University of Groningen

Medical imaging before and after kidney transplantation

Benjamins, Stan

DOI:
[10.33612/diss.157939666](https://doi.org/10.33612/diss.157939666)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2021

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):
Benjamins, S. (2021). *Medical imaging before and after kidney transplantation*. [Thesis fully internal (DIV), University of Groningen]. University of Groningen. <https://doi.org/10.33612/diss.157939666>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

MEDICAL IMAGING BEFORE AND AFTER KIDNEY TRANSPLANTATION

Stan Benjamins

Financial support for the printing of this thesis from the University of Groningen, University Medical Center Groningen, Research Institute GUIDE, Nederlandse Transplantatie Vereniging, and Dutch Heart Foundation is gratefully acknowledged.

Additional financial support for the printing of this thesis was kindly provided by Chiesi Pharmaceuticals BV, ChipSoft BV, Erbe Nederland BV, Noord Negentig accountants en belastingadviseurs, and Tromp Medical BV.

© Copyright 2021 S. Benjamins

All rights reserved. No part of this thesis may be reproduced, copied, modified, stored in a retrieval system, or transmitted in any form or by any means without prior permission of the author, or when applicable, of the publishers of the scientific papers.

Cover design and layout: © evelienjagtman.com

Printing: Ridderprint – ridderprint.nl

rijksuniversiteit
 groningen

MEDICAL IMAGING BEFORE AND AFTER KIDNEY TRANSPLANTATION

Proefschrift

ter verkrijging van de graad van doctor aan de
Rijksuniversiteit Groningen
op gezag van de
rector magnificus prof. dr. C. Wijmenga
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op

woensdag 10 maart 2021 om 16.15 uur

door

Stan Benjamens

geboren op 27 augustus 1994
te Amsterdam

Promotores

Prof. dr. R.H.J.A. Slart

Prof. dr. S.P. Berger

Copromotor

Dr. R.A. Pol

Beoordelingscommissie

Prof. dr. J.P.P.M. de Vries

Prof. dr. M. Prokop

Prof. dr. J.N.M. IJzermans

Paranimfen

Drs. J.E. Emmens

Dhr. S.E. Dulfer

TABLE OF CONTENTS

Chapter 1	General introduction and outline of this thesis	9
Chapter 2	A broader view on transplantation research	27
Part I Renal scintigraphy after kidney transplantation		
Chapter 3	Renal scintigraphy for post-transplant monitoring after kidney transplantation	49
Chapter 4	Can transplant renal scintigraphy predict the duration of delayed graft function? A dual center retrospective study	75
Chapter 5	Limited clinical value of two consecutive post-transplant renal scintigraphy procedures	95
Part II Calcification before and after kidney transplantation		
Chapter 6	[¹⁸ F]-sodium fluoride autoradiography imaging of nephrocalcinosis in donor kidneys and explanted kidney allografts	117
Chapter 7	A high abdominal aortic calcification score by dual x-ray absorptiometry (DXA) is associated with cardiovascular events after kidney transplantation	137
Chapter 8	Bone mineral density and aortic calcification: evidence for a bone-vascular axis after kidney transplantation	155
Chapter 9	Aorto-iliac artery calcification prior to kidney transplantation	179
Chapter 10	Aorto-iliac artery calcification and graft outcomes in kidney transplant recipients	203
Chapter 11	Summary, discussion and future perspectives	225
Appendices		
Appendix I	Living donor hand-assisted laparoscopic nephrectomy in a healthy individual with situs inversus totalis: no need to turn down the donor	241
Appendix II	Kidney allograft cyst infection	251
Appendix III	The fear for contrast-induced nephropathy in kidney transplant recipients: time for a paradigm shift?	261
Addendum		
	Summary in Dutch / Nederlandse samenvatting	271
	List of contributing authors	279
	List of publications	285
	Acknowledgments / Dankwoord	291
	Curriculum Vitae	299

General introduction and outline of this thesis

Adapted from:
Kidney Transplantation and Diagnostic Imaging: The Early Days
and Future Advancements of Transplant Surgery
Diagnostics, 2021
Stan Benjamens, Cyril Moers, Riemer H.J.A. Slart, Robert A. Pol

KIDNEY TRANSPLANTATION: THE EARLY DAYS

One of the tales of Saint Cosmas and Damian, as compiled in the *Legenda Aurea* by Jacob de Voragine, the archbishop of Genoa, Italy in 1265, is an example of the fascination of human beings with transplantation. As described by the archbishop, the twin brothers dedicated their lives to medicine and the church, providing care without the desire for rewards. The most appealing story of their legendary tales is “*The miracle of the transplantation of the black leg*” (Figure 1). In one of the multiple accounts of this legendary tale a caretaker in the Roman church suffers from cancers in his left leg. As the twin brothers are in Rome, they visit this patient by night and amputate the affected leg. Instead of leaving him crippled, the brothers amputate the leg of a recently buried Moor and use this leg for transplantation.¹

Figure 1. Saints Cosmas and Damian: The miracle of the transplantation of the black leg (Jaume Huguet, 15th century).

Centuries passed between the compilation of this legendary tale and the initial quest for successful kidney transplantation, started by the surgeon Emerich Ullmann in Vienna, Austria, 1902. By performing a dog-to-dog kidney transplantation, resulting in five days of kidney transplant function, he motivated European surgeon-scientists to move forward on this medical endeavor.² In the same year, Alexis Carrel, a genius surgeon and a controversial political figure, published his first article on the vascular anastomosis of the jugular vein and carotid artery. His pioneering work on blood vessel anastomosis as well as experimental vessel and organ transplantation, supervised by his mentor Mathieu Jaboulay, is still considered the essential surgical innovation for later human-to-human transplantation. For these efforts he was awarded the Nobel Prize in Physiology

or Medicine in 1912, as the youngest physician-scientist to date.³⁻⁵ The next gigantic step for organ transplantation was taken by the Ukrainian surgeon Yurii Voronoy in 1933. With his surgical team from Kherson in the Ukraine, he performed the first human-to-human deceased donor kidney transplantation. This surgical intervention resulted in hyperacute rejection and death of the 26-years-old recipients just two days after transplantation. The recipient's history of mercury intoxication, the extremely long warm ischemia time of more than 6 hours, and blood group mismatch are considered to be the main causes.^{6,7} However, the potential was recognized and new initiatives and experiments were started. In 1950, Dempster et al. performed successful dog-to-dog transplantations, with the neck as surgical site. This also resulted in the first available diagnostic image of the graft, an X-ray image using the injection of 35% pyelosil contrast fluid (Figure 2).⁸

Figure 2. The first available diagnostic image, an X-ray image at 12 minutes after injection of 35% pyelosil contrast fluid, of a dog-to-dog transplanted kidney (Dempster, *Ann R Coll Surg Engl.* 1950).⁸

The next steps towards successful organ transplantation took more than 15 years. While the disruptive effect of World War II heavily impacted medical sciences, important innovations took place in the field of vascular surgery, going from vascular ligation to arterial repair.⁹ Led by the French surgeon Rene Kuss, a series of kidney transplantations was performed with grafts from convicted felons who were executed by guillotine beheading, and thus used as a donor. While the procedures were a surgical success, the lack of knowledge on immunological matching resulted in transplant rejection in the days following transplantation.¹⁰ In this post-war era, surgeon Richard Lawler from Illinois, USA, and his team performed a human-to-human deceased donor kidney

transplantation in a 49-years-old female recipient with polycystic kidney disease. He described graft function for more than nine months after transplantation and the intervention was considered successful from a surgical perspective. The impact of this transplantation is still the subject of debate, as this patient did not have end-stage renal disease and lived for five years after transplantectomy, without the need for dialysis or re-transplantation. In the nine months prior to transplantectomy, the first post-transplant diagnostic imaging procedure was performed, being a retrograde pyelography which confirmed the diagnosis of a partial stricture of the ureter-bladder anastomosis.^{11,12}

A breakthrough was achieved with the living kidney donation and transplantation from one identical twin to another by surgeon Joseph Murray and colleagues in 1954. In this historic case, diagnostic imaging was also applied, being a urogram with intravenous contrast.¹³

In the following years, the Boston team performed another seven living donor kidney transplantations between identical twins. In six out of these seven cases, normal kidney function was achieved after transplantation, whereas in one recipient surgical failure occurred due to kinking of the renal artery and subsequent thrombosis. The landmark paper of the description of these cases in the *Annals of Surgery* (1958) can be considered the first guideline for living donor kidney transplantation, with donor and recipient screening recommendations and descriptions of post-transplant diagnostic procedures (Figure 3). Pre-transplant donor and recipient imaging included a chest X-ray, a cystogram and a urogram with intravenous contrast (Figure 4), whereas post-transplant diagnostics relied on retrograde pyelography.¹⁴

TABLE 2. *Criteria for Transplantation*

Donor :
(1) Two normal kidneys
(2) Normal lower urinary tract
(3) Absence of infection
(4) Sufficient understanding
Recipient :
(1) Irreversible terminal disease
(2) Normal lower urinary tract
(3) Infection, if present, minimal or controllable
(4) Inactive primary renal disease

Figure 3. Criteria for transplantation, from Murray et al., *Ann Surg*. 1958.¹⁴

Figure 4. Pre-transplant donor imaging with on the left the result of a cystogram, “showing bilateral ureteral reflux, slightly more marked on the right”, and the right the result of an excretory urogram, “showing bilateral normal function. Bladder shadow reflects interstitial cystitis which responded to treatment before transplantation” (Murray et al., *Ann Surg.* 1958).¹⁴

With the introduction of immunosuppressive medication in 1962, starting with 6-mercaptopurine and azathioprine, transplantation with an unrelated living or deceased donor kidney became a viable opportunity.^{15,16} Together with the ongoing fight against the immunological barriers for organ transplantation, urological complications showed to be a tough surgical hurdle to take.¹⁷ In an elaborate description by surgeon Thomas Starzl, often referred to as “the father of modern transplantation”, urological complications of the first 216 consecutive transplant recipients are outlined.¹⁸ In all these cases, urograms with intravenous iodine contrast were performed in the first days following transplantation, with a repetition every three to six months. In a subgroup of patients, cystograms and retrograde pyelograms were performed to identify or exclude possible complications.¹⁹ The progress made between 1962 and 1970 can be considered ground breaking, with a one-year graft survival of 67%, 68%, and 92% for the periods 1962-1964, 1964-1966, and 1966-1968, respectively.²⁰ The role of advancements in immunosuppressive medication and knowledge about human leukocyte antigen (HLA) matching in this progress is beyond dispute, whereas the alignment between transplant surgery and diagnostic imaging is less well known.

The success story of kidney transplantation came to The Netherlands in 1966 with the first living donor kidney transplantation in Leiden.²¹ One-year and three days later, the first kidney transplantation was performed in Groningen, the transplant centre at which half a decade later the projects for this PhD thesis started.

HISTORICAL ALIGNMENT BETWEEN TRANSPLANT SURGERY AND DIAGNOSTIC IMAGING

Kidney transplantation and diagnostic imaging followed a similar time path for their development. In 1895, the German physicist Wilhelm C. Röntgen, who was first expelled from the Technical School in Utrecht, The Netherlands, produced the first X-ray image.²² Only a year later, Antoine H. Becquerel, chairman of Physics of the École Polytechnique in Paris, France, revealed the first evidence for radioactivity.²³ Supervised by Professor Becquerel, Maria Sklodowska-Curie started her eventually ground-breaking work on uranium radiation. Only months after the first dog-to-dog kidney transplantation, Maria Curie, Professor Becquerel and her husband (Pierre Curie) received the Nobel Prize in Physics 1903.^{23,24} In the same era, with the search for applications of X-ray imaging, the first steps for retrograde pyelography were made by Voelcker and von Lichtenberg, generally considered an unintentional discovery.²⁵ The introduction of intravenous urography, taking another 20-years, was enabled by the work of the urologist Moses Swick and others on organically bound iodine (Uroselectan).^{26,27} As described in the reports by Murray and Starzl, these diagnostic imaging techniques proved pivotal for both pre-transplant screening and post-transplantation diagnosis of complications.

In the same year as the first human-to-human kidney transplantation (1933), the scientist Irène Joliot-Curie, daughter of Maria and Pierre Curie, showed that radioactive elements can be produced artificially. This was the essential step toward the discovery of Iodine-131 and Technetium-99m, the cornerstones of nuclear medicine.^{28,29} Although the development of kidney transplantation stagnated during World War II, the innovation of X-ray imaging and radioactivity skyrocketed. Radiotracers became a serious factor in medical practice in the 1960s, the golden era for kidney transplantation innovations.^{30,31} The medical fields of kidney transplantation and nuclear medicine crossed paths in the application of renal scintigraphy (renography). This technique was first performed using Iodine-131-Hippuran (Figure 5), and later replaced by Technetium-99m mercaptoacetyltriglycine (MAG3) in 1988, due to better count statistics and a lower radiation dose.³² With the use of radioactive Xenon (Xenon-133) intra-operative renal blood flow measurements were tested in 1967, injecting the radioactive substance directly in the renal artery, while recording the activity over the entire kidney allograft.³³

While X-ray imaging was already widely available, as documented with the first transplant diagnostic images in 1956, the introduction of ultrasound and computed tomography (CT) did not enter clinical practice until the 1950s and 1970s, respectively.^{34,35}

In a report on postoperative ultrasound examinations, Petrek et al. (1976) described a protocol of ultrasound examinations within 24 hours after transplantation for 135 consecutive kidney transplant recipients (Figure 6). These ultrasound examinations of the kidney graft and surrounding anatomical structures focused on depicting and assessing graft enlargement, renal pelvis dilatation, and perinephric fluid collections. In these days, determining graft enlargement without signs of renal pelvis dilatation was considered a reliable sign for acute rejection.³⁶ In a later prospective study, ultrasound examination showed to be a useful adjunct, with a 81% sensitivity for acute rejection, but to date cannot replace histological examination.³⁷

Figure 5. The result of a normal renal scintigraphy with Iodine-131-Hippuran, presented by Collins and Wilson in *Ann Surg.* 1965: "A. First (vascular) phase of the normal renogram. B. Second (secretory) phase. C. Third (excretory) phase".³³

The specific role of CT-procedures in kidney transplantation had still to be determined, with the first record of its application in 1977.³⁸ Further steps for a broader clinical application took until 1978, with an overview of CT-applications for "*renal transplant problems*" by Kittredge et al., recommending CT-guided intravenous urography as a first line diagnostic modality, to determine the kidney graft's position and the extent of surrounding fluid collections.³⁹ In the following decades, CT-procedures were primarily used for living donor assessment and post-transplant screening for malignancies.⁴⁰

Figure 6. The ultrasound image of a normal transplanted kidney (upper panel) and a transplant with an episode of acute rejection (lower panel), presented by Petrek et al. *Ann Surg.* 1976.³⁶

While joined innovations between transplant surgery and diagnostic imaging slowly decreased after the introduction of ultrasound and CT, imaging innovations in general gained momentum. This included the progress in molecular imaging, with functional magnetic resonance sequences and innovative positron emission tomography tracers, as well as the implementation of artificial intelligence.⁴¹⁻⁴³

KIDNEY TRANSPLANTATION IN THE 21-CENTURY

As an internal medicine resident and PhD candidate, Willem Kolff made his first steps for the development of dialysis in 1943 at the hospital of Kampen and the University of Groningen. In 1960, the introduction of the arteriovenous shunt enabled the use of maintenance haemodialysis for patient with end-stage renal disease.⁴⁴ In the same decade, the first kidney transplantation was performed in The Netherlands (Leiden 1966), followed by the first kidney transplantation in Groningen in 1968. The Groningen Transplant Center reached its highest number of yearly kidney transplantations in 2016 (116 living donor and 84 deceased donor kidney transplantations).⁴⁵

From the start of the century until 2019, a clear increase in living (un)related kidney donation is observed in the Netherlands, with a slight decrease in the past four years. In the same time period, a shift from donation after brain death (DBD) to donation after circulatory death (DCD) is seen, without signs for a stagnation of this trend in the past years (Figure 7).⁴⁶ In the same time period, the mean age of deceased donors in The Netherlands increased, following the gradual ageing of the population. In 2000, the vast majority (73%) of deceased donors for kidney transplantation was younger or equal to 55 years-of-age, compared to 46% in 2019. Where deceased donors in the age group 65 and above were scarce at the start of this century, they now make up 30% of the total number of deceased kidney donors (Figure 8).⁴⁶ Also, the use of expanded criteria deceased donors (older or equal to 60 years-of-age; in the age group 50 to 59 with vascular comorbidities) increased due the ongoing shortage of organ donors.

Not only deceased donor demographics changed, kidney transplant recipients became older and critical comorbidities became increasingly common. Where 68% of deceased donor kidney transplant recipients was younger or equal to 55 years-of-age, this was only 46% in 2019. This trend is fuelled by the increase in transplant recipients in the age group 65 and above, going from 9% in 2001 to 30% in 2019 (Figure 9).⁴⁶ With the improvement of patient survival after transplantation, the need for re-transplantation after chronic transplant failure increased. The number of patients listed for re-transplantation went from 81 in 2000 to 230 in 2019.⁴⁶ For comorbidities prior to transplantation, an ongoing high prevalence of traditional risk factors is observed, with diabetes mellitus, hypertension, hypercholesterolemia, and smoking as the most prevalent.⁴⁷ For transplant candidate-specific risk factors for cardiovascular disease, for example prolonged dialysis burden, chronic kidney disease–mineral bone disorder (CKD-MBD) and hyperparathyroidism, a similar high prevalence is seen.^{48,49} For the period after transplantation, transplant recipient-specific risk factors (i.e. immunosuppressive therapy and progressive

uraemia) impose an ongoing cardiovascular risk. Combined, these factors contribute to atherosclerosis in the pre- and post-transplant situation, resulting in a high incidence of coronary artery disease and a high degree of calcification of the aorto-iliac vascular trajectory. This is of utmost clinical importance, as cardiovascular disease is the leading cause of mortality with a functioning kidney allograft.^{50,51}

Figure 7. Yearly rate of kidney transplantations per donor type (donation after brain death; donation after circulatory death; and living (un)related donation) for the period 2000 until 2019, in The Netherlands (smooth curve, including 95% confidence interval). Data from the *Nederlandse Transplantatie Stichting*.⁴⁶

When summarizing these trends for kidney donation, we see more DCD donors, donors with a higher age, and donors with more comorbidities. These trends have an impact on kidney transplantation outcomes, with lower one-year graft function and graft survival rates for kidneys from older donors compared to kidneys from younger donors, especially in case of DCD donation.^{52,53} Also, delayed graft function (DGF) showed to be an important factor in DCD kidneys, with a lower overall and death-censored graft survival in DCD kidney transplant recipients, compared to kidneys without DGF.⁵⁴ For transplant recipients, we observe increased transplantation of older recipients, with more comorbidities. This increase in recipients' age is associated with inferior patient survival.⁵⁵ These older recipients are more likely to have traditional and transplant candidate-specific risk factors for cardiovascular disease. In combination with post-transplantation risk factors for cardiovascular disease, pre-transplantation traditional and transplant candidate-specific factors negatively influence vascular and kidney allograft calcification. Together with recipient age, this can contribute to inferior patient and transplant survival.⁵⁶⁻⁵⁸

Figure 8. Percentage of deceased donor kidney transplantations per donor age group for the period 2000 until 2019, in The Netherlands. For the year 2000, donations rates were not separately addressed for the age group 65 and above. Data from the *Nederlandse Transplantatie Stichting*.⁴⁶

Figure 9. Percentage of new patients registered on the waiting list for deceased donor kidney transplantation per patient age group for the period 2000 until 2019, in The Netherlands. For the year 2000, donations rates were not separately addressed for the age group 65 and above. Data from the *Nederlandse Transplantatie Stichting*.⁴⁶

OUTLINE OF THIS THESIS

This thesis comprises studies on pre- and post-transplantation diagnostic imaging for kidney function, kidney calcification, and aorto-iliac artery calcification assessment. The preface contains the highlights of four publications on the academic developments in kidney transplantation research. This includes a bibliometric analysis on the trends in publications on imaging and kidney transplantations; a study on gender disparities in authorships and citations in transplantation research; an overview on the impact of Brexit on transplantation research; and a letter on the role of young transplant professionals in guiding the future of solid organ transplantation. *Part I* contains a systematic review and the results of two cohort studies on the assessment of early post-transplant kidney function with renal scintigraphy. These studies provide guidance for the use of renal scintigraphy after kidney transplantation, covering the diagnosis of vascular and urological complications and prediction of early transplant outcomes, including duration of DGF and length of hospital stay. *Part II* contains the results of studies focusing on kidney and vascular calcification. Kidney calcification was studied in a pre-clinical project on non-invasive imaging for the identification of microcalcifications in active nephrocalcinosis. Using the positron emission tomography tracer [^{18}F]-sodium fluoride ([^{18}F]-NaF), known for the visualization of vascular (micro)calcification, a pre-clinical study was performed with donor kidneys and explanted kidney allografts samples. The assessment of aorto-iliac artery calcification is described in studies using dual-energy X-ray absorptiometry (DXA) and non-contrast enhanced CT. The abdominal aortic calcification score, assessed by DXA after kidney transplantation, was used to identify patients at risk for cardiovascular events and to examine the evidence for a bone-vascular axis after kidney transplantation. The aorto-iliac CaScore, assessed by non-contrast enhanced CT prior to kidney transplantation, was used to identify patients at risk for early (cardiovascular) death and to assess if aorto-iliac artery calcification influences kidney graft outcomes, including one-year graft function and graft failure.

Acknowledgments

We thank the following authors and publishers for the kind permission to reproduce several figures: Figure 2 originates from Dempster WJ. Observations on the behaviour of the transplanted kidney in dogs. *Ann R Coll Surg Engl*. 1950;7(4):275-302.; Figure 3 and Figure 3 originate from Murray JE, Merrill JP, Harrison JH. Kidney transplantation between seven pairs of identical twins. *Ann Surg*. 1958;148(3):343-359.; Figure 5 originates from Collins JJ, Wilson RE. Functional Evaluation of Human Kidney Transplants with Renograms. *Ann Surg*. 1965;161(3):428-440.; and Figure 6 originates from Petrek J, Tilney NL, Smith EH, Williams JS, Vineyard GC. Ultrasound in Renal Transplantation. *Ann Surg*. 1977;185(4):441-447.

REFERENCES

1. Fracchia C, de Jong J, Santing C. *One Leg in the Grave Revisited: The Miracle of the Transplantation of the Black Leg by the Saints Cosmas and Damian*. (Zimmerman K, ed.). Maarssen : Barkhuis; 2013.
2. Nagy J. A Note on the Early History of Renal Transplantation: Emerich (Imre) Ullmann. *Am J Nephrol*. 1999;19(2):346-349.
3. Hamilton D. *The First Transplant Surgeon: The Flawed Genius of Nobel Prize Winner, Alexis Carrel*. WORLD SCIENTIFIC; 2016.
4. Sade RM. Transplantation at 100 Years: Alexis Carrel, Pioneer Surgeon. *Ann Thorac Surg*. 2005;80(6):2415-2418.
5. Carrel A, Guthrie CC. Successful transplantation of both kidneys from a dog into a bitch with removal of both normal kidneys from the latter. *Science*. 1906;23(584):394-395.
6. Matevossian E, Kern H, Hüser N, et al. Surgeon Yuri Voronoy (1895-1961) - a pioneer in the history of clinical transplantation: in Memoriam at the 75th Anniversary of the First Human Kidney Transplantation. *Transpl Int*. 2009;22(12):1132-1139.
7. Khadzhyunov D, Peters H. History of nephrology: Ukrainian aspects. *Kidney Int*. 2012;81(1):118.
8. Dempster WJ. Observations on the behaviour of the transplanted kidney in dogs. *Ann R Coll Surg Engl*. 1950;7(4):275-302.
9. Barr J, Cherry KJ, Rich NM. Vascular Surgery in World War II: The Shift to Repairing Arteries. *Ann Surg*. 2016;263(3):615-620.
10. Starzl TE. History of clinical transplantation. *World J Surg*. 2000;24(7):759-782.
11. Lawler RH, West JW, McNulty PH, Clancy EJ, Murphy RP. Homotransplantation of the kidney in the human. *J Am Med Assoc*. 1950;144(10):844.
12. Lawler RH, West JW, McNulty PH, Clancy EJ, Murphy RP. Homotransplantation of the kidney in the human: Supplemental report of a case. *J Am Med Assoc*. 1951;147(1):45.
13. Merrill JP. Successful homotransplantation of the human kidney between identical twins. *J Am Med Assoc*. 1956;160(4):277.
14. Murray JE, Merrill JP, Harrison JH. Kidney transplantation between seven pairs of identical twins. *Ann Surg*. 1958;148(3):343-359.
15. Calne RY, Alezandre GP, Murray JE. A study of the effects of drugs in prolonging survival of homologous renal transplants in dogs. *Ann N Y Acad Sci*. 1962;99(3):743-761.
16. Merrill JP. Successful Transplantation of Kidney from a Human Cadaver. *J Am Med Assoc*. 1963;185(5):347.
17. Palmer JM, Kountz SL, Swenson RS, Lucas ZJ, Cohn R. Urinary Tract Morbidity in Renal Transplantation. *Arch Surg*. 1969;98(3):352.
18. Fung JJ. Obituary of Thomas E. Starzl, MD, PhD. *Am J Transplant*. 2017;17(5):1153-1155.
19. Starzl TE, Groth CG, Putnam CW, et al. Urological complications in 216 human recipients of renal transplants. *Ann Surg*. 1970;172(1):1-22.
20. Starzl TE, Porter KA, Andres G, et al. Long-term survival after renal transplantation in humans: (With Special Reference to Histocompatibility Matching, Thymectomy, Homograft Glomerulonephritis, Heterologous ALG, and Recipient Malignancy). *Ann Surg*. 1970;172(3):437-472.
21. van Saase JL, Mallat MJ, van der Woude FJ, van Bockel JH, van Es LA. [Results of kidney transplantation in Leiden, 1966-1994, and prognostic factors]. *Ned Tijdschr Geneesk*. 1996;140(15):827-832.
22. Riesz PB. The life of Wilhelm Conrad Roentgen. *Am J Roentgenol*. 1995;165(6):1533-1537.
23. Strauss HW, Zaret B, Pieri G, Lahiri A. History corner: Antoine Henri Becquerel. *J Nucl Cardiol*. 2017;24(5):1515-1516.

24. Mould RF. Marie and Pierre Curie and radium: History, mystery, and discovery. *Med Phys.* 1999;26(9):1766-1772.
25. Macalpine JA. Instrumental pyelography and ureterography. In: *Cystoscopy and Urography*. Elsevier; 2013:387-423.
26. Swick M. The discovery of intravenous urography: historical and developmental aspects of the urographic media and their role in other diagnostic and therapeutic areas. *Bull NY Acad Med.* 1966;42(2):128.
27. Beer E. Uroselectan intravenous urography. *Ann Surg.* 1930;92(4):761.
28. Gilmer PJ. Irène Joliot-Curie, a Nobel Laureate in Artificial Radioactivity. In: *Celebrating the 100th Anniversary of Madame Marie Sklodowska Curie's Nobel Prize in Chemistry*. Rotterdam: SensePublishers; 2011:41-57.
29. Anderson CJ, Ling X, Schlyer DJ, Cutler CS. A Short History of Nuclear Medicine. In: *Radiopharmaceutical Chemistry*. Cham: Springer International Publishing; 2019:11-26.
30. Richards P, Tucker WD, Srivastava SC. Technetium-99m: An historical perspective. *Int J Appl Radiat Isot.* 1982;33(10):793-799.
31. Collins JJ, Wilson RE. Functional Evaluation of Human Kidney Transplants with Renograms. *Ann Surg.* 1965;161(3):428-440.
32. Al-Nahhas AA, Jafri RA, Britton KE, et al. Clinical experience with 99mTc-MAG3, mercaptoacetyl triglycine, and a comparison with 99mTc-DTPA. *Eur J Nucl Med.* 1988;14(9-10):453-462.
33. Lewis DH, Bergentz SE, Brunius U, Erman H, Gelin LE, Hood B. Value of Renal Blood Flow Measurement with Xenon-133 at the Time of Kidney Transplantation. *Ann Surg.* 1967;166(1):65-74.
34. Richmond C. Sir Godfrey Hounsfield. *BMJ.* 2004;329(7467):6871.
35. Newman PG, Rozycki GS. The history of ultrasound. *Surg Clin North Am.* 1998;78(2):179-195.
36. Petrek J, Tilney NL, Smith EH, Williams JS, Vineyard GC. Ultrasound in Renal Transplantation. *Ann Surg.* 1977;185(4):441-447.
37. Nicholson ML, Williams PM, Bell A, Donnelly PK, Veitch PS, Bell PRF. Prospective study of the value of ultrasound measurements in the diagnosis of acute rejection following renal transplantation. *Br J Surg.* 1990;77(6):656-658.
38. Sagel SS, Stanley RJ, Levitt RG, Geisse G. Computed Tomography of the Kidney. *Radiology.* 1977;124(2):359-370.
39. Kittredge RD, Brensilver J, Pierce JC. Computed Tomography in Renal Transplant Problems. *Radiology.* 1978;127(1):165-169.
40. Letourneau JG, Day DL, Feinberg SB. Ultrasound and computed tomographic evaluation of renal transplantation. *Radiol Clin North Am.* 1987;25(2):267-279.
41. Rashidi P, Bihorac A. Artificial intelligence approaches to improve kidney care. *Nat Rev Nephrol.* 2020;16(2):71-72.
42. Schutter R, Lantinga VA, Borra RJH, Moers C. MRI for diagnosis of post-renal transplant complications: current state-of-the-art and future perspectives. *Magn Reson Mater Physics, Biol Med.* 2020;33(1):49-61.
43. Saez-Rodriguez J, Rinschen MM, Floege J, Kramann R. Big science and big data in nephrology. *Kidney Int.* 2019;95(6):1326-1337.
44. van Gijn J, Gijssels JP, Nurmohamed SA. [Kolff and the artificial kidney]. *Ned Tijdschr Geneesk.* 2013;157(16):A5711.
45. Berger S, Sanders J, Pol R, de Kleijn R. [Niertransplantatie: Jaarverslag 2019, Universitair Medisch Centrum Groningen].
46. Nederlandse Transplantatie Stichting (NTS). [Jaarverslagen 2000 - 2019].
47. Jardine AG, Gaston RS, Fellstrom BC, Holdaas H. Prevention of cardiovascular disease in adult recipients of kidney transplants. *Lancet.* 2011;378(9800):1419-1427.
48. Gomes-Neto AW, Osté MCJ, Sotomayor CG, et al. Mediterranean Style Diet and Kidney Function Loss in Kidney Transplant Recipients. *Clin J Am Soc Nephrol.* 2020;15(2):238-246.
49. Rangaswami J, Mathew RO, Parasuraman R, et al. Cardiovascular disease in the kidney

- transplant recipient: epidemiology, diagnosis and management strategies. *Nephrol Dial Transplant*. 2019;34(5):760-773.
50. Stoumpos S, Jardine AG, Mark PB. Cardiovascular morbidity and mortality after kidney transplantation. *Transpl Int*. 2015;28(1):10-21.
51. Ying T, Shi B, Kelly PJ, Pilmore H, Clayton PA, Chadban SJ. Death after Kidney Transplantation: An Analysis by Era and Time Post-Transplant. *J Am Soc Nephrol*. September 2020:ASN.2020050566.
52. Pippas M, Jager KJ, Åsberg A, et al. Young deceased donor kidneys show a survival benefit over older donor kidneys in transplant recipients aged 20-50 years: a study by the ERA-EDTA Registry. *Nephrol Dial Transplant*. 2020;35(3):534-543.
53. Peters-Sengers H, Berger SP, Heemskerk MBA, et al. Stretching the Limits of Renal Transplantation in Elderly Recipients of Grafts from Elderly Deceased Donors. *J Am Soc Nephrol*. 2017;28(2):621-631.
54. Lim WH, McDonald SP, Russ GR, et al. Association Between Delayed Graft Function and Graft Loss in Donation After Cardiac Death Kidney Transplants-A Paired Kidney Registry Analysis. *Transplantation*. 2017;101(6):1139-1143.
55. Karim A, Farrugia D, Cheshire J, et al. Recipient Age and Risk for Mortality After Kidney Transplantation in England. *Transplantation*. 2014;97(8):832-838.
56. Jardine AG, Gaston RS, Fellstrom BC, Holdaas H. Prevention of cardiovascular disease in adult recipients of kidney transplants. *Lancet*. 2011;378(9800):1419-1427.
57. Wu DA, Robb ML, Forsythe JLR, et al. Recipient Comorbidity and Survival Outcomes After Kidney Transplantation. *Transplantation*. 2020;104(6):1246-1255.
58. Sotomayor CG, Velde-Keyzer CA te, de Borst MH, Navis GJ, Bakker SJL. Lifestyle, Inflammation, and Vascular Calcification in Kidney Transplant Recipients: Perspectives on Long-Term Outcomes. *J Clin Med*. 2020;9(6):1911.

A broader view on transplantation research

Adapted from:

Have we forgotten imaging prior to and after kidney transplantation?

European Radiology, 2018

Stan Benjamens, Andor W.J.M. Glaudemans,
Stefan P. Berger, Riemer H.J.A. Slart, Robert A. Pol

Gender disparities in authorships and citations in transplantation research

Transplantation Direct, 2020

Stan Benjamens, Louise B.D. Banning, Tamar A.J. van den Berg, Robert A. Pol

Brexit and Transplantation Research: EU Funding and Scientific Collaborations

Transplantation, 2020

Stan Benjamens, Tamar A.J. van den Berg, Vassilios Papalois, Frank J.M.F. Dor, Robert A. Pol

C4: The future of solid organ transplantation from the
perspective of young transplant professionals

American Journal of Transplantation, 2019

Stan Benjamens, Tamar A.J. van den Berg, Robert A. Pol

Background: The scientific field of kidney transplantation is a thriving academic community, with a high scientific productivity. However, several factors can negatively impact this scientific community, such as (1) the lack of interdisciplinary collaborations, (2) gender bias and (3) international developments causing uncertainty.

Methods: In three bibliometric analyses, we reviewed the past decades of kidney transplantation research. Large datasets of published articles were used to examine: (1) the collaboration between the field of medical imaging and kidney transplantation; (2) trends in first and last author gender and gender differences in citations and rewarded research funding; and (3) the potential impact of Brexit on transplantation research.

Results: (1) From the identified publications on kidney transplantation ($n = 31,001$), only a low number covered the field of imaging ($n = 1,730$, 5.6%). These publications on imaging and kidney transplantations are poorly cited and evidence-based recommendations for clinical use are lacking. (2) When reviewing author gender differences in this field, a gender gap was identified (36.2% female first and 30.2% female last authors), with an increase in the percentage of female authors in this 20-year time-period. Also, citation rates and rewarded funding showed to differ between female and male authors. (3) The United Kingdom (UK) receives 10.7% of transplantation research-related European Union (EU)-funding and UK-based researcher collaborate in almost half of all EU-funded projects.

Conclusions: These three publications provide an insight into the factors that can negatively impact scientific productivity and innovation of kidney transplantation. An active approach is required to promote interdisciplinary collaborations, to eliminate gender bias, and to protect the scientific community from negative international developments.

INTRODUCTION

In the past decades, outcomes after kidney transplantation have improved significantly, as shown by a 10.8% decline in 10-year graft failure for deceased donor transplantation between 1995 and 2005.¹ The driving force behind this improvement was a research community producing a large number of high-quality clinical trials and widely accepted clinical guidelines. However, in the research field of pre- and post-kidney transplantation medical imaging, guidelines mainly provide recommendations based on “low level of evidence” and “expert opinions” (Figure 1).^{2–6} New research and publications on imaging techniques for the evaluation of transplant recipients seem to have stagnated, limiting the development of adequate clinical guidelines.

In the same period, there has been a rapid change in the gender ratio of medical doctors, while gender differences in academia remain apparent.^{7–12} Fifty years ago, only 9% of the medical students in the United States were female. Nowadays, the number of female students exceeds the number of male students.¹³ This increase in female doctors is present in all medical specialties, including surgery. In 1980, almost one-quarter of the surgeons were female and since then it has increased up to 35%.¹⁴ Despite this growth, females remain underrepresented in transplantation research, and there is little data concerning changes in proportion of authorship, citation, and funding of female researchers over the past 20 years.

A more recent development is the United Kingdom (UK)’s impending departure from the European Union (EU), referred to as “Brexit”. It has been extensively argued that this will have a major impact on the National Health Service and the scientific community in the UK. The consequences of such a scenario are expected to be severe.^{15–18} Without an agreement on future cooperation and collaborations, prospects are that the UK will lose access to EU research funding programs, which could significantly weaken the British scientific community. With the above in mind, the consequences for the UK’s involvement in transplantation research, in both Europe and globally, remain uncertain. The current literature does not provide an analysis of the UK’s involvement in (transplantation) research projects and scientific publications, or on the consequences of global research collaborations.^{19,20}

In three publications, we focused on factors that can negatively impact this scientific community. The studied topics are: (1) collaboration between the field of medical imaging and the field of kidney transplantation; (2) gender disparities in authorships and citations in transplantation research; and (3) the impact of Brexit on transplantation research. These topics were studied using large publicly available datasets with information on scientific publications, citations, research funding, and first name gender-matching.

Technique	Application	Guidelines
Ultrasound	Renal and cardiac screening	Expert opinion
	Assesment of allograft dysfunction	Intermediate level of evidence
	Annual transplant assesment	Expert opinion
Magnetic Resonance Imaging	Assesment of transplant oxygenation	Not mentioned
Computed Tomography	CT Angiography prior to transplant	Low level of evidence
	Abdominal CT prior to transplant	Not mentioned
Nuclear Medicine	Myocardial perfusion scan	Low level of evidence
	Renal Scintigraphy for allograft function	Not mentioned
	Bone Mineral Density measurement	Low level of evidence

Figure 1. Kidney transplantation guideline recommendations on the use of pre- and post-transplant imaging techniques. Based on recommendations from the following guidelines: (I) European Renal Best Practice (ERBP), (II) Kidney Disease Improving Global Outcome (KDIGO), (III) Kidney Transplantation, European Association of Urology (EAU), (IV) Management of the Failing Kidney Transplant, British Transplantation Society, (V) Post-operative Care in the Kidney Transplant Recipient, The Renal Association.²⁻⁶

METHODS

In three bibliometric analyses, we reviewed the past decades of kidney transplantation research. The information for the bibliometric datasets was extracted from the Web of Science database. For the first topic, articles from the journal categories: 'Transplantation', 'Surgery', 'Urology and Nephrology', 'Radiology', and 'Nuclear Medicine' were extracted for the period 01-01-1996 to 12-31-2016. For the second and third project, article matching the search term '*transplant**' were used in the period 01-01-1999 until 12-31-2018. These original articles had to match the scientific fields 'Transplantation', 'Surgery', 'Immunology', 'Urology Nephrology', 'Gastroenterology Hepatology' or 'Medicine General Internal', and originate from high impact journals (defined as the 1st quartile (Q1) journals in a field).

For topic 1, results were subsequently grouped into four categories: (I) Total number of kidney transplantation publications in clinical journals, (II) number of ultrasonography (US), magnetic resonance imaging (MRI), computed tomography (CT) and nuclear medicine imaging (NM) publications in imaging journals, (III) number of US, MRI, CT and NM publications on kidney transplantation in clinical journals, and (IV) number of kidney transplantation publications in imaging journals.

For topic 2, the software R-Package 'gender' package, a well-established method for first name gender extraction, was used to predict author gender using international databases with name-gender matches: the U.S. Social Security Administration baby name database; the U.S. Census database; the North Atlantic Population Project; and the Kantrowitz corpus of male and female names.^{21,22} Publications for which only initials of first names were published (n = 16,372) and publications for which authors' names were not included in the international databases were excluded from the analyses (n = 1,265). The analyses were performed for first and last authors, with sub-analyses for the USA and Europe, for authors from the top-10 countries based on scientific productivity, and for publications grouped by number of citations.

For topic 3, additional information on research funding was extracted from the Publications Office of the European Union (CORDIS) database.²³ The CORDIS database contains information from 1988, the year in which EU-wide scientific funding started, up to now. A CORDIS search was performed using the query "content type='project' AND ('transplantation') AND period=01-01-1988 to 25-03-2019" at March 25, 2019. Data of 877 projects was extracted and screened for the sole inclusion criterion "projects focusing on solid organ transplantation". All data was analysed using R: A Language and Environment for Statistical Computing, version 3.5.2 (R Foundation for Statistical Computing, Vienna, Austria), with the software R-Packages 'bibliometrix', 'gender', and 'bib2df'.

RESULTS

Topic 1. Have we forgotten imaging prior to and after kidney transplantation?

In the last 21 years, 31,001 kidney transplantation publications were issued in clinical journals, 1730 (5.6%) of which focused on imaging (803 on US, 288 on MRI, 528 on CT, 111 on NM). In the same period 216,661 publications were issued in imaging journals, with 642 (0.3%) reporting on radiological or nuclear imaging and kidney transplantation (210 on US, 181 on MRI, 163 on CT, 88 on NM).

The yearly publication rates within the field of kidney transplantation showed an average increase of 2.3% per year, which is much lower than the yearly increase of 14.8% in the total field of imaging (Figure 2).

While the number of imaging publications in clinical journals showed an average annual increase rate of 6.7% (US 3.2%, MRI 2.1%, CT 39.0%, NM 6.3%), their share of the total number of kidney transplantation publications in clinical journals only rose from 5.6% to 6.5% in 21 years (US 2.4% to 2.7%, MRI 0.9% to 0.9%, CT 0.5% to 2.5%, NM 0.3% to 0.4%). The number of publications on kidney transplantation in imaging journals increased with 4.8% for US, 9.3% for MRI, 5.9 for CT, and decreased with 0.4% for NM, while their proportion dropped from 0.5% to 0.3% in this same period (US 0.7% to 0.5%, MRI 0.3% to 0.2%, CT 0.3% to 0.2%, NM 0.7% to 0.2%).

Topic 2. Gender disparities in authorships and citations in transplantation research

Based on first author gender matching, a total of 15,498 publications were included. From the identified first authors, 5,605 (36.2%) were female and 9,893 (63.8%) male. With gender matching for the last authors, 13,345 name-gender matches were identified, with 4,032 (30.2%) female and 9,313 (69.8%) male authors. An analysis of the data according to year demonstrated an increase in the percentage of female first and last authors in the period 1999 – 2018 (Figure 3). Data for first authors based in the USA and Europe showed a similar trend, with an increase in the percentage of female authors in the past 20 years.

Figure 2. Publications rates on semi-logarithmic scale for the imaging techniques (I) ultrasonography (US), (II) magnetic resonance imaging (MRI), (III) computed tomography (CT) and (IV) nuclear medicine imaging (NM). Showing publications on imaging and kidney transplantation (KTX) separately for clinical and imaging journals, compared to publication rates of total KTX publications in clinical journals and total US/CT/MRI/NM publications in imaging journals.

Figure 3. Percentage of first and last author publications by authors' gender for the years 1999 until 2018.

Overall, female first authors received significantly fewer citations compared to their male colleagues, with median 13 [6, 29] and median 14 [6, 32] ($p < 0.001$) citations per single publication respectively. When stratifying first author publications based on the number of citations, a decline was seen in the percentage of female authors, from 34.6% in the first group (≤ 10 citations) to 20.8% in the fifth group (> 200 citations) ($p < 0.001$). Percentages for all groups are shown in Table 1 and a similar trend was observed for last authors. From all first author name-gender matches, a total of 6,574 (42.4%) publications reported external funding. From these publications, 2,732 (41.6%) had female first authors and 3,842 (58.4%) had male first authors ($p < 0.001$). A total of 823 (5.3%) reported funding by pharmaceutical companies, of which 292 (35.5%) by female first authors and 531 (64.5%) by male first authors ($p = 0.701$). From USA based authors, 1,266 reported funding by the NIH. From these publications, 463 (36.6%) had female first authors and 803 (63.4%) had male first authors ($p < 0.001$).

Topic 3. Brexit and Transplantation Research: EU Funding and Scientific Collaborations

The data of EU-funding for transplantation research clearly shows the dependence of the UK transplant community on EU-funding: From 1988 to 2019, 135 projects focusing on transplantation research received EU-funding, with a total budget of €292 million. The 4 largest EU countries, based on population size, received the largest part of the funding budget, with 26.8% (€78.3 million) going to Germany, 17.1% (€49.8 million) to France, 12.0% (€35.0 million) to Italy, and 10.7% (€31.4 million) supporting UK research. Stratifying projects based on coordinating countries show that the UK is one of the

5 leading coordinators of EU funded projects on transplantation research (Figure 4) (France 17.8%, Germany 14.8%, UK 14.1%, Spain 10.4%, and Italy 9.6%). These numbers indicate the strong link among EU-member states, with the involvement of UK-based researchers in 47.4% of the EU-funded projects (14.1% as coordinating country and 33.3% and participating country). Participation of UK-based researchers was mostly seen in projects coordinated by Germany (28.9%), France (22.2%), and The Netherlands (13.3%) (Figure 5).

Table 1. Number of publications and citation by gender

First author	Total	Female	Citations	Male	Citations	p-values
Total	15,498	5,605 (36.2)	13 [6, 29]	9,893 (63.8)	14 [6, 32]	< 0.001^a
By citation groups						< 0.001^c
≤ 10	6135 (39.6)	2124 (34.6)	-	4011 (65.4)	-	
11 – 50	7483 (48.3)	2532 (33.8)	-	4951 (66.2)	-	
51 – 100	1415 (9.1)	416 (29.4)	-	999 (70.6)	-	
101 – 200	351 (2.3)	100 (28.5)	-	251 (71.5)	-	
> 200	114 (0.7)	24 (20.8)	-	90 (79.2)	-	
External funding	6,574 (42.4)	2,732 (41.6)	-	3,842 (58.4)	-	< 0.001^b
Funding by pharmaceutical companies	823 (5.3)	292 (35.5)	-	531 (64.5)	-	0.701 ^b
Funding by NIH	1,266 (8.2)	463 (36.6)	-	803 (63.4)	-	< 0.001^b

Numbers (%) and median [interquartile range]. Statistical difference in ^a number of citations by Wilcoxon's rank-sum test, ^b number of male and female authors by student t-test and ^c differences for citation group by chi-square test. P-values < 0.05 are significant (presented in bold)

When assessing the scientific output in the transplantation community, the United States has been the largest contributor (17 277 publications) followed by Germany (3179 publications), with the UK ranked third (2766 publications). Approximately 20% of scientific publications by UK primary authors are the result of an international collaboration. When reviewing these international collaborations, the United States (n = 306, 7.6%), Denmark (n = 139, 3.4%), Germany (n = 121, 3.0%), The Netherlands (n = 113, 2.8%), and France (n = 102, 2.5%) were identified as the most relevant scientific allies of UK-based authors, 4 of which are part of the EU.

Figure 4. Percentage of EU-funded projects by coordinating country.

Figure 5. EU-funded projects and coordination/participation by UK-based researchers.

DISCUSSION

Topic 1. Have we forgotten imaging prior to and after kidney transplantation?

While innovative techniques and scientific productivity in the field of medical imaging in general have steadily increased, the contribution of medical imaging around kidney transplantation to this general increase is neglectable. Development of new imaging techniques for the evaluation of transplant recipients have not succeeded in contributing to significant changes in clinical practice or development of clinical guidelines. The steady increase in imaging publications has only had a small effect on publications in clinical journals. Compared to other techniques, publications on CT pre- and post-transplantation have contributed importantly to this increase. Most likely a higher cardiovascular burden among kidney transplant recipients has led to increased abdominal and cardiac CT imaging in order to determine and lower perioperative mortality risk.²⁴ As the rate of general imaging publications has grown extensively in the past 10 years, the share of publications on kidney transplantation has decreased. Interestingly, NM imaging went from being the highest contributor of scientific publications in the field of kidney transplantation to being the smallest. Although there is a reduced but steady number of publications on renal scintigraphy, publications on new and innovative kidney transplant positron emission tomography (PET) tracers, a field with significant clinical potential, are scarce. Citation rates show a similar trend, with a surge for all included publications, but a lower rate for publications combining imaging and kidney transplantation. Studies focusing on PET/CT for the diagnosis of acute allograft rejection showed significant associations in both acute rat kidney allograft models and a clinical study, while the sensitivity remained too low for clinical applications.²⁵⁻²⁸ As the number of patients on waiting lists for transplantation rises, the proportion of extended-criteria donors has been rising.²⁹ Development of imaging techniques for graft evaluation and follow-up could contribute to a more reliable graft assessment. Several studies from the research group of Hueper et al., using a kidney transplantation animal model, have shown promising results for the use of MR after transplantation.³⁰⁻³³ This same research group and several others have initiated studies to translate these outcomes to the clinical setting, showing significant associations between kidney allograft function and several MRI sequences in small and heterogeneous cohorts.³⁴⁻³⁸ These outcomes have not yet been validated in a larger and more homogenous population of kidney transplant recipients and have therefore not yet been introduced to clinical protocols for post-transplant follow-up.

Topic 2. Gender disparities in authorships and citations in transplantation research

This first publication focusing on gender disparities in transplantation research shows that female authors remain underrepresented in transplantation research, with large differences in gender ratios between countries. While reporting less external funding, female first and last authors are especially underrepresented in the share of highly cited publications (> 200 citations). The *Transplantation Society of Australia and New Zealand* summarized several societal and academic factors contributing to the difficulties faced by females in academia, with a focus on transplantation research.³⁹ Earlier in their career, female students face a pervasive bias due to undervaluation of their scientific capabilities⁴⁰. This clear bias towards favouring male colleagues persists throughout their career, with less than a quarter of all professors on medical schools in the USA being female.⁴¹ In a survey among members of the *American Society of Transplant Surgeons*, male transplant surgeons had a higher median number of total (83 versus 26) and first author (23 versus 16) publications. Regarding lifestyle factors, both genders reported the same amount of 70 work hours per week, with 14 days a month on call. The hours per week devoted to childcare differed substantially between the sexes (median 10 for females versus median 4 for males).⁴² Over the past years, it became clear that a *controllable lifestyle*, which means a “control of work hours”, plays a major role in the career specialty preferences.⁴³ In the field of transplantation, there is a so-called *incontrollable lifestyle* given the relative high amount of on calls and work-hours.^{44,45}

The gender ratio for authors presented in this study showed a higher percentage of female authors (35.9%) compared to previous studies in other fields of medicine. A previous analysis of female author representation in high impact medical journals showed a plateaued female representation between 1994 and 2014, with even a decrease in some journals.⁴⁶ Consistent with the literature, this study found that female researchers were less likely to receive external funding for their scientific work. A previous study, reporting on a cross-sectional database analysis of medical schools in the USA, showed that females received less NIH funding compared to their male colleagues (6.8% versus 11.3%).⁴¹ With data on public and philanthropic cancer research funding awarded to UK institutions showing that female principle investigators received 31.0% of the funding budget compared to 69.0% for male principle investigators.⁴⁸ This study supports earlier findings with regard to differences in citation rates between female and male authors. Female authors are especially underrepresented in the share of highly cited publications (> 200 citations). The landmark publication by Larivière et al. in *Nature* reported the results of a global, cross-disciplinary bibliometric analysis, and provided first-time strong evidence of fewer citations for publications with female authors in dominant author positions.¹¹ A recent publication in *The BMJ* by Lerchenmueller et al. provides a partial explanation for the dominance of male authors with regard to citation rates.

With an extensive bibliometric analysis, this study showed that male authors present their results with more positive terms and stronger statements, which was associated with higher citation rates.⁴⁹ Overall, the disparity in citations rates can be explained by differences in presentation of research findings, self-promotion and underrepresentation of females in high impact research consortia.^{11,49} Therefore, we propose an important role for scientific journals and scientific meeting committees to actively approach women to write invited commentaries, sit on panels during conferences and lobby for equal pay and career opportunities. Diversity pledges from scientific journals, in which i.e. all-male panels are rejected and commitments to increase representation of women among editorial staff, peer-review and authors are made, actively help in narrowing the gap.⁵⁰

Topic 3. Brexit and Transplantation Research: EU Funding and Scientific Collaborations

Earlier publications on the effect of the dawning Brexit on international scientific collaborations and research output point to the high probability of a negative impact in the first years after Brexit.^{16–18} In 2017 and 2019, Fahy et al. provided an extensive analysis of 4 possible Brexit scenarios, with (1) a no-deal Brexit; (2) a withdrawal agreement; (3) the Northern Ireland Protocol's backstop, an option assuring that the border between the UK (Northern Ireland) and Ireland remains open; and (4) a political declaration on the future relationship between the UK and EU.^{17,18} Currently, we see three possible options: (1) a withdrawal agreement (deal); (2) no deal; or (3) a revoke of #50 (no Brexit), albeit the latter seems to be improbable. Current evaluations show that all variations of Brexit will have negative consequences for the UK's position in healthcare and health-related research, with the most detrimental effect in case of a no-deal Brexit. As the UK is a significant beneficiary of EU-funding, the UK government announced their efforts to guarantee EU-funding beyond the official Brexit date, at least up to the end of Horizon 2020.⁵² The UK government also announced to explore the possibility of creating an international research fund to fill the gap when EU-funding opportunities are lost after Brexit.⁵³ From a scientific perspective, allowing Britain to participate in the Horizon 2020's successor "Horizon Europe" as an "associated" country is expected to significantly limit Brexit's impact. The status of "associated" country would enable UK-based researchers to be part of European Research Council projects, a status currently held by non-EU countries such as Norway and Switzerland. However, a series of side-deals and compromises will be essential to soften the blow on research and innovation for both the UK and the EU. From a clinical perspective, Brexit could have a forceful impact on organ donation and transplantation in the UK. While international donation and transplantation societies are thriving, UK-based researchers could face difficulties when pursuing international collaborations.¹⁹ Shapey and co-workers detailed the areas of potential impact on clinical transplantation in case of a Brexit extending to (1) existing EU-wide legislation; (2) regulation and governance, with requirements and standards

of quality and safety for organs; (3) existing organ-sharing networks; (4) pan-European initiatives, including EU-funding for research and cross border initiatives to increase donation rates; (5) EU efforts to combat organ trafficking and transplant tourism; and (6) legal status for EU-citizens working as clinicians and/or researchers in UK transplant centres.²⁰

CONCLUSION

These three publications provide an insight into the factors that can negatively impact scientific productivity and innovation of kidney transplantation. An active approach is required to promote interdisciplinary collaborations, to eliminate gender bias, and to protect the scientific community from negative international developments. As addressed in a *Letter to the Editor* (AJT 2019), titled '*C4: The future of solid organ transplantation from the perspective of young transplant professionals*', young transplant surgeons have a key role to play in addressing the many academic questions in this field.⁵⁴⁻⁵⁶ When addressing the future of organ transplantation, the perspective of young transplant professionals is essential. Young transplant researchers, working as clinicians or as PhD candidates, can be closely involved in ground-breaking clinical trials or laboratory achievements. Above all, they can provide new insights in longstanding transplant-related hurdles, making their status as novices an exceptional advantage. While years of experience in this field has many advantages, a fresh set of eyes, not opinionated by prevailing dogmas, can be of additional value.

