RESEARCH PROTOCOL

for non-WMO-applicable research

Official Title: The Impact of Donor and Recipient Sex on Long-term Outcomes Following Living Donor Kidney Transplantation

Brief Title: Sex Impact on Long-term Outcomes of LDKT

NCT number: NCT06016283

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Full title of protocol	The Impact of Donor and Recipient Sex on Long-term Outcomes Following Living Donor Kidney Transplantation
Short title or Acronym	Sex impact on living donor kidney transplantation
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Subsidizing party[5]	*N/A*

Name	Signature	Date
Sponsor or legal representative: *Erasmus MC* **Head of Department:** *Prof.dr. J.M. Hendriks*		
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1. *Coordinating investigator: Investigator who bears the responsibility for the coordination of investigators operating in the various centers participating in multicenter research. Not all multicenter research will have a coordinating investigator. There is no requirement to appoint one. A project leader has the responsibility to develop a research protocol and to complete the study within the predefined goals.*
2. *Principal investigator: Investigator who has the overall responsibility to comply and to complete the study within the predefined goals.*
3. *Multicenter research: as an alternative you can also state that these are specified in the list with participating centers including principal investigator. This separate document with version date must be uploaded under category I1.*
4. *Sponsor: The party that commissions the organization or performance of the research, for example a pharmaceutical company, academic hospital, scientific organization or the investigator's employee. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidizing party.*
5. *Subsidizing party: A party that provides funding for a study but does not commission it.*

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Please note that it is not allowed to remove paragraphs from this template protocol. If a paragraph is not applicable, please mention this in the specific paragraph, preferably with a short motivation.

List of abbreviations and relevant definitions*

CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CTA	Clinical Trial Agreement
De novo biobank	A new data, human material or imaging collection
DGF	Delayed Graft Function
DMP	Data Management Plan
DPIA	Data Protection Impact Assessment
DTA	Data Transfer Agreement
eGFR	Estimated Glomerular Filtration Rate
Exception consent	Form Care for data Template, in Dutch: Formulier uitzondering toestemming
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation in Dutch: Algemene Verordening Gegevensbescherming
IC	Informed Consent
IFU	Instruction For Use
LDKT	Living Donor Kidney Transplantation
MTA	Material Transfer Agreement
NWTC	Non-WMO Review Committee; in Dutch: Niet WMO Toetsingscommissie
PNF	Primary Non Function
UAVG	Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet Algemene Verordening Gegevensbescherming
WMO	Medical Research Involving Human Subjects Act, in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen

**Please add any new definitions that are used in the research protocol*

Summary

Rationale

The impact of donor and recipient sex combinations on kidney transplant outcomes has been widely studied, yet results remain inconclusive. Immunologically, female recipients of male donor kidneys face higher graft failure and mortality risks due to H-Y antigens. Another theory suggests male recipients of smaller female kidneys have worse outcomes due to nephron overload and hyperfiltration. Estrogen's protective effect against ischemia-reperfusion injury (IRI) lowers delayed graft function (DGF) incidence in female recipients, but recent research indicates higher mortality and graft loss rates for female recipients compared to males. These conflicting findings mainly pertain to deceased donor transplants.

For living donor kidney transplants (LDKT), previous study reported that male recipients of male donor kidneys had better graft survival in a study of 30,258 LDKTs from 1990-1999. Another study of 5,716 HLA-identical sibling transplants from 1985-2000 showed female recipients had better graft survival regardless of donor sex. Despite clinical advances, no large-scale studies have explored this issue in the last two decades.

Objective(s)

1. To investigate the impact of different donor and recipient sex combinations on the graft survival following living donor kidney transplantation
2. To investigate the impact of different donor and recipient sex combinations on the graft function (eGFR) following living donor kidney transplantation

Study type
Retrospective cohort study

Study population
Adult patients (n=~2000) undergoing LDKT at Erasmus Medical Center (EMC) and University Medical Center Groningen (UMCG) between January 2010 and December 2020.

