
Intermediate no. eplet mm (14 – 24)	1.00	Reference	
High no. eplet mm (25 – 45)	1.06	1.00 – 1.13	0.066

Values in parentheses are the total number of eplet mismatches (mm). HR, Hazard ratio; 95% CI, 95 per cent confidence interval. *Adjusted for recipient age, recipient sex, black race, ischemia time, recipient/donor weight ratio, donor age, recipient on ventilator, recipient on extracorporeal membrane oxygenation, panel reactive antibodies > 10%, recipient underlying diagnosis: graft failure, congenital or ischemic cardiomyopathy, and era of transplant.



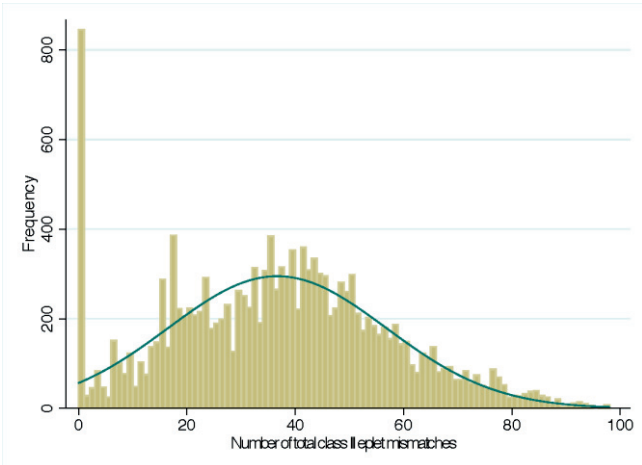


Figure 14 A and B:
Frequencies of total class I and class II HLA eplet mismatches.

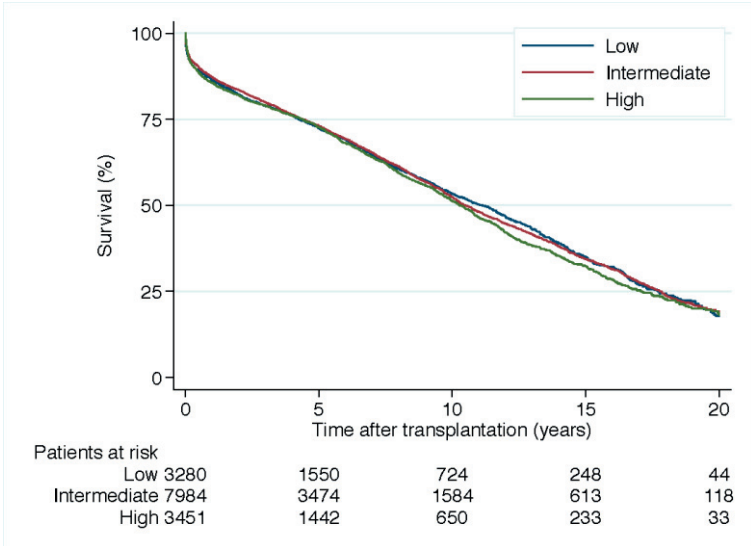


Figure 15:
Kaplan-Meier survival curves by number of class I HLA eplet mismatches. The red solid line shows the observed cumulative survival in the low number cohort, defined as 0 – 25th percentile, corresponding to 0 – 13 class I eplet mismatches. The blue solid line shows survival for transplanted patients in the intermediate number cohort, defined as 25th – 75th percentile, corresponding to 14 – 24 class I eplet mismatches. The green solid line shows survival for transplanted patients in the high number cohort, defined as 75th – 100th percentile, corresponding to 25 – 45 class I eplet mismatches.

As illustrated in Figure 16, the high eplet mismatch group has inferior survival compared with the low and intermediate groups for the class II HLA loci. The significant increase in mortality for the high eplet mismatch was confirmed in both univariable and multivariable analysis, Table 7B. The interaction analysis showed that this was only valid between 0 and 8 years post-transplant (HR: 1.12, 95 % CI 1.04 - 1.20; p = 0.003), but not thereafter (p = 0.788).