REFERENCES

- Hart A, Smith JM, Skeans MA, et al. OPTN/SRTR 2015 Annual Data Report: Kidney. *Am J Transplant.* 2017;17 Suppl 1(Suppl 1):21-116.
- Abramowicz D, Cochat P, Claas FHJ, et al. European Renal Best Practice Guideline on kidney donor and recipient evaluation and perioperative care. *Nephrol Dial Transpl.* 2015;30:1790-1797.
- Kidney Disease: Improving Global Outcomes (KDIGO) Transplant Work Group. KDIGO clinical practice guideline for the care of kidney transplant recipients. *Am J Transplant.* 2009;9(Suppl 3):S1-S157.
- Breda A, Olsburgh J, Budde K, Figueiredo A, García EL, Regele H. EAU guidelines on renal transplantation. Edn. presented at the EAU Annual Congress London 2017. 2017;2017(10-04).
- Andrews PA, Standards Committee of the British Transplantation Society. Summary of the British Transplantation Society Guidelines for Management of the Failing Kidney Transplant. *Transplantation.* 2014;98(11):1130-1133.
- Baker RJ, Mark PB, Patel RK, Stevens KK, Palmer N. Renal association clinical practice guideline in post-operative care in the kidney transplant recipient. *BMC Nephrol.* 2017;18(1):172-174.
- Jagsi R, Silver JK. Gender differences in research reporting. *BMJ.* 2019;367:16692.
- Jagsi R, Guancial EA, Worobey CC, et al. The “Gender Gap” in Authorship of Academic Medical Literature — A 35-Year Perspective. *N Engl J Med.* 2006;355(3):281-287.
- Jena AB, Khullar D, Ho O, Olenski AR, Blumenthal DM. Sex Differences in Academic Rank in US Medical Schools in 2014. *JAMA.* 2015;314(11):1149-1158.
- Witteman HO, Hendricks M, Straus S, Tannenbaum C. Are gender gaps due to evaluations of the applicant or the science? A natural experiment at a national funding agency. *Lancet.* 2019;393(10171):531-540.
- Larivière V, Ni C, Gingras Y, Cronin B, Sugimoto CR. Global gender disparities in science. *Nature.* 2013;504(7479):211-213.
- Lerchenmueller MJ, Sorenson O, Jena AB. Gender differences in how scientists present the importance of their research: observational study. *BMJ.* 2019;367:16573.
- More Women Than Men Enrolled in U.S. Medical Schools in 2017 | AAMC. Association of American Medical Colleges.
- Lyons NB, Bernardi K, Huang L, et al. Gender Disparity in Surgery: An Evaluation of Surgical Societies. *Surg Infect (Larchmt).* 2019;20(5):406-410.
- Torjesen I. Four in 10 European doctors may leave UK after Brexit vote, BMA survey finds. *BMJ.* Published online 2017.
- Begum M, Lewison G, Lawler M, Sullivan R. The value of European immigration for high-level UK research and clinical care: cross-sectional study. *JR Soc Med.* 2019;112(1):29-35.
- Fahy N, Hervey T, Greer S, et al. How will Brexit affect health services in the UK? An updated evaluation. *Lancet.* 2019;393(10174):949-958.
- Fahy N, Hervey T, Greer S, et al. How will Brexit affect health and health services in the UK? Evaluating three possible scenarios. *Lancet.* 2017;390(10107):2110-2118.
- Neuberger J. Brexit and transplantation does anyone win? *Clin Transplant.* 2018;32(8):e13327.
- Shapey IM, Summers AM, Simkin IJ, Augustine T, van Dellen D. When politics meets science: What impact might Brexit have on organ donation and transplantation in the United Kingdom? *Clin Transplant.* 2018;32(8):e13299.
- Ruzycki SM, Fletcher S, Earp M, Bharwani A, Lithgow KC. Trends in the Proportion of Female Speakers at Medical Conferences in the United States and in Canada, 2007 to 2017. *JAMA Netw Open.* 2019;2(4):e192103.
- Abel GJ, Muttarak R, Bordone V, Zagheni E. Bowling Together: Scientific Collaboration

- Networks of Demographers at European Population Conferences. *Eur J Popul.* 2019;35(3):543-562.
23. European Commission. Publications Office of the European Union (CORDIS).
 24. Cianciolo G, Capelli I, Angelini ML, et al. Importance of Vascular Calcification in Kidney Transplant Recipients. *Am J Nephrol.* 2014;39(5):418-426.
 25. Grabner A, Kentrup D, Edemir B, et al. PET with ¹⁸F-FDG-Labeled T Lymphocytes for Diagnosis of Acute Rat Renal Allograft Rejection. *J Nucl Med.* 2013;54(7):1147-1153.
 26. Lovinfosse P, Weekers L, Bonvoisin C, et al. Fluorodeoxyglucose F 18 Positron Emission Tomography Coupled With Computed Tomography in Suspected Acute Renal Allograft Rejection. *Am J Transplant.* 2015;16(1):310-316.
 27. Reuter S, Schnockel U, Edemir B, et al. Potential of Noninvasive Serial Assessment of Acute Renal Allograft Rejection by ¹⁸F-FDG PET to Monitor Treatment Efficiency. *J Nucl Med.* 2010;51(10):1644-1652.
 28. Reuter S, Schnöckel U, Schröter R, et al. Non-invasive imaging of acute renal allograft rejection in rats using small animal F-FDG-PET. Zoccali C, ed. *PLoS One.* 2009;4(4):e5296.
 29. Aubert O, Kamar N, Vernerey D, et al. Long term outcomes of transplantation using kidneys from expanded criteria donors: prospective, population based cohort study. *BMJ.* 2015;351:h3557.
 30. Hueper K, Rong S, Gutberlet M, et al. T2 relaxation time and apparent diffusion coefficient for noninvasive assessment of renal pathology after acute kidney injury in mice: comparison with histopathology. *Invest Radiol.* 2013;48(12):834-842.
 31. Hueper K, Gutberlet M, Bräsen JH, et al. Multiparametric Functional MRI: Non-Invasive Imaging of Inflammation and Edema Formation after Kidney Transplantation in Mice. Joles JA, ed. *PLoS One.* 2016;11(9):e0162705.
 32. Hueper K, Schmidbauer M, Thorenz A, et al. Longitudinal evaluation of perfusion changes in acute and chronic renal allograft rejection using arterial spin labeling in translational mouse models. *J Magn Reson Imaging.* 2017;46(6):1664-1672.
 33. Hueper K, Hensen B, Gutberlet M, et al. Kidney Transplantation: Multiparametric Functional Magnetic Resonance Imaging for Assessment of Renal Allograft Pathophysiology in Mice. *Invest Radiol.* 2016;51(1):58-65.
 34. Hueper K, Khalifa AA, Bräsen JH, et al. Diffusion-Weighted imaging and diffusion tensor imaging detect delayed graft function and correlate with allograft fibrosis in patients early after kidney transplantation. *J Magn Reson Imaging.* 2016;44(1):112-121.
 35. Seif M, Eisenberger U, Binser T, et al. Renal Blood Oxygenation Level-dependent Imaging in Longitudinal Follow-up of Donated and Remaining Kidneys. *Radiology.* 2016;279(3):795-804.
 36. Ren T, Wen C-L, Chen L-H, et al. Evaluation of renal allografts function early after transplantation using intravoxel incoherent motion and arterial spin labeling MRI. *Magn Reson Imaging.* 2016;34(7):908-914.
 37. Fan W, Ren T, Li Q, et al. Assessment of renal allograft function early after transplantation with isotropic resolution diffusion tensor imaging. *Eur Radiol.* 2016;26(2):567-575.
 38. Peperhove M, Vo Chieu VD, Jang M-S, et al. Assessment of acute kidney injury with T1 mapping MRI following solid organ transplantation. *Eur Radiol.* 2018;28(1):44-50.
 39. Dwyer KM, Clark CJ, MacDonald K, et al. Gender Equity in Transplantation: A Report from the Women in Transplantation Workshop of The Transplantation Society of Australia and New Zealand. *Transplantation.* 2017;101(10):2266-2270.
 40. Moss-Racusin CA, Dovidio JF, Brescoll VL, Graham MJ, Handelsman J. Science faculty's subtle gender biases favor male students. *Proc Natl Acad Sci U S A.* 2012;109(41):16474-16479.

41. Jena AB, Khullar D, Ho O, Olenski AR, Blumenthal DM. Sex Differences in Academic Rank in US Medical Schools in 2014. *JAMA*. 2015;314(11):1149-1158.
42. Florence LS, Feng S, Foster CE, et al. Academic careers and lifestyle characteristics of 171 transplant surgeons in the ASTS. *Am J Transplant*. 2011;11(2):261-271.
43. Dorsey ER, Jarjoura D, Rutecki GW. Influence of controllable lifestyle on recent trends in specialty choice by US medical students. *JAMA*. 2003;290(9):1173-1178.
44. McCord JH, McDonald R, Levenson G, et al. Motivation to pursue surgical subspecialty training: is there a gender difference? *J Am Coll Surg*. 2007;205(5):698-703.
45. Iskander KN, Litle VR. Commentary: Parental Leave Policies: Have We Come a Long Way, Baby? *World J Surg*. Published online July 1, 2020.
46. Filardo G, da Graca B, Sass DM, Pollock BD, Smith EB, Martinez MA-M. Trends and comparison of female first authorship in high impact medical journals: observational study (1994-2014). *BMJ*. 2016;352:i847.
47. Witteman HO, Hendricks M, Straus S, Tannenbaum C. Are gender gaps due to evaluations of the applicant or the science? A natural experiment at a national funding agency. *Lancet*. 2019;393(10171):531-540.
48. Zhou CD, Head MG, Marshall DC, et al. A systematic analysis of UK cancer research funding by gender of primary investigator. *BMJ Open*. 2018;8(4):e018625.
49. Lerchenmueller MJ, Sorenson O, Jena AB. Gender differences in how scientists present the importance of their research: observational study. *BMJ*. 2019;367:l6573.
50. The Editors of the Lancet Group. The Lancet Group's commitments to gender equity and diversity. *Lancet*. Published online 2019.
51. Rimmer A. BMA repeats its call for second Brexit referendum. *BMJ*. Published online 2018.
52. HM Treasury, Department for Communities and Local Government, Department for Environment Food & Rural Affairs, Department for Work and Pensions, Department for Exiting the European Union D for BE& IS. Chancellor Philip Hammond Guarantees EU Funding Beyond Date UK Leaves the EU. Published online 2016.
53. Gibney E. UK considers post-Brexit research fund open to world. *Nature*. Published online March 2019.
54. Englesbe MJ, Lynch RJ, Sung RS, Segev DL. Time-trends in publication productivity of young transplant surgeons in the United States. *Am J Transplant*. 2012;12(3):772-778.
55. Englesbe MJ, Sung RS, Segev DL. Young Transplant Surgeons and NIH Funding. *Am J Transplant*. 2011;11(2):245-252.
56. Benjamens S, van den Berg TAJ, Pol RA. C4: The future of solid organ transplantation from the perspective of young transplant professionals. *Am J Transplant*. 2019;19(6):1867-1867.

Part I

Renal scintigraphy after kidney transplantation

Renal scintigraphy for post-transplant monitoring after kidney transplantation

Transplantation Reviews, 2018

Stan Benjamens
Stefan P. Berger
Andor W.J.M. Glaudemans
Jan-Stephan F. Sanders
Robert A. Pol
Riemer H.J.A. Slart

Background: Clinicians use several diagnostic modalities to recognize post-transplant complications, such as acute tubular necrosis, acute rejection, urologic and vascular complications. Currently, there is no consensus about the best procedural approach to evaluate post-transplant renal dysfunction. Renal needle-biopsy is often required; however, this is invasive and may lead to sample errors and complications, and most clinicians prefer using one of the noninvasive diagnostic modalities.

Methods: A systematic literature search was performed using PubMed, EMBASE, the Cochrane Library, MEDLINE (OvidSP), Web of Science, and Google Scholar to identify relevant articles. This review provides a literature overview of the technical aspects, new developments and clinical value of renal scintigraphy (RS), after kidney transplantation. Additionally, the advantages and limitations of RS in comparison to other diagnostic modalities are addressed. The study protocol is registered with PROSPERO, protocol number CRD42017078391.

Results: A total of 32 studies were included. Studies were categorized in the following groups: tracer pharmacokinetics; acute rejection and acute tubular necrosis; vascular complications; urological complications; postoperative fluid collections; early transplant outcomes; one-year transplant outcomes.

Conclusions: Several studies have described the use of RS for the diagnosis of acute rejection, however, differentiating between rejection and acute tubular necrosis remains difficult. For the diagnosis of vascular complications, RS has been described as an alternative for invasive procedures. For urologic complications, studies support the use of RS in combination with routine ultrasonography (US) surveillance. For the diagnosis of postoperative fluid collections, RS provides information to differentiate lymphoceles and urinomas. Altogether, RS should be considered in case of non-acute complications, and if US provides insufficient results.

INTRODUCTION

To recognize post-transplant complications, such as urologic and vascular complications, acute tubular necrosis (ATN) and acute rejection (AR), clinicians can choose from several diagnostic modalities. Currently, there is no consensus regarding the best procedural approach to evaluate post-transplant renal dysfunction. Renal needle-biopsy, which generally is considered the gold standard for de diagnosis AR, is often required. However, this is invasive and may lead to sample errors or complications. Most clinicians prefer using one of the noninvasive methods, such as ultrasonography (US), computed tomography (CT), magnetic resonance imaging (MRI) and renal scintigraphy (RS).

US is a noninvasive and non-nephrotoxic diagnostic test with relatively low costs. However, US results are difficult to interpret and showed to have a high inter-observer variability. CT provides detailed images of the renal transplant anatomy and the surrounding tissue but has limitations due to the nephrotoxic agents used. The use of MRI, for both anatomical and functional evaluation, has increased in the past years, but is mainly described as a second-line diagnostic modality.

RS, first introduced in 1965 for post-transplant assessment, is a non-invasive diagnostic test using radioactive isotopes (Figure 1). The most commonly used technique for RS is a dynamic planar procedure using a radioactive isotope which is imaged on a gamma camera, producing two-dimensional images. Radioactive isotopes (gamma ray-emitting radioactive nuclides) are used to visualize the physiological pathway from the renal artery to the urinary tract. This method is considered a sensitive diagnostic tool for the assessment of renal transplant dysfunction, urological and vascular complications, and postoperative fluid collections, particular in the early post-transplant period.

The aim of this review is to provide a literature overview of the technical aspects, new developments and clinical value of RS, after kidney transplantation. This review also addresses the advantages and limitations of RS in comparison with other imaging modalities.

Figure 1. Kidney transplant anatomy (left) and results of renal scintigraphy with Technetium-99m-labelled mercaptoacetyltri-glycine (^{99m}Tc -MAG₃)

LITERATURE SEARCH

A literature search with predefined search terms was performed using the following databases: PubMed (Biomedical research articles), Embase (Medicine and Pharmacology), the Cochrane Library (Systematic reviews and controlled trials), MEDLINE (OvidSP), Web of Science, and Google Scholar. To identify all relevant publications, we used a search strategy consisting of Medical Subject Headings (MeSH) and “Title and Abstract” (TiAb) terms: “kidney transplantation”, “renal transplantation”, “radioisotope renography”, “renal (transplant) scan”, “radionuclide imaging”, “renogram”, “technetium”, “Tc 99m and 99mTc mertiatide”, and “MAG3”. We used verified electronic search strategies, with database specific formats and the reference lists from the selected articles were manually checked for additional relevant studies. Specific search terms for each database are provided in Supplement 1. In Pubmed, the “related article” algorithm was employed to identify additional articles. Papers written in a non-English language, non-human trials, including < 30 patients, when no full text was available or only supplements were excluded (Figure 2). Additionally, we included the recommendations of the five most relevant guidelines for kidney transplantation.¹⁻⁵ The study protocol is registered with PROSPERO, protocol number CRD42017078391.

A total of 32 studies were included. Studies were categorized in the following groups: tracer pharmacokinetics; acute rejection and acute tubular necrosis; vascular complications; urological complications; postoperative fluid collections; early transplant outcomes; one-year transplant outcomes.

Figure 2. Flowchart, selection of studies.

RADIOPHARMACEUTICALS

Several radiopharmaceuticals are available for the assessment of renal function after kidney transplantation. Technetium-99m-labelled mercaptoacetyltriglycine ($^{99m}\text{Tc-MAG}_3$) is nowadays the most commonly used radiopharmaceutical.⁶ The high plasma protein binding capacity (90%) causes rapid excretion and a low percentage of irrelevant tracer distribution, leading to a high imaging quality.⁷ The second-most used tracer is technetium-99m diethylene-triamine-pentaacetic acid ($^{99m}\text{Tc-DTPA}$). $^{99m}\text{Tc-DTPA}$ is only filtered by glomerular filtration, making it very suitable for the direct measurement of the glomerular filtration rate (GFR).⁸ Despite the lower imaging quality and lower renal extraction fraction (20%) compared to $^{99m}\text{Tc-MAG}_3$ (40-50%), quantitative $^{99m}\text{Tc-DTPA}$ measurements correlate well with short and long-term transplant outcomes.⁹ Iodine-131 labelled o-iodohippuric acid ($\text{I}^{131}\text{-OIH}$) is a tubular excreted tracer, comparable to $^{99m}\text{Tc-MAG}_3$. $\text{I}^{131}\text{-OIH}$ is characterised by high energy emission and concomitant high radiation exposure.⁶ The maximum dose of $\text{I}^{131}\text{-OIH}$ is 30 times lower than $^{99m}\text{Tc-MAG}_3$ due to the long half-life time and high β -emission rate, resulting in a lower count rate and therefore lower imaging quality.⁶ The functioning renal proximal tubular mass and the cortical renal part are most optimally detected by Tc-99m-labelled dimercaptosuccinic acid ($^{99m}\text{Tc-DMSA}$).¹⁰ $^{99m}\text{Tc-DMSA}$ is found to have a high sensitivity for the detection of renal scarring and diffuse structural abnormalities, possibly related to increased tracer uptake in affected renal transplant parenchyma, and is generally used in combination with 3-D single-photon emission computed tomography (SPECT).¹¹

INTERPRETATION

RS is used to assess the structure, blood flow, and function of renal grafts, allowing the imaging of reduced flow, obstructions, or leaks. The various techniques of interpretation, being visual, qualitative and quantitative analysis, describe the three sequential phases of renal function: (I) The vascular phase, or flow study, shows the transit of radiotracer through the blood vessels; (II) the parenchymal or function phase is the period in which the nephrons extract the tracer from the blood and excrete it by glomerular filtration or tubular secretion; (III) the washout or excretory phase is the period during which the tracer drains through the renal pelvis to the bladder.

Visual interpretation

Visual inspection focuses on the arterial patency, perfusion of the native kidneys and excludes occlusion of the ureter, renal transplant artery and/or vein. Fast and high uptake is an indication of good perfusion and the absence of pre-renal complications, fast plasma clearance and parenchymal washout indicates normal tubular function, and rapid excretion from the pelvic region excludes complications in the post-renal structures.¹² Signs of hydronephrosis or progressive RS tracer activity surrounding the ureter indicate respectively urinary tract obstruction and urinary leaks. Some factors can hamper the visual interpretation of the scans. First, visualisation of urological complications can be difficult in dehydrated patients. Second, inspection can be difficult due to procedure artefacts, such as patients' motion, a wrong detector position, and a low dose of the used radiopharmaceutical. Third, the interpretation can be troubled by patient specific characteristics, such as renal transplant cysts and deviations from the standard vascular and urological post-transplant anatomy.

Qualitative assessment

Tracer uptake and perfusion is visualised using time-activity curves derived from graft regions of interest. Through the grading of these curves with the grading scale of Heaf and Iversen, renal function can be assessed qualitatively (Figure 3).¹³ Normal renal transplant function (grade 0) is characterised by a fast-rising curve, reaching its maximum within 5 minutes, and nearly complete excretion within thirty minutes. Reduced renal transplant function, as seen in a majority of renal transplants in the early post-operative period, is characterised by a reduced or absent excretion with normal perfusion (grade 1-2). A time-activity curve during ATN or AR shows a slowly rising curve without a downslope phase (grade 3-4). A minimal uptake of the tracer, as seen in grade 5, reflects a renal transplant with very limited function and/or perfusion.

Quantitative assessment

A quantitative analysis is preferable since it is less affected by inter-observer variability compared to a visual analysis with/without the use of a curve grading scale.¹⁴ Over the years, several methods have been introduced with varying degrees of success, due to lack of diagnostic power and the inability to distinguish AR from ATN.^{12,15} In general, most quantitative indices describe the extraction/uptake and excretion/clearance. The largest retrospective studies have focused on the use of *tubular function slope (TFS)*, reflecting the curves upslope, *MUC10*, representing kidney activity as a fraction of the injected dose, *R20/3*, dividing one-minute time intervals ending at 20 and 3 min, and *GI*, combining both time-based indices and counts-based indices (Table 1).^{16–19}

Figure 3. Renal scintigraphy grading scale. Grade 0: normal renal scintigraphy; Grade 1: normal uptake, reduced excretion; Grade 2: normal uptake, flat excretion curve; Grade 3: rising curve; Grade 4: reduced rate of uptake, rising curve and reduced absolute uptake; Grade 5: minimal uptake.

Table 1. Literature overview renal scintigraphy, grouped according to renal transplant end-points

End-point	First author (Ref)	Publication (Year)	Patients (number)	Index
AR / ATN	Yazici ¹⁵	2013	107	<i>R20/3</i> <i>RS Grade</i>
	El Maghraby ²⁰	1998	43	<i>HI</i> <i>K3/20</i>
	Bajen ²¹	1997	43	<i>MTT</i>
	Li ²²	1994	307	<i>R20/3</i>
	Gedroyc ²³	1986	43	<i>HI</i>
Early renal transplant outcome	Yazici ¹⁹	2016	179	<i>GI</i>
	Majima ²⁴	2012	31	<i>cTER</i>
	Obeidat ²⁵	2011	143	<i>RS Grade</i>
	El Maghraby ¹⁶	2002	42	<i>TFS</i>
	Stevens ¹⁷	2001	256	<i>MUC10</i>
	Heaf ¹³	2000	201	<i>RS Grade</i>
1-year renal transplant outcome	Yazici ¹⁹	2016	179	<i>GI</i>
	Yazici ²⁶	2015	111	<i>R20/3</i> <i>T1/2</i> <i>RS Grade</i>
	Gupta ²⁷	2012	214	<i>HI</i>
	Guignard ²⁸	2011	100	<i>TFS</i>
	Ngai ²⁹	2008	64	<i>HI</i>
	Russell ¹⁸	2000	200	<i>R20/3</i>

KTX - kidney transplantation ^aWorsened renal transplant function defined as sCr >1.5 mg/dL^b Renal transplant survival, number of functioning renal transplants

Days post KTX	Results (sensitivity / specificity)
2	93% / 73% for AR/ATN within 7days. 94% / 86% for AR/ATN within 7 days.
2	Significant difference in patients with slow graft function compared to patients with AR or ATN.
1-3	Significant lower uptake in case of AR/ATN
Suspicion of AR/ATN	Correlation with the clinically assessed severity of AR/ATN.
Suspicion of AR/ATN	No significant difference between AR and cyclosporine nephrotoxicity
2	86% / 86% for period of dialysis-dependence in first week.
4	Significant correlation with period of dialysis-dependence in first week.
1-2	86% / 84% for period of dialysis-dependence in first week.
1	Significant difference in patients with DGF at day 7 compared to patients with immediate renal transplant function.
2	Significant correlation with period of dialysis-dependence in first week.
1-21	76% sensitivity for 10% sCr rise within 2 days.
2	74% / 76% for 1-year renal transplant function. ^a
2	54% / 78% for 1-year renal transplant function. ^a 54% / 79% for 1-year renal transplant function. ^a 31% / 92% for 1-year renal transplant function. ^a
2	73% / 72% for 1-year renal transplant survival. ^b
4	88% / 59% for 1-year renal transplant survival. ^b
3	67% / 67% for 1-year renal transplant survival. ^b
3	90% / 36% for 1-year renal transplant function. ^a
3	Significant correlation with the 1y renal transplant survival.

ASSESSMENT OF COMPLICATIONS

Differentiating acute rejection from acute tubular necrosis

Differentiating between AR and ATN is important though challenging since AR will result in permanent damage if left untreated. Additionally, complicated by the fact that both entities can be present at the same time (Table 2).²⁵ In case of both AR and ATN, the perfusion, uptake, parenchymal transit, and excretion of the renal transplant are all impaired. Needle biopsy is considered the golden standard for evaluating morphologic changes in the renal transplant tissue and diagnosing causes of renal transplant dysfunction such as AR.³⁰ However, needle biopsy cannot always be performed due to several contraindications, such as uncontrolled hypertension, active renal or perirenal infection, hydronephrosis, and an INR ≥ 1.5 .³¹ Combined with a risk of major complications (need for intervention or intensive care admittance) between 1.1% - 1.9%, with a three times increased risk within the first week after transplantation.³¹⁻³³ Additionally, sample errors are seen in 0.5% of US-guided procedures.³⁴ A study including 43 transplant recipients with multiple RS procedures, showed a significant difference in quantitative RS indices between recipients with slow renal transplant function and recipients with renal transplant pathology (both AR and ATN).²⁰ However, quantitative RS analyses (*Hilson's perfusion index* and *Kirchner's kidney/aorta ratio*) could not unequivocally differentiate between renal transplants with ATN and cases of AR. The quantitative index $R_{20/3}$ is the most commonly described quantitative index, which can be calculated for both $^{99m}\text{Tc-MAG3}$ and $^{99m}\text{Tc-DTPA}$ by dividing the renal activity at 20 minutes by the activity at 3 minutes. In a large retrospective cohort of 307 transplant recipients, the index $R_{20/3}$ was shown to have a strong correlation with the severity of both ATN and AR.²² A retrospective study comparing US with the intra-renal resistive index (RI), and RS using the index $R_{20/3}$ and a qualitative grading, found a sensitivity and specificity of respectively 93% and 73% for RS and 76% and 66% for US, for the prediction of renal transplant pathology (both AR and ATN) within seven days post-RS.¹⁵ The clinical relevance of these findings is questionable as needle biopsy remains the 'golden standard'. However, the potential ability of RS to differentiate AR from ATN is important, since unnecessary biopsies and/or treatment procedures of acute rejection should be avoided.

Vascular complications

Vascular complications occur in 10% of renal transplant recipients and are an important cause of renal graft dysfunction and graft loss.³⁵ Transplant renal artery stenosis (TRAS) is the most common vascular complication, followed by transplant renal artery thrombosis (TRAT) and transplant renal vein thrombosis (TRVT) (Table 2).³⁵⁻³⁷ Angiography is the gold-standard invasive modality for the diagnosis of TRAS,

TRAT and TRVT, however, various reports have suggested a role for RS and US.³⁸ The major drawbacks of angiography are the complications such as contrast-induced nephropathy and thromboembolism.³⁶ Colour Doppler US is used to evaluate the renal vascular flow and the *RI* is used to give quantitative results.³⁹ Large studies comparing invasive and non-invasive procedures are lacking. A study in 47 transplant recipients showed that computed tomography angiography (CTA) and gadolinium enhanced magnetic resonance angiography (MRA) are superior to ultrasound and captopril ^{99m}Tc-DTPA RS for diagnosing morphological stenosis.⁴⁰ A study evaluating 133 ^{99m}Tc-DTPA RS procedures, with 7 cases of TRVT and 2 cases of TRAT, concluded that both complications show a similar pattern of photo-deficient areas at the anatomical location of the transplant.⁴¹ Unfortunately, studies comparing non-invasive diagnostic modalities for TRAT and TRVT are lacking.

Urological complications

Urological complications after transplantation generally consist of urinary tract obstruction and/or urinary leaks and can be determined with RS as signs of hydronephrosis or progressive RS tracer activity surrounding the ureter (Supplement 2). There is currently a lack of evidence to express a preference for either RS or US in diagnosing urological complications. However, RS provides dynamic images, which can provide elaborate information about the nature, intensity, and location of an obstruction or leak. Two retrospective studies including 1525 and 965 transplant recipients, with a 4.5% - 4.7% incidence of urinary tract obstruction and a 1.5% - 1.6% incidence of urinary leaks (Table 2), support the use of dynamic renal ^{99m}Tc-DTPA RS in combination with routine US surveillance.^{42,43} A study evaluating 133 ^{99m}Tc-DTPA RS procedures, with 12 cases of urinary leaks and 2 cases of urinary obstruction, described no false-negative and -positive outcomes.⁴¹ While a more recent study including 211 paediatric transplant recipients, with a 7.6% incidence of urological obstructions and extravasations, supports the use of ^{99m}Tc-MAG3.⁴⁴

Postoperative fluid collections

Clinically significant postoperative fluid collections are commonly seen in the earlier and late postoperative period. These collections include urinomas or hematomas in the immediate post-transplant period, and abscesses or lymphoceles, in the following months (Table 2). US is mostly used as primary diagnostic modality and was shown to have a high sensitivity, although the results are nonspecific. Studies comparing the sensitivity of US and RS are lacking, but with visual RS analysis the distinction between lymphoceles and urinomas can be made. However, lymphoceles and urinomas, at the anterior/posterior side of the renal transplant, could be missed with RS due to the planar (2-dimensional) view. In these cases, 3-dimensional images should be obtained using

SPECT. In a retrospective analysis of 2000 transplant recipients undergoing both ^{99m}Tc -DTPA RS and US, lymphoceles were detected in 13 transplant recipients with a similar accuracy.⁴⁵ The visual analyses of these lymphoceles showed photopenic ‘cold’ areas in the first RS phase, representing an area without tracer uptake, contrary to images of urinomas which show ‘hot’ areas with tracer uptake.^{45,46}

Table 2. Complications after kidney transplantation

	Complication	Incidence (%)
Renal complications		
	Acute rejection (AR)	8% – 10% ^{47,48}
	Acute tubular necrosis (ATN)	22% ⁴⁹
Vascular complications		
	Transplant renal artery stenosis (TRAS)	1 – 23% ^{35,36}
	Transplant renal artery thrombosis (TRAT)	0.8% – 3% ^{35,37}
	Transplant renal vein thrombosis (TRVT)	1.0% – 1.8% ^{35,37}
Urological complications		
	Urinary tract obstruction	4.5 – 4.7 ^{42,43}
	Urinary leak	1.5 – 1.6 ^{42,43}
Postoperative fluid collections		
	Urinoma	^a
	Hematoma	4.9% ⁵⁰
	Abscess	0.3% ⁵¹
	Lymphocele	5.2% ⁵²

^ano data available

CORRELATION WITH SHORT- AND LONG-TERM OUTCOMES

In general, early post-transplant RS is performed in cases of delayed graft function (DGF) or suspicion of surgical complications. In addition to their current contribution to clinical decision-making, several studies have focused on the predictive potential of baseline RS procedures for early and one-year transplant outcomes.

Early renal transplant outcome

The quantitative *GI* index has been developed for the analyses of ^{99m}Tc -DTPA, which resulted in a high sensitivity and specificity for the need of dialysis in the first week post-transplant (86% and 86%, respectively) (Table 1).¹⁹ The counts-based indices *tubular function slope (TFS)* and *MUC10* have shown to correlate significantly with the occurrence of dialysis dependence in the first postoperative week.^{16,17} The grading scale of Heaf and Iversen has a sensitivity of 76% regarding the prediction of a 10% rise in serum creatinine levels between two following days.¹³ To date it has not been proven that this early predictive potential of RS is superior to the simple measurement of serum creatinine.

One-year renal transplant outcome

The *GI* index described earlier, showed a moderate sensitivity and specificity for the prediction of renal transplant function, based on serum creatinine, at one year post-transplantation (74% and 76%, respectively) (Table 2).¹⁹ The use of the quantitative index $T_{1/2}$, from RS procedures within two days post-transplantation, yield a relatively poor sensitivity and specificity of 54% and 79% for renal transplant function at one year based on serum creatinine.²⁶ The *Hilson's perfusion index* and the grading scale of Heaf and Iversen showed to have a low predictive value for one-year renal transplant function, with a sensitivity / specificity of respectively 73% / 72% and 31% / 92%.^{26,27} On the contrary, a recent study focusing on long-term transplant function showed no significant correlation between *RI* results of US procedures results and serum creatinine at one year post-transplantation.²⁶ A result which is in line with the conclusion that *RI* results during the first two years post-transplantation are not accurate enough to predict renal transplant outcome, although they reflect recipient characteristics and patient mortality.⁵³

CURRENT GUIDELINE RECOMMENDATIONS

The five reviewed kidney transplantation guidelines did not provide recommendations and did not mention the use of postoperative RS. The use of US was the only mentioned procedure for postoperative transplant imaging. However, recommendations with regard to this topic were all based on low-degree evidence or expert opinions, with level of evidence between 3 and 5 (Oxford Centre for Evidence-Based Medicine, Levels of Evidence).⁵⁴ To reach a balanced recommendation for the use of either RS or US, more elaborate studies comparing both procedures are needed.

DISCUSSION

In this review, we described the technical and clinical aspects of RS after kidney transplantation. The available qualitative curves grading scales, for the different possible shapes of renal time-activity curves, have a high sensitivity and somewhat low specificity for changes in renal transplant function. Moreover, several methods for a quantitative analysis are available from which *GI*, *T_{1/2}*, *R_{20/3}* and *Hilson's perfusion index* were shown to have moderate predictive values for the occurrence of DGF, AR or ATN, and short- or long-term renal transplant outcomes.

RS is used to make a comprehensive dynamic evaluation of renal perfusion, excretion patterns, and leakage, while US is used to evaluate both the internal renal morphology, such as heterogeneity of renal cortex and hypo-echogenicity of renal pyramids. Both procedures provide comprehensive information on pre- and post-renal complications, such as postoperative fluid collections, urological-, and vascular complications. RS is less hampered by inter-observer variability and this advantage of RS leads to a higher sensitivity and specificity for the diagnosis of early graft dysfunction. However, a differentiation between AR and ATN cannot be made by either RS or US alone. For the diagnosis of urologic complications, there is currently no clear preference for either RS or US, partly because a proper comparison between both tests is lacking. However, lymphoceles and urinomas, at the anterior/posterior side of the renal transplant, could be missed with RS due to the planar (2-dimensional) view. In these cases, 3-dimensional images should be obtained using SPECT. The availability and applications of SPECT have increased in the past years. Future studies should provide more information on the advantages of SPECT compared to both conventional RS and US. Possible disadvantages of RS compared to US are higher costs, limited availability of tracers and equipment, and radiation exposure. In more detail, the costs of a RS procedure is approximately 450,00 USD (+/- 400,00 EUR) compared to approximately 175,00 USD (+/-150 EUR) for an US procedure, and approximately USD (+/- EUR) for a SPECT procedure 580,00 USD (+/- 500,00 EUR), in The Netherlands.

FUTURE PERSPECTIVES

Future research should provide more information about the feasibility to differentiate AR from ATN with improved scoring methodologies for RS images. To improve clinical value, future studies should focus on the characterization and value of different curve shapes, reliable quantification techniques, and the introduction and renewing of visualisation techniques. Furthermore, the use of SPECT-measured ^{99m}Tc -DMSA could provide more comprehensive results, however, studies comparing the use of regular RS and SPECT are not available.¹⁰

Various new and promising imaging techniques could be useful for the follow-up of patients after kidney transplantation (Table 3). The introduction of contrast-enhanced ultrasound (CEUS), a combination of microbubble technology and complementary US, could improve the clinical value of post-transplant US.⁵⁵ Further research is necessary to assess the detection of haemodynamic changes and usefulness of quantification techniques, such as the resistive index used for conventional US. Several studies described the use of ultrasound elastography (UE), a method of measuring shear wave velocity to determine the extent of tissue stiffness. However, its use for post-transplant monitoring is still limited to the diagnosis of renal transplant fibrosis, for which UE provides reliable measurements in 70% of patients.⁵⁶ The use of dynamic contrast-enhanced MRI (MR renography) has been explored for renal evaluation with promising results for TRAS and urological obstruction.⁵⁷ Large studies focusing on the accuracy of MR renography for the distinction between AR and ATN are lacking. More recent developments use bodily substances such as haemoglobin for Blood Oxygenation Level-Dependent magnetic resonance imaging (BOLD MRI) and water molecules for Diffusion-Weighted Imaging (DWI MRI).⁵⁸ Promising results have been published using BOLD MRI, showing a significant difference between AR and ATN MRI results.⁵⁹ For DWI MRI, more research is necessary in order to determine whether different causes of renal transplant dysfunction could be differentiated.^{60,61} The wide introduction of positron emission tomography-computer tomography (PET-CT) provides the opportunity for higher-resolution images and more reliable quantitative measurements. Fluor-18 (^{18}F) FDG-labeled T-lymphocytes PET-CT was shown to make a distinction between AR and ATN in a rat model of kidney transplantation.⁶² In a prospective human-trial including 31 patients with suspected AR, (^{18}F) FDG PET-CT was shown to prevent avoidable transplant biopsies.⁶³ Studies focusing on renal blood flow, including healthy volunteers, have been performed with Oxygen-15-water (^{15}O - H_2O) PET-CT ($n = 6$) and rubidium-82 chloride (^{82}Rb) PET-CT ($n = 8$).^{64,65} Ongoing research into tracer binding to intracellular targets or leukocytes could fulfill the demand for methods for non-invasive diagnosis of AR.

Table 3. Future alternatives

	Methods
Ultrasonography (US)	Contract-enhanced ultrasound (CEUS) ⁶⁶ Ultrasound Elastography (UE) ⁵⁶
Magnetic resonance imaging (MRI)	MR Renography ⁵⁷ Blood Oxygenation Level-Dependent MRI (BOLD-MRI) ⁵⁹ Diffusion-Weighted MRI (DWI MRI) ⁶⁰
Positron emission tomography -computer tomography (PET-CT)	Fluor-18 FDG T-lymphocyte PET-CT (¹⁸ F-FDG PET-CT) ⁶⁷ Rubidium-82 chloride PET-CT (⁸² Rb-chloride PET-CT) ⁶⁴ Oxygen-15-water PET-CT (¹⁵ O-H ₂ O PET-CT) ⁶⁵

CONCLUSION

RS has its merits for the evaluation of complications after kidney transplantation, especially for vascular and/or urological complications. However, providing a clear recommendation for the use of either RS or US during the early postoperative period is not possible, based on the available literature. Several studies have described the use of RS for the diagnosis of acute rejection, however, differentiating between rejection and acute tubular necrosis remains difficult. For the diagnosis of vascular complications, RS has been described as an alternative for invasive procedures. For urologic complications, studies support the use of RS in combination with routine ultrasonography (US) surveillance. For the diagnosis of postoperative fluid collections, lymphoceles show areas with lack of tracer uptake in contrast to the accumulated tracer uptake in urinomas. Altogether, RS should be considered in case of non-acute complications, and if US provides insufficient results. Early diagnosis of vascular and urological complications can contribute to a more specific surgical approach and better post-transplant outcomes.

REFERENCES

1. Abramowicz D, Cochat P, Claas FHJ, et al. European Renal Best Practice Guideline on kidney donor and recipient evaluation and perioperative care. *Nephrol Dial Transpl.* 2015;30:1790-1797.
2. Kidney Disease:Improving Global Outcomes (KDIGO) Transplant Work Group. KDIGO clinical practice guideline for the care of kidney transplant recipients. *Am J Transplant.* 2009;9(Suppl 3):S1-S157.
3. Breda A, Olsburgh J, Budde K, Figueiredo A, García EL, Regele H. EAU guidelines on renal transplantation. Edn. presented at the EAU Annual Congress London 2017. 2017;2017(10-04).
4. Baker RJ, Mark PB, Patel RK, Stevens KK, Palmer N. Renal association clinical practice guideline in post-operative care in the kidney transplant recipient. *BMC Nephrol.* 2017;18(1):172-174.
5. Andrews PA, Burnapp L, Manas D, et al. Summary of the British Transplantation Society/Renal Association U.K. guidelines for living donor kidney transplantation. *Transplantation.* 2012;93(7):666-673.
6. Russell CD, Thorstad BL, Stutzman ME, Yester M V, Fowler D, Dubovsky E V. The kidney: imaging with Tc-99m mercaptoacetyltriglycine, a technetium-labeled analog of iodohippurate. *Radiology.* 1989;172(2):427-430.
7. Bubeck B, Brandau W, Weber E, Kälble T, Parekh N, Georgi P. Pharmacokinetics of technetium-99m-MAG3 in humans. *J Nucl Med.* 1990;31(8):1285-1293.
8. Reinig JW, Gordon L, Frey D, Garrick E, Daniel WT. Glomerular filtration rate in transplantation patients: estimation of renal function using Tc-99m DTPA. *Radiology.* 1985;156(2):505-507.
9. Aktaş A, Aras M, Çolak T, Gençoğlu A, Karakayalı H. Comparison of Tc-99m DTPA and Tc-99m MAG3 Perfusion Time-Activity Curves in Patients With Renal Allograft Dysfunction. *Transplant Proc.* 2006;38(2):449-453.
10. Even-Sapir E, Gutman M, Lerman H, et al. Kidney allografts and remaining contralateral donor kidneys before and after transplantation: assessment by quantitative (99m)Tc-DMSA SPECT. *J Nucl Med.* 2002;43(5):584-588.
11. Budihna N V, Milcinski M, Kajtna-Koselj M, Malvorh M. Relevance of Tc-99m DMSA Scintigraphy in Renal Transplant Parenchymal Imaging. *Clin Nucl Med.* 1994;19(9):782-784.
12. el Maghraby TA, van Eck-Smit BL, de Fijter JW, Pauwels EK. Quantitative scintigraphic parameters for the assessment of renal transplant patients. *Eur J Radiol.* 1998;28(3):256-269.
13. Heaf JG, Iversen J. Uses and limitations of renal scintigraphy in renal transplantation monitoring. *Eur J Nucl Med Mol Imaging.* 2000;27(7):871-879.
14. Gupta SK, Lewis G, Rogers KM, et al. Quantitative (99m)Tc DTPA renal transplant scintigraphic parameters: assessment of interobserver agreement and correlation with graft pathologies. *Am J Nucl Med Mol Imaging.* 2014;4(3):213-224.
15. Yazici B, Yazici A, Oral A, Akgün A, Toz H. Comparison of Renal Transplant Scintigraphy With Renal Resistance Index for Prediction of Early Graft Dysfunction and Evaluation of Acute Tubular Necrosis and Acute Rejection. *Clin Nucl Med.* 2013;38(12):931-935.
16. El-Maghraby TAF, Boom H, Camps JAJ, et al. Delayed graft function is characterized by reduced functional mass measured by 99mtechnetium-mercaptoacetyltriglycine renography. *Transplantation.* 2002;74(2):203-208.
17. Stevens H, Klerk JM, Mertens IJ, Rijk PP, Hené RJ. Quantitative baseline renography

- 48 hours after renal transplantation predicts long-term graft survival. *Eur J Nucl Med.* 2001;28(11):1677-1681.
18. Russell CD, Yang H, Gaston RS, Hudson SL, Diethelm AG, Dubovsky E V. Prediction of renal transplant survival from early postoperative radioisotope studies. *J Nucl Med.* 2000;41(8):1332-1336.
 19. Yazici B, Oral A, Gokalp C, Akgün A, Toz H, Hoscoskun C. A New Quantitative Index for Baseline Renal Transplant Scintigraphy With 99mTc-DTPA in Evaluation of Delayed Graft Function and Prediction of 1-Year Graft Function. *Clin Nucl Med.* 2016;41(3):182-188.
 20. El-Maghraby TAF, de Fijter JW, van Eck-Smit BLF, Zwinderman AH, El-Haddad SI, Pauwels EKJ. Renographic indices for evaluation of changes in graft function. *Eur J Nucl Med Mol Imaging.* 1998;25(11):1575-1586.
 21. Bajén MT, Puchal R, González A, et al. MAG3 renogram deconvolution in kidney transplantation: utility of the measurement of initial tracer uptake. *J Nucl Med.* 1997;38(8):1295-1299.
 22. Li Y, Russell CD, Palmer-Lawrence J, Dubovsky E V. Quantitation of renal parenchymal retention of technetium-99m-MAG3 in renal transplants. *J Nucl Med.* 1994;35(5):846-850.
 23. Gedroyc W, Taube D, Fogleman I, Neild G, Cameron S, Maisey M. TC-99m DTPA scans in renal allograft rejection and cyclosporine nephrotoxicity. *Transplantation.* 1986;42(5):494-496.
 24. Majima T, Hattori R, Funahashi Y, et al. 99mTc-mercaptoacetyl Triglycine Renography to Monitor Renal Transplant Function Among Kidneys from Donors After Cardiac Death. *Transplant Proc.* 2012;44(1):49-53.
 25. Obeidat MA, Luyckx VA, Grebe SO, et al. Post-transplant nuclear renal scans correlate with renal injury biomarkers and early allograft outcomes. *Nephrol Dial Transplant.* 2011;26(9):3038-3045.
 26. Yazici B, Oral A, Gokalp C, et al. Evaluation of Renal Transplant Scintigraphy and Resistance Index Performed Within 2 Days After Transplantation in Predicting Long-Term Graft Function. *Clin Nucl Med.* 2015;40(7):548-552.
 27. Gupta SK, Lewis G, Rogers K, Attia J. Quantitative Tc-99m DTPA renal transplant scintigraphy predicts graft survival in the very early postoperative period. *Nucl Med Commun.* 2012;33(12):1292-1299.
 28. Guignard R, Mourad G, Mariano-Goulart D. Utility of postsurgical renal scintigraphy to predict one-year outcome of renal transplants in patients with delayed graft function. *Nucl Med Commun.* 2011;32(4):314-319.
 29. Ngai S, Demetriades T. Prediction of Long-Term Renal Transplant Allograft Function From Day 3 Post-transplant Tc-99m MAG3 Scintigraphy. *Clin Nucl Med.* 2008;33(2):102-105.
 30. Furness PN, Philpott CM, Chorbajian MT, et al. Protocol biopsy of the stable renal transplant: a multicenter study of methods and complication rates. *Transplantation.* 2003;76(6):969-973.
 31. Nankivell BJ, Chapman JR. The significance of subclinical rejection and the value of protocol biopsies. *Am J Transplant.* 2006;6(9):2006-2012.
 32. Morgan TA, Chandran S, Burger IM, Zhang CA, Goldstein RB. Complications of Ultrasound-Guided Renal Transplant Biopsies. *Am J Transplant.* 2016;16(4):1298-1305.
 33. Redfield RR, McCune KR, Rao A, et al. Nature, timing, and severity of complications from ultrasound-guided percutaneous renal transplant biopsy. *Transpl Int.* 2016;29(2):167-172.
 34. Wilczek HE. Percutaneous needle biopsy of the renal allograft. A clinical safety evaluation of 1129 biopsies. *Transplantation.* 1990;50(5):790-797.
 35. Hernández D, Rufino M, Armas S, et al. Retrospective analysis of surgical complications following cadaveric kidney

- transplantation in the modern transplant era. *Nephrol Dial Transplant*. 2006;21(10):2908-2915.
36. Bruno S, Remuzzi G, Ruggenenti P. Transplant Renal Artery Stenosis. *J Am Soc Nephrol*. 2004;15(1):134-141.
 37. Englesbe MJ, Punch JD, Armstrong DR, Arenas JD, Sung RS, Magee JC. Single-Center Study of Technical Graft Loss in 714 Consecutive Renal Transplants. *Transplantation*. 2004;78(4):623-626.
 38. Mangray M, Vella JP. Hypertension After Kidney Transplant. *Am J Kidney Dis*. 2011;57(2):331-341.
 39. Cano H, Castañeda DA, Patiño N, et al. Resistance Index Measured by Doppler Ultrasound as a Predictor of Graft Function After Kidney Transplantation. *Transplant Proc*. 2014;46(9):2972-2974.
 40. Eriksson P, Mohammed AA, De Geer J, et al. Non-invasive investigations of potential renal artery stenosis in renal insufficiency. *Nephrol Dial Transplant*. 2010;25(11):3607-3614.
 41. Lubin E, Shapira Z, Melloul M, Youssim A. Scintigraphic detection of vascular and urological complications in the transplanted kidney: 133 cases. *Eur J Nucl Med*. 1985;10(7-8).
 42. Zavos G, Pappas P, Karatzas T, et al. Urological Complications: Analysis and Management of 1525 Consecutive Renal Transplantations. *Transplant Proc*. 2008;40(5):1386-1390.
 43. Dinckan A, Tekin A, Turkyilmaz S, et al. Early and late urological complications corrected surgically following renal transplantation. *Transpl Int*. 2007;20(8):702-707.
 44. Routh JC, Yu RN, Kozinn SI, Nguyen HT, Borer JG. Urological Complications and Vesicoureteral Reflux Following Pediatric Kidney Transplantation. *J Urol*. 2013;189(3):1071-1076.
 45. Kumar R, Bharathi Dasan J, Choudhury S, Guleria S, Padhy A, Malhotra A. Scintigraphic patterns of lymphocele in post-renal transplant. *Nucl Med Commun*. 2003;24(5):531-535.
 46. Fortenberry EJ, Blue PW, Van Nostrand D, Anderson JH. Lymphocele: the spectrum of scintigraphic findings in lymphoceles associated with renal transplant. *J Nucl Med*. 1990;31(10):1627-1631.
 47. Hart A, Smith JM, Skeans MA, et al. OPTN/SRTR 2015 Annual Data Report: Kidney. *Am J Transplant*. 2017;17 Suppl 1(Suppl 1):21-116.
 48. US Renal Data System. *USRDS 2013 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States*; 2013.
 49. Susantitaphong P, Cruz DN, Cerda J, et al. World incidence of AKI: a meta-analysis. *Clin J Am Soc Nephrol*. 2013;8(9):1482-1493.
 50. Hachem LD, Ghanekar A, Selznern M, Famure O, Li Y, Kim SJ. Postoperative surgical-site hemorrhage after kidney transplantation: incidence, risk factors, and outcomes. *Transpl Int*. 2017;30(5):474-483.
 51. Edelstein HE, McCabe RE, Lieberman E. Perinephric abscess in renal transplant recipients: report of seven cases and review. *Rev Infect Dis*. 11(4):569-577.
 52. Lucewicz A, Wong G, Lam VWT, et al. Management of Primary Symptomatic Lymphocele After Kidney Transplantation: A Systematic Review. *Transplantation*. 2011;92(6):663-673.
 53. Naesens M, Heylen L, Lerut E, et al. Intrarenal Resistive Index after Renal Transplantation. *N Engl J Med*. 2013;369(19):1797-1806.
 54. Howick J, Chalmers I, Glasziou P, et al. Explanation of the 2011 Oxford Centre for Evidence-Based Medicine (OCEBM) Levels of Evidence.
 55. McArthur C, Baxter GM. Current and potential renal applications of contrast-enhanced ultrasound. *Clin Radiol*. 2012;67(9):909-922.
 56. Sommerer C, Scharf M, Seitz C, et al. Assessment of renal allograft fibrosis by transient elastography. *Transpl Int*. 2013;26(5):545-551.
 57. Yamamoto A, Zhang JL, Rusinek H, et al. Quantitative evaluation of acute renal transplant dysfunction with low-dose

- three-dimensional MR renography. *Radiology*. 2011;260(3):781-789.
58. Park SY, Kim CK, Park BK, Kim SJ, Lee S, Huh W. Assessment of early renal allograft dysfunction with blood oxygenation level-dependent MRI and diffusion-weighted imaging. *Eur J Radiol*. 2014;83(12):2114-2121.
 59. Han F, Xiao W, Xu Y, et al. The significance of BOLD MRI in differentiation between renal transplant rejection and acute tubular necrosis. *Nephrol Dial Transplant*. 2008;23(8):2666-2672.
 60. Thoeny HC, De Keyzer F. Diffusion-weighted MR Imaging of Native and Transplanted Kidneys. *Radiology*. 2011;259(1):25-38.
 61. Abou-El-Ghar ME, El-Diasty TA, El-Assmy AM, Refaie HF, Refaie AF, Ghoneim MA. Role of diffusion-weighted MRI in diagnosis of acute renal allograft dysfunction: a prospective preliminary study. *Br J Radiol*. 2012;85(1014):e206-e211.
 62. Grabner A, Kentrup D, Edemir B, et al. PET with ¹⁸F-FDG-Labeled T Lymphocytes for Diagnosis of Acute Rat Renal Allograft Rejection. *J Nucl Med*. 2013;54(7):1147-1153.
 63. Lovinfosse P, Weekers L, Bonvoisin C, et al. Fluorodeoxyglucose F(18) Positron Emission Tomography Coupled With Computed Tomography in Suspected Acute Renal Allograft Rejection. *Am J Transplant*. 2016;16(1):310-316.
 64. Tahari AK, Bravo PE, Rahmim A, Bengel FM, Szabo Z. Initial human experience with Rubidium-82 renal PET/CT imaging. *J Med Imaging Radiat Oncol*. 2013;58(1):25-31.
 65. Kudomi N, Koivuviita N, Liukko KE, et al. Parametric renal blood flow imaging using [¹⁵O]H₂O and PET. *Eur J Nucl Med Mol Imaging*. 2008;36(4):683-691.
 66. Jin Y, Yang C, Wu S, et al. A Novel Simple Noninvasive Index to Predict Renal Transplant Acute Rejection by Contrast-Enhanced Ultrasonography. *Transplantation*. 2015;99(3):636-641.
 67. Reuter S, Schnöckel U, Schröter R, et al. Non-invasive imaging of acute renal allograft rejection in rats using small animal F-FDG-PET. Zoccali C, ed. *PLoS One*. 2009;4(4):e5296.

Can transplant renal scintigraphy predict the duration of delayed graft function? A dual center retrospective study

PLOS ONE, 2018

Stan Benjamins

Robert A. Pol

Lioe-Fee de Geus-Oei

Aiko P.J. de Vries

Andor W.J.M. Glaudemans

Stefan P. Berger [¶]

Riemer H.J.A. Slart [¶]

[¶]These authors contributed equally to this work

Background: This study focused on the value of quantitatively analysed and qualitatively graded renal scintigraphy in relation to the expected duration of delayed graft function after kidney transplantation. A more reliable prediction of delayed graft function duration may result in a more tailored and patient-specific treatment regimen post-transplantation.

Methods: From 2000 to 2014, patients with early transplant dysfunction and a Tc-99m MAG3 renal scintigraphy, within 3 days post-transplantation, were included in a dual centre retrospective study. Time-activity curves of renal scintigraphy procedures were qualitatively graded and various quantitative indices (*R20/3*, *TFS*, *cTER*, *MUC10*) were combined with a new index (*Average upslope*). The delayed graft function duration was defined as the number of days of dialysis-based/functional delayed graft function.

Results: A total of 377 patients were included, with a mean age ($\pm$ SD) of 52 ± 14 years, and 58% were male. A total of 274 (73%) patients experienced delayed graft function ≥ 7 days. Qualitative grading for the prediction of delayed graft function ≥ 7 days had a sensitivity and specificity of respectively 87% and 65%. The quantitative indices with the most optimal results were *cTER* (76% sensitivity, 72% specificity), and *Average upslope* (75% sensitivity, 73% specificity).

Conclusions: Qualitative renal scintigraphy grading and the quantitative indices *cTER* and *Average upslope* predict delayed graft function ≥ 7 days with a high sensitivity. This finding may help to support both clinicians and patients in managing early post-operative expectations. However, the specificity is limited and thus renal scintigraphy does not reliably help to identify patients in whom the course of delayed graft function is longer than anticipated.

INTRODUCTION

Monitoring renal function after kidney transplantation is pivotal to recognize post-transplant complications, such as vascular or urological complications, acute tubular necrosis (ATN) or rejection.¹ Therefore, an imaging modality with a high predictive value for post-transplantation complications may result in a more tailored and patient-specific treatment regimen and possible reduction of early graft failure.

Delayed graft function (DGF) is a common complication after kidney transplantation, with an overall incidence between 2% to 50%, depending on the type of kidney graft (donation after circulatory death (DCD), donation after brain death (DBD) or after living donation) and the definition used for DGF.² The most commonly used definitions for DGF are (1) dialysis-based DGF, the need for postoperative dialysis within the first 7 days after transplantation and (2) functional DGF, defined by a serum creatinine level failing to decrease with 10% on 3 consecutive days following transplantation.^{3,4} The impact of DGF on the short-term outcome after transplantation is a longer duration of hospitalization, increased risk of graft loss and a higher mortality.⁵⁻⁷

Renal scintigraphy (RS) is a common and widely used test to assess graft function and complications after kidney transplantation. Previous studies introduced RS as a tool for the evaluation and/or prediction of DGF.⁸⁻¹¹ However, correct acquisition and interpretation is difficult, since several different radiopharmaceuticals and multiple quantitative indices are available.¹² In this study we focus on Technetium-99m mercaptoacetyltriglycine (MAG3) RS, the most commonly used radiopharmaceutical for post-transplantation evaluation.¹³ We evaluate the qualitative grading scale of Heaf and Iversen and the following four quantitative indices: the ratio of uptake at 20 and 3 minutes (R20/3), the tubular function slope (TFS; counts/second), the corrected tubular extraction rate (cTER; mL/min/1.73 m²) and the uptake within the first 10 minutes, as a fraction of the injected dose (MUC10; counts/second/MBq), which showed to correlate significantly with post-transplantation outcomes, in studies with smaller cohorts.^{8,10,14,15}

Earlier studies showed that RS cannot differentiate reliably between rejection and acute tubulus necrosis (ATN), as a cause of DGF.^{16,17} We postulated that RS is able to predict the expected time course of recovery from DGF and may provide useful information to identify patients with additional allograft pathology. Improving the predictive value and clinical applicability of qualitative and quantitative RS indices could result in improved identification of the cause and expected duration of early graft dysfunction. This may facilitate to take image guided treatment decisions, subsequently leading to a reduction in the number of diagnostic biopsies and faster treatment.

We performed a dual centre retrospective study to evaluate the prognostic performance of RS, with regard to the duration of DGF after kidney transplantation, by using qualitative and quantitative analysis methodology.

PATIENTS AND METHODS

Patients were included in the University Medical Center Groningen (UMCG) from 2000 to 2014 ($n = 177$) and in Leiden University Medical Center (LUMC) from 2011 to 2014 ($n = 200$). All available post-transplantation RS procedures performed within 3 days after transplantation were included and patients' charts were screened for baseline characteristics and renal function within the first year, using the electronic hospital registries. At the UMCG cohort, RS was performed when transplant dysfunction was clinically suspected. In the LUMC cohort, RS was performed according to the standard post-transplantation protocol, including cases with suspicion of vascular or urological complications and with ongoing DGF. Primary outcome was the duration of DGF, analysed as a continuous and dichotomous variable. For dichotomous analyses, patients of both centres were divided into two groups based on graft function: (I) DGF, dialysis-based delayed graft function or functional delayed graft function for ≥ 7 days ($n = 274$); (II) non-DGF, no dialysis-based delayed graft function or functional delayed graft function for ≥ 7 days ($n = 103$). In both centres, kidney biopsies were performed per protocol if DGF persisted for more than 7-10 days without sign of improvement. As acute rejection is generally not thought to subside spontaneously it seems improbable that a significant number of rejections were missed with this approach. Patient data were processed and electronically stored according to the declaration of Helsinki Ethical principles for medical research involving human subjects. The local Medical Ethical Committees of both participating medical centres gave approval for this study and waived the need for written informed consent (Medical Ethical Committee, University Medical Center Groningen 2015/338 and Medical Ethical Committee, Leiden University Medical Center P15.372). The clinical and research activities are consistent with the Principles of the Declaration of Istanbul as outlined in the 'Declaration of Istanbul on Organ Trafficking and Transplant Tourism'.

All Tc-99m MAG3 RS procedures were performed using an intravenous administration dose in a range of 80-100 MBq (2.16-2.70 mCi) and results were reanalysed for the purpose of this study. Dynamic images were acquired with 1-second frames for two minutes (perfusion phase) followed by 20 seconds frames for 28 minutes (clearance phase). Reconstructed RS data were processed using Syngovia (Siemens Medical Systems, TN, U.S.A.) and regions of interest (ROIs) were drawn manually. Correction for patients' motions was applied when needed. The renal ROI was drawn proximal to the renal cortex and the background ROI was drawn as a crescent shape, opposite of the renal artery and vein, resulting in a background corrected time-activity curve, from start of the procedure up to 20 minutes. Patients were excluded if RS procedures could not be reanalysed since re-analysis of the original data, to derive all quantitative indices, was necessary for this study.

Figure 1. Qualitative renal scintigraphy grading scale for post-transplantation Tc-99m MAG₃ renal scintigraphy. *Grade 1*, a normal renal scintigraphy curve, with fast uptake and excretion. *Grade 2*, normal uptake with flat excretion curve / plateau phase. *Grade 3*, rising curve without excretion phase. *Grade 4*, reduced absolute uptake without excretion phase.

Time-activity curves were qualitatively graded using the adjusted qualitative grading scale of Heaf and Iversen (Figure 1). This qualitative grading scale divides RS curves based on uptake and excretion curve shapes.¹⁴ For this study we combined the first two and final two grades of the qualitative grading scale of Heaf and Iversen, since the difference between these grades was small with an equal distribution of DGF and non-DGF patients. This resulted in the following grades: *Grade 1*, a normal RS curve, with fast uptake and excretion; *Grade 2*, normal uptake with flat excretion curve; *Grade 3*, rising curve without excretion phase; *Grade 4*, reduced absolute uptake without excretion phase.

Quantitative assessment of RS curves was performed with the four most common indices *R20/3*, *TFS*, *cTER*, *MUC10*, including a newly developed index *Average upslope* (Figure 2). The *R20/3* index is a quantitative relationship of retention to uptake ratio, which can be calculated by dividing the Tc-99m MAG3 uptake at 20 minutes by the uptake at 3 minutes.¹⁸⁻²⁰ The tubular function slope (*TFS*) reflects the Tc-99m MAG3 uptake by renal tubular cells during the first minutes after Tc-99m MAG3 injection, presented as the linear fit of the curve between 50 and 110 seconds.^{8,11} The corrected tubular extraction rate (*cTER*), in mL/min/1.73m², reflects the Tc-99m MAG3 uptake corrected for the body surface using the renal uptake rate for 1 to 2 minutes after injection and the following regression equation: $9.825X + 11.258$ (wherein X is the renal uptake).¹⁵ *MUC10* is an index describing the Tc-99m MAG3 uptake, within the first 10 minutes, as a fraction of the injected dose.¹⁰ Since we hypothesised that the upslope of the RS curve is most influenced by the early graft function, we introduced a new quantitative index, *Average upslope*, reflecting fast rise of the RS curve. The upslope of the RS curve ended between 2 and 3 minutes in the current cohort of Tc-99m MAG3 procedures and this resulted in the following formula: (counts at 3 minutes - counts at 20 seconds) / 160 seconds).

Figure 2. Quantitative indices for post-transplantation renal scintigraphy shown for a normal curve, with the corresponding planar RS images. Tubular Function Slope (*TFS*), the linear fit of the curve between 50 and 110 seconds. Corrected tubular extraction rate (*cTER*), the renal uptake rate for 1 to 2 minutes corrected for body surface. *Average upslope*, the slope between counts at 20 seconds and counts at 3 minutes. *MUC10* resembles the uptake within 10 minutes as a fraction of the injected dose. *R20/3*, retention to uptake ratio, dividing uptake at 20 minutes by the uptake at 3 minutes.

Statistical analysis

Baseline characteristics and clinical follow-up results are presented as mean and standard deviation (SD) when normal distribution was assumed by means of a Q-Q plot or histogram, as median and interquartile range (IQR) for skewed data and as frequency and percentage when data were categorical. We compared baseline characteristics of both centres, by performing student-t test or chi-square test. We investigated the correlation of qualitative grades and quantitative indices and duration of DGF, by performing a Spearman coefficient of correlation Test. To assess the predictive value of the adjusted qualitative grading scale of Heaf and Iversen, we calculated the number of patients with DGF for each grade and the sensitivity / specificity, and positive / negative predictive value. To assess differences in distribution of the quantitative indices between the DGF patients and the non-DGF patients we performed a Mann-Whitney U test for each index (two-sided p-value < 0.05 = significant), since the quantitative indices were not normally distributed. A receiver-operating characteristic (ROC) curve analysis was used for each significant quantitative parameter, considering an AUC between 0.5 – 0.7 as a poor level of discrimination, between 0.7 – 0.8 as an acceptable level of discrimination, and above 0.8 as a reliable level of discrimination.²¹ The highest value of the Youden-Index (YI) (sensitivity + specificity-1) was considered as the optimal cut-off point (YI of 1 represents a perfect diagnostic test and YI of 0 indicates that the diagnostic test is not effective).²² The Kaplan–Meier method was used to visualize the differences between patients with RS-values below or above the optimal cut-off. Differences between Kaplan-Meier curves were determined with the use of log-rank tests. Final results are presented as sensitivity / specificity, and positive / negative predictive value. All statistical analyses were performed with the Statistical Package for the Social Sciences (IBM SPSS Statistics Version 22).

RESULTS

Baseline characteristics

In the UMCG, a total of 1690 kidney transplantations were performed from 2000 to 2014 and in the LUMC a total of 511 kidney transplantations were performed from 2011 to 2014. A total of 232 (13.7%) out of the 1690 patients in the UMCG and 200 (39.1%) out of 511 patients in the LUMC fitted the inclusion criteria. Subsequently, 377 patients were included after exclusion of 55 patients due to missing original RS data. Mean age ($\pm$ SD) was 49 ± 14 and 55 ± 13 years, 57% and 59% were male, 17 (10%) and 24 (12%) patients underwent a pre-emptive transplantation, and the median (IQR) time of (haemo)dialysis prior to transplantation was 32.0 (48.50 – 54.5) and 36.4 (14.3 – 57.3) months, respectively, for the UMCG cohort and LUMC cohort (Table 1). In the UMCG cohort, 17% of patients were transplanted after living donation, 41% after donation after brain death (DBD), and 42% after donation after circulatory death (DCD). In the LUMC cohort, living donation was seen in 16%, DBD in 25%, DCD in 47%, and simultaneous kidney-pancreas donation in 12% of patients. A total of 143 (81%) and 131 (66%) patients developed DGF for a duration of > 7 days respectively, in the UMCG cohort and LUMC cohort. Hundred-twenty-six patients (46.0%) were stratified in the DGF > 7 days group based on dDGF and 148 (54.0%) patients were stratified in this group based on fDGF. Renal needle biopsies were performed in 35 out of 177 patients (18%) patients in the UMCG cohort and in 21 out of 200 patients, in the LUMC cohort, within the first 7 days. Within the first year after transplantation, a total of 137 (77%) and 129 (65%) of patients received a renal biopsy (including ≤ 14 days biopsies), respectively, in the UMCG cohort and LUMC cohort. In the UMCG cohort, rejection was proven by renal biopsy in 14 out of 177 patients (8%) within the first 7 days and in 57 patients (32%) during the first year (including ≤ 14 days rejections). In the LUMC cohort, rejection was proven in 9 out of 200 (5%) patients within the first 7 days and in 36 patients (18%) during the first year. A significant difference in baseline characteristics was found for the variables ‘age’ ($p < 0.001$), ‘BMI’ ($p = 0.025$), ‘DGF > 7 days after transplantation’ ($p < 0.001$), and biopsy results for 14 days and 1 year after transplantation, other baseline characteristics were not significantly different.

Qualitative grading and DGF prediction

Results of qualitative grading of the RS curves are shown in Table 2. *Grade 1* pattern was seen in 38 patients (10%), *grade 2* in 65 patients (17%), *grade 3* was seen in 180 patients (48%) and *grade 4* in 94 patients (25%). The duration of DGF increased significantly ($p < 0.001$) with higher Heaf and Iversen grades (Spearman correlation of $r = 0.54$). The proportion of patients with DGF ≥ 7 increased with higher Heaf and Iversen grades: 10 out of 38 (26%) patients with *Grade 1*, 26 out of 65 (40%)

patients with *Grade 2*, 146 (81%) out of 180 patients with *grade 3*, and in 92 (98%) out of 94 patients with *grade 4* pattern experienced DGF ≥ 7 . Kaplan-Meier curves (Figure 3), for patients with a qualitative grade above and below the optimal cut-off, were significantly different (log-rank $p < 0.001$). Qualitative grading for the prediction of DGF ≥ 7 days, with $> \text{grade } 2$ as cut-off, had a sensitivity and specificity of respectively 87% and 65%, with a positive and negative predictive value of 87% and 65%.

Table 1. Patient characteristics grouped according to cohort

Variables	UMCG cohort (n = 177)	LUMC cohort (n = 200)	P-value
Male ^a	101 (57)	116 (59)	0.993 ^c
Age ^b	49 $\pm$ 14	55 $\pm$ 13	<0.001 ^d
BMI ^b	25.6 $\pm$ 4.6	26.6 $\pm$ 3.7	0.025 ^d
Pre-emptive KTX ^a	17 (10)	24 (12)	0.456 ^c
Duration pre-KTX dialysis, months ^e	32.0 (48.50 – 54.5)	36.4 (14.3 – 57.3)	0.425 ^c
Type of donation			
Living-(un)related	31 (17)	32 (16)	
DBD	72 (41)	50 (25)	
DCD	74 (42)	94 (47)	
Kidney-Pancreas	0 (0)	24 (12)	
Side of transplant, right ^a	144 (81)	153 (77)	0.168 ^c
Duration of DGF, days ^b	7 (4 – 14)	5 (0 – 9)	0.722 ^c
DGF > 7 days after KTX ^a	143 (81)	131 (66)	<0.001 ^c
Biopsies performed ^a			
7 days after KTX	35 (18)	21 (11)	0.012 ^c
14 days after KTX	87 (49)	45 (23)	<0.001 ^c
1 year after KTX	137 (77)	129 (65)	<0.001 ^c
Rejection ^a			
7 days after KTX	14 (8)	9 (5)	0.167 ^c
14 days after KTX	40 (23)	17 (9)	<0.001 ^c
1 year after KTX	57 (32)	36 (18)	<0.001 ^c

KTX = kidney transplantation; DBD = donation after brain death; DCD = donation after circulatory death; DGF = delayed graft function

^a n (%) ^b mean $\pm$ standard deviation (SD) ^c P-value by chi-square test

^d P-value by student-t test ^e median (IQR)

Quantitative analysis and DGF prediction

Differences in mean values for patients with ($n=274$) or without DGF ($n=103$) were statistically significant for *cTER*, *Average upslope*, *TFS*, *MUC10* ($p < 0.001$) and *R20/3* ($p = 0.042$) (Table 3). The duration of DGF increased significantly ($p < 0.001$) with lower quantitative results: *TFS* ($r = -0.39$), *cTER* (Spearman correlation of $r = -.46$), *MUC10* ($r = -0.39$), *Average Upslope* ($r = -0.47$). *R20/3* results did not significantly correlate with the duration of DGF ($p = 0.848$). *cTER* and *Average slope* provided a reliable discrimination with AUC values of respectively 0.80 and 0.82 for DGF ≥ 7 (Table 2). The levels of discrimination of *TFS* and *MUC10* were considered acceptable, with an AUC of respectively 0.75 and 0.75 for DGF ≥ 7 , and *R20/3* had a poor level of discrimination, with an AUC of 0.57. The optimal cut-off values for parameters with a reliable or acceptable level of discrimination are presented in Table 2. AUC values were not significantly different between patients from the UMCG cohort and LUMC cohort for *TFS* ($p = 0.226$), *cTER* ($p = 0.089$), *MUC10* ($p = 0.758$), and *Average Upslope* ($p = 0.096$). Log-rank tests comparing Kaplan-Meier curves (Figure 3), for patients with a quantitative grade above and below the optimal cut-off, were significant ($p < 0.001$) for the indices *TFS*, *cTER*, *MUC10*, and *Average Upslope*. YI values of *cTER* and *Average upslope*, for predicting DGF ≥ 7 days, was 0.48 for both indices, with a sensitivity 76% and a specificity of 72% for *cTER*, and a sensitivity of 75% and specificity of 73% for *Average upslope*. The YI for *TFS* and *MUC10*, for predicting DGF ≥ 7 days, was 0.41 for both indices, with a sensitivity 80% and a specificity of 61% for *TFS*, and a sensitivity of 64% and specificity of 77% for *MUC10*. The corresponding positive and negative predictive values for all indices are shown in Table 2.

Differentiating ATN and rejection

Based on previously published studies, we tried to reproduce whether patients with a DGF course longer than anticipated were more likely to have an additional pathology such as rejection. This group did not show more cases of rejection between day 7 and 14 compared to the group with expected DGF ≥ 7 days, based on qualitative grading: 4 (11%) patients with rejection out of the 36 patients below the cut-off (*grade 2*) and 24 (10%) patients with rejection out of the 238 patients above the cut-off. Excluding all cases of rejection between day 7 and 14, did not result in a better performance for predicting DGF ≥ 7 days, with an AUC of 0.76 (interval = 0.70-0.81) for *TFS*, 0.80 (0.74-0.85) for *cTER*, 0.75 (0.70-0.81) for *MUC10*, 0.82 (0.78-0.87) *Average Upslope*, and a sensitivity and specificity for qualitative grading of 87% and 63%, respectively.

Figure 3. Kaplan-Meier curves, showing the incidence of delayed graft function within the first month after kidney transplantation (censored for patients with DGF > 30 days, $n = 10$) for (A) qualitative grading, (B) *TFS*, (C) *cTER*, (D) *MUC10*, and (E) *Average upslope*. Higher curves for the group of patients with an expected longer duration of delayed graft function and lower curves for the group of patients with in expected shorter duration of delayed graft function, based on quantitative analyses and qualitative grading.

Table 2. Qualitative and quantitative analysis (sensitivity / specificity / PPV / NPV / ROC) for ≥ 7 days delayed graft function

Index	Cut-off	Sensitivity, %	Specificity, %	YI
Qualitative				
RS Grade	>2	87	65	0.52
Quantitative				
TFS	0.64	80	61	0.41
cTER	262	76	72	0.48
MUC ₁₀	825	64	77	0.41
Average upslope	0.47	75	73	0.48

RS Grade = Renal scintigraphy grade from 0 to 4; DGF = delayed graft function; YI = Youden-Index; PPV = positive predictive value; NPV = negative predictive value; AUC = area under the curve; TFS = Tubular function slope; cTER = corrected tubular extraction rate; MUC₁₀ = first 10 minutes uptake as a fraction of the injected dose; Average upslope = reflecting the slope during counts at 20 seconds and counts at 3 minutes

Table 3. Distribution of index values for delayed graft function

Index	DGF (n = 274)	Non-DGF (n = 103)	P-value
TFS	0.30 (0.13 – 0.53)	0.75 (0.38 – 1.43)	<0.001
cTER	144 (70 – 257)	444 (237 – 768)	<0.001
MUC ₁₀	644 (393 – 1069)	1249 (830 – 2271)	<0.001
Average upslope	0.23 (0.09 – 0.48)	0.88 (0.46 – 1.49)	<0.001
R _{20/3}	1.68 (1.20 – 2.09)	1.57 (1.02 – 1.91)	0.042

P-value by Mann-Whitney U test; median (IQR); significant values (two-tailed) are presented in bold; R_{20/3} = uptake at 20 minutes divided by the uptake at 3 minutes; TFS = Tubular function slope; cTER = corrected tubular extraction rate; MUC₁₀ = first 10 minutes uptake as a fraction of the injected dose; Average upslope = reflecting the slope during counts at 20 seconds and counts at 3 minutes

PPV, %	NPV, %	AUC	95% Confidence interval	
			Lower limit	Upper limit
87	65	-	-	-
84	53	0.75	0.70	0.81
88	52	0.80	0.75	0.85
88	44	0.75	0.69	0.80
88	52	0.82	0.78	0.86

DISCUSSION

This study shows that a DGF duration of ≥ 7 days post-transplantation can be predicted by using qualitative grading of RS curves and RS quantitative indices (*cTER* and *Average upslope*). The RS grades and indices showed a high sensitivity and positive predictive value for predicting a DGF duration longer than 7 days. However, the specificity of DGF prediction is insufficient to reliably identify patients with longer than expected DGF duration and a higher risk of AR. These findings can facilitate clinical management and help to inform patients on the expected course after kidney transplantation. RS does not help to identify patients with an increased risk of rejection and requiring renal biopsy.

Several previous studies described the value of qualitative and quantitative RS analyses using Tc-99m MAG3 or Tc-99m DTPA. However, only three of these studies focused on the prediction or correlation of RS curve grading and transplantation outcomes.^{14,17,19} In 2000 the qualitative grading scale of Heaf and Iversen for Tc-99m MAG3 RS was introduced first and found a 76% sensitivity for predicting a 10% serum creatinine rise within two days.¹⁴ In two more recent studies, the applicability of a perfusion curve grading scale for Tc-99m DTPA RS was described in which a sensitivity and specificity of respectively 94% and 44% was reported for the prediction of rejection within the first week after transplantation and a sensitivity and specificity of 39.5% and 93.7% for the prediction of a serum creatinine rise at three months after transplantation.^{17,19} Due to differences in Tc-99m DTPA and Tc-99m MAG3 curve shapes, caused by the higher renal extraction rate of MAG3 compared to DTPA, we could not apply this grading scale of Tc-99m DTPA in our study.

Three previous publications focused on DGF in which, among others, a significant correlation between the *MUC10* index and the occurrence of DGF within the first week post-transplantation was reported, concluding that a *MUC10* value below the cut-off indicates a poorer graft prognosis.^{8,10} In 2002, the *TFS* index was introduced for the use of predicting DGF. In a small prospective study in 42 patients, the authors demonstrated a significant difference in *TFS* values for patients with DGF and patients with immediate graft function.⁸ A more recent article by Yazici et al., described a high sensitivity and specificity (86.1% and 86.2%) for the identification of DGF by using the relatively new index *graft index (GI)* in 179 patients.⁹ Since our study was focusing on Tc-99m MAG3 RS, we were again unable to apply this new Tc-99m DTPA RS curve index. However, the presence of DGF is generally detected by serum creatinine measurement and RS is only of additional value if it can provide information on the cause of DGF or the prognosis. In the current study, we focused on the prediction of the duration of DGF which showed to have a sensitivity and specificity of the *TFS* for DGF ≥ 7 days of 80% and 61%.

Some limitations of our study need to be addressed. First, the retrospective design of this study creates the risk of selection bias. RS procedures were performed at the physicians' discretion in the UMCG cohort which contributed to a selection of patients with generally worse early post-transplant outcomes compared to the whole renal transplant population. This was confirmed by the higher number of rejections at day 14 post-transplantation together with a higher percentage of patients with DGF after all types of donation, both showing a significant difference compared to the LUMC cohort. The high number of patients with DGF influenced the positive and negative predictive values, which should therefore be judged together with the provided sensitivity and specificity. Second, we had to exclude 55 patients, because the RS-results could not be retrieved, due to incomplete data. However, we do not expect that this has altered our results since the exclusion was due to technical problems and thus not leading to extra selection bias. Third, the long period of inclusion, 2000 – 2014, contributed to a variety of RS protocols. The main differences were the duration of the scan, between 20 and 40 minutes, and the software used for the RS-analysis. This shortcoming was addressed by shortening the analysed procedure time, maximum analysed procedure duration of 20 minutes, and by using the new method for the quantitative analyses. Finally, we do not expect large differences in the injected dose of the radiopharmaceutical since the deviation in administered doses were considered small. Additionally, we minimised the effect of the administered doses by correcting the quantitative indices for the administered dose.

In conclusion, the qualitative RS-grading and the RS quantitative indices *cTER* and *Average upslope* predict a DGF duration longer than 7 days with a high sensitivity. This finding may help to support both clinicians and patients in managing expectations in the early post-operative period. However, due to the low specificity for DGF ≥ 7 days RS does not contribute to the identification of patients with prolonged DGF due to superimposed rejection.

REFERENCES

1. Cornell LD, Smith RN, Colvin RB. Kidney Transplantation: Mechanisms of Rejection and Acceptance. *Annu Rev Pathol Mech Dis*. 2008;3:189-220.
2. Perico N, Cattaneo D, Sayegh MH, Remuzzi G. Delayed graft function in kidney transplantation. *Lancet*. 2004;364(9447):1814-1827.
3. Sharif A, Borrows R. Delayed graft function after kidney transplantation: The clinical perspective. *Am J Kidney Dis*. Published online 2013.
4. Mallon DH, Summers DM, Bradley JA, Pettigrew GJ. Defining delayed graft function after renal transplantation: simplest is best. *Transplantation*. 2013;96(10):885-889.
5. Yarlagadda SG, Coca SG, Formica RN, Poggio ED, Parikh CR. Association between delayed graft function and allograft and patient survival: a systematic review and meta-analysis. *Nephrol Dial Transplant*. 2008;24(3):1039-1047.
6. Butala NM, Reese PP, Doshi MD, Parikh CR. Is delayed graft function causally associated with long-term outcomes after kidney transplantation? Instrumental variable analysis. *Transplantation*. 2013;95(8):1008-1014.
7. Johnston O, O'Kelly P, Spencer S, et al. Reduced graft function (with or without dialysis) vs immediate graft function—a comparison of long-term renal allograft survival. *Nephrol Dial Transplant*. 2006;21(8):2270-2274.
8. El-Maghraby TAF, Boom H, Camps JAJ, et al. Delayed graft function is characterized by reduced functional mass measured by (99m)Technetium-mercaptoacetyl triglycine renography. *Transplantation*. 2002;74(2):203-208.
9. Yazici B, Oral A, Gokalp C, Akgün A, Toz H, Hoscoskun C. A New Quantitative Index for Baseline Renal Transplant Scintigraphy With 99mTc-DTPA in Evaluation of Delayed Graft Function and Prediction of 1-Year Graft Function. *Clin Nucl Med*. 2016;41(3):182-188.
10. Stevens H, Klerk JM, Mertens IJ, Rijk PP, Hené RJ. Quantitative baseline renography 48 hours after renal transplantation predicts long-term graft survival. *Eur J Nucl Med*. 2001;28(11):1677-1681.
11. Guignard R, Mourad G, Mariano-Goulart D. Utility of postsurgical renal scintigraphy to predict one-year outcome of renal transplants in patients with delayed graft function. *Nucl Med Commun*. 2011;32(4):314.
12. El-Maghraby TAF, De Fijter JW, Wasser MNJM, Pauwels EKJ. Diagnostic imaging modalities for delayed renal graft function: A review. *Nucl Med Commun*. 1998;19(10):915-936.
13. Russell CD, Dubovsky E V, Taylor AT. Prediction of urinary excretion of technetium-99m-MAG3. *J Nucl Med*. 1998;39(7):1257-1259.
14. Heaf JG, Iversen J. Uses and limitations of renal scintigraphy in renal transplantation monitoring. *Eur J Nucl Med*. 2000;27(7):871-879.
15. Majima T, Hattori R, Funahashi Y, et al. 99mTc-mercaptoacetyl triglycine renography to monitor renal transplant function among kidneys from donors after cardiac death. *Transplant Proc*. 2012;44(1):49-53.
16. El-Maghraby TAF, De Fijter JW, Van Eck-Smit BLF, Zwinderman AH, El-Haddad SI, Pauwels EKJ. Renographic indices for evaluation of changes in graft function. *Eur J Nucl Med*. 1998;25(11):1575-1586.
17. Yazici B, Yazici A, Oral A, Akgün A, Toz H. Comparison of Renal Transplant Scintigraphy With Renal Resistance Index for Prediction of Early Graft Dysfunction and Evaluation of Acute Tubular Necrosis and Acute Rejection. *Clin Nucl Med*. 2013;38(12):931-935.

18. Russell CD, Yang H, Gaston RS, Hudson SL, Diethelm AG, Dubovsky E V. Prediction of renal transplant survival from early postoperative radioisotope studies. *J Nucl Med.* 2000;41(8):1332-1336.
19. Yazici B, Oral A, Gokalp C, et al. Evaluation of Renal Transplant Scintigraphy and Resistance Index Performed Within 2 Days After Transplantation in Predicting Long-Term Graft Function. *Clin Nucl Med.* 2015;40(7):548-552.
20. Li Y, Russell CD, Palmer-Lawrence J, Dubovsky E V. Quantitation of renal parenchymal retention of technetium-99m-MAG₃ in renal transplants. *J Nucl Med.* 1994;35(5):846-850.
21. Hosmer D, Lemeshow S, Sturdivant R. *Applied Logistic Regression.* 3th ed. Chichester: Wiley; 2013.
22. Shan G. Improved Confidence Intervals for the Youden Index. Rapallo F, ed. *PLoS One.* 2015;10(7):e0127272.

Limited clinical value of two consecutive post-transplant renal scintigraphy procedures

European Radiology, 2020

Stan Benjamins
Robert A. Pol
Stefan P. Berger
Andor W.J.M. Glaudemans
Petra Dibbets-Schneider
Riemer H.J.A. Slart
Lioe-Fee de Geus-Oei

Background: Duration of delayed graft function (DGF) and length of hospital stay (LOS) are outcomes of interest in an era that warrants increased efficacy of transplant care whereas renal allografts originate increasingly from marginal donors. While earlier studies investigate the predictive capability of a single renal scintigraphy, this study focuses on the value of both DGF duration and LOS of consecutively performed scintigraphies.

Methods: From 2011 to 2014, renal transplant recipients referred for a Tc-99m MAG3 renal scintigraphy were included in a single-centre retrospective study. Primary endpoints were DGF duration and LOS. Both the first (≤ 3 days) and second scintigraphy (3-7 days after transplantation) were analysed using a 4-grade qualitative scale and quantitative indices (*TFS*, *cTER*, *MUC10*, *Average Upslope*).

Results: We evaluated 200 first and 108 (54%) consecutively performed scintigraphies. Kaplan-Meier curves for DGF duration and qualitative grading of the first and second scintigraphy showed significant differences between the grades ($p < 0.01$). Kaplan-Meier curve for the delta grades between these procedures (lower, equal, or higher grade) did not show significant differences ($p = 0.18$). Multivariate analysis showed a significant association between the qualitative grades, from the first and second scintigraphy, and DGF duration, HR 1.8 (1.4-2.2, $p < 0.01$) and 2.8 (1.8-4.3, $p < 0.01$), respectively.

Conclusions: Qualitative grades of single renal scintigraphies, performed within 7 days after transplantation, can be used to make a reliable image-guided decision on the need for dialysis and to predict LOS. A consecutive renal scintigraphy, however, did not show an additional value in the assessment of DGF.

INTRODUCTION

The duration of delayed graft function (DGF) and the length of hospital stay (LOS) are outcomes of interest in an era that warrants increased efficacy of transplant care whereas renal allografts originate increasingly from marginal donors, being allografts from extended criteria and donation after circulatory death (DCD) donors.

DGF describes the failure of the renal transplant to function immediately after transplantation.¹ DGF is associated with renal allograft failure in the first year after donation after brain death (DBD) transplantation; however, allografts with DGF still provide survival benefit compared to maintenance dialysis.² Moreover, DGF is associated with higher incidence of biopsy-proven acute rejection and increased LOS.³ The current trend of using marginal donors is associated with more DGF, longer hospital stay, and subsequently higher transplant-related costs.⁴⁻⁶ Predicting the duration of DGF and the LOS provides clinicians with the opportunity to optimize timing of renal biopsies and post-transplant dialysis. For this purpose, research focus has been on urinary and blood biomarkers for DGF, such as urinary tissue inhibitor of metalloproteinases-2 (TIMP-2), and quantitative/qualitative renal scintigraphy indices.^{7,8}

Renal scintigraphy is an imaging biomarker of renal function, reflecting perfusion, reabsorption and excretion. It may help predicting DGF and LOS.^{9,10} Results of renal scintigraphy can be interpreted qualitatively, differentiating in six- (*Heaf and Iversen Grading scale*) or in four-curve types, and quantitatively, using several time-activity indices.¹¹⁻¹⁶ Several studies showed promising results for the use of renal scintigraphy to predict the course of DGF; however, these studies did not adjust for clinical variables associated with DGF.^{13,17-20} Moreover, previous studies focused primarily on the qualitative and quantitative interpretation of renal scintigraphy parameters from single procedures, whereas, clinicians may focus more on consecutively performed imaging.

In this centre, Technetium-99m mercaptoacetyltriglycine (Tc-99m MAG3) renal scintigraphies were performed consecutively in the first week after transplantation in all patients with ongoing DGF, according to a standard post-transplant protocol. The present study was initiated to determine if two consecutive renal scintigraphies improved the prediction of DGF and LOS.

METHODS

Study design and participants

We studied all patients receiving a renal transplant at the Leiden University Medical Center, between 2011 and 2014, who underwent a Tc-99m MAG3 renal scintigraphy within 3 days after transplantation. These patients are all part of a larger dual-centre retrospective cohort, resulting in an earlier publication focusing on the predictive value of a single renal scintigraphy for the duration of DGF > 7 days after transplantation.²¹ Patients were not included in case of receiving a dual renal transplant or both a renal and pancreas transplant, and when under 18 years of age at moment of transplantation. All clinical data for this study were retrieved from our national transplant research database, the Netherlands Organ Transplant Registry (NOTR). Missing data and information on possible peri- and post-operative complications, was retrieved by screening patients' charts retrospectively. Patient data were processed and electronically stored according to the Declaration of Helsinki Ethical principles for medical research involving human subjects, and approval for this study was given by the Leiden University Medical Center ethics committee. The clinical and research activities being reported are consistent with the Principles of the Declaration of Istanbul as outlined in the "Declaration of Istanbul on Organ Trafficking and Transplant Tourism".

Outcome assessment

We defined DGF as the need for dialysis after transplantation (dialysis-based DGF) and as the failure of serum creatinine to decrease with $\geq 10\%$ / day during 3 consecutive days (functional DGF), which is in accordance with the majority of studies on DGF.²² Based on these definitions, we described early transplant function using four groups, namely immediate graft function (IGF), a serum creatinine decrease of $\geq 10\%$ / day during 3 consecutive days or no need for dialysis; slow graft function (SGF), DGF between day 3-6 after transplantation; delayed graft function (DGF), DGF for more than 7 days after transplantation; primary non-function (PNF), immediate graft failure with the need of dialysis. We defined LOS as the number of days between transplantation and initial discharge.

Clinical covariates

The following covariates were examined: (i) recipient factors (gender; age (years); Body Mass Index (BMI, in kg/m²), diabetes mellitus, duration of pre-transplant dialysis (months)); (ii) donor factors (age (years), living(un)related (L(U)RD) donation, donation after circulatory death (DCD), DBD); (iii) transplant factors (pre-emptive transplantation, number of human leukocyte antigen (HLA)-mismatches); (iiii) acute rejection, defined as renal biopsy proven acute rejection (BPAR) or as non-BPAR, being an acute rejection treatment episode without BPAR according to Banff 2015 criteria²³.

Renal scintigraphy

All included patients underwent renal scintigraphy for analysis of DGF, discerning possible acute tubular necrosis from vascular or urological complications. In our centre, a second renal scintigraphy was performed in case of ongoing DGF or suspicion of vascular/urological complications. Renal scintigraphies were performed using a bolus intravenous injection of 100 MBq Tc-99m MAG3. Two phase digital dynamic images were obtained and processed using Syngo.via (Siemens Healthineers): (i) 1-second frames for two minutes; (ii) 20-seconds frames for 28 minutes. To calculate the renal scintigraphy time-activity curves, renal transplant regions-of-interest (ROIs) were drawn manually surrounding the renal transplant and the background ROIs were drawn crescent-shaped, opposite of the renal vessels. Analysis of the renal scintigraphy data was performed blinded to all clinical variables by a single researcher.

Qualitative analysis of the time-activity curves was performed using a four-curve type differentiation (Figure 1 and Supplement Figure 1A & B), with a normal renal function with fast uptake and excretion (Grade 1), a normal uptake with flat excretion curve (Grade 2), a rising curve without excretion phase (Grade 3), and a reduced absolute uptake without excretion phase (Grade 4). Furthermore, renal scintigraphy results were stratified in four groups, being peri-transplant fluid collections, vascular complications, urological complications, and no complications.

Quantitative analysis was performed using four indices reflecting renal perfusion, reabsorption and excretion. The *tubular function slope (TFS)* is a linear fit of the Tc-99m MAG3 curve between 50 and 110 seconds, reflecting the tracer uptake by renal tubular cells (counts/second).^{13,24} *MUC10* reflects the uptake within the first 10 minutes, as a fraction of the injected dose (counts/second/MBq).¹⁹ The *corrected tubular extraction rate (cTER)* is the tracer uptake between procedure start and two minutes, corrected for the body surface (mL/min/1.73 m²).¹⁷ The *Average upslope* reflecting the curve during the upslope period (counts at 3 minutes - counts at 20 seconds) / 160 seconds, in counts/second).²¹

Statistical analysis

Baseline descriptive statistics and clinical characteristics are presented as mean $\pm$ SD or median (range) for continuous variables and counts with percentages for categorical variables. Mann-Whitney test and One-way ANOVA were used to describe the variance of continuous variables between groups. Two-sided p-values of less than 0.05 were considered to indicate statistical significance. Correlations were assessed by means of Pearson's or Spearman's analysis. Univariate and multivariate Cox proportional hazards analysis and Kaplan-Meier curves with log-rank tests, were used to examine

the associations. The hazard ratios (HRs) and their corresponding 95% confidence intervals (CIs) are reported. The added value of renal scintigraphy indices was assessed by examining the change in $-2 \log$ likelihood. We used Package for the Social Sciences (IBM® SPSS Statistics® Version 22) for all statistical analyses and GraphPad Prism, version 5.04 (GraphPad Software), for graph presentation.

Figure 1. Qualitative Renal Scintigraphy grading: Grade 1, a normal renal function with fast uptake and excretion; Grade 2, a normal uptake with flat excretion curve; Grade 3, a rising curve without excretion phase; Grade 4, a reduced absolute uptake without excretion phase.²¹

RESULTS

Patient characteristics

Patients' characteristics of the 200 included patients are displayed in Table 1. Median age was 52 ± 13 years, 59% were male, 12% underwent pre-emptive renal transplantation, and median (IQR) duration of pre-transplant dialysis was 36.4 (14.3 – 57.3) months. Seventy-four (37%) patients received a DBD transplant, 94 (47%) a DCD transplant, and 32 (16%) a living donor transplant. The median (IQR) LOS was 15 (11 – 21) days.

Table 1. Patient characteristics

Variable	Patients (n = 200)
Male ^a	116 (59)
Age, years ^b	55 ± 13
BMI ^b	26.6 ± 3.7
Pre-emptive Tx ^a	24 (12)
Duration pre-Tx dialysis, months ^c	36.4 (14.3 – 57.3)
Type of donation	
Living-(un)related	32 (16)
DBD	74 (37)
DCD	94 (47)
DGF > 7 days after Tx ^a	131 (66)
Length of hospital stay, days ^c	15 (11 – 21)
Rejection ^a	
7 days after Tx	22 (11)
14 year after Tx	37 (18.5)

Tx = kidney transplantation; DGF = delayed graft function

^a n (%); ^b mean $\pm$ standard deviation (SD); ^c median (IQR)

For 161 (81%) patients, the indication for the first renal scintigraphy was a suspected acute tubular necrosis as cause of DGF. For 39 (19%) patients, the indication was a suspicion for fluid collections, vascular, or urological complications. Only 3 out of these 39 patients experienced a vascular or urological complication needing surgical intervention within 2 weeks after transplantation.

The study population was stratified into four groups, based on early transplant function, as shown in Table 2. From the 131 patients experiencing either DGF or PNF, 108 patients underwent a second renal scintigraphy within 7 days after transplantation (Figure 2).

Table 2. Early graft function for different types of kidney donation

Type of donation	Early graft function			
	IGF (n = 39)	SGF (n = 30)	DGF (125)	PNF (n = 6)
Living-(un)related	20 (63%)	2 (6%)	7 (22%)	3 (9%)
DBD	16 (22%)	16 (22%)	41 (55%)	1 (1%)
DCD	3 (3%)	12 (13%)	77 (82%)	2 (2%)

IGF = immediate graft function; SGF = slow graft function; DGF = delayed graft function; PNF = primary non-function; DBD = donation after brain death; DCD = donation after circulatory death

Figure 2. Flowchart included patients (KTX = kidney transplantation; RS = renal scintigraphy).

Qualitative grades and DGF duration

The qualitative grades of the first renal scintigraphy did significantly ($p < 0.01$) differ between the groups of early graft dysfunction. DGF was observed in 75 (81%) out of 93 patients with Grade 3 and in 35 (85%) out of 41 patients with Grade 4, while IGF was noticed in 16 (88%) out of 18 patients with Grade 1 and in 19 (40%) out of 48 patients with Grade 2 (Supplement Table 1).

Based on the qualitative grades, the anticipated moment of DGF ending was calculated in a subset of patients without IGF (Table 3): Grade 1 and 2 of the first renal scintigraphy correspond with a median (IQR) of 5.0 (2.0-7.0) days DGF; Grade 3 with 7.0 (6.3-10.0) days DGF; Grade 4 with 11.0 (7.5-19.5) days DGF. Outcomes corresponding with the qualitative grading of the second renal scintigraphy are presented in Table 3.

The Kaplan-Meier curves (Figure 3) for the duration of DGF and qualitative grading of the first and second renal scintigraphy showed a significant difference in DGF duration between Grade 2, Grade 3, and Grade 4 ($p < 0.01$) and between Grade 3

and 4 ($p < 0.01$), respectively. The Kaplan-Meier curve for delta qualitative grades between the first and second renal scintigraphy did not show significant differences between grades ($p = 0.18$).

Using univariate Cox proportional hazard analysis, qualitative grades of both the first and second renal scintigraphy were significantly associated with the DGF duration. The delta qualitative grades between the first and second renal scintigraphy was not significantly associated with the duration of DGF (Table 4).

Table 3. Duration of delayed graft function and length of hospital stay based on qualitative grading

	Patients, n	Duration of DGF		Length of hospital stay	
		Median	IQR	Median	IQR
First scintigraphy					
Grade 1 and 2	30	5.0	2.0 – 7.0	11.0	12.0 – 19.0
Grade 3	89	7.0	6.3 – 10.0	15.0	12.0 – 19.0
Grade 4	41	11.0	7.5 – 19.5	20.0	14.0 – 28.5
Second scintigraphy					
Grade 2	25	7.0	7.0 – 7.5	14.0	12.0 – 16.5
Grade 3	52	8.0	7.0 – 10.0	15.0	12.3 – 19.0
Grade 4	31	15.0	10.0 – 20.0	22.0	15.8 – 29.3

Results based on a subset of the included patients, after exclusion of all patients with immediate graft function

Quantitative indices and DGF duration

Quantitative indices *TFS*, *cTER*, and *Average Upslope* of the first renal scintigraphy were significantly different between IGF and SGF, whereas *MUC10* did not show a significant difference. All indices were significantly different between SGF and DGF, whereas no significant difference was observed between DGF and PNF (Supplement Table 1 and Supplement Figure 2).

For the first renal scintigraphy, there was a significant association between the quantitative indices and DGF duration: *TFS*, $r = -0.44$, $p < 0.01$; *MUC10*, -0.46 , $p < 0.01$; *cTER*, -0.44 , $p < 0.01$; *Average Upslope*, -0.45 , $p < 0.01$. Analysis of the second renal scintigraphy showed a weaker, but still significant association between the quantitative indices and DGF duration: *TFS*, $r = -0.32$, $p = 0.01$; *MUC10*, -0.30 , $p = 0.02$; *cTER*, -0.32 , $p = 0.01$; *Average Upslope*, -0.33 , $p = 0.01$. Analysis of the delta quantitative indices between the first and second renal scintigraphy showed an even weaker association: *TFS*, $r = -0.24$, $p = 0.01$; *MUC10*, -0.26 , $p < 0.01$; *cTER*, -0.24 , $p = 0.01$; *Average Upslope*, -0.25 , $p = 0.01$.

Figure 3. Kaplan Meier curves for qualitative grading (KTX = kidney transplantation; RS = renal scintigraphy).

Using univariate Cox proportional hazard analysis, quantitative indices of both the first and second renal scintigraphy were significantly associated with the duration of DGF. The deltas of the quantitative indices *TFS* and *cTER*, between the first and second renal scintigraphy, were significantly associated with the duration of DGF, HR 0.4 (0.4-0.8, $p < 0.01$) and HR 1.0 (1.0-1.0, $p < 0.01$) respectively (Table 4).

Qualitative grades and length of hospital stay

Using univariate Cox proportional hazard analysis, qualitative grades of the first renal scintigraphy were significantly associated with LOS (Supplement Table 3). Based on the qualitative grades, the anticipated LOS was calculated (Table 3): Grade 1 and 2 of the first renal scintigraphy correspond with a median (IQR) of 11.0 (12.0-19.0) days of hospitalization; Grade 3 with 15.0 (12.0-19.0) days of hospitalization; Grade 4 with 20.0 (14.0-28.5) days hospitalization. Outcomes corresponding with the qualitative grading of the second renal scintigraphy, and the delta between the first and second renal scintigraphy are presented in Table 3.

Table 4. Cox proportional hazard analysis for quantitative / qualitative indices and duration of delayed graft function

Indices	First renal scintigraphy (n = 200)			
	Univariate		Multivariate	
	Hazard ratio	p-value	Hazard ratio	p-value
Quantitative				
TFS	0.5 (0.4-0.6)	< 0.01	1.3 (0.3-6.0)	0.77
MUC10	0.2 (0.1-0.4)	< 0.01	0.5 (0.2-1.2)	0.12
cTER	0.6 (0.5-0.7)	< 0.01	0.8 (0.2-2.6)	0.68
Average Upslope	0.6 (0.5-0.8)	< 0.01	0.9 (0.7-1.2)	0.59
Qualitative grading (0 – 4)	2.0 (1.7-2.4)	< 0.01	1.8 (1.4-2.2)	< 0.01

Data in parentheses are 95% confidence intervals. Hazard ratios are per Log-unit of change for the quantitative indices.

Multivariate analysis consists of all quantitative/qualitative indices, recipients age, gender, Body Mass Index, Diabetes Mellitus, DCD donation, pre-emptive transplantation, donor age, HLA-mismatches, and duration of pre-transplant dialysis.

Quantitative indices and length of hospital stay

For the first renal scintigraphy, there was a significant, however, weak correlation between LOS and the quantitative indices: *TFS*, $r = -0.23$, $p < 0.01$; *MUC10*, -0.28 , $p < 0.01$; *cTER*, -0.23 , $p < 0.01$; *Average Upslope*, -0.19 , $p < 0.01$. Analysis of the second renal scintigraphy also showed a weak but significant correlation between LOS and the quantitative indices: *TFS*, $r = -0.24$, $p = 0.02$; *MUC10*, -0.26 , $p < 0.01$; *cTER*, -0.24 , $p = 0.02$; *Average Upslope*, -0.23 , $p = 0.02$. Using univariate Cox proportional hazard analysis, quantitative indices (*TFS*, *MUC10*, and *cTER*) of the first renal scintigraphy were significantly associated with LOS (Supplement Table 3).

Multivariate analysis

Covariates with a significant association with DGF were DCD donation, HR 1.9 (1.4 – 2.5, $p < 0.01$); pre-emptive transplantation, HR 0.5 (0.3 – 0.7, $p < 0.01$); and duration of pre-transplant dialysis, HR 1.1 (1.0-1.1, $p < 0.01$) (Supplement Table 3). The clinical covariates recipient gender, recipient age, recipient BMI, pre-transplantation diabetes mellitus, donor age, and HLA-mismatches did not contribute to a significant hazard ratio (Supplement Table 2). Outcomes corresponding with the LOS are presented in Supplement Table 2.

Second renal scintigraphy (n = 108)				Delta renal scintigraphy (n = 108)			
Univariate		Multivariate		Univariate		Multivariate	
Hazard ratio	p-value	Hazard ratio	p-value	Hazard ratio	p-value	Hazard ratio	p-value
0.6 (0.4-0.8)	< 0.01	2.3 (0.4-12.4)	0.34	0.4 (0.2-0.8)	< 0.01	0.4 (0.1-1.4)	0.16
0.4 (0.2-0.8)	< 0.01	1.0 (0.2-3.9)	0.97	1.0 (1.0-1.0)	0.09	1.0 (1.0-1.0)	0.88
0.6 (0.4-0.8)	< 0.01	0.3 (0.1-2.1)	0.45	1.0 (1.0-1.0)	< 0.01	1.0 (1.0-1.0)	0.16
0.5 (0.3-0.8)	< 0.01	0.9 (0.4-2.1)	0.73	1.0 (1.0-1.0)	0.83	1.0 (1.0-1.0)	0.77
2.2 (1.6-3.0)	< 0.01	2.8 (1.8-4.3)	< 0.01	1.3 (1.0-1.8)	0.08	1.4 (0.9-2.2)	0.15

TFS = Tubular function slope; MUC₁₀ = first 10 min uptake as a fraction of the injected dose; cTER = corrected tubular extraction rate; Average upslope = reflecting the slope during counts at 20 sec and counts at 3 min.

In a multivariate analysis, including all other quantitative indices, the qualitative grading scale, and the clinical covariates, the association between the qualitative grading of the first renal scintigraphy and the duration of DGF was significant for Grade 3, HR 2.3 (1.3-4.2, $p < 0.01$), and Grade 4, HR 3.4 (1.7-7.1, $p < 0.01$). The association between the qualitative grading of the second renal scintigraphy and the duration of DGF was significant for Grade 4, HR 4.1 (1.9-8.8, $p < 0.01$) (Table 4).

In a multivariate analysis, including all other quantitative indices, the qualitative grading scale and the clinical covariates, the association between the qualitative grading of the first renal scintigraphy and LOS was HR 1.3 (1.0-1.6, $p = 0.04$). Multivariate analysis of the quantitative indices, the qualitative grading scale of the second renal scintigraphy and LOS did not result in significant associations (Supplement Table 2).

Predictive performance of qualitative grades for the duration of DGF

When assessing the predictive performance of the clinical variables, the -2 log likelihood improved significantly when including the qualitative grades from the first renal scintigraphy (1623.6 to 1583.7, $p < 0.01$). The predictive performance of the model with clinical variables, did not show a significant improvement after including the qualitative grades of the second renal scintigraphy (766.0 to 737.9, $p < 0.01$).

DISCUSSION

Our analysis of Tc-99m MAG₃ renal scintigraphies indicates that qualitative grades of two separately analysed procedures, at ≤ 3 and ≤ 7 days after transplantation respectively, are significantly associated with the duration of DGF and the LOS. However, the delta of qualitative grades and the changes of quantitative indices between these sequential performed renal scintigraphies are not associated with the duration of DGF and the LOS. These findings underline the strength of qualitative analysis of single renal scintigraphy for the prediction of DGF duration and LOS. Conversely, there is no additional value of performing repetitive renal scintigraphy procedures to assess duration of DGF and LOS.

Our study confirms the findings of previous studies, which indicated the applicability of the quantitative indices *TFS*, *MUC10*, *cTER* and *Average Upslope*, and of qualitative grading with four or six grades for the evaluation of DGF.^{12,13,17–20} A previous study, focusing on *TFS* at 48 hours after transplantation, showed the capability of this index to separate patients with DGF from patients with IGF¹³. In our study, *TFS* differed significantly between types of early transplant function and was associated with a longer duration of DGF, HR 0.5 (0.4–0.6, $p < 0.01$) and HR 0.6 (0.4–0.8, $p < 0.01$) respectively for the first and second renal scintigraphy. For *MUC10* from a renal scintigraphy performed within 48 hours after transplantation, a previous study showed significant differences between DGF and non-DGF patients, which is in line with the results of our analysis, showing a significant difference in *MUC10* values between the SGF and DGF group.¹⁹ For *cTER* from a renal scintigraphy performed ≤ 4 days after transplantation, a previous study showed a significant correlation with the period of dialysis dependence ($r = -0.68$, $p < 0.01$), which is slightly stronger than the correlation found in this study ($r = -0.44$, $p < 0.01$).¹⁷ In a previous study, a four grade index was introduced for renal scintigraphy at ≤ 3 days after transplantation, using this four grade index an independent association between a longer duration of DGF and the qualitative grades was shown, HR 1.8 (1.4–2.2, $p < 0.01$), which is consistent with the results of studies using both four and six grade indices.^{12,21}

Although previous studies have described the applicability of a first renal scintigraphy at ≤ 48 or 62 hours after transplantation, this study is the first comprehensive analysis of a second renal scintigraphy at ≤ 7 days after transplantation. Quantitative indices of the second renal scintigraphy were associated with the duration of DGF, however, not when adjusted for clinical covariates. Multivariate analysis of the first and second renal scintigraphy showed an independent significant association between the qualitative grades and duration of DGF, HR 1.8 (1.4–2.2, $p < 0.01$) and HR 2.8 (1.8–4.3, $p < 0.01$) respectively. The delta qualitative grades between the procedures were not significantly associated with the duration of DGF in multivariate analysis.

The presented results should be evaluated in light of non-imaging biomarkers for DGF, such as the urinary biomarker TIMP-2 and neutrophil gelatinase-associate lipocalin (NGAL). The predictive value of TIMP-2 was assessed in a population of DCD transplant recipients ($n = 74$), showing an area-under-the-curve (AUC) of 0.89 (95% CI 0.78 – 0.99) for >7 days functional DGF.⁷ For urinary NGAL, the AUC for >7 functional and dialysis based DGF was 0.75 (95% CI 0.65 – 0.84) in a population of both DBD and DCD transplant recipients ($n = 176$).⁸ Renal scintigraphy, performed within 3 days post-transplantation to predict ≥ 7 functional and dialysis based DGF, showed to have an 87% sensitivity and 65% specificity when analysed qualitatively and an AUC of 0.82 (95% CI 0.78 – 0.86) when analysed quantitatively.²¹ Further prospective studies are needed to establish the clinical value of qualitative and quantitative renal scintigraphy analysis in light of emerging non-imaging biomarkers for DGF.

Previous studies focusing on the use of renal scintigraphy after transplantation did not use LOS as one of the endpoints. However, this is important since with the increased use of renal allografts from extended criteria and DCD donors, a prolonged hospital stay, and subsequent higher transplant-related costs are reported.⁴ In addition, an increased focus on patient-related outcome measures (PROMS) shows the significance of informing patients on the clinical path during and after hospitalization, urging for a reliable indication of moment of DGF ending and the LOS. The results of our study are in line with the literature, with 47% of the transplants coming from DCD donors, a median length of stay of 15 (11-21) days and in 66% of patients a DGF duration of > 7 days and provide insight in the expected moment of hospital discharge.

Due to the retrospective design, a clinical selection bias resulted in cohort of patients with a high incidence of DGF and a minimal number of patients with IGF, this selection further increased when analysing patients with a second renal scintigraphy. On the contrary, the relatively large number of patients with a sequential renal scintigraphy ≤ 7 days after transplantation contributes to a reliable analysis. Thereby, we performed an extensive multivariate analysis to adjust for possible confounders, including all qualitative and quantitative scintigraphy indices in a single model. Presenting a single-centre study with a small timeframe for inclusion, we can expect a uniformity in transplant care, similarity in renal scintigraphies. Furthermore, analysing the results quantitatively and qualitatively decreases the impact of inter-observer variability, while our blinded renal scintigraphy analysis decreases the risk of bias.

In conclusion, a reliable indication of the duration of DGF and the LOS can be provided by qualitative analysis of single renal scintigraphies, whereas the qualitative and quantitative change between sequential performed renal scintigraphies does not strengthen the prediction of DGF duration and LOS. Qualitative grades of single renal scintigraphies can be used to provide clinicians and patients with a reliable indication of the need for dialysis after transplantation and the expected duration of hospitalization, while the additional value of performing a consecutive renal scintigraphy for the assessment of DGF was not found.

REFERENCES

1. Perico N, Cattaneo D, Sayegh MH, Remuzzi G. Delayed graft function in kidney transplantation. *Lancet*. 2004;364(9447):1814-1827.
2. Gill J, Dong J, Rose C, Gill JS. The risk of allograft failure and the survival benefit of kidney transplantation are complicated by delayed graft function. *Kidney Int*. 2016;89:1331-1336.
3. Wu WK, Famure O, Li Y, Kim SJ. Delayed graft function and the risk of acute rejection in the modern era of kidney transplantation. *Kidney Int*. 2015;88(10):851-858.
4. Saidi RF, Elias N, Kawai T, et al. Outcome of kidney transplantation using expanded criteria donors and donation after cardiac death kidneys: realities and costs. *Am J Transplant*. 2007;7(12):2769-2774.
5. Englesbe MJ, Ads Y, Cohn JA, et al. The effects of donor and recipient practices on transplant center finances. *Am J Transplant*. 2008;8(3):586-592.
6. Machnicki G, Lentine KL, Salvalaggio PR, Burroughs TE, Brennan DC, Schnitzler MA. Kidney transplant Medicare payments and length of stay: associations with comorbidities and organ quality. *Arch Med Sci*. 2011;7(2):278-286.
7. Bank JR, Ruhaak R, Soonawala D, et al. Urinary TIMP-2 predicts the presence and duration of delayed graft function in donation after circulatory death kidney transplant recipients. *Transplantation*. Published online 2018.
8. Hollmen ME, Kyllönen LE, Inkinen KA, Lalla MLT, Salmela KT. Urine neutrophil gelatinase-associated lipocalin is a marker of graft recovery after kidney transplantation. *Kidney Int*. 2011;79(1):89-98.
9. Yazici B, Gunay E, Akgün A, Sen S, Ozbek SS. Scintigraphic and Sonographic Findings of Subcapsular Cortical Necrosis in a Transplanted Kidney. *Clin Nucl Med*. 2018;43(7):542-545.
10. Yazici B, Oral A, Akgün A. Contribution of SPECT/CT to evaluate urinary leakage suspicion in renal transplant patients. *Clin Nucl Med*. 2018;43(10):e378-e380.
11. Benjamins S, Berger SP, Glaudemans AWJM, Sanders JSF, Pol RA, Slart RHJA. Renal scintigraphy for post-transplant monitoring after kidney transplantation. *Transplant Rev*. 2018;32(2):102-109.
12. Heaf JG, Iversen J. Uses and limitations of renal scintigraphy in renal transplantation monitoring. *Eur J Nucl Med*. 2000;27(7):871-879.
13. El-Maghraby TAF, Boom H, Camps JAJ, et al. Delayed graft function is characterized by reduced functional mass measured by (99m)Technetium-mercaptoacetyl triglycine renography. *Transplantation*. 2002;74(2):203-208.
14. Yazici B. Practical Method for Calculation of Graft Index in Renal Transplant Scintigraphy: A Technical Note. *Clin Nucl Med*. 2018;00(00):1.
15. Yazici B, Yazici A, Oral A, Akgün A, Toz H. Comparison of renal transplant scintigraphy with renal resistance index for prediction of early graft dysfunction and evaluation of acute tubular necrosis and acute rejection. *Clin Nucl Med*. 2013;38(12):931-935.
16. Yazici B, Oral A, Gokalp C, et al. Evaluation of Renal Transplant Scintigraphy and Resistance Index Performed Within 2 Days After Transplantation in Predicting Long-Term Graft Function. *Clin Nucl Med*. 2015;40(7):548-552.
17. Majima T, Hattori R, Funahashi Y, et al. 99mTc-mercaptoacetyl triglycine renography to monitor renal transplant function among kidneys from donors after cardiac death. *Transplant Proc*. 2012;44(1):49-53.
18. Obeidat MA, Luyckx VA, Grebe SO, et al. Post-transplant nuclear renal scans correlate with renal injury biomarkers and early allograft outcomes. *Nephrol Dial Transplant*. 2011;26(9):3038-3045.

19. Stevens H, Klerk JM, Mertens IJ, Rijk PP, Hené RJ. Quantitative baseline renography 48 hours after renal transplantation predicts long-term graft survival. *Eur J Nucl Med*. 2001;28(11):1677-1681.
20. Yazici B, Oral A, Gokalp C, Akgün A, Toz H, Hoscoskun C. A New Quantitative Index for Baseline Renal Transplant Scintigraphy With ^{99m}Tc -DTPA in Evaluation of Delayed Graft Function and Prediction of 1-Year Graft Function. *Clin Nucl Med*. 2016;41(3):182-188.
21. Benjamins S, Pol RA, de Geus-Oei L-F, et al. Can transplant renal scintigraphy predict the duration of delayed graft function? A dual center retrospective study. *PLoS One*. 2018;13(3):e0193791.
22. Yarlagadda SG, Coca SG, Garg AX, et al. Marked variation in the definition and diagnosis of delayed graft function: a systematic review. *Nephrol Dial Transplant*. 2008;23(9):2995-3003.
23. Loupy A, Haas M, Solez K, et al. The Banff 2015 Kidney Meeting Report: Current Challenges in Rejection Classification and Prospects for Adopting Molecular Pathology. *Am J Transplant*. 2017;17(1):28-41.
24. Guignard R, Mourad G, Mariano-Goulart D. Utility of postsurgical renal scintigraphy to predict one-year outcome of renal transplants in patients with delayed graft function. *Nucl Med Commun*. 2011;32(4):314.

Part II

**Calcification before and
after kidney transplantation**

[¹⁸F]-sodium fluoride autoradiography imaging of nephrocalcinosis in donor kidneys and explanted kidney allografts

Scientific Reports, 2021

Stan Benjamins
Ines F. Antunes
Jan-Luuk Hillebrands
Melanie Reijrink
Marian L.C. Bulthuis
Stefan P. Berger
Cyril Moers
Martin H. de Borst
Riemer H.J.A. Slart
Robert A. Pol

Background: Nephrocalcinosis is present in up to 43% of kidney allograft biopsies at one-year after transplantation and is associated with inferior graft function and poor graft survival.

Methods: We studied [^{18}F]-sodium fluoride ([^{18}F]-NaF) imaging of microcalcifications in donor kidneys ($n = 7$) and explanted kidney allografts ($n = 13$). Three μm paraffin-embedded serial sections were used for histological evaluation of calcification (Alizarin Red; Von Kossa staining) and ex-vivo [^{18}F]-NaF autoradiography. The images were fused to evaluate if microcalcification areas corresponded with [^{18}F]-NaF uptake areas.

Results Based on histological analyses, tubulointerstitial and glomerular microcalcifications were present in 19/20 and 7/20 samples, respectively. Using autoradiography, [^{18}F]-NaF uptake was found in 19/20 samples, with significantly more tracer activity in kidney allograft compared to deceased donor kidney samples ($p = 0.019$). Alizarin Red staining of active microcalcifications demonstrated good correlation (Spearman's rho of 0.81, $p < 0.001$) and Von Kossa staining of consolidated calcifications demonstrated significant but weak correlation (0.62, $p = 0.003$) with [^{18}F]-NaF activity.

Conclusions: This correlation between ex-vivo [^{18}F]-NaF uptake and histology-proven microcalcifications, is the first step towards an imaging method to identify microcalcifications in active nephrocalcinosis. This may lead to better understanding of the aetiology of microcalcifications and its impact on kidney transplant function.

INTRODUCTION

Nephrocalcinosis, defined as parenchymal and tubular deposition of calcium-oxalate (CaOx) or calcium-phosphate (CaPhos), is an important factor in the decline of graft function after kidney transplantation.¹⁻³ Deposition of CaOx and CaPhos are attributed to high levels of serum oxalate, serum calcium and serum phosphate in patients with chronic kidney disease and/or hyperparathyroidism.^{4,5} The incidence of nephrocalcinosis, based on kidney allograft biopsies, is 6% at 6 months, 25 - 43% at one-year and up to 79% at 10-years after kidney transplantation.⁵⁻⁷ Studies focusing on kidney allograft biopsies within one-year after transplantation demonstrated an association between the early presence of calcium depositions and both inferior graft function and poor graft survival.^{4,8,9} However, whether these microcalcifications play an active role in allograft dysfunction or are merely indicators of deregulated mineral metabolism remains unclear.

To date, kidney allograft biopsies are the only diagnostic method to evaluate post-transplant microcalcifications. A non-invasive and reliable diagnostic approach would allow for repeated measurements, facilitating tracking of calcifications over time and intervention studies with potential improvement of outcomes for graft function and graft survival after kidney transplantation.¹⁰

Positron emission tomography / computed tomography (PET/CT) imaging with [¹⁸F]-sodium fluoride ([¹⁸F]-NaF) is used in clinical practice and experimental studies as skeletal and vascular imaging modality.¹¹⁻¹⁵ While reliable visualization of vascular microcalcifications is hampered with CT imaging, results of ex-vivo [¹⁸F]-NaF microPET imaging correlated well with histology-proven microcalcifications in human and animal vascular samples.^{16,17} Moreover, the results of several prospective clinical studies showed that early vascular [¹⁸F]-NaF activity relates well to both macrocalcifications, detected by CT imaging, and more interestingly, the increase in size of these calcifications.¹⁸⁻²⁰

Here, we present the results of a proof of concept autoradiography study for ex-vivo use of [¹⁸F]-NaF imaging of nephrocalcinosis in donor kidneys and transplanted kidney allografts as a diagnostic approach for the visualization of microcalcifications.

METHODS

Human kidney samples

Kidney tissue was obtained after transplantectomy ($n = 13$) and from discarded kidneys from deceased donors ($n = 7$) in case of decline of the donor kidney by affiliated transplant centres. Clinical data from kidney transplant recipients and donors was retrieved from the patients' medical records, including presence of hyperparathyroidism, hyperphosphatemia and diabetes mellitus prior to transplantectomy, and clinical pathology assessment of kidney allograft samples reported using the *Banff Classification of Renal Allograft Pathology*.²¹ Research activities were performed in line with the declaration of Helsinki Ethical principles for medical research involving human subjects and the Institutional review board gave approval for this study (Medical Ethical Committee *University Medical Center Groningen* 2015/501). Informed consent was obtained from all transplant recipients and pre-donation approval was given for the use of discarded kidneys from deceased donors for research purposes. The clinical and research activities were consistent with the Principles of the Declaration of Istanbul as outlined in the 'Declaration of Istanbul on Organ Trafficking and Transplant Tourism'.

The Standard Operating Procedure (SOP) for tissue sampling was as follows: Samples were obtained at surgical theatre, immediately following kidney extraction (for transplantectomy) or at arrival of the discarded donor kidney in our transplant centre. The samples used for this experiment were taken in the transverse plane of the upper pole, including cortical and medullary kidney tissue, with a size between 25mm x 15mm and 20mm x 40mm. These samples were formalin-fixed and paraffin-embedded. Using a microtome precision cutting instrument, three μm paraffin-embedded sections were made. These sections were mounted on adhesion microscope glass (TOMO, Matsunami Glass, USA). Serial sections, i.e. adjacent sections that reveal sequential layers of the kidney tissue samples, were used for autoradiography and histological analysis. Prior to tracer incubation and histological staining, sections were de-paraffinized, rehydrated in demineralized water, and temporarily stored in phosphate-buffered saline (PBS).

[^{18}F]-NaF incubation and autoradiography

The ex-vivo experiment was based on the protocol used in the previous studies by Hop et al. and Reijrink et al.^{22,23} The first study is an autoradiography study on [^{18}F]-NaF uptake in carotid plaques from stroke patients undergoing surgery, with CT-based indication of calcification as comparison. The second study is an autoradiography study on [^{18}F]-FDG uptake in adipose tissue samples, with immunohistochemistry of serial sections as comparison. One of each three serial sections was bathed in a solution of [^{18}F]-NaF, at a concentration of 618 millibecquerel (MBq) in 300mL PBS, at room temperature, for

30 minutes. Washing was performed by three times rinsing with cold PBS and twice with cold water. Samples were dried in open air and a blanked phosphor screen was placed over the samples. Exposure was performed overnight, for a period of 24 hours and 30 minutes. Determining tissue activity distribution and intensity was performed on a phosphor screen using the Amersham Typhoon Biomolecular Imager (GE healthcare, Marlborough, USA).

Alizarin red and Von Kossa staining

Alizarin red staining was performed to visualize calcium accumulation in early stage calcification. Samples were incubated in 2% w/v Alizarin red (dissolved in demineralized water) solution, pH 4.2 for 5 minutes.²⁴ Washing was performed by 20 times rinsing in 1:1 acetone/xylene and 20 times rinsing in 100% xylene. Von Kossa staining was performed to visualize presence of inorganic phosphate molecules in consolidated calcifications. Briefly, samples were incubated in 1% silver nitrate (AgNO₃) solution for 1 hour, while being exposed to sunlight.²⁴ Alternated by three times rinsing in demineralized water, incubation was performed with sodium thiosulfate for 5 minutes, followed by incubation in nuclear fast red for 3 minutes.

Image processing and analyses

Stained tissue sections were digitalized with the Hamamatsu Nanozoomer 2.0HT (Hamamatsu Photonics, Hamamatsu, Japan), and images were inspected using Aperio ImageScope software version 12.1.0.5029 (Leica Microsystems CMS GmbH, Wetzlar, Germany). Autoradiography and quantitative computerized image analyses were performed with ImageJ software, version 1.51 (NIH, Bethesda, Maryland, <http://fiji.sc>).

Background subtraction was applied for all histology images, inserting an outline at the border of the histology sample. Semi-automated detection of calcifications in the Alizarin red and Von Kossa staining was performed with the ImageJ - BioVoxxel Toolbox.²⁵ Thresholds were applied for all samples and detected areas of calcification were dilated with 6 iterations, to optimize visualization. For the autoradiography images, rotation and re-sizing was performed based on the original histology samples. Thresholds were applied for all samples, resulting in areas of tracer uptake, following the approach described by Irkle et al.: images were thresholded using the Otsu method, followed by Gaussian blurring and thresholding with the Li method.¹⁶ Overlay images were acquired by matching of the outlines from the adjacent histology images, with subsequent matching of the histology and autoradiography results (Figure 1). Pixel-based quantification of calcification and tracer area (as percentage of the sample area) was performed with the ImageJ – Analyze Particles tool.

Statistical analyses

Statistics were performed with the Statistical Package for the Social Sciences version 23 (IBM Corporation, Armonk N.Y., USA), graphs were made with GraphPad Prism 7.02 for Windows (GraphPad Software, San Diego, USA) and figures were produced using Adobe Illustrator CS6 (Adobe Systems, San Jose, USA). Results were presented as median and interquartile range (IQR) for skewed data, and as frequency and percentage when data were categorical. We compared groups using Mann-Whitney U tests (two-tailed p -value < 0.05 = significant) and correlation analyses were performed using the Spearman rank test (two-tailed p -value and Spearman's ρ). True and false positive rates were based on the pixel-based quantification of tracer and histology findings. To assess the interobserver reproducibility for semi-automated detection of calcifications, the intraclass correlation coefficient (ICC) with 95% confidence intervals (CI) were calculated.

Figure 1. Autoradiography image of [¹⁸F]-sodium fluoride ([¹⁸F]-NaF) uptake (A), with the overlay image of [¹⁸F]-NaF uptake with Alizarin red staining of microcalcifications (B) and Von Kossa staining of calcifications (C).

RESULTS

Sample cohort

Thirteen kidney samples were obtained after transplantectomy, at a median of 33 [IQR 9 – 66] months after transplantation, from transplant recipients with a median age of 51 [IQR 30 – 64] years. Transplantectomy was performed for allograft rejection (Banff IIA and IIB) in seven patients, chronic allograft arteriopathy in two patients, allograft infection in two patients, primary nonfunction one patient, and interstitial fibrosis and tubular atrophy Grade III in one patient. Eight out of 13 (61.5%) patients had hyperparathyroidism, seven (53.8%) had hyperphosphatemia, five (38.5%) had diabetes mellitus and none of these patients had signs of nephrocalcinosis on clinical ultrasound or a history of kidney stones (Table 1). Seven deceased donor kidney samples were obtained, from donors with a median age of 65 [IQR 64 – 71] years. Four out of seven (57.1%) donors had a history of cardiovascular disease, none had diabetes mellitus and six out of seven (85.7%) had atherosclerosis of the kidney vasculature on visual inspection, which are potential factors for declining these donor kidneys (Table 2).

Autoradiography and histology

With autoradiography, [^{18}F]-NaF uptake was identified in 19 out of 20 samples, with a median tracer surface area of 0.81% [IQR 0.27 – 5.19%]. A tracer surface area of $\leq 5\%$ was seen in 15 (75%) and of $> 5\%$ in 5 (25%) out of 20 samples (Figure 1 and Figure 2 – A). The number of [^{18}F]-NaF areas was significantly higher in kidney allograft samples compared to deceased donor kidney samples, with a median of 7.0 [IQR 4.0 – 15.5] and 2.0 [IQR 1.0 – 6.0], respectively ($p = 0.019$).

Tubulointerstitial microcalcifications, visualised with Alizarin red staining, were present in 19 out 20 samples, with a median of 5.5 [IQR 2 – 10.3] per sample, and glomerular microcalcifications were seen in seven out 20 samples, with a median of 1 [IQR 1 – 3] per sample (Figure 2 – B). A significantly higher number of microcalcifications was observed in kidney allograft samples compared to deceased donor kidney samples, with a median of 9.0 [IQR 5.0 – 20.5] and 4.0 [IQR 1.0 – 4.0], respectively ($p = 0.046$). With Von Kossa staining, tubulointerstitial calcifications were seen in 18 out 20 samples, with a median of 2.5 [IQR 1 – 5] per sample, and glomerular calcifications were seen in seven out 20 samples, with a median of 1 [IQR 0 – 3] per sample (Figure 2 – C). The number of calcifications was not significantly different between kidney allograft samples and deceased donor kidney samples, with a median of 4.0 [IQR 2.0 – 6.0] and 2.0 [IQR 2.0 – 7.0], respectively ($p = 0.311$). The interobserver reproducibility was identified between visual and semi-automated detection of calcifications, with an ICC of 0.94 (95% CI 0.85 – 0.98) for Alizarin red staining and of 0.77 (95% CI 0.42 – 0.91) for Von Kossa staining.

Table 1. Kidney transplant recipient characteristics

	Age	Gender	Months post- transplant	Hyper- para- thyroidism	Hyper- phospha- temia	Diabetes mellitus	Pathology assessment*
I	67	Male	7	no	no	yes	Primary non-function
II	21	Male	35	yes	yes	no	Vascular rejection (type IIB)
III	43	Female	13	no	no	yes	Vascular rejection (type IIA) & infection
IV	30	Female	74	no	yes	yes	Interstitial fibrosis & cellular rejection (type IIA)
V	27	Female	10	yes	no	no	Vascular rejection (type IIB) & ischemic nephropathy
VI	57	Male	6	yes	yes	no	FSGS & vascular rejection (type IIB) & CaOx depositions
VII	28	Male	43	yes	yes	no	Chronic allograft arteriopathy
VIII	78	Male	1	yes	yes	yes	IFTA Grade III
IX	64	Female	146	yes	no	no	Chronic allograft arteriopathy & IFTA Grade III
X	37	Male	31	no	yes	no	Acute rejection (type IIB) & IFTA Grade III
XI	52	Female	215	yes	yes	yes	Infection & extensive ischemic damage
XII	37	Female	55	yes	no	no	Acute rejection (type IIB)
XIII	63	Female	22	no	no	no	Infection & extensive ischemic damage

No cases of hypercalcemia is this cohort; *classification of Banff Classification of Allograft Pathology – 2018 ²¹; FSGS = Focal segmental glomerulosclerosis; IFTA = Interstitial fibrosis and tubular atrophy; Corresponding autoradiography and histology results are presented as supplementary of the original manuscript

Table 2. Deceased kidney donor characteristics

	Age	Gender	Cause of death	Cardiovascular disease*	Atherosclerosis**
I	71	Male	Cardiac arrest	yes	Massive
II	63	Male	Intra cerebral bleeding	yes	Massive
III	47	Male	Cardiac arrest	no	Unknown
IV	71	Male	Cardiac arrest	no	Moderate
V	65	Male	Intra cerebral bleeding	yes	Massive
VI	71	Female	Sub Arachnoid bleeding	yes	Massive
VII	54	Male	Traumatic brain injury	no	Moderate

*Cardiovascular disease defined as cerebrovascular event, myocardial infarction, vascular disease requiring invasive intervention; **as noted by the procurement team; Corresponding autoradiography and histology results are presented as supplementary of the original manuscript; no donors with diabetes mellitus

Overlay images of autoradiography and histology results showed co-localization of [^{18}F]-NaF uptake with Alizarin red staining for microcalcification (Figure 1 – B). In comparison, overlay images of [^{18}F]-NaF uptake and Von Kossa staining of calcifications showed uptake in areas without histology-proven calcifications (Figure 1 – C). The false positive [^{18}F]-NaF uptake, as the percentage of histology-proven (micro)calcification areas outside the overlay, was median 0% [IQR 0 – 25%] for Alizarin red areas and median 100% [65 – 100%] for Von Kossa areas. Alizarin Red staining of microcalcifications demonstrated good correlation with [^{18}F]-NaF uptake (Spearman's rho of 0.81 (95% CI 0.57 – 0.92), $p < 0.001$), whereas Von Kossa staining demonstrated weak correlation with [^{18}F]-NaF uptake (0.62 (95% CI 0.24 – 0.84), $p = 0.003$) (Figure 3). Significant differences in the number of (micro)calcifications, by Alizarin red (median 4.0 [IQR 3.0 – 8.0] versus 34.0 [IQR 19.0 – 41.0], $p < 0.001$) and Von Kossa (median 2.0 [IQR 2.0 – 4.0] versus 6.0 [5.5 – 9.5], $p = 0.004$) staining, were observed between samples with $\leq 5\%$ and $> 5\%$ [^{18}F]-NaF uptake (Figure 4).

Figure 2. Overlay image of [^{18}F]-sodium fluoride ([^{18}F]-NaF) uptake and (Alizarin red) microcalcifications (A), with Alizarin red staining for microcalcifications (B) and Von Kossa staining for calcifications (C).

Figure 3. Correlation of the number of $[^{18}\text{F}]$ -sodium fluoride ($[^{18}\text{F}]$ -NaF) areas with Alizarin red stained microcalcifications (A) and Von Kossa stained calcifications (B).

Figure 4. Differences between two groups, $\leq 5\%$ and $> 5\%$ $[^{18}\text{F}]$ -sodium fluoride ($[^{18}\text{F}]$ -NaF) tracer surface area, for the number of Alizarin red stained microcalcifications (A) and Von Kossa stained calcifications (B) (median, interquartile range).

Recipient characteristics at time of transplantectomy

No differences were observed between kidney allograft samples from patients with (n = 7) or without hyperphosphatemia (n = 6), for the number of [¹⁸F]-NaF areas (median 5.0 [IQR 2.0 – 11.8] versus 7.0 [7.0 – 23.0], p = 0.170), Alizarin red (median 7.5 [IQR 5.0 – 14.0] versus 12.0 [3.0 – 34.0], p = 0.390), and Von Kossa (median 5.0 [IQR 3.5 – 6.3] versus 3.0 [2.0 – 5.0], p = 0.247). Comparing kidney allograft samples from patients with (n = 8) or without (n = 5) hyperparathyroidism did not show differences, for the number of [¹⁸F]-NaF areas (median 7.0 [IQR 4.0 – 7.5] versus 7.5 [4.0 – 23.0], p = 0.459), Alizarin red (median 9.0 [IQR 6.0 – 11.0] versus 10.0 [3.5 – 32.8], p = 0.769), and Von Kossa (median 4.0 [IQR 1.5 – 5.0] versus 4.5 [2.0 – 6.8], p = 0.505). Significant differences were not identified between kidney allograft samples from patients with (n = 5) or without (n = 8) diabetes mellitus, for the number of [¹⁸F]-NaF areas (median 5.5 [IQR 2.5 – 19.3] versus 7.0 [6.5 – 15.5], p = 0.524), Alizarin red (median 10.0 [IQR 5.5 – 24.8] versus 9.0 [4.0 – 8.5], p = 0.833), and Von Kossa (median 3.0 [IQR 2.0 – 5.8] versus 4.0 [3.5 – 8.5], p = 0.284). For the time between transplantation and transplantectomy (months) no significant correlations were observed with the number of [¹⁸F]-NaF areas (p = 0.586), Alizarin red (p = 0.667) and Von Kossa (p = 0.459). For the age at time of transplantation no significant correlations were observed with the number of [¹⁸F]-NaF areas (p = 0.902), Alizarin red (p = 0.798) and Von Kossa (p = 0.334). Similarly, no significant correlations were observed between the dialysis duration (months) after kidney allograft failure and number of [¹⁸F]-NaF areas (p = 0.481), Alizarin red (p = 0.565) and Von Kossa (p = 0.174).

DISCUSSION

In this proof of concept study, ex-vivo [^{18}F]-NaF uptake in human kidney parenchyma samples demonstrated good correlation with histology-proven microcalcifications. Uptake of [^{18}F]-NaF correlated stronger with Alizarin red staining for active microcalcifications compared to Von Kossa staining of consolidated calcifications, suggesting a role for [^{18}F]-NaF imaging in the identification of early stage areas of nephrocalcinosis.

The presented samples represent a case mix of donor kidneys and explanted kidney allografts. These two sample types differed with regard to tracer binding, with more [^{18}F]-NaF uptake observed in explanted kidney allograft samples. This is in line with previous publications, reporting an increase of microcalcifications after kidney transplantation.⁵⁻⁷ The association of nephrocalcinosis with inferior graft function at time of biopsy and at one-year after transplantation is reported in two kidney allograft biopsy studies, with 258 and 149 kidney transplant recipients respectively.^{8,9} Moreover, in a longitudinal cohort with 97 kidney transplant recipients, a significantly worse 1-year and 12-year graft survival is shown in patients with calcium-oxalate depositions (i.e. a graft survival of 98.6% versus 72.5% and 79.3% versus 49.7%, respectively). In multivariate logistic regression analysis, the presence of calcium-oxalate depositions was independently associated with graft loss, in a model including among others creatinine at time of biopsy and donor type.⁴ The specific role of microcalcifications in this cascade of kidney allograft function decline remains unclear. In the pre-transplantation clinical setting, CaOx depositions are attributed to high levels of serum oxalate, a molecule not efficiently removed during dialysis.⁴ Whereas CaPhos depositions are most commonly seen in patients with hyperparathyroidism and high levels of serum calcium, due to a disordered mineral metabolism pre- and post-transplantation.⁵ We found no differences in histology and autoradiography results for hyperphosphatemia, hyperparathyroidism, diabetes mellitus, time between transplantation and transplantectomy and the dialysis duration after kidney allograft failure.

For the development of novel treatment strategies for active microcalcifications in nephrocalcinosis, its clinical relevance with regard to (chronic) transplant failure should be understood more comprehensively. With the identification of a target population of high-risk kidney transplant recipients, clinical studies can be designed to explore treatment strategies.²⁶ A possible focus could be found in the up-regulation of endogenous calcification inhibitors, with a specific emphasis on osteopontin.³

Given the high incidence of nephrocalcinosis and its association with kidney allograft function, better understanding of the aetiology of microcalcifications is warranted. A non-invasive and reliable diagnostic approach to identify and quantify microcalcifications in early nephrocalcinosis could enable studies on impact of nephrocalcinosis on kidney transplant function. [¹⁸F]-NaF imaging is of interest, since [¹⁸F]-NaF uptake in human and animal vascular samples is increased in biologically active areas of calcification, before these areas can be visualised by micro-computed tomography.^{16,17,22} In recent clinical PET/CT studies, [¹⁸F]-NaF uptake demonstrated to be related to the progression of vascular calcification, providing clinical evidence for the use of [¹⁸F]-NaF for detecting areas with biologically active calcification.^{18–20} The observations in this study are in line with these previous findings in the field of vascular imaging. A good correlation between [¹⁸F]-NaF uptake and the histology-proven microcalcifications was demonstrated, while the correlation with consolidated calcifications was clearly weaker.

As stated, co-localization between [¹⁸F]-NaF uptake and Alizarin red positive microcalcifications was identified, while [¹⁸F]-NaF uptake areas did not co-localize with Von Kossa staining of consolidated calcifications. These calcified areas, with consolidated calcifications and no corresponding [¹⁸F]-NaF uptake might be considered areas of inactive calcification, where there is no ongoing process of mineralization, as earlier described by Irkle et al. in an ex-vivo study of carotid calcification.¹⁶ Although only validated for vascular calcifications and not for kidney parenchyma calcifications, it is supported by the binding properties of [¹⁸F]-NaF to the outer surface of calcifications and limited penetration in solid calcifications. Therefore, [¹⁸F]-NaF uptake will be higher in microcalcifications, with a large surface area, compared to macrocalcifications, with a high volume but relatively small surface area.^{13,16}

This proof of concept study also has some limitations. First, inherent to the study design, the results represent a single time point, lacking clinical follow-up of calcification progression. Second, this proof of concept study consists of a relatively low number of kidney samples, as transplantectomy after late transplant failure is only performed on clinical indication and the availability of declined donor kidneys for research use is limited.²⁷ Therefore, a comprehensive analysis of the aetiology of calcium depositions should be considered beyond the scope of this project. Third, clinical translation of the results is hampered by the lack of knowledge on in-vivo [¹⁸F]-NaF distribution in kidneys and the effect of kidney tracer clearance, i.e. a low target-to-background activity could limit visualization of calcifications. To tackle this problem, the optimal moment of [¹⁸F]-NaF PET imaging should be assessed, i.e. at which [¹⁸F]-NaF is renally excreted and remaining activity comes from binding to calcified areas. Possibly, this can be achieved with delayed imaging exceeding three hours, as performed for assessment

of coronary calcifications.²⁸ The reduced glomerular filtration rate found in transplant recipients could result in a prolonged excretion phase, requiring a longer period between tracer injection and PET imaging, but current guidelines do not indicate potential nephrotoxicity of the [^{18}F]-NaF tracer.^{11,29} A potential step between the here presented proof of concept study and in-vivo imaging can be ex-vivo [^{18}F]-NaF PET imaging of normothermic perfused kidneys or in-vivo [^{18}F]-NaF microPET animal studies.^{30,31} Fourth, clinical application of this technique could be hampered by the costs for performing a [^{18}F]-NaF PET/CT procedure and the inherent radiation exposure. However, the new digital PET camera systems with a higher sensitivity may reduce both, due to shorter scanning time and lower injected activity dose.

The strength of this proof of concept study lies in the detailed one-to-one comparison of microcalcifications identified by [^{18}F]-NaF autoradiography and histology. The [^{18}F]-NaF uptake in different samples could be compared without need for further adjustments, as all samples were incubated in the same [^{18}F]-NaF solution. Moreover, this study provides the first data showing a visual and statistical match between [^{18}F]-NaF uptake and histology-proven microcalcifications in kidney samples.

To conclude, we provide the first data for the use of [^{18}F]-NaF in an autoradiography study to identify microcalcifications in active nephrocalcinosis after kidney transplantation. This ex-vivo [^{18}F]-NaF proof of concept study is the first step towards the potential application of clinical [^{18}F]-NaF PET/CT in kidney transplant recipients.

REFERENCES

- 1 Gwinner W, Suppa S, Mengel M, Hoy L, Kreipe HH, Haller H, et al. Early calcification of renal allografts detected by protocol biopsies: Causes and clinical implications. *Am J Transplant* 2005;5(8):1934–41.
- 2 Shavit L, Jaeger P, Unwin RJ. What is nephrocalcinosis? *Kidney Int* 2015;88(1):35–43.
- 3 Palioian NJ, Leaf EM, Giachelli CM. Osteopontin protects against high phosphate-induced nephrocalcinosis and vascular calcification. *Kidney Int* 2016;89(5):1027–36.
- 4 Pinheiro HS, Camara NOS, Osaki KS, De Moura LAR, Pacheco-Silva A. Early Presence of Calcium Oxalate Deposition in Kidney Graft Biopsies is Associated with Poor Long-Term Graft Survival. *Am J Transplant* 2005;5(2):323–9.
- 5 Evenepoel P, Lerut E, Naesens M, Bammens B, Claes K, Kuypers D, et al. Localization, Etiology and Impact of Calcium Phosphate Deposits in Renal Allografts. *Am J Transplant* 2009;9(11):2470–8.
- 6 Nankivell BJ, Borrows RJ, Fung CL-S, O'Connell PJ, Allen RDM, Chapman JR. The Natural History of Chronic Allograft Nephropathy. *N Engl J Med* 2003;349(24):2326–33.
- 7 Schwarz A, Mengel M, Gwinner W, Radermacher J, Hiss M, Kreipe H, et al. Risk factors for chronic allograft nephropathy after renal transplantation: A protocol biopsy study. *Kidney Int* 2005;67(1):341–8.
- 8 Bagnasco SM, Mohammed BS, Mani H, Gandolfo MT, Haas M, Racusen LC, et al. Oxalate deposits in biopsies from native and transplanted kidneys, and impact on graft function. *Nephrol Dial Transplant* 2009;24(4):1319–25.
- 9 Snijders MLH, Hesselink DA, Claahsen-Van Groningen MC, Roodnat JL. Oxalate deposition in renal allograft biopsies within 3 months after transplantation is associated with allograft dysfunction. *PLoS One* 2019;14(4).
- 10 Benjamens S, Glaudemans AWJM, Berger SP, Slart RHJA, Pol RA. Have we forgotten imaging prior to and after kidney transplantation? *Eur Radiol* 2018;28(8):3263–7.
- 11 Beheshti M, Mottaghy FM, Payche F, Behrendt FFF, Van den Wyngaert T, Fogelman I, et al. 18F-NaF PET/CT: EANM procedure guidelines for bone imaging. *Eur J Nucl Med Mol Imaging* 2015;42(11):1767–77.
- 12 Dweck MR, Chow MWL, Joshi N V, Williams MC, Jones C, Fletcher AM, et al. Coronary Arterial 18F-Sodium Fluoride Uptake. *J Am Coll Cardiol* 2012;59(17):1539–48.
- 13 Dweck MR, Jenkins WSA, Vesey AT, Pringle MAH, Chin CWL, Malley TS, et al. 18F-Sodium Fluoride Uptake Is a Marker of Active Calcification and Disease Progression in Patients With Aortic Stenosis. *Circ Cardiovasc Imaging* 2014;7(2):371–8.
- 14 Derlin T, Richter U, Bannas P, Begemann P, Buchert R, Mester J, et al. Feasibility of 18F-sodium fluoride PET/CT for imaging of atherosclerotic plaque. *J Nucl Med* 2010;51(6):862–5.
- 15 Bucerius J, Dijkgraaf I, Mottaghy FM, Schurgers LJ. Target identification for the diagnosis and intervention of vulnerable atherosclerotic plaques beyond 18F-fluorodeoxyglucose positron emission tomography imaging: promising tracers on the horizon. *Eur J Nucl Med Mol Imaging* 2019;46(1):251–65.
- 16 Irkle A, Vesey AT, Lewis DY, Skepper JN, Bird JLE, Dweck MR, et al. Identifying active vascular microcalcification by 18F-sodium fluoride positron emission tomography. *Nat Commun* 2015;6(May).
- 17 Creager MD, Hohl T, Hutcheson JD, Moss AJ, Schlotter F, Blaser MC, et al. 18 F-Fluoride Signal Amplification Identifies Microcalcifications Associated With Atherosclerotic Plaque Instability

- in Positron Emission Tomography/Computed Tomography Images. *Circ Cardiovasc Imaging* 2019;12(1). Doi: 10.1161/CIRCIMAGING.118.007835.
- 18 den Harder AM, Wolterink JM, Bartstra JW, Spiering W, Zwakenberg SR, Beulens JW, et al. Vascular uptake on 18F-sodium fluoride positron emission tomography: precursor of vascular calcification? *J Nucl Cardiol* 2020.
 - 19 Derlin T, Tóth Z, Papp L, Wisotzki C, Apostolova I, Habermann CR, et al. Correlation of inflammation assessed by 18F-FDG PET, active mineral deposition assessed by 18F-fluoride PET, and vascular calcification in atherosclerotic plaque: A dual-tracer PET/CT study. *J Nucl Med* 2011;52(7):1020–7.
 - 20 Kitagawa T, Yamamoto H, Nakamoto Y, Sasaki K, Toshimitsu S, Tatsugami F, et al. Predictive value of 18F-sodium fluoride positron emission tomography in detecting high-risk coronary artery disease in combination with computed tomography. *J Am Heart Assoc* 2018;7(20).
 - 21 Roufosse C, Simmonds N, Clahsen-Van Groningen M, Haas M, Henriksen KJ, Horsfield C, et al. A 2018 Reference Guide to the Banff Classification of Renal Allograft Pathology. *Transplantation* 2018;1795–814.
 - 22 Hop H, de Boer SA, Reijrink M, Kamphuisen PW, de Borst MH, Pol RA, et al. 18F-sodium fluoride positron emission tomography assessed microcalcifications in culprit and non-culprit human carotid plaques. *J Nucl Cardiol* 2019;26(4):1064–75.
 - 23 Reijrink M, de Boer SA, Antunes IF, Spoor DS, Heerspink HJL, Lodewijk ME, et al. [18F]FDG Uptake in Adipose Tissue Is Not Related to Inflammation in Type 2 Diabetes Mellitus. *Mol Imaging Biol* 2020.
 - 24 Shao J-S, Sierra OL, Cohen R, Mecham RP, Kovacs A, Wang J, et al. Vascular Calcification and Aortic Fibrosis A Bifunctional Role for Osteopontin in Diabetic Arteriosclerosis. *Arter Thromb Vasc Biol* 2011;31:1821–33.
 - 25 Brocher J. Qualitative and Quantitative Evaluation of Two New Histogram Limiting Binarization Algorithms. *Int J Image Process* 2014;8(2):30–48.
 - 26 Bäck M, Aranyi T, Cancela ML, Carracedo M, Conceição N, Leftheriotis G, et al. Endogenous Calcification Inhibitors in the Prevention of Vascular Calcification: A Consensus Statement From the COST Action EuroSoftCalcNet. *Front Cardiovasc Med* 2019;5.
 - 27 Johnston O, Rose C, Landsberg D, Gourlay WA, Gill JS. Nephrectomy after transplant failure: Current practice and outcomes. *Am J Transplant* 2007;7(8):1961–7.
 - 28 Kwiecinski J, Berman DS, Lee SE, Dey D, Cadet S, Lassen ML, et al. Three-hour delayed imaging improves assessment of coronary 18F-sodium fluoride PET. *J Nucl Med* 2019;60(4):530–5.
 - 29 Bucerius J, Hyafil F, Verberne HJ, Slart RHJA, Lindner O, Sciagra R, et al. Position paper of the Cardiovascular Committee of the European Association of Nuclear Medicine (EANM) on PET imaging of atherosclerosis. *Eur J Nucl Med Mol Imaging* 2016;43(4):780–92. Doi: 10.1007/s00259-015-3259-3.
 - 30 Buchs J-B, Buehler L, Moll S, Ruttimann R, Nastasi A, Kasten J, et al. DCD pigs' kidneys analyzed by MRI to assess ex vivo their viability. *Transplantation* 2014;97(2):148–53.
 - 31 Pool M, Eertman T, Sierra Parraga J, 't Hart N, Roemeling-van Rhijn M, Eijken M, et al. Infusing Mesenchymal Stromal Cells into Porcine Kidneys during Normothermic Machine Perfusion: Intact MSCs Can Be Traced and Localised to Glomeruli. *Int J Mol Sci* 2019;20(14).

A high abdominal aortic calcification score by dual x-ray absorptiometry (DXA) is associated with cardiovascular events after kidney transplantation

Nephrology Dialysis Transplantation, 2018

Stan Benjamins
Robert A. Pol
Andor W.J.M. Glaudemans
Ivanka Wieringa
Stefan P. Berger
Stephan J.L. Bakker
Riemer H.J.A. Slart

Background: Aortic calcification is associated with an increased risk for cardiovascular events in renal transplant recipients. This study focused on the association of abdominal aortic calcification (AAC) and cardiovascular events assessed using a dual X-ray absorptiometry (DXA) scoring methodology for AAC.

Methods: From 2008 to 2014, renal transplant recipients referred for a DXA procedure within 6 months after transplantation were included in a retrospective, single-centre study. Primary endpoint was the occurrence of cardiovascular events, defined as myocardial infarction, cerebrovascular accident or transient ischemic attack, after transplantation. AAC was quantified using an 8-score scale system and patients were divided into 3 groups; a control group (AAC = 0), a low-AAC group (AAC = 1 – 3), and a high-AAC group (AAC = 4 – 8).

Results: We evaluated 701 patients, 267 (38.1%) had detectable calcifications (low-AAC 190 patients, high-AAC 77 patients) and 434 (61.9%) had no calcifications. Cardiovascular events were seen in 37 (8.5%) patients in the control group, in 18 (9.5%) in the low-AAC, and in 20 (26.0%) in the high-AAC group. Univariate Cox proportional hazard analysis of the high-AAC score showed a hazard ratio (HR) of 4.23 (95% CI 2.44 – 7.33, $p < 0.01$) for cardiovascular events, while results were not significant for the low-AAC score. Multivariate analysis showed an independent significant association between a high-AAC score and cardiovascular events, HR 2.78 (95% CI 1.05 – 7.64, $p = 0.04$). Assessment of the continuous net reclassification index (NRI), comparing the combined clinical variables with a model of both AAC-scoring and clinical variables, showed a NRI of 0.76 (95% CI 0.65 – 0.86), $p < 0.01$.

Conclusions: An independent association between a high-AAC score, assessed by DXA, and cardiovascular events was identified and provides an opportunity for physicians for early cardiovascular risk stratification in renal transplant recipients.

INTRODUCTION

The risk of cardiovascular events increases substantially with end-stage renal disease, and cardiovascular death is the leading cause of mortality after kidney transplantation.¹ The cumulative incidence of a myocardial infarction (MI) is 11.1% at 36 months after kidney transplantation and 6.8% for de novo cerebrovascular disease, including cerebrovascular accident (CVA) and transient ischemic attack (TIA).^{2,3} Cardiovascular risk factors such as hypercholesterolemia, hypertension, and diabetes mellitus are common in end-stage renal disease, which contributes to a higher prevalence of cardiovascular disease when compared to the general population.⁴ In addition, kidney transplant-specific risk factors, such as the duration of pre-transplant dialysis, are important factors to consider in this patient population.⁵

The Kidney Disease: Improving Global Outcomes (KDIGO) 2017 clinical practice guideline recommends bone mineral density testing in the first 3 months following transplantation in patients with an estimated glomerular filtration rate >30 ml/min/1.73 if they receive corticosteroids or have risk factors for osteoporosis.⁶ An interesting but often neglected feature of dual energy X-ray absorptiometry (DXA) is that the degree of calcification of the abdominal aorta can be assessed in the image that is obtained for vertebral fracture assessment.

Earlier studies showed that abdominal aortic calcification (AAC) data derived from DXA procedures are associated with risk of future development of cardiovascular events in the general population.⁷ A retrospective, single-centre study with patients from the general population referred for bone mineral density screening showed a significantly higher risk for cardiovascular events in patients with an increased AAC score compared to the control group with no visual AAC.⁸

Studies in which vascular calcification was assessed using computed tomography (CT), lumbar X-ray or cardiac-CT found significant associations of vascular calcification in both the aorta and coronary arteries with cardiovascular events in renal transplant recipients.⁹⁻¹³

We performed a retrospective, single-centre study to evaluate the association between AAC assessed by DXA and cardiovascular events after kidney transplantation.

METHODS

Patients

Renal transplant recipients referred for a DXA procedure within 6 months after transplantation at the University Medical Center Groningen (UMCG), The Netherlands between 2008 and 2014 were included in the study. Patients' charts were screened for baseline characteristics, prior cardiovascular events (MI, CVA, TIA), and cardiovascular events after transplantation. Pre-transplant hypertension was defined as blood pressure $>140/90$ mmHg or current antihypertensive medication, pre-transplant hypercholesterolemia was defined as total cholesterol levels >200 mg/dL or current use of lipid-lowering agents, pre-transplant diabetes mellitus (DM) was defined as the use of anti-diabetic medication, and patients were considered smokers if they smoked at the time of transplant wait-listing admission.¹⁴ The serum creatinine-based Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was used to calculate the estimated Glomerular Filtration Rate (eGFR). The primary endpoint of this study was the occurrence of fatal and nonfatal cardiovascular events, defined as MI, CVA or TIA, after transplantation. Causes of death during follow-up were subdivided in cardiovascular event (MI, CVA or TIA), cardiac failure, pulmonary disease, infection, cancer, or unknown.

Patient data were processed and electronically stored according to the Declaration of Helsinki ethical principles for medical research involving human subjects and the institutional ethics review board gave approval for this study (Medical Ethical Committee UMCG 2017/457). The clinical and research activities were consistent with the principles of the Declaration of Istanbul as outlined in the "Declaration of Istanbul on Organ Trafficking and Transplant Tourism".

DXA procedure and AAC scoring

Lateral single-energy images of the lumbar spine were obtained on a Discovery DXA System (Hologic, Bedford, MA, USA). Two independent imaging specialists, blinded to the patients' medical history, analysed the DXA images to quantify AAC, using a visual 8-point scale, as described by Schousboe et al.¹⁵ This scale reflects the total length of calcification on the anterior and posterior aortic walls at the level of the L1-L4 vertebral bones. A point was given for a single-sided calcification with an aggregate length up to the height of one vertebra, subsequent points were given when calcifications reached the level of the three other vertebrae. The total score was the summation of anterior and posterior calcification scores and ranged from 0 to 8 (Figure 1). Based on AAC scoring outcomes, patients were stratified into three groups: the control group with an AAC score of 0; the low-AAC group with an AAC score between 1 and 3; and the high-AAC group with an AAC score between 4 and 8.

Figure 1. Example of a vertebral fracture assessment image from DXA with the lumbar vertebral bones L1-L4. This image shows double-sided aortic calcifications with an aggregate length up to the height of all four vertebrae, resulting in an AAC score of 8.

Statistical analysis

Baseline characteristics and clinical follow-up results were presented as mean and standard deviation (SD) when normal distribution was assumed by means of a Q-Q plot or histogram, as median and interquartile range [IQR] for skewed data, and as frequency and percentage when data were categorical. We compared patient characteristics between the control group, low-AAC group, and high-AAC group, using chi-square test and one-way analysis of variance (significant at a two-sided p-value < 0.05). Cardiovascular events were shown for the three defined groups as numbers and percentages. We used univariate and multivariable Cox regression to evaluate the association between the clinical covariates and the used AAC-scoring system. Multivariable analyses were performed with recipient age, recipient gender, pre-transplant DM, pre-transplant hypertension, pre-transplant hypercholesterolemia, duration of pre-transplant dialysis, history of cardiovascular events, and eGFR at 3 months post-transplantation as potential confounders. The hazard ratios (HRs) and their corresponding 95% confidence intervals (Cis) are reported. The event-free and overall survival were visualised using Kaplan-Meier survival curves. Differences between Kaplan-Meier curves were calculated using

log-rank tests. The added value of the AAC-scoring system for estimating the risk of cardiovascular events was assessed by examining increase in Harrell's C-index and the change in -2 Log Likelihood. In addition, we assessed the continuous net reclassification index (NRI) for category-independent and time-dependent model-based risk estimates of risk of cardiovascular events.^{16,17} Statistical analyses were performed with the Statistical Package for the Social Sciences (version 23; Armonk, NY, USA), STATA-SE (version 15; StataCorp, TX, USA), and R (version 3.4.3 for Mac OS X; R Foundation for Statistical Computing, Vienna, Austria). Univariate- and multivariate analyses and the NRI analysis were performed using the survival, survC1, and survIDINRI packages from the R statistics program.

RESULTS

Characteristics

We evaluated 921 renal transplant recipients, of which 701 (76%) were referred for a DXA procedure within 6 months after transplantation and formed the basis for this analysis. At baseline, there were no significant differences between the patients with (n = 219) and without (n = 701) DXA with regard to sex (p = 0.78), age (p = 0.08), Body Mass Index (BMI) (p = 0.08), smoking status (p = 0.74), pre-transplant diabetes mellitus (p = 0.63), duration of pre-transplantation dialysis (p = 0.64), and history of cardiovascular events (p = 0.49). Of the included recipients, a total of 267 (38.1%) patients had detectable calcifications and 434 (61.9%) patients had no calcifications. Patients with detectable calcifications were stratified according to the AAC score into a low-AAC group, consisting of 190 patients, and a high-AAC group of 77 patients. Patient characteristics according to AAC status are shown in Table 1. The mean age was significantly different between the groups (p < 0.01), with 47 (13) years in the control group, 57 (11) years in the low-AAC group, and 60 (13) years in the high-AAC group. A significant difference between the groups was also seen for prevalence of hypercholesterolemia (p < 0.01) and prevalence of diabetes mellitus (p = 0.03), with the highest prevalence in the high AAC group. No significant differences were seen between the control group, low-AAC group, and high AAC group for gender (p = 0.56), hypertension (p = 0.31), BMI (p = 0.52), eGFR at 3 months post-transplantation (p = 0.53), and type of immunosuppressive therapy (p = 0.38), and smoking (p = 0.50). The percentage of patients undergoing a pre-emptive transplantation did not differ between groups (p = 0.66), while differences in the median duration of pre-transplantation dialysis were significant (p = 0.02) with durations of 34 [20 – 51], 42 [26 – 62], and 50 [26 – 66] months, for the control group, low-AAC group, and high-AAC group respectively. There was a significant difference regarding history of MI with 7.6%, 11.6%, and 24.7% (p < 0.01), but not for CVA or TIA, with 5.8%, 7.9%, and 6.5% (p = 0.74), for the control group, low-AAC group, and high-AAC group, respectively.

Follow-up

The median follow-up for cardiovascular events was 5.4 [3.9 – 7.2] years. During follow-up, 75 cardiovascular events occurred, with a MI in 57 patients and a CVA or TIA in 18 patients. The cumulative incidence of cardiovascular events after transplantation was 8.5% in the control group, 9.5% in the low-AAC group, and 26.0% in the high-AAC group (p < 0.01) (Table 2). A total of 137 (19.5%) patients died after transplantation: 14 (10.2%) due to cardiovascular events (10 due to a MI and 4 due to a CVA); 19 (13.9%) due to cardiac failure; 9 (6.6%) due to pulmonary disease, 13 (9.5%) due to infections; 21 (15.3%) deaths were cancer related; 5 (3.6%) due to other causes;

and in 56 patients (40.9%) the cause of death was unknown. Eight (1.8%) patients in the control group, 3 (1.6%) in the low-AAC group, and 3 (3.9%) in the high-AAC group died of cardiovascular events ($p = 0.44$).

Table 1. Patient characteristics grouped according to AAC-group

Variables	Control group (n = 434)	Low-AAC (n = 190)	High-AAC (n = 77)	p-value
Male ^a	263 (58.4)	114 (60.0)	40 (52.0)	0.56 ^d
Age, years ^b	47 ± 13	57 ± 11	60 ± 13	<0.01 ^e
Hypertension ^a	344 (79.3)	160 (84.2)	64 (83.1)	0.31 ^d
Hypercholesterolemia ^a	166 (38.2)	99 (52.1)	37 (48.1)	<0.01 ^d
Diabetes mellitus ^a	49 (11.3)	28 (14.7)	17 (22.1)	0.03 ^d
Body Mass Index ^b	25 ± 4	26 ± 4	25 ± 4	0.52 ^e
Smoking ^a	92 (21.2)	35 (18.4)	19 (24.7)	0.50 ^d
Pre-emptive transplantation ^a	157 (36.2)	76 (40.0)	29 (37.7)	0.66 ^d
Duration pre-transplant dialysis, months ^c	34 [20 – 51]	42 [26 – 62]	50 [26 – 66]	0.02 ^e
eGFR 3 months post-transplant ^c	50 [43 – 60]	51 [40 – 59]	50 [38 – 64]	0.53 ^e
Immunosuppressive therapy ^a				0.38 ^d
Cyclosporin + MMF + Corticosteroids	198 (45.6)	78 (41.1)	38 (49.4)	
Tacrolimus + MMF/MPS + Corticosteroids	216 (49.8)	100 (52.6)	38 (49.4)	
Others	20 (4.6)	12 (6.3)	1 (1.3)	
History of MI ^a	33 (7.6)	22 (11.6)	19 (24.7)	<0.01 ^d
History of CVA / TIA ^a	25 (5.8)	15 (7.9)	5 (6.5)	0.74 ^d

AAC = abdominal aortic calcification; eGFR = estimated Glomerular Filtration Rate; MMF = Mycophenolate Mofetil, MPS = Mycophenolate Sodium; MI = myocardial infarction, CVA = cerebrovascular accident, TIA = transient ischemic attack; values presented as ^a numbers (%); ^b mean ± standard deviation (SD); ^c median (interquartile range); ^d p-value by chi-square test; ^e p-value by One-way ANOVA

Table 2. Number and percentage of cardiovascular events grouped according to AAC-group

	Control group	Low-AAC	High-AAC
MI	28 (6.4)	15 (7.9)	14 (18.1)
CVA / TIA	9 (2.1)	3 (1.6)	6 (7.8)
Combined cardiovascular endpoint	37 (8.5)	18 (9.5)	20 (26.0)

Values presented as numbers (%); AAC = abdominal aortic calcification; MI = myocardial infarction; CVA = cerebrovascular accident; TIA = transient ischemic attack

Association between AAC-scoring and cardiovascular events

Using univariate Cox proportional hazard analysis, a high-AAC score was associated with a higher cardiovascular risk [HR 4.23 (95% CI 2.44 – 7.33); $p < 0.01$] (Table 3). There was a significant difference in cumulative event-free survival between the control group, low-AAC group, and high-AAC group ($p < 0.01$) (Figure 2). A low-AAC score was not associated with a higher risk cardiovascular risk [HR 1.26 (0.72 – 2.21); $p = 0.43$]. Additional covariates with a significantly higher risk were age [HR 1.05 (1.03 – 1.07); $p < 0.01$], hypercholesterolemia [HR 1.99 (1.26 – 3.15); $p < 0.01$], hypertension [HR 2.06 (1.02 – 4.15); $p = 0.04$], duration of pre-transplant dialysis [HR 1.01 (1.00 – 1.01); $p = 0.03$], a history of cardiovascular events [HR 2.73 (1.63 – 4.58); $p < 0.01$], and eGFR at 3 months post-transplantation [HR 0.96 (0.94 – 0.98); $p < 0.01$]. The covariates gender and diabetes mellitus did not contribute to a significantly higher risk. In a multivariable analysis with the a priori selected clinical variables (Table 4), a low-AAC score was not associated with an increased risk [HR 1.02 (0.42 – 2.59); $p = 0.95$], while the high-AAC score was associated with increased an increased risk for a cardiovascular event [HR 2.78 (1.05 – 7.64); $p = 0.04$].

Table 3. Univariate Cox regression analysis of clinical covariates

Covariates	Hazard ratio	95% CI	p-value
AAC			
Low	1.26	0.72 – 2.21	0.43
High	4.23	2.44 – 7.33	<0.01
Gender	1.27	0.79 – 2.03	0.32
Age	1.05	1.03 – 1.07	<0.01
Hypercholesterolemia	2.06	1.02 – 4.15	<0.01
Diabetes mellitus	1.65	0.90 – 3.00	0.10
Hypertension	2.06	1.02 – 4.15	0.04
Duration pre-transplant dialysis	1.01	1.00 – 1.01	0.03
History of cardiovascular event	2.73	1.63 – 4.58	<0.01
eGFR 3 months post-transplant	0.96	0.94 – 0.98	<0.01

Hazard ratio; 95% confidence interval (95% CI); p-value by Cox regression analysis; eGFR = estimated Glomerular Filtration Rate

Table 4. Multivariate Cox regression analysis comparing control group, Low-AAC, and High-AAC for cardiovascular events

	Control group	Low-AAC			High-AAC		
	Hazard ratio	Hazard ratio	95% CI	p-value	Hazard ratio	95% CI	p-value
Multivariate	1.00	1.02	0.42 – 2.59	0.95	2.78	1.05 – 7.64	0.04

Hazard ratio; 95% confidence interval (95% CI); p-value by Cox regression analysis; multivariate analysis consists of age, gender, history of diabetes mellitus, history of hypertension, history of hypercholesterolemia, duration of pre-transplant dialysis, history of cardiovascular events, and estimated Glomerular Filtration Rate 3 months post-transplant.

Figure 2. Kaplan-Meier curve for cumulative event-free survival for the control group, low-AAC group, and high-AAC group, with a significant difference between the high-AAC group and both the low-AAC and control group ($p < 0.01$).

Prognostic performance of the AAC-scoring system for cardiovascular events

The combined clinical variables, as used for the multivariable analysis, had a C-index of 0.700 (95% CI 0.631 – 0.770) for estimating the risk of cardiovascular events. After implementation of the AAC-scoring results, the C-index of this model increased to 0.731 (95% CI 0.663 – 0.800), resulting in a C-index gain of 0.031 (95% CI -0.003 – 0.065); $p = 0.07$). When investigating change in -2 Log Likelihood of the clinical variable model with and without the AAC-scoring results, the -2 Log Likelihood improved significantly with AAC-scoring included in the model (from a -2 Log Likelihood of 505.8 to 494.9; $p < 0.01$). Assessment of the continuous NRI, comparing the combined clinical variables with the model of both AAC-scoring and clinical variables showed a NRI of 0.76 (95% CI 0.65 – 0.86; $p < 0.01$).

Association between AAC-scoring and overall survival

Using univariate Cox proportional hazard analysis, both a low-AAC score and a high-AAC score were associated with a lower overall survival [HR 1.92 (1.03 – 2.83); $p < 0.01$ and HR 4.01 (2.62 – 6.12); $p < 0.01$ respectively]. The cumulative overall survival for the control group, low-AAC group, and high-AAC group, was significantly different between the three groups ($p < 0.01$) (Figure 3). Additional covariates with a significant higher risk were age [HR 1.07 (1.05 – 1.09); $p < 0.01$], hypercholesterolemia [HR 1.82 (1.30 – 2.55); $p < 0.01$], diabetes mellitus [HR 1.68 (1.09 – 2.57); $p = 0.02$], duration of pre-transplant dialysis [HR 1.01 (1.00 – 1.01); $p < 0.01$], history of cardiovascular events [HR 2.84 (1.97 – 4.09); $p < 0.01$], and eGFR at 3 months post-transplantation [HR 0.97 (0.96 – 0.98); $p < 0.01$]. The covariates gender and history of hypertension did not contribute to a significantly higher hazard ratio. In a multivariable analysis including the a priori selected clinical variables, the association between a low-AAC score and all-cause mortality was not significant [HR 1.46 (0.84 – 2.57); $p = 0.19$], while the association with a high-AAC score remained significant [HR 2.64 (1.28 – 4.98); $p < 0.01$].

Figure 3. Kaplan-Meier curve for cumulative overall survival for the control group, low-AAC group, and high-AAC group, was significantly different between the three groups ($p < 0.01$).

DISCUSSION

This study shows an association between high-AAC score, acquired on DXA for routine clinical evaluation of bone density, and both cardiovascular events and overall survival after kidney transplantation. The independent association between a high-AAC score and cardiovascular events provides an opportunity for physicians for early cardiovascular risk stratification in renal transplant recipients. In patients with a high risk for cardiovascular events, interventions such as lipid control, treatment of hypertension, and antiplatelet therapy could possibly lead to a risk reduction.¹⁴ In addition, a cumulative incidence of cardiovascular events after transplantation of 26.0% urges for a more thorough cardiovascular follow-up in patients with a high-AAC score.

The wide accessibility, extended experience and very low radiation burden (< 0.01 mSv) contribute to the routine use of DXA procedures after kidney transplantation. Due to the KDIGO 2017 clinical practice guideline recommendation to evaluate the bone density in the first 3 months following transplantation, a wide range of renal transplant recipients are eligible for DXA procedures.⁶ Therefore, implementation of this 8-point AAC scale system will not lead to a greater patient burden while it provides the opportunity for early cardiovascular risk stratification and prevention.

Several previous studies described the use of DXA images for the assessment of AAC in the general population. These studies reported a low inter-observer variation, with an intra-class correlation coefficient of 0.9 (CI 95% 0.8 – 0.9), and a good intra-observer reliability, with an intra-class correlation coefficient of 0.9 (CI 95% 0.8 – 0.9).^{15,18} With a significant association between cardiovascular events and both low AAC scores [HR 4.2 (95% CI 1.2 – 15.4); $p = 0.03$] and high AAC scores [HR 6.1 (95% CI 1.7 – 21.6); $p < 0.01$], the association between a high-AAC score and cardiovascular events in our study is comparable with these earlier outcomes.⁸ However, we did not find an association between cardiovascular events and a low-AAC score. In addition, it has been shown that in dialysis patients there is a significant correlation between aortic calcification as assessed using DXA images and aortic calcification as assessed using CT images.¹⁹

We found a significant association between a high AAC score assessed by DXA scoring and future development of cardiovascular events in renal transplant recipients, which is in line with results of earlier studies in which AAC was assessed by other methods (i.e. CT and lumbar X-ray).^{9,12} Similar results were seen for the association between coronary artery calcification and cardiovascular events, in renal transplant recipients

with increased coronary calcification.^{10,11} Our results with regard to overall survival were comparable to those of a study in which iliac artery calcification was assessed by means of CT, wherein 3-year survival in the non-calcification group was 94% versus 87% in the calcification group.¹³

A study evaluating pre-transplantation evaluation modalities and the occurrence of cardiovascular events within one-year after transplantation, reported a significant association with a history of cardiovascular events [HR 2.06 (95% CI 1.1 – 4); $p = 0.03$], echocardiographic left ventricular hypertrophy [HR 2.04 (95% CI 1.0 – 4.0); $p = 0.04$], and abnormal myocardial perfusion testing [HR 2.25 (95% CI 1.1 – 6.0); $p = 0.03$]. The pre-transplantation risk stratification methods showed that HRs were comparable to the AAC score. However, these were associations with cardiovascular events within one-year after transplantation, whereas AAC scores were associated with events within a median follow-up of 5.4 [3.9 – 7.2] years.

When assessing the prognostic performance of AAC-scoring for cardiovascular events, the Harrell's C-index was higher for the clinical variable model with AAC-scoring included compared to the model without these scores. This difference did not reach statistical significance ($p = 0.07$). It should be realized, however, that Harrell's C-statistics are relatively insensitive for detection of differences between models. To avoid injudicious discarding of otherwise promising markers, it is currently recommended to perform additional analyses in which -2 Log Likelihood ratios are compared.²⁰ Using the -2 Log Likelihood analyses, we observed a significant ($p < 0.01$) improvement of the model with the AAC-scoring included. When comparing the clinical variable model with the model of both AAC-scoring and clinical variables, the NRI showed to be 0.76 (95% CI 0.65 – 0.86), indicating an improved prognostic performance when implementing AAC-scoring.

Some limitations of our study need to be addressed. First, the retrospective design of this study creates the risk of an underestimation of the total number of cardiovascular events due to incomplete patient records. The incidence of cardiovascular events is low compared to the literature in which a cumulative incidence of 11.1% for MI and of 6.8% for CVA or TIA is reported at 36 months after kidney transplantation.²³ The low rate of pre-transplantation diabetes mellitus (13.4%), compared to 44.7% in the study mentioned before, could be seen as an explanation for this discrepancy.² Thus we do not expect that this has altered our results since most patients were regularly seen in our clinic and the analysis has been based on a between-group comparison. Second, the study only included patients from a single centre. Therefore, the presented results are not necessarily generalizable to a broader population of renal transplant recipients. Third, the patients in the high-AAC group had a significantly greater baseline risk for future cardiovascular

events compared to the control group, with a longer duration of pre-transplant dialysis, a higher age, and a higher percentage of patients with pre-transplant diabetes mellitus and hypercholesterolemia. We performed multivariable analyses, adjusting for these clinical variables, and we presented the predictive value of both the clinical variables alone and the combination of AAC-scoring and clinical variables. Both these statistical analyses showed the independent value of AAC-scoring after transplantation. Fourth, the number of patients in the high-AAC group was small, hindering the inclusion of more clinical covariates in the performed multivariable analyses. Furthermore, the number of fatal cardiovascular events was low. The reason of death could not be defined for 22.8% of patients who died within 36 months after transplantation, and for 40.9% of patients who died during the entire follow-up. Nevertheless, we have shown a significant association in a large cohort of renal transplant recipients, with no signs for selection bias.

In conclusion, an independent association between a high-AAC score and cardiovascular events was identified. The combination of this DXA scoring methodology for AAC and the described clinical variables can be useful for early post-transplantation cardiovascular risk stratification and prevention. Due to the routine use and wide availability of DXA for evaluation of bone density after transplantation, AAC-scoring can be added as a low-cost and easy-to-implement method for cardiovascular screening.

REFERENCES

1. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu C-Y. Chronic Kidney Disease and the Risks of Death, Cardiovascular Events, and Hospitalization. *N Engl J Med*. 2004;351(13):1296-1305.
2. Lentine KL, Brennan DC, Schnitzler MA. Incidence and predictors of myocardial infarction after kidney transplantation. *JASN*. 2005;16(2):496-506.
3. Lentine KL, Rocca Rey LA, Kolli S, et al. Variations in the risk for cerebrovascular events after kidney transplant compared with experience on the waiting list and after graft failure. *CJASN*. 2008;3(4):1090-1101.
4. Jardine AG, Gaston RS, Fellstrom BC, Holdaas H. Prevention of cardiovascular disease in adult recipients of kidney transplants. *Lancet*. 2011;378(9800):1419-1427.
5. Cianciolo G, Capelli I, Angelini ML, et al. Importance of Vascular Calcification in Kidney Transplant Recipients. *Am J Nephrol*. 2014;39(5):418-426.
6. KDIGO 2017 Clinical Practice Guideline Update for the Diagnosis, Evaluation, Prevention, and Treatment of Chronic Kidney Disease–Mineral and Bone Disorder (CKD-MBD). *Kidney Int*. 2017;7(1).
7. Schousboe JT, Lewis JR, Kiel DP. Abdominal aortic calcification on dual-energy X-ray absorptiometry: Methods of assessment and clinical significance. *Bone*. 2017;104:91-100.
8. Golestani R, Tio R, Zeebregts CJ, et al. Abdominal aortic calcification detected by dual X-ray absorptiometry: A strong predictor for cardiovascular events. *Ann Med*. 2010;42(7):539-545.
9. DeLoach SS, Joffe MM, Mai X, Goral S, Rosas SE. Aortic calcification predicts cardiovascular events and all-cause mortality in renal transplantation. *Nephrol Dial Transpl*. 2009;24(4):1314-1319.
10. Nguyen PT, Henrard S, Coche E, Goffin E, Devuyst O, Jadoul M. Coronary artery calcification: a strong predictor of cardiovascular events in renal transplant recipients. *Nephrol Dial Transpl*. 2010;25(11):3773-3778.
11. Roe P, Wolfe M, Joffe M, Rosas SE. Inflammation, coronary artery calcification and cardiovascular events in incident renal transplant recipients. *Atherosclerosis*. 2010;212(2):589-594.
12. Claes KJ, Heye S, Bammens B, et al. Aortic calcifications and arterial stiffness as predictors of cardiovascular events in incident renal transplant recipients. *Transpl Int*. 2013;26(10):973-981.
13. Davis B, Marin D, Hurwitz LM, et al. Application of a Novel CT-Based Iliac Artery Calcification Scoring System for Predicting Renal Transplant Outcomes. *Am J Roentgenol*. 2016;206(2):436-441.
14. Piepoli MF, Hoes AW, Agewall S, et al. 2016 European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice. *Eur Hear J*. 2016;37(29):2315-2381.
15. Schousboe JT, Wilson KE, Kiel DP. Detection of Abdominal Aortic Calcification With Lateral Spine Imaging Using DXA. *J Clin Densitom*. 2006;9(3):302-308.
16. Pencina MJ, D'Agostino RB, Steyerberg EW. Extensions of net reclassification improvement calculations to measure usefulness of new biomarkers. *Stat Med*. 2011;30(1):11-21.
17. Leening MJG, Vedder MM, Witteman JCM, Pencina MJ, Steyerberg EW. Net reclassification improvement: Computation, interpretation, and controversies: A literature review and clinician's guide. *Ann Intern Med*. 2014;160(2):122-131.

18. Schousboe JT, Wilson KE, Hangartner TN. Detection of Aortic Calcification during Vertebral Fracture Assessment (VFA) Compared to Digital Radiography. *PLoS One*. 2007;2(8):e715.
19. Toussaint ND, Lau KK, Strauss BJ, Polkinghorne KR, Kerr PG. Determination and validation of aortic calcification measurement from lateral bone densitometry in dialysis patients. *Clin J Am Soc Nephrol*. 2009;4(1):119-127.
20. Cook NR. Use and Misuse of the Receiver Operating Characteristic Curve in Risk Prediction. *Circulation*. 2007;115(7):928-935.

Bone mineral density and aortic calcification: evidence for a bone-vascular axis after kidney transplantation

Transplantation, 2020

Camilo G. Sotomayor [¶]

Stan Benjamins [¶]

Antônio W. Gomes-Neto

Robert A. Pol

Dion Groothof

Charlotte A. te Velde-Keyzer

Guillermo Chong

Andor W.J.M. Glaudemans

Stefan P. Berger

Stephan J.L. Bakker

Riemer H.J.A. Slart

[¶]These authors contributed equally to this work

Background: Chronic kidney disease mineral and bone disorders (CKD-MBD) and vascular calcification are often seen in kidney transplantation recipients. This study focused on the bone-vascular axis hypothesis, the pathophysiological mechanisms driving both bone loss and vascular calcification, supported by an association between lower bone mineral density (BMD) and higher risk of vascular calcification.

Methods: Patients referred for a dual-energy X-ray absorptiometry (DXA) procedure within 6-months post-transplantation were included in a cross-sectional study (2004–2014). Areal BMD was measured at the proximal femur, and abdominal aortic calcification (AAC) was quantified (8-points score) from lateral single-energy images of the lumbar spine. Patients were divided into three AAC-categories (negative-AAC: AAC zero; low-AAC: AAC 1-3; and high-AAC: AAC 4-8). Multivariable-adjusted multinomial logistic regression models were performed to study the association between BMD and AAC.

Results: We included 678 kidney transplant recipients (51 ± 13 years-old, 58% males), 366 (54%) had BMD disorders and 266 (39%) had detectable calcification. High-AAC was observed in 9%, 11% and 25% of patients with normal BMD, osteopenia, and osteoporosis, respectively ($p < 0.001$). Higher BMD (T-score, continuous) was associated with lower risk of high-AAC (OR 0.60, 95% CI 0.43–0.85; $p = 0.001$), independent of age, sex, eGFR, and immunosuppressive therapy. kidney transplant recipients with osteoporosis, patients with normal BMD were less likely to have high-AAC (OR 0.23, 95% CI 0.08–0.65; $p = 0.01$).

Conclusions: BMD disorders are highly prevalent in kidney transplant recipients. The independent inverse association between BMD and AAC may provide evidence to point towards the existence, while highlighting the clinical and epidemiological relevance, of a bone-vascular axis post-kidney transplantation.

INTRODUCTION

Chronic kidney disease (CKD) is an independent risk factor for cardiovascular disease. Cardiovascular disease, in turn, leads the burden of morbidity and premature mortality in chronic kidney disease and end-stage renal disease patients.¹ Whilst kidney transplantation is the gold-standard treatment for end-stage renal disease, cardiovascular disease continues to portray the major risk in the post-kidney transplantation setting, which continues to challenge improvement of long-term survival after kidney transplant.^{2,3}

A large body of evidence underscores the relevance of vascular calcification as being independently associated with major adverse cardiovascular events and mortality due to cardiovascular disease in kidney transplant recipients.^{4–9} Linking vascular disease with bone disease, chronic kidney disease mineral and bone disorders (CKD-MBD) constitute a syndrome codified by the KDIGO more than a decade ago, in which vascular calcification is associated with CKD due to disruption of the complex systems biology encompassing the kidney, skeleton, and cardiovascular system.^{10–17}

Kidney transplantation aims to restore renal function, as well as mineral regulating hormones and overall homeostasis of mineral metabolites. However, disturbed bone and mineral metabolism persists after kidney transplantation.¹⁸ Upon pre-transplant renal osteodystrophy and persistent metabolic bone disorders, maintenance immunosuppressive therapy appends an additional transplant-specific hazard for altered bone turnover, mineralization and volume.^{19,20} Indeed, post-transplant bone disease is considered significantly different to that observed within the context of pre-transplant CKD-MBD.²¹ The substantial epidemiological relevance of post-transplant bone disease is being ever-increasingly acknowledged and, accordingly, actively addressed among clinicians.^{21,22,31,23–30} Recommendations of bone mineral density (BMD) testing after transplantation have been formally incorporated in the KDIGO 2017 clinical practice guidelines.²⁸ Non-invasive, relatively accurate and cost-effective, dual-energy X-ray absorptiometry (DXA) is the imaging method of choice for bone mass screening early after kidney transplantation.²⁸

While the link between bone disease and vascular calcification arising from primary disturbance of calcium phosphate homeostasis has long been acknowledged in native chronic kidney disease, there is in contrast a paucity of studies devoted to investigate the postulated independent association between bone disease and risk of vascular calcification in the post-kidney transplantation setting.^{10,12,15–17,32–35} We hypothesized that, in kidney transplant recipients, BMD is independently and inversely associated with

risk of vascular calcification. Evidence of this association would further support the existence of a bone-vascular axis, it would provide data to evaluate its epidemiological relevance post-kidney transplantation, and it would point towards otherwise overlooked therapeutic opportunities to potentially decrease the high cardiovascular burden in kidney transplant recipients.

In a large cohort of kidney transplant recipients, we aimed to investigate BMD disorders as assessed by a DXA scan, in line with the KDIGO guidelines, and study the potential independent association between BMD and risk of abdominal aortic calcification (AAC) post-kidney transplantation.

METHODS

Study Design

We performed a single-centre cross-sectional cohort study in a university setting (University Medical Center Groningen, Groningen, The Netherlands) (Supplement 1). All adult patients referred for a DXA scan within 6 months after a first kidney transplantation between 2004-2014 were considered eligible. The study protocol regarding patient data processing and storage for medical research involving human subjects, was approved by the Institutional Review Board (Medical Ethical Committee 2017/457) and conducted in accordance with declarations of Helsinki and Istanbul.

Medical history including transplant characteristics, and medication use were extracted from patients' medical records. As described elsewhere,³⁶ standard immunosuppression consisted of the following: cyclosporine (target trough levels 175–200 mg/L in the first 3 months, 100 mg/L thereafter), prednisolone (starting with 20 mg/day and tapering to 10 mg/day) and mycophenolate mofetil (2 g/day), and for kidney transplant recipients with no complications, cyclosporine was slowly withdrawn from 1 year after transplantation onward. In 2012 cyclosporine was replaced by tacrolimus, and kidney transplant recipients continued triple-immunosuppressive therapy with prednisolone (20 mg/day, tapering to 5 mg/day), tacrolimus (target trough levels 8–12 mg/L in the first 3 months, 6–10 mg/L until month 6, and 4–6 mg/L from 6 months onward), and mycophenolate mofetil (starting with 2 g/day, tapering to 1 g/day).

We investigated and documented clinical data as following. Pre-transplant hypertension was defined as blood pressure > 140/90 mmHg or current anti-hypertensive medication. Pre-transplant hypercholesterolemia was defined as total cholesterol levels > 200 mg/dL or current use of lipid-lowering agents. Following the WHO guidelines - *International statistical classification of diseases and related health problems* – cardiovascular events were defined as the occurrence of a myocardial infarction (International Statistical Classification of Diseases and Related Health Problems (ICD)-10: I21), both ST-elevation myocardial infarction and non-ST-elevation myocardial infarction, instable angina pectoris (ICD-10: I20), a cerebrovascular accident (ICD I60-I66), or a transient ischemic attack (ICD-10 G45). Information with regard to the definition used to prospectively collect data on cardiovascular events post-kidney transplantation in this patient cohort, and the analyses on the association between abdominal aortic calcification and risk of cardiovascular events can be found elsewhere⁹. As described elsewhere,³⁷ pre-transplant diabetes mellitus was defined according to the guidelines of the American Diabetes Association, when at least one of the following criteria was met: symptoms of diabetes plus casual plasma glucose concentration $\geq$ 200 mg/dL (11.1 mmol/L), or fasting

plasma glucose ≥ 126 mg/dL (7.0 mmol/L), or 2-hours postload glucose ≥ 200 mg/dL (11.1 mmol/L) during an oral glucose tolerance test; or the use of anti-diabetic medication.³⁸ Smoking status was considered active if patients were current smokers at the time of transplant wait-listing admission.³⁹ Cardiovascular disease history was considered positive if patients had a myocardial infarction, cerebrovascular accidents or transient ischemic attack. Either history of hyperparathyroidism or use of cinacalcet was used to indicate pre-transplant hyperparathyroidism. Estimated Glomerular Filtration Rate (eGFR) was calculated applying the serum creatinine-based Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.⁴⁰

DXA scan, bone mineral density and abdominal aortic calcification scoring

Lateral single-energy images of the lumbar spine were obtained on a Discovery DXA System (Hologic, Bedford, MA, USA). DXA images were analysed by two blinded independent imaging specialists. Areal BMD was measured at the proximal femur and expressed as a T-score (Figure 1.). In keeping with the World Health Organization, BMD was then classified into osteoporosis with a T-score of ≤ -2.5 or less; osteopenia with a T-score between -2.5 and -1.0 ; or normal BMD with a T-score higher than -1.0 . AAC was quantified by means of a visual 8-point scale, as previously described by Schousboe et al.⁴¹ This scale reflects the total length of calcification on the anterior and posterior aortic walls between L1 and L4 vertebral bones. The scale system assigns 1-point for a single-sided calcification with an aggregate length up to the height of one vertebra. Additional scoring-points are given when calcifications reached the level of the three other vertebrae. The total score was the summation of anterior and posterior calcification scores and ranged from 0 to 8, as described before.⁹ Based on AAC-scoring, patients were stratified into three AAC-categories (i) negative finding, (ii) low-AAC, and (iii) high-AAC; according to AAC-scores 0, 1-3 and 4-8, respectively.

Statistical Analyses

Data were analysed using IBM SPSS software version 23.0 (SPSS Inc., Chicago, IL, USA), STATA 14.1 (STATA Corp., College Station, TX), and R version 3.2.3 (R Foundation for Statistical Computing, Vienna, Austria). Data are expressed as mean $\pm$ standard deviation (SD) for normally distributed variables, and as median (interquartile range; IQR) for skewed variables. Categorical data are expressed as *n* (percentage). The percentage of missing data was 0.003% for immunosuppressive therapy, and 32 and 38% for risk-of-fracture and dialysis vintage, respectively. Differences in baseline characteristics among categories of BMD were evaluated by using the Kruskal-Wallis test for skewed variables, the analysis of variance (ANOVA) for normally distributed variables, and Chi-squared test for categorical data. In all analyses, a two-sided $p < 0.05$ was considered significant.

To study the association of BMD with risk of low- and high-AAC, multinomial logistic models were fitted to the data, with adjustment for age, sex, body mass index, eGFR, and immunosuppressive therapy (model 1); history of hyperparathyroidism, history of parathyroidectomy, use of calcium supplements, use of vitamin D supplements, use of cinacalcet pre- and post-transplantation, and use of biphosphonates (model 2); and, calcium, phosphate, aspartate aminotransferase, gamma glutamyl transpeptidase, and alkaline phosphatase (model 3). In order to comprehensively study these associations, odds ratios and 95% confidence intervals were calculated with BMD as continuous variable and as categorical variable according to clinical categories (normal BMD and osteopenia, with osteoporosis as reference). Potential heterogeneity on the association of BMD with AAC by age, sex, body mass index, eGFR, diabetes, smoking history, cardiovascular history, hyperparathyroidism, use of cinacalcet pre- and post-transplantation, and immunosuppressive therapy were tested by fitting models containing both main effects and their cross-product terms. $P_{\text{interaction}} < 0.05$ was considered to indicate significant heterogeneity.

Figure 1. Example of a lateral single-energy image of the lumbar spine, with the lumbar vertebral bones L1 to L4, and a proximal femur image for areal BMD assessment from dual energy X-ray absorptiometry (DXA).

RESULTS

We included 678 kidney transplant recipients (51 ± 13 years-old, 58% males, eGFR 51 ± 15 mL/min/1.73 m², proximal femur T-score -1.1 ± 1.1), of whom 366 (54%) had BMD disorders, *i.e.*, 301 (44%) had osteopenia and 65 (10%) had osteoporosis. In turn, 266 (39%) had detectable aortic calcification (AAC-score ≥ 1). Additional baseline characteristics, overall and by categories of bone mineral density are shown in Table 1. Distribution of AAC-categories was significantly different across subgroups of patients according to BMD ($p < 0.001$), with *e.g.*, high-AAC observed in 9%, 11% and 25% of patients with normal bone mineral density, osteopenia, and osteoporosis, respectively. Patients with osteoporosis were older, mostly women, and had lower body mass index, higher general and hip-specific risk of fracture, higher aspartate aminotransferase, gamma glutamyl transpeptidase and alkaline phosphatase.

In unadjusted logistic regression analyses, we found that relatively higher BMD (T-score, continuous) was consistently associated with lower risk of low-AAC (OR 0.71, 95% CI 0.60–0.84; $p < 0.001$) or high-AAC (OR 0.66, 95% CI 0.52–0.84; $p = 0.001$). When we analysed BMD as a categorical variable, we found that in comparison to patients with osteoporosis, those with normal BMD (OR 0.26, 95% CI 0.12–0.52; $p < 0.001$) or osteopenia (OR 0.39, 95% CI 0.19–0.79; $p = 0.01$) were less likely to have high-AAC. These findings remained materially unaltered in further models with for *e.g.*, adjustment for history hyperparathyroidism, history of parathyroidectomy, use of calcium and vitamin D supplements, use of cinacalcet, and use of bisphosphonates (Model 2; Table 2). We observed no heterogeneity for the association of BMD and AAC by age, sex, body mass index, eGFR, diabetes, smoking history, cardiovascular history, hyperparathyroidism, use of cinacalcet and immunosuppressive therapy ($P_{\text{interaction}} > 0.05$ for all). Figure 2 represents the association of femoral T-score with risk of AAC, data were fitted by logistic regression using median femoral T-score as reference value.

Figure 2. Association of femoral T-score with risk of AAC. Data were fitted by logistic regression using median femoral T-score as reference value and presented for the unadjusted outcomes (A), model 1 (B), model 2 (C) and model 3 (D). The black line represents the odds ratio and the grey area represents the 95% confidence interval.

Table 1. Baseline characteristics, overall and by BMD categories according to T-score measured by DXA at the proximal femur

Baseline characteristics	
	Total (n = 678)
Demographics	
Age, years, mean (SD)	51 (13)
Gender, male, n (%)	394 (58)
Body mass index, kg/m ² , mean (SD)	25.5 (4.2)
eGFR, mL/min/1.73 m ² , mean (SD)	51 (15)
Hypertension, n (%)	547 (81)
Hypercholesterolemia, n (%)	291 (43)
Diabetes mellitus, n (%)	92 (14)
Smoking history, n (%)	142 (21)
Cardiovascular history, n (%)	112 (17)
Hyperparathyroidism, n (%)	145 (21)
Post-parathyroidectomy, n (%)	38 (6)
Dialysis vintage (months), median (IQR) ^b	39 (21–55)
Dual-energy X-ray absorptiometry	
General risk-of-fracture, median (IQR) ^a	5.8 (3.6–10.0)
Hip risk-of-fracture, median (IQR) ^a	1.1 (0.3–2.8)
Vertebral fractures, n (%)	122 (18)
Thoracic, n (%)	65 (10)
Lumbar, n (%)	26 (4)
Thoracic and lumbar, n (%)	10 (2)
AAC-score, median (IQR)	0 (0–2)
AAC-score, categories	
No calcification, n (%)	412 (61)
Low AAC-score (1–3), n (%)	190 (28)
High AAC-score (4–8), n (%)	76 (11)
Renal transplant and immunosuppressive therapy	
Immunosuppressive therapy	
Use of corticosteroids, n (%) ^c	661 (98)
Corticosteroids dose, mg/day, median (IQR)	17.5 (10.0–20.0)
Calcineurin inhibitors	
Use of cyclosporine, n (%) ^c	309 (46)
Use of tacrolimus, n (%) ^c	186 (27)
Proliferation inhibitors	
Use of azathioprine, n (%) ^c	14 (2)

BMD, categories			
Normal (n = 312)	Osteopenia (n = 301)	Osteoporosis (n = 65)	p-value
50 (13)	52 (13)	54 (12)	0.02
199 (64)	171 (57)	24 (37)	<0.001
26.6 (4.2)	24.7 (3.9)	23.6 (4.3)	<0.001
52 (15)	52 (15)	46 (15)	0.07
259 (83)	237 (79)	51 (79)	0.36
131 (42)	130 (43)	30 (46)	0.82
42 (14)	36 (12)	14 (22)	0.12
62 (20)	63 (21)	17 (26)	0.53
55 (18)	44 (15)	13 (20)	0.44
67 (22)	60 (20)		0.59
22 (7)	12 (4)	4 (6)	0.28
39 (19–53)	35 (22–56)	46 (27–65)	0.06
4.2 (2.4–6.5)	7.0 (4.5–10.0)	17.0 (11.0–23.8)	<0.001
0.3 (0.1–0.9)	1.9 (0.8–3.8)	8.1 (3.6–15.0)	<0.001
49 (16)	58 (19)	15 (23)	0.28
28 (9)	28 (10)	7 (11)	0.90
15 (5)	8 (3)	3 (5)	0.39
6 (2)	3 (1)	1 (2)	0.66
0 (0–1)	0 (0–2)	1 (0–4)	0.001
			<0.001
212 (68)	168 (56)	32 (49)	
73 (23)	100 (33)	17 (26)	
27 (9)	33 (11)	16 (25)	
302 (97)	295 (98)	64 (99)	0.82
17.5 (10.0–20.0)	17.5 (10.0–19.4)	17.5 (15.0–20.0)	0.81
146 (47)	124 (41)	39 (60)	0.02
87 (28)	86 (29)	13 (20)	0.36
3 (1)	9 (3)	2 (3)	0.18

Table 1. Continued

Baseline characteristics	
	Total (n = 678)
Use of myfortic, n (%) ^d	237 (35)
Combined immunosuppressive therapy ^c	
Cyclosporine+MMF+corticosteroids, n (%)	307 (45)
Tacrolimus+MMF/MPS+corticosteroids, n (%)	332 (49)
Others, n (%)	37 (6)
Medication	
Use of calcium supplements, n (%)	87 (13)
Use of vitamin D supplements, n (%)	97 (14)
Use of biphosphonates, n (%)	16 (2)
Use of cinacalcet pre-transplantation, n (%)	60 (9)
Use of cinacalcet post-transplantation, n (%)	26 (4)
Laboratory measurements	
Haemoglobin, mmol/L, mean (SD)	7.7 (1.1)
Leukocyte count, x 10 ⁹ /L, mean (SD)	7.5 (3.2)
Total cholesterol, mmol/L, mean (SD)	5.4 (1.3)
Low density lipoprotein cholesterol, mmol/L, mean (SD)	2.26 (0.74)
Calcium, mmol/L, mean (SD)	2.4 (0.2)
Phosphate, mg/dL, mean (SD)	0.9 (0.2)
ASAT, U/L, mean (SD)	23 (10)
ALAT, U/L, median (IQR)	19 (15–26)
Gamma glutamyl transpeptidase, U/L, median (IQR)	30 (21–50)
Alkaline phosphatase, U/L, median (IQR)	81 (63–109)

Differences in baseline characteristics among categories of bone mineral density were evaluated by using the Kruskal-Wallis test for skewed variables, the analysis of variance (ANOVA) for normally distributed continuous variables, and Chi-squared test for categorical data. Data available in ^a455, ^b420, ^c676, and patients. BMD, bone mineral density; AAC, abdominal aortic calcification; ALAT, alanina aminotransferase; ASAT, aspartato aminotransferase; eGFR, estimated Glomerular Filtration Rate; MMF, mycophenolate mofetil.

BMD, categories			
Normal (n = 312)	Osteopenia (n = 301)	Osteoporosis (n = 65)	p-value
103 (33)	115 (38)	19 (29)	0.26
145 (47)	123 (41)	39 (60)	0.02
150 (48)	160 (53)	22 (34)	0.02
15 (5)	18 (6)	4 (6)	0.80
35 (11)	43 (15)	8 (12)	0.45
50 (16)	38 (13)	9 (14)	0.55
4 (1)	7 (2)	3 (5)	0.09
27 (9)	22 (7)	11 (17)	0.05
10 (3)	13 (4)	3 (5)	0.73
7.7 (1.1)	7.6 (1.1)	7.7 (1.1)	0.43
7.6 (3.4)	7.5 (3.4)	7.2 (3.0)	0.69
5.4 (1.2)	5.5 (1.3)	5.7 (1.1)	0.18
228 (72)	226 (78)	216 (69)	0.53
2.4 (0.2)	2.4 (0.2)	2.5 (0.1)	0.13
0.9 (0.2)	0.9 (0.2)	0.9 (0.2)	0.92
22 (7)	23 (11)	26 (16)	0.02
20 (15–26)	19 (14–26)	19 (17–28)	0.70
20 (28–46)	31 (21–55)	38 (26–56)	0.01
78 (60–98)	82 (64–116)	89 (69–138)	0.004

Table 2. Association between BMD by DXA scan and risk of low- and high-AAC

BMD	Categories of AAC					
	Low- Calcification			High- Calcification		
	OR	(95% CI)	p-value	OR	(95% CI)	p-value
Unadjusted						
T-score, continuous	0.71	0.60 to 0.84	<0.001	0.66	0.52 to 0.84	0.001
Categories						
Normal BMD	0.65	0.34 to 1.24	0.19	0.26	0.12 to 0.52	<0.001
Osteopenia	1.13	0.59 to 2.13	0.72	0.39	0.19 to 0.79	0.01
Osteoporosis		Ref.			Ref.	
Model 1[†]						
T-score, continuous	0.67	0.53 to 0.84	0.001	0.61	0.42 to 0.88	0.008
Categories						
Normal BMD	0.55	0.24 to 1.27	0.16	0.24	0.08 to 0.72	0.01
Osteopenia	0.92	0.41 to 2.10	0.85	0.44	0.16 to 1.23	0.12
Osteoporosis		Ref.			Ref.	
Model 2[‡]						
T-score, continuous	0.71	0.60 to 0.84	<0.001	0.67	0.52 to 0.85	0.001
Categories						
Normal BMD	0.62	0.32 to 1.20	0.16	0.26	0.13 to 0.55	<0.001
Osteopenia	1.09	0.57 to 2.09	0.79	0.42	0.20 to 0.87	0.02
Osteoporosis		Ref.			Ref.	
Model 3[§]						
T-score, continuous	0.72	0.60 to 0.86	<0.001	0.73	0.57 to 0.94	0.02
Categories						
Normal BMD	0.65	0.33 to 1.29	0.22	0.31	0.15 to 0.67	0.003
Osteopenia	1.12	0.58 to 2.19	0.73	0.42	0.20 to 0.90	0.03
Osteoporosis		Ref.			Ref.	

Multivariable-adjusted multinomial logistic regression analyses. [†]Model 1 was adjusted for age, sex, body mass index, estimated Glomerular Filtration Rate, and immunosuppressive therapy. [‡]Model 2 was adjusted for history of hyperparathyroidism, history of parathyroidectomy, use of calcium and vitamin D supplements, use of cinacalcet pre- and post-transplantation, and use of bisphosphonates. [§]Model 3 was adjusted for calcium, phosphate, aspartate aminotransferase, gamma glutamyl transpeptidase, and alkaline phosphatase. Odds ratios and 95% confidence intervals were calculated with BMD (T-score) as continuous variable and as categorical variable according to clinical categories (normal BMD and osteopenia, with osteoporosis as reference). AAC, abdominal aortic calcification; BMD, bone mineral density.

DISCUSSION

Our study shows an independent inverse association between BMD and risk of AAC, which supports the hypothesis of the existence of a bone-vascular axis after kidney transplantation. These findings underscore a non-traditional and modifiable —yet rather underestimated— risk factor for excess cardiovascular disease and premature cardiovascular mortality of kidney transplant recipients.

The presented data underlines that BMD disorders are substantially prevalent after kidney transplantation, with a ratio higher than 1 out of 2 kidney transplant recipients, as assessed by a DXA scan within 6 months after kidney transplantation. This is in line with recently published studies and international guidelines focusing on post-transplant bone disease.^{21,22,31,23–30} As soon as 6 months post-kidney transplantation, BMD declines 4–10%, with a prevalence of BMD disorders of at least 50% within the first year after transplantation.^{22,42–44} Recently, Keronen et al. provided valuable DXA scan data to show that in comparison to a baseline pre-transplant examination, femoral neck T-score was significantly lower 2 years post-kidney transplantation. In addition, whereas the rate of abnormal mineralization of patients that remained in dialysis decreased after 2 years of follow-up, patients that underwent kidney transplantation depicted a relative increase in abnormal mineralization rates during the same follow-up period.³⁰ It is relevant to note that low bone turnover increases by 100% within 2 years post-kidney transplant.^{30,45} Two major recent studies further support that bone turnover tends to decline post-kidney transplantation.^{27,29} These findings underscore that transplantation itself is a hallmark for additional hazards for bone health. The latter partly explains that post-transplant bone disease is considered significantly different to that observed within the context of pre-transplant CKD-MBD.²¹

The most widely studied clinical consequence hereof is the post-kidney transplantation risk of fracture. In the US Renal Data System, 22.5% of kidney transplant recipients showed to have a fracture within 5 years after transplantation ($n=68814$ kidney transplant recipients). However, despite a large body of evidence accounting for the relationship between bone mineralization and calcium deposition in the vascular wall of native CKD patients,^{10–14,17,46} bone disease in kidney transplant recipients as risk factor for an increased risk of vascular calcification is underrepresented in literature.

Vascular calcification is an active cell-mediated process that resembles developmental osteogenesis, and it is made worse by disturbances in calcium phosphate metabolism with involvement of mediators of bone mineralization.^{16,47–49} Bone demineralization and abnormal bone remodelling seen in CKD promotes vascular calcification via multiple

mechanisms (reviewed in detail in references 14–17,47). By leading to release of circulating nucleational complexes, bone turnover plays a key pathophysiological role linking BMD disorders with vascular calcification.^{51–53} Whereas low-turnover bone disease appears to account for the greatest vascular calcification risk,^{12,45} severe high-turnover bone disease has also been linked with vascular calcification.^{24,51,52,54,55} Bisphosphonates, aiming at reduction of bone resorption, have been reported to prevent vascular calcification in haemodialysis patients, although the exact mechanism of inhibition remains unclear.⁵⁶ In kidney transplant recipients, an inverse association was recently shown between the use of bisphosphonates and hard endpoints post-kidney transplant such as graft and patient survival.⁵⁷ Regrettably, however, the data collected by Song et al. do not allow to evaluate the potential explanatory involvement of the bone-vascular axis for such findings.⁵⁷ As first observed by Malluche et al., and recently emphasized by Seifert et al., in the post-kidney transplantation setting, there is no evidence encompassing relation of bone disease with vascular calcification.^{15,22} Yet, vascular calcification is associated with adverse cardiovascular outcomes, which, in turn, leads the burden of premature mortality of kidney transplant recipients.^{2,3,9} By underscoring the substantial prevalence of osteoporosis and osteopenia in kidney transplant recipients, and describing its independent association with AAC, we emphasize the multi-fold nature of clinical hazards derived from bone disease, particularly, post-kidney transplantation.

Because a previous study showed that treatment of hyperparathyroidism with cinacalcet—a calcimimetic agent that activates the calcium-sensing receptors in parathyroid glands—may negatively impact thyroid function,⁵⁸ we aimed to study whether cinacalcet use pre- and post-transplantation may interact with the association between BMD and AAC. In agreement with observations of a large and double-blind randomized study, in which no beneficial impact of cinacalcet on BMD was shown,⁵⁹ we found that there is no significant interaction of cinacalcet use pre- or post-transplantation on the association of BMD with increased risk of AAC.

Assessment of bone health by means of a DXA scan is a limitation of the current study on the basis that quantitative histomorphometric analysis of a bone biopsy with use of the TMV (turnover, mineralization, volume) system is the gold-standard for evaluation of bone alterations.^{30,60} DXA scans, among other imaging techniques such as magnetic resonance imaging, high-resolution peripheral quantitative computed tomography (CT), and ¹⁸F-sodiumfluoride positron emission tomography, are meaningful help to non-invasively assess bone health, yet it is unlikely that these techniques may thoroughly substitute bone biopsies.²⁵ Nevertheless, it should be realized that in daily clinical practice, bone biopsies are not part of routine diagnostic tools nor used for long-term follow-up of patients, being only exceptionally performed in specific cases.²⁵ Furthermore,

bone biopsy studies in kidney transplant recipients, beyond being logistically hampered by the invasive nature of the procedure, have long delivered limited conclusions due to small sample sizes that lack statistical power to comprehensively study clinical impacts of bone disease. The latter explains the fact that KDIGO bone biopsy recommendations are not graded.²⁸ Future combined efforts to collaboratively perform adequately powered studies are warranted.^{25,30,31} The routine use of DXA scans post-kidney transplantation, on the contrary, is supported by KDIGO guidelines. The current study, performed in a large cohort of kidney transplant recipients, provides data derived from DXA scans, a routinely accessible imaging technique for the assessment of bone alterations early post-kidney transplantation. This large dataset allowed us to study the independent association of BMD disorders with risk of vascular calcification in kidney transplant recipients, which was robust to adjustment for several potential confounders including body mass index,^{61,62} eGFR, and immunosuppressive therapy. This observation is particularly relevant by taking into account that patients under the aforementioned regimen indeed showed a significantly lower prevalence of osteoporosis, whereas an alternative regimen (corticosteroids + mycophenolate mofetil + cyclosporine) seemed to relate with a significantly higher prevalence of osteoporosis; yet, the increased risk of AAC observed in relation to a relatively lower BMD was not modified by the use of either immunosuppressive regimen. Taken together, these data may suggest that underlying mechanisms linking vascular disease with bone disease may persist post-kidney transplant. The latter is concerning when contrasting the relatively scant attention given to bone disease in this particular clinical setting in current international guidelines on CKD-MBD (e.g. KDIGO²⁸), in spite of the opportunity it may offer to aid on managing vascular calcification-associated risk for cardiovascular events post-kidney transplant⁴⁻⁹. These findings point towards a rather underestimated, yet epidemiologically relevant and potentially modifiable, non-traditional cardiovascular risk factor after kidney transplantation, which urges collaborative clinical and scientific attention.

While electron beam CT and multislice CT are considered the gold-standard imaging techniques for quantitative evaluation of vascular calcifications, DXA-based quantification showed to be associated with cardiovascular endpoints in several studies. Studies using improved sensitivity of imaging modalities may be of particular interest to study the progression of vascular calcifications longitudinally. Whereas studies focusing on CT quantification usually are of limited clinical extrapolation due to cost-effectiveness constraints and lead to a significant radiation burden, DXA-based screening is inexpensive, a single combined procedure of BMD and AAC assessment, and easy to interpret by the attending nephrologist or physician and associates a low radiation burden.^{63,64}

The cross-sectional design of the current study should be considered as its main limitation, hampering hard conclusions about the temporal nature of the bone vascular-axis. Achieving further understanding of whether it is bone loss driving vascular calcification, or vascular calcification driving bone loss through impaired blood and nutrient supply to the bones, or rather a vicious circle of these pathophysiological mechanisms occurring concurrently, warrants future studies. Considering that we measured BMD at a single site, the proximal femur, and that we were limited in differentiation of pre- from post-transplant bone-vascular disease, comprehensive longitudinal assessments starting from pre-transplant stages are essential. Given the potential therapeutic opportunity that the bone vascular-axis may point towards to aid on managing vascular calcification-associated cardiovascular risk post-kidney transplant, which is the leading individual cause of long-term mortality in this population, the current findings hold the plea for future studies in which such analyses are performed.

In conclusion, BMD disorders are highly prevalent in kidney transplant recipients and BMD assessed by DXA scan is inversely and independently associated with risk of AAC. These findings point towards the existence of a bone-vascular axis, evidenced, for the first time, post-kidney transplantation. Our findings are in line with previous studies, which have separately emphasized the post-transplantation milieu as an additional hazard for the complex biology system enclosed by the kidney, skeleton and cardiovascular system. Further studies are warranted to evaluate whether focused preventive management of CKD-BMD early after kidney transplantation may represent a material therapeutic target to reduce the high cardiovascular burden post-kidney transplantation.

REFERENCES

- Go AS, Chertow GM, Fan D, McCulloch CE, Hsu C-Y. Chronic Kidney Disease and the Risks of Death, Cardiovascular Events, and Hospitalization. *N Engl J Med*. 2004;351(13):1296-1305.
- Jardine AG, Gaston RS, Fellstrom BC, Holdaas H. Prevention of cardiovascular disease in adult recipients of kidney transplants. *Lancet*. 2011;378(9800):1419-1427.
- Lamb KE, Lodhi S, Meier-Kriesche HU. Long-term renal allograft survival in the United States: A critical reappraisal. *Am J Transplant*. 2011;11(3):450-462.
- DeLoach SS, Joffe MM, Mai X, Goral S, Rosas SE. Aortic calcification predicts cardiovascular events and all-cause mortality in renal transplantation. *Nephrol Dial Transpl*. 2009;24(4):1314-1319.
- Nguyen PT, Henrard S, Coche E, Goffin E, Devuyst O, Jadoul M. Coronary artery calcification: a strong predictor of cardiovascular events in renal transplant recipients. *Nephrol Dial Transpl*. 2010;25(11):3773-3778.
- Roe P, Wolfe M, Joffe M, Rosas SE. Inflammation, coronary artery calcification and cardiovascular events in incident renal transplant recipients. *Atherosclerosis*. 2010;212(2):589-594.
- Claes KJ, Heye S, Bammens B, et al. Aortic calcifications and arterial stiffness as predictors of cardiovascular events in incident renal transplant recipients. *Transpl Int*. 2013;26(10):973-981.
- Davis B, Marin D, Hurwitz LM, et al. Application of a Novel CT-Based Iliac Artery Calcification Scoring System for Predicting Renal Transplant Outcomes. *Am J Roentgenol*. 2016;206(2):436-441.
- Benjamins S, Pol RA, Glaudemans AWJM, et al. A high abdominal aortic calcification score by dual X-ray absorptiometry is associated with cardiovascular events after kidney transplantation. *Nephrol Dial Transplant*. 2018;33(12):2253-2259.
- Braun J, Oldendorf M, Moshage W, Heidler R, Zeitler E, Luft FC. Electron beam computed tomography in the evaluation of cardiac calcifications in chronic dialysis patients. *Am J Kidney Dis*. 1996;27(3):394-401.
- Goodman WG, Goldin J, Kuizon BD, et al. Coronary-artery calcification in young adults with end-stage renal disease who are undergoing dialysis. *N Engl J Med*. 2000;342(20):1478-1483.
- London GM, Marty C, Marchais SJ, Guerin AP, Metivier F, de Vernejoul M-C. Arterial calcifications and bone histomorphometry in end-stage renal disease. *J Am Soc Nephrol*. 2004;15(7):1943-1951.
- Moe SM. Vascular calcification and renal osteodystrophy relationship in chronic kidney disease. *Eur J Clin Invest*. 2006;36(SUPPL. 2):51-62.
- Moe SM, Drüeke T, Lameire N, Eknoyan G. Chronic Kidney Disease-Mineral-Bone Disorder: A New Paradigm. *Adv Chronic Kidney Dis*. 2007;14(1):3-12.
- Seifert ME, Hruska KA. The kidney-vascular-bone axis in the chronic kidney disease-mineral bone disorder. *Transplantation*. 2016;100(3):497-505.
- Mizobuchi M, Towler D, Slatopolsky E. Vascular calcification: the killer of patients with chronic kidney disease. *J Am Soc Nephrol*. 2009;20(7):1453-1464.
- Thompson BB, Towler DA. Arterial calcification and bone physiology: role of the bone-vascular axis. *Nat Rev Endocrinol*. 2012;8:529-543.
- Perrin P, Caillard S, Javier RM, et al. Persistent hyperparathyroidism is a major risk factor for fractures in the five years after kidney transplantation. *Am J Transplant*. 2013;13(10):2653-2663.

19. Julian BA, Laskow DA, Dubovsky J, Dubovsky E V, Curtis JJ, Quarles LD. Rapid loss of vertebral mineral density after renal transplantation. *N Engl J Med*. 1991;325(8):544-550.
20. Monier-Faugere MC, Mawad H, Qi Q, Friedler RM, Malluche HH. High prevalence of low bone turnover and occurrence of osteomalacia after kidney transplantation. *J Am Soc Nephrol*. 2000;11(6):1093-1099.
21. Bouqueneau A, Salam S, Delanaye P, Eastell R, Khwaja A. Bone Disease after Kidney Transplantation. *Clin J Am Soc Nephrol*. 2016;11:CJN.11371015.
22. Malluche HH, Monier-Faugere M-C, Herberth J. Bone disease after renal transplantation. *Nat Rev Nephrol*. 2010;6(1):32-40.
23. Neves CL, Dos Reis LM, Batista DG, et al. Persistence of bone and mineral disorders 2 years after successful kidney transplantation. *Transplantation*. 2013;96(3):290-296.
24. Iyer SP, Nikkel LE, Nishiyama KK, et al. Kidney Transplantation with Early Corticosteroid Withdrawal: Paradoxical Effects at the Central and Peripheral Skeleton. *J Am Soc Nephrol*. 2014;25:1331-1341.
25. Evenepoel P, D'Haese P, Bacchetta J, et al. Bone biopsy practice patterns across Europe: the European renal osteodystrophy initiative—a position paper. *Nephrol Dial Transplant*. 2017;32(10):1608-1613.
26. Perrin P, Kiener C, Javier R-M, et al. Recent Changes in Chronic Kidney Disease-Mineral and Bone Disorders and Associated Fractures After Kidney Transplantation. *Transplantation*. 2017;101(8):1897-1905.
27. Evenepoel P, Behets GJ, Viaene L, D'Haese PC. Bone histomorphometry in de novo renal transplant recipients indicates a further decline in bone resorption 1 year posttransplantation. *Kidney Int*. 2017;91(2):469-476.
28. KDIGO 2017 Clinical Practice Guideline Update for the Diagnosis, Evaluation, Prevention, and Treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD). *Kidney Int Suppl*. 2017;7(1):1-59.
29. Marques IDB, Araújo MJCLN, Gracioli FG, et al. A Randomized Trial of Zoledronic Acid to Prevent Bone Loss in the First Year after Kidney Transplantation. *J Am Soc Nephrol*. 2019;30(2):355-365.
30. Keronen S, Martola L, Finne P, Burton IS, Kröger H, Honkanen E. Changes in Bone Histomorphometry after Kidney Transplantation. *Clin J Am Soc Nephrol*. 2019;14(6):894-903.
31. Drüeke TB, Evenepoel P. The Bone after Kidney Transplantation. *Clin J Am Soc Nephrol*. 2019;14(6):795-797.
32. Veit Barreto D, De F, Barreto C, et al. Association of Changes in Bone Remodeling and Coronary Calcification in Hemodialysis Patients: A Prospective Study. *Am J Kidney Dis*. 2008;52:1139-1150.
33. London GM, Marchais SJ, Guérin AP, Boutouyrie P, Métivier F, de Vernejoul M-C. Association of Bone Activity, Calcium Load, Aortic Stiffness, and Calcifications in ESRD. *J Am Soc Nephrol*. Published online 2008.
34. Adragao T, Herberth J, Monier-Faugere M-C, et al. Low Bone Volume-A Risk Factor for Coronary Calcifications in Hemodialysis Patients. *Clin J Am Soc Nephrol*. 2009;4:450-455.
35. Asci G, Ok E, Savas R, et al. The link between bone and coronary calcifications in CKD-5 patients on haemodialysis. *Nephrol Dial Transplant*. Published online 2011.
36. Gomes-Neto AW, Osté MC, Sotomayor C, et al. Fruit and Vegetable Intake and Risk of Posttransplantation Diabetes in Renal Transplant Recipients. *Diabetes Care*. 2019;42(9):1644-1652.
37. Sotomayor CG, Gomes-Neto AW, Eisenga MF, et al. Consumption of fruits and vegetables and cardiovascular mortality in renal transplant recipients: a prospective cohort study. *Nephrol Dial Transplant*. 2020;35(2):357-365.
38. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the expert committee on the diagnosis and

- classification of diabetes mellitus. *Diabetes Care*. 2003;26 Suppl 1:S5-20.
39. Piepoli MF, Hoes AW, Agewall S, et al. 2016 European Guidelines on cardiovascular disease prevention in clinical practice. *Eur Heart J*. 2016;37(29):2315-2381.
 40. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150(9):604-612.
 41. Schousboe JT, Wilson KE, Kiel DP. Detection of Abdominal Aortic Calcification With Lateral Spine Imaging Using DXA. *J Clin Densitom*. 2006;9(3):302-308.
 42. Marcén R, Caballero C, Uriol O, et al. Prevalence of Osteoporosis, Osteopenia, and Vertebral Fractures in Long-Term Renal Transplant Recipients. *Transplant Proc*. 2007;39(7):2256-2258.
 43. Durieux S, Mercadal L, Orcel P, et al. Bone mineral density and fracture prevalence in long-term kidney graft recipients. *Transplantation*. 2002;74(4):496-500.
 44. Mazzaferro S, Diacinti D, Proietti E, et al. Morphometric X-ray absorptiometry in the assessment of vertebral fractures in renal transplant patients. *Nephrol Dial Transplant*. 2006;21(2):466-471.
 45. Hutchison AJ, Whitehouse RW, Boulton HF, et al. Correlation of bone histology with parathyroid hormone, vitamin D₃, and radiology in end-stage renal disease. *Kidney Int*. 1993;44(5):1071-1077.
 46. Giachelli CM. Vascular calcification mechanisms. *J Am Soc Nephrol*. Published online 2004.
 47. Ducy P, Karsenty G. The family of bone morphogenetic proteins. *Kidney Int*. 2000;57(6):2207-2214.
 48. Moe SM, O'Neill KD, Duan D, et al. Medial artery calcification in ESRD patients is associated with deposition of bone matrix proteins. *Kidney Int*. 2002;61(2):638-647.
 49. Cheng S-L, Shao J-S, Charlton-Kachigian N, Loewy AP, Towler DA. MSX2 promotes osteogenesis and suppresses adipogenic differentiation of multipotent mesenchymal progenitors. *J Biol Chem*. 2003;278(46):45969-45977.
 50. Moe SM, Chen NX. Pathophysiology of Vascular Calcification in Chronic Kidney Disease. *Circ Res*. 2004;95(6):560-567.
 51. Price PA, Faus SA, Williamson MK. Bisphosphonates alendronate and ibandronate inhibit artery calcification at doses comparable to those that inhibit bone resorption. *Arterioscler Thromb Vasc Biol*. 2001;21(5):817-824.
 52. Price PA, June HH, Buckley JR, Williamson MK. Osteoprotegerin inhibits artery calcification induced by warfarin and by vitamin D. *Arterioscler Thromb Vasc Biol*. 2001;21(10):1610-1616.
 53. Price PA, Caputo JM, Williamson MK. Bone origin of the serum complex of calcium, phosphate, fetuin, and matrix Gla protein: Biochemical evidence for the cancellous bone-remodeling compartment. *J Bone Miner Res*. Published online 2002.
 54. Price PA, Buckley JR, Williamson MK. The Amino Bisphosphonate Ibandronate Prevents Vitamin D Toxicity and Inhibits Vitamin D-Induced Calcification of Arteries, Cartilage, Lungs and Kidneys in Rats. *J Nutr*. 2001;131(11):2910-2915.
 55. Price PA, June HH, Buckley JR, Williamson MK. SB 242784, a Selective Inhibitor of the Osteoclastic V-H⁺-ATPase, Inhibits Arterial Calcification in the Rat. *Circ Res*. 2002;91(6):547-552.
 56. Nitta K, Akiba T, Suzuki K, et al. Effects of cyclic intermittent etidronate therapy on coronary artery calcification in patients receiving long-term hemodialysis. *Am J Kidney Dis*. 2004;44(4):680-688.
 57. Song S, Huh K, Choi H, et al. Bisphosphonates Effectively Improve the Long Term Renal Transplant Outcomes; A Single Center Retrospective Study [abstract]. *Am J Transplant*. 2019;19(suppl 3).
 58. Ritter C, Miller B, Coyne DW, et al. Paricalcitol and cinacalcet have disparate actions on parathyroid oxyphil cell content in patients

- with chronic kidney disease. *Kidney Int.* 2017;92(5):1217-1222.
59. Evenepoel P, Cooper K, Holdaas H, et al. A randomized study evaluating cinacalcet to treat hypercalcemia in renal transplant recipients with persistent hyperparathyroidism. *Am J Transplant.* 2014;14(11):2545-2555.
 60. Gal-Moscovici A, Sprague SM. Bone Health in Chronic Kidney Disease-Mineral and Bone Disease. Published online 2007.
 61. Ribot C, Tremollieres F, Pouilles JM, Bonneau M, Germain F, Louvet JP. Obesity and postmenopausal bone loss: The influence of obesity on vertebral density and bone turnover in postmenopausal women. *Bone.* 1987;8(6):327-331.
 62. Siris ES, Miller PD, Barrett-Connor E, et al. Identification and fracture outcomes of undiagnosed low bone mineral density in postmenopausal women: Results from the National Osteoporosis Risk Assessment. *J Am Med Assoc.* 2001;286(22):2815-2822.
 63. Dequeker J. Osteoporotic fractures, ageing, and the bone density T-score. *Clin Rheumatol.* 2000;19(3):171-173.
 64. Adragão T, Frazão JM. Cardiovascular risk in dialysis patients: an X-ray vision on vascular calcifications. *Kidney Int.* 2008;74:1505-1507.

Aorto-iliac artery calcification prior to kidney transplantation

Journal of Clinical Medicine, 2020

Stan Benjamens

Elsaline Rijkse

Charlotte A. te Velde-Keyzer

Stefan P. Berger

Cyril Moers

Martin H. de Borst

Derya Yakar

Riemer H. J. A. Slart

Frank J. M. F. Dor

Robert C. Minnee

Robert A. Pol

Background: As vascular calcification is common in kidney transplant candidates, aorto-iliac vessel imaging is performed for surgical planning. The aim of the present study was to investigate whether a novel non-contrast enhanced computed tomography-based quantification technique for aorto-iliac calcification can be used for cardiovascular risk stratification prior to kidney transplantation.

Methods: In this dual-centre cohort study, kidney renal transplant recipients referred for non-contrast enhanced CT within 3 years prior to transplantation were included (2005–2018). Aorto-iliac calcification was quantified using a semi-automated tool, as the aorto-iliac CaScore - a modified Agatston score. The primary outcome measure was all-cause mortality and the secondary outcome measures were cardiovascular mortality and cardiovascular events.

Results: We included 547 kidney transplant recipients, during a median (interquartile range) follow-up of 3.1 (1.4, 5.2) years after transplantation, 80 (14.7%) patients died, of which 32 (40.0%) died due to cardiovascular causes, and 84 (15.5%) patients had a cardiovascular event. Kaplan-Meier survival curves showed significant differences between the CaScore tertiles for cumulative overall-survival (Log-rank test $p < 0.0001$), cardiovascular survival ($p < 0.0001$), and cardiovascular event-free survival ($p < 0.001$). In multivariable Cox regression, the aorto-iliac CaScore was associated with all-cause mortality (hazard ratio 1.53, 95% CI 1.14–2.06, $p = 0.005$), cardiovascular mortality (2.04, 1.20–3.45, $p = 0.008$), and cardiovascular events (1.35, 1.01–1.80, $p = 0.042$).

Conclusions: These independent associations of the aorto-iliac CaScore with the outcome measures can improve the identification of patients at risk for (cardiovascular) death and those who could potentially benefit from stringent cardiovascular monitoring to improve their prognosis after transplantation.

INTRODUCTION

Kidney transplantation improves survival of patients with end-stage renal disease (ESRD) and reduces cardiovascular disease burden.^{1,2} Despite the improved prognosis after transplantation, patients remain vulnerable, with cardiovascular disease as the leading cause of death.^{2,3} A high overall prevalence of cardiovascular disease in the first year after kidney transplantation is reported, with myocardial infarction (MI) in 5.1%, cerebrovascular accident (CVA)/transient ischemic attack (TIA) in 7.3%, and peripheral artery disease (PAD) in 15.8% of patients.^{2,4}

Multidisciplinary evaluation, including consultation of a vascular surgeon, to evaluate the cardiovascular status of kidney transplant candidates could improve the identification of patients at risk for (cardiovascular) death and those who could potentially benefit from interventions to improve their prognosis after transplantation.⁵⁻⁷ When screening kidney transplant candidates, evaluation generally includes traditional risk factors, as used in risk prediction scores for the general population (i.e., the Framingham score), and transplant candidate-specific risk factors, with among others chronic kidney disease–mineral bone disorder (CKD-MBD).^{8,9} As ESRD accelerates vascular calcification and kidney transplantation incompletely decreases this acceleration, cardiovascular risk stratification is not always straightforward in this population.^{10,11}

Prior studies have identified imaging-proven vascular calcification as a prognostic factor for inferior survival and cardiovascular events in kidney transplant recipients.^{12,13} For the applied imaging studies, a distinction can be made between coronary artery calcification (CAC) and aorto-iliac calcification, as well as between assessment of calcification prior to and after transplantation. CAC, expressed as Agatston score by non-contrast enhanced cardiac computed tomography (CT), demonstrated progression within four years in prevalent kidney transplant recipients and is associated with myocardial infarction and mortality.¹⁴⁻¹⁸ Aorto-iliac calcification, by plain pelvic X-ray, dual X-ray absorptiometry, or semi-quantitative CT assessment, was associated with inferior overall patient survival.^{12,19-24}

To date, CT imaging of the aorto-iliac vessels prior to kidney transplantation is primarily performed for surgical planning, while the available imaging data are not widely used for cardiovascular risk stratification in clinical practice.^{25,26} As the imaging data are available, non-contrast enhanced CT-based quantification of aortic calcification can be used for cardiovascular risk stratification of patients screened for kidney transplantation, without the need for additional procedures.^{27,28}

Here, we present the results of a dual-centre cohort study for the quantification of aorto-iliac calcification prior to kidney transplantation and its association with all-cause mortality, cardiovascular mortality, and cardiovascular events, using the aorto-iliac calcium score (CaScore)—an adapted Agatston score.

METHODS

Subjects and study design

Patients referred for a non-contrast enhanced CT-procedure, as part of the screening of high-risk patients prior to kidney transplantation, were included in a dual-centre cohort study (University Medical Center Groningen and Erasmus MC University Medical Center, The Netherlands). In line with the pre-transplant screening protocol in both transplant centres, a CT-procedure was required for transplant screening if either of the following were present: age > 50 years, dialysis vintage > 2 years, a history of PAD or signs and/or symptoms of PAD, diabetes mellitus, or prior surgery in the iliac fossa. Patients were referred for pre-transplant cardiac evaluation if the following were present: symptoms and signs of cardiac disease, a history of cardiac disease, reduced exercise tolerance, PAD, or diabetes mellitus. All adult patients undergoing kidney transplantation between January 2005 to December 2018, in which a CT-procedure was performed within three years prior to transplantation, were considered eligible. Transplant-related data was extracted from the Dutch Organ Transplant Registry and the electronic patient records were screened for baseline and follow-up variables. The study was performed in line with the Declaration of Helsinki and the principles outlined in the Declaration of Istanbul on Organ Trafficking and Transplant Tourism. The Medical Ethical Committee gave approval (University Medical Center Groningen 2017/523) for this dual-centre cohort study.

Outcome measures and clinical variables

The primary outcome measure of this study was all-cause mortality and the secondary outcome measures were cardiovascular mortality and cardiovascular events (including cardiovascular mortality and events). Cardiovascular events were defined as MI (International Statistical Classification of Diseases and Related Health Problems (ICD)-10: I21), CVA (ICD I60-I66), TIA (ICD-10 G45), or new onset PAD (ICD-10 I73) Fontaine III or IV. Cardiovascular mortality was defined as death due to a cardiovascular event or heart failure (ICD-10 I50). Baseline characteristics consisted of the following covariables at time of transplantation: gender, age, diabetes mellitus (fasting glucose ≥ 7.0 mmol/L, casual plasma glucose ≥ 11.1 mmol/L + diabetes symptoms, or glucose tolerance test with 2-hour plasma glucose ≥ 11.1 mmol/L), Body Mass Index (BMI in kg/m²), smoking status (non, former, current), total cholesterol level (mmol/L), hypercholesterolemia (total cholesterol level > 5.2 mmol/L or use of lipid lower medication), systolic and diastolic blood pressure (mmHg), and use of antihypertensive medication. History of cardiovascular disease consisted of MI, CVA, TIA, or PAD. Comorbidities were scored using the Charlson Comorbidity Index and the 10-year risk for MI was estimated with the Framingham score.^{8,29} Transplant-related data consisted of date of transplantation,

type of donation (living-donation, donation after circulatory death, and donation after brain death), number of previous transplantations, type of dialysis (pre-emptive, haemodialysis, or peritoneal dialysis), and dialysis vintage pre-transplantation. Data at one-year post-transplant consisted of estimated-Glomerular Filtration Rate (eGFR), calcium, phosphate, albumin, parathyroid hormone (PTH), and use of calcineurin inhibitors (cyclosporin or tacrolimus).

Quantification of aorto-iliac calcification

Non-contrast enhanced CT images of the aorto-iliac trajectory were extracted from the imaging databases of both hospitals. Quantification of aorto-iliac calcification was performed for three vascular segments, being the abdominal aorta inferior of the renal arteries, the common iliac artery, and the external iliac artery on the side of the subsequent transplant, by an examiner blinded for the patients' medical details (Figure 1). The selected areas of calcification were reviewed on the axial, sagittal, and coronal views, and adjusted if necessary.²⁸ Using the syngo.CT CaScoring software (Siemens Healthineers, Erlangen, Germany), with the standard calcification threshold of 130 Hounsfield Units (HU), the Agatston score adapted for the aorto-iliac trajectory (aorto-iliac CaScore) was obtained for the three segments.²⁷

Statistical analysis

Based on the aorto-iliac CaScore, patients were stratified into tertiles of equal size: low CaScore (0–859 HU), medium CaScore (860–5600 HU), and high CaScore (> 5600 HU). The data are presented as mean (standard deviation, (SD)) in case of normal distribution, median (interquartile range, (IQR)) for skewed data, and number (percentage, %) for categorical data, for the full cohort and the aorto-iliac CaScore tertiles. Tertiles were compared using a chi-square test or one-way analysis of variance. The median (IQR) time of follow-up was calculated with the reversed Kaplan-Meier method, with the date of transplantation as the start of the follow-up. The cumulative overall, event-free survival, and cardiovascular survival were calculated using Kaplan-Meier survival curves, comparing aorto-iliac CaScore tertiles with Log-rank testing. Univariable and multivariable Cox proportional hazards regression analysis were used to evaluate the association of the aorto-iliac CaScore, as a categorical and continuous variable, with all-cause mortality, cardiovascular events, and cardiovascular mortality (Tables S1–S3). Additional survival analysis was performed for time between transplantation and death with a functioning graft (censored for return to dialysis and re-transplantation). To avoid overfitting of the multivariable model with the a priori selected covariables, the results are presented in six steps, as presented in Table 4. Additional analyses of the interaction with covariables were performed by univariable subgroup analysis and analysis of interaction terms for aorto-iliac CaScore (continuous) and all-cause mortality,

with groups based on the mean or median for continuous variables. The p-values of interaction terms were considered significant when <0.05 (Keyzer et al.) or <0.20 (Ramos et al.).^{11,30} Skewed variables were transformed to the natural log ($\log(x + 1)$) for the regression analyses and results are presented as hazard ratio (HR) and corresponding 95% confidence interval (95% CI). The additional discriminative capacity of the aorto-iliac CaScore compared to a basic model (gender and age), the Charlson Comorbidity Index, and the Framingham score was assessed using C-statistics (Harrell's concordance index), a change in C-statistics, and the integrated discrimination improvement (IDI). The IDI is a statistical method to describe the predictive performance of biomarkers in extensive regression models, by comparing an initial model with a model including this new biomarker.³¹ A p-value of <0.05 was considered statistically significant. Statistical analyses were performed with R: A Language and Environment for Statistical Computing, version 1.0.153 for Mac (R Foundation for Statistical Computing, Vienna, Austria).

Figure 1. Graphical image of the kidney transplant anatomy and the aorto-iliac calcium score (CaScore) assessment performed in three vascular segments, being (I) the abdominal aorta inferior of the renal arteries, (II) the common iliac artery, and (III) the external iliac artery on the side of the subsequent transplant.

RESULTS

Study population

This dual-centre cohort consisted of 547 kidney transplant recipients, with a mean time between CT and transplantation of 0.91 (0.72) years. In this cohort, 336 (61.4%) patients were male, the median age at time of transplantation was 60 (51, 68) years, 173 (31.6%) patients had diabetes mellitus, and 350 (64.0%) were on dialysis. Additional baseline characteristics are summarized in Table 1, variables at one-year post-transplant are summarized in Table 2.

Aorto-iliac CaScore and all-cause mortality

After a median follow-up of 3.1 (1.4, 5.2) years after transplantation, 80 (14.7%) patients had died (Table 3). The Kaplan-Meier survival curve for overall survival showed a significant difference between the tertiles ($p < 0.0001$) (Figure 2).

In univariable Cox regression analysis, the continuous CaScore was significantly associated with all-cause mortality (HR 2.02, 95% CI 1.49–2.56, $p < 0.0001$), with consistent HRs in the subgroup analysis (Table 4 and Figure 3). Based on the p -value cut-off by Keyzer et al. (< 0.05), no significant interaction terms were found. With the cut-off by Ramos et al. (< 0.20), the variables age ($p = 0.191$) and diabetes mellitus ($p = 0.141$) showed a significant interaction. In multivariable Cox regression analysis, the association between the CaScore (continuous) and all-cause mortality remained significant after adjustment in six models, with HR 1.53 (95% CI 1.14–2.06, $p = 0.005$) in model 5 and HR 1.72 (95% CI 1.22–2.43, $p = 0.002$) in model 6. A considerably higher HR was observed for the high CaScore (HR 2.59, 95% CI 1.22–5.51) compared to the medium CaScore (HR 1.27, 95% CI 0.60–2.66), with the low CaScore as reference, with $P(\text{trend}) = 0.004$ (model 5) (Table 4). When censoring patients with graft failure, the association between the CaScore (continuous) and all-cause mortality ($n = 47$) remained significant, with HR 2.32 (95% CI 1.52–3.55, $p < 0.0001$) after adjustment in five models.

Aorto-iliac CaScore and cardiovascular mortality

During the follow-up period, in 32 (40.0%) out of the 80 patients who died, mortality was due to cardiovascular causes (Table 3). For the remaining patients who died: 17 (21.2%) out of 80 died due to infection, 15 (18.8%) due to an oncologic cause, three (3.8%) due to pulmonary disease, and for 13 (16.2%), the cause of death could not be established. The Kaplan-Meier survival curve for cardiovascular survival showed a significant difference between the tertiles ($p < 0.0001$) (Figure 4).

Figure 2. Kaplan-Meier curve for cumulative overall survival for the low aorto-iliac calcium score (CaScore tertile), the medium CaScore tertile, and the high CaScore tertile. The overall survival at the median follow-up time was 94.9% (95% CI 91.5–98.5) in the low, 90.5% (95% CI 85.4–95.9) in the medium, and 71.0% (95% CI 62.7–80.3) in the high CaScore tertile.

In univariable Cox regression analysis, a significant association with cardiovascular mortality was observed for the continuous CaScore (HR 2.27, 95% CI 1.55–3.34, $p < 0.0001$). In multivariable Cox regression analysis, the association between the CaScore and cardiovascular mortality remained significant after adjustment in six models, HR 2.04 (95% CI 1.20–3.45, $p = 0.008$) (continuous) in model 5 and HR 2.13 (95% CI 1.32–3.43, $p = 0.002$) in model 6. A considerably higher HR was observed for the high CaScore (HR 4.46, 95% CI 1.19–16.71) compared to the medium CaScore (HR 1.64, 95% CI 0.46–5.76), with the low CaScore as reference, with $P(\text{trend}) = 0.014$ (model 5) (Table 4).

Aorto-iliac CaScore and cardiovascular events

During the follow-up period, a total of 84 (15.5%) patients had one or more cardiovascular events, as described in detail in Table 3. The Kaplan-Meier survival curve for cardiovascular event-free survival showed a significant difference between the tertiles ($p < 0.001$) (Figure 4).

Table 1. Patient characteristics.

Variables	Total (n = 547)
Male gender ^a	336 (61.4)
Age, years ^c	60 (51, 68)
Diabetes mellitus ^a	173 (31.6)
Body Mass Index, kg/m ² ^b	26.8 (4.7)
Smoker ^a	
Non	154 (28.2)
Former	332 (60.7)
Current	61 (11.2)
Total cholesterol, mmol/L ^b	4.7 (1.3)
Hypercholesterolemia ^a	153 (28.0)
Systolic blood pressure, mmHg ^b	145 (22)
Diastolic blood pressure, mmHg ^b	80 (14)
Use of antihypertensive medication ^a	439 (80.3)
Type of donation ^a	
Living-donation	301 (55.0)
Donation after circulatory death	128 (23.4)
Donation after brain death	118 (21.6)
Previous transplants ^a	
Non	514 (94.0)
One	22 (4.0)
Two	11 (2.0)
Type of dialysis ^a	
Pre-emptive	197 (36.0)
Haemodialysis	250 (45.7)
Peritoneal dialysis	100 (18.3)
Dialysis vintage pre-transplantation, months ^c	12 (0, 28)
History of MI ^a	82 (15.0)
History of CVA ^a	40 (7.3)
History of TIA ^a	23 (4.2)
History of PAD ^a	78 (14.3)
Charlson Comorbidity Index ^b	6.4 (2.5)
Framingham score, % ^c	9.6 (3.4, 18.8)

MI = myocardial infarction; CVA = cerebrovascular accident; TIA = transient ischemic attack; PAD = peripheral artery disease; ^a number (%); ^b mean ± standard deviation (SD); ^c median (interquartile range); ^d *p*-value by chi-square test; ^e *p*-value by One-way ANOVA; ^f *p*-value by Kruskal-Wallis test; *p*-values in bold indicate statistical significance.

Low (n = 183)	Medium (n = 182)	High (n = 182)	p-Value
98 (53.6)	108 (57.8)	131 (72.0)	0.001^d
48 (39, 56)	62 (55, 68)	66 (60, 71)	<0.001^e
56 (30.6)	61 (33.5)	56 (30.8)	0.798 ^d
26.0 (5.1)	27.7 (4.6)	26.7 (4.1)	0.002^e
			<0.001^d
74 (40.4)	45 (24.7)	35 (19.2)	
89 (48.6)	117 (64.3)	126 (69.2)	
20 (10.9)	20 (11.0)	21 (11.5)	
4.7 (1.2)	4.8 (1.5)	4.5 (1.2)	0.066 ^e
37 (20.2)	59 (32.4)	57 (31.3)	0.016^d
141 (20)	145 (22)	148 (24)	0.014^e
82 (13)	80 (13)	78 (14)	0.018^e
132 (72.1)	150 (82.4)	157 (86.3)	0.002^d
			0.200 ^d
110 (60.1)	105 (57.7)	86 (47.3)	
39 (21.3)	36 (19.8)	53 (29.1)	
34 (18.6)	41 (22.5)	43 (23.6)	
			0.161 ^d
167 (93.3)	176 (96.7)	171 (94.0)	
10 (5.5)	4 (2.2)	8 (4.4)	
6 (3.3)	2 (1.1)	3 (1.6)	
			0.196 ^d
71 (38.8)	71 (39.0)	55 (30.2)	
77 (42.1)	85 (46.7)	88 (48.4)	
36 (19.1)	26 (14.3)	39 (21.4)	
10 (0, 23)	12 (0, 29)	16 (0, 33)	0.017^f
9 (4.9)	30 (16.5)	43 (23.6)	<0.001^d
7 (3.8)	11 (6.0)	22 (12.1)	0.007^d
4 (2.2)	11 (6.0)	8 (4.4)	0.183 ^d
18 (9.8)	24 (13.2)	36 (19.8)	0.022^d
5.1 (2.0)	6.3 (1.9)	7.7 (2.7)	<0.001^e
3.5 (0.6, 9.5)	10.2 (4.1, 20.2)	16.6 (9.4, 23.8)	<0.001^f

Table 2. One-year post-transplant variables

Variables	Total (n = 547)	Low (n = 183)	Medium (n = 182)	High (n = 182)	p-Value
Laboratory values ^a					
eGFR ^b	51.3 (20.6)	55.9 (22.2)	50.2 (18.1)	47.68 (20.82)	0.001 ^e
Calcium, mmol/L ^b	2.43 (0.14)	2.40 (0.14)	2.45 (0.13)	2.45 (0.14)	0.001 ^e
Phosphate, mmol/L ^b	0.93 (0.21)	0.94 (0.23)	0.95 (0.19)	0.89 (0.19)	0.014 ^e
Albumin, g/L ^b	43.3 (3.1)	43.2 (3.4)	43.6 (2.9)	43.0 (3.0)	0.237 ^e
PTH, pmol/L ^c	10.4 (7.4, 15.6)	9.4 (6.5, 12.8)	10.6 (7.8, 16.3)	11.6 (8.0, 16.4)	0.020 ^f
CNI ^d	535 (97.8)	178 (97.3)	179 (98.4)	178 (97.8)	0.779 ^g
Ciclosporin ^d	42 (7.7)	16 (8.7)	13 (7.1)	13 (7.1)	0.803 ^g
Tacrolimus ^d	494 (90.3)	163 (89.1)	166 (91.2)	165 (90.7)	0.773 ^g

eGFR = estimated-Glomerular Filtration Rate, eGFR-CKD-EPI (mL/min per 1.73 m²); PTH = parathyroid hormone; CNI = calcineurin inhibitors; ^a one-year post-transplant data available for 462 patients; ^b mean ± standard deviation (SD); ^c median (interquartile range); ^d number (%); ^e p-value by student-t test; ^f p-value by Mann-Whitney U test; ^g p-value by chi-square test; p-values in bold indicate statistical significance.

Table 3. Number (%) of cardiovascular events and deaths.

	Total (n = 547)	Low (n = 183)	Medium (n = 182)	High (n = 182)	p-Value
Median follow-up, years	3.1 (1.4, 5.2)	3.6 (2.0, 6.0)	2.6 (1.3, 4.9)	2.7 (1.2, 4.2)	-
Mortality					
All-cause	80 (14.7)	14 (7.7)	22 (12.1)	44 (24.3)	<0.001
Cardiovascular	32 (5.9)	4 (2.2)	10 (5.5)	18 (9.9)	0.007
Cardiovascular events					
MI	46 (8.4)	7 (3.8)	17 (9.3)	22 (12.1)	0.015
CVA	16 (2.9)	6 (3.3)	7 (3.8)	3 (1.6)	0.434
TIA	2 (0.4)	0 (0.0)	0 (0.0)	2 (1.1)	0.134
PAD	30 (5.7)	7 (3.8)	9 (4.9)	15 (8.2)	0.166
Combined	84 (15.5)	18 (9.8)	30 (16.5)	36 (19.8)	0.027

Median follow-up after transplantation (interquartile range); cardiovascular events and deaths presented as numbers (%), p-value by chi-square test MI = myocardial infarction; CVA = cerebrovascular accident; TIA = transient ischemic attack; PAD = new onset peripheral artery disease, Fontaine III or IV; p-values in bold indicate statistical significance.

In univariable Cox regression analysis, a significant association with cardiovascular events was observed for the continuous CaScore (HR 1.56, 95% CI 1.25–1.95, $p < 0.0001$). In multivariable Cox regression analysis, the association between the CaScore and cardiovascular events remained significant after adjustment in six models, HR 1.35 (95%

CI 1.01–1.80, $p = 0.042$) (continuous) in model 5 and HR 1.45 (95% CI 1.09–1.92, $p = 0.010$) in model 6. No significant increase in HR was observed for the high CaScore (HR 1.98, 95% CI 0.97–4.07) compared to the medium CaScore (HR 1.47, 95% CI 0.76–2.85), with the low CaScore as reference, with $P(\text{trend}) = 0.060$ (model 6) (Table 4).

Figure 3. Forest plot of hazard ratios for the univariable subgroup analysis and p-values of interaction analysis for aorto-iliac calcium score (CaScore) (continuous) and all-cause mortality.

Discriminative capacity of the aorto-iliac CaScore

The aorto-iliac CaScore alone had a C-statistic of 0.66 (95% CI 0.49–0.72) for all-cause mortality. When added to a basic model of gender and age, a significant improvement of the c-statistic (0.07, 95% CI 0.01–0.14, $p = 0.042$) and a positive and significant IDI (4.1%, $p < 0.0001$) were observed. Addition of the CaScore to the Charlson Comorbidity Index and the Framingham score, resulted in c-statistics of 0.75 (95% CI 0.67–0.83) and 0.76 (95% CI 0.69–0.83), respectively. The improvement in c-statistics were not significant, whereas the IDI's were positive and significant for the addition of the CaScore to the Charlson Comorbidity Index (IDI 4.2, $p < 0.0001$) and the Framingham score (IDI 2.5, $p < 0.001$) (Table S4). C-statistics of the CaScore alone for cardiovascular mortality and cardiovascular events were 0.72 (95% CI 0.63–0.81) and 0.58 (95% CI 0.52–0.64), respectively. Changes in c-statistics and IDI's for these outcome measures are presented in Table S4.

Table 4. Multivariable adjusted associations of CaScore with all-cause mortality, cardiovascular events, and cardiovascular mortality.

	Low	Medium	
	Hazard Ratio	Hazard Ratio	95% CI
All-cause mortality			
Univariate	1.0 (Ref)	2.12	1.08–4.15
Model 1	1.0 (Ref)	2.20	1.12–4.32
Model 2	1.0 (Ref)	1.61	0.80–3.25
Model 3	1.0 (Ref)	1.39	0.67–2.88
Model 4	1.0 (Ref)	1.28	0.61–2.70
Model 5	1.0 (Ref)	1.27	0.60–2.66
Model 6	1.0 (Ref)	1.50	0.64–3.51
Cardiovascular mortality			
Univariate	1.0 (Ref)	3.42	1.07–10.91
Model 1	1.0 (Ref)	3.45	1.08–11.05
Model 2	1.0 (Ref)	2.40	0.72–8.00
Model 3	1.0 (Ref)	1.91	0.56–6.59
Model 4	1.0 (Ref)	1.65	0.47–5.74
Model 5	1.0 (Ref)	1.64	0.46–5.76
Model 6	1.0 (Ref)	2.30	0.64–8.32
Cardiovascular events			
Univariate	1.0 (Ref)	2.13	1.19–3.84
Model 1	1.0 (Ref)	2.20	1.22–3.98
Model 2	1.0 (Ref)	1.88	1.00–3.53
Model 3	1.0 (Ref)	1.69	0.89–3.20
Model 4	1.0 (Ref)	1.48	0.76–2.88
Model 5	1.0 (Ref)	1.47	0.76–2.85
Model 6	1.0 (Ref)	1.47	0.76–2.87

Data are presented as hazard ratio and 95% confidence interval (CI) for the tertiles (low, medium, high) and the continuous (natural log transformed) CaScore outcome; *p*-values in bold indicate statistical significance. Model 1: adjusted for transplant centre and time between computed tomography and transplantation; Model 2: adjusted for model 1 plus age at time of transplantation, and gender; Model 3: adjusted for model 2 plus BMI, total cholesterol level, diabetes mellitus, smoking status, and systolic blood pressure;

High			Continuous		
Hazard Ratio	95% CI	p-value (trend)	Hazard Ratio	95% CI	p-value
5.22	2.84–9.58	<0.0001	2.02	1.59–2.56	<0.0001
5.45	2.94–10.07	<0.0001	2.02	1.60–2.56	<0.0001
3.40	1.69–6.85	<0.001	1.72	1.31–2.28	<0.001
3.31	1.60–6.87	<0.001	1.75	1.31–2.33	<0.001
2.84	1.34–6.00	0.002	1.59	1.19–2.14	0.002
2.59	1.22–5.51	0.006	1.53	1.14–2.06	0.005
3.12	1.34–7.26	0.006	1.72	1.22–2.43	0.002
7.86	2.63–23.47	<0.0001	2.27	1.55–3.34	<0.0001
7.69	2.55–23.14	<0.0001	2.24	1.53–3.29	<0.0001
4.66	1.38–15.74	0.009	1.94	1.24–3.04	0.004
4.32	1.22–15.38	0.014	1.92	1.19–3.09	0.007
4.40	1.19–16.24	0.013	2.03	1.20–3.42	0.008
4.46	1.19–16.71	0.014	2.04	1.20–3.45	0.008
55.67	1.68–8.32	0.004	2.13	1.32–3.43	0.002
2.86	1.62–5.08	<0.001	1.56	1.25–1.95	<0.0001
2.61	1.46–4.64	<0.001	1.60	1.28–1.99	<0.0001
2.45	1.25–4.81	0.010	1.48	1.13–1.93	0.004
2.58	1.20–4.77	0.013	1.48	1.12–1.94	0.005
2.13	1.05–4.35	0.034	1.39	1.04–1.85	0.025
1.98	0.97–4.07	0.060	1.35	1.01–1.80	0.042
2.17	1.08–4.37	0.028	1.45	1.09–1.92	0.010

Model 4: adjusted for model 3 plus type of dialysis (pre-emptive, haemodialysis, peritoneal dialysis), dialysis vintage pre-transplantation, and number of previous transplantations; Model 5: adjusted for model 4 plus Charlson Comorbidity Index; Model 6: adjusted for model 2 plus one year-post transplant variables (eGFR, calcium, phosphate, albumin, PTH).

Figure 4. Kaplan-Meier curve for (A) cardiovascular survival and (B) cardiovascular event-free survival for the low aorto-iliac CaScore tertile, the medium CaScore tertile, and the high CaScore tertile. The cardiovascular survival at the median follow-up time was 98.5% (95% CI 96.4–100.0) in the low, 96.3% (95% CI 92.8–99.9) in the medium, and 86.4% (95% CI 79.5–94.0) in the high CaScore tertile. The cardiovascular event-free survival at the median follow-up time was 89.3% (95% CI 84.6–94.4) in the low, 81.0% (95% CI 74.2–88.5) in the medium, and 75.2% (95% CI 67.0–85.5) in the high CaScore tertile.

DISCUSSION

Non-contrast enhanced CT-based quantification of the aorto-iliac vessels during screening prior to kidney transplantation, as an adapted Agatston score, is a novel approach in transplant medicine. In the kidney transplant recipient cohort presented, the aorto-iliac CaScore showed to be associated with all-cause and cardiovascular mortality, and cardiovascular events after adjustment for traditional and transplant candidate-specific risk factors. The identified association was consistent in subgroup and interaction analyses, and the CaScore showed to have a significant discriminative capacity for all-cause and cardiovascular mortality.

The baseline differences, as compared for the aorto-iliac CaScore tertiles, are in line with previous studies on cardiovascular risk stratification prior to kidney transplantation.² The traditional risk factors, i.e., male gender, age, BMI, current or former smoker, hypercholesterolemia, and hypertension, and the transplant candidate-specific risk factor dialysis vintage pre-transplantation, were more prevalent in the medium and high CaScore tertiles. To note, there were no differences between the tertiles regarding diabetes mellitus prior to transplantation. The relatively high Charlson Comorbidity Index pointed towards more comorbidities and the Framingham score showed more expected cardiovascular events in the medium and high CaScore tertiles. The incidence of cardiovascular events was high ($n = 84$, 15.5%) compared to previously published outcomes of kidney transplant recipients in the Netherlands (8.5–11%, in 5.4 and 7 years, respectively), which was expected since the indication for a pre-transplant CT-scan was defined by cardiovascular risk factors.^{12,32}

To establish the clinical relevance of the aorto-iliac CaScore, a comparison was made with clinical variables available at the time of screening prior to transplantation. The discriminative capacity of the Charlson Comorbidity Index and Framingham score for all-cause and cardiovascular mortality significantly increased when the CaScore was added. The c-statistics and IDI's of the CaScore for cardiovascular events were considerably lower compared to those for all-cause and cardiovascular mortality.

The quantification technique for vascular calcification is considered to be reproducible and reliable, with an interobserver agreement of ≥ 0.99 and an inter-scanner agreement of ≥ 0.97 .^{33,34} In this study we focused on the total calcium volume in the aorto-iliac trajectory; the advantages of this approach are the quantification after non-contrast enhanced CT procedures and the use of CT procedures with varying slice-thicknesses.²⁷ In the general population, application of this technique resulted

in a significant difference in five-year cardiovascular event-free survival between the quartiles of abdominal aorta calcification, outperforming the Framingham score (area under the curve of 0.76 versus 0.64, $p = 0.013$).²⁸

Two relatively small studies focused on non-contrast enhanced aorto-iliac CT prior to kidney transplantation, the first ($n = 131$) with a 14-point visual grading scale and the second with quartiles of calcification, demonstrating correlations with surgical complexity and cardiovascular surgery during follow-up.^{23,24} In contrast to the independent association identified in the current study, the two earlier studies did not find significant associations with all-cause mortality analyses, in cohorts with 21 and four events, respectively. The results of a more recent study, focusing on aorto-iliac stenosis on contrast-enhanced CT prior to transplantation and all-cause mortality, were in line with our findings for all-cause mortality.²²

The literature on abdominal aorta calcification (AAC) assessment by plain pelvic X-ray or dual X-ray absorptiometry, using visual grading scales, is more extensive.¹³ Studies evaluating this method showed results similar to our findings, with significant associations of a high-AAC with all-cause mortality and cardiovascular events in multivariable Cox regression analyses.^{12,20,21} Timing of the imaging procedure in previous studies is an important difference compared with the current study, as imaging procedures were performed after transplantation and at the physician's discretion, instead of CT procedures during the screening prior to transplantation.

For CAC prior to and after kidney transplantation, identified by non-contrast CT, several studies have shown the association with cardiovascular events and mortality.^{17,18} The first prospective study showing the association of CAC after transplantation and cardiovascular events is from Nguyen et al., with a mean follow-up of 2.3 years and a total of 31 events.¹⁷ In a small study ($n = 112$) with asymptomatic transplant recipients, Roe et al. showed that laboratory markers of inflammation are associated with the severity of CAC.¹⁸

To our knowledge, this is the largest kidney transplant recipient cohort with CT imaging prior to transplantation available for re-evaluation. Strengths of this study include the median follow-up of 3.1 years, the representative case-mix of pre-emptive (36.0%) and dialysis-dependent (64.0%) patients, and the relatively large number of outcome measures studied for all-cause mortality, cardiovascular mortality, and cardiovascular events, compared to earlier studies. Additionally, the quantification technique for vascular calcification is considered to be reproducible and reliable, with an interobserver agreement of ≥ 0.99 and an inter-scanner agreement of ≥ 0.97 .³³

For the number of included variables in the multivariable analyses, we followed the recommendation by Vittinghoff and McCulloch of 5–9 events per predictor variable.³⁵ This recommendation could be followed for the outcome measures all-cause mortality and cardiovascular events; however, there is a risk for overfitting in model 4 and model 5 with the outcome measure cardiovascular mortality. The main limitation of this study is the selection of patients based on the availability of CT images prior to transplantation, resulting in a cohort of patients with a relatively high age at transplantation (median 60 years), more comorbidities (mean Charlson Comorbidity Index of 6.4), and a subsequent higher mortality risk. While the Charlson Comorbidity Index was high compared to a previously published Dutch kidney transplant cohort, with a mean of 2.8 (1.0), the median Framingham score of 9.6 (3.4, 18.8) was comparable to earlier studies.^{8,36} The in- and exclusion criteria limit conclusions regarding the assessment of patient's suitability for transplantation, as the results of this study cannot be generalized to the overall transplant candidate population. As the tertiles are based on equal-size groups, the reported CaScore ranges per tertile are highly population dependent. External validation of these CaScore tertile ranges is therefore required.

CONCLUSIONS

In conclusion, in this dual-centre kidney transplant recipient cohort, we demonstrated an independent association of the aorto-iliac CaScore, extracted from commonly performed non-contrast enhanced CT procedures prior to transplantation, with all-cause mortality, cardiovascular mortality, and cardiovascular events. These findings can improve the identification of patients at risk for (cardiovascular) death and those who could potentially benefit from stringent cardiovascular monitoring to improve their prognosis after transplantation.

REFERENCES

1. Tonelli M, Wiebe N, Knoll G, et al. Systematic Review: Kidney Transplantation Compared With Dialysis in Clinically Relevant Outcomes. *Am J Transplant*. 2011;11(10):2093-2109.
2. Rangaswami J, Mathew RO, Parasuraman R, et al. Cardiovascular disease in the kidney transplant recipient: epidemiology, diagnosis and management strategies. *Nephrol Dial Transplant*. 2019;34(5):760-773.
3. Harhay MN, Rao MK, Woodside KJ, et al. An overview of frailty in kidney transplantation: measurement, management and future considerations. *Nephrol Dial Transplant*. Published online March 19, 2020.
4. National Institute of Health; National Institute of Diabetes and Digestive and Kidney Diseases. *United States Renal Data System 2017 USRDS Annual Data Report: Epidemiology of Kidney Disease in the United States*; 2017.
5. Hart A, Weir MR, Kasiske BL. Cardiovascular risk assessment in kidney transplantation. *Kidney Int*. 2015;87(3):527-534.
6. Kulu Y, Fathi P, Golriz M, et al. Impact of Surgeon's Experience on Vascular and Haemorrhagic Complications After Kidney Transplantation. *Eur J Vasc Endovasc Surg*. 2019;57(1):139-149.
7. Matia I, Adamec M, Varga M, Janousek L, Lipar K, Viklicky O. Aortoiliac Reconstruction with Allograft and Kidney Transplantation as a One-stage Procedure: Long Term Results. *Eur J Vasc Endovasc Surg*. 2008;35(3):353-357.
8. Silver SA, Huang M, Nash MM, Prasad GVR. Framingham Risk Score and Novel Cardiovascular Risk Factors Underpredict Major Adverse Cardiac Events in Kidney Transplant Recipients. *Transplantation*. 2011;92(2):183-189.
9. Sotomayor CG, Benjamens S, Gomes-Neto AW, et al. Bone Mineral Density and Aortic Calcification: Evidence for a Bone-Vascular Axis After Kidney Transplantation. *Transplantation*. Published online March 2020:1.
10. Manoushagian S, Meshkov A. Evaluation of Solid Organ Transplant Candidates for Coronary Artery Disease. *Am J Transplant*. 2014;14(10):2228-2234.
11. Keyzer CA, de Borst MH, van den Berg E, et al. Calcification Propensity and Survival among Renal Transplant Recipients. *J Am Soc Nephrol*. 2016;27(1):239-248.
12. Benjamens S, Pol RA, Glaudemans AWJM, et al. A high abdominal aortic calcification score by dual X-ray absorptiometry is associated with cardiovascular events after kidney transplantation. *Nephrol Dial Transplant*. 2018;33(12):2253-2259.
13. Rijkse E, Dam JL, Roodnat JJ, Kimenai HJAN, IJzermans JNM, Minnee RC. The prognosis of kidney transplant recipients with aorto-iliac calcification: a systematic review and meta-analysis. *Transpl Int*. 2020;33(5):483-496.
14. Maréchal C, Coche E, Goffin E, et al. Progression of Coronary Artery Calcification and Thoracic Aorta Calcification in Kidney Transplant Recipients. *Am J Kidney Dis*. 2012;59(2):258-269.
15. Schankel K, Robinson J, Bloom RD, et al. Determinants of coronary artery calcification progression in renal transplant recipients. *Am J Transplant*. 2007;7(9):2158-2164.
16. DeLoach SS, Joffe MM, Mai X, Goral S, Rosas SE. Aortic calcification predicts cardiovascular events and all-cause mortality in renal transplantation. *Nephrol Dial Transpl*. 2009;24(4):1314-1319.
17. Nguyen PT, Henrard S, Coche E, Goffin E, Devuyt O, Jadoul M. Coronary artery calcification: a strong predictor of cardiovascular events in renal transplant

- recipients. *Nephrol Dial Transplant*. 2010;25(11):3773-3778.
18. Roe P, Wolfe M, Joffe M, Rosas SE. Inflammation, coronary artery calcification and cardiovascular events in incident renal transplant recipients. *Atherosclerosis*. 2010;212(2):589-594.
19. Aalten J, Dekker HM, van der Vliet JA, Hoitsma AJ. Does a plain X-ray of the pelvis predict arterial complications in renal transplantation? A prospective study. *Nephrol Dial Transplant*. 2011;26(6):2007-2012.
20. Lewis JR, Wong G, Taverniti A, Vucak-Dzumhur M, Elder GJ. Association between Aortic Calcification, Cardiovascular Events, and Mortality in Kidney and Pancreas-Kidney Transplant Recipients. *Am J Nephrol*. 2019;50(3):177-186.
21. Claes KJ, Heye S, Bammens B, et al. Aortic calcifications and arterial stiffness as predictors of cardiovascular events in incident renal transplant recipients. *Transpl Int*. 2013;26(10):973-981.
22. Rijkse E, Kimenai HJAN, Roodnat JJ, et al. Impact of Aortoiliac Stenosis on Graft and Patient Survival in Kidney Transplant Recipients Using the TASC II Classification. *Transplantation*. 2019;103(10):2164-2172.
23. Davis B, Marin D, Hurwitz LM, et al. Application of a Novel CT-Based Iliac Artery Calcification Scoring System for Predicting Renal Transplant Outcomes. *Am J Roentgenol*. 2016;206(2):436-441.
24. Chavent B, Maillard N, Boutet C, Albertini J-N, Duprey A, Favre J-P. Prognostic Value of Aortoiliac Calcification Score in Kidney Transplantation Recipients. *Ann Vasc Surg*. 2017;44:245-252.
25. Chadban SJ, Ahn C, Axelrod DA, et al. Summary of the Kidney Disease: Improving Global Outcomes (KDIGO) Clinical Practice Guideline on the Evaluation and Management of Candidates for Kidney Transplantation. *Transplantation*. 2020;104(4):708-714.
26. Benjamens S, Glaudemans AWJM, Berger SP, Slart RHJA, Pol RA. Have we forgotten imaging prior to and after kidney transplantation? *Eur Radiol*. 2018;28(8):3263-3267.
27. Saboo SS, Abbara S, Rybicki FJ, Chatzizisis YS. Quantification of aortic calcification – How and why should we do it? *Atherosclerosis*. 2015;240(2):469-471.
28. O'Connor SD, Graffy PM, Zea R, Pickhardt PJ. Does Nonenhanced CT-based Quantification of Abdominal Aortic Calcification Outperform the Framingham Risk Score in Predicting Cardiovascular Events in Asymptomatic Adults? *Radiology*. 2019;290(1):108-115.
29. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: Development and validation. *J Chronic Dis*. 1987;40(5):373-383.
30. Ramos LF, Shintani A, Ikizler TA, Himmelfarb J. Oxidative Stress and Inflammation Are Associated with Adiposity in Moderate to Severe CKD. *J Am Soc Nephrol*. 2008;19(3):593-599.
31. Hayashi K, Eguchi S. The power-integrated discriminant improvement: An accurate measure of the incremental predictive value of additional biomarkers. *Stat Med*. 2019;38(14):2589-2604.
32. van Dijk M, van Roon AM, Said MY, et al. Long-term cardiovascular outcome of renal transplant recipients after early conversion to everolimus compared to calcineurin inhibition: results from the randomized controlled MECANO trial. *Transpl Int*. 2018;31(12):1380-1390.
33. Ghadri JR, Goetti R, Fiechter M, et al. Inter-scan variability of coronary artery calcium scoring assessed on 64-multidetector computed tomography vs. dual-source computed tomography: a head-to-head comparison. *Eur Heart J*. 2011;32(15):1865-1874.

34. Mori S, Takaya T, Kinugasa M, et al. Three-dimensional quantification and visualization of aortic calcification by multidetector-row computed tomography: A simple approach using a volume-rendering method. *Atherosclerosis*. 2015;239(2):622-628.
35. Vittinghoff E, McCulloch CE. Relaxing the Rule of Ten Events per Variable in Logistic and Cox Regression. *Am J Epidemiol*. 2007;165(6):710-718.
36. Laging M, Kal-van Gestel JA, van de Wetering J, et al. A High Comorbidity Score Should Not be a Contraindication for Kidney Transplantation. *Transplantation*. 2016;100(2):400-406.

Aorto-iliac artery calcification and graft outcomes in kidney transplant recipients

Journal of Clinical Medicine, 2021

Stan Benjamens
Saleh Z. Alghamdi
Elsaline Rijkse
Charlotte A. te Velde-Keyzer
Stefan P. Berger
Cyril Moers
Martin H. de Borst
Riemer H.J.A Slart
Frank J.M.F. Dor
Robert C. Minnee
Robert A. Pol

Background: While the association of vascular calcification with inferior patient outcomes in kidney transplant recipients is well-established, the association with graft outcomes has received less attention. With this study we aimed to determine the clinical impact of recipient pre-transplant aorto-iliac calcification and the association with kidney graft outcomes.

Methods: We performed a dual-centre cohort study in kidney transplant recipients who underwent screening with non-contrast enhanced CT-imaging within three years prior to transplantation (2005-2018). Aorto-iliac calcification was quantified by using the adjusted Agatston score (aorto-iliac CaScore). The association of the CaScore with estimated-Glomerular Filtration Rate (eGFR) at one-year after transplantation, death-censored graft failure, overall graft failure (including death), death with a functioning graft and graft function decline (doubling of serum creatinine or graft failure) were tested in linear and Cox regression analyses.

Results: We included 547 patients (61.4% male, age 60 [interquartile range 51-68] years), with a median follow-up of 3.1 [1.4-5.2] years after transplantation. The aorto-iliac calcification score (CaScore) was inversely associated with one-year eGFR in univariate linear regression analysis (standard β -3.3 (95% CI -5.1 to -1.5, $p < 0.0001$), but not after adjustment for potential confounders, including donor and recipient age ($p = 0.077$). In multivariable Cox regression analyses, a high CaScore was associated with overall graft failure ($p = 0.004$) and death with a functioning graft ($p = 0.002$), but not with death-censored graft failure and graft function decline.

Conclusions: This study demonstrated that pre-transplant aorto-iliac calcification is associated with one-year eGFR in univariate, but not in multivariable linear regression analyses. Moreover, this study underlines that transplantation in patients with a high CaScore does not result in earlier transplant function decline or worse death censored graft survival, although ongoing efforts for the prevention of death with a functioning graft remain essential.

INTRODUCTION

Atherosclerosis is an important pathophysiological process responsible for an increased disease burden in kidney transplant candidates and recipients, and is associated with decreased overall survival and increased cardiovascular morbidity and mortality.¹⁻³ Currently, there is limited published data on the effect of recipient pre-transplant aorto-iliac calcification on kidney graft outcomes.

Kidney graft outcomes can be divided in short-term outcomes, such as early failure or one-year graft function, and long-term outcomes, such as (death-censored) graft failure and graft function decline.⁴ Death-censored graft failure, as return to dialysis or retransplantation, is seen in 10-12.5% of kidney transplant recipients at 5-6.2 years after transplantation.^{5,6} Overall graft failure, including death with a functioning graft, is seen at a rate of 5% each year of follow-up, of which 40-60% is attributed to death with a functioning graft.⁷⁻¹¹ Graft function decline, as doubling of serum creatinine or graft failure, is seen in 18.8% of kidney transplant recipients at 5.4 years after transplantation.¹² Short and long-term graft outcomes are strongly associated and both closely linked to patient-specific and transplantation-related risk factors.¹³⁻¹⁶

A recent meta-analysis on pre-transplant aorto-iliac calcification found an association with decreased overall survival and overall graft failure. Statistical significance was lacking for the association with death-censored graft failure and graft function. This lack of statistical power with regard to kidney graft outcomes can be attributed to the limited number of studies available, the various methods used for identification of calcification and the small sample sizes of the existing publications.¹⁷ Hence, studies including higher numbers of transplant recipients, focusing on various kidney graft outcomes and applying reliable methods of calcifications assessment, are deemed pivotal.

We previously reported that the aorto-iliac CaScore – the adjusted Agatston score, was independently associated with patient outcomes, e.g., risk of early (cardiovascular) death and cardiovascular events.¹

In the present study, we aimed to determine whether the aorto-iliac CaScore, measured as an adjusted Agatston score on pre-transplant CT-imaging, is associated with kidney graft function at one-year after transplantation, death-censored graft failure, overall graft failure (including death), death with a function graft, and graft function decline.

METHODS

Study design

We performed a dual-centre cohort study in The Netherlands (University Medical Center Groningen (UMCG) and Erasmus University Medical Center (Erasmus MC)), in adult kidney transplant recipients. All patients who underwent screening prior to transplantation by means of a non-contrast enhanced CT between January 2005 and December 2018 were included. CT procedures were performed according to the pre-transplant screening protocol in both transplant centres; selecting patients based on age (>50 years); comorbidities (diabetes mellitus and peripheral artery disease) and dialysis vintage (>2 years). Exclusion criteria were age < 18 years at the time of transplantation and an interval of more than three years between CT and transplantation. In the inclusion period, both transplant centres participated in the Eurotransplant Senior Program (ESP), a Eurotransplant allocation scheme matching deceased donors above 65 years-of-age to recipients in a similar age range.¹⁸ The primary outcome measure of this study was estimated-glomerular filtration rate (eGFR) at one-year after transplantation. Secondary outcome measures were death-censored graft failure, defined as return to dialysis or retransplantation (censoring at time of death), overall graft failure (including death with a functioning graft), death with a functioning graft, and graft function decline, defined as doubling in serum creatinine or graft failure (censoring at time of death). This study was approved by the institutional review board of the UMCG (2017/523) and performed in line with the *Declaration of Helsinki* and the *Declaration of Istanbul on Organ Trafficking and Transplant Tourism*.

Computed tomography assessment of aorto-iliac calcification

The applied method for quantifying aorto-iliac calcification, as the Agatston score adjusted for the aorto-iliac trajectory (aorto-iliac CaScore) for non-enhanced CT images, has been described previously.¹ In short, CaScore (syngo.CT CaScoring software, Siemens Healthineers, Erlangen, Germany) was quantified for the abdominal aorta below the origin of the renal arteries, the common iliac artery, and the external iliac artery for the side of the transplant anastomosis. The standard calcification threshold of 130 Hounsfield Units (HU) was used.

Clinical variables

Details of this cohort, including baseline cardiovascular risk factors, with history and follow-up of cardiovascular disease, have been published previously.¹ The recipient variables included in the present analyses were gender, age, pre-transplant diabetes mellitus (fasting glucose ≥ 7.0 mmol/L, casual plasma glucose ≥ 11.1 mmol/L + diabetes symptoms, or glucose tolerance test with 2-hours plasma glucose ≥ 11.1 mmol/L), Body Mass

Index (BMI in kg/m²), smoking status (non, former, or current), hypercholesterolemia (total cholesterol level > 5.2 mmol/L or use of lipid lower medication), total cholesterol level (mmol/L), systolic blood pressure (mmHg), use of antihypertensive medication, dialysis status (pre-emptive, haemodialysis, or peritoneal dialysis), dialysis vintage (months), and number of previous kidney transplantations. The transplant variables included the type of donation (living-donation, donation after circulatory death (DCD), or donation after brain death (DBD)), donor gender and age, number of total HLA-mismatches, warm ischemia time (minutes), and cold ischemia time (minute). Follow-up variables included eGFR at six-months and one-year, calculated with the CKD-EPI equation (mL/min per 1.73 m²), laboratory values at one-year after transplantation (serum haemoglobin, calcium, phosphate, albumin, glucose, parathyroid hormone (PTH), and urinary protein excretion), and primary cytomegalovirus (CMV) infection.¹⁹ Short-term outcomes were early graft failure (death-censored), defined as return to dialysis in 30-days after transplantation, delayed graft function, defined as dialysis requirement in the first week after transplantation, acute rejection, defined as biopsy-proven acute rejection (BPAR) or an acute rejection treatment episode without BPAR (non-BPAR, Banff 2015 criteria).^{4,20}

Statistical analyses

For baseline characteristics, patients were stratified into two groups based on the aorto-iliac CaScore, being a low CaScore (0 – 5600 HU) and a high CaScore (> 5600 HU). These cut-off values are based on the initial aorto-iliac CaScore analyses.¹ Data were expressed as mean (standard deviation, SD) for normally distributed variables, as median [interquartile range, IQR] for variables with non-normal distribution, and as number (percentage, %) for categorical variables. For regression analyses, non-normally distributed variables were transformed to the natural log (ln(x + 1)). Results of linear regression were presented as standardized Beta coefficients (Standard β) and in proportional hazards regression analysis as hazard ratios (HRs), both with 95% confidence intervals (95%CI). The reversed Kaplan-Meier method was used to calculate the median [IQR] follow-up, considering the date of transplantation as the start of follow-up.²¹ In all analyses, a p-value < 0.05 was considered significant.

(Death-censored) graft failure-free and graft function decline-free were calculated using Kaplan-Meier survival curves. The low and high aorto-iliac CaScores were compared for living and deceased-donor kidney transplant recipients with Log-rank testing. Cox proportional hazards regression analysis, for living and deceased-donor kidney transplant recipients, was used to establish the association of the high aorto-iliac CaScore with (death-censored) graft failure, death with a functioning graft and graft function decline. Linear regression was used to establish the association of the aorto-

iliac CaScore (continuous) with one-year eGFR. Models were built with *a priori* selected covariables. In Model 1, we adjusted for transplant centre (UMCG or Erasmus MC) and time between CT and transplantation. In model 2, we adjusted for model 1 plus donor gender, cold ischemia time, number of HLA mismatches, recipient gender, donor type (living donation, DCD or DBD), diabetes mellitus, smoking, dialysis vintage, number of previous transplantations. In model 3, we adjusted for model 2 plus recipient age and in model 4 for model 3 plus donor age. For linear regression, laboratory results at one year after transplantation (serum phosphate, serum calcium, serum glucose, serum haemoglobin, proteinuria) and an episode of acute rejection were included as additional variables in model 2. Analysis of interaction terms for the association of the aorto-iliac CaScore (continuous) with one-year eGFR by the covariables recipient age, gender, diabetes, donor age, gender, and donor type, was performed. To quantify the magnitude of confounding by donor age on the association between the aorto-iliac CaScore and one-year eGFR, we used the regression methodology as proposed by Janes et al.²²

Statistical analyses were performed with R: A Language and Environment for Statistical Computing, version 1.0.153 for Mac (R Foundation for Statistical Computing, Vienna, Austria), using the software R-Packages “MASS”, “mediation”, “survival”, “ggplot2”, and “survminer”.

RESULTS

Characteristics and short-term graft outcomes

A total of 547 patients were included, of which 446 in the UMCG and 101 in the Erasmus MC (61.4% male, age 60 [51, 68] years). The mean time from CT to transplantation was 0.91 (0.72) years and the median follow-up was 3.1 [1.4, 5.2] years after transplantation. Baseline patient characteristics for the total cohort and CaScore groups are shown in Table 1. In the high compared to the low CaScore group, transplant recipients were older (66 [60, 71] versus 55 [47, 64]) (Figure S1), patients had a higher donor age (59 (12) versus 52 (14) years), there were fewer living-donor (47.3% versus 58.9%), and more DCD (29.1% versus 20.5%) and DBD (23.6% versus 20.5%) transplantation procedures. In the first year of follow-up, laboratory values ($n = 462$) of the CaScore groups differed for eGFR at six-months and one-year, calcium, phosphate, glucose, PTH, and urinary protein excretion (Table 1). For short-term graft outcomes, more early graft failure (death-censored) events were observed in the high compared to the low CaScore group ($n = 9$ (4.9) versus $n = 5$ (1.4), $p = 0.027$), but no differences in delayed graft function or acute rejections (Table 2). In nine out of the 14 patients with early graft failure, it was suspected that graft failure was due to a vascular cause. In the supplementary material of the original manuscript, patient characteristics are presented for living- and deceased donation separately.

Aorto-iliac CaScore and one-year kidney transplant function

Mean eGFR at one-year after transplantation ($n = 462$) was 51 (21), with 48 (21) in the high and 53 (20) in the low CaScore group. The CaScore (continuous) was inversely associated with one-year eGFR, with a standard β -3.5 (95% CI -5.3 to -1.7, $p < 0.0001$) when adjusting for transplant centre and time CT to transplantation (model 1). When adjusting for patient, transplantation and follow-up covariables, with the exception of donor age, this inverse association remained significant (model 2, standard β -4.1 (95% CI -4.9 to -2.4, $p < 0.0001$), & model 3 (including recipient age), standard β -2.7 (95% CI -4.6 to -0.7, $p = 0.008$)). This association lost statistical significance after adjustment for donor age (model 4, standard β -1.7 (95% CI -3.6 to 0.2, $p = 0.077$)) (Table 3). There was a significant interaction for the univariable association of CaScore with one-year eGFR by donor age ($p < 0.0001$), but not for the covariables recipient age, gender, diabetes, donor gender, and donor type ($p > 0.05$). When quantifying the magnitude of confounding, the direct effect of the aorto-iliac CaScore on one-year eGFR was -3.3 (95% CI -5.1 to -1.5) and the effect on the confounder (donor age) was 5.0 (95% CI 3.8 to 6.2). The direct effect of the confounder on one-year eGFR was -0.6 (95% CI -0.7 to -0.5), with a bootstrapped indirect effect of -2.9 (95% CI -4.0 to -1.9). Donor age was identified as a confounder of the association between the aorto-iliac CaScore and one-year eGFR, resulting in an 87.2%

of the association (Figure 1). In the supplementary material of the original manuscript, the linear regression for one-year eGFR is presented for living- and deceased donation separately.

Table 1. Characteristics

Variables	Total (n = 547)	Low (n = 365)	High (n = 182)	p-value
Patient				
Male gender, <i>n</i> (%) ^a	336 (61.4)	205 (56.2)	131 (72.0)	<0.001^b
Age, <i>years</i> ^c	60 [51, 68]	55 [47, 64]	66 [60, 71]	<0.001^d
Diabetes mellitus, <i>n</i> (%) ^a	173 (31.6)	117 (32.1)	56 (30.8)	0.836 ^b
Body Mass Index, <i>kg/m</i> ² ^b	26.8 (4.7)	26.9 (4.9)	26.7 (4.1)	0.670 ^c
Smoker, <i>n</i> (%) ^a				0.004^b
Non	154 (28.2)	119 (32.6)	35 (19.2)	
Former	332 (60.7)	206 (56.4)	126 (69.2)	
Current	61 (11.2)	40 (11.0)	21 (11.5)	
Hypercholesterolemia, <i>n</i> (%) ^a	153 (28.0)	96 (26.3)	57 (31.3)	0.258 ^b
Total cholesterol, <i>mmol/L</i>	4.7 (1.3)	4.8 (1.5)	4.5 (1.2)	0.031^c
Systolic blood pressure, <i>mmHg</i>	145 (22)	143 (21)	148 (24)	0.014^c
Use of antihypertensive medication, <i>n</i> (%)	439 (80.3)	282 (77.3)	157 (86.3)	0.017^b
Type of dialysis, <i>n</i> (%) ^a				0.109 ^b
Pre-emptive	197 (36.0)	142 (38.9)	55 (30.2)	
Haemodialysis	250 (45.7)	162 (44.4)	88 (48.4)	
Peritoneal dialysis	100 (18.3)	61 (16.7)	39 (21.4)	
Dialysis vintage, <i>months</i> ^c	12 [0, 28]	11 [0, 25]	16 [0, 33]	0.012^d
Previous transplants, <i>n</i> (%) ^a				0.872 ^b
Non	514 (94.0)	343 (94.0)	171 (94.0)	
One	22 (4.0)	14 (3.8)	8 (4.4)	
Two	11 (2.0)	8 (2.2)	3 (1.6)	
Aorto-iliac CaScore, <i>HU</i>	2994 [294, 7888]	856.20 [46, 2994]	9732 [7918, 14315]	<0.001^d
Transplantation				
Type of donation, <i>n</i> (%) ^a				0.025^b
Living-donation	301 (55.0)	215 (58.9)	86 (47.3)	
Donation after circulatory death	128 (23.4)	75 (20.5)	53 (29.1)	
Donation after brain death	118 (21.6)	75 (20.5)	43 (23.6)	
Male gender donor, <i>n</i> (%)	253 (46.3)	160 (43.8)	93 (51.1)	0.130 ^b
Donor age, <i>years</i>	54 (14)	52 (14)	59 (12)	<0.001^c
No. of HLA-mismatches, <i>n</i>	3.5 (1.5)	3.4 (1.5)	3.6 (1.4)	0.127 ^c

Table 1. Continued

Variables	Total (n = 547)	Low (n = 365)	High (n = 182)	p-value
Warm ischemia time, <i>minutes</i>	42 (36)	42 (44)	41 (13)	0.676 ^c
Cold ischemia time, <i>minutes</i>	433 (340)	415 (343)	470 (331)	0.076 ^c
Follow-up^e				
eGFR at six-months ^a	50 (18)	51 (19)	46 (17)	0.004^c
eGFR at one-year ^a	51 (21)	53 (20)	48 (21)	0.007^c
Haemoglobin at one-year, <i>mmol/L</i>	8.3 (3.8)	8.3 (4.6)	8.2 (1.1)	0.811 ^c
Calcium at one-year, <i>mmol/L</i>	2.43 (0.14)	2.43 (0.14)	2.45 (0.14)	0.034^c
Phosphate at one year, <i>mmol/L</i>	0.93 (0.21)	0.95 (0.21)	0.89 (0.19)	0.004^c
Albumin at one-year, <i>g/L</i>	43 (3)	43 (3)	43 (3)	0.234 ^c
Glucose at one-year, <i>mmol/L</i>	5.9 [5.1, 7.3]	5.7 [5.1, 7.2]	6.2 [5.4, 7.6]	0.005^d
PTH at one-year, <i>pmol/L</i>	10 [7, 16]	10 [7, 15]	12 [8, 16]	0.064^d
Protein excretion at one-year, <i>g/24h</i>	0.2 [0.1, 0.3]	0.1 [0.1, 0.2]	0.2 [0.1, 0.3]	0.002^d
Cytomegalovirus infection, <i>n (%)</i>	29 (5.3)	17 (4.7)	12 (6.6)	0.453 ^b

eGFR = estimated-Glomerular Filtration Rate; PTH = parathyroid hormone; ^a eGFR – CKD-EPI (mL/min per 1.73 m²); ^b p-value by chi-square test; ^c p-value by student-t test; ^d p-value by Mann-Whitney U test; ^e data available for 462 patients

Figure 1. Graphical illustration of the magnitude of confounding for one-year estimated-Glomerular Filtration Rate (eGFR), showing the regression coefficients for the associations (***) indicating a p-value < 0.0001). The direct effect of the aorto-iliac CaScore on one-year eGFR was -3.3 (95%CI -5.1 to -1.5) and the effect on the confounder (donor age) was 5.0 (95%CI 3.8 to 6.2). The direct effect of the confounder on one-year eGFR was -0.6 (95%CI -0.7 to -0.5), with a bootstrapped indirect effect of -2.9 (95%CI -4.0 to -1.9).

Table 2. Short and long-term graft outcomes (numbers, %)

	Total (n = 547)	Low (n = 365)	High (n = 182)	p-value
Median follow-up, years	3.1 (1.4, 5.2]	3.2 (1.5, 5.6]	2.7 (1.2, 4.2]	-
Short-term graft outcomes				
Early graft failure (death-censored)	14 (2.6)	5 (1.4)	9 (4.9)	0.027
Delayed graft function	155 (28.3)	100 (27.4)	55 (30.2)	0.555
Acute rejection (first-year)	70 (12.8)	47 (12.9)	23 (12.6)	1.000
Long-term graft outcomes				
Death-censored graft failure	64 (11.7)	41 (11.2)	23 (12.6)	0.734
Overall graft failure	118 (21.6)	64 (17.5)	54 (29.7)	0.002
Death with a functioning graft	54 (9.9)	23 (6.3)	31 (17.0)	<0.001
Graft function decline	81 (14.8)	51 (14.0)	30 (16.5)	0.515

Median follow-up after transplantation (interquartile range]; number of short-term and long-term graft outcomes (%); p-value by chi-square test

Table 3. Multivariable adjusted associations of the CaScore with one-year eGFR

	Standard β	95% CI	p-value
One-year eGFR			
Univariate	-3.3	-5.1 to -1.5	<0.0001
Model 1	-3.5	-5.3 to -1.7	<0.0001
Model 2	-4.1	-5.9 to -2.4	<0.0001
Model 3	-2.7	-4.6 to -0.7	0.008
Model 4	-1.7	-3.6 to 0.2	0.077

Linear regression analysis, data available for 462 patients; data are presented as hazard ratio and 95% confidence interval (CI) for the continuous CaScore (natural log transformed); model 1: adjusted for transplant centre and time between computed tomography and transplantation; model 2: adjusted for model 1 plus donor gender, donor type (living donation, DCD or DBD), cold ischemia time, no. of HLA mismatches, recipient gender, diabetes mellitus, smoking, dialysis vintage, no. of previous transplantations, statin use, laboratory results at one year after transplantation (serum phosphate, serum calcium, serum glucose, serum haemoglobin, proteinuria), and episode of acute rejection; model 3: adjusted for model 2 plus recipient age; model 4: adjusted for model 3 plus donor age

Aorto-iliac CaScore and death-censored graft failure

A total of 64 (11.7%) events of death-censored graft failure occurred, with 23 (12.6%) in the high and 41 (11.2%) in the low CaScore group (Table 2). Kaplan-Meier survival analysis for death-censored graft failure, with the low and high CaScore groups stratified by living and deceased donor kidney transplantation, showed earlier graft failure for the deceased donor – high CaScore group ($p < 0.001$) (Figure 2). Kaplan-Meier survival analysis stratified by transplant recipient age, below or above 65 years-of-age, did not show significant differences ($p = 0.078$, Figure S2). In Cox regression analysis, no significant associations were observed for a high CaScore and death-censored graft failure (model 4, HR 1.1 (95% CI 0.6 to 2.0, $p = 0.711$)) (Table 4). No differences were observed when stratifying for living- and deceased donation.

Aorto-iliac CaScore and overall graft failure

A total of 118 (21.6%) events of overall graft failure occurred, with 54 (29.7%) in the high and 64 (17.5%) in the low CaScore group (Table 2). Kaplan-Meier survival analysis for overall graft failure, with the low and high CaScore group stratified by living and deceased donor kidney transplantation, showed earlier graft failure for the living and deceased donor – high CaScore groups ($p < 0.0001$) (Figure 2). Kaplan-Meier survival analysis stratified by transplant recipient age showed, a lower survival for the recipients ≥ 65 years-of-age – high CaScore group ($p < 0.0001$) (Figure S2). In Cox regression analysis, a significant association was observed for a high CaScore and graft failure after adjusting for patient and transplantation covariables, including recipient and donor age (model 4, HR 1.9 (95% CI 1.2 to 2.9, $p = 0.006$)) (Table 4). When stratifying for donor status, the association remained significant for deceased donation (model 4, $p = 0.048$) and for living-donation (model 4, $p = 0.0036$).

Aorto-iliac CaScore and death with a functioning graft

A total of 54 (9.9%) events of death with a functioning graft occurred, with 31 (17.0%) in the high and 23 (6.3%) in the low CaScore group (Table 2). Kaplan-Meier survival analysis for death with a functioning graft, with the low and high CaScore group stratified by living and deceased donor kidney transplantation, showed earlier graft failure for the living and deceased donor – high CaScore groups ($p < 0.0001$) (Figure 3). Kaplan-Meier survival analysis stratified by transplant recipient age showed, a lower survival for the recipients ≥ 65 years-of-age – high CaScore group ($p < 0.0001$) (Figure S3). In Cox regression analysis, a significant association was observed for a high CaScore and death with a functioning graft after adjusting for patient and transplantation covariables, including recipient and donor age (model 4, HR 2.7 (95% CI 1.4 to 5.0, $p = 0.024$)) (Table 4). When stratifying for donor status, the association remained significant for both living- and deceased-donation (model 4, $p = 0.015$ and $p = 0.042$, respectively).

Table 4. Multivariable adjusted associations of the CaScore with death-censored graft failure, overall graft failure, death with a functioning graft and graft function decline

	Low CaScore	High CaScore		
	Hazard ratio	Hazard ratio	95% CI	p-value
Death censored graft failure				
Univariate	1.0 (Ref)	1.5	0.9 to 2.5	0.125
Model 1	1.0 (Ref)	1.6	0.9 to 2.7	0.082
Model 2	1.0 (Ref)	1.4	0.8 to 2.4	0.211
Model 3	1.0 (Ref)	1.1	0.6 to 2.0	0.697
Model 4	1.0 (Ref)	1.1	0.6 to 2.0	0.711
Overall graft failure				
Univariate	1.0 (Ref)	2.4	1.7 to 3.5	<0.0001
Model 1	1.0 (Ref)	2.5	1.7 to 3.7	<0.0001
Model 2	1.0 (Ref)	2.4	1.6 to 3.5	<0.0001
Model 3	1.0 (Ref)	1.8	1.2 to 2.7	0.008
Model 4	1.0 (Ref)	1.9	1.2 to 2.9	0.006
Death with a functioning graft				
Univariate	1.0 (Ref)	3.8	2.2 to 6.5	<0.0001
Model 1	1.0 (Ref)	3.9	2.3 to 6.9	<0.0001
Model 2	1.0 (Ref)	4.0	2.3 to 7.1	<0.0001
Model 3	1.0 (Ref)	2.8	1.5 to 5.2	0.002
Model 4	1.0 (Ref)	2.7	1.4 to 5.0	0.004
Graft function decline				
Univariate	1.0 (Ref)	1.6	1.0 to 2.6	0.038
Model 1	1.0 (Ref)	1.8	1.1 to 2.8	0.018
Model 2	1.0 (Ref)	1.6	1.0 to 2.6	0.043
Model 3	1.0 (Ref)	1.3	0.8 to 2.1	0.368
Model 4	1.0 (Ref)	1.4	0.8 to 2.4	0.223

Cox proportional hazards regression analysis. Data are presented as hazard ratio and 95% confidence interval (CI) for the two aorto-iliac CaScore groups (low and high). Model 1: adjusted for transplant centre and time between computed tomography and transplantation; model 2: adjusted for model 1 plus donor gender, donor type (living donation, DBD or DCD), cold ischemia time, no. of HLA mismatches, recipient gender, diabetes mellitus, dialysis vintage, and no. of previous transplantations, statin use; model 3: adjusted for model 2 plus recipient age; model 4: adjusted for model 3 plus donor age.

Figure 2. Kaplan-Meier survival curve for death-censored and overall graft failure free-survival for the low and high aorto-iliac CaScore group, stratified by living and deceased donor kidney transplantation and including a life table applicable for both graphs.

Aorto-iliac CaScore and graft function decline

A total of 81 (14.8%) events of graft function decline occurred, with 51 (14.0%) in the high and 30 (16.5%) in the low CaScore group (Table 2). Kaplan-Meier survival analysis for graft function decline, with the low and high CaScore group stratified by living and deceased donor kidney transplantation, showed a lower time-to-graft function decline for deceased donor – high CaScore group ($p < 0.0001$) (Figure 4). Kaplan-Meier survival analysis stratified by transplant recipient age, showed a lower survival for the recipients ≥ 65 years-of-age – high CaScore group ($p = 0.014$) (Figure S4). In Cox regression analysis, no significant associations were observed between the high CaScore and graft function

decline after adjusting for patient and transplantation covariables, including recipient and donor age (model 4, HR 1.4 (95% CI 0.8 to 2.4, $p = 0.223$) (Table 4). No differences were observed when stratifying for living- and deceased donation (model 4).

Figure 3. Kaplan-Meier survival curve for death with a functioning graft free-survival for the low and high aorto-iliac CaScore group, stratified by living and deceased donor kidney transplantation.

Figure 4. Kaplan-Meier survival curve for graft function decline free-survival for the low and high aorto-iliac CaScore group, stratified by living and deceased donor kidney transplantation.

DISCUSSION

In this dual centre cohort study, we identified a non-causal inverse association between pre-transplant aorto-iliac calcification and one-year eGFR, after adjustment for patient-specific, transplantation-related, and follow-up covariables, with the exception of donor age. For this association, the increased donor age in older recipients, due to the allocation of older donor kidneys to older recipients in the Eurotransplant Senior Program, was a significant confounder. For long-term graft outcomes, no association could be established between a high aorto-iliac CaScore and an increased risk of death-censored graft failure or graft function decline, in both living and deceased donor kidney transplantation. Whereas the identified association between a high aorto-iliac CaScore and graft failure (death-censored) remained significant after adjustment for various covariables, including donor and recipient age. Also, the high aorto-iliac CaScore was associated with death with a functioning graft.

Our study has both similarities and important differences compared to four previously published studies on pre-transplant imaging-based visualization of calcification. In the largest study ($n = 374$), aorto-iliac stenosis on CT-imaging was not an independent risk factor for graft failure, but was associated with an increased risk of overall graft failure.²³ In a relatively small study ($n = 119$) with lateral lumbar radiography-based calcification assessment, transplant recipients with and without calcifications differed with regard to graft failure-free survival.²⁴ In a study on pre-transplant CT-based quantification of calcification ($n = 100$), no statistically significant difference in eGFR at one-year after transplantation was found for the quartiles of aorto-iliac calcification. Also, survival analysis for graft failure, with nine events after a median follow-up of 2.6 years, did not show differences between the quartiles.²⁵ In a cohort of 93 transplant recipients, Aitken et al. showed that visual two group stratification of vascular calcification on pelvic X-ray did result in graft survival differences, but not in differences for one-year creatinine.²⁶ Compared to these imaging studies, the current study has the largest sample size and a relatively long period of follow-up. The results of our study were comparable for living- and deceased-donation, with an exception for the outcome measure graft function decline. In univariate analyses, there was a significant association between the CaScore and graft function decline for deceased-donation, whereas the association was not significant for living-donation. A possible explanation is the significant age difference and difference in diabetes mellitus prevalence between the living- and deceased-donation patients.

The outcomes of our study are in line with previous non-imaging studies on calcification. In a histology-based cohort study ($n = 90$), differences in graft failure were observed for transplant recipients with and without intimal microcalcification of the iliac artery; however, these differences were not tested in multivariable analyses. For graft function decline, an independent association with the combination of intimal microcalcification with positive intimal fetuin-A was identified.²⁷ In post-transplant blood serum analysis ($n = 699$), Calciprotein particle maturation time (T50), a measure for blood calcification propensity, showed to be associated with all-cause mortality, cardiovascular mortality, and graft failure.²⁸

A global development in transplant programs is a steady increase in donor and transplant candidate age.²⁹ With this trend, the causal relation of recipient age with vascular calcification and the confounding effect of donor age on the association between calcification and outcomes, can be important factors affecting transplant outcomes. Efforts could go to the optimization of kidney function and patient survival for transplant recipients with a high degree of aorto-iliac calcification. The kidney function of grafts from older donors may be improved, by decreasing cold ischemia time, reducing kidney rewarming during transplantation, and optimizing the use of hypothermic and normothermic machine perfusion.^{30–33} To improve patient survival in general and death with a functioning graft specially, focus might go to the reduction of modifiable risk factors for cardiovascular disease in kidney transplant candidates and recipients. This risk reduction, pre- and post-transplantation, could be achieved by focusing on prevention of new-onset diabetes, preventive measures for mineral and bone disorders, and reduction of hyperlipidaemia and hypertension.³⁴ Medical strategies in line with these focus points are age-adapted immunosuppressive therapy, stringent use of statins and antihypertensive medication, and general lifestyle interventions.^{35–37}

Our study has a few limitations that need to be addressed. First, we retrospectively selected patients in which a CT procedure was already performed based on predefined criteria, making the study prone to selection bias and confounding. Although the presented findings are applicable to the majority of transplant recipients with comparable baseline characteristics (median age of 60 [51–68] years), they should not be extrapolated to relatively young patients with limited comorbidities. Second, the two transplant centres in this cohort study are part of Eurotransplant Senior Program.^{18,38} This particular allocation scheme could have influenced the association between the aorto-iliac CaScore and donor age, given the causal relationship between vascular calcification and recipient age. Third, the period of follow-up of 3.1 years (median) is relatively long compared to previous imaging studies on vascular calcification but on the short side compared to large registry studies on the topic of kidney graft outcomes. Fourth, for a

relatively large proportion of patients ($n = 85$; of which 31 patients experienced overall graft failure with the first year) laboratory values at one-year after transplantation were not available. Complying with general standards for dealing with missing data, a complete case analysis was found to most reliable. Fifth, the interpretation of the implications of a statistically significant difference should always include a reflection of the clinical significance of the observed difference. In this study, a mean difference in eGFR of 5 mL/min per 1.73 m² was found between a high and a low aorto-iliac CaScore (mean 48 (21) versus 53 (20)). With a mean eGFR of 51 in the total cohort, this is a 10% difference in one-year graft function.

Nonetheless, this study has its unique strengths, which justify attention. This is the first study evaluating the association between aorto-iliac calcification and one-year graft function in multivariable analysis, including patient, transplantation, and follow-up covariables. Second, the aorto-iliac CaScore is an easy-to-implement assessment, as the software is readily available in standard imaging analysis packages and pre-transplant CT-imaging is advised for high-risk transplant candidates.^{39,40} Third, the discrepancy between the results of Kaplan-Meier survival analysis and multivariable Cox regression analyses highlights the importance of relatively large cohort studies that enable adjustment for important covariables. Kaplan-Meier survival analysis indicated significant differences based on the aorto-iliac CaScore for (death-censored) graft failure and graft function decline, whereas a significant independent association in Cox regression analysis was only found for overall graft failure and death with a function graft.

In conclusion, this study demonstrated that pre-transplant aorto-iliac calcification is associated with one-year eGFR in univariate, but not in multivariable linear regression analyses. The increased donor age in older recipients is a significant confounder for this association. A high CaScore was not associated with death-censored graft failure and graft function decline, whereas independent associations with overall graft failure and death with a functioning graft were identified. These results underline that transplantation in patients with a high CaScore does not result in earlier transplant function decline or worse death censored graft survival, although ongoing efforts for the prevention of death with a functioning graft remain essential.

REFERENCES

- 1 Benjamins S, Rijkse E, te Velde-Keyzer CA, Berger SP, Moers C, de Borst MH, et al. Aorto-Iliac Artery Calcification Prior to Kidney Transplantation. *J Clin Med* 2020;9(9):2893. Doi: 10.3390/jcm9092893.
- 2 Rangaswami J, Mathew RO, Parasuraman R, Tantisattamo E, Lubetzky M, Rao S, et al. Cardiovascular disease in the kidney transplant recipient: epidemiology, diagnosis and management strategies. *Nephrol Dial Transplant* 2019;34(5):760–73. Doi: 10.1093/ndt/gfz053.
- 3 Cianciolo G, Capelli I, Angelini ML, Valentini C, Baraldi O, Scolari MP, et al. Importance of Vascular Calcification in Kidney Transplant Recipients. *Am J Nephrol* 2014;39(5):418–26. Doi: 10.1159/000362492.
- 4 Hamed MO, Chen Y, Pasea L, Watson CJ, Torpey N, Bradley JA, et al. Early Graft Loss After Kidney Transplantation: Risk Factors and Consequences. *Am J Transplant* 2015;15(6):1632–43. Doi: 10.1111/ajt.13162.
- 5 De Vusser K, Lerut E, Kuypers D, Vanrenterghem Y, Jochmans I, Monbaliu D, et al. The predictive value of kidney allograft baseline biopsies for long-term graft survival. *J Am Soc Nephrol* 2013;24(11):1913–23. Doi: 10.1681/ASN.2012111081.
- 6 Issa N, Lopez CL, Denic A, Taler SJ, Larson JJ, Kremers WK, et al. Kidney Structural Features from Living Donors Predict Graft Failure in the Recipient. *J Am Soc Nephrol* 2020;31(2):415–23. Doi: 10.1681/ASN.2019090964.
- 7 Burton H, Iyamu Perisanidou L, Steenkamp R, Evans R, Mumford L, Evans KM, et al. Causes of renal allograft failure in the UK: trends in UK Renal Registry and National Health Service Blood and Transplant data from 2000 to 2013. *Nephrol Dial Transplant* 2019;34(2):355–64. Doi: 10.1093/ndt/gfy168.
- 8 Naesens M, Kuypers DRJ, De Vusser K, Evenepoel P, Claes K, Bammens B, et al. The histology of kidney transplant failure: a long-term follow-up study. *Transplantation* 2014;98(4):427–35. Doi: 10.1097/TP.000000000000183.
- 9 Van Loon E, Senev A, Lerut E, Coemans M, Callemeyn J, Van Keer JM, et al. Assessing the Complex Causes of Kidney Allograft Loss. *Transplantation* 2020. Doi: 10.1097/TP.0000000000003192.
- 10 Van Loon E, Bernards J, Van Craenenbroeck AH, Naesens M. The Causes of Kidney Allograft Failure: More Than Alloimmunity. A Viewpoint Article. *Transplantation* 2020;104(2):e46–56. Doi: 10.1097/TP.0000000000003012.
- 11 Lamb KE, Lodhi S, Meier-Kriesche H-U. Long-term renal allograft survival in the United States: a critical reappraisal. *Am J Transplant* 2011;11(3):450–62. Doi: 10.1111/j.1600-6143.2010.03283.x.
- 12 Sotomayor CG, Gomes-Neto AW, Eisenga MF, Nolte IM, Anderson JL, de Borst MH, et al. Consumption of fruits and vegetables and cardiovascular mortality in renal transplant recipients: a prospective cohort study. *Nephrol Dial Transplant* 2020;35(2):357–65. Doi: 10.1093/ndt/gfy248.
- 13 Schnitzler MA, Lentine KL, Gheorghian A, Axelrod D, Trivedi D, L'Italien G. Renal function following living, standard criteria deceased and expanded criteria deceased donor kidney transplantation: impact on graft failure and death. *Transpl Int* 2012;25(2):179–91. Doi: 10.1111/j.1432-2277.2011.01395.x.
- 14 Opelz G, Döhler B. Influence of Time of Rejection on Long-Term Graft Survival in Renal Transplantation. *Transplantation* 2008;85(5):661–6. Doi: 10.1097/TP.0b013e3181661695.
- 15 Gaston RS, Fieberg A, Hunsicker L, Kasiske BL, Leduc R, Cosio FG, et al. Late graft failure after kidney transplantation as the consequence of late versus early events. *Am J Transplant* 2018;18(5):1158–67. Doi: 10.1111/ajt.14590.

- 16 Kasiske BL, Israni AK, Snyder JJ, Skeans MA, Patient Outcomes in Renal Transplantation (PORT) Investigators. The relationship between kidney function and long-term graft survival after kidney transplant. *Am J Kidney Dis* 2011;57(3):466–75. Doi: 10.1053/j.ajkd.2010.10.054.
- 17 Rijkse E, Dam JL, Roodnat JJ, Kimenai HJAN, IJzermans JNM, Minnee RC. The prognosis of kidney transplant recipients with aorto-iliac calcification: a systematic review and meta-analysis. *Transpl Int* 2020;33(5):483–96. Doi: 10.1111/tri.13592.
- 18 Schachtner T, Otto NM, Reinke P. Two decades of the Eurotransplant Senior Program: the gender gap in mortality impacts patient survival after kidney transplantation n.d. Doi: 10.1093/cjk/sfz118.
- 19 Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, Feldman HI, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009;150(9):604–12. Doi: 10.7326/0003-4819-150-9-200905050-00006.
- 20 Loupy A, Haas M, Solez K, Racusen L, Glotz D, Seron D, et al. The Banff 2015 Kidney Meeting Report: Current Challenges in Rejection Classification and Prospects for Adopting Molecular Pathology. *Am J Transplant* 2017;17(1):28–41. Doi: 10.1111/ajt.14107.
- 21 Clark TG, Bradburn MJ, Love SB, Altman DG. Survival Analysis Part I: Basic concepts and first analyses. *Br J Cancer* 2003;89:232–8. Doi: 10.1038/sj.bjc.6601118.
- 22 Janes H, Dominici F, Zeger S. On quantifying the magnitude of confounding. *Biostatistics* 2010;11(3):572–82. Doi: 10.1093/biostatistics/kxq007.
- 23 Rijkse E, Kimenai HJAN, Roodnat JJ, Ten Raa S, Bijdevaate DC, van Dam JL, et al. Impact of Aortoiliac Stenosis on Graft and Patient Survival in Kidney Transplant Recipients Using the TASC II Classification. *Transplantation* 2019;103(10):2164–72. Doi: 10.1097/TP.0000000000002635.
- 24 Munguia P, Caramelo R, Rubio M V, Sahdala L, Arnaud L, Paul J, et al. Pre-Transplant Assessment of Vascular Calcification as a Risk Factor of Mortality, Graft Loss, and Cardiovascular Events in Renal Transplant Recipients. *Transplant Proc* 2015;47(8):2368–70. Doi: 10.1016/j.transproceed.2015.08.038.
- 25 Chavent B, Maillard N, Boutet C, Albertini J-N, Duprey A, Favre J-P. Prognostic Value of Aortoiliac Calcification Score in Kidney Transplantation Recipients. *Ann Vasc Surg* 2017;44:245–52. Doi: 10.1016/j.avsg.2017.03.180.
- 26 Aitken E, Ramjug S, Buist L, Kingsmore D. The Prognostic Significance of Iliac Vessel Calcification in Renal Transplantation. *Transplant Proc* 2012. Doi: 10.1016/j.transproceed.2012.06.058.
- 27 Hwang HS, Lim SW, Sun IO, Yang KS, Yoon HE, Chung BH, et al. Clinical significance of preexisting microcalcification in the iliac artery in renal transplant recipients. *Transplantation* 2015;99(4):811–7. Doi: 10.1097/TP.0000000000000409.
- 28 Keyzer CA, De Borst MH, Van Den Berg E, Jahnhen-Dechent W, Arampatzis S, Farese S, et al. Calcification Propensity and Survival among Renal Transplant Recipients. *J Am Soc Nephrol* 2016;27(1):239–48. Doi: 10.1681/ASN.2014070670.
- 29 Veroux M, Grosso G, Corona D, Mistretta A, Giaquinta A, Giuffrida G, et al. Age is an important predictor of kidney transplantation outcome. *Nephrol Dial Transplant* 2012;27(4):1663–71. Doi: 10.1093/ndt/gfr524.
- 30 Benjamins S, van den Berg TAJ, Kuipers TGJ, Moers C, Berger SP, Leuvenink HGD, et al. Kidney temperature during living donor kidney transplantation is associated with short-term measured glomerular filtration rate - a prospective study. *Transpl Int* 2020;33(2):174–80. Doi: 10.1111/tri.13528.
- 31 O'Neill S, Srinivasa S, Callaghan CJ, Watson CJ, Dark JH, Fisher AJ, et al. Novel Organ Perfusion and Preservation Strategies in Transplantation - where are we going in the UK? *Transplantation* 2020. Doi: 10.1097/TP.0000000000003106.

- 32 Gill J, Dong J, Eng M, Landsberg D, Gill JS. Pulsatile perfusion reduces the risk of delayed graft function in deceased donor kidney transplants, irrespective of donor type and cold ischemic time. *Transplantation* 2014;97(6):668–74. Doi: 10.1097/01.TP0000438637.29214.10.
- 33 Peters-Sengers H, Houtzager JHE, Idu MM, Heemskerk MBA, van Heurn ELW, Homan van der Heide JJ, et al. Impact of Cold Ischemia Time on Outcomes of Deceased Donor Kidney Transplantation: An Analysis of a National Registry. *Transplant Direct* 2019;5(5):e4448. Doi: 10.1097/TXD.0000000000000888.
- 34 Jardine AG, Gaston RS, Fellstrom BC, Holdaas H. Prevention of cardiovascular disease in adult recipients of kidney transplants. *Lancet* 2011;378(9800):1419–27. Doi: 10.1016/S0140-6736(11)61334-2.
- 35 Krenzien F, ElKhal A, Quante M, Rodriguez Cetina Biefer H, Hirofumi U, Gabardi S, et al. A Rationale for Age-Adapted Immunosuppression in Organ Transplantation. *Transplantation* 2015;99(11):2258–68. Doi: 10.1097/TP0000000000000842.
- 36 Sotomayor CG, Velde-Keyzer CA te, de Borst MH, Navis GJ, Bakker SJL. Lifestyle, Inflammation, and Vascular Calcification in Kidney Transplant Recipients: Perspectives on Long-Term Outcomes. *J Clin Med* 2020;9(6):1911. Doi: 10.3390/jcm9061911.
- 37 Sotomayor CG, Benjamens S, Gomes-Neto AW, Pol RA, Groothof D, te Velde-Keyzer CA, et al. Bone Mineral Density and Aortic Calcification: Evidence for a Bone-Vascular Axis After Kidney Transplantation. *Transplantation* 2020:1. Doi: 10.1097/TP0000000000003226.
- 38 Peters-Sengers H, Berger SP, Heemskerk MBA, Al Arashi D, Homan van der Heide JJ, Hemke AC, et al. Stretching the Limits of Renal Transplantation in Elderly Recipients of Grafts from Elderly Deceased Donors. *J Am Soc Nephrol* 2017;28(2):621–31. Doi: 10.1681/ASN.2015080879.
- 39 Benjamens S, Glaudemans AWJM, Berger SP, Slart RHJA, Pol RA. Have we forgotten imaging prior to and after kidney transplantation? *Eur Radiol* 2018;28(8):3263–7.
- 40 Chadban SJ, Ahn C, Axelrod DA, Foster BJ, Kasiske BL, Kher V, et al. Summary of the Kidney Disease: Improving Global Outcomes (KDIGO) Clinical Practice Guideline on the Evaluation and Management of Candidates for Kidney Transplantation. *Transplantation* 2020;104(4):708–14. Doi: 10.1097/TP0000000000003137.

**Summary,
discussion
and future perspectives**

SUMMARY

In the past century, kidney transplantation became the optimal treatment opportunity for patients with end stage renal disease (ESRD). For decades, major improvements were reported for kidney graft and patient survival; however, this progress in patient outcomes stagnated in the past years. At the start of the 21st century, a shift from donation after brain death (DBD) kidney transplantation to living (un)related donation and donation after circulatory death (DCD) kidney transplantation was observed in The Netherlands. Together with these changes, there was an increase in donor and kidney transplant recipient age, accompanied by a high cardiovascular burden in transplant candidates. This thesis describes the role of diagnostic imaging in the quest to optimize kidney transplantation outcomes.

The introduction of this thesis, **chapter 1**, provides an insight into the historical alignment between transplant surgery and diagnostic imaging. This is followed by a brief overview of the changing demographics of living and deceased kidney donors and kidney transplant recipients. The preface, **chapter 2**, summarizes the results of three bibliometric analyses, on (1) the lack of interdisciplinary collaborations, (2) gender bias, and (3) international developments causing uncertainty. For the first topic, it was demonstrated that the number of publications on imaging and kidney transplantation is low. These publications are poorly cited, as compared with other fields of imaging. Whereas there is a clinical need for evidence-based recommendations and innovative advances for the use of imaging in the field of kidney transplantation. For the second topic, it was found that the percentage of female authors increased in the field of transplantation research, with large differences in gender ratios between countries. Female authors reported less external funding and were especially underrepresented in the share of highly cited publications (>200 citations). The third topic highlighted the potential impact of Brexit for the United Kingdom (UK) and the European Union (EU). The UK received 10.7% of transplantation research-related EU-funding and UK-based researcher collaborate in almost half of all EU-funded projects. Also, approximately 20% of scientific publications by UK primary authors are the result of an international collaboration, in many cases with EU-based partner institutions.

In **Part I**, the use of renal scintigraphy is examined in three chapters. A systematic review, **chapter 3**, describes the use of renal scintigraphy for non-acute vascular and urologic complications and the assessment of postoperative fluid collections. The literature did not support the use of renal scintigraphy for the differentiation between acute rejection and acute tubular necrosis. The results of a collaboration between the Groningen Transplant Center and Leiden Transplant Center, **chapter 4**, were used to

describe the role of renal scintigraphy for the prediction of the duration of delayed graft function (DGF) after transplantation. In this cohort of 377 kidney transplant recipients, several renal scintigraphy parameters had a high sensitivity for the prediction of DGF ≥ 7 days; however, the low specificity limits their use for the identification of patients in whom the course of DGF is longer than anticipated. In the Leiden Transplant Center cohort, **chapter 5**, the role of a second renal scintigraphy within 3 to 7 days after transplantation was examined. This study demonstrated an independent association between qualitative scintigraphy grades of the first and second procedure with DGF duration and length of hospital stay (LOS). However, for the delta grades between these procedures, no differences in DGF and LOS were found.

In **Part II**, kidney and vascular calcification prior to and after kidney transplantation is extensively studied. **Chapter 6** focused on a pre-clinical study for non-invasive imaging for the identification of microcalcifications in active nephrocalcinosis. Using autoradiography, [^{18}F]-sodium fluoride (^{18}F)-NaF uptake was visualised in the donor kidney and explanted kidney allograft samples. A correlation between ex-vivo [^{18}F]-NaF uptake and histology-proven microcalcifications was identified. For **chapter 7**, a cohort of 701 kidney transplant recipients was selected. In this cohort, a high degree of aorta calcification, assessed by a dual-energy X-ray absorptiometry (DXA) procedure within 6 months after transplantation, was independently associated with cardiovascular events. With the same cohort, **chapter 8**, the association between bone mineral disorder (BMD) and aorta calcification was examined. This study provided evidence pointing towards the existence of a bone-vascular axis after kidney transplantation. For **chapter 9**, a cohort of 547 kidney transplant recipients was selected from the Groningen Transplant Center and Erasmus MC transplant centre, a project that was performed in collaboration with Imperial College Renal and Transplant Centre. In this chapter, the aorto-iliac CaScore was introduced, a modified Agatston score, for non-contrast enhanced computed tomography (CT)-based quantification of calcification prior to transplantation. With this study, an independent association between the CaScore and (cardiovascular) mortality and cardiovascular events was identified. With the same cohort, **chapter 10**, the association between the aorto-iliac calcification and kidney graft outcomes was studied. This study demonstrated that pre-transplant aorto-iliac calcification is associated with one-year eGFR in univariate, but not in multivariable linear regression analyses. The increased donor age in older recipients is a significant confounder for this association. A high CaScore was not associated with death-censored graft failure and graft function decline, whereas independent associations with overall graft failure and death with a functioning graft were identified.

DISCUSSION

A broad view on science, in this case transplantation research, is pivotal for further innovation. Lessons can be learned from historical achievements and recent developments, by using previously found solutions as an advantage and identifying mistakes at an early timepoint. The historical alignment between transplant surgery and diagnostic imaging can be an example to promote interdisciplinary collaborations. As most opportunities to innovate are outside of the regular scope of transplant surgery and nephrology, such as artificial intelligence, magnetic resonance and three-dimensional imaging and bioartificial organs for transplantation, these connections are essential to ensure future progress. For these innovations, an open academic environment is critical. Addressing potential causes of academic bias, such as gender, racial and financial bias, will support the identification of problems to be tackled. In general terms, diverse representative bodies can result in less academic disparities. Expert groups, editorial boards and meeting committees should have an equal male-female ratio, represent all ethnical backgrounds and have a representation from various continents. Regarding these aims, the research group involved in the studies presented in this thesis is not a good example. Also, financial structures, such as governmental funding agencies and industry funding, should be examined. Financial ties between research groups and third industry partners should be addressed and funding should be rewarded based on unbiased assessments of the candidate's merits and quality of the research idea.

As shown in a pan-European study on short- and long-term graft survival after kidney transplantation between 1986 and 2015, the improvement of transplant survival slowed down in the past years. This trend indicates that innovation should regain priority, enabling further improvement of patient and transplant survival.¹

At the start of this PhD trajectory, we aimed to re-evaluate the assessment of renal scintigraphy procedures after kidney transplantation; however, first and second renal scintigraphy procedures could not be used to reliably predict prolonged DGF and LOS. The results of these studies indicate that the role of renal scintigraphy for the prediction of early post-transplantation outcomes is limited. For other indications, such as suspicion of vascular complications, urological complications, and postoperative fluid collections, renal scintigraphy in planar view or with three-dimensional images (single-photon emission computed tomography, SPECT) is a reliable diagnostic procedure.

In the pre-clinical autoradiography study, we demonstrated good correlation between [¹⁸F]-NaF uptake in human kidney parenchyma samples and histology-proven microcalcifications. Tracer uptake was higher in explanted kidney allograft samples

compared to donor kidney samples, which is in line with reports on the increase of microcalcifications after transplantation.²⁻⁴ Imaging with [¹⁸F]-NaF is of interest, as its uptake is increased in biologically active areas of calcification, before these areas can be visualised by computed tomography.⁵ For kidney transplantation, a non-invasive and reliable diagnostic approach to identify and quantify microcalcifications in early nephrocalcinosis could enable studies on impact of nephrocalcinosis on transplant function.⁶ A potential step between the here presented feasibility study and in-vivo imaging can be ex-vivo [¹⁸F]-NaF PET imaging of normothermic perfused kidneys.

With the project on DXA procedures after kidney transplantation, we retrieve additional clinically relevant data from regularly performed procedures. The 8-point abdominal aorta calcification (AAC) score presented in these studies is ready to be implemented in daily practice, enabling assessment of bone density and vascular calcification with one procedure. Studies with larger patient groups should investigate whether incorporation of the AAC score in cardiovascular risk prediction models increases their reliability. In addition, this project provided evidence supporting the existence of a bone-vascular axis after kidney transplantation. The hypothesis supported by these findings, being a close relationship between decrease in bone density and increase in vascular calcification, warrants attention for preventive management of chronic kidney disease-BMD (CKD-BMD) early after kidney transplantation.

With the project on aorto-iliac artery calcification prior to kidney transplantation, we introduced a non-contrast enhanced CT-based quantification tool for aorta-iliac artery calcification. The calculated aorto-iliac CaScore, a novel approach in transplant medicine, was associated with all-cause and cardiovascular mortality, and cardiovascular events after adjustment for traditional and transplant candidate-specific risk factors. The high-risk patients identified in this study, with a high CaScore and multiple comorbidities, could possibly benefit from interventions prior to transplantation. Some of the modifiable risk factors prior to transplantation, such as a high BMI, current smoking and frailty, could be addressed in prehabilitation programs. In other patient populations, the interaction between vascular calcification and frailty has been identified and prehabilitation prior to kidney transplantation has shown favourable outcomes in a preliminary study.^{7,8} Also, attention should go to treatment of common comorbidities such as hypertension, hypercholesterolemia and diabetes mellitus. This aorto-iliac CaScore project provided a rationale behind the unadjusted association of vascular calcification with one-year estimated glomerular filtration rate (eGFR), graft failure and graft function decline. Regarding the first, we demonstrated that the increased donor age in older recipients is a significant confounder for the association between pre-transplant aorto-iliac calcification and one-year eGFR. The Eurotransplant Senior Program can be potential

explanation for this finding, as kidneys from older donors tend to have a lower one-year eGFR.⁹ Regarding the other outcome measures, a high CaScore was not associated with death-censored graft failure and graft function decline, whereas independent associations with deceased donor overall graft failure and death with a functioning graft (living and deceased) were identified. These results underline that transplantation in patients with a high CaScore does not lead to earlier transplant function decline or worse death censored graft survival, although ongoing efforts for the prevention of death with a functioning graft remain essential.

In summary, the importance of this thesis is not determined by the implementation of novel diagnostic imaging techniques, but by the knowledge gained on widely available diagnostic procedures. It's pivotal that indications and implications for the use of diagnostic imaging procedures prior to and after kidney transplantation will get attention in future international guidelines. At this moment, these guidelines do not provide guidance on the indications for diagnostic imaging and lack specificity on the type of imaging to use. Only when the use of these procedures is standardized, their implications for clinical practice in kidney transplantation can be studied adequately.

FUTURE PERSPECTIVE

It is expected that ageing of the population will continue in the coming decades and that the medical challenges caused by comorbidities, like obesity and diabetes mellitus, will not be over soon. Knowing this, one can expect that cardiovascular disease and vascular calcification will be a growing concern prior to and after kidney transplantation. Identifying patients who are at risk for major adverse events, due to cardiovascular disease and/or a high degree of vascular calcification, will be essential to improve patients' outcomes after kidney transplantation. The projects presented in this thesis confirm the importance of diagnostic imaging in the screening for vascular calcification. The advancements in screening for vascular calcification should still be followed by innovations on interventions to reduce calcification, as the identification of risk should entail treatment options for high-risk patients. Other advancements in the field of diagnostic imaging are expected to provide a more in-depth view on the extent and progression of calcification. For this purpose, research efforts should focus on the application of [^{18}F]-NaF PET imaging of active calcifications in transplant candidates and recipients. The outcomes of these [^{18}F]-NaF PET procedures showed to be useful in prediction acceleration and assessing vulnerability of vascular calcification in the general population; however, its applicability for in-vivo visualization of nephrocalcinosis has not yet been studied.¹⁰ Using this technique in combination with modalities for anatomical imaging, such as CT and magnetic resonance imaging (MRI), will enable precise localization of areas with active calcifications.

Over time, with important immunosuppressive innovations, the one-year incidence of acute rejection has gone from nearly 100% to approximately 10%.¹¹ Still, acute rejection is an important factor associated with inferior long-term transplant and patient survival.¹² Research efforts should go to early and reliable diagnosis of acute rejection episodes, to ensure prompt and adequate therapy. With the pitfalls of kidney allograft biopsy, the current gold standard for the diagnosis of acute rejection, development of a non-invasive diagnostic tool for this diagnosis can be important for both clinical practice and patient comfort.¹³ First steps have been taken in this direction, with the use of [^{18}F]-fluorodeoxyglucose ([^{18}F]-FDG) PET for the diagnosis of subclinical rejection in human and [^{18}F]-FDG-labelled T lymphocytes for the diagnosis of acute rat kidney allograft rejection.^{14,15} A significant part of this PhD trajectory has been dedicated to a project on non-invasive diagnosis of acute rejection. This, still ongoing, prospective proof of concept study evaluates the applicability and feasibility of visualizing infiltrating T lymphocytes with [^{18}F]-Fluorobenzoyl-Interleukin-2 ([^{18}F]-FB-IL2) PET.¹⁶ It's the first study using this innovative non-invasive diagnostic tool, radiolabelled interleukin-2 PET, for the diagnosis of acute rejection after kidney transplantation. The study design

includes a one-to-one comparison of the [^{18}F]-FB-IL2 PET results with biopsy findings and results of blood/urine T cell quantification, including Kidney Solid Organ Response Test (kSORT) analysis for acute rejection risk.^{17,18}

Besides the diagnosis of acute rejection episodes, the identification of reduced kidney allograft perfusion remains of clinical importance. Renal scintigraphy is not suitable for in-depth quantification of kidney perfusion and the studies presented in this thesis show its lack of accuracy for post-transplantation outcomes. Several other diagnostic imaging procedures could provide more in-depth information on kidney perfusion, such as oxygen-15-labeled water ([^{15}O]- H_2O) PET and contrast-enhanced ultrasound (CEUS).^{19–21} The advantage of [^{15}O]- H_2O PET is the opportunity for quantification of renal microvascular function, with a three-dimensional image and low interobserver variability.¹⁹ For CEUS, the advantage is the wide availability of ultrasound equipment, the lack of radiation exposure and the relatively low costs. However, the high interobserver variability and low availability of CEUS-trained professionals limits its clinical application.²¹

To ensure the continuation of this fruitful collaboration between the fields of transplant surgery, nephrology and diagnostic imaging, our team-initiated preparations for an international prospective study on imaging and kidney transplantation in 2018. This resulted in a comprehensive study proposal, combining functional MRI technology and artificial intelligence-based analyses to detect kidney allograft dysfunction after kidney transplantation. Several studies have explored the use of MRI techniques, both functional and anatomical, prior to and after kidney transplantation.^{22,23} The positive findings in these studies did not yet convince the broader transplant community to implement the use of MRI in daily transplant practise.²⁴ To provide solid evidence on the relevance and clinical applicability of functional MRI, we aim to demonstrate the capability of an artificial intelligence algorithm to adequately identify post-transplant complications.

Besides attention for international collaboration, efforts went into the initiation of the Dutch Kidney Transplant Study Group (DKTSG), an initiative for kidney transplant surgery research in The Netherlands.²⁵ This initiative is supported by all Dutch academic medical centres and the aim is to build and solidify fruitful research collaborations between Dutch transplant surgeons. The joined project between the Groningen Transplant Center and the Erasmus MC transplant centre (chapter 9 & chapter 10) can be seen as a first step. In following years, we will start projects with all other Dutch transplant centres.

In conclusion, there are several diagnostic techniques that can be considered promising innovations in the field of kidney transplantation. From the perspective of nuclear medicine, the most promising techniques are [^{18}F]-NaF PET/CT for vascular imaging and [^{18}F]-FB-IL2 PET for kidney imaging. From the radiology perspective, high clinical impact is expected from functional MRI innovations in combination with artificial intelligence. To answer these research questions, studies with relatively large samples sizes are needed, making national and international collaborations pivotal.

REFERENCES

- Coemans M, Süsal C, Döhler B, et al. Analyses of the short- and long-term graft survival after kidney transplantation in Europe between 1986 and 2015. *Kidney Int.* 2018;94(5):964-973.
- Evenepoel P, Lerut E, Naesens M, et al. Localization, Etiology and Impact of Calcium Phosphate Deposits in Renal Allografts. *Am J Transplant.* 2009;9(11):2470-2478.
- Nankivell BJ, Borrows RJ, Fung CL-S, O'Connell PJ, Allen RDM, Chapman JR. The Natural History of Chronic Allograft Nephropathy. *N Engl J Med.* 2003;349(24):2326-2333.
- Schwarz A, Mengel M, Gwinner W, et al. Risk factors for chronic allograft nephropathy after renal transplantation: a protocol biopsy study. *Kidney Int.* 2005;67(1):341-348.
- Irkle A, Vesey AT, Lewis DY, et al. Identifying active vascular microcalcification by 18F-sodium fluoride positron emission tomography. *Nat Commun.* 2015;6(May).
- Bäck M, Aranyi T, Cancela ML, et al. Endogenous Calcification Inhibitors in the Prevention of Vascular Calcification: A Consensus Statement From the COST Action EuroSoftCalcNet. *Front Cardiovasc Med.* 2019;5.
- Guaraldi G, Raggi P. Atherosclerosis in frailty: Not frailty in atherosclerosis. *Atherosclerosis.* 2017;266:226-227.
- McAdams-DeMarco MA, Ying H, Van Pilsum Rasmussen S, et al. Prehabilitation prior to kidney transplantation: Results from a pilot study. *Clin Transplant.* 2019;33(1):e13450.
- Peters-Sengers H, Berger SP, Heemskerk MBA, et al. Stretching the Limits of Renal Transplantation in Elderly Recipients of Grafts from Elderly Deceased Donors. *J Am Soc Nephrol.* 2017;28(2):621-631.
- den Harder AM, Wolterink JM, Bartstra JW, et al. Vascular uptake on 18F-sodium fluoride positron emission tomography: precursor of vascular calcification? *J Nucl Cardiol.* 2020.
- Cooper JE. Evaluation and Treatment of Acute Rejection in Kidney Allografts. *Clin J Am Soc Nephrol.* 2020;15(3):430-438.
- Clayton PA, McDonald SP, Russ GR, Chadban SJ. Long-Term Outcomes after Acute Rejection in Kidney Transplant Recipients: An ANZDATA Analysis. *J Am Soc Nephrol.* 2019;30(9):1697-1707.
- Köhnke R, Kentrup D, Schütte-Nütgen K, et al. Update on imaging-based diagnosis of acute renal allograft rejection. *Am J Nucl Med Mol Imaging.* 2019;9(2):110-126.
- Hanssen O, Weekers L, Lovinfosse P, et al. Diagnostic yield of 18F-FDG PET/CT imaging and urinary CXCL9/creatinine levels in kidney allograft subclinical rejection. *Am J Transplant.* 2020;20(5):1402-1409.
- Grabner A, Kentrup D, Edemir B, et al. PET with 18F-FDG-labeled T lymphocytes for diagnosis of acute rat renal allograft rejection. *J Nucl Med.* 2013;54(7):1147-1153.
- Benjamins S, Slart RH, Glaudemans AW, et al. IL2 Imaging in Renal Transplantation - ClinicalTrials.gov.
- Roedder S, Sigdel T, Salomonis N, et al. The kSORT assay to detect renal transplant patients at high risk for acute rejection: results of the multicenter AART study. *PLoS Med.* 2014;11(11):e1001759.
- Crespo E, Roedder S, Sigdel T, et al. Molecular and Functional Noninvasive Immune Monitoring in the ESCAPE Study for Prediction of Subclinical Renal Allograft Rejection. *Transplantation.* 2017;101(6):1400-1409.
- Päivärinta J, Oikonen V, Räisänen-Sokolowski A, et al. Renal vascular resistance is increased in patients with kidney transplant. *BMC Nephrol.* 2019;20(1):437.
- Päivärinta J, Koivuviita N, Oikonen V, et al. The renal blood flow reserve in healthy humans and patients with atherosclerotic renovascular disease measured by positron

- emission tomography using $[^{15}\text{O}]\text{H}_2\text{O}$. *EJNMMI Res.* 2018;8(1):45.
21. Morgan TA, Jha P, Poder L, Weinstein S. Advanced ultrasound applications in the assessment of renal transplants: contrast-enhanced ultrasound, elastography, and B-flow. *Abdom Radiol.* 2018;43(10):2604-2614.
22. Longchamp A, Klauser A, Songeon J, et al. Ex Vivo Analysis of Kidney Graft Viability Using ^{31}P Magnetic Resonance Imaging Spectroscopy. *Transplantation.* July 2020.
23. Bane O, Hectors SJ, Gordic S, et al. Multiparametric magnetic resonance imaging shows promising results to assess renal transplant dysfunction with fibrosis. *Kidney Int.* 2020;97(2):414-420.
24. Schutter R, Lantinga VA, Borra RJH, Moers C. MRI for diagnosis of post-renal transplant complications: current state-of-the-art and future perspectives. *Magn Reson Mater Physics, Biol Med.* 2020;33(1):49-61.
25. Pol RA, Minnee RC, van den Berg TAJ. Dutch Kidney Transplant Study Group (DKSTG).

Appendices

Living donor hand-assisted laparoscopic nephrectomy in a healthy individual with situs inversus totalis: no need to turn down the donor

BMJ Case Reports, 2020

Stan Benjamens
Tamar A.J. van den Berg
Johan F.M. Lange
Robert A. Pol

A 70-year-old healthy male individual offered to undergo a living donor hand-assisted laparoscopic nephrectomy to enable kidney transplantation for a close relative. As required for all living transplant donor candidates, extensive screening was performed to exclude potential contraindications for donation. Tests revealed a situs inversus totalis, meaning a complete transposition of the thoracic and abdominal organs in the sagittal plane. As other contraindications for living kidney donation were absent, the feasibility of this procedure was determined multidisciplinary. A successful donation procedure was performed without surgical complications for the donor and good short-term transplant outcomes. In line with current developments that have resulted in more liberal criteria for potential living kidney donors, major anatomical deviations should not automatically be a contraindication. With multidisciplinary efforts and thorough surgical preparation at a high-volume transplant centre, this procedure is feasible and safe.

INTRODUCTION

Living kidney donation could be considered to be in disagreement with the general medical principle of doing no harm, and elaborate screening of living transplant donor candidates is pivotal.^{1,2} Standard living donor nephrectomy has proven to be without major risk, with 2.6% postsurgical complications and a perioperative mortality of 3.1 per 10,000 donors.³⁻⁵ Laparoscopic (hand-assisted) nephrectomy is standard practice in most centres, showing similarly low complication rates even for more challenging cases.⁶ While 25% of donors experience chronic pain after hand-assisted laparoscopic nephrectomy, the post-donation quality of life is reported as equal or better than that of the general population.^{7,8} A worldwide estimated total of 27,000 living donor transplantations are performed each year (2006), showing that 39% of all kidney transplantations are enabled by living donation.⁹ For the recipient, receiving a transplant from a living donor comes with the advantage of a shorter period on the waiting list and better long-term survival compared to deceased donor transplantation.¹⁰ While the literature on potential donor candidate refusal is scarce, a single-centre descriptive study shows that over half of potential donors do not pass the initial health screening and 10% is refused based on anatomical information obtained by contrast-enhanced computed tomography (CT).¹¹ Donor safety is of the utmost importance in any living donation programme, therefore a thorough pre-donation screening is required. With the ongoing long times on waiting lists and the increasing demand for donor organs, more liberal selection criteria for potential living kidney donors, such as donors with anatomical deviations, have become common practice.^{2,12,13} Several reports describe the potential harms of refusing to allow living donation, showing the emotional consequences of turning down a potential donor candidate.^{14,15}

CASE PRESENTATION

General information

A 70-year-old healthy male individual was considered fit to donate his left kidney to a close relative. Medical history showed no prior abdominal surgical procedures and no history of renal insufficiency or other medical conditions, with the exception of controlled hypertension. In-depth psychological assessment did not show contraindications for donation, confirming good motives, commitment and understanding of the balance between the procedure's risk and potential benefits for the recipient. Standard pre-donation screening included chest X-ray and contrast-enhanced CT, revealing situs inversus totalis (Figure 1 and Figure 2). This situs inversus totalis was not accompanied by Kartagener syndrome, the underlying condition of primary ciliary dyskinesia seen in 25% of these individuals.¹⁶ There were no additional anatomical deviations (e.g. no vascular abnormalities) or suspicion for malignancy; the donor candidate had a body mass index (BMI) of 30.2 kg/m² (Figure 3).

Figure 1. Chest X-ray prior to living donor hand-assisted laparoscopic nephrectomy

Investigations

In line with the current guidelines for screening of living transplant donor candidates, this patient underwent a chest X-ray (Figure 1) and contrast-enhanced CT (Figure 2). The chest X-ray shows the cardiac apex pointing to the right and a right-sided aortic arch, indicating dextrocardia, and free air under the right-side of the diaphragm, indicating

a right-sided stomach. The contrast-enhanced CT shows complete transposition of the abdominal organs in the sagittal plane. Assessment of the kidneys showed a small cystic lesion on the right kidney, with an accessory renal artery. Signs of a focal parenchymal lesion were seen on the left kidney, possibly due to calcification and/or ischaemic damage.

Outcomes and follow-up

Hand-assisted laparoscopic donor nephrectomy using a transperitoneal approach is standard practice in our centre. The procedure was performed with the patient in right-sided position and a suprapubic transverse incision was made to provide hand access. The first trocar (10 mm) was placed in the left iliac fossa, the second trocar (10 mm) periumbilically as a video port, and the third trocar (5 mm) subcostally. After successful nephrectomy of the left kidney, the suprapubic fascia was closed with running absorbable suture.⁷ To assess the success of the donation procedure there was a 30-day follow-up. The donor had a procedure-related complication during this follow-up period, a seroma at the incision site. This surgical complication was classified as *Clavien-Dindo Grade I*, not requiring a medical intervention.¹⁷ The donor did not experience other complications in this period, although he did have pain at the surgical wound site in the first days after donation. Postoperative pain scores were assessed by a visual analogue scale (VAS) score. Postoperative VAS was 3/10 at rest and he was discharged on the third postoperative day. At two weeks follow-up VAS score was 0/10 at rest and 1-2/10 during mild exercise, with some remaining irritation at the suprapubic wound site. At 30 days follow-up the donor reported a VAS score of 0/10 at rest and 1/10 during mild exercise. His creatinine was 72 $\mu\text{mol/L}$ preoperatively, 119 $\mu\text{mol/L}$ on the second postoperative day and 69 $\mu\text{mol/L}$ on the sixth postoperative day.

Figure 2. Contrast-enhanced CT, showing a transversal and frontal image of the abdomen prior to living donor hand-assisted laparoscopic nephrectomy

Figure 3. Intraoperative image, showing the renal vasculature during living donor hand-assisted laparoscopic nephrectomy

DISCUSSION

Situs inversus totalis is the total reversal of both thoracic and abdominal organs in the sagittal plane, and was first described by Leonardo da Vinci in the 15th century. It is seen in about 1 person in 10,000.¹⁸ This anatomical deviation complicates surgical procedures, as it requires a complete shift of the team's anatomical perspective. However, in the current report we describe a case of a successful living donor hand-assisted laparoscopic nephrectomy on a 70-year-old male with situs inversus totalis, showing that this major anatomical deviation does not pose an increased threat to the donor's safety.

Living kidney donors are extremely motivated to undergo a donor nephrectomy procedure enabling kidney transplantation for a family member or friend in need. Not being able to donate due to anatomical deviations discovered during pre-donation screening is a tremendous disappointment for these potential donors. Although previous case reports have been published on living donor nephrectomy in a donor with situs inversus totalis, the perspective of the potential donor has been underexposed. Qualitative research on emotional status after screening for living kidney donation revealed negative emotional consequences in a subgroup of candidates who were turned down for donation.¹⁵ This is in line with the Viewpoint article written by Allen and colleagues, addressing the expanded view of risks and benefits for potential living kidney donor candidates. Their systematic analysis of risks and benefits in living kidney donation describes an underestimation of the harms of being turned down for donation.¹⁴

We conducted a systematic search to identify similar cases in the literature (PubMed search: (“transplant* nephrectomy” OR “donor nephrectomy”) AND “situs inversus”), and found six earlier case reports. Even when combining the experience gained in these six performed procedures, the data on donation procedures in situs inversus donors remains limited. While most case reports discuss the transplant outcomes, five out of six did not report data on donor follow-up.^{19–23} The case report addressing donor outcomes reports creatinine levels within normal range and fast recovery without need for additional pain medication.²⁴

In line with current developments, namely more liberal criteria for potential living kidney donors, major anatomical deviations should not automatically be a contraindication. Multidisciplinary teamwork is currently the cornerstone of these kind of procedures, and this, together with thorough surgical preparation, should enable high-volume transplant centres to perform living donor hand-assisted laparoscopic nephrectomy in a safe and feasible way for donors with situs inversus totalis. This would enable centres to lower their refusal rates for potential donors based on their anatomical deviations, without additional hazards for the donor.

REFERENCES

1. Reese PP, Boudville N, Garg AX. Living kidney donation: outcomes, ethics, and uncertainty. *Lancet (London, England)*. 2015;385(9981):2003-2013.
2. Lentine KL, Kasiske BL, Levey AS, et al. KDIGO Clinical Practice Guideline on the Evaluation and Care of Living Kidney Donors. *Transplantation*. 2017;101(8S Suppl 1):S7-S105.
3. Chen J, Bhattacharya S, Sirota M, et al. Assessment of Postdonation Outcomes in US Living Kidney Donors Using Publicly Available Data Sets. *JAMA Netw open*. 2019;2(4):e191851.
4. Lentine KL, Lam NN, Segev DL. Risks of living kidney donation: Current state of knowledge on outcomes important to donors. *Clin J Am Soc Nephrol*. 2019;14(4):597-608.
5. Segev DL, Muzaale AD, Caffo BS, et al. Perioperative mortality and long-term survival following live kidney donation. *JAMA*. 2010;303(10):959-966.
6. Ahearn AJ, Posselt AM, Kang SM, Roberts JP, Freise CE. Experience with laparoscopic donor nephrectomy among more than 1000 cases: Low complication rates, despite more challenging cases. *Arch Surg*. 2011;146(7):859-864.
7. Zorgdrager M, van Londen M, Westenberg LB, et al. Chronic pain after hand-assisted laparoscopic donor nephrectomy. *BJS*. Published online 2019;bjs.11127.
8. Gross CR, Messersmith EE, Hong BA, et al. Health-Related Quality of Life in Kidney Donors From the Last Five Decades: Results From the RELIVE Study. *Am J Transplant*. 2013;13(11):2924-2934.
9. Horvat LD, Shariff SZ, Garg AX. Global trends in the rates of living kidney donation. *Kidney Int*. Published online 2009.
10. Davis CL, Delmonico FL. Living-donor kidney transplantation: A review of the current practices for the live donor. *J Am Soc Nephrol*. 2005;16(7):2098-2110.
11. Perlis N, Connelly M, D'A Honey JR, Pace KT, Stewart R. Evaluating potential live-renal donors: Causes for rejection, deferral and planned procedure type, a single-centre experience. *Can Urol Assoc J*. 2013;7(1-2):41-45.
12. Bia MJ, Ramos EL, Danovitch GM, et al. Evaluation of living renal donors: The current practice of US transplant centers. *Transplantation*. 1995;60(4):322-327.
13. Andrews PA, Burnapp L, Manas D, et al. Summary of the British Transplantation Society/Renal Association U.K. guidelines for living donor kidney transplantation. *Transplantation*. 2012;93(7):666-673.
14. Allen MB, Abt PL, Reese PP. What are the harms of refusing to allow living kidney donation? An expanded view of risks and benefits. *Am J Transplant*. 2014;14(3):531-537.
15. Reese PP, Allen MB, Carney C, et al. Outcomes for individuals turned down for living kidney donation. *Clin Transplant*. 2018;32(12):e13408.
16. Leigh MW, Pittman JE, Carson JL, et al. Clinical and genetic aspects of primary ciliary dyskinesia/kartagener syndrome. *Genet Med*. 2009;11(7):473-487.
17. Clavien PA, Barkun J, De Oliveira ML, et al. The Clavien-Dindo classification of surgical complications: Five-year experience. *Ann Surg*. 2009;250(2):187-196.
18. Sharma S. Situs Inversus Totalis (Dextroversion) - An Anatomical Study. *Anat Physiol*. 2012;02(05).
19. Sonawane G, Moorthy K, Pillai B. Complete situs inversus - is it a contraindication for organ donation? *Indian J Urol*. 2019;35(4):303.
20. Gahagan J V, Whealon MD, Reddy U, Foster CE, Ichii H. Hand-Assisted Laparoscopic Donor Nephrectomy in Complete Situs Inversus. *J Endourol Case Reports*. 2016;2(1):108-110.
21. Gonzalez-Heredia R, Garcia-Roca R, Benedetti E. Hand-assisted robotic right

- donor nephrectomy in patient with total situs inversus: A case report. *Int J Surg Case Rep.* 2016;23:44-46.
22. Hoffmann D, Gunselman J, Chewaproug D, et al. Living-related Donor Nephrectomy in a Patient With Complete Situs Inversus. *J Exp Clin Med.* 2010;2(6):305-306.
 23. Black PC, Porter JR, Charpentier KP, Bakthavatsalam R, Marsh CL. Hand-assisted laparoscopic right-donor nephrectomy in a patient with situs inversus. *Transplantation.* 2003;76(10):1530.
 24. Berber I, Gures N, Gurluler E, Alim A, Cakir U, Gurkan A. Total Laparoscopic Donor Nephrectomy In Situs Inversus Totalis: A Case Report. *Exp Clin Transplant.* 2013;11(2):195-198.

Kidney allograft cyst infection

Kidney International Reports, 2020

Geraldo R. Ramos de Freitas

Stan Benjamens

Priscila Dias Gonçalves

Maria L. Castelli de Azevedo

Evandro Reis da Silva Filho

José O. Medina-Pestana

Robert A. Pol

Thiago Reis

INTRODUCTION

Due to the persistent shortage of kidney allograft available for transplantation, living donor selection criteria have been expanded in recent years. As an example, kidney cysts found during pre-donation imaging screening are no longer a contraindication for living donor kidney donation.^{1,2}

Kidney cysts are characterised under the Bosniak classification system.³ The most common and of benign morphology is the *Bosniak grade 1*, usually referred to as simple cyst. As part of the pre-donation screening, potential living kidney donors undergo contrast-enhanced computed tomography (CT) imaging. This pre-donation imaging screening reveals asymptomatic and usually clinically irrelevant anatomical deviations.⁴ The literature available on this topic suggests that simple cysts are found in approximately 10% of living donors.^{5,6} The prevalence of simple cysts increases progressively with age and the majority of them remains asymptomatic.^{7,8} Even though kidney allografts with simple cysts have been shown to be associated with reduced transplant function, transplantation of kidney allografts with simple cysts is commonly performed.⁵ Cyst unroofing or excision of the cyst is generally not necessary, however, it can be performed if the cyst is large and superficial.⁶

The case reported here highlights a potential clinical challenge related to living donor kidney transplantation of a kidney allograft with a simple cyst and shows that recurrent cyst infections should be considered as part of the differential diagnosis of fever in kidney transplant patients.

CASE REPORT

A 34-year-old woman with chronic kidney disease due to diabetic nephropathy received a pre-emptive living donor kidney transplant from her healthy 32-year old husband. During pre-donation screening a cyst was identified in the right kidney on contrast-enhanced CT. This 5.9-cm cyst had an imperceptible wall, was round, had a benign appearance and was classified as a *Bosniak grade 1* kidney cyst (Figure 1). Mismatch results for HLA antigen showed 1, 2 and 1 mismatch for HLA-A, -B, and -DR antigens, respectively. Pre-transplantation panel reactive antibody (PRA) for both class I and II was zero. After discussion at our multidisciplinary conference, the transplantation was cleared to proceed despite the renal cyst. This was based on the recommendations in the *KDIGO Clinical Practice Guidelines on the Evaluation and Care of Living Kidney Donors*.¹ The cyst was unroofed following donor nephrectomy, after which the transplant procedure was performed. The donor's right kidney was implanted in the recipient's right iliac fossa and the postoperative period was uncomplicated for both donor and recipients. Induction regimen included thymoglobulin 3mg/kg single dose and methylprednisolone 1000 mg. The patient was on tacrolimus, azathioprine, and prednisolone for maintenance therapy. During the first year of post-transplant follow-up, there were no transplant-related complications and the kidney allograft function remained stable.

Figure 1. Computed tomography images of the donor, showing a 5.9-cm renal cyst (A), with an imperceptible wall and benign appearance (B), classified as *Bosniak grade 1*.

Fourteen months after transplantation, the transplant recipient presented with pain in the right iliac fossa, without fever or signs of a urinary infection. Laboratory tests were unremarkable and plasma Beta HCG was negative. An abdominal CT did not reveal the source of the symptoms. However, the kidney allograft showed a recurrence of the previously unroofed simple cyst (Figure 2). Without a clear diagnosis regarding the lower

abdominal pain, analgesics were prescribed (i.e. paracetamol 1 gram / 4 times a day and metamizole (dipyrone) 1 gram up to 4 times a day) which provided adequate pain relief and the patient was subsequently discharged.

Figure 2. Computed tomography image of the transplant recipient at one-year after transplantation, showing the initially unroofed cyst which regained full size due to allograft encapsulation (A) and an ultrasound of the kidney allograft with signs of simple cyst infection (B).

Six days after discharge, the patient presented again with worsening pain and fever (39°C), but without any urinary symptoms. Laboratory testing revealed leucocytosis with left shift, acute kidney injury *KDIGO 1*, normal urine analysis and urine culture was negative.⁹ Doppler ultrasound of the kidney allograft showed normal perfusion, however, heterogeneity and debris in the simple cyst was observed (Figure 2), leading to the possibility that a cyst infection was present.

Empirical treatment with intravenous ciprofloxacin 400mg two times a day was initiated. A partial clinical response on this antibiotic therapy was observed: Fever lasted for 48 hours, after which the body temperature normalized. Sustained leucocytosis was observed for three days after therapy initiation. Pain relief and reduction of asthenia was initially achieved in 24 hours, but both returned after 72 hours. Thereafter, a percutaneous CT-guided drainage of the infected cyst resulted in removal of 170mL purulent secretion. A pigtail drainage catheter was left in the cyst (Figure 3). Contrast-enhanced CT showed no connection of the cyst with the urinary tract (Figure 4). Serum creatinine was 1.53 mg/dl and cyst creatinine were 1.41 mg/dl. Analysis of the cyst fluid revealed 656,000 cells/mm³ with 98% neutrophils and the fluid culture was negative. The pigtail catheter was maintained for seven days, with continued antibiotic treatment. Kidney allograft function and other laboratory parameters returned to baseline and ciprofloxacin treatment was stopped after catheter removal.

Figure 3. Percutaneous computed tomography guided drainage of the simple cyst (A) with purulent secretion (B).

Figure 4. Contrast-enhanced computed tomography study showing no connection between cyst and urinary tract.

DISCUSSION

In the context of a clinical picture of an infection without clear source in a transplant patient with a kidney cyst, cyst infection should be considered in the differential diagnosis. This diagnosis is infrequent but can lead to serious illness and worsening kidney allograft function in case of diagnostic delay (Table 1).

Table 1. Teaching points

1.	Simple kidney cysts are no contraindication for living donor kidney donation
2.	Cyst unroofing or excision is generally not necessary, but can be performed if the cyst is large and on the parenchymal surface
3.	Previously unroofed kidney allograft cysts can regain full size due to allograft encapsulation
4.	Recurrence of an unroofed cyst, in combination with abdominal pains and fever, points towards a cyst infection
5.	Decision for antibiotic regimen should be based on drug penetration
6.	Antibiotic therapy should be combined with cyst drainage

With an increase in the age of living kidney donors, due to more liberal donor inclusion criteria, one can expect to identify more simple cysts during pre-donation imaging screening.

Imaging studies showed an increase in the prevalence of simple cyst in the older population. Ultrasound screening of asymptomatic individuals, grouped as 30-49, 50-70 and over 70 years of age, revealed simple cysts in 1.7%, 11.5% and 22.1%, respectively.¹⁰ CT screening identified an even higher prevalence of 8.2%, 27.5%, 49% and 60.6% in individuals between 17-39, 40-59, 60-80 and over 80 years, respectively.¹¹ Prevalence is higher in male individuals, individuals with a history of smoking, patients with hypertension and chronic kidney disease.⁴ In living kidney donor candidates, simple cysts prevalence ranges from 10-43%.⁴⁻⁶

Despite the high prevalence, simple cysts related complications are rarely reported in native kidneys and include flank pain, haematuria, intracystic haemorrhage, and pelvicalyceal obstruction in a small percentage of patients.¹² The most common symptoms requiring intervention on simple cysts are related to increase in cyst size and compression of the surrounding structures. Cyst aspiration, sclerotherapy obstruction and laparoscopic excision are the interventions usually performed.

The experience with treating cyst infections derives from patients with autosomal dominant polycystic kidney disease (ADPKD) where this complication is not uncommon. The preferred imaging modalities to diagnose cyst infection are magnetic resonance imaging (MRI) and positron emission tomography–CT (PET-CT).¹³ The choice of antibiotic for cyst infections should be based on the ability to penetrate into the cyst cavity. Quinolones are usually used as first line treatment, but co-trimoxazole, chloramphenicol and aminoglycosides may also provide adequate cyst penetration.¹⁴ In addition to antibiotic treatment opportunities, drainage of the cyst under CT or ultrasound guidance is usually necessary.

In the case presented, initial symptoms and subsequent laboratory findings did not raise suspicion for cyst infection during the patient's first presentation. However, during the second admission after six days, ultrasound findings pointed towards the diagnosis of kidney allograft cyst infection. This diagnosis was confirmed by the drainage of purulent fluid, showing neutrophils. The treatment approach with a combination of antibiotic therapy and drainage resulted in prompt clinical improvement. The exact cause for the cyst infection was not determined but we hypothesize this could result from transient asymptomatic bacteraemia; contrast-enhanced CT showed no connection of the cyst with the urinary tract.

To our knowledge, this is the first report of a simple kidney allograft cyst infection. If performed with the precautions outlined in international guidelines, transplantation of kidney allografts with simple, *Bosniak grade 1*, kidney cysts can be performed safely. However, cysts may recur despite unroofing and rarely present with infection.

REFERENCES

1. Lentine KL, Kasiske BL, Levey AS, et al. KDIGO Clinical Practice Guideline on the Evaluation and Care of Living Kidney Donors. *Transplantation*. 2017;101(8S Suppl 1):S7-S105.
2. Minnee RC, Kimenai HJAN, Verhagen PC, et al. Algorithm for Bosniak 2F Cyst in Kidney Donation. *Am J Case Rep*. 2017;18:733-738.
3. Israel GM, Bosniak MA. An update of the Bosniak renal cyst classification system. *Urology*. 2005;66(3):484-488.
4. Rule AD, Sasiwimonphan K, Lieske JC, Keddiss MT, Torres VE, Vrtiska TJ. Characteristics of renal cystic and solid lesions based on contrast-enhanced computed tomography of potential kidney donors. *Am J Kidney Dis*. 2012;59(5):611-618.
5. Qiu W, Jiang Y, Wu J, et al. Simple Cysts in Donor Kidney Contribute to Reduced Allograft Function. *Am J Nephrol*. 2017;45(1):82-88.
6. Grotemeyer D, Voiculescu A, Iskandar F, et al. Renal Cysts in Living Donor Kidney Transplantation: Long-Term Follow-up in 25 Patients. *Transplant Proc*. 2009;41(10):4047-4051.
7. Eknayan G. A clinical view of simple and complex renal cysts. *J Am Soc Nephrol*. 2009;20(9):1874-1876.
8. Simms RJ, Ong ACM. How simple are "simple renal cysts"? *Nephrol Dial Transplant*. 2014;29(suppl 4):iv106-iv112.
9. Kellum JA, Lameire N, Aspelin P, et al. Kidney disease: Improving global outcomes (KDIGO) acute kidney injury work group. KDIGO clinical practice guideline for acute kidney injury. *Kidney Int Suppl*. 2012;2(1):1-138.
10. Ravine D, Gibson RN, Donlan J, Sheffield LJ. An Ultrasound Renal Cyst Prevalence Survey: Specificity Data for Inherited Renal Cystic Diseases. *Am J Kidney Dis*. 1993;22(6):803-807.
11. Carrim ZI, Murchison JT. The prevalence of simple renal and hepatic cysts detected by spiral computed tomography. *Clin Radiol*. 2003;58(8):626-629.
12. Terada N, Arai Y, Kinukawa N, Yoshimura K, Terai A. Risk factors for renal cysts. *BJU Int*. 2004;93(9):1300-1302.
13. Sallée M, Rafat C, Zahar JR, et al. Cyst infections in patients with autosomal dominant polycystic kidney disease. *Clin J Am Soc Nephrol*. 2009;4(7):1183-1189.
14. Lantinga MA, Casteleijn NF, Geudens A, et al. Management of renal cyst infection in patients with autosomal dominant polycystic kidney disease: A systematic review. *Nephrol Dial Transplant*. 2017;32(1):144-150.

The fear for contrast-induced nephropathy in kidney transplant recipients: Time for a paradigm-shift?

Transplant International, 2018

Stan Benjamens
Derya Yakar
Riemer H.J.A. Slart
Jan-Stephan F. Sanders
Robert A. Pol

DEAR EDITORS,

A recently published article in the Lancet (AMACING trial) challenges a long-standing clinical consensus: intravenous (i.v.) volume expansion with isotonic saline as a prophylactic measure for iodinated contrast-induced nephropathy (CIN).¹ The randomized trial showed that — in patients with an estimated glomerular filtration rate (eGFR) of 30–59 mL/min/1.73m², undergoing an elective procedure — as compared to i.v. hydration, no prophylaxis is non-inferior in preventing CIN.

While caring for kidney transplant recipients, we are often challenged by the widely applied clinical practice of using iodinated contrast for diagnostic and interventional purposes, such as computed tomography (CT), CT angiography, and endovascular procedures. On paper, the current reluctance to avoid iodinated contrast after transplantation may delay diagnosis of post-transplant complications. This could possibly be avoided, since the median (2.5-97.5 percentile) measured GFR (mGFR) at 10 weeks post-transplant is 51 (29 – 78), indicating that most kidney transplant recipients have a mGFR comparable to the patients enrolled in the AMACING trial.² Previous retrospective cohort studies have reported on the incidence of CIN in kidney transplant recipients, showing an incidence of 2.9-13.3% after infusion of iodinated contrast for CT, 12.9% after cardiac catheterization, 8.1% after renal graft catheter arteriography, and 12.5% after endovascular aortic aneurysm repair (Table 1).³⁻⁷ This, compared to the most recent meta-analysis on CIN in the general population, reporting a 7.2% incidence of CIN after iodinated contrast CT.⁸ Even though the studies with kidney transplant recipients are small and retrospective, based on these data, the *Contrast Medium Safety Committee* of the *European Society of Urogenital Radiology*, concluded that there is insufficient evidence to substantiate a higher incidence of CIN in kidney transplant recipients than in the non-transplant population.⁹ Hereby, kidney transplant recipients should be treated in concordance with non-transplant recipients with a similar eGFR. As such, the paradigm-shift on prophylaxis for CIN in the general population, supported by the AMACING trial, could also be applied to kidney transplant recipients: for patients with an eGFR above 59, the risk for CIN is low and prophylaxis is not required. Moreover, for patients with an eGFR between 30–59, no prophylaxis can be considered as a safe alternative for elective procedures using iodinated-contrast. Meanwhile, for patients with an eGFR under 30, prophylaxis with i.v. sodium chloride or i.v. sodium bicarbonate is advised. However, these patients are underrepresented in the literature.

Table 1. Literature overview on the incidence of contrast-induced nephropathy in kidney transplant recipients

	Jawdeh (2017) ³	Fananapazir (2016) ⁴
Sample size	76	104
Inclusion period	2000-2014	2005-2015
Intervention	CT (59%); Cardiac cath (41%)	CT
Prophylaxis		
IV volume expansion	53%	85%
N-acetylcysteine	36%	14%
Definition of CIN	Increase in sCr of ≥ 0.3 or $\geq 25\%$ drop in eGFR	Increase in sCr of (i) ≥ 0.3 or (ii) 0.5
Incidence of CIN	CT: 6 (13.3%) Cath: 4 (12.9%)	(i) 7 (6.7%) or (ii) 3 (2.9%)
Resulting in dialysis	None	None

CT = computed tomography; Cardiac cath = cardiac catheterization; sCr = serum creatinine in mg/dL

Based on the current literature, a clear recommendation for either i.v. sodium chloride or i.v. sodium bicarbonate cannot be stated. The results of the PRESERVE trial, performed in patients with an eGFR of 15–44.9 or 45–59.9 in diabetic patients, were presented in a recent NEJM article. When comparing patients receiving either i.v. sodium bicarbonate or i.v. sodium chloride, there was no significant difference in the incidence of CIN after angiography, indicating that both types of prophylaxis lead to similar outcomes.¹⁰

In short, there has been a paradigm-shift on prophylaxis for CIN in the general population: going from prophylaxis in patients with an eGFR under 60 to prophylaxis for only those high-risk patients with an eGFR under 30. This beckons the discussion to equate kidney transplant recipients to non-transplant patients in contrast administration procedures, with the suggestion to make an eGFR based decision on the need for prophylaxis for each specific kidney transplant recipients. Implementation of these recommendations will arguably result in faster and more accurate diagnosis of post-transplant complications, since contrast-enhanced diagnostic and intervention procedures are, in many cases, the standard of care.

Bostock (2016) ⁵	Fananapazir (2016) ⁶	Haider (2015) ⁷
40	37	124
2003-2014	2006-2014	2002-2013
Endovascular aortic aneurysm repair	Renal graft catheter arteriography	CT (77%); Cardiac cath (5%); pulmonary angiogram (18%)
Not mentioned	58%	70%
	15%	30%
Increase in sCr of ≥ 0.5	Increase in sCr of ≥ 0.5	Increase in sCr of ≥ 0.5 or $\geq 25\%$ drop in eGFR
5 (12.5%)	3 (8.1%)	7 (5.6%)
1 (2.5%)	None	None

REFERENCES

1. Nijssen EC, Rennenberg RJ, Nelemans PJ, et al. Prophylactic hydration to protect renal function from intravascular iodinated contrast material in patients at high risk of contrast-induced nephropathy (AMACING): a prospective, randomised, phase 3, controlled, open-label, non-inferiority trial. *Lancet*. 2017;389(10076):1312-1322.
2. Salvador CL, Hartmann A, Åsberg A, Bergan S, Rowe AD, Mørkrid L. Estimating Glomerular Filtration Rate in Kidney Transplant Recipients: Comparing a Novel Equation With Commonly Used Equations in this Population. *Transplant direct*. 2017;3(12):e332.
3. Abu Jawdeh BG, Leonard AC, Sharma Y, et al. Contrast-Induced Nephropathy in Renal Transplant Recipients: A Single Center Experience. *Front Med*. 2017;4:64.
4. Fananapazir G, Troppmann C, Corwin MT, Nikpour AM, Naderi S, Lamba R. Incidences of acute kidney injury, dialysis, and graft loss following intravenous administration of low-osmolality iodinated contrast in patients with kidney transplants. *Abdom Radiol*. 2016;41(11):2182-2186.
5. Bostock IC, Zarkowsky DS, Hicks CW, et al. Outcomes of Endovascular Aortic Aneurysm Repair in Kidney Transplant Recipients: Results From a National Quality Initiative. *Am J Transplant*. 2016;16(8):2395-2400.
6. Fananapazir G, Troppmann C, Corwin MT, Bent CK, Vu CT, Lamba R. Incidence of Contrast-Induced Nephropathy After Renal Graft Catheter Arteriography Using Iodine-Based Contrast Medium. *Am J Roentgenol*. 2016;206(4):783-786.
7. Haider M, Yessayan L, Venkat KK, Goggins M, Patel A, Karthikeyan V. Incidence of Contrast-induced Nephropathy in Kidney Transplant Recipients. *Transplant Proc*. 2015;47(2):379-383.
8. Aycock RD, Westafer LM, Boxen JL, Majlesi N, Schoenfeld EM, Bannuru RR. Acute Kidney Injury After Computed Tomography: A Meta-analysis. *Ann Emerg Med*. 2018;71(1):44-53.e4.
9. van der Molen AJ, Reimer P, Dekkers IA, et al. Post-contrast acute kidney injury – Part 1: Definition, clinical features, incidence, role of contrast medium and risk factors. *Eur Radiol*. February 2018:1-11.
10. Weisbord SD, Gallagher M, Jneid H, et al. Outcomes after Angiography with Sodium Bicarbonate and Acetylcysteine. *N Engl J Med*. 2018;378(7):603-614.

Addendum

Summary in Dutch /
Nederlandse samenvatting
List of contributing authors
List of publications
Acknowledgments / Dankwoord
Curriculum Vitae

**Summary in Dutch /
Nederlandse samenvatting**

NEDERLANDSE SAMENVATTING

Na de eerste geslaagde niertransplantatie in 1954, hebben decennia van innovatie ertoe geleid dat dit de behandeling van voorkeur werd voor patiënten met eindstadium nierfalen. Deze vooruitgang in zorg voor nierpatiënten lijkt echter te stagneren sinds de start van de 21^{ste} eeuw. Dit is een trend die in Nederland samengaat met de toename van niertransplantatie na donatie bij leven. Tevens neemt donatie na overlijden ten gevolge van een circulatiestop toe ten opzichte van donatie na overlijden ten gevolge van hersendood. De leeftijd van zowel de donoren als de niertransplantatie ontvangers neemt toe, met daarbij samengaan hart- en vaatziekten.

In dit proefschrift wordt de rol beschreven van beeldvormende diagnostiek bij het verbeteren van de uitkomsten na niertransplantatie. Dit wordt weergegeven in tien hoofdstukken, die in de hierop volgende paragrafen zijn samengevat.

In **hoofdstuk 1**, de inleiding van dit proefschrift, wordt de geschiedenis van de transplantatiechirurgie en beeldvormende diagnostiek beschreven. Hierbij ligt de nadruk op de gedeelde geschiedenis van deze twee medische stromingen, als een voorbeeld van innovatie door samenwerking. Daarnaast geeft dit hoofdstuk een overzicht van de stand van zaken met betrekking tot orgaandonatie en niertransplantatie in Nederland. **Hoofdstuk 2** gaat over een bredere blik naar het wetenschappelijke veld rondom (nier)transplantatie. In een samenvatting van drie bibliometrische studies, oftewel analyses van grote groepen wetenschappelijke publicaties, worden de volgende aspecten beschreven: (1) een gebrek aan samenwerking tussen verschillende medische disciplines; (2) verschillen tussen mannelijke en vrouwelijke wetenschappers met betrekking tot publicatie aantallen, citaties en wetenschappelijke financiering; (3) het effect van grote internationale veranderingen op de academische samenwerking tussen landen. Met betrekking tot het eerste onderdeel konden wij concluderen dat er weinig publicaties zijn over niertransplantatie en beeldvormende diagnostiek. Tevens werden de beschikbare publicaties weinig geciteerd. Hernieuwde aandacht voor dit onderwerp is belangrijk, omdat de huidige klinische richtlijnen onvoldoende wetenschappelijke onderbouwing hebben. Het tweede onderdeel geeft een beeld van de toename van vrouwelijke wetenschappers binnen de transplantatiechirurgie, met een relatief lager aantal citaties en minder verworven financiering ten opzichte van hun mannelijke collega's. In het derde onderdeel wordt de noodzaak voor een blijvende samenwerking tussen de landen van de Europese Unie (EU) en het Verenigd Koninkrijk beargumenteerd. Het Verenigd Koninkrijk ontvangt 10.7% van al het transplantatie-gerelateerde EU-

budget en participeert in meer dan de helft van de EU-onderzoeksprojecten. We konden op basis hiervan concluderen dat er een grote impact van Brexit op zowel het transplantatieonderzoek in het Verenigd Koninkrijk als op het onderzoek in de overige EU-landen wordt verwacht.

In **onderdeel I** wordt het gebruik van het renogram na niertransplantatie beschreven. **Hoofdstuk 3** is het resultaat van een systematisch literatuuronderzoek over het gebruik van het renogram voor het vaststellen van niet-acute vasculaire en urologische complicaties en het beoordelen van postoperatieve vochtcollecties. In de literatuur werd geen bewijs gevonden voor het gebruik van het renogram voor het diagnosticeren van transplantaatafstoting. De resultaten van de samenwerking tussen het Groninger Transplantatie Centrum en het Transplantatiecentrum Leiden worden beschreven in **hoofdstuk 4**. In een groep van in totaal 377 niertransplantatie ontvangers werd gevonden dat het vertraagd op gang komen van de nierfunctie na transplantatie kan worden beoordeeld met een renogram. Deze methode heeft een hoge sensitiviteit en een lage specificiteit voor het voorspellen van het aantal dagen waarna de nierfunctie opgang komt. **Hoofdstuk 5** gaat uitgebreider in op de patiënten van het Transplantatiecentrum Leiden. In deze groep werd het initiële renogram herhaald na drie tot zeven dagen. De uitkomsten van dit tweede renogram waren geassocieerd met het opgang komen van de nier en duur van de ziekenhuisopname, maar de vergelijking tussen het eerste en het tweede renogram resulteerde niet in een verbeterde voorspelling.

In **onderdeel II** worden de verschillende onderzoeken naar nier- en vaatverkalking beschreven. **Hoofdstuk 6** gaat over de radioactieve tracer [18-Fluor]-sodium fluoride ($[^{18}\text{F}]\text{-NaF}$). Deze tracer die bindt aan micro-verkalkingen in afgekeurde donornieren en in geëxplanteerde transplantatie nieren. Dit is een eerste stap voor het onderzoeken van $[^{18}\text{F}]\text{-NaF}$ PET-beeldvorming in niertransplantatie ontvangers. Deze techniek kan mogelijk worden gebruikt voor het vaststellen van verkalking van zowel de nier en de bloedvaten. In **hoofdstuk 7** wordt beschreven in welke mate verkalking van de buikslagader zichtbaar is, met DXA-beeldvorming, binnen zes maanden na transplantatie. In een groep van 701 patiënten werd vastgesteld dat de mate van vaatverkalking verband houdt met acute hart- en vaatziekte gerelateerde problematiek. **Hoofdstuk 8** gaat over de onderliggende oorzaken van vaatverkalking voor en na niertransplantatie. Hierbij werd een relatie gevonden tussen botontkalking en vaatverkalking na niertransplantatie. De resultaten van een samenwerking tussen het Transplantatiecentrum Groningen, het Transplantatiecentrum van het Erasmus MC en het Imperial College Renal and Transplant Centre worden beschreven in **hoofdstuk 9**. In een groep van 547 patiënten werd een nieuwe methode getest om de mate van vaatverkalking vast te stellen. Hierbij werd gebruik gemaakt van CT-beeldvorming en het resultaat wordt de “aorto-iliac

CaScore” genoemd. Het werd aangetoond dat deze aorto-iliac CaScore verband houdt met overlijden en acute hart- en vaatziekte gerelateerde problematiek. **Hoofdstuk 10** gaat over de relatie tussen een hogere aorto-iliac CaScore en een verlaagde nierfunctie op een jaar na transplantatie. De oudere leeftijd van donoren voor oudere ontvangers, binnen het *Eurotransplant Senior Programma*, was een belangrijke factor voor deze relatie. Een verband tussen de CaScore en zowel transplantaat falen (exclusief patiënt overlijden) en transplantatie functie achteruitgang werd niet gevonden, terwijl er wel een verband was van de CaScore met transplantaat falen (inclusief patiënt overlijden) en overlijden met een functionerend transplantaat.

DISCUSSIE

In de eerste periode van dit promotietraject is er gewerkt aan het standaardiseren van de beoordeling van het renogram. Met deze benadering kon worden aangetoond dat de uitkomsten van het renogram niet kunnen worden gebruikt voor een betrouwbare voorspelling van het moment van opgang komen van de nierfunctie of van de duur van de ziekenhuisopname. Echter blijft er een rol weggelegd voor het renogram bij de vaststellen van mogelijke vasculaire of urologische complicaties, dan wel voor de beoordeling van vochtcollecties na de operatie. Met het project over [^{18}F]-NaF PET-beeldvorming konden de mogelijkheden van deze techniek worden getoond voor het aantonen van verkalking van de nier. Deze techniek kan mogelijk worden ingezet om het effect van nier verkalking op nierfunctie na transplantatie te bepalen. Tevens kan deze techniek worden gebruikt voor het vaststellen van vaatverkalking. Voor DXA-beeldvorming en CT-beeldvorming heeft de nieuwe gestandaardiseerde beoordeling geleid tot een betrouwbare meting van vaatverkalking voor en na niertransplantatie. Hierbij werd aangetoond dat er een verband bestaat tussen een afname in botdichtheid en een toename in vaatverkalking na transplantatie. De in dit proefschrift gepresenteerde resultaten kunnen bijdragen aan de beoordeling van hart- en vaatziekten voorafgaand aan de niertransplantatie en in de periode daaropvolgend. Het identificeren van patiënten met een hoog risico op morbiditeit en mortaliteit ten gevolge van hart- en vaatziekten kan leiden tot patiënt-specifieke interventies. Hierbij kan worden gedacht aan een pre-habilitatie programma gericht op gewichtsverlies, stoppen met roken en/of het reduceren van algehele kwetsbaarheid, of extra aandacht voor de behandeling van een verhoogde bloeddruk, een verhoogd cholesterol en diabetes mellitus.

De kracht van dit proefschrift is niet de introductie van alleen nieuwe beeldvormende diagnostiek, maar de verkregen kennis over veel gebruikte diagnostische procedures. Hierbij is aandacht besteed aan het uitlichten van de beperkingen en mogelijke nieuwe toepassingen van diagnostische procedures binnen niertransplantatie programma's. In toekomstige medische richtlijnen dient een plek te zijn voor de indicaties en implicaties van het gebruik van beeldvormende diagnostiek voorafgaand en na niertransplantatie. Door de inzet van beeldvormende diagnostiek te standaardiseren kan de impact adequaat worden beoordeeld en vervolgens worden verbeterd.

VOORUITKIJKEND

Het ligt in de lijn der verwachtingen dat de vergrijzing van de bevolking zal voorzetten en dat de medische strijd tegen hart- en vaatziekten nog niet snel gewonnen is. Hart- en vaatziekten, en meer specifiek vaatverkalking, zullen een steeds groter probleem worden voorafgaande en na niertransplantatie. Om dit probleem het hoofd te bieden zal de inzet van beeldvormende diagnostiek essentieel zijn. Een innovatieve techniek beschreven in dit proefschrift, [^{18}F]-NaF PET-beeldvorming, kan de mogelijkheid bieden om versnelde ontwikkeling van vaatverkalking vast te stellen.

Voor het voorkomen en behandelen van acute transplantaatafstoting is in de afgelopen decennia veel vooruitgang geboekt. De huidige diagnostiek, middels een nier biopsie, is echter niet optimaal voor zowel patiënten als artsen. Een belangrijk gedeelte van het hier beschreven promotietraject is gericht geweest op een alternatief voor het nier biopsie middels beeldvormende diagnostiek. Dit niet-invasieve onderzoek, met Interleukin-2 PET-beeldvorming bij verdenking op transplantaatafstoting, zal in de komende jaren worden afgerond.

Om de samenwerking tussen de afdelingen chirurgie, nefrologie en beeldvormende diagnostiek te continueren, werkt ons team aan het opzetten van een prospectief internationaal onderzoek. In dit onderzoek zal worden gekeken naar het gebruik van functionele MRI-beeldvorming in combinatie met kunstmatige intelligentie. De nadruk zal liggen op het trainen van een algoritme om complicaties na transplantatie vast te stellen op basis van functionele MRI-beeldvorming. Dit onderzoek zal worden uitgevoerd binnen een consortium met het Transplantatiecentrum Groningen, de Hannover Medical School en Cercare Medical ApS.

Tot slot zal niet alleen gewerkt worden aan het versterken van internationale samenwerkingen, maar ook naar het consolideren van de samenwerking binnen Nederland. De oprichting van de Nederlandse niertransplantatie studiegroep (Dutch Kidney Transplant Study Group - DKSTG) heeft reeds geresulteerd in een succesvolle samenwerking tussen het Transplantatiecentrum Groningen en het Transplantatiecentrum van het Erasmus MC. Deze samenwerking zal naar verwachting een opmaat zijn voor vervolgprojecten met meerdere Nederlandse transplantatiecentra.

List of contributing authors

LIST OF CONTRIBUTING AUTHORS

Saleh Z. Alghamdi

Department of Surgery, Division of Transplant Surgery, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Ines F. Antunes

Department of Nuclear Medicine and Molecular Imaging, Medical Imaging Center, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Stephan J.L. Bakker

Department of Medicine, Division of Nephrology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Louise B.D. Banning

Department of Surgery, Division of Vascular Surgery, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Tamar A.J. van den Berg

Department of Surgery, Division of Transplant Surgery, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Stefan P. Berger

Department of Medicine, Division of Nephrology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Martin H. de Borst

Department of Medicine, Division of Nephrology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Marian L.C. Bulthuis

Department of Pathology and Medical Biology, Division of Pathology, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands

Maria L. Castelli de Azevedo

Department of Nephrology and Kidney Transplantation, Clínica de Doenças Renais de Brasília (CDRB), Brasília, Brazil

Guillermo Chong

Department of Radiology, Clínica Alemana De Santiago, Universidad del Desarrollo, Santiago, Chile

Priscila Dias Gonçalves

Department of Nephrology and Kidney Transplantation, Clínica de Doenças Renais de Brasília (CDRB), Brasília, Brazil

Petra Dibbets-Schneider

Department of Radiology, Division of Nuclear Medicine, Leiden University Medical Center, Leiden University, Leiden, The Netherlands

Frank J. M. F. Dor

Imperial College Renal and Transplant Centre, Hammersmith Hospital, Imperial College Healthcare NHS Trust, London, United Kingdom

Lioe-Fee de Geus-Oei

Department of Radiology, Division of Nuclear Medicine, Leiden University Medical Center, Leiden University, Leiden, The Netherlands

Andor W.J.M. Glaudemans

Department of Nuclear Medicine and Molecular Imaging, Medical Imaging Center, University Medical Center Groningen, Groningen, University of Groningen, The Netherlands

António W. Gomes-Neto

Department of Medicine, Division of Nephrology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Dion Groothof

Department of Medicine, Division of Nephrology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Jan-Luuk Hillebrands

Department of Pathology and Medical Biology, Division of Pathology, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands

Johan F.M. Lange

Department of Surgery, Division of Transplant Surgery, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

José O. Medina-Pestana

Department of Nephrology, Hospital do Rim e Hipertensão, Federal University of São Paulo, São Paulo, Brazil

Robert C. Minnee

Department of Surgery, Division of HPB & Transplant Surgery, Erasmus Medical Center (MC), Erasmus University, Rotterdam, The Netherlands

Cyril Moers

Department of Surgery, Division of Transplant Surgery, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Vassilios Papalois

Imperial College Renal and Transplant Centre, Hammersmith Hospital, Imperial College Healthcare NHS Trust, London, United Kingdom

Robert A. Pol

Department of Surgery, Division of Transplant Surgery, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Geraldo R. Ramos de Freitas

Department of Nephrology and Kidney Transplantation, Clínica de Doenças Renais de Brasília (CDRB), Brasília, Brazil

Melanie Reijrink

Department of Medicine, Division of Vascular Medicine, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Thiago Reis

Department of Nephrology and Kidney Transplantation, Clínica de Doenças Renais de Brasília (CDRB), Brasília, Brazil

Evandro Reis da Silva Filho

Department of Nephrology and Kidney Transplantation, Clínica de Doenças Renais de Brasília (CDRB), Brasília, Brazil

Elsaline Rijkse

Department of Surgery, Division of HPB & Transplant Surgery, Erasmus Medical Center (MC), Erasmus University, Rotterdam, The Netherlands

Jan-Stephan F. Sanders

Department of Medicine, Division of Nephrology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Riemer H.J.A. Slart

Department of Nuclear Medicine and Molecular Imaging, Medical Imaging Center, University Medical Center Groningen, Groningen, University of Groningen, The Netherlands

Camilo G. Sotomayor

Department of Medicine, Division of Nephrology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Charlotte A. te Velde-Keyzer

Department of Medicine, Division of Nephrology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Aiko P.J. de Vries

Department of Medicine, Division of Nephrology, Leiden University Medical Center, Leiden University, Leiden, The Netherlands

Ivanka Wieringa

Department of Medicine, Division of
Nephrology, University Medical Center
Groningen, University of Groningen,
Groningen, The Netherlands

Derya Yakar

Department of Radiology, Medical Imaging
Center, University Medical Center
Groningen, Groningen, University of
Groningen, The Netherlands

List of publications

LIST OF PUBLICATIONS

Publications included in this thesis

- Benjamens S, Berger SP, Glaudemans AWJM, Sanders JSF, Pol RA, Slart RHJA. Renal scintigraphy for post-transplant monitoring after kidney transplantation. *Transplant Rev.* 2018;32(2):102-109.
- Benjamens S, Glaudemans AWJM, Berger SP, Slart RHJA, Pol RA. Have we forgotten imaging prior to and after kidney transplantation? *Eur Radiol.* 2018;28(8):3263-3267.
- Benjamens S, Pol RA, de Geus-Oei L-F, et al. Can transplant renal scintigraphy predict the duration of delayed graft function? A dual center retrospective study. *PLoS One.* 2018;13(3):e0193791.
- Benjamens S, Yakar D, Slart RHJA, Sanders J-SF, Pol RA. The fear for contrast-induced nephropathy in kidney transplant recipients: time for a paradigm shift? *Transpl Int.* 2018;31(9):1050-1051.
- Benjamens S, Pol RA, Glaudemans AWJM, et al. A high abdominal aortic calcification score by dual X-ray absorptiometry is associated with cardiovascular events after kidney transplantation. *Nephrol Dial Transplant.* 2018;33(12):2253-2259.
- Benjamens S, van den Berg TAJ, Pol RA. C4: The future of solid organ transplantation from the perspective of young transplant professionals. *Am J Transplant.* 2019;19(6):1867-1867.
- Benjamens S, Pol RA, Berger SP, et al. Limited clinical value of two consecutive post-transplant renal scintigraphy procedures. *Eur Radiol.* 2020;30(1):452-460.
- Benjamens S, van den Berg TAJ, Papalois V, Dor FJMF, Pol RA. Brexit and Transplantation Research. *Transplantation.* 2020;104(1):6-9.
- Benjamens S, van den Berg TAJ, Lange JFM, Pol RA. Living donor hand-assisted laparoscopic nephrectomy in a healthy individual with situs inversus totalis: no need to turn down the donor. *BMJ Case Rep.* 2020;13(1):e233523.
- Sotomayor CG, Benjamens S, Gomes-Neto AW, et al. Bone Mineral Density and Aortic Calcification: Evidence for a Bone-Vascular Axis After Kidney Transplantation. *Transplantation.* Published online March 2020:1.
- Ramos de Freitas GR, Benjamens S, Gonçalves PD, et al. Kidney Allograft Cyst Infection. *Kidney Int Reports.* 2020;5(7):1114-1117.
- Benjamens S, Rijkse E, te Velde-Keyzer CA, et al. Aorto-Iliac Artery Calcification Prior to Kidney Transplantation. *J Clin Med.* 2020;9(9):2893.
- Benjamens S, Banning LBD, van den Berg TAJ, Pol RA. Gender Disparities in Transplantation Research. *Transplant Direct.* 2020;6(11):e614.
- Benjamens S, Moers C, Slart RHJA, Pol RA. Kidney Transplantation and Diagnostic Imaging: The Early Days and Future Advancements of Transplant Surgery. *Diagnostics.* 2021;11(1):47.

- Benjamens S, Antunes IF, Hillebrands JL, et al. [18F]-sodium fluoride autoradiography imaging of nephrocalcinosis in donor kidneys and explanted kidney allografts. *Scientific Reports*. 2021.
- Benjamens S, Alghamdi SZ, Rijkse E, et al. Aorto-Iliac Artery Calcification and Graft Outcomes in Kidney Transplant Recipients. *J Clin Med*. 2021;10(2):325.

Other publications

- Benjamens S, Dullaart RPF, Sluiter WJ, Rienstra M, van Gelder IC, Links TP. The clinical value of regular thyroid function tests during amiodarone treatment. *Eur J Endocrinol.* 2017;177(1):9-14.
- Benjamens S, Leemkuil M, Margreiter C, Huurman VA, Leuvenink HG, Pol RA. A steady decline in pancreas transplantation rates. *Pancreatology.* 2019;19(1).
- Benjamens S, van den Berg TAJ, Kuipers TGJ, et al. Kidney temperature during living donor kidney transplantation is associated with short-term measured glomerular filtration rate - a prospective study. *Transpl Int.* 2020;33(2):174-180.
- Janssen YF, van der Plas WY, Benjamens S, Kruijff S. Cancer in low and middle income countries - The same disease with a different face. *Eur J Surg Oncol.* 2020;46(1):1-2.
- Benjamens S, Leemkuil M, Pol RA. Pancreas transplantation in patient with long-standing diabetes mellitus: How to judge fitness for transplant? *Clin Transplant.* 2019;33(11).
- Benjamens S, Weel J, Bosch B. [Cardiologische afwijkingen bij patiënten met Lymeboorrelie]. *Huisarts Wet.* 2019;62(5):51-52.
- van der Plas WY, Benjamens S, Kruijff S. The increased need for palliative cancer care in Sub-Saharan Africa. *Eur J Surg Oncol.* 2020;46(7):1373-1376.
- van der Plas W, Benjamens S, Janssen Y, van de Vijver S, Kruijff S. [Palliatieve geneeskunde in sub-Sahara Afrika]. *Huisarts Wet.* 2020;63(7):49-51.
- Meyer VE, Benjamens S, El Moumni M, Lange JFM, Pol RA. Global Overview of Response Rates in Patient and Health Care Professional Surveys in Surgery. *Ann Surg.* Published online July 7, 2020.
- Reijrink M, de Boer SA, ..., Benjamens S, et al. [18F]FDG Uptake in Adipose Tissue Is Not Related to Inflammation in Type 2 Diabetes Mellitus. *Mol Imaging Biol.* Published online September 4, 2020.
- Benjamens S, Dhunnoo P, Meskó B. The state of artificial intelligence-based FDA-approved medical devices and algorithms: an online database. *npj Digit Med.* 2020;3(1):118.
- Benjamens S, de Meijer VE, Pol RA, Haring MPD. Are all voices heard in the COVID-19 debate? *Scientometrics.* Published online 2020.
- van de Kuit A, Benjamens S, Sotomayor CG, et al. Postoperative Ultrasound in Kidney Transplant Recipients: Association Between Intrarenal Resistance Index and Cardiovascular Events. *Transplant Direct.* 2020;6:e581.

Dankwoord

DANKWOORD

In de zomer van 2013 stond ik voor het eerst bij een niertransplantatie, als jonge student van het Prometheus Nierteam. Op dat moment had ik niet kunnen bedenken dat ik bijna acht jaar later een promotietraject over datzelfde onderwerp zou hebben doorlopen. In deze jaren heb talloze mensen ontmoet en een aantal van hen wil ik persoonlijk bedanken.

Dr. Robert A. Pol, beste Robert, door jouw enthousiasme voor onderwijs en wetenschap ben ik in dit onderzoek verzeild geraakt. Je grootse wetenschappelijke dromen en je optimisme over elk nieuw project hebben mijn promotie tot een succes gemaakt. De aandacht die jij hebt voor de sociale kant van begeleiden is bewonderenswaardig, met etentjes bij je thuis en het op sleeptouw nemen van alle onderzoekers bij congressen. Bij dit alles kan ik natuurlijk je sarcastische grappen, je verschrikkelijke oog voor detail en je soms lachwekkende smetvrees niet vergeten. We hebben in de afgelopen jaren samen veel bereikt en hopelijk zullen er nog talloze gezamenlijke projecten volgen.

Professor dr. Riemer H.J.A. Slart, beste Riemer, een snellere en meer betrokken promotor kan niemand zich wensen. Gedurende mijn gehele promotietraject stond je klaar om mijn vragen te beantwoorden en om adviezen te geven. Langer dan een dag heb ik nooit op een antwoord moeten wachten. Je liet me vrij bij het vormgeven van mijn eigen promotie en hebt me alle – soms nuttige en soms irrelevante – zijpaden laten bewandelen.

Professor dr. Stefan P. Berger, beste Stefan, gedurende mijn promotietraject ben je zowel hoogleraar als afdelingshoofd geworden en toch heb je steeds de tijd gevonden voor mijn projecten. Je kritische blik en aandacht voor de klinische relevantie hebben dit proefschrift naar een hoger niveau gebracht. Voor de toekomst hoop ik nog vaak gebruik te mogen maken van deze eigenschappen.

Graag wil ik de leescommissie bedanken voor de interesse in mijn proefschrift. **Professor dr. Jean-Paul P.M. de Vries**, **Professor dr. Mathias Prokop** en **Professor dr. Jan N.M. IJzermans**, dank voor het commentaar dat jullie hebben gegeven: het is een eer dat jullie onderdeel willen zijn van mijn promotie.

Professor dr. Andor W.J.M. Glaudemans en **dr. Jan-Stephan F. Sanders**, beste Andor en Jan-Stephan, jullie zijn een onmisbaar onderdeel van onze onderzoeksgroep, met altijd een positieve blik en een enorm enthousiasmerende passie voor jullie vakgebieden. Tot mijn grote spijt is het ons niet gelukt om het IL2-PET/CT-project de kers op de taart van dit proefschrift te laten zijn, dus gelden de simpele woorden: dit wordt vervolgd.

Professor dr. Martin H. de Borst, Professor dr. Jan-Luuk Hillebrands, Professor dr. Stephan J.L. Bakker, dr. Aiko P.J. de Vries, dr. Cyril Moers, dr. Derya Yakar en Dr. Johan F.M. Lange, beste Martin, Jan-Luuk, Stephan, Aiko, Cyril, Derya en Johan, dank voor jullie bijdrages aan de verschillende hoofdstukken van mijn proefschrift. Jullie frisse blik, ongezouten commentaar en creatieve oplossingen hebben elk afzonderlijk hoofdstuk sterker gemaakt. Ik heb veel van jullie kunnen leren en het was een groot plezier om met jullie samen te werken.

Professor dr. Lioe-Fee de Geus-Oei, beste Lioe-Fee, jij ontving mij met open armen op de Leidse afdeling nucleaire geneeskunde. De periode van twee maanden non-stop data verzamelen vloog voorbij en het resultaat vormt de uiteindelijke basis van dit proefschrift. Hopelijk kruisen onze paden nog vaker!

Dr. Robert C. Minnee en drs. Elsaline Rijkse, beste Robert en Elsaline, dank voor jullie warme welkom in Rotterdam. Na onze eerste gezamenlijke projecten en met mijn huidige stap naar Rotterdam, hoop ik nog vele projecten met jullie te mogen uitvoeren.

Professor dr. Frank J.M.F. Dor, beste Frank, tot mijn grote spijt heeft de periode van drie maanden intensief samenwerken in Londen door een plotselinge pandemie niet kunnen plaatsvinden. Met je wereldwijde en met name Europese inzet voor de transplantatiechirurgie ben je voor mij een groot voorbeeld. Ik hoop in de komende jaren de ruimte te vinden om een periode in Londen met je samen te werken.

Lisa Emmens en Sebastiaan Dulfer, mijn paranimfen en beste vrienden, al sinds ons eerste jaar zijn wij de drie musketiers, een onafscheidelijk drietal. Samen hebben we vroeg in onze studie besloten dat een MD/PhD-traject ons eerste doel moest worden. Met een gedeelde passie voor onderzoek hebben we elkaar volop gesteund om dit doel te bereiken. Ik ben enorm trots dat ik met jullie steun mijn proefschrift mag verdedigen en ik zal even trots zijn als ik jullie je verdediging zie voeren.

Dr. Willemijn van der Plas, vanaf de eerste dag van onze stagewetenschap zijn we als een team opgetrokken. Samen hebben we ons door het eerste jaar onderzoek heen geslagen, waarna we voor onze coschappen beide in Zwolle terechtkwamen. We besloten samen om naar het Nkhoma Missie Ziekenhuis in Malawi af te reizen en we beklommen *en passant* de Kilimanjaro. Deze samenwerking heeft ons geen windeieren gelegd, aangezien jij in februari reeds je proefschrift hebt mogen verdedigen. Laten we elkaar in de toekomst nog veel steunen, want onze wegen gaan nog talloze keren kruisen.

Tamar van den Berg en **Wiesje Banning**, we hebben drie totaal verschillende promotie-onderwerpen en vormden desondanks een onderzoeksgroep onder leiding van Robert. We hebben de afgelopen jaren vooral veel samen gelachen en voor onderzoekers is dat de beste uitlaatklep. Van Robert leerden we dat onderzoek toch vooral een sociale bezigheid moet zijn. Deze les hebben we tot uitvoering gebracht door zoveel mogelijk congressen te bezoeken, om vooral veel nieuwe steden te ontdekken. Het ontdekken van de kroegen in Seoul werd ons door de pandemie ontnomen, dus dat moet nog zeker volgen!

Martijn Haring en **Bobby Pranger**, de jongens van het dag en nacht werken. Gezamenlijk hebben we ontelbare avond- en weekenduren doorgebracht in het Triadegebouw. Afgewisseld door een voortzetting van het studentenleven, met late uurtjes in de stad. Hoe blij ik ook ben met het afronden van dit proefschrift, er zal toch altijd heimwee blijven naar de Triadeborrels en Gyas schuimparty's.

Dr. Inês F. Antunes, **Marja van Dijk** en **Marian Bulthuis**, zonder jullie hulp was ik op de afdelingen nucleaire geneeskunde, nefrologie en pathologie geen stap verder gekomen. Inês, Marja en Marian, dank dat jullie altijd klaar stonden om te helpen, om geduldig uitleg te geven en om slimme oplossing aan te dragen. Jullie ervaring in het UMCG was voor mij onmisbaar.

Anouk van de Kuit, **Donika Zogaj**, **Evelien Quint**, **Floris Jolink**, **Ivanka Wieringa**, **Megan Bruins**, **Saleh Alghamdi** en **Jeroen Kelderman**, jullie hebben mij laten zien hoe leuk het is om (mede)studenten te begeleiden. Ik hoop dat ik mijn enthousiasme over wetenschappelijk onderzoek op jullie heb kunnen overbrengen.

Al mijn mede-onderzoekers bij de chirurgie, nefrologie en nucleaire geneeskunde: **Melanie Reijrink**, **Merel Pool**, **Marjolein Leemkuil**, **Rosa Lammerts**, **Leonie Venema**, **Isabel Brüggenwirth**, **Sanne Verkuyl**, **Tina Jager**, **Charlotte te Velde-Keyzer**, **Camilo Sotomayor** en **António Gomes-Neto**, jullie wil ik bedanken voor de fantastische jaren, de steun die we elkaar hebben geboden en de mooie congressen die we samen hebben meegemaakt.

Het Prometheus Nierteam: De studenten en alle betrokkenen, waaronder **Drs. Sijbrand Hofker**, **Drs. Christina Krikke** en **Professor dr. Henri G.D. Leuvenink**, wil ik bedanken voor de vliegende start bij de Chirurgie. Als achttienjarige student kreeg ik de mogelijkheid om met jullie niertransplantaties bij te wonen. Jullie warme ontvangst heeft uiteindelijk tot dit promotietraject binnen de transplantatiechirurgie geleid.

Mede-onderzoekers verspreid door het hele ziekenhuis: **Anouk de Wit, Charlotte Vollenbrock, Joost Beusekamp, Kim van der Sloot, Nick Schubert** en iedereen die verder beschikbaar was voor de tiende kop koffie of de zoveelste onderzoekmijdende wandeling. Koffie en gezelligheid zijn volgens mij de motor voor succesvol onderzoek, dus ook jullie hulp was onmisbaar voor deze race naar een proefschrift.

Amarins, Martijn, Nariman en Sadaf, jullie wil ik bedanken voor ons turbulente jaar als Master Academy commissie bij de Vereniging Chirurgie voor Medisch Studenten (VCMS). Een commissie met een serieus doel leidde tot nieuwe vriendschappen, in een jaar van ups en downs. Samen hebben we ons door dit project heen geslagen, met een mooi eindresultaat.

Huisgenoten door de jaren heen, in **Huize Haring, Diezercasa** en uiteindelijk in Helpman, zonder gezellige huisgenoten en een fijne plek om te ontspannen was dit niet gelukt. Jullie hebben kunnen lachen om mijn onvermogen tot uitslapen na een nacht drinken, jullie hebben mijn gezeur moeten aan horen na saaie coschapdagen en jullie hebben me 's avonds chagrijnig mijn onderzoek zien uitvoeren op de bank. Die steun en gezelligheid had ik nooit willen missen!

Bart, Erik, Evert, Max, Pepijn, Robin, Sebastiaan en Tim, de jongens van mijn jaarclub. We zijn een bonte verzameling van verschillende karakters, waarmee we een bijzondere groep vormen. Samen hebben we een prachtige studententijd doorgemaakt, waarna de meeste nu hardwerkende jongens zijn. Voor jullie was dit hele promotietraject nooit een verassing, de geneeskundenerds waren voorbestemd om eindeloos te studeren. Toch sluit ook ik hiermee eindelijk de studententijd officieel af.

Anique, Hester, Kalong, Kevin en Lotte, vrienden van Geneeskunde, maar toch vooral vrienden buiten geneeskunde. Een groep waarmee we eindeloos kunnen lachen, avonden spellen kunnen spelen, kunnen drinken en kunnen huilen. Langzaamaan verspreiden we ons steeds verder over het land, maar het voelt met jullie altijd als thuiskomen.

Lieve Lies, mijn liefste zus, door jou ben ik in Groningen beland. Niet omdat je er zelf studeerde, maar omdat je mij op het hart drukte om de wereld buiten Amsterdam te ontdekken. We hoeven elkaar niet wekelijks te zien om toch een hele hechte band te hebben. Je leert me steeds weer dat er meer is dan hard werken, dat het belangrijk is om te dromen en vooral te genieten. Allebei hebben we ons pad gezocht en gevonden. Het is niet in woorden te vatten hoe trots ik op je ben.

Allerliefste ouders, lieve Jan en Cocky, jullie zijn toch wel de ultieme aanstichters van dit alles en daar ben ik jullie enorm dankbaar voor. Jullie hebben ons leren dromen en steunen ons altijd in het behalen van onze doelen. Wij zijn op deze wereld gezet met de overtuiging dat hard werken altijd wordt beloond en dat je uit moet gaan van je eigen kracht. De interesse die jullie toonden in mijn projecten was onuitputtelijk en mijn onvermogen om het in normaal Nederlands uit te leggen was vaak lachwekkend. Toch is het jullie gelukt om altijd precies op de hoogte te zijn en die steun is onbeschrijfelijk bijzonder.

Mijn lieve Martijn, in clichétermen toch wel mijn steun en toeverlaat, maar vooral mijn relaterend thuisfront. Voor jou was dit promotietraject een abstracte aaneenschakeling van medische terminologie en politieke ‘spelletjes’ binnen het ziekenhuis. Telkens kon jij het terugbrengen tot de kern met de simpele woorden “richt jij je maar op *after kidney transplantation*”. Als team kunnen wij de wereld aan en die gedachte is samen te vatten als onvoorwaardelijk liefde.

Curriculum vitae

CURRICULUM VITAE

Stan Benjamins was born on 27th August 1994 in Amsterdam, The Netherlands. He received his secondary education at the Montessori Lyceum Amsterdam in Amsterdam. Following his graduation, he was admitted to study Medicine at the University of Groningen. After the first year, Stan joined the Prometheus Nierteam, a student team for kidney transplantation and organ donation. In São Paulo, Brazil, he spent two months at Hospital do Rim e Hipertensão, world's largest kidney transplant hospital. For his master thesis, which he conducted in Groningen and at the Leiden University Medical Center, Stan joined the transplant imaging research group. In 2016, he successfully applied for the MD/PhD trajectory at the University of Groningen, providing him with opportunity to perform his research during his medical internships. During this trajectory, he presented his work at several (inter)national conferences and he received multiple study and travel grants for his research. He worked for three months at the Nkhoma Mission Hospital in Malawi and finished his medical internships at the departments of Surgery from the Erasmus MC and Maasstad Hospital in Rotterdam. Stan received his master's degree in Medicine at the start of 2021 and will start working as a junior doctor at the department of Surgery of the Maasstad Hospital in Rotterdam from April 2021.