Methods
Retrospective data from our transplant database were used to identify patients undergoing LDKT between January 2010 and December 2020. LDKTs were categorized into male-to-male, female-to-male, male-to-female, and female-to-female groups based on donor-recipient sex combinations. The primary outcome was death-censored graft survival. The secondary outcomes included patient survival, delayed graft function (DGF), acute rejection and graft function.

Burden and risks
N/A (no intervention involved)

Recruitment and consent
Informed consent has been waived based on the unreasonable effort due to the large size and its observational nature.

1. Introduction and rationale

The impact of donor and recipient sex combination on kidney transplant outcomes has been studied extensively, but with inconclusive results. From an immunologic perspective, the transplantation of male donor kidneys into female recipients was associated with an increased risk of graft failure and mortality due to H-Y minor histocompatibility antigens.[1, 2] Another theory, based on nephron overload and hyperfiltration, suggests that male recipients of female donor kidneys tend to experience worse outcomes due to the smaller size of female kidneys.[3] Additionally, the influence of sex hormones has also been explored. Aufhauser et al.[4] found that estrogen acts as a protective factor against ischemia-reperfusion injury (IRI), resulting in a significantly lower incidence of delayed graft function (DGF) in female recipients compared to male recipients. However, recent research by Vinson et al.[5] demonstrates that female recipients have higher excess mortality rates and graft loss rates than males. These findings seem conflicting, and primarily focus on deceased donor kidney transplantation.

In terms of living donor kidney transplantation (LDKT), Kayler et al.[6] analyzed a transplant database encompassing 30,258 LDKTs between 1990 and 1999. Their study revealed a significant advantage in graft survival for male recipients of male donor kidneys compared to the other combinations. Another study involving 5,716 HLA-identical sibling kidney transplantations between 1985 and 2000 demonstrated that female recipients tend to have better graft survival rates regardless of the donor's sex.[3] Despite advancements in clinical practice, no such large-scale studies exploring this question have been conducted in the subsequent two decades.

2. Objective(s)

I. To investigate the impact of different donor and recipient sex combinations on the graft survival following living donor kidney transplantation

II. To investigate the impact of different donor and recipient sex combinations on the graft function (eGFR) following living donor kidney transplantation

3. Study type

3.1. **Study type**

☒ Retrospective

☐ Prospective

☐ Combination Retrospective/Prospective

3.2. **Single center / Multicenter**

☐ Single center

☒ Multicenter

3.3 **Check all the applicable boxes**

☒ Medical records (re-use of data from healthcare, including AI)

- ☐ Case report
- ☐ Re-use data from research
- ☐ Evaluations of quality of healthcare (retrospective)
- ☐ Research with additional use of residual material from regular healthcare
- ☐ Research with re-use of human material from research or existing biobank
- ☐ De novo biobank
- ☐ Phase IV research
- ☐ Healthcare evaluation research (prospective)
- ☐ Research with medical devices
- ☐ Research with In Vitro Diagnostic Tests
- ☒ Other research

4. Study population

4.1. Study population

☒ Adults (16 years and older)

☐ Minors (younger than 16 years)

☐ Incapacitated adults (16 years and older)

☐ Incapacitated minors (younger than 16 years)

4.2. Population (base)

Adult patients (n=~2000) undergoing LDKT at Erasmus Medical Center (EMC) and University Medical Center Groningen (UMCG) between January 2010 and December 2020.

4.3. Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

Adult patients undergoing LDKT at EMC and UMCG between January 2010 and December 2020.

4.4. Exclusion criteria

Patients who lost the follow-up.

4.5. Sample size calculation

To acquire enough data to make the research results robust, the estimated subject number is ~2000 patients.

5. Methods

5.1. Research methods

Retrospective data from our transplant database were used to identify patients undergoing LDKT between January 2010 and December 2020. LDKTs were categorized into male-to-male, female-to-male, male-to-female, and female-to-female groups based on donor-recipient sex combinations. The primary outcome was death-censored graft survival. The secondary outcomes included patient survival, delayed graft function (DGF), acute rejection and graft function.

5.2. Standard clinical care versus extra for research

No extra intervention are applied on the patients due to its retrospective observational nature.

5.3. Burden and risks

Not applicable because of no intervention in this study.