Table 7B.
Cox multivariate logistic regression analysis for class II (n = 14,811)

Variables	HR	95% CI	p-value
Unadjusted			
Low no. mm (0 – 21)	1.00	0.95 – 1.07	0.823
Intermediate no. mm (22 – 50)	1.00	Reference	
High no. mm (51 – 98)	1.10	1.03 – 1.17	0.004
Adjusted for Age and Gender			
Low no. mm (0 – 21)	1.01	0.95 – 1.07	0.774
Intermediate no. mm (22 – 50)	1.00	Reference	
High no. mm (51 – 98)	1.10	1.03 – 1.17	0.005
Adjusted for 14 covariates*			
Low no. mm (0 – 21)	1.01	0.95 – 1.07	0.819
Intermediate no. mm (22 – 50)	1.00	Reference	
High no. mm (51 – 98)	1.10	1.03 – 1.17	0.004

Values in parentheses are the total number of eplet mismatches (mm). HR, Hazard ratio; 95% CI, 95 per cent confidence interval. *Adjusted for recipient age, recipient sex, black race, ischemia time, recipient/donor weight ratio, donor age, recipient on ventilator, recipient on extracorporeal membrane oxygenation, panel reactive antibodies > 10%, recipient underlying diagnosis: graft failure, congenital or ischemic cardiomyopathy, and era of transplant.

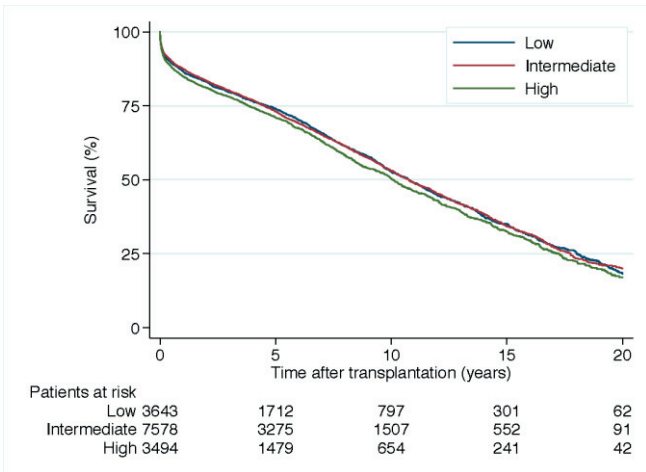


Figure 16:
Kaplan-Meier survival curves by number of class II HLA eplet mismatches. The red solid line shows the observed cumulative survival in the low number cohort, defined as 0 – 25th percentile, corresponding to 0 – 21 class II eplet mismatches. The blue solid line shows survival for transplanted patients in the intermediate number cohort, defined as 25th – 75th percentile, corresponding to 22 – 50 class II eplet mismatches. The green solid line shows survival for transplanted patients in the high number cohort, defined as 75th – 100th percentile, corresponding to 51 – 98 class II eplet mismatches.

The Kaplan Meier curves in Figure 17 illustrate the survival for class I and class II HLA loci combined. Recipients in the high eplet mismatch group experienced a HR for mortality of 1.21 (95 % CI 1.08 – 1.36; p = 0.001) compared with recipients in the intermediate group, Table 7C. In Figure 17, we also saw a trend to higher mortality for the low eplet mismatch group, which was significant between 0 and 2 years post-transplant (HR: 1.22, 95 % CI 1.02 - 1.45; p = 0.031). To further investigate this finding, we divided the transplants in five groups based on the quintiles cut-offs of the distribution of the total number eplet mismatches in class I and/or in class II antigens. We could not identify any difference for class I or class I and II combined antigens group. However, for the class II only antigens we could see a higher mortality for the quintile 1 eplet mismatch group versus the quintile 2 mismatch group. According to these findings patient with 18 up to 31 class II HLA loci eplet mismatch have the most favorable outcome after heart transplantation, Table 8. The multivariable analysis confirmed the results. Furthermore, we divided the transplants into two groups based on a cut-off < 10 total number eplet mismatches in class I and/or in class II. This cut-off did not result in any significantly difference between the groups.

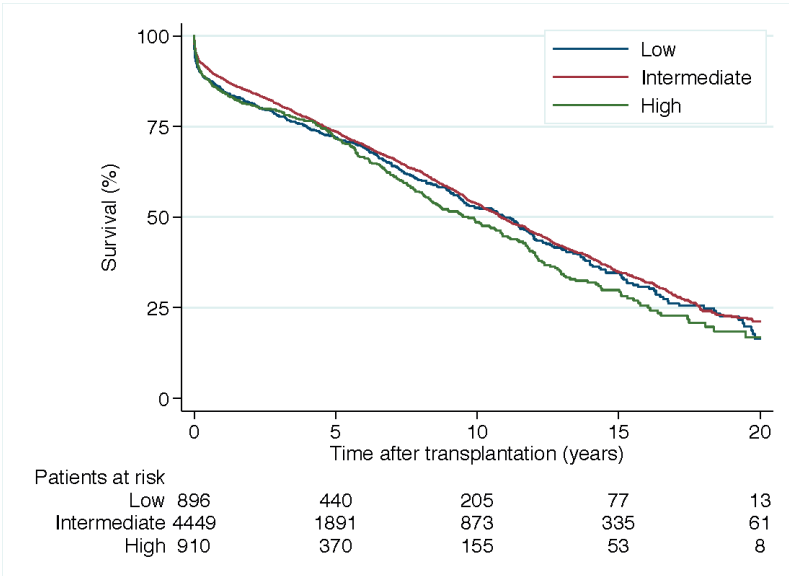


Figure 17:
Kaplan-Meier survival curves by number of class I and class II HLA eplet mismatches. The red solid line shows the observed cumulative survival in the low number cohort, defined as 0 – 25th percentile, corresponding to 0 – 13 class I and 0 – 21 class II eplet mismatches. The blue solid line shows survival for transplanted patients in the intermediate number cohort, defined as 25th – 75th percentile, corresponding to 14 – 24 class I and 22 – 50 class II eplet mismatches. The green solid line shows survival for transplanted patients in the high number cohort, defined as 75th – 100th percentile, corresponding to 25 – 45 class I and 51 – 98 class II eplet mismatches.

Table 7 C.
Cox multivariate logistic regression analysis for class I and class II (n = 6,290)

Variables	HR	95% CI	p-value
Unadjusted			
Low no. mm (0 – 13 / 0 – 21)	1.08	0.97 – 1.20	0.168
Intermediate no. mm (14 – 24 / 22 – 50)	1.00	Reference	
High no. mm (25 – 45 / 51 – 98)	1.18	1.05 – 1.32	0.004
Adjusted for Age and Gender			
Low no. mm (0 – 13 / 0 – 21)	1.07	0.96 – 1.20	0.204
Intermediate no. mm (14 – 24 / 22 – 50)	1.00	Reference	
High no. mm (25 – 45 / 51 – 98)	1.18	1.06 – 1.33	0.003
Adjusted for 14 covariates*			
Low no. mm (0 – 13 / 0 – 21)	1.06	0.95 – 1.19	0.289
Intermediate no. mm (14 – 24 / 22 – 50)	1.00	Reference	
High no. mm (25 – 45 / 51 – 98)	1.21	1.08 – 1.35	0.001

Values in parentheses are the total number of eplet mismatches (mm), class I and class II, respectively. HR, Hazard ratio; 95% CI, 95 per cent confidence interval. *Adjusted for recipient age, recipient sex, black race, ischemia time, recipient/donor weight ratio, donor age, recipient on ventilator, recipient on extracorporeal membrane oxygenation, panel reactive antibodies > 10%, recipient underlying diagnosis: graft failure, congenital or ischemic cardiomyopathy, and era of transplant.

