Research in the domain of nocturnal home hemodialysis (NHD): Long-term clinical outcomes of NHD patients compared to conventional hemodialysis (CHD) patients post renal transplantation

by

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Robert Pauly was responsible for the study design, including the specific research question and analysis plan, in collaboration with Christopher Chan, Andreas Pierratos and James Hanley. Robert Pauly prepared the research ethics board submissions, and completed the data collection. Robert Pauly conducted the statistical analysis under the supervision of James Hanley, and was responsible for the interpretation of the results and preparation of the manuscript in collaboration with the aforenamed co-authors. Reem Asad assisted in data collection, Jeffrey Zaltzman assisted in subject identification and interpretation of the results, and Anne Chery was responsible for patient matching.

LIST OF ABBREVIATIONS

4-D Study	Die Deutsche Diabetes Dialyse Studie
ACE	angiotensin converting enzyme
ADEMEX Study	Adequacy of Peritoneal Dialysis in Mexico Study
ARF	acute renal failure
BDI	Beck's Depression Inventory
CHD	conventional hemodialysis
CHF	congestive heart failure
CI	confidence interval
CKD	chronic kidney disease
CORR	Canadian Organ Replacement Registry
CrCl	creatinine clearance
CRF	chronic renal failure
CRP	C-reactive protein
CVD	cardiovascular disease
DGF	delayed graft function
ECF	extracellular fluid
eGFR	estimated glomerular filtration rate
EPC	endothelial progenitor cells
EPO	erythropoetin
ESRD	end-stage renal disease
HDL	high density lipoprotein
HEMO Study	Hemodialysis Study
HR	hazard ratio
Il	interleukin
KDQOL-SF	Kidney Disease Quality of Life – Short Form
LDL	low-density lipoprotein
LVH	left ventricular hypertrophy
LVMI	left ventricular mass index

MDRD formula	Modification of Diet in Renal Disease formula
MICS	malnutrition-inflammation complex syndrome
NHD	nocturnal home hemodialysis
NIH	National Institutes of Health
PD	peritoneal dialysis
PTH	parathyroid hormone
RCT	randomized controlled trial
RRT	renal replacement therapy
TNF-α	tumor necrosis factor-alpha
UNOS	United Network of Organ Sharing
USRDS	United States Renal Data System

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Abstract

Nocturnal home hemodialysis (NHD) is a novel type of renal replacement therapy (RRT) whereby patients administer their own dialysis on 5 to 7 nights per week, each session lasting between 5 and 8 hours. NHD is known to effectively reverse and even normalize many of the physiological aberrations of uremia. However, at present, renal transplantation remains the gold standard RRT and NHD patients continue to be referred for transplantation. Unfortunately, on account of such small numbers of patients undergoing NHD worldwide, little is known about the short- and long-term clinical outcomes of NHD patients after kidney transplantation. It was hypothesized that the incidence of delayed graft function (DGF), patient and graft survival, and post-transplant estimated glomerular filtration rate (eGFR), is better among conventional hemodialysis (CHD)-transplanted individuals than among those having received NHD. Of 231 past and current NHD patients in the Toronto NHD program, 36 underwent renal transplantation between January 1, 1994 and October 31, 2006. These 36 patients were matched to 68 transplanted CHD patients with a maximum follow-up of 11.7 years to test these hypotheses in the setting of a retrospective cohort study. The incidence of DGF was not significantly greater in the NHD group than in the CHD group [NHD: 15/35 (42.9%) vs. CHD: 25/68 (36.8%) $p = 0.43$] with an unadjusted OR of 1.41 (95% CI 0.60-3.27). Estimated (e)GFR was predicted by pre-transplant weight, donor age and recipient race; and assessed at baseline; at 1, 3, 6, and 12 months post-transplantation; and annually thereafter. Dialysis modality prior to transplantation did not influence the level of eGFR post-transplantation ($p = 0.34$), nor the rate of eGFR decline. Post-transplant hemoglobin was also not affected by pre-transplant dialysis modality ($p = 0.12$). Post-transplant systolic blood pressure was 4.8 mmHg lower in the CHD group ($p = 0.04$), though these patients were receiving significantly more anti-hypertensive medications during the first two years after transplantation. Patient survival was no different among NHD and CHD groups (log rank $p = 0.91$), though deceased donor graft survival favored CHD (log-rank $p = 0.01$) with an unadjusted HR of 0.14 (95% CI 0.02-1.27); however, this latter result is based on only 5 events and should be interpreted with caution. Based on this analysis the incidence of DGF was similar between NHD- and CHD-transplanted patients and pre-

transplant modality did not impact on the level or rate of deterioration of post-transplant eGFR.

Résumé

L'hémodialyse nocturne à domicile (HDN) est un nouveau type de suppléance rénale (SR) où les patients s'administrent eux-mêmes leur dialyse 5 à 7 nuits par semaine, chaque session durant entre 5 et 8 heures. L'HDN est reconnue pour renverser, et même corriger, plusieurs des aberrations physiologiques de l'urémie. Cependant, à ce jour, la transplantation rénale demeure la modalité de SR de choix et les patients sous HDN continuent d'être référés pour une transplantation. Malheureusement, en raison du faible nombre de patients sous HDN dans le monde, nous savons peu de choses au sujet de leur devenir à court et long terme après une transplantation rénale. Des hypothèses ont été formulées à l'effet que l'incidence de fonction retardée du greffon (FRG), la survie du patient et du greffon, ainsi que le taux de filtration glomérulaire estimé (TFGe) post-transplantation étaient meilleurs chez les patients greffés ayant reçu de l'hémodialyse traditionnelle (HDT) que chez ceux ayant reçu de l'HDN. Des 231 patients anciens et actuels du programme d'HDN de Toronto, 36 ont subi une transplantation entre 1994 et le 31 octobre 2006. Pour vérifier ces hypothèses, une étude de cohorte rétrospective a comparé ces 36 patients à 68 patients transplantés qui recevaient de l'HDT avec un suivi maximal de 11.7 ans. L'incidence de FRG n'était pas significativement plus grande dans le groupe d'HDN que dans le groupe d'HDT [HDN : 15/35 (42.9%) vs. HDT : 25/68 (36.8%) p = 0.43] avec un *odds ratio* non-ajusté de 1.41 (IC 95% 0.60-3.27). Le TFGe était prédit par le poids pré-transplantation, l'âge du donneur et la race du receveur; la modalité de dialyse avant transplantation n'influençait pas le niveau de TFGe post-transplantation (p = 0.34), non plus que la vitesse de diminution du TFGe. L'hémoglobine post-transplantation n'était pas non plus affectée par la modalité de dialyse pré-transplantation (p = 0.12). La pression artérielle systolique post-transplantation était 4.8 mmHg inférieure dans le groupe d' HDT (p = 0.04), mais ces patients recevaient significativement plus de médications antihypertensives pendant les deux premières années après la transplantation. La survie des patients n'était pas différente entre les groupes d'HDN et d'HDT (log rank p = 0.91), mais la survie des greffons provenant de donneurs cadavériques favorisait l'HDT (log rank p = 0.01) avec un *hazard ratio* non-ajusté de 0.14 (IC 95% 0.02-1.27); cependant, ce résultat est basé sur

seulement 5 événements et devrait être interprété avec prudence. Il semble que l'incidence de FRG est similaire entre les patients transplantés provenant de l'HDN et de l'HDT et que la modalité pré-transplantation n'influence pas le niveau ou la vitesse de détérioration du TFGe post-transplantation.

Chapter 1: Background

INTRODUCTION

Nocturnal home hemodialysis (NHD) has become the subject of much research over the past decade owing to the increasing evidence linking this form of renal replacement therapy (RRT) to the reversal of physiological perturbations of uremia, and the pervasive feeling in the nephrology community that conventional dialysis modalities have reached a plateau in effectiveness. NHD involves patients self-administering their dialysis typically 5 to 6 nights per week with each session lasting between 6 and 8 hours. Thus, patients receive between 2 to 4 times the amount of dialysis as they would if they were receiving conventional hemodialysis (CHD) or peritoneal dialysis (PD), though direct comparisons are difficult to make and depend on the definition of dialysis dose.

However, NHD is still in its infancy and the proportion of patients in North America using this modality is less than 0.5 % [1]. Given the small number of centers with experience in NHD and the relatively few patients within each program, research in the domain of NHD has a number of challenges. Experimental and cohort studies are limited in power and subject to bias that is difficult to overcome with limited sample sizes. Quasi-experimental studies are better suited to NHD research but also have their drawback. Currently, there is no published framework for conducting or reviewing research involving NHD.

Despite the limitations of the existing research, a growing body of knowledge suggests that NHD effectively reverses, and in many cases completely normalizes, some of the pathophysiological sequelae (or the 'unphysiology' [2,3]) of uremia to an extent that traditional dialysis modalities cannot. This raises an interesting question: how will NHD fit into the cadre of end-stage renal disease (ESRD) treatment options, particularly relative to renal transplantation which is widely considered the gold standard RRT? At present, NHD patients are routinely referred for kidney transplantation given the convincing evidence that survival is improved with transplantation over CHD, and the lack of any direct comparison between transplantation and NHD. It is unknown whether short- or long-term transplant outcomes differ between patients having been on NHD versus CHD immediately prior to their transplantation.

The purpose of this thesis is [a] to develop a structural framework defining biases associated with research in the domain of NHD and suggest strategies to overcome these shortcomings, and [b] to examine the short- and long-term clinical outcomes of transplantation for patients previously on NHD.

LITERATURE REVIEW

Historical Context

Until the mid-1960s end-stage renal disease (ESRD) was an almost certain death sentence for patients with deteriorating kidney function. Though short-term hemodialysis had been demonstrated by the mid-1940s and had gained increasing popularity in the 1950s and early 1960s for the successful treatment of reversible acute renal failure (ARF), the absence of reliable vascular access prohibited its use for chronic renal failure (CRF) [4]. In these early days of RRT, PD was also just evolving into a potential treatment option, but remained largely inaccessible due to the exorbitant expense of the treatment system, the necessity of repeated abdominal puncture and the significant risk of infection [5]. Kidney transplantation, which had been successfully carried out in 1954 between identical twins, quickly became the gold standard RRT in the 1960s, but has always been limited by availability of donor organs and by the fact that many patients with ESRD have co-existing medical conditions that preclude transplantation. It was not until Belding Scribner and Wayne Quinton developed a reliable, repeated-use vascular access system in 1960, innovations in vascular access surgery in the late 1960s, and the replacement of the original coil dialyzers with hollow fiber dialyzers in the early 1970s, that hemodialysis emerged as the predominant treatment for patients with ESRD [4].

In the early era of hemodialysis, routine treatments typically lasted 8 hours or longer, 2 to 3 times per week, usually in a hospital setting. However, in 1964, Scribner's group is credited with developing the first unattended, overnight home hemodialysis, that was self-administered by the patient with the help of a nonmedically trained assistant [4]. Because of the cost-savings and convenience of this approach, home hemodialysis became so widely accepted that by the 1970s over a third of ESRD patients in the United

States were being treated at home [6]. However, this approach went out of favor by the early 1980s with more generous government funding, the overly optimistic reliance in urea kinetic modeling and the belief that adequate dialysis could be delivered with three times-a-week hemodialysis where each session lasts 3 to 4 hours [7,8]. The result was that the trend toward home hemodialysis reversed and treatment moved back into designated hemodialysis units.

However, not everyone was convinced that reducing the amount of dialysis resulted in optimum patient outcomes and several European groups have never abandoned their original long, intermittent hemodialysis regimen of 8 hours dialysis, 3 times-a-week. Justification included superior phosphate and blood pressure control along with improved survival [9,10]. An alternate intensive dialysis strategy that also has its origins in the late 1960s is short daily hemodialysis. This typically consists of 2 to 2.5 hour sessions of dialysis 6 days per week. For a variety of logistic and financial reasons this approach, too, has mostly fallen by the wayside in North America, though is still championed by a small number of European and Canadian centers who have reported on the clinical and biochemical advantages of this strategy over conventional hemodialysis (CHD) [11,12].

Though it is apparent that the constituent features of NHD are not novel, this treatment strategy is the first to combine the three elements of long, intermittent treatment duration, frequent (*ie*. daily) treatment sessions, conducted in the home setting. Additional evidence also suggests that the nocturnal (versus during the day) nature of the regimen has important physiologic and social implications. Uldall and colleagues were the first to implement this strategy in 1994, whereby patients underwent 8 hours of hemodialysis on 5 to 7 nights per week [13].

Interest in this treatment modality has markedly increased over the past decade for two primary reasons. Firstly, there is mounting evidence that NHD is able to reverse many of the physiological perturbations of uremia better than other forms of dialysis. Secondly, evidence suggests that PD and CHD have attained their maximum effectiveness and that relatively minor differences in small solute clearances, as assessed by urea *Kt/V*, miss the bigger picture that uremic clearance as achieved by conventional dialysis therapies is still grossly inadequate [14,15]. This latter point is based on the results

of two randomized controlled trials: the Hemodialysis (HEMO) Study and the Adequacy of Peritoneal Dialysis in Mexico (ADEMEX) Trial, where patients received equilibrated *Kt/V* of 1.16 versus 1.53 or peritoneal *Kt/V* of 1.62 versus 2.13, respectively. No benefit to increasing dialysis dose was demonstrated in either study suggesting that traditional dialysis modalities have likely reached a plateau in their ability to affect morbidity and mortality [16]. The fact that the annual mortality among ESRD patients is approximately 20% and has remained relatively unchanged for the past 2 decades is a sobering reminder of the limitations of traditional dialysis modalities [17]. Cardiovascular disease (CVD) accounts for nearly half of all deaths [17]. However, with typical *Kt/V* values of 2 to 2.5 *per session* and the highest weekly urea clearance of any renal replacement strategy, NHD is arguably the most physiological relative to traditional dialysis modalities. Whether NHD will appreciably impact mortality remains to be seen; a head-to-head trial comparing NHD to either CHD or PD has yet to be done.

Reversing the "unphysiology" of ESRD with NHD

Many clinical and physiological benefits of NHD over CHD are well documented and continue to be active areas of research. Even though there are no direct comparisons between NHD and PD, it is presumed that the superior uremic clearance of NHD will impart similar benefits of NHD over PD.

Cardiovascular system

The multitude of beneficial effects of intensive hemodialysis on the cardiovascular system have been reviewed in detail elsewhere [18]. Briefly, early data from Pierratos *et al*. showed that NHD patients consistently demonstrated a reduction in blood pressure with the average number of antihypertensive drugs per patient decreasing from 2.67 to 1.67 within one year of commencing NHD [14]. In fact, only half of those patients on medication at the time of conversion from CHD to NHD remained so after a year, the rest having been able to discontinue their drugs completely. This finding has been replicated in virtually all studies involving NHD patients since then, and comparable results were also reported for short daily hemodialysis [19]. Additionally, Chan and colleagues reported a 22% reduction in left ventricular mass index (LVMI) after three years in 28 patients

receiving NHD, while control patients receiving CHD demonstrated further progression of their left ventricular hypertrophy (LVH) by 6% [20]. In fact, patients with known congestive heart failure (CHF) and moderately-severe echocardiographically-documented systolic dysfunction, had a 46% improvement in their ejection fraction after conversion from conventional dialysis to NHD [21]. With respect to other traditional cardiovascular risk factors, conversion from CHD to NHD was also associated with an increase in high density lipoprotein (HDL) cholesterol and reduction in homocysteine [22,23]. Overall, emerging evidence also suggests there to be fewer hospitalizations among NHD patients when compared to CHD patients [24] and Kjellstrand *et al.* recently demonstrated a mortality advantage with short-daily hemodialysis patients [25], though both reports are thus far published only in abstract form.

Extracellular fluid volume

A number of mechanisms are thought to underlie these improved cardiovascular parameters and the presumed morbidity and mortality benefit derived from them. Firstly, uremia and ESRD result in volume overload from salt and water retention and pressure overload from increased peripheral vascular resistance and arterial stiffness. Together these factors culminate in hypertension, concentric and eccentric LVH and ultimately in CHF [26]. It is well established that both LVH and CHF are independent predictors of mortality in the dialysis population. Perhaps most obviously, the increased frequency and duration of NHD is characterized by more stable extracellular fluid (ECF) status, and thus avoids the often huge volume fluctuations characteristic of CHD. This volume-related mechanism likely contributes to the findings of Chan and others and may be a pathway for circumventing the detrimental cascade of fluid overload, left ventricular remodeling, LVH, CHF and ultimately cardiomyopathy.

Anemia

Enhanced anemia management with NHD may prove to be another avenue to off-load cardiac work in the ESRD population as anemia has been associated with progression of LVH [27], *de novo* development of CHF and cardiovascular mortality [28]. Though treatment with erythropoietin (EPO) has been the mainstay of anemia management for over two decades, significant variability in EPO sensitivity remains, and

ample data suggest that hormone resistance is at least in part related to inadequate dialysis and the inflammatory milieu of ESRD [29-34]. In comparison to conventional dialysis, NHD has been associated with an 8% improvement in hemoglobin levels with concomitant 27% reduction in EPO requirements at one year, independent of iron status [35]. In a cross-sectional study of 14 NHD patients age and co-morbidity-matched with CHD patients, Yuen *et al*. found a direct association between EPO requirements and highly sensitive C-reactive protein (hsCRP) with lower CRP and interleukin (Il)-6 levels in the NHD patients [36]. EPO resistance has also been tied to secondary hyperparathyroidism, a condition that also improves with NHD. Elevated parathyroid hormone (PTH) may have a direct inhibitory effect on EPO synthesis [37,38] and cause marrow fibrosis resulting in decreased erythrogenesis [39]. Regardless, medical or surgical management of hyperparathyroidism has consistently improved hematocrits and EPO sensitivity [40]. For its part, NHD has repeatedly been shown to decrease the prevalence of secondary hyperparathyroidism in association with dramatic improvement in EPO sensitivity [41,42]. Taken all together, the evidence suggests that NHD normalizes the balance between various pro- and anti-erythrogenic factors resulting in more efficient red cell production.

Vascular calcification

Vascular calcification is believed to play an important contributory role to CVD in ESRD and with the recent demonstration that calcium phosphate crystals can activate macrophages and induce pro-inflammatory cytokine secretion *in vitro*, a compelling link has been made between abnormal Ca/PO_4 metabolism, inflammation and atherogenesis [43]. Further *in vitro* evidence suggests that tumor necrosis factor (TNF)-α is able to directly promote vascular calcification by inducing osteoblastic transformation of vascular smooth muscle cells [44]. Additionally, a substantial body of evidence suggests that ESRD is associated with an imbalance between promoters and inhibitors of calcification including fetuin-A, osteopontin and others [45,46]. In a recent study of the natural history of coronary artery calcification in 38 NHD patients, Chan *et al.* found no significant increase in coronary artery calcification score over a one-year period irrespective of whether patients had a high or low baseline calcific burden [42]. The same group presented a case report of a patient with severe peripheral arterial disease and metastatic calcification who had

profound improvement in limb claudication as well as lower extremity duplex Doppler ultrasound upon conversion to NHD suggesting significant regression of arterial calcification with this treatment modality [21]. It has been well established that NHD results in improved Ca/PO_4 control with increased weekly phosphate clearance, decreased Ca-based phosphate binder usage, liberalization of dietary phosphate intake, and reduction in the Ca x PO_4 product [41,42]. How circulating levels of specific calcification promoters and inhibitors respond to intensive dialysis has yet to be investigated, though we hypothesize that on balance, NHD results in a more normalized ratio of these competing factors.

Progenitor cell function

Yet another mechanism whereby NHD may prove to play a pivotal role in normalizing the "unphysiology" of ESRD and cardiovascular morbidity is in its effect on endothelial progenitor cells (EPCs). These marrow-derived cells function to repair vascular injury by proliferating and differentiating at the site of tissue damage in order to restore normal endothelial integrity and function [47]. The number of EPCs inversely correlate with cardiovascular risk [48,49] and EPCs exhibit impaired differentiation and function with reduced survival in the presence of CRP concentrations known to pose cardiovascular risk [50]. Predictably, EPCs are diminished in ESRD with significantly impaired migratory capacity [51]. Indeed, it was recently demonstrated that EPC number and function are not corrected with three times-a-week hemodialysis while NHD was associated with normalization of not only EPC number but also their migratory function [52]. These observations, again, provide very compelling evidence that the physiology of NHD is completely different from that of conventional dialysis, and more closely approximates the norm.

Other

A number of other physiological aberrations are also subject to correction with NHD and are likely to positively impact cardiovascular health. NHD has been shown to correct ESRD-associated sleep apnea common in dialysis patients [53], and it was demonstrated that this finding is accompanied by normalization of autonomic nervous system activity and improved oxygenation [54]. In fact, NHD is not only associated with

improvement of systolic blood pressure, but also with decreased total peripheral resistance, increased arterial compliance, lowered circulating catecholamines and normalized endothelium-dependent vasodilation [55,56]. All of these physiological parameters have been correlated with adverse cardiovascular outcomes.

Role of Renal Transplantation

Despite the growing body of evidence pointing to the physiologic restorative properties of NHD, correcting the uremic milieu with renal transplantation has, appropriately, been the gold standard RRT for the nearly half a century. This was underscored in the late 1990s by the seminal study of Wolfe and colleagues demonstrating that the number of years of life with transplantation exceeds that with conventional dialysis modalities for all age groups studied, independent of diabetes status [57]. This study included a broad population of patients (all causes of ESRD, between 0 and 74 years of age); however, data was not stratified according to dialysis modality. Despite these optimistic findings, it must be remembered that even after renal transplantation, the majority of graft recipients have a significant burden of CKD corresponding to an eGFR of approximately 50%, or CKD Stage 3 [58]. One-year deceased donor graft survival has steadily improved over the years: 85.3% in 1995 to 89.5% in 2003, and 91.7% versus 95.3% for living donor grafts in the same time period [17]. However, long-term graft survival has changed remarkably little with graft survival half-life (contingent on 1-year survival) being 11.4 years in 1995 and 10.5 years in 2002 for deceased donor grafts, and 18.4 years and 19.1 years for living donor grafts in the same years [17]. Over the past decade much emphasis has been placed on understanding the predictors of short- and long-term graft survival; review of this extensive literature is beyond the scope of this writing and has been reviewed elsewhere [59-61].

Of relevance to this thesis is that dialysis modality immediately prior to transplantation is one variable that has received some attention as a possible predictor of transplant outcomes. The majority of studies exploring this association have focused on CHD versus PD; only a single study compared post-transplant outcomes between CHD and NHD. The short-term complications of transplantation that are most frequently reported include acute rejection, delayed graft function (DGF), graft thrombosis and

infection. In a study by Snyder *et al.* comparing 22,776 renal transplant recipients, PD prior to transplantation was found to be associated with 1.33 times increased risk of death-censored graft failure within the first three months of transplantation as compared to CHD (95% CI 1.16 – 1.53); the hazard ratio was adjusted for a wide range of donor-, recipient, and procurement-related covariates [62]. Only the incidence of graft thrombosis was increased among PD-transplanted patients, while the incidences of hyperacute rejection, acute rejection, primary non-function, infections, and surgical/urological complications were no different between the dialysis modalities; the incidence of DGF was lower in patients previously on PD. This latter observation confirms the findings of 10,584 transplant recipients from the United Network of Organ Sharing (UNOS) registry where PD was found to be protective for both absence of urine production in the first 24 hours post-transplantation and the need for dialysis within the first week post-transplantation [63]. The preponderance of graft thrombosis within 30 days of transplantation in PD-transplanted patients was also noted in a large case-control study based on UNOS/USRDS data [64].

However, outcomes from smaller non-registry studies are conflicting with some indicating PD versus CHD is associated with an increase [65], or no difference [66] in renovascular graft thrombosis; an increase [67], or no difference [68] in acute rejection; a decrease [68-70] or no difference [71] in DGF; and an increase [72] or no difference [67,68,73] in post-transplant infection rates. These studies have a great deal of variability and tend to be limited by small samples sizes, different durations of follow-up, and include patients from various transplant eras using different immunosuppressive regimens.

Long-term outcomes, as assessed by death-censored graft loss and overall patient survival, tend to suggest that outcomes between PD- and CHD-transplanted patients are similar [62,68,71,73,74]. However, the largest study of 92,844 USRDS patients specifically aimed to addressed the questions of allograft and recipient survival found that PD immediately prior to transplantation was associated with a 3% lower risk of graft failure and a 6% lower risk of death [75]. The results were similar when analyzing survival relative to the predominant RRT modality, defined as the modality used for more than 50% of the total ESRD time. The duration of follow-up in this study exceeded 10 years.

At present, no study reports on the long-term outcomes for NHD-transplanted patients compared to other dialysis strategies. The only study to investigate any clinical outcomes of transplantation among patients previously receiving NHD involved 15 NHD patients matched to 29 CHD subjects on the basis of age, era of transplantation, duration of ESRD and transplant type (*ie*. living, deceased donor or kidney-pancreas) [76]. The authors reported a trend toward increased incidence of DGF ($p = 0.15$), but similar 1-year Cockcroft-Gault equation-estimated creatinine clearance (CrCl). Not surprisingly, pre-transplant systolic and diastolic blood pressures were lower among the NHD group, though this difference did not persist greater than 5 months post-transplantation. Post-transplant thrombosis, acute rejection and infection rates were not reported, presumably because a total sample of 44 subjects may have been too small to see even a single occurrence of these complications. Because of its small sample size and limited duration of follow-up, this study leaves the question of whether pre-transplant NHD affects post-transplant outcomes ambiguous.

Conclusion

NHD is a novel form of RRT that combines the advantages of long duration dialysis with frequent sessions. It represents the most intensive form of hemodialysis and is associated with improvement or even normalization of many physiological aberrations of uremia, more so than traditional dialysis modalities. The majority of NHD-related research has been conducted in a small number of centers but this is changing as more dialysis programs are adding NHD into their range of RRT services. As such, more investigators are faced with the challenges of designing studies with small sample sizes and limited power, and more reviewers are faced with critically appraising this literature. Having a guide to interpreting NHD studies would be useful to assist both researchers and readers.

With the current paucity of literature, patients initiating NHD are routinely referred for renal transplantation. At present, the short- and long-term outcomes of renal transplantation for individuals having previously undergone NHD are not clearly delineated. Because the clinical and biochemical outcomes of NHD are so different as

compared to CHD, it cannot be assumed that the post-transplant course of NHD patients is the same as for CHD patients.

OBJECTIVES

The objectives of the thesis were:

1. to synthesize a coherent framework for critically appraising and designing research in the domain of NHD.

   Specific aims:

   a. to identify the limitations of traditional experimental and observational study designs as they relate to NHD research,
   b. to discuss the unique role of quasi-experimental study designs in NHD research, and
   c. to outline the major limitations of quasi-experimental studies as they relate to NHD research including strategies to limit bias.

2. to compare short- and long-term outcomes of renal transplantation between graft recipients having previously received NHD versus those having received CHD.

   Specific aims:

   a. to determine whether pre-transplant hemodialysis modality influences the incidence of DGF,
   b. to determine whether pre-transplant hemodialysis modality predicts either post-transplant eGFR or the rate of eGFR decline, and
   c. to compare post-transplant blood pressure, antihypertensive medication use and hemoglobin concentration between NHD and CHD.

Chapter 2: Methodological Considerations for Research in Nocturnal Home Hemodialysis (NHD)

Methodological Considerations for Research in Nocturnal Home Hemodialysis (NHD)

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Running Title: Methodological considerations in NHD research

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ABSTRACT

Nocturnal hemodialysis (NHD) is a form of frequent intensive renal replacement therapy, which has been suggested to offer multiple physiological benefits over conventional hemodialysis (CHD). Though the evidence supporting these advantages is steadily increasing, significant methodological challenges exist in conducting research in this area. Our review highlights these important considerations and potential biases within the present NHD literature and suggests future research opportunities.

Keywords: Nocturnal Hemodialysis, Quasi-experimental Designs, Methodology

INTRODUCTION

Nocturnal hemodialysis (NHD) is a form of intensive renal replacement therapy whereby patients self-administer their dialysis at home on 4-7 nights per week for 5-8 hours per night. Patients routinely achieve 30-40 hours of dialysis weekly, an amount approximately 3 times that of patients receiving conventional hemodialysis (CHD). The myriad of physiological and psychosocial benefits of this form of intensive dialysis have been described elsewhere [77,78] and underlie the increasing interest in this modality. Though the body of evidence supporting the beneficial effects of NHD is steadily expanding, several key methodological challenges exist in conducting rigorous investigations in this area. The purpose of this review is to highlight these important considerations in the conduct and interpretation of research in the domain of NHD.

COHORT STUDIES AND RANDOMIZED CONTROLLED TRIALS (RCTS)

To date, the total estimated number of end-stage renal disease (ESRD) patients being treated with home hemodialysis (both conventional home hemodialysis and NHD) in North America is approximately 1500, a value less than 0.5% of all hemodialysis treated individuals [1]. This statistic underscores the fact that home hemodialysis in

general, and NHD in particular, is in its infancy despite encouraging clinical outcomes for the latter. With such small numbers of subjects utilizing this modality, it stands to reason that cohorts large enough to yield meaningful research are few in number. To our knowledge, only a few retrospective and concurrent NHD cohorts with greater than 75 patients exist worldwide. In addition to small numbers of NHD patients is the substantial limitation of obtaining meaningful control patients similar enough to the NHD group, differing only in their modality of renal replacement therapy (*ie.* similar subjects who would otherwise be candidates for NHD but are being treated with a different modality). This requires a sufficiently organized system of collecting and accessing data on all ESRD patients to allow for efficient and convenient matching of NHD patients to controls. Furthermore, as with all cohort studies, the occurrence of the outcome under study must be frequent enough to give the study adequate power to detect a clinically significant difference between those exposed and not exposed to an intervention such as NHD, given the small sample size available to most investigators. While changes in surrogate outcomes may be substantial enough to result in meaningful changes in the setting of a small, well-controlled cohort study, this is unlikely to be the case for harder outcomes such as cardiovascular events or mortality.

The most desirable study design to determine the effectiveness of any intervention is a randomized controlled trial (RCT), which, if properly executed, minimizes systematic error more than any other design. However, there are often ethical, financial, or practical reasons making an RCT unfeasible or even impossible. To date, there have been no published RCTs comparing NHD to other ESRD treatment modalities, leaving the question of whether NHD definitively improves ESRD outcomes, and by how much, unanswered. This paucity of evidence has certainly contributed to the lack of widespread acceptance and financial underwriting of NHD programs [79]. In an effort to rectify this gap in trial data the National Institutes of Health (NIH) and the Centers of Medicare and Medicaid Services are funding an RCT comparing NHD to CHD (either in-centre or at home). The primary outcomes are a composite of 1-year mortality and change in health-related quality-of-life, or a composite of 1-year mortality and change in left ventricular mass. Review of the recently published protocol highlights some of the challenges of conducting an RCT with NHD patients [80]. Even with composite outcomes as defined by

the protocol, a sample size of 250 has been estimated to yield a clinically meaningful result; however, achieving this sample size in the time frame of the proposed study may prove difficult. Had mortality alone been the primary outcome, several thousand patients would have to be included in each arm in order to yield a meaningful result. Owing to the relatively small number of NHD programs in North America and the modest number of patients at each center, conducting a trial with only clinically hard outcomes would ultimately prove exorbitantly expensive and is currently unachievable.

QUASI-EXPERIMENTAL STUDIES

With such small numbers of incident NHD patients at any given centre, investigators have needed to rely on study designs more prone to bias and less supportive of causal inference. A commonly used methodological paradigm used in NHD research is the quasi-experimental study. These studies consist of designs whereby investigators non-randomly assign interventions to patients according to pre-specified study protocols or where investigators are bystanders who take advantage of a change in patients' state to study an outcome hypothesized to be influenced by the change in that state (*ie*. the intervention may be NHD while the change in state may be the conversion from CHD to NHD). The distinction between a quasi-experiment and a true experiment is that the in the former the study team does not have control over all (or possibly any) aspects of intervention allocation or timing [81]. Most modern epidemiology texts make little mention of these study designs, if at all; however, they serve as the mainstay of research methodology in the intensive dialysis literature due to their inherent simplicity and accommodation of small sample sizes. However, a recent systematic review of the effects of NHD on blood pressure, left ventricular geometry, Ca/PO_4 metabolism, anemia, and health-related quality-of-life highlighted both the promising effects of this renal replacement modality as well as the short-comings of quasi-experimental designs [77]. Of the 14 studies included in the review, all used some variation of the pre/post-NHD conversion design paradigm.

Quasi-experimental designs are typically of three forms: the *one-group comparison* where the experimental unit (*ie*. the patient) serves as his or her own control

before the introduction of the intervention (*ie*. conversion to NHD), the *multiple-group comparison* where the experimental unit exposed to the intervention is compared to a control group not exposed to the intervention, or a combination of these approaches where the experimental unit is observed before and after the intervention but is simultaneously compared to an external control group which does not undergo the intervention (Table 1) [82]. Understanding the major limitations of these study designs will aid in the interpretation of the NHD literature, in addition to guiding prospective researchers to create rational study protocols.

Table 1 – Quasi-experimental designs

Design	Paradigm
One-group comparison	O - X - O
Multiple-group comparison	X - O - O
Combination	O - X - O O - - O

O represents a measurement or observation
X represents an intervention
NB. Other variations on these paradigms exist

Threats to Internal Validity

Internal validity refers to the degree an inference from an experiment is warranted given the methodology and populations under study [81] and threats to internal validity (*ie*. biases) can wholly or in part provide an alternate explanation to the observed results. Though initially described for quasi-experimental designs in the social science literature in the early 1960s, these threats are equally applicable to NHD-related research and warrant consideration. Campbell and Stanley outlined 7 sources of bias that may render a study internally invalid: history, maturation, testing, instrumentation, statistical regression, selection bias, and differential loss of respondents (Table 2) [83]. The degree to which these affect the interpretation of a study will depend on the choice of paradigm (one-group comparison, multi-group comparison, or a combination of both) and the nature of the study itself (*ie*. threats to a study measuring left ventricular geometry before and after conversion to NHD will be different than threats to a study assessing quality of

Table 2 – Sources of invalidity in quasi-experimental study designs

Sources of invalidity	Definition
History	Changes in the experimental unit occurring as a result of a concurrent secular trend or unintended co-intervention
Maturation	Changes in the experimental unit occurring as a function of the passage of time such as a normal developmental process, subjects aging, or gaining experience
Testing	Changes in post-intervention observations occurring as a result of the pre-intervention test itself
Instrumentation	Changes in post-intervention observations occurring as a result of changes in observers or the instruments used to make observations
Statistical regression	Changes in post-intervention observations due to extreme pre-intervention observation tending to the mean with a second measurement (*ie*. regression to the mean)
Selection bias	Bias occurring when experimental units in the intervention group differ from those in the control group in some systematic way
Differential losses	Bias occurring when experimental units in the intervention group are lost to follow-up in a systematically different manner than from the comparator group
Variable ascertainment timing	Lack of comparability between measurements within an individual experimental unit or between comparator groups due to inconsistent timing of ascertainment (with respect to an intervention or calendar time *ie.* time of day/week/month etc.)

life before and after conversion using a questionnaire).

History threats refer to any unintended event or co-intervention that affects the outcome of observations in conjunction with the experimental intervention, that may affect the interpretation of those observations. This presents a particular dilemma to the pre-post one-group comparison design since there is no way to know with certainty the extent to which changes in management strategies over time affect the outcome independent of the intervention. When the intervention is NHD and the outcome is the dramatic reduction of blood pressure one may have some confidence in attributing this beneficial effect to some aspect of NHD (either changes in volume status or as a result of improved uremia clearance) when no other change in hypertension management (besides the removal of antihypertensive medications) has occurred during the study period. However, any beneficial effect of NHD on a variable such as parathyroid hormone levels, must be viewed with much more caution since changes in diet, phosphate binders, or dialysate calcium concentrations may change in conjunction with NHD and all can affect secondary hyperparathyroidism. Even the addition of a control group, which serves to minimize a history threat from changes in secular trends in ESRD treatment in general, cannot control for changes in management as a result of NHD, changes that have down-stream effects but are not intrinsically associated with NHD itself.

Maturation threats can alter outcome measures as a result of uncontrolled physiological or behavioral changes within individuals that occur with the passage of time and not necessarily as a result of a specific intervention. This is typically a concern in neonatal or pediatric studies where children's natural development can interfere with the inferences regarding an intervention. However, with NHD this may also be a potentially important threat when studying outcomes that may vary with patients' experience and technique comfort over time. Maturation threats are reduced by minimizing the interval between the intervention and the pre- and post-intervention observations, or by statistical techniques to adjust for confounding.

Testing threats can occur when a pre-intervention test familiarizes the participants with the testing instrument enough to change the outcome of the post-intervention test if the same assessment tool is used in both instances. Studies involving repeated surveys, questionnaires, or interviews are most susceptible to this type of threat, which is best dealt with by including a control group that receives the same testing schedule as the experimental group, but without the intervention.

Instrumentation threats may occur when either the observers or their means of observation change between the pre- and post-intervention periods, or when the observers or observation instruments differ between the experimental group and the control group. This source of bias will need to be considered in the future when, owing to the modest numbers of NHD patients per centre, investigators collaborate by pooling their resources and patient populations. Assurance needs to be made that the data collection instruments are identical across all study sites and that study personnel apply these instruments in the same fashion, both within and between centres [84].

Taken together, testing and instrumentation threats constitute forms of measurement error, which may affect comparator groups differently. Differential misclassification of events due to problems in measurement of outcomes may ensue if outcome ascertainment (or classification) is somehow associated with the intervention status of the subjects in some systematic way. The result is an artificially exaggerated or diminished measure of association between the intervention and the outcome measure. Imagine, for example, that the measurement of left ventricular mass (which, if assessed echocardiographically, is relatively operator-dependent) is consistently over-estimated by

unblinded personnel in individuals on conventional dialysis relative to NHD patients; the resulting association between dialysis intensity and cardiac geometry is biased by the consistent misclassification of conventionally dialyzed patients as having more severe cardiac disease than they may have in reality. On the other hand, if misclassification is non-differential (*ie*. affecting exposed and unexposed individuals in a similar manner), the measure of association will generally tend towards the null hypothesis (*ie*. no difference between the pre- and post-intervention observations). Either form of error represents a bias and may affect internal validity.

Statistical regression refers to uniquely extreme observations naturally tending to the mean upon repeat ascertainment. This is commonly called *regression to the mean* and is often a subtle threat to internal validity in quasi-experimental designs. A number of design features can mitigate the effects of this problem: making serial observations (*ie*. the time-series experiment), averaging several observations made at the same time, studying variables/outcomes with intrinsically low biological variability, and incorporating an internal control with predictable responses to the presence or absence of the intervention. Ascertainment of left ventricular mass index can serve as a convenient internal control in NHD research since it is well documented to regress with intensive dialysis while continuing to progress with conventional dialysis [20].

Selection bias may be the most significant threat to internal validity in quasi-experimental studies involving NHD patients. This bias can occur when the subjects receiving the intervention differ systematically from those serving as controls. The NHD literature has been criticized for the presence of such bias since patients undergoing home dialysis tend to be younger, healthier, and a greater proportion being Caucasian than other ESRD patients [14,19]. This issue underscores the importance and difficulty of choosing appropriate controls. Identifying adequate control subjects requires a reliable means to access a pool of conventional dialysis patients. Ideally, NHD patients are matched with others eligible for NHD but who remain on traditional dialysis modalities. Matching criteria should include the most important variables, such as age, sex, duration of ESRD and comorbidities, and statistical adjustment made for known confounders for which matching is not feasible. Despite rigorous selection of a control group, the limitation of this approach remains the inability to adjust for unknown (and thus unmeasured) or under

appreciated (and perhaps imperfectly measured) confounders which can potentially bias the observed magnitude of effect that the intervention is presumed to exert.

A bias from *differential losses* may occur when members of the intervention or control groups drop out of the study; internal validity is violated if the characteristics of these individuals are different from those remaining in the study. An alternate way to conceptualize this problem is by considering differential losses as a *selecting out bias*, as could be the case with losses to follow-up or censoring due to kidney transplantation in a cohort study of ESRD patients. To date, this has not been a problem in the NHD literature owing to the small sample sizes of the studies, with relatively short durations of follow-up and the subsequent ease of following a select group of patients. In principle, however, subjects dropping out need to be accounted for and, where possible, their characteristics described in relation to those participants remaining under investigation, recognizing that this only alerts readers to the possible presence of bias, while not actually correcting it.

An additional threat to internal validity which is not adequately covered by the Stanley and Campbell classification system, but is very germane to dialysis-related research is the potential bias introduced by *variable ascertainment timing*. That is, the timing of measurement with respect to dialysis treatments can be critically important. Blood pressure recordings, left ventricular mass determinations, or serum phosphate levels (to name but a few examples) can fluctuate greatly in the inter- and intradialytic periods, so that *when* these measurements are made relative to a dialysis session is very relevant. This issue is certainly not unique to NHD research but is broadly applicable to ESRD investigations, and also highlights the limitation of using a single numeric value to represent a dynamic variable. For example, the choice of systolic blood pressure, systolic and diastolic blood pressures, or mean arterial pressure taken pre-dialysis, post-dialysis, or in the interdialytic period are all arbitrary and an analysis adjusting for any of these will inadequately encompass the complex biological effects of this hemodynamic phenomenon. To mitigate this bias all variables should be ascertained in a consistent manner for all comparator groups with respect to dialysis session timing, and ideally, multiple measurements ought to be made and averaged over the course of serial dialysis runs. Though this strategy aims to minimize the bias caused by inconsistent measurement

timing it cannot correct for the residual confounding created when single values are meant to represent dynamic variables.

Concerns with Random Error

Random error is a function of a study's sample size and the inherent variability in the outcome measure. The latter is, to some extent, influenced by the precision of the measurement instrument. Sample size in a quasi-experimental study is ideally determined in the same manner as for any other observational or experimental study: estimating the change in the parameter under study in the non-intervention population, estimating the magnitude of change with NHD, and deciding *a priori* the amount of tolerable type I and type II error. A type I error occurs when the analysis suggests a difference between the intervention and control groups exists when in reality none does, whereas a type II error occurs when the analysis suggests a difference does not exist when in reality it does. Unfortunately, given the constraints of a limited sample size in most programs, even including all possible NHD patients may still be associated with an appreciable risk of committing a type II error: all things being equal, a study with a small sample size may be insufficiently powered to detect a difference in outcome between NHD and CHD, when in reality a difference exists.

Threats to External Validity

That NHD patients have tended to be younger and healthier that other dialysis patients was previously mentioned as a threat to internal validity when comparing these patients to a control group within the study itself. However, when trying to extrapolate the results of NHD outcomes to the ESRD population in general, the concern is one of external validity. Also called generalizability, external validity refers to the unbiased inference to the broader target population beyond the study participants, that follow from the results [81], bearing in mind that a study must be internally valid as a prerequisite to being generalizable. Inherent to appreciating the scope to which NHD results are applicable to all dialysis-dependent patients is understanding patient eligibility for home hemodialysis; these criteria vary greatly from one centre to another. This is particularly

true for more experienced programs where much sicker patients are specifically recruited for NHD as a type of salvage therapy where other forms of renal replacement therapy have been deemed suboptimal. Thus, it is important to know the types of patient selected for NHD at a given centre (information not readily available from research studies) and note whether NHD studies include all available patients, consecutive incident patients, or only a subpopulation of the NHD patient pool. Greater transparency in reporting would aid the reader in judging generalizability for any given study in this context.

NHD RESEARCH DOMAINS – MOVING FORWARD

The basic hypothesis underlying the favorable results seen with NHD is that substantially enhanced uremia clearance reverses the "unphysiology" of ESRD. Indeed, the effects we observe on blood pressure, cardiac geometry, or endothelial progenitor cells are undoubtedly the end result of complex multi-step pathways affected by the uremic milieu. Our own group is exploring various strategies to ensure maximal internal validity in the context of the pre/post one-group comparison paradigm by targeting very proximal steps in the presumed pathways by which uremia affects biological systems. Using microarray technology we propose to identify patients' genomic signatures while they undergo conventional dialysis and comparing these to their genomic signatures after a period of substantially augmented uremic clearance with NHD. Thus, modifiable candidate genes responsive to the uremic milieu can potentially be identified and studied using an approach that is broadly applicable to the many pathways perturbed by uremia. With this strategy, patients still serve as their own control, but since standardized protocols are used for CHD, NHD, and biological sample ascertainment and handling, threats to internal validity are minimized.

Qualitative research methods also lend themselves well to studies limited by small sample sizes. Utilization of this approach is often underappreciated, but may serve as a powerful tool to gain insight into the complex psychosocial aspects of chronic medical conditions. Use of qualitative analysis to identify themes that impact patient education or policy development can be easily adapted to quasi-experimental paradigms. The desired

outcome is modification of the process of care to better meet the needs of the patients on, or considering, NHD.

Whereas qualitative methodology can help identify patient barriers to wide-scale acceptance of NHD, such as concerns regarding the dialysis hardware and software, the technological interface between patient and machine is itself a domain of active research. Aims here are the design and implementation of monitoring technology, safety mechanisms, and software usability in order to minimize patients' perceived intrusion of NHD as a daunting and highly technological form of life-sustaining therapy. Again, simple pre/post one-group comparison studies serve as the experimental study of choice in assessing the feasibility and effectiveness of these research outcomes.

However, demonstrating the capacity of NHD to improve physiological parameters, and even understanding and then addressing patient barriers to NHD does not ensure large-scale adoption of this form of renal replacement therapy to a broader ESRD population. Needed now are well-controlled studies with sufficient power and long enough follow-up to look at hard outcomes such as mortality and cardiovascular event rates; soft outcomes such as biochemical surrogates of those hard outcomes and quality-of-life indices; as well as determination of the economic sensibility of expanding the proportion of ESRD patients treated with NHD. To that end, comparisons between NHD patients and CHD, peritoneal dialysis, and even renal transplant patients are also necessary. Though individual centers can effectively investigate many of these issues, it seems clear that to advance this field with studies involving many more patients than are currently enrolled in any individual program, collaborative research networks need to form. Such networks would allow for prospective data collection on a wider range of investigative themes and allow researchers to address questions which are currently not feasible.

CONCLUSION

There is clearly a need for randomized trials to assess various aspects of NHD compared to other forms of ESRD management, though the discussed limitations make their application nearly prohibitive at the present time. Even large cohort studies will

have little feasibility until centres accrue sufficient NHD patients to pool their populations into cohorts of meaningful size. Thus, even though quasi-experiment studies cannot replace these more rigorous designs, they clearly have a role to play in NHD research where the most important limitation is a restricted sample size. Understanding the constraints of this experimental approach on internal and external validity, allows for more comprehensive interpretation of the NHD literature and for more purposeful designs of future studies.

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Chapter 3: Long-term clinical outcomes of nocturnal hemodialysis patients compared to conventional hemodialysis patients post renal transplantation

Long-term clinical outcomes of nocturnal hemodialysis patients compared to conventional hemodialysis patients post renal transplantation

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Running Title: Clinical outcomes of renal transplantation post-NHD

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ABSTRACT

Nocturnal home hemodialysis (NHD) is a novel dialysis strategy associated with multiple physiological advantages over conventional hemodialysis (CHD). However, owing to the small number of patients undergoing this modality worldwide, little is known about the short and long-term clinical outcomes of NHD patients after kidney transplantation. We hypothesized that the incidence of delayed graft function (DGF), patient and graft survival, and post-transplant estimated glomerular filtration rate (eGFR), is better among CHD-transplanted individuals than among those having received NHD. Of 231 past and current NHD patients in our combined programs, 36 underwent renal transplantation between January 1, 1994 and October 31, 2006. This allowed us to conduct a retrospective cohort study of these 36 patients matched to 68 transplanted CHD patients with a maximum follow-up of 11.7 years to test these hypotheses. The incidence of DGF was not significantly greater in the NHD group than in the CHD group [NHD: 15/35 (42.9%) vs. CHD: 25/68 (36.8%) $p = 0.43$] with an unadjusted OR of 1.41 (95% CI 0.60-3.27). Estimated (e)GFR was predicted by pre-transplant weight, donor age and recipient race and determined at baseline; at 1, 3, 6, and 12 months post-transplantation; and annually thereafter. Dialysis modality prior to transplantation did not influence the level of eGFR post-transplantation ($p = 0.34$), nor the rate of eGFR decline. Post-transplant hemoglobin was also not affected by pre-transplant dialysis modality ($p = 0.12$). Post-transplant systolic blood pressure was 4.8 mmHg lower in the CHD group ($p = 0.04$), though these patients were receiving significantly more anti-hypertensive medications during the first two years after transplantation. Patient survival was no different among NHD and CHD groups (log-rank $p = 0.91$), though deceased donor graft survival favored CHD (log-rank $p = 0.01$) with an unadjusted HR of 0.14 (95% CI 0.02-1.27); however, this latter result is based on only 5 events and should be interpreted with caution. Based on this analysis the incidence of DGF was similar between NHD- and CHD-transplanted patients and pre-transplant modality did not impact on the level or rate of change of post-transplant eGFR.

Keywords: nocturnal hemodialysis, kidney transplantation, delayed graft function, estimated glomerular filtration rate, conditional logistic regression, repeated measures analysis, survival analysis

INTRODUCTION

Nocturnal home hemodialysis (NHD) is a form of intensive renal replacement therapy whereby patients self-deliver their dialysis on 4 to 6 nights per week, with each session lasting 5 to 8 hours. This typically results in 30 to 40 hours of weekly hemodialysis, an amount approximately 3 times that of conventional hemodialysis (CHD). The physiological benefits associated with the conversion from CHD to NHD are well documented and include regression of left ventricular mass [20], reduction in blood pressure associated with decreased dependence on antihypertensive medications and improved vascular responsiveness [55], amelioration of phosphate control despite liberalization of diet and reduction in phosphate binder dose [14], and improvement in erythropoetin sensitivity resulting in its dose reduction and augmentation of hemoglobin concentrations [36] to highlight just a few examples. Patients' quality of life also improves with intensive uremia clearance [85] and we have recently demonstrated that patients undergoing NHD experience fewer and shorter hospitalizations than matched patients receiving CHD [24]; mortality data of NHD versus other forms of renal replacement therapy is still unavailable.

Not withstanding the advances in dialysis therapies, renal transplantation remains the standard of care for the treatment of ESRD [57]. However, little is known about the short- or long-term clinical outcomes of transplanted patients who previously received NHD and if these differ from patients having received CHD. Just as lower delayed graft function (DGF) rates have been reported for peritoneal dialysis (PD) versus CHD (presumably secondary to an expanded extracellular fluid volume compartment of PD patients at the time of transplantation) [75], one could surmise that NHD may also influence transplant outcomes on account of its unique hemodynamic characteristics relative to CHD. Though a single study, by our own group, has suggested that one-year graft function and survival were similar among NHD and CHD patients, the incidence of DGF

may indeed have been increased in the NHD group (p = 0.15) [76]. However, this study was limited by a very small sample size (n = 15 NHD patients) with a mean follow-up time of 2.1 years. At present, the incidence of DGF post-transplantation among NHD patients remains uncertain, and the rate of post-transplant estimated glomerular filtration rate (eGFR) deterioration and long-term patient and graft survival remain unknown.

The current investigation was a retrospective matched cohort study aimed to better define these short- and long-term clinical outcomes of NHD patients after renal transplantation. Our program was uniquely able to address these issues since, to our knowledge, the Toronto unit has the largest NHD cohort world-wide with the greatest experience at transplanting NHD patients. The primary outcomes of interest were post-transplant DGF, eGFR and rate of eGFR decline; these were hypothesized to be better among renal transplant recipients having received CHD versus NHD prior to transplantation. Patient or graft survival as well as post-transplantation hemoglobin levels, systolic and diastolic blood pressures, and antihypertensive usage were also reported.

METHODS

Study Population

All transplanted NHD patients between January 1, 1994 and October 31, 2006 were matched with up to two transplanted patients having received CHD on the basis of date of birth (± 5 years), sex, diabetes status at the initiation of ESRD, date of transplantation (± 2 years) and type of transplant (deceased donor, living, or combined deceased-donor kidney-pancreas) using the Toronto Region Dialysis Registry.

Data Extraction

Race, smoking status, and date of dialysis start were also obtained from the same registry. Race was heath care provider reported and smoking status was only known at the start of ESRD and not at time of transplantation. Date of wait-listing, date of transplantation, post-transplant vital signs and medication use, and long-term transplant

outcomes were obtained using the clinical transplant database of the Toronto General Hospital. Blood pressure was collected at the time of hospital admission (*ie*. pre-operatively) on the day of transplantation and at 1, 3, and 6 months, followed by annual recordings thereafter. Information on the post-transplant clinical course, all laboratory investigations and pathology reports were obtained using the institutional paper and electronic records. Laboratory values were collected at the same time interval as mentioned previously. For the first post-transplant year data was chosen closest to the pre-specified time points within 2 weeks; for subsequent time points data was extracted closest to the pre-specified time point within 2 months. Where incomplete, effort was made to ascertain data from ancillary and corroborating sources within patients' records to minimize missing values. DGF was defined as necessitating hemodialysis within the first 72 hours post-transplantation. Estimated (e)GFR was calculated using the Modification of Diet in Renal Disease (MDRD) formula [86].

The study protocol was approved by the research ethics boards of the University Health Network – Toronto General Hospital, Humber River Regional Hospital, and Saint Michael's Hospital, all in Toronto, Canada.

Data Analysis

Of 36 transplanted NHD patients, two matches were found for 32, and only a single match for the remaining 4. Thus, a matched group (or set) usually contained 3 and occasionally 2 subjects and wherever possible, matched analyses were conducted taking into account the variable number of matches for each NHD individual. Differences in means of continuous variables presumed to be normally distributed were assessed using a variation of the paired sample Student t-test where the t-statistic was calculated for the difference between the value of the NHD subject and the mean of the values of the one or two matches. A similar approach was used to compare continuous variables not following a normal distribution using a Wilcoxon Signed Ranks test for differences of medians. Binary outcomes were compared using the Mantel-Haenszel test, a variation of the McNemar Chi-square test, using each matched set as a stratum. An exact test of a common odds ratio of one across sets was used when the expected number of individuals with a given outcome was less than 5.

The initial report that NHD may affect the frequency of DGF was published in March 2004; this may have resulted in a subsequent institutional change in management of NHD patients at time of transplantation. In order to determine whether era of transplantation (before or after March 2004) influenced the occurrence of DGF, patients were stratified according to era, and outcomes compared by Chi-square or by a two-sided Fisher's exact test. Because it was previously thought that a lower intra-operative blood pressure nadir may have contributed to the incidence of DGF, the systolic and diastolic blood pressure nadirs as well as the amount of fluid administered intra-operatively were also analyzed according to era using the Student t-test. Furthermore, univariate and multivariate conditional logistic regression (ie. taking matching into account) was used to complement the above analysis and investigate the predictors of DGF in this dataset.

Unmatched patient and graft survival of deceased donor transplant recipients were compared using Kaplan-Meier curves and the log-rank test. Cox proportional hazard regression taking patient matching into account was used to determine which variables predict graft and/or patient survival. Due to the small number of deaths and graft failures, regression was limited to univariate models.

Repeated measures analysis using a random effects model was used to compare serial levels of systolic and diastolic blood pressures, hemoglobin, and eGFR between the NHD and CHD groups from 6 months to a maximum of 9 years post-transplantation. Variables potentially influencing the rate of eGFR decline over time among the two groups were assessed by including those potential predictor variables in an interaction term with time. A significant interaction implies that the putative variable influences the rate of eGFR deterioration. The rational for excluding the first 6 months of data was to increase the likelihood that patients would have adequately recovered from their surgery and that graft function would have sufficient time to achieve steady state. A random effects model was chosen since this approach allows for otherwise unexplained between-patient variability and simultaneously gives more weight to patients with more follow-up data. This analysis does not allow for the 1:2 or 1:1 matching so the analysis was performed unmatched. The rate of eGFR deterioration over time and the rate of 1/Creatinine decline were compared as an indicator of internal consistency and robustness of the data.

All statistical analysis was conducted using SAS version 9.1 (Cary, NC, USA). Specifically, repeated measures analysis was conducted using the *proc mixed* procedure.

RESULTS

Of the 231 dialysis patients enrolled in the NHD programs at the Toronto General and Humber River Regional Hospitals between 1994 and October 31, 2006, 36 patients underwent renal transplantation at one of two regional transplant centres. These patients were matched as described with 68 patients having previously undergone CHD; baseline characteristics are described in Table 1. The mean patient ages at the start of their ESRD, the mean duration of ESRD at the time of transplantation, mean duration spent on the transplant waiting list and proportion smokers were similar between the two groups. The racial distribution did differ significantly with a greater proportion of the NHD group being Caucasian. The number of NHD patients requiring the use of phosphate binders, or anti-hypertensive medications was significantly lower when compared to the CHD group (Tables 2 and 8).

Table 1. Characteristics of NHD and CHD patients prior to transplantation.

Demographic features	Match Groups	NHD	CHD	*p*
Matched variables				
Male		20/36 (55.6%)	40/68 (58.8%)	
Diabetes		3/36 (8.3%)	6/68 (8.8%)	
Mean age at transplantation (years, SD, range)	36	45.0 ± 9.8 (25.4-66.3)	45.6 ± 9.7 (26.0-66.8)	
Type of transplant				
Deceased donor kidney only		36/36 (72.2%)	51/68 (75.0%)	
Deceased donor kidney and pancreas		2/36 (5.6%)	4/68 (5.9%)	
Living donor		8/36 (22.2%)	13/68 (19.1%)	
Mean age at start of ESRD (years, SD, range) [A]	34	37.5 ± 9.8 (16.2-55.1)	39.7 ± 10.6 (17.4-58.7)	0.17
Mean duration of ESRD (years, SD, range) [A]	34	7.0 ± 5.4 (0.6-27.6)	6.0 ± 4.5 (0.1-21.8)	0.28
Mean duration of NHD (years, SD, range) [A]	36	3.2 ± 2.3 (0.4-11.0)	n/a	
Mean duration on waiting list (years, SD, range)[A]	29	5.0 ± 3.3 (0-13.0)	4.6 ± 3.6 (0.1-18.5)	0.52
Smokers at start of ESRD [B]		2/34 (5.9%)	10/67 (9.9%)	0.15
Race				
Caucasian [B]		25/36 (69.4%)	35/68 (51.5%)	0.05
Asian [C]		3/36 (8.3%)	6/68 (8.8%)	1.00
Black [B]		5/36 (13.9%)	13/68 (19.1%)	0.44
Indian sub-continent [C]		2/36 (5.6%)	13/68 (19.1%)	0.13
Pacific islander [C]		1/36 (2.8%)	0/68	0.67
Middle eastern [C]		0/36	1/68 (1.5%)	1.00

[A] One sample Student t-test for within-matched-set differences
[B] Mantel-Haenszel test
[C] Exact test of the common odds ratio of unity

Table 2. Calcium binder and vitamin D usage among NHD and CHD patients prior to transplantation.

Ca-PO_4 balance among patients with immediate graft function	NHD	CHD	*p*
Number of patients taking $CaCO_3$ prior to transplant [A]	4/33 (12.1%)	47/65 (72.3%)	<0.01
Number of patients taking sevelamer prior to transplant [B]	0/33	6/65 (9.2%)	0.15
Number of patients taking activated vitamin D prior to transplant [A]	10/33 (30.3%)	24/65 (36.9)	0.525

[A] Mantel-Haenszel test
[B] Exact test of the common odds ratio of unity

Delayed Graft Function

Table 3 summarizes the outcomes of NHD and CHD transplanted patients in the immediate post-transplant period. The crude incidence of DGF appears to be similar in both groups over the entire study period ($p = 0.43$). In order to determine whether an era effect exists in the incidence of DGF within the two dialysis cohorts, the occurrence of DGF during two time periods was compared. Among NHD patients there were 9 episodes of DGF among 16 deceased donor transplantations (56.2%) before March 2004, and 3 among 11 thereafter (27.3%); thus suggesting at most a weak association between era and occurrence of DGF ($p = 0.14$). Additionally, intra-operative systolic and dialysis blood pressures were no different among cadaver-transplanted NHD patients in either era ($p = 0.24$ and $p = 0.69$, respectively), nor was there a difference in the amount of fluid administered intra-operatively in either era ($p = 0.31$). The incidence of DGF among 6 living donor transplants before March 2004 was 50% versus 0% among 2 living donors thereafter ($p = 0.46$) in the NHD cohort. For CHD patients having received deceased donor transplantation the incidence of DGF before versus after March 2004 was 15/32 (46.9%) and 10/23 (43.5%) respectively ($p = 0.80$); there were no episodes of DGF among the 13 living donor transplants among CHD patients regardless of era.

Table 3. Transplantation characteristics and short-term transplantation outcomes among NHD and CHD patients.

DGF and risk factors	Match Groups	NHD	CHD	*p*
≥ 1 previous transplant [B]		6/36 (16.7%)	10/68 (14.7%)	0.85
Median of peak percent panel reactive antigen level (IQR [C])	31	5 (0-40)	5 (0-77.5)	0.25
Mean donor age (years, SD) [D]	27	42.6 ±17.1	41.6 ±13.8	0.91
Mean cold ischemic time (hours, SD) [D]	21	18.7 ± 7.4	16.4 ± 9.3	0.26
Mean warm ischemic time (minutes, SD) [D]	22	34.3 ± 9.8	33.7 ± 9.9	0.71
Mean intra-op volume admin (mL, SD) [D]	28	2835 ±1207	2583 ±1119	0.15
Primary non-function [A]		1/36 (2.8%)	0/68 (0%)	1.00
DGF [B]		15/35 (42.9%)	25/68 (36.8%)	0.43
Biopsy performed for DGF		4/15	5/25	
DGF biopsy diagnosis				
Acute tubular necrosis		3/4	5/5	
Acute vascular rejection		1/4	0/5	

[A] Exact test of the common odds ratio of unity
[B] Mantel-Haenszel test
[C] IQR = interquartile range (ie. 1st to 3rd quartile)
[D] One sample Student t-test for within-matched-set differences

Conditional logistic regression confirms the lack of association between dialysis modality and DGF with NHD having an unadjusted OR of 1.41 (95% CI 0.60-3.27). Univariate analysis suggests only cold ischemia time and intra-operative blood pressure nadir are predictive of DGF. Both variables remained significant in a multivariate analysis with cold ischemia time having an adjusted OR of 1.16 per hour (95% CI 1.01-1.32) and a 1 mmHg higher level of intra-operative systolic blood pressure nadir being associated with an adjusted OR of 0.92 (95% CI 0.85-1.00); dialysis modality remained non-significant.

Patient and Graft Survival

The mean duration of follow-up for the NHD cohort was 3.1 years with a range from 0 to 9.7 years, as compared to 3.6 years with a range of 0 to 11.7 years for the CHD cohort (Table 4). The causes of graft loss are summarized in the table. The Kaplan-Meier curve of patient survival for the entire study population is shown in Figure 1. The log-rank test of equality between NHD and CHD failed to demonstrate any difference (p = 0.61); this was unchanged when considering patient survival for deceased donor transplant recipients only (p = 0.91). However, deceased donor death-censored graft survival alone did differ significantly between these modalities, favoring CHD (Figure 2; p = 0.01). Figure 3 shows the Kaplan-Meier curve for the combined outcome of patient survival and graft loss among deceased donor graft recipients; again, the log-rank test reveals an advantage of CHD over NHD though less than graft survival alone (p = 0.04).

Table 4. Long-term transplant outcomes among NHD and CHD patients.

Transplant outcomes	Match Groups	NHD	CHD	*p*
De novo or recurrent glomerulopathy [A]		2/36 (5.6%)	0/68 (0%)	0.22
Loss to follow-up [A]		0/36 (0%)	3/68 (4.4%)	0.44
Graft loss from chronic allograft nephropathy [A]		3/36 (8.3%)	1/68 (1.5%)	0.15
Death with functioning graft [A]		2/36 (5.6%)	4/68 (5.9%)	1.00
Mean duration of follow-up (years, SD, range) [B]	36	3.1 ± 2.5 (0-9.7)	3.6 ± 2.6 (0-11.7)	0.16
Median duration of follow-up (IQR [C]) [D]	36	2.7 (0.8-4.5)	3.1 (1.2-5.6)	0.13

[A] Exact test of the common odds ratio of unity
[B] One sample Student t-test for within-matched-set differences
[C] IQR = interquartile range (ie. 1st to 3rd quartile)
[D] Wilcoxon Signed Ranks test for matched group differences in medians

A univariate Cox model of deceased donor graft survival taking into account patient matching revealed a hazard ratio (HR) of 0.14 in favor of CHD, though this result was not statistically significant (95% CI 0.02-1.27). Due to the limited number of graft failures (5 in all), further adjustment was not feasible, suggesting this result be interpreted with caution.

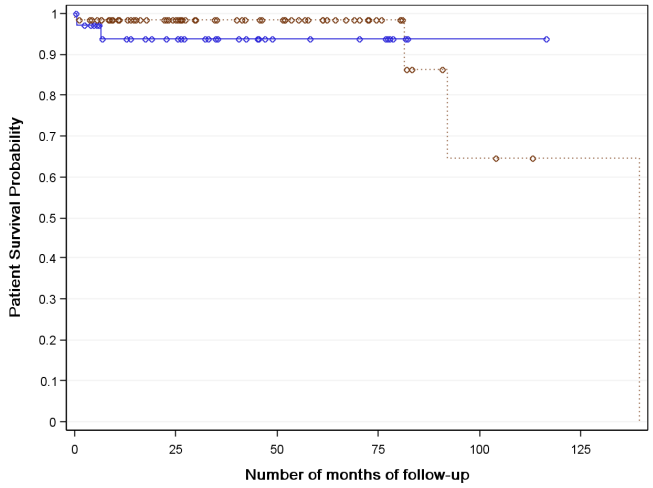

Figure 1. Kaplan-Meier curves of patient survival following renal transplantation (living and deceased donor) for patients previously receiving NHD (solid blue line) versus those previously receiving CHD (dashed red line); log-rank *p* = 0.61.

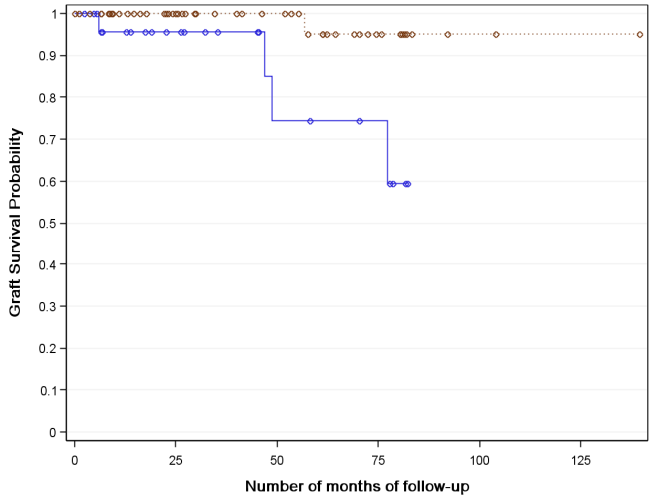

Figure 2. Kaplan-Meier curves of deceased donor graft survival following renal transplantation for patients previously receiving NHD (solid blue line) versus those previously receiving CHD (dashed red line); log-rank *p* = 0.01.

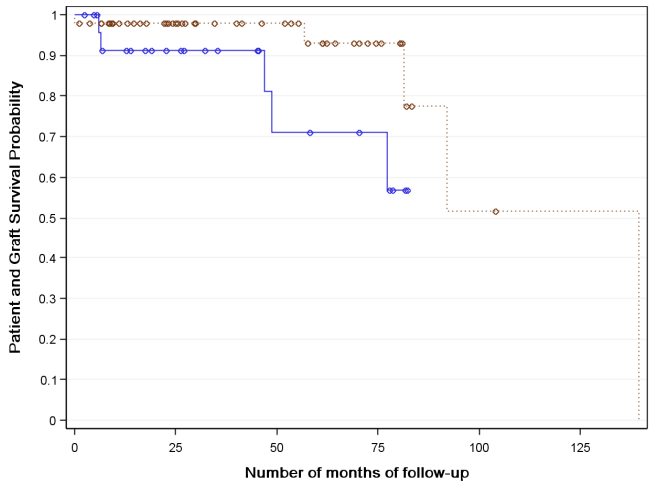

Figure 3. Kaplan-Meier curves of combined patient and graft survival following deceased donor renal transplantation for patients previously receiving NHD (solid blue line) versus those previously receiving CHD (dashed red line); log-rank $p = 0.04$.

Long-term Outcomes

Table 5 summarizes the eGFR of the two groups. The baseline weights were approximately 8 kg greater in the NHD cohort than in the CHD cohort. Not surprisingly, the baseline creatinine of the NHD patients at the time of transplantation was substantially lower. Estimated GFR at the various time intervals are listed in the table and confirm that even successful transplantation results in CKD Stage 3 regardless of the preceding dialysis modality. Repeated measures analysis of eGFR between 6 months and a maximum of 9 years post-transplantation suggest that modality of dialysis preceding transplantation by itself does not influence the level of eGFR post transplantation (p = 0.34). An analysis with 1/Creatinine versus time yields virtually identical results (p = 0.37), suggesting internal consistency of this analysis. Transplant type (deceased donor

Table 5. Estimated GFR after transplantation among NHD and CHD patients.

Estimated GFR (MDRD formula)	Match Groups	NHD	CHD	*p*
Weight at time of transplantation (kg ± SD) [A, B]	35	77.6 ± 22.3	69.6 ± 16.0	0.06
Creatinine at baseline (µmol/L ± SD) [A]	32	490 ± 211	728 ± 231	<0.01
eGFR (ml/min ± SD) [A]				
1 month post-transplant	32	43.9 ± 15.2	51.4 ± 19.1	0.09
3 month post-transplant	24	45.5 ± 15.9	50.6 ± 16.0	0.38
6 month post-transplant	21	45.0 ± 16.5	49.9 ± 15.6	0.48
1 year post-transplant	17	46.1 ± 19.0	50.3 ± 15.1	0.32
2 year post-transplant	13	48.5 ± 21.6	45.7 ± 18.2	0.36

[A] One sample Student t-test for within-matched-set differences
[B] Admission weight

versus living), and presence or absence of delayed graft function by themselves may be predictive of the level of eGFR over time (both having *p*-values of 0.13). Pre-transplant weight, cold and warm ischemic times, and donor age appear to be strong predictors of eGFR (*p*-values ranging from 0.01 to 0.04). Interaction terms added to the individual models suggest that dialysis modality, Caucasian versus non-Caucasian race and higher systolic blood pressure on the day of transplantation may predict a more rapid decline in eGFR over time (*p*-values of 0.09, 0.09, and 0.06 respectively). In multivariate repeated measures analysis many of the variables and interactions identified previously were no longer significant suggesting important covariance and/or confounding was eliminated (Table 6). According to the final model, eGFR falls by 2.2 ml/min per year after transplantation with significant deleterious impact derived from recipient weight at time of transplantation and older donor age. Non-Caucasians have a predicted slower rate of deterioration than Caucasians. That is, a 70 kg Caucasian recipient of a 40 year-old donor organ is expected to have an eGFR of 56.4 ml/min six months post-transplantation, falling by 2.2 ml/min per year thereafter; a non-Caucasian would have a predicted eGFR of 54.7 ml/min but deteriorate at a slower rate of 0.2 ml/min per year thereafter.

Table 6. Final model predicting the behavior of post-transplant eGFR.

Variable	Parameter Estimate	Standard Error	*p*
Intercept	84.7 ml/min	8.5	<0.0001
Time post-transplantation (yrs)	-2.2	0.6	0.0007
Weight pre-transplantation (kg) [A]	-0.3	0.1	0.0020
Donor age (yrs)	-0.3	0.1	0.0023
Race (Caucasian=0, Non-Caucasian=1)	-2.8	3.5	0.4277
Time * Race	2.0	1.1	0.0545

[A] Admission weight

Repeated measures analysis (without time interactions) for serial hemoglobin concentrations (also between 6 months and 9 years) suggests that dialysis modality has no long-term impact on post-transplantation hemoglobin levels (*p*-value for dialysis modality of 0.12); the average hemoglobin concentration for the combined NHD and CHD cohort was 123 g/L over 9 years of follow-up. Comparisons of hemoglobin concentrations at

various time points are listed in Table 7; there is no statistical difference at any discrete point in time.

Table 7. Hemoglobin concentrations after transplantation among NHD and CHD patients.

Hemoglobin outcomes	Match Groups	NHD	CHD	*p*
Hemoglobin (g/L ± SD) [A]				
Baseline	32	124.7 ± 16.1	123.4 ± 16.4	0.56
1 month post-transplant	32	109.2 ± 15.1	108.5 ± 14.7	0.80
3 month post-transplant	24	119.3 ± 18.3	119.2 ± 16.6	0.31
6 month post-transplant	22	120.3 ± 21.8	128.4 ± 15.3	0.15
1 year post-transplant	17	122.8 ± 17.9	129.4 ± 19.7	0.51
2 year post-transplant	13	126.3 ± 16.0	125.5 ± 16.7	0.72

[A] One sample Student t-test for within-matched-set differences

An analysis of blood pressure related variables reveals that the median number of antihypertensive medications among NHD patients at the time of transplantation was significantly lower than among CHD patients ($p < 0.01$; Table 8), and this persisted through the first year of transplantation ($p = 0.02$), becoming less significant by the second post-transplant year ($p = 0.07$). Repeated measures analysis of blood pressure measured 6 months to 2 years post transplantation revealed a 4.8 mmHg lower systolic blood pressure among the CHD (127.2 mmHg) group than in the NHD group (132.0 mmHg; $p = 0.04$), while diastolic pressures did not differ significantly (79.6 mmHg; $p = 0.07$).

Table 8. Blood pressure parameters and antihypertensive medication use before and after transplantation among NHD and CHD patients.

Blood pressure outcomes	Match Groups	NHD	CHD	*p*
Mean systolic blood pressure (mm Hg ± SD) [A]				
Transplant day	32	128.6 ± 19.1	139.3 ± 22.0	0.06
Intra-operative nadir	31	90.4 ± 15.0	93.6 ± 12.2	0.18
1 month post-transplant	27	126.2 ± 16.0	131.1 ± 17.2	0.15
3 month post-transplant	29	128.3 ± 14.2	127.4 ± 16.7	0.88
6 month post-transplant	26	130.7 ± 15.7	129.0 ± 16.7	0.83
1 year post-transplant	19	134.2 ± 14.6	124.5 ± 10.1	0.01
2 year post-transplant	15	127.4 ± 12.8	127.6 ± 13.1	0.87
Mean diastolic blood pressure (mm Hg ± SD) [A]				
Transplant day	32	80.4 ± 13.3	82.5 ± 11.6	0.59
Intra-operative nadir	28	50.2 ± 10.4	53.3 ± 10.1	0.12
1 month post-transplant	27	76.9 ± 9.9	77.0 ± 10.7	0.81
3 month post-transplant	29	78.5 ± 6.8	78.1 ± 9.0	0.75
6 month post-transplant	26	78.6 ± 10.6	77.7 ± 11.1	0.78
1 year post-transplant	19	81.4 ± 9.7	76.9 ± 8.0	0.09
2 year post-transplant	15	79.2 ± 9.4	77.5 ± 7.9	0.84
Mean number of antihypertensive drugs prior to transplantation (range)		0.3 (0-2)	2.0 (0-5)	
Median number of antihypertensive drugs prior to transplantation (IQR [B]) [C]		0 (0-0)	2 (1-3)	<0.01
Mean number of antihypertensive drugs at 1 year (range)		0.7 (0-2)	1.2 (0-3)	
Median number of antihypertensive drugs at 1 year (IQR [B])		1 (1-2)	1 (0-2)	0.02
Mean number of antihypertensive drugs at 2 year (range)		0.9 (0-2)	1.3 (0-3)	
Median number of antihypertensive drugs at 2 year (IQR [B])		1 (0-1)	1.5 (1-2)	0.07

[A] One sample Student t-test for within-matched-set differences
[B] IQR = interquartile range (ie. 1st to 3rd quartile)
[C] Wilcoxon Signed Ranks test for matched group differences in medians

DISCUSSION

Two important conclusions can be derived from this study: [a] we find no evidence that NHD is associated with an increased incidence of DGF in the immediate post-transplant period and [b] both the level of eGFR and the rate of eGFR deterioration over time are not affected by the type of hemodialysis modality prior to transplantation.

Additionally, overall patient survival also appears unaffected by pre-transplant dialysis modality, though deceased donor graft survival and the composite outcome of deceased donor graft plus patient survival appear to favor CHD. However, these latter findings are based on a very low event rate and thus must be interpreted with caution.

With substantially increased sample size corresponding to 4 years of additional data, we were not able to demonstrate a higher incidence of DGF in patients having received NHD versus CHD. Indeed, an adjusted analysis confirms that only cold ischemic time and intra-operative blood pressure nadir predict the occurrence of DGF in our cohort, two known predictors of this outcome [59,61]. Of the multiple other donor-, recipient- and procurement-related risk factors of DGF (reviewed in greater detail elsewhere [60]), none was found significant in our study, likely owing to the still relatively small sample size of the present study and the fact that many of these variables could not be assessed. The notion that dialysis modality may influence the rate of DGF occurrence was prompted by earlier findings that the incidence of DGF is lower among patients having received PD prior to transplantation as compared to patients having received CHD [62]. A number of hypotheses have been put forward to explain this observation including the possible positive impact of better preserved residual renal function seen with PD, or the relatively expanded extracellular fluid (ECF) volume compartment in PD patients which may encourage a better post-transplant perfusion pressure [75]. By extension, it was thought by some that the incidence of DGF may be increased after NHD on account of a contracted ECF volume and an already diminuate peripheral vascular resistance. The presumed pre- and intra-operative hypotension might further be aggravated by the induction of anesthesia, all resulting in potentially reduced perfusion pressure of the implanted graft. Arguing against this is the observation that post-dialysis ECF and stroke volumes remain unchanged when patients convert from CHD to NHD, while at the same time, vascular responsiveness to endothelial-dependent and independent stimuli, as well as baroreceptor sensitivity improve [20,55,56]. Theoretically, NHD patients may actually be able to withstand the hemodynamic stress of renal transplantation better than their CHD counterparts. Thus, despite NHD patients tending to have a lower blood pressure prior to transplantation, being on fewer antihypertensive medications and tending to have a lower intra-operative blood pressure nadir, it is reassuring to see that the incidence of DGF in

the present cohort of NHD patients is not greater than the incidence among CHD recipients, notwithstanding that data on pre-operative fluid administration and intra-operative vasopressor support was not available and could result in residual confounding.

The present study represents the longest post-transplant follow-up of NHD patients, with almost a decade of follow-up for the earliest transplants. During this time there have been 2 cases of *de novo* or recurrent glomerulopathy, 2 deaths with functioning grafts and 3 graft losses due to chronic allograft nephropathy among the 36 transplanted NHD patients. Given these results, any survival analysis must be interpreted with caution, given the potential for confounding that cannot be statistically controlled for on account of the small number of events. Having said this, in the present study, no significant differences in overall patient survival were demonstrated. However, we found a combined patient plus graft, and a graft-only survival advantage to CHD by the Kaplan-Meier method, as well as a trend to the same using an unadjusted Cox regression model; further adjustment was not possible for the aforementioned reason.

A number of variables can be hypothesized to confound the association between dialysis modality and graft survival. Firstly, some transplanted NHD patients may have been be sicker than their CHD counterparts. Indeed, our own unpublished observations suggest that NHD can be such a successful salvage therapy that previously non-transplantable patients become transplantable. This change in transplantability status arising from NHD therapy is likely based on regression of left ventricular mass index and improved ejection fraction which are well established with NHD [20,87]. Of the 7 NHD patients with events (death or graft loss) 3 patients fall into this category; excluding these patients from the analysis of death or deceased donor graft loss changes the log-rank test from marginally significant ($p = 0.04$) to non-significant ($p = 0.07$). Though this change is modest, it does raise the possibility that NHD patients have a slightly different comorbidity distribution relative to CHD patients, which may influence survival. Matching or otherwise adjusting for comorbidities (particularly cardiovascular disease) was not feasible in the current study. Secondly, NHD patients in the current cohort had longer durations of ESRD and transplant wait-list times than their CHD matches; both variables are known to predict poor graft and patient survival [88,89]. Thirdly, we demonstrate that post-NHD-transplanted patients have a systolic blood pressure 4.8

mmHg higher than do patients having received CHD and that they are receiving fewer antihypertensive medications even two years after transplantation; the type of antihypertensives prescribed is not available. These factors may also be important confounders since it is well established that post-transplant hypertension is a strong predictor of long-term graft outcome [90], and the type of antihypertensive treatment may also be important: early evidence suggests that blockade of the renin-angiotensin-aldosterone system (RAAS) may be an underappreciated contributor to preserving graft function [91]. Finally, no data was available for the current cohort regarding their specific immunosuppression therapy. Use of specific immunosuppressive regimens may well contribute to long-term outcomes: it is believed that calcineurin inhibitor sparing strategies, or tacrolimus versus cyclosporine A-based regimens are associated with a reduced occurrence of chronic allograft nephropathy and presumably graft loss (reviewed extensively elsewhere [92]). Without the ability to control for these potential confounders, it is premature to assume that NHD portends a disadvantageous outcome to renal transplantation.

It is unlikely that any one of the aforementioned factors alone can explain the apparently worse graft survival of the NHD cohort, but the combination of any or all these variables may negate or even reverse the association between graft survival and hemodialysis modality. Since survival analysis is dependent on the number of events (ie. death or graft failure), a low event rate limits the usefulness of this analysis given the limited ability to control for confounding. In light of this we used a random effects repeated measures regression analysis to model the relationship between post-transplant eGFR and a variety of patient and procedure-related variables. Modeling eGFR and the rate of eGFR decline after transplantation indirectly yields valuable information on anticipated graft survival. This approach is particularly suited for the present analysis: firstly, it incorporates the entire dataset since it does not rely on a small number of deaths or graft losses, as is the case with Cox regression (ie. the analysis is independent of a low event rate). Secondly, it simultaneously models eGFR and the slope of eGFR (ie. the rate of eGFR deterioration), assuming the slope is linear over time. The result is a more comprehensive evaluation of the behavior of post-transplant eGFR within a single unifying model, as opposed to modeling eGFR and its slope separately. Thirdly, it is self-

weighting so that data is not excluded from patients with fewer than an arbitrary number of observations (ie. proportionately more weight is given to patients with more follow-up data, but patients with little follow-up data are not eliminated from the analysis). Finally, this approach of hierarchical modeling takes into account the relative imprecision of slopes calculated from only a few data points versus slopes calculated from many data points, something that cannot be done when slopes for each patient is calculated individually [93].

Though a number of variables predicted eGFR and its slope when modeled separately, combining these variables into a multivariable repeated measures model suggests that eGFR was best predicted by the time since transplantation, the pre-transplant weight, donor age, and race. Only race affected the rate of eGFR decline over time with Caucasians having a more rapid decline than non-Caucasians. Though examining measures of post-transplant renal function have been reported previously for unselected renal transplant populations, regression of renal function (creatinine clearance (CrCl) or eGFR) and their rate of decline have always been modeled separately [94,95]. For their part, Kasiske *et al.* examined post-transplant renal function in 10,278 recipients and reported unadjusted 6-month eGFRs of 51.3 to 55.5 ml/min between 1984 to 2002, with unadjusted rates of eGFR decline ranging from –1.9 to –2.8 ml/min/yr over the same period [95]. This compares favorably with a 6-month eGFR of 56.4 ml/min for a 70 kg Caucasian recipient of a 40-year-old donor organ with a rate of eGFR decline of –2.2 ml/min/yr from the present study. The 6-month eGFR for a non-Caucasian recipient with the same characteristics is expected to be 54.7 ml/min with a rate of decline of –0.2 ml/min/yr. The slope of eGFR decline of non-Caucasians may be spuriously modest due to combining all non-Caucasian races into one group for the purposes of analysis. This was done on account of the small sample sizes of each non-Caucasian racial group in the current cohort, even though evidence suggests that post-transplant eGFR does not behave the same among all non-Caucasian populations [95]. Gourishankar *et al.* reported a 6-month Cockcroft-Gault creatinine clearance (CrCl) of 64.6 ml/min with a rate of change of –1.4 ml/min/yr and described a number of variables from univariate analysis that predicted 6-month CrCl including donor age, cold ischemic time and DGF [94] – the same variables we also found significant with univariate modeling of eGFR in the present

study. These authors found that transplant era, higher blood pressure, female recipient and any episode of rejection predicted a more rapid rate of loss of CrCl. Episodes of rejection were strongly correlated with the combination of azathioprine and cyclosporine versus mycophenolate mofetil and tarcrolimus. Unfortunately, many of these variables were not available to include in our analysis of post-transplant NHD patients so unavoidable residual confounding of the current model cannot be excluded.

A number of secondary outcomes were analyzed in this study to determine what effect, if any, pre-transplant NHD may have played in determining post-transplant course. Not surprisingly, post-transplant hemoglobin concentration is independent of previous dialysis modality, and presumably, is more likely related to post-transplant eGFR (which was the same for both groups). However, post-transplant systolic blood pressure did differ according to pre-transplant dialysis modality, with higher pressures noted for patients having previously received NHD. Given that NHD patients are also prescribed fewer antihypertensive agents post-transplantation, the difference in systolic blood pressures more likely reflects different pharmacological management of hypertension rather than a true biological effect of hemodialysis modality extending several years beyond the time of transplantation. Why NHD-transplanted patients are prescribed fewer drugs to control their blood pressure is unknown, but may reflect some degree of therapeutic inertia: those patients already receiving a number of antihypertensive agents are less likely to have them withdrawn, while those patients not receiving antihypertensive agents are less likely to have them initiated until some arbitrary clinical threshold is reached.

As with all observational studies, this investigation is subject to a number of limitations. Firstly, all data was collected from pre-existing records thereby creating the potential for information bias. It cannot be assumed that health records (particularly the paper charts from the 1990s) are highly sensitive or specific for obtaining essential patient data, thus resulting in possible misclassification. However, there is no reason to suspect that this bias affects the NHD and CHD groups differently; the result is a tendency toward the null hypothesis (ie. no difference between NHD and CHD). Secondly, even though patients were matched on their date of birth and date of transplantation, they could not feasibly be matched on date of starting ESRD. As mentioned earlier, NHD patients were

of a slightly earlier ESRD vintage, which may bias a survival analysis against this cohort. Thirdly, information on a number of potentially important variables discussed above was not available. Whether the absence from analysis of these variables has minimized or exaggerated the influence of dialysis modality on the outcomes under study cannot be predicted. Fourthly, even though this is the largest study of post-NHD renal transplant recipients the sample size remains relatively small. This limits the power of the present study to detect a difference in clinical outcomes of NHD and CHD patients after transplantation, even if a true difference was to exist (ie. a type 2 error). This is reflected by the large confidence intervals around the resulting odds or hazard ratios we describe. At present, this is unavoidable given the small overall number of patients enrolled in NHD programs worldwide and the even smaller number who are eventually transplanted. Given that the Toronto unit has the longest NHD experience of any program, the current estimates are the best available for the foreseeable future. An additional consequence of a small sample size is the resultant mathematical limitation of adjustment for potential confounders. This is particularly relevant for logistic regression and survival analysis since the number of variables that can reasonably be adjusted for in a model is limited by an even smaller event rate. Consequently, in the current study we chose to match NHD patients for those variables most likely to be potential confounders (ie. age, sex, diabetes status, era of transplantation and transplant type) in an effort to control for as much confounding as was feasible *a priori*. As outlined in the text above, other potential confounding variables can be hypothesized, though information on these variables was not available. This suggests that residual confounding is likely. Notwithstanding these limitations, the predictors of DGF, and graft survival as identified in the present study are consistent with the literature.

CONCLUSION

We present the largest and longest experience of post-transplant outcomes among patients having undergone NHD as compared to CHD, in a retrospective matched cohort study. It appears as though early graft function, and long-term eGFR and rate of eGFR decline are all independent of pre-transplant hemodialysis modality. These results are

reassuring for nephrologists and for NHD patients awaiting renal transplantation. However, patients having undergone NHD prior to transplantation do exhibit a significantly higher systolic pressure and are treated with fewer antihypertensive drugs, suggesting greater attention ought to be given to the management of post-transplant hypertension. Future studies are needed to better define graft and patient survival.

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DISCLOSURES

None.

[End of manuscript]

Chapter 4: Conclusions

SUMMARY OF CONTRIBUTIONS

With traditional dialysis strategies having achieved their maximum efficacy in controlling the uremia of ESRD, and renal transplantation being limited by an inadequate supply of donor organs, NHD is emerging as an important addition to the spectrum of RRT. However, many questions remain unanswered regarding the risks and benefits of this intensive dialysis modality. In an effort to facilitate a better understanding for the challenges faced by investigators and reviewers of NHD research, this thesis has outlined a framework for appreciating and overcoming the limitations of NHD investigations. The drawbacks of experimental and observational studies were discussed and much emphasis was placed on understanding the biases of quasi-experiment studies, since this approach is commonly described in the NHD literature but is frequently overlooked in discussing the limitations of epidemiologic studies. Though framed around quasi-experimental designs, the threats to internal and external validity are broadly applicable to observational studies as well. It is hoped this framework will provide guidance for the appropriate design and interpretation of this literature.

With this in mind, a retrospective cohort study was designed to address the question of whether short- and long-term clinical outcomes of renal transplantation differ between patients having received NHD or CHD immediately prior to transplantation. Based on this investigation it appears as though pre-transplant hemodialysis modality does not influence the incidence of DGF, nor eGFR or its rate of decline. These results reassure dialysis providers and transplant teams alike that patients previously on NHD appear to fare as well after transplantation as patients having been treated with a more traditional dialysis modality. Post-transplant hemoglobin and diastolic blood pressure were also not found to differ according to pre-transplant dialysis modality. Systolic blood pressure, on the other hand, was determined to be lower among patients having previously received CHD, however, it was noted that CHD patients were prescribed more antihypertensive medications even two years after their transplant. This result suggests that transplant recipients having undergone NHD could benefit from greater attention to post-transplant hypertension management. Overall this study contributes to a more

thorough understanding of post-transplant clinical outcomes among a growing subpopulation of transplant recipients who previously underwent NHD.

FUTURE DIRECTIONS

The present thesis has begun to explore the relationship between NHD, CHD, and renal transplantation. This work has laid the foundation for a much broader theme of investigation: how to view NHD in the overall cadre of treatment options for ESRD, particularly with respect to transplantation. In order to address this objective it will be important to bring together data on hard clinical outcomes, economic analyses, and patient preferences into a global analysis of ESRD care. Outlined below are a number of studies, employing a variety of investigative approaches, which ultimately aim to identify the ESRD subgroup for which NHD is most appropriate and at what time during the course of their illness the benefit of this dialysis therapy would be optimal.

1. To date, no published reports compare NHD to renal transplantation with respect to mortality, cardiovascular events or hospitalizations. A matched cohort study comparing the past and current Canadian NHD population to transplant recipients from the USRDS and the Canadian Organ Replacement Registry (CORR) can address this gap in knowledge. The advantage of pooling Canadian NHD populations is that the number of patients and the duration of follow-up optimize the power of the study to detect clinically meaningful differences in outcomes. Additionally, USRDS and CORR provide a much larger pool for matching transplant patients than would be feasible at any individual center. The major limitation of this study would be the difficulty controlling for differences in a Canadian NHD population and an American transplant population in the case of a USRDS-based comparison.
2. An analogous study to above, comparing morbidity and mortality between NHD and CHD, PD and transplant-wait-listed individuals will provide a better basis for comparing dialysis modalities than is currently available. Similar limitations to above apply. This type of study is needed since a direct comparison between NHD and the

other dialysis modalities in the form of an RCT investigating clinically relevant hard outcomes is not likely forthcoming.

3. An extensive literature describes the quality of life benefits of renal transplantation relative to traditional dialysis modalities. To date, however, no comparison exists between quality of life indicators of NHD and renal transplantation. Validated instruments such as the Kidney Disease Quality of Life Short Form (KDQOL-SF) questionnaire, the Illness Intrusiveness Scale and Beck's Depression Inventory (BDI), can be used in a cross-sectional or longitudinal study design to elucidate important differences between NHD and transplant patients' perception and satisfaction with RRT. This line of investigation also has the potential to identify patient issues amenable to modification through education or process of care adaptations.
4. Over the past decade a number of NHD patients from the Toronto program have undergone renal transplantation who, while originally receiving CHD, were not considered appropriate transplant candidates on account of their poor cardiac status. NHD has well documented cardiovascular restorative properties and this consequence of NHD-mediated cardiac rehabilitation has not previously been described and requires documentation in the form of a case series.
5. Approximately 20% of the prevalent patients in the Toronto General Hospital NHD program have expressed a desire to forego transplantation or even work-up for kidney transplantation despite being reasonable transplant candidates. Anecdotally, the reasons cited include fear in body image changes associated with steroid use, fear of immunosuppression-induced malignancy, desire to become pregnant while still on NHD, and good quality of life with NHD, bad experience with transplantation in the past, among others. This phenomenon has not previously been studied and lends itself to qualitative methods for identification of patient- and modality-related factors that underlie this decision. Understanding the rationale behind patients' wishes will allow caregivers to better meet the needs of those patients and identify issues amenable to modification.
6. With increasing numbers of patients undergoing kidney transplantation, there are inevitably more patients returning to dialysis after graft failure. In fact, of the 99 past and present NHD patients followed at the Toronto General Hospital, 38% had at least

one previous transplant and approximately one third of those have had at least 2 (unpublished data). This provides an opportunity to study the patient- and modality-related factors that motivate individuals to choose one form of RRT over another after they have experienced the gold standard therapy of transplantation. Such investigations also lend themselves well to qualitative methodology.

7. By taking into account the results of (1-6), it will be instructive to conduct a decision analysis to delineate the role of NHD relative to renal transplantation in ESRD. By taking into account the risks and benefits of NHD versus transplantation (*eg*. probability of mortality or a cardiovascular event), as well as the variables identified to affect patient preferences (ie. affecting utility), a decision analysis may add valuable information to define the therapy (NHD versus transplantation) best suited to which subpopulation (*eg*. the elderly, diabetic, etc.) and at what time in the natural history in their ESRD.

The aforementioned studies will contribute to a body of knowledge whose aim is to understand the appropriate use of NHD versus renal transplantation. The work of thesis has begun to advance this goal.

SUMMATION

Findings from the current investigation suggest that pre-transplant dialysis modality has no effect on DGF immediately post-transplantation or on post-transplant eGFR. Systolic blood pressures over the first two years following transplantation appears better controlled among CHD-transplanted patients; however, these patients were prescribed significantly more antihypertensive medications than their NHD counterparts. This is the likely explanation for the observed difference, rather than some inherent biological difference between the groups that persists once transplanted.

As the popularity in NHD continues to grow, it will become necessary to better define the risks and benefit of this treatment modality in the spectrum of ESRD therapy, particularly with regards to transplantation, which, to date, remains the standard of care.

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APPENDIX [A]: RESEARCH ETHICS BOARD APPROVAL DOCUMENTATION

University Health Network
Research Ethics Board
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Notification of REB Approval for Access to Retrospective Data for Research Purposes

Date: September 6, 2006

To: Dr. Chris Chan
NU 8-842, TGH

Re: 06-0624-AE
Long-term Clinical Outcomes of Renal Transplantation Following Nocturnal versus Conventional Hemodialysis (NHD vs. CHD) (Chart Review)

REB Review Type:	Expedited
REB Initial Approval Date:	September 2, 2006
REB Expiry Date:	September 2, 2007
Note:	

Data Range of requested data approved: April 1, 1994 to September 2, 2006.

We wish to remind you that access to personal health records for research purposes without patient consent is a privilege granted by the REB. Please be sure to adhere at all times to the UHN Policy on Information and Data Security as noted in the Confidentiality Agreement signed as part of this submission.

If, during the course of the research, there are any confidentiality concerns, changes in the approved project, or any new information that must be considered with respect to the project, these should be brought to the immediate attention of the REB. In the event of a privacy breach, you are responsible for reporting the breach to the UHN REB and the UHN Corporate Privacy Office (in accordance with Ontario health privacy legislation – Personal Health Information Protection Act, 2004). Additionally, the UHN REB requires reports of inappropriate/unauthorized use of the information.

Please note that approval for this study will expire on this date unless the UHN REB is otherwise notified.

The UHN Research Ethics Board operates in compliance with the Tri-Council Policy Statement, ICH/GCP Guidelines and Part C, Division 5 of the Food and Drug Regulations of Health Canada.

Sincerely,

Ronald Heslegrave, Ph.D.
Chair, University Health Network Research Ethics Board

RH/tt

Research Ethics Board
Research Ethics Office
Telephone: (416) 864-6060 Ext. 2557
Facsimile: (416) 864-6043
E-mail: patelkl@smh.toronto.on.ca

Leading with Innovation
Serving with Compassion

ST. MICHAEL'S HOSPITAL
A teaching hospital affiliated with the University of Toronto

February 07, 2007

Dr. Jeffrey Zaltzman
Division of Nephrology
St. Michael's Hospital

Dear Dr. Zaltzman,

Re: REB # 06-352C: Long-term clinical outcomes of renal transplantation following nocturnal versus conventional hemodialysis (NHD vs CHD)

REB APPROVAL:	**Original Approval Date**	**February 07, 2007**
	Annual Review Date	**February 07, 2008**

Thank you for your application submitted on December 21, 2006 detailing the above research project. The study has been reviewed through an expedited process (not by full Board review). The views of the St. Michael's Hospital (SMH) Research Ethics Board (REB) have been documented and resolved.

The REB approves the study as it is found to comply with relevant research ethics guidelines, as well as the Ontario Personal Health Information Protection Act (PHIPA), 2004. The REB hereby issues approval for the above named study for a period of twelve months from the date of this letter. Continuation beyond that date will require further review of REB approval.

This letter serves as approval by the SMH REB for conduct of this study; however, additional approvals are required as outlined on the Research Administration Authorization Check List form. Enclosed is a copy of this check list and REB authorization is in the appropriate space. Also, the Clinical Trial Agreements have to be submitted to the Research Office for review and approval. The remainder of the approvals **must be** coordinated through the Research Office prior to initiation of this research. All drug dispensing must be coordinated through the Research Pharmacy at 416-864-5413.

During the course of this investigation, any significant deviations from the approved protocol and/or unanticipated developments or significant adverse events should immediately be brought to the attention of the REB.

The SMH REB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans, the Ontario Personal Health Information Protection Act, 2004, and ICH Good Clinical Practice Consolidated Guideline E6, Health Canada's Regulations Amending the Food and Drug Regulations (1024 - Clinical Trials). Furthermore, all investigational drug trials at SMH are conducted by Qualified Investigators (as defined in the latter document).

Good luck with your investigations.

With best wishes,

☐ Dr. Julie Spence
Chair, Research Ethics Board

☑ Dr. Brenda McDowell
Vice Chair, Research Ethics Board

JS/BJM/jm

30 Bond Street
Toronto, Ontario
M5B 1W8
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Humber River
REGIONAL HOSPITAL

Research Ethics Board
200 Church Street, CB 21
Weston, Ontario
M9N 1N8

Phone: (416) 243-4562

Office for Human Research Protections Federalwide Assurance: FWA00005993

Humber River Regional Hospital REB Initial Approval (Expedited)

October 30, 2003

Dr. C. Chan
Toronto General Hospital
8N-842, 200 Elizabeth Street
Toronto, Ontario
M5G 2C4

Dear Dr. Chan:

Re: **REB File Number: 2006-024-ME**
Protocol Title: Long-term Clinical Outcomes of Renal Transplantation Following Nocturnal Hemodialysis versus Conventional Hemodialysis (NHD vs CHD)

REB Original Approval Date: **30 October, 2006**
REB Expiry Date: **30 October, 2007**

The above named protocol was reviewed and received expedited approval on **October 30, 2006** by the Humber River Regional Hospital Research Ethics Board.

The following is approved from an ethical standpoint for **12** months:

- **Application Form**

Should your study continue beyond **30 October, 2007** you are responsible for ensuring the study receives continuing REB review and re-approval. Please ensure that your Continued Review Submission is forwarded to the REB in sufficient time for review to avoid a lapse in REB approval.

As Principal Investigator you are responsible for the ethical conduct of this study and for submitting all documents related to this approval. During the course of this study the following is to be immediately brought to the attention of the Research Ethics Board:

- any significant deviations from the approved protocol (***that is, any deviation which would lead to an increase in risk or a decrease in benefit to human subjects)***
- amendments to the approved protocol
- revisions to the informed consent
- any new information that must be considered with respect to the study
- any unanticipated developments
- any protocol violations waivers or study issues
- any drug discrepancies
- any concerns regarding confidentiality issues
- privacy breaches
- all serious adverse events
- accrual completion
- site closure, termination or withdrawal of this study
- study publications
- final study report

Investigators of this study are responsible for ensuring that a copy of the signed informed consent is inserted in the patient's health record, a copy is given to the patient and the original filed with the regulatory documents.

Any failure to comply with REB policy and procedure could result in Research Ethics Board approval for the study being immediately suspended or terminated.

Humber River Regional Hospital Research Ethics Board acknowledges receipt of the following documents:

- **REB Approval Letter University Health Network, dated 6 September 2006**
- **Project Summary**
- **Data Collection Form**

Humber River Regional Hospital Research Ethics Board operates in compliance with the Tri-council Policy Statement, ICH/GCP Guidelines and Part C, