

Pancreas and Kidney Transplantation in Patients with  
Type 1 Diabetes and End-Stage Renal Disease:  
Long-Term Outcomes

PhD Thesis  
Jørn Petter Hanto Lindahl  
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Oslo University Hospital, Rikshospitalet  
Department of Transplantation Medicine  
Section of Nephrology



University of Oslo  
Faculty of Medicine  
Institute of Clinical Medicine

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## **Summary**

**Background/objectives:** This thesis examines transplant modality and long-term outcomes in type 1 diabetic patients with end-stage renal disease (ESRD), who may be offered a pancreas and kidney transplant simultaneously from the same deceased donor (SPK), or a single kidney transplant from a living donor (LDK) or a deceased donor (DDK) to replace lost kidney function. LDK transplantation has been first priority at our hospital due to better long-term patient and kidney graft survival rates compared with DDK transplantation, and it also reduces the wait list for the latter. Patients with ESRD, particularly those with diabetes, are at high risk of cardiovascular disease (CVD). A successful SPK transplantation restores glycaemic control, and may therefore protect against or halt the development of diabetic complications post-transplant. Previous research has shown conflicting results in terms of superior long-term outcomes with either SPK transplantation or LDK transplantation. The inconsistent results may stem from a combination of factors, including differences in age and comorbidities, immunosuppressive regimens, surgical techniques, as well as small sample sizes or short follow-up. Since we were largely able to overcome these shortcomings, we raised the following four research questions: Compared with kidney transplantation alone (KTA), particularly LDK transplantation, does SPK transplantation (1) improve patient and kidney graft survival rates, (2) improve cardiovascular death rates, (3) prevent progression of coronary artery disease (CAD), and (4) prevent recurrence of diabetic glomerular changes in the kidney graft post-transplant? This thesis advances our understanding of the potential benefits of restoring glycaemic control post-transplant, and enables us to better choose a transplant modality for type 1 diabetic patients with ESRD.

**Methods:** A retrospective observational cohort study was conducted on all kidney transplants performed in Norway between 1983 and 2012 on patients with type 1 diabetes. We collected

data from the Norwegian Renal Registry and hospital records. Multivariate Cox regression was used to assess associations between transplant modality and (1) patient and graft survival in 630 patients transplanted with SPK ( $n=222$ ), LDK ( $n=171$ ), or DDK ( $n=237$ ) grafts between 1983 and 2010, and (2) all-cause and CVD- and CAD-related death in 486 patients transplanted with SPK ( $n=256$ ) or LDK ( $n=230$ ) grafts from 1983 to the end of 2012. In a subgroup of 42 (25 SPK/17 LDK) recipients receiving grafts between 1983 and 2003 still functioning at median 10.1 years post-transplant, angiographic progression of CAD between baseline and follow-up was studied, and kidney graft biopsies were performed at follow-up to examine for occurrence of diabetic glomerular lesions (e.g., glomerular basement membrane [GBM] thickening and mesangial expansion).

**Results:** The mean follow-up time was 7.1 years in the main cohort of type 1 diabetic kidney transplant recipients transplanted in the period 1983-2010. In multivariate Cox regression, SPK transplantation was associated with superior patient survival compared with both LDK transplantation (hazard ratio [HR] 0.70, 95% confidence interval [CI] 0.52, 0.95;  $p=0.02$ ) and DDK transplantation, which in turn was inferior to LDK transplantation (HR 1.41, 95% CI 1.04, 1.93;  $p=0.029$ ). SPK superiority over LDK was dependent on donor age. Adjusted kidney graft survival did not differ between SPK and LDK recipients (HR 0.99, 95% CI 0.73, 1.37;  $p=0.99$ ), while kidney graft survival in DDK recipients was poorer than in LDK recipients (HR 1.45, 95% CI 1.08, 1.96;  $p=0.014$ ). Pancreas graft survival also improved after year 2000, with a 5-year graft survival rate of 78% versus 61% in previous years (1988-1999).

The median follow-up length was 7.9 years in patients transplanted in the period 1983-2012 and studied for CVD-related deaths. The adjusted HR for CVD-related deaths in SPK recipients compared with LDK recipients was 0.63 (95% CI 0.40, 0.99;  $p=0.047$ ), while the

HRs for all-cause and CAD-related mortality were 0.81 (95% CI 0.57, 1.16;  $p=0.25$ ) and 0.63 (95% CI 0.36, 1.12;  $p=0.12$ ), respectively.

In the subgroup consisting of 25 SPK and 17 LDK patients, median duration of follow-up was 10.1 years. The mean HbA<sub>1c</sub> levels during follow-up were  $5.5\pm 0.4\%$  and  $8.3\pm 1.5\%$  in the SPK and LDK group, respectively ( $p<0.001$ ). The progression of CAD occurred at similar rates (10 of 21 cases in the SPK and 5 of 14 cases in the LDK group;  $p=0.49$ ). Compared with SPK recipients, LDK recipients had wider GBM ( $369\pm 109$  nm versus  $281\pm 57$  nm;  $p=0.008$ ) and increased mesangial volume fraction (median 0.23 [range 0.13-0.59] versus 0.16 [0.10-0.41];  $p=0.007$ ) at follow-up. Absolute estimated glomerular filtration rate (eGFR) change from baseline was  $-11\pm 21$  and  $-23\pm 15$  ml/min/1.73 m<sup>2</sup> ( $p=0.060$ ), and eGFR slope was  $-1.1$  (95% CI  $-1.7, -0.5$ ) and  $-2.6$  (95% CI  $-3.1, -2.1$ ) ml/min/1.73 m<sup>2</sup> per year in the SPK and LDK group, respectively ( $p=0.001$ ).

**Conclusions:** We conclude that in type 1 diabetic patients with ESRD, SPK transplantation was associated with better long-term patient survival compared with both LDK and DDK transplantation, but the difference in survival between SPK and LDK transplantation depended on donor age. Long-term kidney graft survival was inferior in DDK recipients compared with both SPK and LDK recipients, in whom there was no difference in kidney graft survival. There was an association between LDK transplantation and increased risk of cardiovascular death compared with SPK transplantation. Compared with LDK transplantation, SPK transplantation did not slow the progression of CAD, but kidney graft ultrastructure and function were better preserved post-transplant in a subgroup of patients with long-term functioning grafts.



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Oslo, October 2016  
Jørn Petter Hanto Lindahl





## List of Abbreviations

<b>ACE</b>	Angiotensin-Converting Enzyme
<b>ARB</b>	Angiotensin Receptor Blocker
<b>CAC</b>	Coronary Artery Calcification
<b>CAD</b>	Coronary Artery Disease
<b>CAG</b>	Coronary Angiography
<b>CI</b>	Confidence Interval
<b>CNI</b>	Calcineurin Inhibitor
<b>CT</b>	Computed Tomography
<b>CTS</b>	Collaborative Transplant Study
<b>CVD</b>	Cardiovascular Disease
<b>DCCT</b>	Diabetes Control and Complications Trial
<b>DDK</b>	Deceased Donor Kidney
<b>EDIC</b>	Epidemiology of Diabetes Interventions and Complications
<b>eGFR</b>	Estimated Glomerular Filtration Rate
<b>ESRD</b>	End-Stage Renal Disease
<b>GBM</b>	Glomerular Basement Membrane
<b>GFR</b>	Glomerular Filtration Rate
<b>HLA</b>	Human Leukocyte Antigen
<b>HR</b>	Hazard Ratio
<b>IPTR</b>	International Pancreas Transplant Registry
<b>IQR</b>	Interquartile Range
<b>KTA</b>	Kidney Transplantation Alone
<b>LDK</b>	Living Donor Kidney
<b>LV</b>	Left Ventricular
<b>OPTN</b>	Organ Procurement and Transplantation Network
<b>PAK</b>	Pancreas After Kidney
<b>PCR</b>	Protein/Creatinine Ratio
<b>SD</b>	Standard Deviation
<b>SPK</b>	Simultaneous Pancreas and Kidney
<b>SRTR</b>	Scientific Registry of Transplant Recipients
<b>UNOS</b>	United Network for Organ Sharing
<b>USRDS</b>	United States Renal Data System



## List of Papers

The present work is based on the following papers:

1. Lindahl JP, Hartmann A, Horneland R, Holdaas H, Reisæter AV, Midtvedt K, Leivestad T, Øyen O, Jenssen T (2013) Improved patient survival with simultaneous pancreas and kidney transplantation in recipients with diabetic end-stage renal disease. *Diabetologia* 56:1364-1371
2. Lindahl JP, Hartmann A, Aakhus S, Endresen K, Midtvedt K, Holdaas H, Leivestad T, Horneland R, Øyen O, Jenssen T (2016) Long-term cardiovascular outcomes in type 1 diabetic patients after simultaneous pancreas and kidney transplantation compared with living donor kidney transplantation. *Diabetologia* 59:844-852
3. Lindahl JP, Massey RJ, Hartmann A, Aakhus S, Endresen K, Günther A, Midtvedt K, Holdaas H, Leivestad T, Horneland R, Øyen O, Jenssen T (2016) Cardiac assessment of patients with type 1 diabetes median 10 years after successful simultaneous pancreas and kidney transplantation compared with living donor kidney transplantation. *Transplantation* doi:10.1097/TP.0000000000001274 [published online ahead of print]
4. Lindahl JP, Reinholt FP, Eide IA, Hartmann A, Midtvedt K, Holdaas H, Dorg LT, Reine TM, Kolset SO, Horneland R, Øyen O, Brabrand K, Jenssen T (2014) In patients with type 1 diabetes simultaneous pancreas and kidney transplantation preserves long-term kidney graft ultrastructure and function better than transplantation of kidney alone. *Diabetologia* 57:2357-2365



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# **1. Introduction to the Thesis**

## **1.1 Type 1 Diabetes and End-Stage Renal Disease**

Type 1 diabetes is characterised by loss of insulin-producing beta cells in the pancreas resulting in a chronic state of hyperglycaemia. Consequently, long-term, continuous hyperglycaemia may lead to adverse complications affecting both small (microvascular) and large (macrovascular) blood vessels. In non-uraemic patients with type 1 diabetes, studies have shown that tight blood glucose control is associated with reduced risk for development of diabetic microvascular complications,<sup>1-5</sup> that is, retinopathy, nephropathy, and neuropathy, and probably also non-fatal and fatal cardiovascular disease (CVD),<sup>6</sup> which are macrovascular complications of diabetes. Diabetic nephropathy is one of the most common causes of end-stage renal disease (ESRD) worldwide.<sup>7, 8</sup> Uraemic patients, particularly those with diabetes, suffer higher rates of cardiovascular morbidity and mortality than the general population.<sup>9-12</sup> Kidney transplantation is in general the treatment of choice, offering both improved quality of life<sup>13-15</sup> and increased survival rates<sup>16-18</sup> compared with continuous dialysis.

In patients with type 1 diabetes and ESRD, there are two alternative ways to replace the lost kidney function by transplantation: one is receiving a simultaneous pancreas and kidney (SPK) transplant, and the other is receiving a kidney transplant alone from either a living (LDK) or a deceased donor (DDK). LDK transplantation is superior to DDK transplantation due to better patient and kidney graft survival rates.<sup>7, 19-21</sup> First priority in our country has therefore been to transplant a kidney from a living donor, if available. Adding a pancreas transplant to a kidney transplant from a deceased donor has become an established therapeutic option along with improved surgical technique and immunosuppressive protocols.<sup>22, 23</sup> A successful SPK transplantation restores normal blood glucose control, and could theoretically protect from development of diabetic microvascular and macrovascular complications post-transplant.

## **1.2 Problem Statement**

The four studies in this thesis derive from the main question: Does SPK transplantation in type 1 diabetic patients with ESRD lower the risk of post-transplant diabetic complications and improve patient survival compared with kidney transplantation alone (KTA)? A major obstacle when comparing outcomes after SPK transplantation with KTA in recipients with type 1 diabetes has been an imbalance in risk factors between patient populations, particularly due to the selection of younger patients with less comorbidity for SPK transplantation. This has complicated direct post-transplant patient comparison. However, at our centre we have also promoted the use of LDK transplantation for patients with type 1 diabetes.<sup>24, 25</sup> In 2014, 25% of all our kidney transplants were from living donors;<sup>8</sup> patients tend to be younger and with lower comorbidity than those selected for transplantation of a kidney from a deceased donor. Thus, as a group, LDK recipients may serve as a suitable comparison to recipients receiving an SPK transplant. To date, there is still an unresolved question in the literature whether a pancreas transplant in addition to a kidney transplant actually improves long-term outcomes compared with KTA, particularly compared with a kidney from a living donor.

## **1.3 Purpose and Scope of the Thesis**

The purpose of this thesis is to investigate associations between transplant modality (SPK transplantation or KTA) and long-term outcomes in uraemic patients with type 1 diabetes. The scope of the study is limited to examining post-transplant patient and graft survival rates, cardiovascular outcomes and recurrence of early glomerular diabetic lesions and kidney function in patients undergoing SPK transplantation versus KTA, particularly LDK transplantation. It is not within the scope of this thesis to examine patients with type 2 diabetes and ESRD or patients who have received a pancreas after kidney transplant (PAK),



pancreas transplant alone (PTA), islet transplant alone (ITA), or islet after kidney transplant (IAK).

#### **1.4 Potential Impact of the Thesis**

One intended outcome of the thesis, on a theoretical level, is to extend knowledge and understanding of long-term outcomes after pancreas and kidney transplantation in patients with type 1 diabetes. On a practical level, a second intended outcome of the study is to clarify for the transplant community and local nephrologists what transplant modality is best for individual patients with type 1 diabetes who need a transplant to replace lost kidney function.

#### **1.5 Overview of the Thesis**

This thesis consists of another seven chapters. Chapter Two presents additional background information on transplant options at first transplantation in type 1 diabetic patients with ESRD. Glycaemic control and the risk for secondary diabetic micro- and macrovascular complications are also discussed and related to outcomes after transplantation in type 1 diabetic patients with ESRD. Based on the literature review, the most pressing gaps in the literature are then identified and our specific research questions are posed in Chapter Three. Chapter Four deals with the research strategy, the tools for collecting, presenting, and analysing data. The result of data analyses on long-term outcomes comparing pancreas and kidney transplantation with kidney-only transplantation are presented in Chapter Five. Chapter Six contains the discussion on the research strategy, key findings, and strengths and limitations. Chapter Seven contains the conclusions. Finally, Chapter Eight addresses the implications of the thesis and suggests further research agendas.

## **2. Background of the Thesis**

### **2.1 Epidemiology of Type 1 Diabetes**

Diabetes is a major and increasing global health problem. It is estimated that 415 million people or one in 11 adults in the world have diabetes, and that 642 million people will have diabetes by 2040.<sup>26</sup> Of all cases of diabetes, type 2 diabetes is estimated to represent 90% or more. Type 1 diabetes may present at any age, but it most commonly manifests early in life. In Norway, the incidence rate among children aged 0-14 years was about 33 per 100,000 person-years during 2004-2012.<sup>27</sup> Based on calculations from the Norwegian Prescription Database, it can be estimated that about 28,000 people have type 1 diabetes in Norway.<sup>28</sup>

### **2.2 Glucose as a Risk Factor in Type 1 Diabetes**

Diabetes-related morbidity and mortality is due to microvascular and macrovascular disease, as well as acute metabolic complications.<sup>29</sup> In type 1 diabetic patients, the goal is to keep the concentration of blood glucose as close to normal as possible because tight glycaemic control is associated with a reduced risk of diabetic complications. Studies first showed an association between poor glycaemic control and microvascular complications.<sup>1-4</sup> The causality of this association was confirmed in the prospective Diabetes Control and Complications Trial (DCCT),<sup>5</sup> which showed that intensive insulin therapy to reduce glycaemia resulted in decreased rates of retinopathy, nephropathy, and neuropathy in type 1 diabetic patients. The importance of tight glycaemic control for protection against macrovascular disease in patients with type 1 diabetes has also been established in the DCCT/Epidemiology of Diabetes Interventions and Complications (EDIC) study.<sup>6</sup> In the EDIC follow-up study of the DCCT, intensive insulin therapy seemed to decrease long-term fatal and non-fatal cardiovascular events.

### **2.3 Type 1 Diabetes and Cardiovascular Disease**

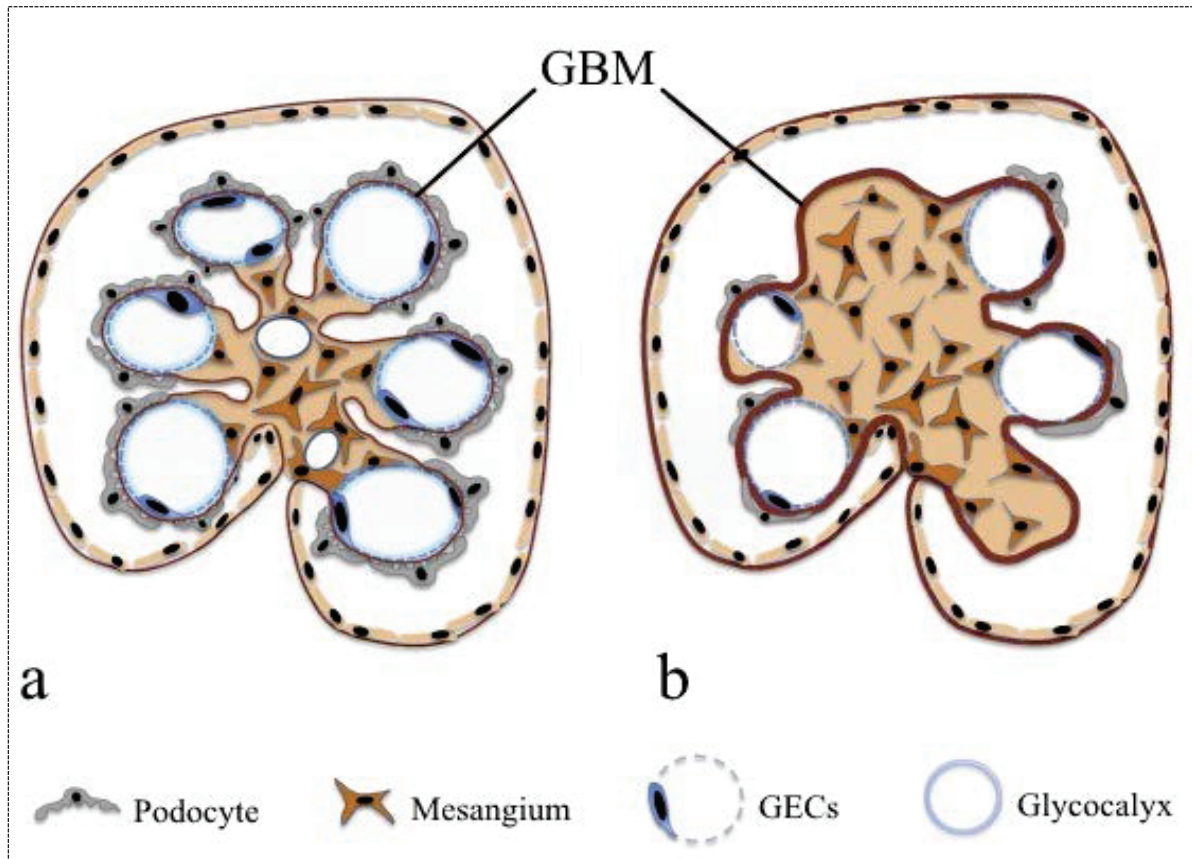
Type 1 diabetes is associated with higher risk of CVD,<sup>30-32</sup> the main macrovascular complication of diabetes, primarily caused by atherosclerosis. Atherosclerosis may result in coronary artery disease (CAD), cerebrovascular disease, or peripheral artery disease, often manifesting as non-fatal and fatal myocardial infarction, stroke, and intermittent claudication, diabetic wounds, or gangrene respectively. CVD events are more common and occur earlier in patients with type 1 diabetes than in the non-diabetic population.<sup>32-34</sup> Furthermore, type 1 diabetes is associated with an increased risk of mortality compared with individuals without diabetes.<sup>35-38</sup> Diabetic nephropathy is a complication of type 1 diabetes that is strongly linked to CVD. Developing diabetic nephropathy is a turning point in the life of a type 1 diabetic patient. The risk of all-cause mortality increases with the severity of diabetic kidney disease, from moderately increased albuminuria (formerly called microalbuminuria)<sup>39</sup> to severely increased albuminuria (formerly called macroalbuminuria)<sup>39</sup> to ESRD.<sup>38, 40, 41</sup>

### **2.4 Type 1 Diabetes and Diabetic Nephropathy**

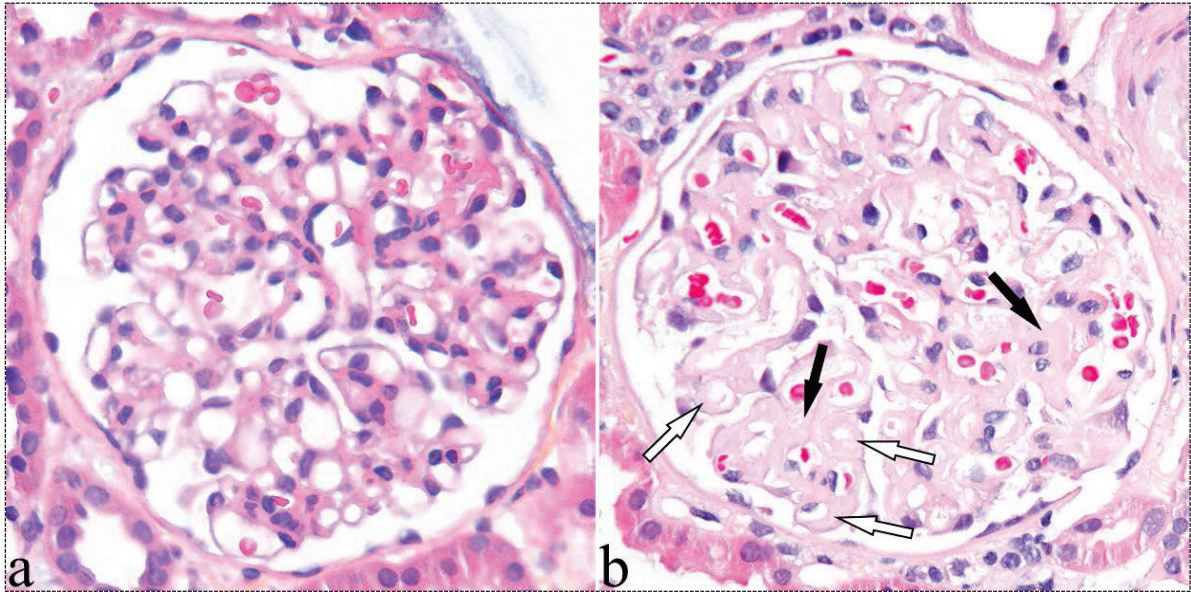
Diabetic nephropathy in type 1 diabetes reflects injury to the kidney as a consequence of chronic long-standing hyperglycaemia. Moderately increased albuminuria (microalbuminuria) will eventually develop in about 50% of patients with type 1 diabetes. In type 1 diabetes, previous studies have reported a cumulative incidence of diabetic nephropathy of 25-40% after 25 years of disease duration.<sup>42-45</sup> The cumulative incidence of diabetic nephropathy has been reported to have decreased to approximately 15% over 20-25 years in more recent cohorts.<sup>46, 47</sup>

Diabetic nephropathy is defined by characteristic structural and functional changes. Structural diabetic glomerular changes include thickening of the glomerular basement membrane

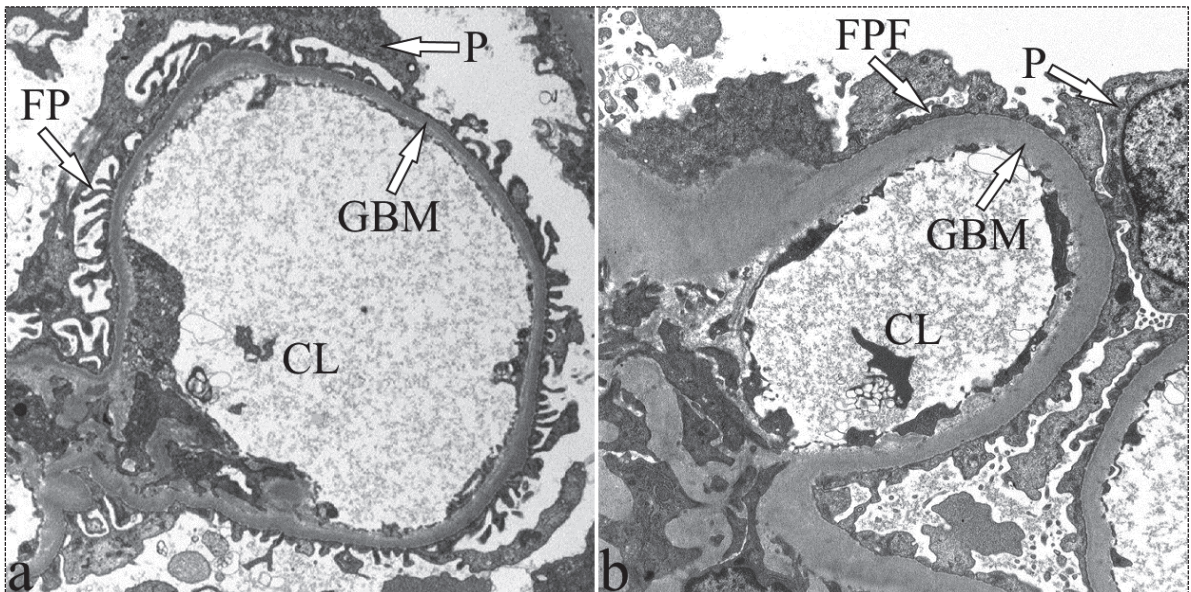
(GBM), mesangial expansion, and podocyte changes and loss.<sup>48-51</sup> These characteristic glomerular changes are illustrated with a schematic view in Fig. 1<sup>52</sup> and with light and electron micrographs in Figs. 2 and 3 respectively. Thickening of the GBM is the first change



**Figure 1.** (a) Schematic structure of a normal glomerulus. Cells of the glomerular tuft and extracapillary glomerulus are shown, along with the glomerular basement membrane (GBM). (b) Schematic structure of a diabetic glomerulus. Diabetic glomerular changes include thickening of the GBM, mesangial expansion, podocyte injury, and also impairment in the glycocalyx<sup>53</sup> and glomerular endothelial cells<sup>54</sup> (GECs). Reprinted from Trends in Endocrinology & Metabolism, Gnudi L et al.,<sup>52</sup> Diabetic Nephropathy: Perspective on Novel Molecular Mechanisms, (In Press), 2016, with permission from Elsevier



**Figure 2. (a)** Light micrograph illustrating a normal glomerulus. **(b)** Light micrograph (at same magnification) illustrating a diabetic glomerulus showing thickening of capillary walls (white arrows) and mesangial matrix increase (black arrows). Photo by courtesy of Professor Reinholt FP, Department of Pathology, Oslo University Hospital, Rikshospitalet, Oslo, Norway



**Figure 3. (a)** Electron micrograph illustrating a portion of a normal glomerulus (CL, capillary lumen; GBM, glomerular basement membrane; P, podocyte; FP, foot process). **(b)** Electron micrograph (at same magnification) illustrating a diabetic glomerulus. A glomerular lobule shows a thickened GBM and foot process fusion (FPF). Photo by courtesy of Professor Reinholt FP, Department of Pathology, Oslo University Hospital, Rikshospitalet, Oslo, Norway

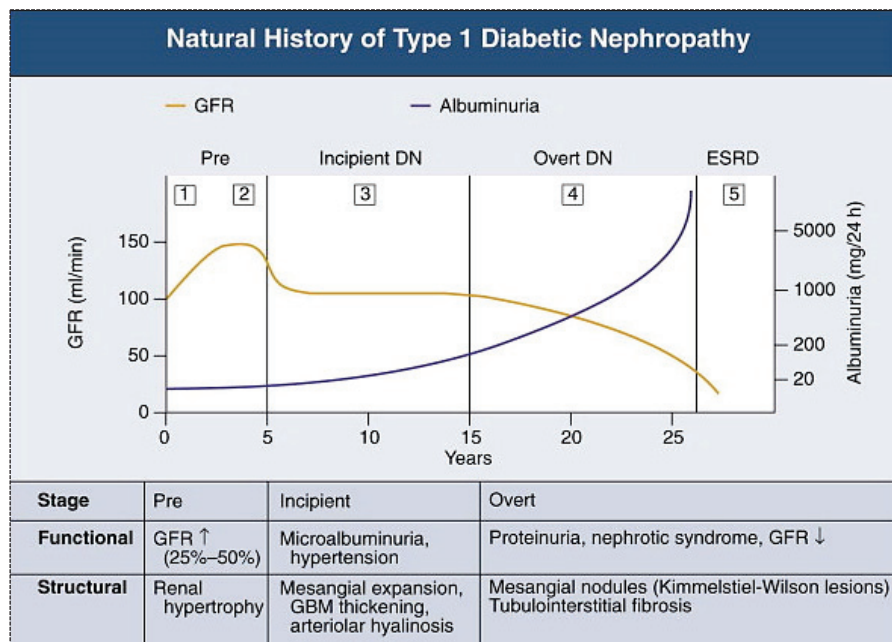
or histological hallmark of diabetic nephropathy, and may occur as early as 1 to 2 years after the onset of diabetes.<sup>49, 55</sup> The second hallmark of diabetic nephropathy is the expansion of cellular and matrix components in the mesangium, which can occur as early as 4 to 5 years after the onset of type 1 diabetes.<sup>49</sup> If at least one Kimmelstiel-Wilson lesion (nodular sclerosis) is present, this indicates a more advanced form of diabetic nephropathy. Tubular basement thickening<sup>56</sup> and arteriolar hyalinosis,<sup>57</sup> predominantly of the efferent arteriole, is a relatively specific finding for diabetic nephropathy. A pathological classification of diabetic nephropathy<sup>58</sup> was proposed in 2010 as shown in Fig. 4. In this classification system, diabetic nephropathy is divided into four classes based on progressively more serious glomerular lesions. In diabetic nephropathy, there appears to be a close relationship between the functional or clinical manifestations of diabetic nephropathy and structural changes in the kidney.<sup>51, 59</sup>

Functionally, diabetic nephropathy in type 1 diabetes is characterised by the development of persistent severely increased albuminuria (macroalbuminuria), that is, urinary excretion of more than 300 mg albumin/24 hours (urine albumin/creatinine ratio (ACR) >30 mg/mmol) or 500 mg protein/24 hours (urine protein/creatinine ratio (PCR) >50 mg/mmol), which can be diagnosed clinically in the presence of diabetic retinopathy and absence of evidence of other kidney disease. The presence of moderately increased albuminuria (microalbuminuria) predicts the development of diabetic nephropathy in type 1 diabetes.<sup>60</sup> The clinical hallmarks for development of diabetic nephropathy are progressive degree of albuminuria and subsequent proteinuria, a steady decline in glomerular filtration rate (GFR), and elevated blood pressure.<sup>61, 62</sup> Fig. 5 shows clinical course of the development and progression of diabetic nephropathy.<sup>63, 64</sup>

Class	Description	Inclusion Criteria
I	Mild or nonspecific LM changes and EM-proven GBM thickening	Biopsy does not meet any of the criteria mentioned below for class II, III, or IV GBM > 395 nm in female and >430 nm in male individuals 9 years of age and older <sup>a</sup>
IIa	Mild mesangial expansion	Biopsy does not meet criteria for class III or IV Mild mesangial expansion in >25% of the observed mesangium
IIb	Severe mesangial expansion	Biopsy does not meet criteria for class III or IV Severe mesangial expansion in >25% of the observed mesangium
III	Nodular sclerosis (Kimmelstiel-Wilson lesion)	Biopsy does not meet criteria for class IV At least one convincing Kimmelstiel-Wilson lesion
IV	Advanced diabetic glomerulosclerosis	Global glomerular sclerosis in >50% of glomeruli Lesions from classes I through III

LM, light microscopy.  
<sup>a</sup>On the basis of direct measurement of GBM width by EM, these individual cutoff levels may be considered indicative when other GBM measurements are used.

**Figure 4.** Glomerular classification of diabetic nephropathy. Republished with permission of American Society of Nephrology, from Pathologic Classification of Diabetic Nephropathy, Tervaert TWC et al.,<sup>58</sup> 21, 2010; permission conveyed through Copyright Clearance Center, Inc



**Figure 5.** Functional and structural manifestations of type 1 diabetic nephropathy. Numbers 1 through 5 indicate different stages of nephropathy as described by Mogensen CE.<sup>63</sup> (Microalbuminuria and proteinuria as denoted in the figure are moderately and severely increased albuminuria, respectively, in the new nomenclature). Reprinted from Comprehensive Clinical Nephrology, 4<sup>th</sup> Edition, Ritz E, Wolf G, Pathogenesis, Clinical Manifestations, and Natural History of Diabetic Nephropathy, p 367, 2010,<sup>64</sup> with permission from Elsevier

## **2.5 A Brief History of Transplantation**

### **2.5.1 Kidney Transplantation**

Joseph E. Murray performed the world's first successful kidney transplantation between identical twins in December 1954 at the Peter Bent Brigham Hospital in Boston.<sup>65-68</sup> This success was, however, of limited practical value since identical twins are rare. Accordingly, the success with unrelated kidney transplants was poor in the early era of kidney transplantation both without any immunosuppression at all or with corticosteroids,<sup>69</sup> or using total-body irradiation<sup>70</sup> in addition to corticosteroids. In the early 1960s, with the introduction of azathioprine coupled with available corticosteroids, transplantation of unrelated donor kidneys also became a viable treatment for advanced renal failure. The success rates of kidney transplantation soon improved, with approximately 50% of the kidneys still functioning after 1 year.<sup>71, 72</sup> In the early 1980s, with the arrival of cyclosporine,<sup>73, 74</sup> 1-year kidney graft survival rose significantly<sup>75, 76</sup> compared with a azathioprine and corticosteroids based regimen. Current kidney graft survival rates are more than 90 % 1 year after transplantation<sup>7</sup>.

### **2.5.2 Pancreas Transplantation**

Richard C. Lillehei, together with William D. Kelly, performed the world's first clinical pancreas transplantation, simultaneously with a kidney graft, in December 1966, to treat a patient with type 1 diabetes and ESRD at the University of Minnesota Hospital.<sup>77</sup> Initially, the success rate with pancreas transplantation was low,<sup>78</sup> but increased in the 1980s along with advances in surgical technique and immunosuppression therapy, leading to increased application.<sup>22, 23</sup> In the 2010-2014 cohorts, SPK transplant pancreas graft survival rates are now just over 89% and 82% at 1 and 3 years respectively.<sup>79</sup>



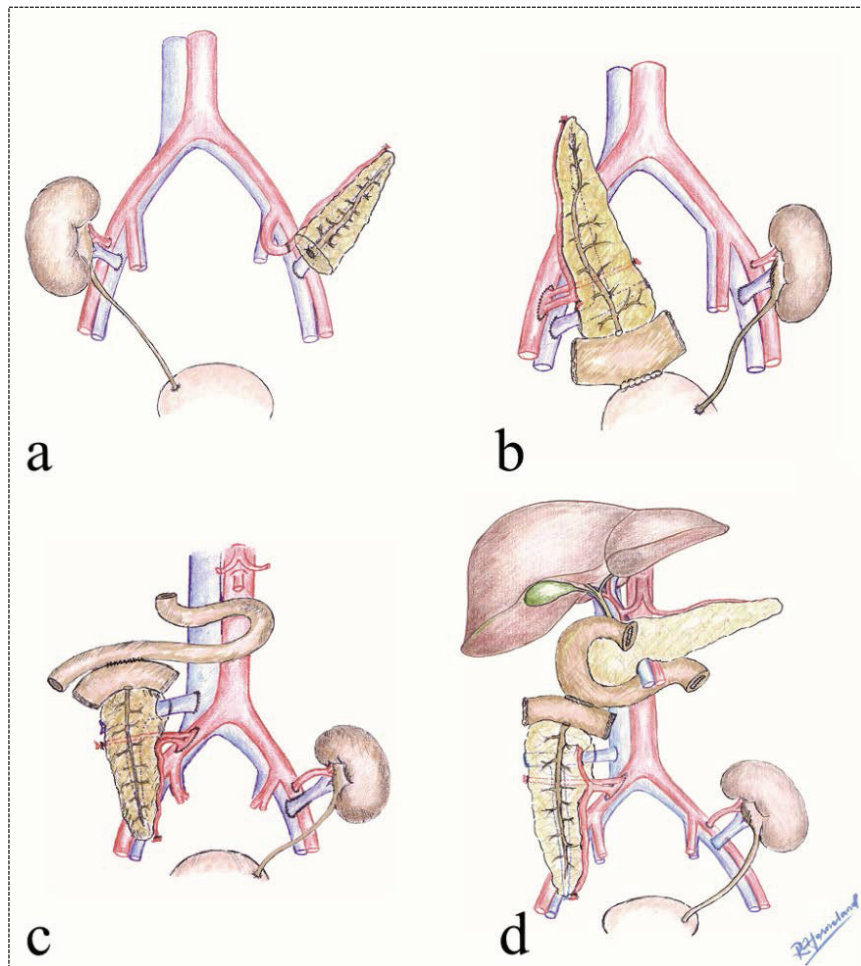
### **2.5.3 Kidney and Pancreas Transplantation: The Norwegian Experience**

The Norwegian experience with kidney transplantation started at the National Hospital in Oslo in August 1956 when Leif Efskind transplanted a kidney from an unrelated donor to a patient with uraemia.<sup>80-82</sup> From 1969, the beginning of the national transplant program in Norway,<sup>81</sup> and through 2015 a total of 8,103 consecutive patients have received a renal transplant, of which 2,901 were from living donors. During 2014, a total of 521 new patients entered renal replacement therapy in Norway. Among these new patients, 88 (17%) had diabetic nephropathy as the cause of ESRD.<sup>8</sup> Twenty-nine of those patients with diabetic nephropathy were registered with type 1 diabetes and 59 with type 2 diabetes. Seventy-nine patients with primary renal disease other than diabetic nephropathy had diabetes as a comorbidity. Accordingly, 167 (32%) of new patients with ESRD in 2014 had diabetes.

From 1983, all organ transplantations in Norway have been performed at a single centre:<sup>81</sup> Oslo University Hospital, Rikshospitalet, Oslo, which serves the entire population of just over 5.2 million inhabitants (January 2016). Medical key information on transplant patients is annually reported to the Norwegian Renal Registry, which was constituted in 1994 as collaboration between the Norwegian Society of Nephrology and Oslo University Hospital, Rikshospitalet. National data on renal replacement therapy has been collected within the Norwegian Society of Nephrology since 1980, and the transplant centre has stored data on transplanted patients since the late 1960s.

The introduction of cyclosporine in 1983 made the transplantation of organs other than kidneys (e.g., pancreas, heart, lungs, liver) a clinical reality in Norway. The Norwegian pancreas transplantation program was initiated by the transplant surgeon Professor Inge B. Brekke in June 1983.<sup>83</sup> Initially, a duct-occluded segmental pancreas was used for

transplantation.<sup>83</sup> In April 1988, the surgical technique was changed from duct-occluded segmental pancreas to whole pancreas grafting with duodenocystostomy.<sup>83</sup> After March 1998, bladder drainage was substituted with enteric drainage through a donor-derived duodenal segment attached to the proximal jejunum.<sup>84</sup> Since September 2012, the duodenal part of the transplant has been anastomosed to the recipient's duodenum rather than the jejunum to obtain endoscopic biopsies from both the duodenum and pancreas allografts.<sup>85</sup> The surgical techniques by era are shown in Fig. 6.<sup>86</sup> By the end of December 2015, a total of 411



**Figure 6.** Surgical techniques for pancreas transplantation. (a) Duct-occluded segmental pancreas. (b) Whole pancreas grafting with duodenocystostomy. (c) Whole pancreas grafting with intestinal (jejunum) drainage. (d) Whole pancreas grafting with intestinal (duodenum) drainage. Reprinted from *Diabetes Research and Clinical Practice*, 105, Lindahl JP et al.,<sup>86</sup> Long-term outcomes after organ transplantation in diabetic end-stage disease, 14-21, 2014, with permission from Elsevier. Illustration by courtesy of Horneland R, Department of Transplant Medicine, Oslo University Hospital, Rikshospitalet, Oslo, Norway

pancreas transplantations have been performed in Norway, 316 simultaneously with a kidney transplant.

## **2.6 Literature Review for the Thesis**

The content of the literature review (section 2.6) is partly reported in my review article ‘Long-term outcomes after organ transplantation in diabetic end-stage renal disease’ published in *Diabetes Research and Clinical Practice* (2014).<sup>86</sup>

A number of approaches were taken for the literature search of this thesis: (1) systematic (finding all relevant material), (2) retrospective (finding the most recent material and working backwards), (3) citation (following up references from useful articles), and (4) targeted (restricting the topic and focussing on a narrow area of the literature). The following search terms (separately or in combination) were used to search PubMed for relevant articles: ‘type 1 diabetes’, ‘diabetic nephropathy’, ‘end-stage renal disease’, ‘kidney transplantation’, ‘pancreas transplantation’, ‘patient survival’, ‘graft survival’, ‘cardiovascular disease’, ‘coronary artery disease’, ‘coronary artery calcification’, and ‘diabetic glomerulopathy’.

### **2.6.1 Patient Survival**

When insulin became available for clinical use in 1922,<sup>87</sup> the life expectancy of type 1 diabetic patients changed dramatically. Because long-term hyperglycaemia is associated with high risk of serious complications such as diabetic nephropathy and CVD, patients with type 1 diabetes got to live long enough with insulin therapy to experience the problem of ESRD. Patients with diabetes and advanced renal failure were not even considered candidates for dialysis or transplantation because of poor prognosis as recently as the 1970s. However, the survival rate of diabetic patients on chronic dialysis has improved and approached that of non-

diabetic patients on chronic dialysis<sup>88</sup> along with better clinical care for CVD and options for renal replacement therapy.

Comparison of survival rates between patients on dialysis treatment and transplant recipients is hampered by selection bias. Patients with low comorbidity and of younger age are more likely to be referred for transplantation, whereas older patients and those with more comorbidity are referred for chronic dialysis. To avoid selection bias, studies have been performed on patients already accepted on the wait list for transplantation, comparing those who receive a transplant with those who remain listed, assuming that the two groups are otherwise at comparable risk. Given these limitations, kidney transplantation is associated with better patient survival long-term compared with continuous dialysis.<sup>16, 89, 90</sup> Patients with type 1 diabetes on long-term dialysis therapy are at particularly high risk for CVD disease.<sup>16</sup> Type 1 diabetic patients also have a higher mortality risk after kidney transplantation compared with non-diabetic patients.<sup>91</sup>

The survival rates of type 1 diabetic patients with ESRD who have received SPK transplants have continuously improved over the last decades, along with advances in surgical technique and immunosuppression therapy. Data from the United Network for Organ Sharing (UNOS)/International Pancreas Transplant Registry (IPTR)<sup>79</sup> show that US SPK transplant unadjusted patient survival rates at 1, 3, and 5 years post-transplant are 96%, 95%, and 90%, respectively. The unadjusted patient survival rates for all causes of ESRD (numbers in parentheses are corresponding patient survival rates for ESRD due to diabetes not differentiated between type 1 and type 2 diabetes) in LDK transplantation are 99% (98%), 96% (93%), 93% (86%), and 77% (58%) at 1, 3, 5, and 10 years, respectively and in DDK

transplantation 96% (95%), 90% (88%), 84% (77%), and 62% (50%) at 1, 3, 5, and 10 years, respectively according to data from the United States Renal Data System (USRDS) registry.<sup>92</sup>

*Studies with Follow-Up 10 Years or Shorter*

When analysing patient survival rates across transplant modalities for type 1 diabetic patients, it is important to take into account what time period the patients received their transplants and the length of follow-up. Earlier studies with short follow-up were in favour of KTA alone.<sup>93-95</sup> More recent studies have shown equal<sup>22, 96-101</sup> or better<sup>102-116</sup> survival rates for SPK transplant patients compared with DDK recipients, particularly with increased follow-up time. However, studies comparing SPK transplantation to LDK transplantation are not consistent for patient survival. These reports show worse,<sup>95, 99</sup> equal,<sup>22, 101, 103, 105, 106, 108</sup> or better<sup>112</sup> (the latter assuming SPK transplant pancreas graft survival at 1 year) outcome when observation time is 10 years or less after transplantation. Table 1 summarises survival data of SPK compared with KTA (LDK or DDK) in studies with an observation time of 10 years or less. Overall, these studies demonstrate no survival benefit of SPK over LDK recipients.

**Table 1.** Studies, sorted by year of publication, comparing survival in SPK recipients to that in KTA recipients with a maximum follow-up of 10 years or shorter

First author	Year	Source	Patients	Follow-up	Patient survival
Cheung <sup>93</sup>	1992	Single centre (US)	n=128	Up to 2 years	DDK $\geq$ SPK <sup>a</sup>
Douzdjian <sup>94</sup>	1994	Single centre (US)	n=153	Up to 5 years	DDK $\geq$ SPK <sup>b</sup>
Manske <sup>95</sup>	1995	Single centre (US)	n=173	Up to 3 years	DDK=LDK>SPK
Douzdjian <sup>96</sup>	1996	Single centre (US)	n=124	Up to 5 years	DDK=SPK

**Table 1.** Continued from previous page

First author	Year	Source	Patients	Follow-up	Patient survival
Rayhill <sup>103</sup>	2000	Single centre (US)	<i>n</i> =805	Up to 10 years	SPK=LDK>DDK
Tydén <sup>105</sup>	2000	Single centre (EU)	<i>n</i> =515	Up to 10 years	SPK=LDK>DDK
Ojo <sup>106</sup>	2001	SRTR/USRDS (Registry data)	<i>n</i> =13,467	Up to 10 years	SPK=LDK>DDK
Sutherland <sup>22</sup>	2001	Single centre (US)	<i>n</i> =396	Up to 4 years	SPK=LDK=DDK
La Rocca <sup>107</sup>	2001	Single centre (EU)	<i>n</i> =351	Up to 7 years	SPK>DDK
Bunnapradist <sup>97</sup>	2003	UNOS (Registry data)	<i>n</i> =6,016	Up to 6 years	SPK=DDK
Reddy <sup>108</sup>	2003	UNOS (Registry data)	<i>n</i> =18,549	Up to 10 years	SPK=LDK>DDK
Waki <sup>98</sup>	2006	UNOS (Registry data)	<i>n</i> =1,088	Up to 10 years	SPK=DDK
Young <sup>99</sup>	2009	OPTN/UNOS (Registry data)	<i>n</i> =11,362	Up to 7 years	LDK>SPK=DDK
Weiss <sup>112</sup>	2009	SRTR (Registry data)	<i>n</i> =8,281	Up to 7 years	SPK+P>LDK>DDK LDK>SPK-P=DDK
Weiss <sup>100</sup>	2009	SRTR (Registry data)	<i>n</i> =8,453	Up to 5 years	SPK=DDK
Norman <sup>115</sup>	2011	OPTN/SRTR (Registry data)	<i>n</i> =6,282	Up to 8 years	SPK+P>SPK-P
Huang <sup>101</sup>	2011	OPTN/UNOS (Registry data)	<i>n</i> =2,192	Up to 10 years	SPK=LDK=DDK

SPK+P/SPK-P denote functioning/not functioning pancreas graft at 1 year post-transplant; US, United States; EU, Europe; SRTR, Scientific Registry of Transplant Recipients; USRDS, United States Renal Data System; UNOS, United Network for Organ Sharing; OPTN, Organ Procurement and Transplantation Network

<sup>a</sup> Survival rates were similar in recipients <45 years old but lower in SPK recipients ≥45 years old

<sup>b</sup> Survival rates were higher in the DDK group at 1 year but similar in both groups at 5 years

*Studies with Follow-Up more than 10 Years*

Table 2 summarises survival data of SPK transplant recipients compared with recipients of a kidney transplant alone (LDK or DDK) in studies with an observation time of more than 10 years. Overall, these studies demonstrate a survival benefit of SPK transplantation over both LDK and DDK transplantation.

**Table 2.** Studies, sorted by year of publication, comparing survival in SPK recipients to that in KTA recipients with a maximum follow-up of more than 10 years

First author	Year	Source	Patients	Follow-up	Patient survival
Smets <sup>102</sup>	1999	Single centre (EU)	n=415	Up to 12 years	SPK>DDK
Becker <sup>104</sup>	2000	Single centre (US)	n=642	Up to 33 years	SPK>LDK>DDK
Mohan <sup>109</sup>	2003	Single centre (EU)	n=101	Up to 11 years	SPK>DDK
Marroquin <sup>110</sup>	2005	OPTN (Registry data)	n=15,655	Up to 15 years	SPK>DDK
Morath <sup>111</sup>	2008	CTS (Registry data)	n=11,420	Up to 18 years	SPK>LDK>DDK
Sollinger <sup>113</sup>	2009	Single centre (US)	n=2,100	Up to 22 years	SPK>LDK>DDK
Morath <sup>114</sup>	2010	CTS (Registry data)	n=15,118	Up to 20 years	SPK>LDK>DDK
Sung <sup>116</sup>	2015	SRTR (Registry data)	n=11,253	Up to 12 years	SPK>DDK

EU, Europe; US, United States; OPTN, Organ Procurement and Transplantation Network; CTS, Collaborative Transplant Study; SRTR, Scientific Registry of Transplant Recipients

Two recent major publications<sup>111, 112</sup> presented long-term outcomes in favour of better patient survival with SPK transplantation even when compared with LDK recipients. Data from the international Collaborative Transplant Study (CTS)<sup>111</sup> showed that SPK recipients had superior survival beyond the 10th year post-transplant than those receiving an LDK or DDK

transplant alone. A study from the Scientific Registry of Transplant Recipients (SRTR) in the US by Weiss et al.<sup>112</sup> also showed superior patient survival among SPK recipients compared with both LDK and DDK recipients. However, only SPK recipients with a functioning pancreas graft 1 year after transplantation were included in their analysis. These observations are in contrast to the findings of Young and colleagues,<sup>99</sup> who analysed Organ Procurement and Transplantation Network/United Network of Organ Sharing (OPTN/UNOS) database registry data. In multivariate analysis, LDK transplantation was associated with lower adjusted risk versus SPK transplantation over a period of 72 months' follow-up with respect to patient death. Furthermore, Knoll et al.<sup>117</sup> examined the optimal strategy for type 1 diabetic recipients with ESRD using a decision model. In this analysis, LDK recipients had the best long-term life expectancy compared with both SPK and DDK (poorest outcome) recipients. Sollinger et al.<sup>113</sup> published a single-centre study including SPK recipients followed for more than 20 years. They reported superior recipient survival with SPK compared with LDK transplantation. The differences in survival outcomes between the various studies may partly be due to lack of sufficient follow-up time. It has been reported that the benefit of a functioning pancreas graft on the kidney is first recognised after 5-10 years.<sup>118</sup> It also takes more than 10 years to lower the risk of CVD with improved glycaemic control in type 1 diabetes.<sup>6</sup> In addition, pancreas transplantation appears to have metabolic effects beyond improved glucose control. SPK transplantation leads to improved blood pressure and lipid control compared with kidney-only transplantation in patients with type 1 diabetic end-stage renal disease.<sup>119</sup> These findings emphasise the importance of long-term follow-up data to assess any benefit of combined pancreas and kidney transplantation.

### **2.6.2 Kidney Graft Survival**

The prognosis for patients who receive kidney transplants with or without diabetes is



generally good, taking into account the risks associated with transplant surgery and allograft rejection. Data for type 1 diabetic patients studied separately also show promising results. The OPTN/SRTR 2012 annual data report<sup>120</sup> show that US SPK transplant kidney graft survival rates (not censored for patient death) at 1, 5, and 10 years post-transplant are 97%, 80%, and 63% respectively. The non-censored kidney graft survival rates for all causes of ESRD in LDK transplantation are 97%, 85%, and 61% at 1, 5, and 10 years respectively, and in DDK transplantation 93%, 74%, and 46% at 1, 5, and 10 years respectively.<sup>92</sup> In patients with diabetes (not differentiated by type 1 and type 2 diabetes) and ESRD the non-censored graft survival rates for LDKs are 97%, 80%, and 48% at 1, 5, and 10 years respectively, and the corresponding numbers for DDKs are 92%, 69%, and 38% at 1, 5, and 10 years respectively.<sup>92</sup>

As a result of improved immunosuppression, first azathioprine in the 1960s and second and most importantly cyclosporine in the 1980s, 1-year kidney graft survival rates have also improved substantially over the years.<sup>21, 92</sup> In patients with ESRD from all causes, living donor transplants have significantly better graft survival rates compared with those for deceased donor transplants.<sup>92, 121</sup> Somewhat contradictory to this, in type 1 diabetic transplant patients with ESRD several studies have reported equal<sup>22, 101, 103, 104, 108, 111</sup> kidney graft survival rates comparing SPK transplant kidney grafts with LDK grafts. Indeed, registry data presented by Morath et al.<sup>114</sup> reported superior long-term SPK transplant kidney graft survival even compared with an LDK transplant after 20 years of follow-up. There are several studies<sup>22, 93, 94, 96-101, 103, 104, 108-112, 114-116, 122</sup> that have assessed SPK kidney graft survival compared with KTA. Tables 3 and 4 summarise kidney graft survival data of SPK transplantation compared with KTA (LDK or DDK transplantation) in studies with an observation time of 10 years or less or more than 10 years, respectively.

**Table 3.** Studies, sorted by year of publication, comparing kidney graft survival in SPK recipients to that in KTA recipients with a maximum follow-up of 10 years or shorter

First author	Year	Source	Patients	Follow-up	Kidney graft survival
Cheung <sup>93</sup>	1992	Single centre (US)	<i>n</i> =128	Up to 2 years	DDK≥SPK <sup>a</sup>
Douzdjian <sup>94</sup>	1994	Single centre (US)	<i>n</i> =153	Up to 5 years	DDK=SPK
Douzdjian <sup>96</sup>	1996	Single centre (US)	<i>n</i> =124	Up to 5 years	DDK=SPK
Rayhill <sup>103</sup>	2000	Single centre (US)	<i>n</i> =805	Up to 10 years	SPK=LDK>DDK
Sutherland <sup>22</sup>	2001	Single centre (US)	<i>n</i> =396	Up to 4 years	SPK=LDK>DDK
Bunnapradist <sup>97</sup>	2003	UNOS (Registry data)	<i>n</i> =6,016	Up to 6 years	SPK=DDK
Reddy <sup>108</sup>	2003	UNOS (Registry data)	<i>n</i> =18,549	Up to 10 years	SPK=LDK>DDK
Waki <sup>98</sup>	2006	UNOS (Registry data)	<i>n</i> =1,088	Up to 10 years	SPK=DDK
Young <sup>99</sup>	2009	OPTN/UNOS (Registry data)	<i>n</i> =11,362	Up to 7 years	LDK>SPK=DDK
Weiss <sup>112</sup>	2009	SRTR (Registry data)	<i>n</i> =8,281	Up to 7 years	SPK+P>LDK>DDK LDK>SPK–P=DDK
Weiss <sup>100</sup>	2009	SRTR (Registry data)	<i>n</i> =8,453	Up to 5 years	SPK=DDK
Norman <sup>115</sup>	2011	OPTN/SRTR (Registry data)	<i>n</i> =6,282	Up to 8 years	SPK+P>SPK–P
Huang <sup>101</sup>	2011	OPTN/UNOS (Registry data)	<i>n</i> =2,192	Up to 10 years	SPK=LDK=DDK

**SPK+P/SPK–P denote functioning/not functioning pancreas graft at 1 year post-transplant; US, United States; UNOS, United Network for Organ Sharing; OPTN, Organ Procurement and Transplantation Network; SRTR, Scientific Registry of Transplant Recipients**

<sup>a</sup> Kidney graft survival rates were similar in recipients <45 years old but lower in SPK recipients ≥45 years old

**Table 4.** Studies, sorted by year of publication, comparing kidney graft survival in SPK recipients to that in KTA recipients with a maximum follow-up of more than 10 years

First author	Year	Source	Patients	Follow-up	Kidney graft survival
Becker <sup>104</sup>	2000	Single centre (US)	<i>n</i> =642	Up to 33 years	SPK=LDK>DDK
Mohan <sup>109</sup>	2003	Single centre (EU)	<i>n</i> =101	Up to 11 years	SPK=DDK
Israni <sup>122</sup>	2005	OPTN/UNOS/SRTR (Registry data)	<i>n</i> =8,323	Up to 12 years	SPK>DDK
Marroquin <sup>110</sup>	2005	OPTN (Registry data)	<i>n</i> =17,048	Up to 15 years	SPK>DDK
Morath <sup>111</sup>	2008	CTS (Registry data)	<i>n</i> =11,420	Up to 18 years	SPK=LDK>DDK
Morath <sup>114</sup>	2010	CTS (Registry data)	<i>n</i> =15,118	Up to 20 years	SPK>LDK>DDK
Sung <sup>116</sup>	2015	SRTR (Registry data)	<i>n</i> =11,253	Up to 12 years	SPK>DDK

US, United States; EU, Europe; OPTN, Organ Procurement and Transplantation Network; UNOS, United Network for Organ Sharing; SRTR, Scientific Registry of Transplant Recipients; CTS, Collaborative Transplant Study

### 2.6.3 Pancreas Graft Survival

Short- and long-term SPK pancreas graft survival rates have increased along with improvement in surgical technique and immunosuppressive therapy.<sup>22, 23</sup> From December 16, 1966 to December 31, 2014 the outcome of more than 48,000 pancreas transplantations have been reported to the IPTR.<sup>79</sup> In the period 2010-2014 compared with 2005-2009, US SPK transplant pancreas graft survival rates (not censored for patient death) at 1 and 3 years post-transplant increased from 85% to 89% and 79% to 82% respectively.<sup>79</sup> The OPTN/SRTR 2013 annual data report<sup>123</sup> revealed a 1- and 5-year non-censored pancreas graft survival rate for the 2008 cohort of 86% and 74% respectively. UK Transplant Registry data<sup>124</sup> (2006-2016)

for SPK transplant, censored for patient death, showed pancreas graft survival rates 1 and 5 years post-transplant of 87% and 75% respectively.

In paper 1, with data from a large single-centre medical practice in Europe, we present long-term outcomes on patient and graft survival rates in patients with type 1 diabetes after transplantation comparing SPK transplantation with both LDK and DDK transplantation.

#### **2.6.4 Cardiovascular Outcomes**

Patients with diabetes and ESRD are at particularly high risk of cardiovascular complications and death. The question whether cardiovascular outcomes can be improved by transplanting a pancreas simultaneously with a kidney is still debated. Most research on outcomes after kidney transplantation in patients with type 1 diabetes has focused on all-cause mortality, in which death from CVD is dominant. There are few studies that have actually assessed cardiovascular outcomes comparing SPK with KTA. In a small study, Biesenbach et al.<sup>125</sup> showed a reduction in risk factors for the development of macroangiopathy but failure to halt progression of macrovascular diseases, that is, coronary artery, cerebrovascular, and peripheral artery disease. They studied 11 SPK recipients compared with ten KTA (from deceased donors) recipients with at least 2-year functioning grafts and a follow-up time of nearly 6 years. However, later on and with a longer follow-up time, the same group<sup>126</sup> showed that the progression of macrovascular diseases was significantly slower in recipients with a functioning SPK graft compared with KTA recipients. La et al.<sup>107</sup> addressed cardiovascular mortality following transplantation and showed a significant reduction in cardiovascular mortality when 130 SPK recipients were compared with 25 patients (originally enrolled on a waiting list for SPK transplantation) who received a KTA and were followed for 7 years. Ojo et al.<sup>106</sup> analysed registry data and revealed that SPK transplantation was associated with a

reduced risk for cardiovascular death compared with both LDK and DDK transplantation. The same conclusion could also be drawn from a large registry data analysis by Morath et al.<sup>111</sup>

Until now, to our knowledge, only one study has been published evaluating the progression of CAD angiographically in SPK versus kidney-only recipients post-transplant. Jukema et al.<sup>127</sup> compared progression of atherosclerosis (measured by mean-segment diameter loss on coronary angiography) in 26 patients with and six patients without a functioning pancreas after SPK transplantation. The mean follow-up time was 3.9 years. They concluded that the progression of atherosclerosis in patients with successful SPK transplantation was reduced compared with recipients with pancreas graft failure.

In paper 2, with data from a large single-centre medical practice in Europe, we present all-cause mortality as well as CVD- and CAD-related mortality in patients with type 1 diabetes post-transplant, comparing SPK recipients with LDK recipients. In paper 3, we present what is to our knowledge the largest study with the longest follow-up on progression of CAD following SPK transplantation compared with LDK transplantation.

### **2.6.5 Recurrence of Diabetic Nephropathy in the Kidney Graft**

Only few studies<sup>128-131</sup> have addressed whether successful SPK transplantation with long-term normoglycaemia could prevent recurrence of diabetic glomerular lesions in the kidney transplant. Shortcomings of these studies have been small patient groups, suboptimal normalization of plasma glucose, and short follow-up time (mostly less than 5 years). However, recurrent diabetic glomerular lesions have been found in the transplanted kidneys as early as 2 years post-transplant in kidney-alone transplant recipients,<sup>132-134</sup> but it takes several years to develop glomerulosclerosis with impairment of renal function. After successful

transplantation of a single pancreas it takes up to 10 years to reverse diabetic lesions in native kidneys.<sup>118</sup>

Bohman et al.<sup>128</sup> reported that kidney graft biopsies from two SPK patients were devoid of diabetic changes 2 years post-transplant, when compared with six diabetic patients who received a single kidney. They concluded that combined transplantation appeared to prevent the recurrence of diabetic nephropathy. However, the study was flawed by the small number of patients, a short follow-up time, and a poor metabolic control in the KTA group. Wilczek et al.<sup>129</sup> presented a larger cohort of 20 SPK and 30 KTA patients (18 deceased and 12 living donor kidneys). Repeat kidney graft biopsies of SPK and KTA recipients were obtained 1-6.5 and 1-6.8 years after transplantation, respectively. They concluded that a functioning pancreas graft prevented development of diabetic nephropathy. Somewhat in contrast to these findings, Nyberg et al.<sup>130</sup> found that despite successful SPK transplantation, recurrence of early diabetic glomerular changes could be seen after 2-4 years follow-up in 11 recipients of a combined segmental pancreas and kidney graft. These recipients (SPK) had, however, impaired glucose control with a mean HbA<sub>1c</sub> of 7.2%, and only two out of 11 SPK recipients were categorised with normal glycaemic control. Bilous et al.<sup>131</sup> reported data on 12 recipients who underwent pancreas after kidney (PAK) transplantation. Pancreas transplantation was performed 1.0-7.2 years (a mean of 4.2 years) after KTA in this study. Renal biopsies were performed prior to successful pancreas transplantation (PAK) and at a mean follow-up time of 4.4 years post-transplant. These biopsies were compared with biopsies obtained after similar follow-up time in KTA recipients. Mesangial volume fraction was lower in the PAK than in the KTA recipients. However, GBM thickness was not significantly different between the two groups. This could be due to the relatively short follow-up time. One must also remember that these transplanted kidneys had been exposed to 1.0-7.2 years of hyperglycaemia prior to PAK

transplantation. This may also have contributed to an aborted effect of subsequent normoglycaemia. The evidence up to the present suggests, however, that successful pancreas transplantation ameliorates the development of diabetic glomerulopathy in the transplanted kidney.

In paper 4, we examine recurrence of diabetic glomerular lesions in the kidney allograft post-transplant comparing successful SPK transplantation with successful LDK transplantation. The sample and follow-up time is expanded compared with previous studies. This is the only study that compares an SPK kidney with long-term changes in a kidney derived from a living donor.

### **3. Aims of the Thesis and Specific Research Questions**

#### **3.1 Aims of the Thesis**

In the first study, the aim was to determine whether SPK transplantation could improve patient and kidney graft survival rates in type 1 diabetic patients compared with KTA, that is, with a kidney from either a living or deceased donor. The purpose of the second study was to assess whether SPK transplantation could improve long-term cardiovascular death rates compared with LDK transplantation alone. In the third study, the aim was to identify whether normoglycaemia achieved by a successful SPK transplantation could beneficially affect progression of CAD when compared with transplantation of single kidney from a living donor. The aim of the fourth and last study was to assess whether long-term normoglycaemia achieved by successful SPK transplantation could preserve kidney graft structure and function better than LDK transplantation alone.

#### **3.2 Specific Research Questions:**

**Paper 1:** Does SPK transplantation improve patient and kidney graft survival rates compared with KTA from either a living (LDK) or deceased donor (DDK)?

**Paper 2:** Does SPK transplantation improve cardiovascular death rates compared with LDK transplantation alone?

**Paper 3:** Does successful SPK transplantation prevent progression of CAD compared with LDK transplantation alone?

**Paper 4:** Does successful SPK transplantation preserve kidney graft structure and function better than LDK transplantation alone?



## **4. Research Strategy**

### **4.1 Study Design**

A study should be designed to minimise the influence of extraneous variables. A confounder, whether measured (known) or unmeasured (unknown), is a variable whose presence affects the variables being studied so that the results can be explained in more than one way; that is, it is difficult to tell whether the difference is due to the treatment itself or because of other differences between the groups. The confounding variable is associated both with the outcome and the treatment group or risk factor. Consequently, controlling for confounding variables can help to determine a causal relationship.

In selecting the study design for the present thesis, a randomised design was therefore the preferable approach. There are two main reasons to randomise: one is to eliminate selection bias and the other is to minimise confounding from known and, more importantly, unknown confounders. However, for ethical reasons, it is highly unlikely that a design in which patients with type 1 diabetes are randomised to either an SPK or kidney-only transplantation could be conducted. Therefore, the four papers of this thesis are based on observational cohort studies with a retrospective design and long-term follow-up. Accordingly, we must rely on statistical methods to adjust for potential confounding effects, and the retrospective design is limited to only addressing associations between treatment modality and long-term outcomes and not causal relationships.

### **4.2 Research Validity**

Validity indicates whether or not a study is appropriately designed and provides results that are suitable to be generalised to a wider population. Internal validity denotes how well a study is conducted and to what extent the study can draw warranted conclusions. Bias is one of the

three major threats to internal validity aside from confounding and random error. Systematic error, or bias, can be classified as selection bias, information bias, and confounding bias.

In this quantitative research design, the internal validity is influenced by the type of research design chosen and possible threats that may have changed the results. External validity is also important and closely related to the generalisability of the findings, that is, whether the findings apply to individuals whose place, time, and circumstances differ from those of the participants studied.

The validity of each of the four studies in this thesis will be further discussed in the discussion section 6.1 along with the reliability (the repeatability of the findings).

### **4.3 Study Population**

#### **4.3.1 Paper 1**

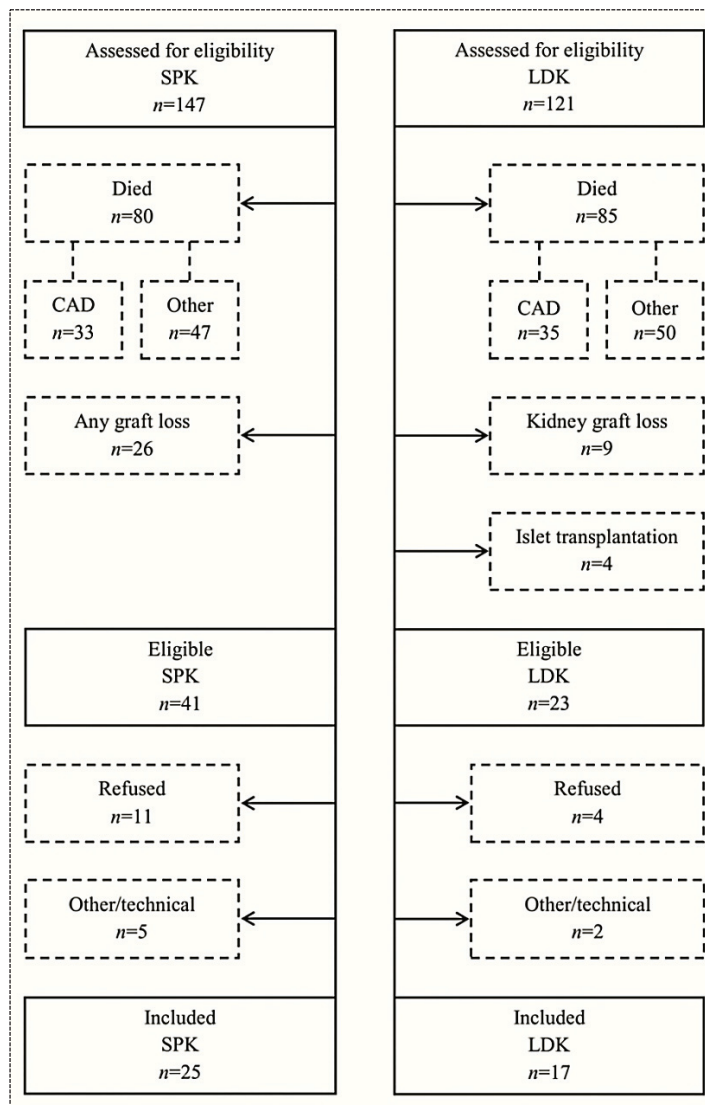
In the first paper, all patients with type 1 diabetes and ESRD engrafted with SPK ( $n=222$ ), LDK ( $n=171$ ) or DDK ( $n=237$ ) transplants between 1983 and 2010 were identified from the Norwegian Renal Registry and assessed for eligibility. No patient was lost to follow-up.

#### **4.3.2 Paper 2**

In the second paper, all patients with type 1 diabetes and ESRD engrafted with SPK ( $n=256$ ) or LDK ( $n=230$ ) transplants between 1983 and 2012 were identified from the Norwegian Renal Registry and assessed for eligibility. No patient was lost to follow-up.

### 4.3.3 Papers 3 and 4

The study participants in the third and fourth paper were all patients with type 1 diabetes and ESRD who had undergone SPK or LDK transplantation between 1983-2003 and were still alive with functioning grafts more than seven years post-transplant. They were identified from the Norwegian Renal Registry, assessed for eligibility, and invited to participate in an extensive follow-up study of the heart (third paper) and kidney graft (fourth paper). Patient disposition for papers three and four is shown in Fig. 7.<sup>135</sup>



**Figure 7.** Patient disposition for papers 3 and 4. Transplantation (2016) doi:10.1097/TP.000000000000 [published online ahead of print].<sup>135</sup> Wolters Kluwer Health Lippincott Williams & Wilkins©

## **4.4 Data Collection**

All explanatory variables judged as clinically relevant and/or possible confounders to the outcome variables were defined before any statistical analysis was performed.

### **4.4.1 Paper 1**

In paper 1, the outcome variables were patient and graft survival rates. Data on transplant type, recipient age, sex, time on dialysis, donor age, cold ischaemia time, human leukocyte antigen (HLA) mismatches, and era of transplantation—that is, the explanatory variables—were collected from the Norwegian Renal Registry. Date of transplantation, patient-related clinical events according to annual registry reports, that is, dates for kidney and/or pancreas graft losses or death, were retrieved to be included in the survival analysis. Kidney graft loss was defined as the need for dialysis treatment or repeat transplantation or death with a functioning graft. Pancreas graft loss was defined as the need for insulin treatment, HbA<sub>1c</sub> levels  $\geq 6.5$  %, or death with a functioning graft. Cases were closed for analyses on June 30, 2011.

### **4.4.2 Paper 2**

The outcome measures or variables in the second paper were all-cause and CVD- and CAD-related death. Data on transplant type, recipient age, sex, time on dialysis, donor age, HLA mismatches, and transplant year (the explanatory variables) were obtained from the Norwegian Renal Registry. Hospital or medical records were studied in particular to obtain more complete comorbidity data on the duration of diabetes; relevant medication, e.g., immunosuppressive and antihypertensive drugs and aspirin or statin therapy; history of coronary artery, cerebrovascular, or peripheral arterial disease; and smoking habits pre-transplant. Dates of transplantation, graft loss, and dates and causes of death were also

collected from the Norwegian Renal Registry to be included in the survival analysis. The end of follow-up was predefined to be the end of December 2014.

#### **4.4.3 Paper 3**

In paper 3, the main outcome variable was progression of CAD judged by differences between baseline and follow-up angiography. Coronary artery calcification (CAC) scores and left ventricular (LV) function at follow-up were two other outcome measures. Data on date of transplantation, transplant type, recipient age, sex, duration of dialysis pre-transplant, and donor age were retrieved from the Norwegian Renal Registry. Data on the duration of diabetes, cardiovascular events (myocardial infarction, percutaneous coronary intervention, and/or coronary artery bypass grafting), immunosuppressive and antihypertensive agents and aspirin and/or statin use, and smoking history were obtained after questioning each study patient at follow-up and/or using hospital records to acquire complete data. Annual HbA<sub>1c</sub> levels, cyclosporine and tacrolimus trough levels, systolic and diastolic blood pressure recordings, estimated glomerular filtration rate (eGFR) measurements, and lipids were collected either from the Norwegian Renal Registry, hospital records, and/or by examining the study patient at follow-up. Data on coronary artery luminal diameter stenosis, CAC scores, and echocardiographic variables were assessed by examination of patients with coronary angiography, computed tomography (CT) scans, and echocardiography respectively.

#### **4.4.4 Paper 4**

The outcome variables in the fourth and last paper were kidney graft structure, particularly GBM and podocyte foot process widths and mesangial volume fraction and function, that is, eGFR. Data on the date of transplantation, transplant type, recipient age, sex, donor age, and HLA mismatches were obtained from the Norwegian Renal Registry. Data on diabetes

duration; kidney graft rejection episodes; immunosuppressive and antihypertensive agents, including angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs); and statin use were retrieved by questioning each study patient at follow-up and/or the use of hospital records to obtain complete data. Annual recordings of HbA<sub>1c</sub> levels, cyclosporine or tacrolimus trough levels, and eGFR measurements were collected either from the Norwegian Renal Registry, hospital records, and/or by examining the study patient at follow-up. Data on lipids, alanine transaminase (ALT), urinary protein/creatinine ratio (PCR), systolic and diastolic blood pressure, and body mass index (BMI) were drawn either from the Norwegian Renal Registry, hospital records, or by examining the study patient at follow-up. Data on kidney graft structure were obtained from transplant kidney biopsies examined with light and electron micrographs.

## **4.5 Specific Investigations in the Thesis**

### **4.5.1 Cardiac Work-Up Before Transplantation**

For all type 1 diabetic patients who are potential candidates for kidney transplantation at our centre, coronary angiography has been a routine part of the cardiac work-up since 1999. Before 1999, type 1 diabetic patients were screened for CAD with a non-invasive cardiac stress test, and, if judged clinically relevant, coronary angiography was performed. Patients with an ejection fraction of less than 30% are not accepted for transplantation according to our kidney transplant protocol.

### **4.5.2 Post-Transplant Kidney Graft Function and Surveillance of Glycaemic Control**

The study participants in papers 3 and 4 were recruited from the same pool. The blood creatinine concentrations were measured in a stable phase approximately three months after transplantation and annually thereafter. Annual blood creatinine values following

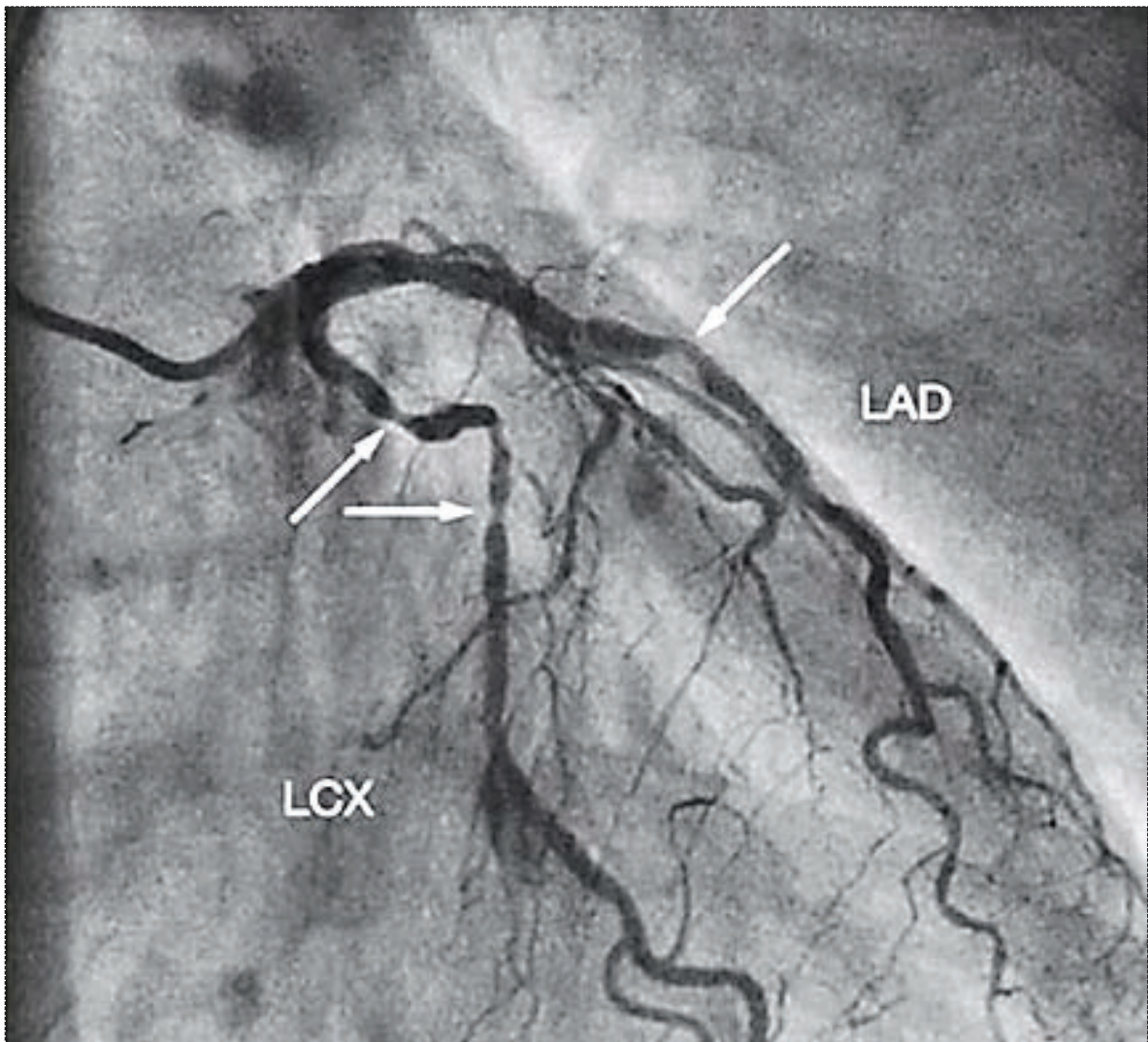
transplantation were either retrieved from the Norwegian Renal Registry or from local hospitals. Blood creatinine values obtained before June 2003, which were analysed using the old or conventional non-isotope dilution mass spectrophotometry (IDMS)-standardised assay, were converted to the new IDMS-traceable creatinine values. The old non-IDMS assay methods overestimate<sup>136</sup> true blood creatinine values by up to 20% compared with the IDMS method. Estimated GFRs were calculated by applying the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation,<sup>137</sup> which is based on IDMS-traceable creatinine analysis.

HbA<sub>1c</sub> values were analysed according to the DCCT standard and were used to assess glycaemic control in SPK and LDK recipients annually after transplantation. These were either retrieved from the Norwegian Renal Registry or from local hospitals. In paper 4, diabetic kidney disease in the donor kidneys was, as far as possible, ruled out by performing oral glucose tolerance tests (OGTTs) in living donors before approval for kidney donation. The absence of diabetes in the deceased donor is absolutely mandatory to be accepted for pancreas donation.

#### **4.5.3 Coronary Angiography**

Selective coronary angiography (CAG) was performed using standard projections of both left and right coronary arteries. A single experienced cardiologist, blinded to the patient's clinical information, evaluated all images by 'eyeball' estimates of the percentage of diameter stenosis. The progression of CAD was determined after comparing the results of baseline and follow-up CAG. Significant CAD was defined as coronary artery luminal diameter stenosis of 50% or greater in at least one of the segments using a 16-segment American Heart Association coronary artery classification.<sup>138</sup> Progression of CAD was defined as follows: (1)

development of new coronary artery lesions with luminal obstruction of 50% or greater, or (2) any increase in a luminal diameter stenosis ( $\geq 50\%$ ) from baseline to a higher degree of severity, that is,  $\geq 70\%$  to  $< 90\%$ ,  $\geq 90\%$  to  $< 100\%$  or 100% luminal obstruction. For more details, see the methods section of paper 3. Fig. 8<sup>139</sup> illustrates coronary artery luminal obstruction as judged by coronary angiography.



**Figure 8.** Coronary angiogram illustrating coronary artery luminal diameter stenosis of more than 70% in the mid left anterior descending (LAD) and mid circumflex (CX) coronary arteries (arrows). Right coronary artery (RCA) not shown. Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Cardiology, Möhlenkamp S et al., 2007<sup>139</sup>



#### **4.5.4 Coronary Artery Calcification Scores**

CAC was assessed with non-contrast CT scans. An experienced radiologist blinded to the patient's transplant type calculated the amount of CAC as an Agatston score<sup>140</sup> using a computerised program. An Agatston score of less than 400 is classified as mild to moderate, while 400 or greater is considered a high degree of CAC.<sup>141, 142</sup> See also the methods section of paper 3 for more information.

#### **4.5.5 Echocardiography**

Parasternal and apical imaging views were performed to measure LV myocardial wall thickness, chamber dimensions, and LV ejection fraction.<sup>143</sup> LV mass was calculated using the Devereux formula.<sup>144</sup> Cardiac output was calculated from LV outflow blood flow velocities multiplied by aortic annular diameter, assuming a circular shape. All recordings were performed by a single experienced research sonographer and analysed in dedicated software by a single observer blinded for patient status. The methods section of paper 3 provides more detail about the methods.

#### **4.5.6 Kidney Graft Structure**

A more detailed presentation of the methods used is given in the methods section of paper 4. Ultrasound guided percutaneous kidney graft core needle biopsies were sampled and glomerular structures in the kidney graft were examined by light and electron microscopy. Semi-quantitative estimation of kidney graft pathology was performed by light microscopy in line with the Banff scheme<sup>145</sup> and subsequent use of the chronic allograft damage index (CADI).<sup>146</sup> Glomerular structure morphometric measurements were performed by a single researcher blinded to treatment groups. The maximal profile area (MPA) method<sup>147</sup> was used to measure the glomerular area, from which glomerular volume was calculated, assuming the

glomerulus to be a sphere. GBM width was obtained from electron micrographs measuring the distance of a line orthogonal from the edge of the endothelial side to the edge of the epithelial side.<sup>148</sup> The volume fraction of glomerular mesangium was obtained from electron micrographs by point counting.<sup>149</sup> The podocyte foot process widths were measured by the method described by Deegens et al.<sup>150</sup>

#### **4.6 Statistical Analysis**

Statistical analysis was conducted always using the newest versions of IBM SPSS Statistics for Windows (IBM, Armonk, NY, USA) or Stata (StataCorp LP, College Station, TX, USA). All reported *p* values are two-tailed; *p* values <0.05 are considered significant.

For each paper in this thesis, baseline (at the time of transplantation) patient demographic data or characteristics were summarised and grouped by transplant type. Continuous variables are reported as the mean (standard deviation [SD]) or median (range or interquartile range [IQR]). Categorical data are described using frequencies (%). Depending on normal distribution, group differences were assessed using Student's *t* test for independent samples or the Mann-Whitney *U* (Wilcoxon) test was used to compare continuous variables, as appropriate. The Pearson  $\chi^2$  test was applied to compare categorical variables. Fisher's exact test was used if the number of observations per cell was fewer than five.

Survival analysis was used to analyse time-to-event data, that is, the length of time before an event (e.g., graft loss or death) occurred in paper 1 and 2. The Kaplan-Meier method<sup>151, 152</sup> was used to estimate the probability of survival as a function of time from a starting point (e.g., from the date of transplantation). The Cox proportional hazards regression analysis<sup>153</sup>

was used to calculate unadjusted and adjusted HRs for graft loss (not censored for patient death) and death. The proportional hazards assumption was tested with graphical checks.<sup>152</sup>

In research, it is rare to obtain complete data from every case. Missing data are observations a researcher intended to make but did not, and can reduce the power of a study and may also lead to biased results.<sup>154</sup> There are several statistical techniques to handle missing data.<sup>155</sup> The ways in which data are missing matters. Deleting from the analysis those cases in the dataset that had any data missing would constitute a so-called complete case analysis. If data are missing completely at random, that is, the chance of data being missing is unrelated to any of the variables in the analysis, a complete case analysis does not lead to bias.<sup>155, 156</sup> This is seldom the case, and complete participant analysis is usually not advisable unless the percent of patients with incomplete data is less than 5-10%.<sup>156, 157</sup> In paper 2, 9% of the cases had missing data on patient smoking history. The main survival analyses were performed after replacing missing data for smoking using multiple imputation.<sup>155, 156, 158, 159</sup> Missing values were estimated based on known explanatory and outcome variables, and 20 sets were created and pooled for analysis.

To determine the rate of decline of eGFR for the SPK and LDK group (papers 3 and 4), all measured eGFR values during annual follow-up were used in a repeated measures analysis treating time as a continuous variable (linear growth model). The eGFR slopes were derived for each group and compared for any difference. To compare any relationships between structural (e.g., GBM width, mesangial volume fraction) and functional (proteinuria) variables in the SPK and LDK groups, Spearman's correlations were calculated in paper 4.

#### **4.7 Research Ethics**

All studies were conducted according to the principles of the Declaration of Helsinki and approved by the Regional Committee for Medical and Health Research Ethics.

All study participants gave their written informed consent for papers 3 and 4.

## 5. Results

### 5.1 Paper 1

This study ('Improved patient survival with simultaneous pancreas and kidney transplantation in recipients with diabetic end-stage renal disease') assessed the associations between transplant type (SPK [ $n=222$ ], LDK [ $n=171$ ], or DDK [ $n=237$ ]) and long-term patient and kidney and pancreas graft survival in 630 patients with type 1 diabetes and ESRD transplanted from 1983 and to the end of 2010, and were followed to the end of June 2011.

The mean follow-up time after transplantation was 7.1 years. Median actuarial patient survival was 14.0 years for SPK, 11.5 years for LDK, and 6.7 years for DDK recipients respectively. In a multivariate Cox regression analysis adjusting for recipient age, sex, transplant modality, time on dialysis, and transplant era, we found that SPK transplantation was associated with improved survival compared with LDK transplantation (HR 0.70, 95% CI 0.52, 0.95;  $p=0.02$ ). This significance disappeared when we adjusted for donor age (HR 0.84, 95% CI 0.60, 1.18;  $p=0.32$ ). DDK transplantation was associated with inferior patient survival compared with both SPK and LDK transplantation. The most striking effect on patient survival was visible in transplant era. Patients transplanted after 2000 had an HR of 0.40 (95% CI 0.30, 0.55;  $p<0.001$ ) for all-cause mortality compared with recipients transplanted from 1983 to the end of 1999.

The median kidney graft survival was 11.0 (SPK), 9.3 (LDK), and 5.9 (DDK) years respectively. In a multivariate Cox regression analysis adjusting for recipient age, sex, transplant modality, time on dialysis, donor age, cold ischaemia time, HLA-DR mismatches, and transplant era, DDK recipients had inferior kidney graft survival (HR 1.45, 95% CI 1.08,

1.96;  $p=0.014$ ), using LDK recipients as a reference. Kidney graft survival did not differ between SPK and LDK recipients (HR 0.99, 95% CI 0.73, 1.37;  $p=0.99$ ).

Pancreas graft survival was significantly different between all three periods of transplantation (1983-1987, 1988-1999, and 2000-2010). Pancreas graft survival improved by the turn of the century, with a 5-year graft survival rate of 78% versus 61% in previous years (1988–1999). The most recent era showed superior pancreas graft survival compared with the middle era (HR 0.58, 95% CI 0.36, 0.94;  $p=0.029$ ). In a univariate Cox regression analysis, the only significant factor associated with pancreas graft survival was the era of transplantation. Inferior survival was seen in the first era, using the middle era as a reference (HR 2.82, 95% CI 1.84, 4.32;  $p<0.001$ ).

Our findings suggest that SPK transplantation is superior for patient survival compared with both LDK and DDK transplantation, but reliant on donor age in comparison with LDK transplantation. Kidney graft survival is equal between SPK and LDK transplantation, and both are superior to DDK transplantation. Pancreas graft survival has improved over the three eras.

## **5.2 Paper 2**

This paper ('Long-term cardiovascular outcomes in type 1 diabetic patients after simultaneous pancreas and kidney transplantation compared with living donor kidney transplantation') examined the associations between transplant type (SPK [ $n=256$ ] or LDK [ $n=230$ ]) and all-cause mortality and CVD- and CAD-related mortality.

The median follow-up time after transplantation was 7.9 years. There were 228 (SPK,  $n=100$ ; LDK,  $n=128$ ) deaths from all causes, of which 136 (60%) (SPK,  $n=57$  [42%]; LDK,  $n=79$  [58%]) were caused by CVD. Among CVDs, CAD was the cause of 86 (38%) deaths (SPK,  $n=36$  [42%]; LDK,  $n=50$  [58%]). In multivariate Cox proportional hazards models with adjustment for transplant type, recipient age, sex, duration of diabetes, number of antihypertensive drugs, aspirin and statin use, cardiovascular comorbidity, smoking habits, duration of dialysis, and transplant year, HRs and 95% CIs for all-cause and CAD-related mortality in SPK recipients were 0.76 (0.57, 1.03;  $p=0.074$ ) and 0.66 (0.41, 1.06;  $p=0.084$ ) respectively, using LDK recipients as reference. CVD-related deaths were significantly decreased: HR was 0.64 (95% CI 0.44, 0.93;  $p=0.019$ ) for SPK compared with LDK recipients. After additional adjustment for donor age, a significant difference was still apparent between SPK and LDK recipients for CVD-related mortality (HR 0.63, 95% CI 0.40, 0.99;  $p=0.047$ ). Compared with those who received their grafts between 1983 and 1999, patients transplanted after 2000 had adjusted HRs of 0.51 (95% CI 0.34, 0.77;  $p=0.001$ ), 0.39 (95% CI 0.22, 0.70;  $p=0.002$ ) and 0.37 (95% CI 0.17, 0.78;  $p=0.009$ ) for all-cause and CVD- and CAD-related mortality respectively.

Forty-four SPK recipients had lost their pancreas graft within 12 months post-transplant. Those who had a functioning ( $n=212$ ) or failing ( $n=44$ ) pancreas graft 1 year post-transplant were compared with the LDK recipients ( $n=230$ ) for mortality outcomes. SPK patients with a functioning pancreas graft 1 year post-transplant had reduced all-cause (HR 0.65 [95% CI 0.44, 0.96];  $p=0.029$ ) and CVD- (HR 0.46 [95% CI 0.28, 0.76];  $p=0.003$ ) and CAD-related (HR 0.46 [95% CI 0.24, 0.86];  $p=0.015$ ) mortality compared with the LDK group, after adjusting for potential confounders. SPK patients with a functioning pancreas graft did better than SPK patients with a failing pancreas graft for all-cause and CVD-related mortality (HR

0.35 [95% CI 0.23, 0.56],  $p < 0.001$ ; and HR 0.30 [95% CI 0.17, 0.54],  $p < 0.001$ ), respectively, but the difference was not statistically significant for CAD-related mortality (HR 0.24 [95% CI 0.06, 1.04];  $p = 0.057$ ). Compared with LDK recipients, all-cause mortality was higher in SPK patients with a failing pancreas graft 1 year post-transplant (HR 1.61 [95% CI 1.01, 2.57];  $p = 0.044$ ). This comparison was not significantly different for CVD- (HR 1.42 [95% CI 0.80, 2.52];  $p = 0.23$ ) and CAD-related (HR 1.52 [95% CI 0.75, 3.09];  $p = 0.25$ ) mortality.

In conclusion, we found that SPK transplantation was associated with reduced CVD-related mortality compared with LDK transplantation. SPK patients with a functioning pancreas graft had reduced mortality compared with LDK patients. Deaths from CVD are predominant after kidney transplantation in type 1 diabetes.

### **5.3 Paper 3**

The main objective of this study ('Cardiac assessment of patients with type 1 diabetes median 10 years after successful simultaneous pancreas and kidney transplantation compared with living donor kidney transplantation') was to search for associations between transplant type (SPK [ $n = 25$ ] or LDK [ $n = 17$ ] transplantation) and the progression of CAD, judged by angiography, between the time of transplant and follow-up median 10.1 years later. CAC scores and LV function at follow-up were also assessed.

We found that there was no difference in CAD-related deaths between SPK and LDK recipients in those assessed for eligibility. Mean HbA<sub>1c</sub> levels during follow-up were  $5.5 \pm 0.4\%$  and  $8.3 \pm 1.5\%$  in the SPK and LDK group, respectively ( $p < 0.001$ ). Forty-one (24 SPK/17 LDK) out of 42 patients underwent follow-up CAG. Baseline (pre-transplant) CAG data were evaluable in 35 patients (21 SPK/14 LDK). CAG demonstrated CAD in ten out of



21 SPK and one out of 14 LDK recipients at baseline ( $p=0.023$ ). Follow-up CAG revealed a progression of CAD in 10 out of 21 SPK patients and 5 out of 14 LDK patients ( $p=0.49$ ). The median CAC scores were high in both groups (1767 [IQR 321, 4035] and 1045 [IQR 807, 2643], for the SPK and LDK group respectively;  $p=0.59$ ). There were no significant differences between the SPK and LDK groups in terms of LV wall thickness, ejection fraction, or cardiac output at follow-up.

Our findings suggest that successful SPK transplantation is not sufficient to slow the progression of CAD compared with LDK transplantation median 10.1 years post-transplant. High CAC scores are a prominent feature in this type of patients.

#### **5.4 Paper 4**

The objective of this study ('In patients with type 1 diabetes simultaneous pancreas and kidney transplantation preserves long-term kidney graft ultrastructure and function better than transplantation of a kidney alone') was to assess associations between transplant type (SPK [ $n=25$ ] or LDK [ $n=17$ ]) and the development of early diabetic glomerular lesions and kidney transplant function in type 1 diabetic patients with intact graft functions more than 7 years post-transplant.

The median duration of follow-up was 10.1 years. Mean HbA<sub>1c</sub> levels during follow-up were  $5.5\pm 0.4\%$  and  $8.3\pm 1.5\%$  in the SPK and LDK group, respectively ( $p<0.001$ ). In the LDK recipients, the GBM width was significantly wider ( $369\pm 109$  nm versus  $281\pm 57$  nm;  $p=0.008$ ) and the mesangial volume fraction was significantly higher ( $0.23$  [0.13-0.59] versus  $0.16$  [0.10-0.41];  $p=0.007$ ) than in SPK recipients at follow-up. There were no statistical

differences in podocyte foot process width between SPK and LDK recipients at follow-up (507 [381-777] nm versus 523 [447-1,268] nm;  $p=0.12$ ).

Three months after transplantation there was no significant difference in the eGFR, which was  $78\pm 17$  and  $69\pm 13$  ml/min/1.73 m<sup>2</sup> in SPK and LDK recipients respectively ( $p=0.059$ ). From baseline to follow-up the eGFR significantly differed between the SPK and LDK recipients at follow-up ( $67\pm 24$  versus  $46\pm 14$  ml/min/1.73 m<sup>2</sup>;  $p=0.002$ ) but the absolute fall in eGFR ( $\Delta$ eGFR from baseline to follow-up) was not statistically different ( $p=0.060$ ). The slope of eGFR over the actual observation periods was  $-1.1$  ml/min/1.73 m<sup>2</sup> per year in SPK recipients, which was significantly better than  $-2.6$  ml/min/1.73 m<sup>2</sup> per year in the LDK group ( $p=0.001$ ).

We conclude that SPK transplantation compared with LDK transplantation was associated with better preservation of kidney graft ultrastructure and function after transplantation.

## **6. Discussion**

### **6.1 Research Strategy**

All scientific studies have certain limitations due to their research strategies. There are two main types of errors, random and systematic, which could affect the reproducibility and the validity of a study. Random errors may affect the reproducibility and systematic errors (bias) could threaten the validity. Important causes of systematic errors are selection bias, information bias, and confounding. Selection bias is a systematic difference in characteristics between those who are selected for study and those who are not. Information bias is information that is erroneous or misclassified by placing someone in the wrong category. Confounding means the mixing or blurring of effects; the researcher believes he or she is measuring the effect of an exposure on the outcome, while actually measuring the effect of another confounding factor. The confounding factor obscures the real effect of an exposure.

As mentioned above, due to a lack of a randomised design for all four papers in this thesis, associations can only be drawn from transplant type and long-term outcomes. A randomised design would better control for known and unknown confounders that may have biased the outcome. However, possible confounders were identified and controlled for in the statistics.

Selecting a relevant control group is important when comparing an outcome between different therapies. To properly test a therapy with a control group, it is critical that the groups are matched for other variables that may confound the results. For our patient population, the LDK group appeared to be the best control group rather than a DDK group for comparison with the SPK group. However, there are nonetheless differences in recipient, donor, and transplant factors between the SPK and LDK groups that could obscure the interpretation of the results.

Given the limitation that treatment cannot be selected randomly for type 1 diabetic patients with ESRD, a more proper control group could have been (1) a sample of type 1 diabetic patients who were originally waitlisted for SPK transplants but ended up receiving DDK transplants, or (2) DDK recipients with type 1 diabetes receiving the contralateral kidney from the same deceased donor as an SPK recipient. However, this design would present a problem of obtaining a large enough sample, at least in a single-centre study.

Missing data and data placed in wrong category in a registry may also affect the results. Data on panel reactive antibodies (PRA), otherwise relevant for comparing adjusted graft survival rates between the groups, were only available for more contemporary cohorts and therefore not included in the analysis (due to too much missing data). The definition of pancreas graft loss could also be questioned. HbA<sub>1c</sub> levels have been reported since 1997 in the Norwegian Renal Registry. Oral glucose lowering agents, insulin dosage, and C-peptide levels have not been reported routinely in annual registry reports. Patients with a partially functioning pancreas transplant may therefore have been classified with graft loss.

The study participants in papers 3 and 4 were recruited from the same pool, and were a selection of survivors with intact long-term graft functions post-transplant. Only 42 of 64 (66%) available patients were finally included. (The reasons for study refusal were not systematically recorded, but long distances to the hospital and time spent on examinations were two important barriers to participation.) Thus, a selection bias is obvious from the original 268 patients assessed for eligibility. To evaluate the degree of selection bias, the characteristics of those who participated should be compared with those who were eligible to participate and did not. Unfortunately, in paper 3 we did not have any complete data on the status of CAD during follow-up for those who were eligible to participate and did not. As a

compromise, we compared deaths due to CAD between SPK and LDK patients among those who were assessed for eligibility. The intention of paper 3 was, however, to study long-term evolution with normoglycaemia (SPK) versus hyperglycaemia (LDK) and not SPK transplantation versus LDK transplantation as methods.

In paper 4 the scenario was different than in paper 3 in that the kidney grafts had not been exposed to hyperglycaemia before transplantation as was the case for patient coronary arteries in paper 3. In paper 4, a selection bias was also present and we had no knowledge of the status of kidney graft structure in those patients who were eligible to participate and did not.

Furthermore, taking into account the biological variation in the ultrastructure (e.g., GBM width) of the kidneys, only analysing two glomeruli of the kidney graft at follow-up and lacking GBM widths at the time of transplantation may have biased the results. However, one might expect random errors to be fairly evenly distributed among SPK and LDK kidney graft biopsies, and it is less likely that there were systematic morphometric differences between the SPK and LDK donor kidneys at the time of transplantation. On the other hand, annual eGFR measurements post-transplant could also have been collected from those patients who were eligible to participate and did not in order to describe the eGFR slope post-transplant more accurately.

The reproducibility or reliability in papers 3 and 4 could be questioned as there is no measure (coefficient) of the reproducibility or reliability for evaluating coronary artery lumen diameter stenosis and kidney graft structure variables respectively. Coronary angiograms were evaluated by an experienced cardiologist blinded to patient status. A potential weakness of this study is that a second cardiologist did not evaluate the same films for verification. For the evaluation of kidney graft structure variables, however, there was another experienced

pathologist who examined some of the micrographs as a quality check to ensure the reproducibility and consistency of the estimates in the study. Also, half of the cases were examined twice by the primary examiner as a control. Accordingly, the reliability of the CAC scores and echocardiographic measurements may also be questioned, and lack of baseline data makes it impossible to quantify changes. On the other hand, since two groups were compared, one might expect random errors to be fairly evenly distributed across the SPK and LDK recipients and affect the two groups in a similar way. Also, all these measurements are well-established methods that are known to have generally high reliability.

Statistical power is the probability of detecting an effect given that the effect is actually present. In other words, it is the probability of rejecting the null hypothesis when it is in fact false. For example, in paper 4 GBM widths at follow-up were compared between SPK and LDK kidney grafts. If receiving successful SPK grafts truly is effective, the statistical power is the probability of finding a difference between the SPK and LDK groups. In this study, a power of 0.9 means that 90% of the time a statistically significant difference between the SPK group and the LDK group would be obtained. This also means that 10% of the times that this study were to be run, there would be no statistically significant difference between the two groups, even though there is a real difference present (type II error). Power was calculated for the clinical endpoint GBM width prior to examination of kidney graft biopsies in paper 4. In two-tailed analysis, provided the standard deviation was less than 25% of the average value in the sample and a meaningful difference in outcome parameters was 20%, the study had more than 90% power to detect a difference at an alpha error level of 5%. Unfortunately, power analysis was not performed prior to paper 3 (on the angiographic progression of CAD), and the probability of making a type II statistical error is indeed present.

## **6.2 Results**

The main finding of this thesis was that SPK transplantation was associated with a beneficial effect on patient survival, cardiovascular death rates, and recurrent diabetic disease in the SPK transplant kidney graft compared with LDK transplantation. These results could possibly be due to better glucose control in recipients of SPK transplants compared with LDK transplants, but more favourable recipient and donor factors in the SPK group make direct post-transplant comparison difficult.

### **6.2.1 Patient Survival**

In type 1 diabetic patients, the results from our single-centre study demonstrated that receiving SPK transplants compared with either an LDK or a DDK transplant was directly associated with better patient survival after transplantation. The survival benefit in SPK recipients compared with LDK recipients was dependent on donor age.

Other reports on patient survival rates after transplantation in type 1 diabetic patients across the three transplant treatment modalities—SPK, LDK, or DDK—have shown divergent results. The inconsistent results may stem from a combination of factors, including the use of an international registry versus single-centre analyses, date of transplantation, differences in age and comorbidities, immunosuppressive regimens, surgical techniques, as well as small sample sizes or short follow-up times.

Patient survival in SPK transplantation is closely related to long-term functioning of the pancreas graft. Studies have shown that SPK transplant pancreas graft survival at one year post-transplant improves patient survival long-term compared with that of an LDK transplant. In the early years of pancreas transplantation, SPK transplant pancreas graft survival rates

were poor compared with more contemporary SPK transplant populations, which have benefitted from advances in surgical technique and immunosuppressive therapy. Therefore, more contemporary patient populations with SPK transplants probably reflect more accurately the impact of pancreas transplantation on patient survival compared with KTA.

Low donor age is associated with better outcomes after kidney transplantation.<sup>160-162</sup> A low donor age is a prerequisite and an inherent part of the SPK transplant method. It could thus be debated whether it is appropriate to adjust for donor age in the statistical analysis. Morath et al.<sup>111</sup> do not support donor age adjustment. Therefore, donor age adjustment may be less relevant comparing the real outcome of SPK transplantation with single kidney transplantation. On the other hand, adjusting for donor age is more appropriate when evaluating the effect of normalised metabolic control after successful SPK transplantation compared with kidney-only transplantation.

### **6.2.2 Graft Survival**

There was no difference between SPK transplant and LDK transplant kidney graft survival rates in the population studied. This could possibly be due to more favourable donor characteristics in the SPK group compared with the group receiving KTA. Kidney graft survival rates with LDK are regularly better compared with deceased donor kidney transplants.<sup>7, 19-21</sup> A possible direct association between better glycaemic control and improved kidney graft survival in SPK recipients compared with LDK recipients is less likely since kidney graft loss due to recurrent diabetic nephropathy in the kidney graft is expected to take up to two decades or more to manifest.



### 6.2.3 Cardiovascular Outcomes

In type 1 diabetic patients, receiving an SPK transplant compared with an LDK transplant was directly associated with reduced risk of death secondary to CVD following transplantation.

Most studies report all-cause mortality in patients with type 1 diabetes after kidney transplantation and not cause-specific mortality such as CVD- and CAD-related mortality. However, all-cause mortality indirectly reflects death due to CVD, which is the predominant cause of death among kidney transplant recipients. Recipients of kidney transplants may also die from infection and malignant disease, which are competing risks of death.

In our study, no difference was evident in the progression of CAD after transplantation when comparing SPK recipients with LDK recipients. This is in contrast to the study by Jukema et al.<sup>127</sup> and could possibly be viewed as an unexpected finding given that successful SPK transplantation normalise glucose control and has been shown to improve cardiovascular risk factors compared with KTA. On the other hand, SPK recipients, like LDK recipients, have been exposed to hyperglycaemia for more than 20 years as well as uraemia of various durations. Both are important risk factors for CVD prior to transplantation. In addition, patients receiving SPK transplantation have historically received heavier loads of immunosuppression post-transplant. Both these pre-transplant and post-transplant factors may have moderated the possibly beneficial effect of restoring normal glucose control in SPK recipients.

The finding of no difference in progression of CAD between SPK and LDK patients is somewhat in contrast to our finding of reduced cardiovascular death in SPK recipients compared with LDK recipients. This could possibly be explained by the fact that CVD also

includes cerebrovascular disease and heart failure, aside from CAD, which also are important causes of death in type 1 diabetic patients. Another issue is that in patients with type 1 diabetes and established CAD, hypoglycaemia following use of insulin might increase the risk of death due to cardiovascular autonomic neuropathy,<sup>163</sup> resulting in abnormalities in heart rate control and vascular dynamics. Furthermore, a meta-analysis<sup>164</sup> showed that cardiovascular autonomic neuropathy was associated with an increased risk of mortality in patients with diabetes. In an editorial from the same group,<sup>165</sup> the conclusion from the Action to Control Cardiovascular Risk in Diabetes (ACCORD) study<sup>166</sup> is that autonomic dysfunction is a significant risk factor for CVD. Thus, a lack of difference in CAD progression or mortality between SPK patients and LDK patients may be due to another competing risk of CVD and death.

#### **6.2.4 Diabetic Graft Nephropathy and Kidney Graft Function**

Compared with LDK transplant recipients, SPK transplant recipients with functioning pancreas grafts after transplantation were more likely to preserve kidney graft ultrastructure with fewer diabetic glomerular changes. This falls in line with previous intervention studies with intensified glucose control in patients with type 1 diabetes.<sup>1, 4, 5</sup>

Metabolic (hyperglycaemia) and haemodynamic (glomerular hypertension and hyperfiltration) derangements play a major role among several pathophysiologic mechanisms involved in the development and progression of the histopathological changes seen in diabetic nephropathy.<sup>167</sup> Hyperglycaemia leads to increased glycolysis with subsequent upregulation of the polyol and hexosamine pathways, production of advanced glycation end products (AGEs), and activation of protein kinase C (PKC).<sup>168</sup> These pathological processes that occur in susceptible kidneys exposed to hyperglycaemia may eventually manifest as diabetic

nephropathy. Tight glucose control reduces the risk of developing diabetic kidney complications. An elevation in the GFR (hyperfiltration) is frequently seen in the early course of type 1 diabetes,<sup>169</sup> and may lead to glomerular hypertrophy and increased renal size.<sup>170</sup> These changes to glomerular haemodynamics and structure are important and associated with the development and progression of diabetic nephropathy.<sup>171</sup> Dilation of the afferent glomerular arteriole due to increased sodium-glucose absorption in the proximal tubule and decreased sodium flow near macula densa is an important factor contributing to hyperfiltration.<sup>169, 172</sup> This is believed to take place through a tubuloglomerular feedback mechanism.<sup>173</sup> Furthermore, vasoconstriction of the efferent arterioles results in glomerular hyperfiltration and is mediated through the renin-angiotensin-aldosterone system (RAAS). ACE inhibitors<sup>174</sup> and ARBs<sup>175</sup> have renoprotective effects, in that they reduce progression of albuminuria, increase in creatinine, and eventually the occurrence of ESRD.

Thickening of the GBM may also occur in hypertensive nephropathy and nephropathy due to calcineurin inhibitor (CNI)-toxicity, as well as in the early phase of antibody mediated rejection or chronic T-cell mediated rejection. In these cases, the ultrastructural thickening of the GBM is characterised by thickening of the lamina rara interna secondary to an injury to the endothelium. This is in contrast with thickening of the GBM in diabetes (the most specific glomerular change of diabetes), in which the lamina densa part of the GBM is thickened. The late phase of chronic rejection is characterised by formation a double contour of the GBM. The other typical change in diabetic nephropathy is nodular glomerulosclerosis (nodular mesangium sclerosis), which is not frequently seen in early changes of diabetic nephropathy. CNI-toxicity of the kidney manifests most often as global glomerulosclerosis. Hypertensive nephropathy can imitate the diabetic-related increase in the mesangium. There were no differences between the SPK and LDK group in the mean of the annual blood pressure

measurements during follow-up or in the frequency of kidney rejections. Also, the levels of CNIs have historically been higher in SPK transplantation compared with LDK transplantation.

The slope of eGFR over time in the SPK kidney grafts was better than in LDK kidney grafts. However, there are potential confounding factors in that result. An obvious fact is that donor age for SPK kidney grafts was lower compared with LDK kidney grafts; increased donor age is inversely associated with kidney graft survival. Different use of CNIs and ACE inhibitors or ARBs could also explain a group difference in eGFR slope. Dosage of CNIs has, however, historically been higher in SPK transplantation compared with kidney-only transplantation, and this would instead favour a steeper decline in eGFR in the SPK group. More LDK patients used ACE inhibitors or ARBs, which could also have had a negative short-term effect on eGFR but a more favourable long-term effect on eGFR. Increased recipient blood pressure is inversely associated with kidney graft survival. There was, however, no difference in blood pressure between SPK and LDK patients. Recurrence of diabetic glomerular lesions in the LDK kidney grafts could partly explain the difference in eGFR slope compared with SPK kidney grafts, but it is less likely to be a main cause given the long time span required to develop a decline in GFR secondary to diabetes.

### **6.3 Strengths and Limitations**

The strengths of the studies in papers 1 and 2 are that they represent a large single-centre experience with long-term follow-up time, and that no patient was lost to follow-up.

Therefore, the selection bias for comparing SPK with LDK patients may have been less pronounced than in studies from larger international registries. Also, paper 2 had detailed data available on cardiovascular risk factors at the time of transplantation. The strengths of papers

3 and 4 are that they are the only studies that have compared SPK with LDK recipients, and at present they represent the largest studies on comparison of CAD progression and occurrence of glomerular diabetic lesions post-transplant respectively.

The limitations of the present studies are their retrospective and non-randomised design. Apart from a few exceptions, these are cohorts of white individuals only, and data on cardiovascular risk factors at the time of transplantation were incomplete, especially for paper 1. A selection bias is present in papers 3 and 4 as well as small patient samples, and the results should be interpreted with caution. The intra- and inter-reliability of papers 3 and 4 could also be questioned.

## 7. Conclusions

Below are the answers to the specific research questions posed in section 3.2.

**Paper 1:** SPK transplantation was associated with superior patient survival rates compared with both LDK and DDK transplantation, although the SPK transplant advantage over LDK transplantation was dependent on donor age. Furthermore, no difference was found in kidney graft survival rates between SPK and LDK transplantation, whereas kidney graft survival rates in DDK transplantation was inferior compared with both SPK transplantation and LDK transplantation alone.

**Paper 2:** Compared with LDK transplantation, SPK transplantation was associated with a long-term reduced risk of CVD-related death compared with LDK transplantation alone.

**Paper 3:** In SPK and LDK recipients with functioning grafts at median 10.1 years after transplantation, SPK transplantation was not associated with a slowing of the progression of CAD compared with LDK transplantation alone.

**Paper 4:** In SPK and LDK recipients with functioning grafts at median 10.1 years after transplantation, SPK transplantation was associated with better preserved kidney graft ultrastructure and function compared with LDK transplantation alone.

## **8. Implications and Future Perspectives**

### **8.1 Implications**

This study provides data that are of potential importance to clinicians and patients helping to select the most appropriate therapeutic option for patients with type 1 diabetes and advanced chronic kidney disease. This research has shown that an SPK transplant does not jeopardise, but on the contrary may improve patient survival even when compared with LDK transplantation. SPK transplantation should be considered a preferable treatment option for a selected group of type 1 diabetic patients with low comorbidity and some degree of failure in insulin-based therapy preventing acute diabetic complications. LDK transplantation should still be encouraged. If a living donor is available, particularly in patients with well-regulated type 1 diabetes, LDK transplantation shows good long-term outcomes. Furthermore, LDK transplantation contributes to reducing the wait list for kidney transplantation.

### **8.2 Future Perspectives**

Randomised controlled trials are probably unlikely to be conducted for the purpose of comparing outcomes in SPK transplantation compared with LDK transplantation. Furthermore, several Western countries (e.g., Poland, Finland, Italy, Belgium, and Germany) do not encourage kidney donation from living donors for ethical reasons. Future studies should include large patient samples from contemporary populations, and long-term follow-ups of more than 10 years should be advocated to verify whether SPK transplantation provides better outcomes than LDK transplantation. It takes time before the added surgical risk of the more complex SPK procedure is outweighed by the benefit of glycaemic control attributable to a functioning pancreas allograft. Advances in CNI-free and steroid-free immunosuppression and in diagnosis of pancreas rejection/immune monitoring, including donor-specific antibody as a non-invasive biomarker of rejection, will likely lead to even

better pancreas graft outcomes and likely better patient survival. Studies on immunosuppressive drugs with less diabetogenic effects (e.g., belatacept) and studies on pancreas transplant rejection markers should therefore be emphasised in the future. Postoperative surgical complications, particularly graft thrombosis (both arterial and venous), are still major threats to pancreas graft survival. Future studies with microdialysis, a minimally-invasive sampling technique used for continuous monitoring of molecules in the extracellular fluid, may be helpful in detecting early ischaemia (thrombosis) and rejection in pancreas transplants, and may eventually improve pancreas graft survival in the early post-surgical period.<sup>176, 177</sup> An expansion of SPK recipients to also include selected patients with type 2 diabetes and older recipients may be a possibility. In order to more reliably prove the concept that SPK transplantation is able to prevent the development of early diabetic glomerular lesions, only well-designed future studies in which kidney biopsies are performed at the time of transplantation and by protocol at defined follow-up times (e.g., 5, 10, and 15 years) will improve our current knowledge.



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