Table 8.
Cox multivariate logistic regression analysis for class II (n = 14,811)

Variables	HR	95% CI	p-value
Unadjusted			
Quintile #1 (0 – 17)	1.12	1.03 – 1.21	0.007
Quintile #2 (18 – 31)	1.00	Reference	
Quintile #3 (32 – 41)	1.10	1.02 – 1.19	0.019
Quintile #4 (42 – 52)	1.13	1.04 – 1.23	0.003
Quintile #5 (53 – 98	1.17	1.08 – 1.27	<0.001
Adjusted for age and gender			
Quintile #1 (0 – 17)	1.12	1.03 – 1.22	0.006
Quintile #2 (18 – 31)	1.00	Reference	
Quintile #3 (32 – 41)	1.10	1.01 – 1.19	0.021
Quintile #4 (42 – 52)	1.13	1.04 – 1.23	0.004
Quintile #5 (53 – 98	1.17	1.08 – 1.27	<0.001
Adjusted for 14 covariates*			
Quintile #1 (0 – 17)	1.12	1.03 – 1.21	0.007
Quintile #2 (18 – 31)	1.00	Reference	
Quintile #3 (32 – 41)	1.10	1.02 – 1.20	0.017
Quintile #4 (42 – 52)	1.14	1.05 – 1.23	0.003
Quintile #5 (53 – 98	1.18	1.09 – 1.28	<0.001

Values in parentheses are the total number of eplet mismatches. HR, Hazard ratio; 95% CI, 95 per cent confidence interval. *Adjusted for recipient age, recipient sex, black race, ischemia time, recipient/donor weight ratio, donor age, recipient on ventilator, recipient on extracorporeal membrane oxygenation, panel reactive antibodies > 10%, recipient underlying diagnosis: graft failure, congenital or ischemic cardiomyopathy, and era of transplant.

Subgroup analyses with interaction testing were performed to determine whether the increase in the HR for death (adjusting for the same covariates as in the main Cox regression model) for the high eplet mismatch groups was consisted across nine immunological relevant subgroups for the class I and class II HLA loci, respectively. No significant interactions were observed for PRA > 10%, previous blood transfusion, recipient age > 55 years, recipient gender, recipient with ventricular assist device, HLA-A or HLA-B compatibility grafts. However, in recipient's matched with a HLA-DR compatible graft there was a trend to higher HR for the high eplet mismatch class II group (HR: 3.40, 95 % CI 1.00 – 11.6; p = 0.050) compared with HLA-DR mismatch graft (HR: 1.10, 95 % CI 1.03 – 1.17; p = 0.006). Although, the interaction test did not reach significance, p = 0.070. In one of the subgroups there was an interaction between the high eplet mismatch groups for class II antigen and patient who were re-transplanted due to graft failure. The HR for the re-transplanted patients were 0.68 (95 % CI 0.47 – 1.00; p = 0.047) compared with 1.11 (95 % CI 1.04 – 1.19; p = 0.001) for the novo transplanted, p = 0.012.

Chapter 5 General Discussion

Systematic review of the available evidence

In study I, a systematic review and meta-analysis was performed to evaluate the importance of HLA matching in heart transplantation. To our knowledge, this is the first study of its kind in the field of heart transplantation. Most of the trial results support the conclusion that HLA matching increases graft survival and reduces the incidence of graft rejection. However, the association between HLA matching and overall patient survival have been less clear. Meta-analysis showed that matching at the HLA-DR locus has protective effect on the prevention of graft failure and incidence of graft rejection. The pooled results from trials that compared 0–1 mismatches with 2 mismatches showed a statistically significant reduction in graft failure and graft rejection at 1 year. HLA-DR matching could improve graft survival by 9 % and reduce the incidence of graft rejection by 19 %. This should be balanced against the increased cost and logistical burden of HLA matching and the longer cold-ischemic times that may result from reliance on tissue typing.

Better understanding of HLA matching in heart transplantation may be accompanied by less dependence on immunosuppression and lead to a reduced rate of infections and malignancies. In heart transplantation, patients often require an urgent transplantation. Even with the introduction of mechanical circulation support, this situation has not changed much. In contrast to kidney transplantation in heart transplantation there is a shortage of critical donors and the current preservation techniques limit acceptable duration of ischemia (to <4 h) [133]. In the current clinical practise, matching of age, gender, and size have higher priority than HLA matching [67, 148]. Previously serological HLA typing methods were used. This has now been replaced by DNA-based methods. DNA-based HLA typing methods, utilizing sequencing-based typing (SBT) and the technologies of sequence-specific primers (SSP) and sequence-based oligonucleotides (SSO) are more precise than the serological methods used previously and provide sequencing results within hours. Therefore, HLA typing results can be obtained within the time limit of permissive ischemia time, making it feasible in the clinical settings of a heart transplantation [149].

Because of the polymorphism of HLA, obtaining a complete HLA match just by chance is unlikely. According to Opelz et al., the probability of achieving a complete match between a 'random' donor and a recipient is less than 1.5%. However, in a pool of 1,000 recipients it was possible to find over 60 % of donor-recipient pairs with two mismatches or less [141]. To determine whether prospective HLA matching is possible, DiSesa et al. [105] performed hypothetical matching by analyzing the HLA type of the recipients in their heart transplant list (n=47) and in all potential heart donors in the geographic area east of the Mississippi River. When broad specificities were used, they found that 94 % of the recipients on the list had at least one potential donor with at least 4 matches (out of 6). They concluded that prospective HLA matching is feasible.

Methodological considerations (I)

The greatest limitation of this systematic review was the lack of randomized data. Retrospective studies are known to be sensitive to confounding. The level, detail, and quality of the original reports determines the quality of the systematic review. Lack of standardization in the studies for many of the variables considered, such as follow-up, outcome, and HLA mismatch, limited the number of reports that could be included in the meta-analysis. Where significant heterogeneity in the available trials were found, expressed as an I^2 value of more than 60 %, the results should be interpreted with caution. We are well aware of introducing heterogeneity by combining studies from different centers in different geographic locations with different treatment protocols. It should also be noted that only one multicenter study and only 3 single- center studies have been published since 2000. With the improvements in other aspects of post-transplant care and therefore in survival with time, it is reasonable to expect that any absolute benefit from matching, found in this review, might be reduced in a modern cohort.

It is possible that allelic disparities at the four-digit level could explain the conflicting results regarding outcome, found in the studies included in this systematic review [150, 151].

The link between HLA and outcome

In study II we tested the hypothesis that HLA-A matching might have more or less favorable effect in the long term depending in the match status of the other HLA loci. We used data on adult heart transplant recipient from the ISHLT registry. We found an association between increased mortality in the late post-transplant period and higher degree of HLA-A matching in patients with HLA–B and/or -DR incompatible grafts. Evaluation of HLA compatibility as a potential risk factor for survival beyond 15 years post-transplantation has not been done before [152, 153]. Also, analysis of interactions between the different HLA-loci offers a new way of thinking. We believe that a possible immunologic cause for the improved survival

explains our findings, as chronic rejection was the outcome primarily related to HLA-A matching.

It could be speculated whether our results may be explained by the existence of a gene involved in the induction of tolerance across a class I disparity. Actually, the possibility of such a gene was found likely in swine model[154], but no gene able to induce tolerance to class I mismatched grafts has been evaluated in cardiac transplantation in humans to date.

Our results may agree with the proposed interactive effect of the HLA-A class I region and the HLA class II region on the regulation of the immune response. Briefly, the incompatibility between donor and recipient for class I HLA-A related antigens in association/linkage disequilibrium with HLA-A alloantigens induces a down-regulatory reaction on the immune response to incompatible HLA-B and HLA-DR antigens.[155, 156] However, in our study the number of patients at risk after 15 years post-transplant was small, which should prompt caution in interpreting the results.

Study V is the first study of the association between donor-recipient structural HLA matching and outcome in adult heart transplantation. We demonstrated that the total number of eplet mismatches in class I and class II HLA loci influence survival after heart transplantation. A higher number of eplet mismatches is associated with increased mortality. Furthermore, it seems that class I loci has an impact in the later time periods after transplantation, whereas class II loci has an impact in the early time periods after transplantation. Our results further indicated that eplet mismatch in class II loci had a stronger impact on survival risk than class I loci. When class I and class II loci were combined the impact of the eplet mismatch on mortality risk was potentiated with an increase in HR.

We saw an increase in mortality in the lowest number eplet mismatched class II antigens group (no. eplet mismatch < 18). Our finding that patients with 18 up to 31 class II antigen eplet mismatch have the most favorable outcome after heart transplantation compared with the high eplet mismatch groups is expected since lower eplet loads correlate with lower frequencies of HLA antibody responses. The opposite result in the lowest quintile group was unexpected. A possible explanation for the higher early mortality in the lowest eplet group may be the amount of immunosuppression. Although there was no difference in the immunosuppression regimes (i.e. the drug used), we do not have any information on the doses, which may differ and influenced the survival.

Another explanation could be that certain HLA-phenotypes are more antigenic than others and more prone to elicit an antibody response. It has been found in kidney transplants that the HLA phenotype of the recipient does affect the immunogenicity of the donor HLA antigens [157]. Various allelic class II major histocompatibility

(MHC) molecules differ in their ability to bind different antigenic peptides and therefore to stimulate specific helper T-cells [24, 25]. Likewise, some HLA-phenotypes might be more prone to bind protein fragments shed by specific donor antigens. In our subgroup analyses of different HLA phenotypes, we found a trend that the HLA-DR matched graft in the high eplet mismatch class II group have an even higher HR, and in the high eplet mismatch groups for class II antigen in patient who were re-transplanted due to graft failure a lower HR. Future studies should focus on exploring the mechanisms behind the impact of these HLA-phenotypes and combinations. Additionally, as recently been described by Duquesnoy, for each allele a higher number of mismatched eplet increase the likelihood of antibodies with reactivity patterns restricted to a few epitopes [85]. Each epitope is defined by a nonself eplet generally surrounded by self residues as potential contact sites for the CDRs of antibody. According to the nonself-self algorithm of eplet immunogenicity, these antibodies originate from B-cells with Ig-receptors for self HLA epitopes. Such B-cells can only be activated and produce antibodies when exposed to alleles with nonself eplets surrounded by self residues. Accordingly, alleles with too many mismatched eplets close together can be expected to be less immunogenic [85]. This finding gives us a novel insight about the complexity of HLA matching at the molecular structural level.

The influence of immunotherapy on outcome

In study III, we showed that induction with BAS was associated with higher all-cause mortality and higher infection-, cardiovascular-, and graft failure- related deaths, compared with induction with ATG.

A few studies have evaluated BAS and ATG use in heart transplantation. Mehra et al.[158] concluded, in their randomized controlled study, that BAS was well tolerated and exhibited a safety profile not significantly different to placebo. In a study by Carrier et al non-inferiority of BAS vs rabbit ATG in terms of immunosuppression effect was not achieved, with the latter showing a lower incidence of rejection at 6 months.[159] In another retrospective study, Flaman et al showed that rabbit ATG was more effective than BAS for prevention of rejection episodes after heart transplantation.[160]

We found in this study that in patients previously transplanted or re-transplanted due to graft failure, BAS performed similarly to ATG. This may be explained by the findings of Regan et al who demonstrated that active thymoglobulin levels are influenced by the degree of sensitization (anti-ATG) and offers an explanation through the pharmacokinetic profile of ATG.[161] Re-transplanted patients may have previously been treated with ATG and therefore possessed anti-ATG antibodies. BAS may have other clearance pathways than anti-BAS antibodies, and therefore not affected as much in a re-treatment setting. Studies are needed to prove or refute this hypothesis. According to latest ISHLT registry report, polyclonal ALG/ATG

use is more common in re-transplants, whereas IL-2R antagonists are more common in primary transplants.[162]

In the subgroup of patients where no corticosteroid was administered, those receiving ATG had a considerably better prognosis than those receiving BAS. When we evaluated this cohort further we found that most deaths in the BAS group occurred in the immediate postoperative period, which may indicate that BAS was given to the more seriously ill patients. It should be noted that, while BAS was used in 34 % of the cohort we examined, its use in heart transplantation is considered off-label.[95] Adoption of BAS use in heart transplantation may have been enhanced by expectations that this (as compared to ATG) more selective immunosuppressive agent might decrease the frequency of post-transplant malignancy, a leading cause of mortality late after transplant.[163] And in fact, our study did show a trend for lower rate of malignancy in BAS treated patients late after transplant. It is possible, however, that BAS use in heart transplantation may pose additional risks that may result in higher morbidity and mortality early after transplant. A recent warning by the European Medicines Agency Pharmacovigilance Committee brings further attention to this possibility.[164]

The difference in survival between the ATG and BAS groups started after the first year post-transplantation and became clearly evident toward the end of the follow-up, suggesting that ATG brings lower risk of chronic graft rejection. While increasing immunosuppression generally renders patients more susceptible to opportunistic infections, this did not translate into higher mortality rates in the ATG group. The practical implication of this study, if confirmed in a randomized setting, are that we should reframe the way we think about induction treatment in heart transplantation and reconsider the routine use of BAS.

Like study III in adult heart recipients, study IV demonstrated that BAS was associated with higher long-term mortality compared with ATG in pediatric heart transplantation. The discrepancy in mortality appeared towards the end of the follow-up.

Approximately 30 % of the patients in recent years received BAS in our study population. This rate is similar to the 25 % rate, of those receiving any induction, in pediatric heart transplant patients receiving interleukin-2 receptor antagonists reported by the Registry of the International Society for Heart and Lung Transplantation.[163] Our data also demonstrated that the use of BAS has risen. In the unadjusted analysis, there was a marked separation between the survival curves. The higher mortality of BAS remained significant after multivariable adjustment. There was no interaction with any of the relevant clinical variables, suggesting that in no subgroup in particular would BAS use be preferred over ATG.

A few studies have evaluated ATG or BAS use in pediatric heart transplantation.[165-169] None of these studies, however, compared BAS with ATG.

In this study, the distinct immunosuppressive mechanisms of the two drugs did not translate into differences in mortality related to potential drug-induced adverse effects, i.e. cardiovascular disease, infection or malignancy. It is possible that the relative rareness of cancers in childhood stopped us from observing even large increases in risk.

It is well known that acute early rejection is a risk factor for mortality in pediatric heart transplantation.[163] As stated earlier, experience in adult heart transplantation has demonstrated an advantage of ATG, compared with BAS, in preventing early post-transplant rejection episodes. Study IV, similarly to adult heart transplantation, shows that BAS and ATG also seem to differ in their impact on chronic rejection. Interestingly differences in mortality was found only for mortality related to graft failure, and not for the other causes of death. Also worth mentioning, is the fact that Daclizumab, another an interleukin-2 receptor antagonist, was found to be associated with an increase in mortality in a randomized, double blind, placebo controlled trial[170] and its production was discontinued for the United States market in 2009 following a diminished market demand.[47]

Methodological considerations

The studies were limited by the retrospective nature of the analyses. Known differences between comparison groups were adjusted for but unknown or unmeasured differences in baseline clinical characteristics and immunosuppression treatment of our populations may have influenced our results. We used multiple imputation technique to handle missing values. This technique is probably the best method available today. Head to head drug comparisons are best performed in randomized controlled trials. However, although randomized controlled trials eliminate bias and confounding they may have limited generalizability and may be complemented by rigorous registry studies with greater power.

In study II, one of the limitations was that we do not know to what extent the donors in the individual transplant centers were allocated based on HLA matching. Therefore, the distribution of HLA matching may not represent random chance but influenced by unknown factors, not accounted for. Furthermore, in study II, we had no information on donor specific antibodies. To limit the confounding effect of pre-existing donor-specific antibodies, recipients that had undergone cardiac surgery, including ventricular assist device, or previous transplantation were excluded. To limit this even further we also excluded patients with PRA ≥10%, the cutoff value above which PRA is associated with worse survival after transplantation.

In study V there was no information on DRw antigen in the UNOS database. We wanted to see how much this position influenced the total number of eplet

mismatches. Therefore, we tried some different HLA antigens in this position, and found that the total number of eplet mismatches did not change. There were also missing values in HLA donor data. However, missing donor data arise randomly and the proportion of missing values are much smaller compared with recipient missing data. This is because many of the donors are multi-donors and for example in kidney transplantation more complete donor HLA data is required. Excluding these patients would limit the statistical power. To evaluate how much the missing donor HLA data influenced our results, we performed a subgroup analysis, excluding the donor HLA missing values, and found that the HRs only changed slightly in univariable and multivariable analyses without a change in the interpretation of the results.

Chapter 6 Conclusions

The major conclusions reached in the studies included in this thesis were:

I. Systematic review and meta-analysis of the available evidence show that most of the trial results support the conclusion that HLA matching increases graft survival and reduces the incidence of graft rejection. The pooled results from trials that compared 0–1 mismatches with 2 HLA-DR mismatches showed a statistically significant reduction in graft failure and graft rejection at 1 year. HLA-DR matching could improve graft survival by 9% and reduce the incidence of graft rejection by 19%.

II. An association was found between increased mortality in the late post-transplant period and higher degree of HLA-A matching in patients with HLA–B and/or -DR incompatible grafts. The fact that HLA-A mismatching was associated with lower mortality related to chronic rejection indicated a possible immunological cause for the improved survival.

III. In the ISHLT Registry experience, use of ATG rather than basiliximab as induction therapy appears to be associated with better long-term survival. Use of basiliximab was associated with higher risk of death related to graft failure, cardiovascular events, and infection, but not malignancy.

IV. In pediatric heart transplant patients, the use of basiliximab for induction therapy was associated with an increased risk of mortality, when compared with those receiving ATG. At 10 years post-transplant survival with the use of basiliximab was 49 % versus 65 % for ATG ($P < 0.001$).

V. Structurally based HLA mismatching may aid in identifying recipients at increased risk of long-term mortality, which may have important clinical consequences for survival after heart transplantation. However, that the intermediate eplet mismatch group turned out to have the most favorable outcome after heart transplantation was unexpected. This finding gives us a novel insight about the complexity of HLA matching at the molecular structural level.

Future perspectives

Research in heart transplantation presents great opportunities to improve outcomes for the heart transplant patients. First and foremost, this research should focus on immunological factors that play a role in the development of chronic rejection.
We have shown that induction treatment with ATG is associated with better long-term survival compared with basiliximab, despite the fact the latter is a more selective immunosuppressive drug. A prospective randomized trial with an adequately long follow-up comparing the two drugs is warranted. But it is equally important to compare induction treatment with no induction treatment, as today there is a need for more support for a beneficial role of induction treatment at all. The potential effect of ATG in inducing immunological tolerance[75] is very exciting and should be explored further. Not only can it explain why ATG treated patients have better survival than basiliximab treated patients, but it can also constitute a model for future drug development in organ transplantation.

We have shown that the interplay between HLA and chronic rejection is more complex than just the number of mismatches. HLA-A compatible and incompatible grafts have different prognosis depending on the HLA-B and –DR status of the patients. This indicates an interaction between the HLA loci and the possibility of tolerance genes is likely. Identification of these tolerance promoting genes, cells and molecules and their role in up or down regulating the immune system is the key factor to understand to explain how a donated graft can be accepted in a foreign environment represented by the recipient´s body. This knowledge will also enable us to develop treatments that promotes tolerance in order to avoid chronic rejection and improve patient survival.

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