5.4. Medical device(s) / In vitro diagnostic tests

Not applicable because no medical device is involved in this study

6. Incidental findings

6.1. Chance of incidental findings

Is there a chance of incidental findings?

☐ Yes

☒ No

6.2. Procedures

Not applicable, there is no change of incidental findings.

7. Statistical analysis

7.1 Main study parameters/endpoints

The primary outcome was death-censored graft survival.

7.2 Secondary study parameters/endpoints

The secondary outcomes included patient survival, delayed graft function (DGF), acute rejection and graft function. Graft function is defined by estimated glomerular filtration rate (eGFR) using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.

7.3 Other study parameters

Patient age, gender, medical history, surgical details and follow-up data.

7.4 Analysis

Continuous variables were reported as medians with interquartile ranges (IQRs), and categorical variables were reported as frequencies with percentages. The Kruskal-Wallis test was used to compare continuous variables, and the chi-squared test was used to compare categorical variables. Kaplan-Meier curves and the log-rank test were used to compare patient and graft survival rates. Cox-regression was performed to identify the risk factors for death-censored graft failure. Variables with a p-value < 0.1 in the univariate analysis were included in the multivariate analysis. The proportional hazards assumption was evaluated through Schoenfeld residuals. A two-sided p-value < 0.05 was considered statistically significant. All statistical analyses were conducted using R version 4.2.2.

8. Ethical considerations

8.1 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki (64th World Medical Association General Assembly, Fortaleza, Brazil, October 2013). The following guidelines also apply to this study;

- Gedragscode Gezondheidszorgonderzoek 2022.
- Privacy regulation of participating hospitals
- EU General Data Protection Regulation and the Dutch Act on Implementation of the General Data Protection Regulation (in Dutch: Uitvoeringswet ‘Algemene Verordening Gegevensbescherming’ (UAVG)).

This study has been exempted by the Medical Research Ethics Committee (MREC); in Dutch: Non-WMO Review Committee (NWTC). Following review of the protocol, the MREC concluded this study is not subject to the Medical Research Involving Human Subjects Act (WMO). They concluded that the study is a medical/ scientific research, but no patients are subjected to procedures or are required to follow rules of behavior.

8.2 Informed consent

Will the subjects be asked for informed consent?

☐ Yes *(Upload Participant Information Letter and Informed Consent)*

☐ No, consent already given in previous study *(Upload Participant Information Letter and Informed Consent previous study)*

☒ No, this research will be performed under the exception consent *(Upload form Care for data Template, in Dutch: Formulier uitzondering toestemming)*

☐ Other (e.g. partly, indirectly) *Please describe the situation.*

8.3 Recruitment and informed consent procedures

Not applicable, patients will not be asked to consent to their data being used for the current study.

8.4 Exception consent

All cases in the transplant database have already provided informed consent for their data to be shared with the Dutch Transplant foundation for future research. The informed consent is asked before a recipient is placed on the transplant waiting list.

9. Handling and storage of data / images / sound recordings / photos / film recordings

9.1 Data / images / sound recordings / photos / film recordings

Patient clinical data, including demographics, surgical information and follow-up information, will be used. These data are obtained for regular healthcare purposes.

9.2 Privacy protection

To ensure the subject's privacy is protected, each patient will be assigned a unique code that does not include any personal identifiers such as patient ID or date of birth. This code will be used throughout the study to identify the data associated with each subject. A designated data manager (the principal investigator), is responsible for the assignment of unique codes and ensure that the coding process is consistently applied. The key table linking the unique codes to the personal identifiers will be stored in a secure file that is separate from the research data. Only authorized personnel, such as the principal investigator, will have access to this database.

The handling of personal data has to comply with the EU General Data Protection Regulation and the Dutch Act on Implementation of the General Data Protection Regulation (in Dutch: Algemene Verordening Gegevensbescherming en Uitvoeringswet Algemene Verordening Gegevensbescherming).

9.3 Handling and storage of data

Research data will be stored in the format of SPSS in Erasmus MC departmental X drive with limited access to the PI and co-investigators, and will be handled confidentially. Only authorized personnel can view research data that can be traced to individual participants. These persons are the members of the research team, members of the health care inspection, and members of

the Medical Research Ethics Committee of the participating hospital. Data review may be necessary in order to ensure the reliability and quality of the research. The handling of personal data is in compliance with the GDPR General Data Protection Regulation (in Dutch AVG 25-05-2018) and the privacy regulation of the Erasmus MC. In line with Erasmus MC guidelines, data will be kept 10 years after it is collected.

9.4 **Handling and storage of images / sound recordings / photos / film recordings**

Not applicable, this study does not involve images, sound recordings, photos, or film recordings

9.5 **Approval of access to data / images / sound recordings / photos / film recordings**

For each study site, access to data has been approved by the department head or board of directors, as applicable.

10. Handling and storage of human material

10.1 **Human material**

Not applicable, this study does not involve human material

10.2 **Check all the boxes which are applicable to the human material origin:**

☐ Residual material from regular healthcare

☐ Research (material acquired from a previous study).
Add the reference of the study i.e., MEC-number Erasmus MC.

☐ Re-use of human material from existing biobank
Describe whether the human material originates from research into the same disease.

☐ Other, *please specify*

☒ Not applicable, this study does not involve human material.

10.3 **Handling and storage of human material**

☐ Anonymous, i.e. the material can never be traced back to an individual subject

☐ Pseudonymized/Coded

☐ Identifiable

☒ Not applicable, this study does not involve human material.

10.4 **Biobank**

Not applicable, this study does not involve biobank.

10.5 **Approval of access to human material**

Not applicable, this study does not involve human material.

11. Exchange, sharing or transfer of data and/or human material and/or images

We will receive the data from University Medical Center Groningen, a non-profit educational and research organization within the Netherlands. The data transfer will be conducted via SURF and stored in Erasmus MC departmental X drive with limited access to the PI and co-investigators. A Data Transfer Agreement is available.

12. Amendments

Amendments are changes made to the research after a favorable opinion by the NWTC has been given.

All amendments must be submitted to the NWTC that gave the favorable opinion.

Substantial amendments must be approved by the Niet WMO Toetsingscommissie before they can be implemented.

13. End of study report

Within one year after the end of the study a final study report will be submitted with the results of the study, including any publications/abstracts of the study.

14. Publication

Do you have the intention to submit the study results in a manuscript for publication in a journal:

☒ Yes

☐ No, *please motivate*

The final study report will be submitted by the end of 2025

15. References

1. Gratwohl A, Dohler B, Stern M, Opelz G. H-Y as a minor histocompatibility antigen in kidney transplantation: a retrospective cohort study. Lancet. 2008;372(9632):49-53.
2. Kim SJ, Gill JS. H-Y incompatibility predicts short-term outcomes for kidney transplant recipients. J Am Soc Nephrol. 2009;20(9):2025-33.
3. Zeier M, Dohler B, Opelz G, Ritz E. The effect of donor gender on graft survival. J Am Soc Nephrol. 2002;13(10):2570-6.
4. Aufhauser DD, Jr., Wang Z, Murken DR, Bhatti TR, Wang Y, Ge G, et al. Improved renal ischemia tolerance in females influences kidney transplantation outcomes. J Clin Invest. 2016;126(5):1968-77.
5. Vinson AJ, Zhang X, Dahhou M, Susal C, Dohler B, Melk A, et al. A multinational cohort study uncovered sex differences in excess mortality after kidney transplant. Kidney Int. 2023;103(6):1131-43.
6. Kayler LK, Rasmussen CS, Dykstra DM, Ojo AO, Port FK, Wolfe RA, et al. Gender imbalance and outcomes in living donor renal transplantation in the United States. Am J Transplant. 2003;3(4):452-8.

16. Attachments

☐ Participant information letter and Informed consent document

☒ Care for data Template – Formulier uitzondering toestemming

☐ Questionnaires

☐ Data Management Plan

☒ Data Transfer Agreement

☐ Material Transfer Agreement

☐ Clinical Trial (Site) Agreement

☐ Other, *please describe*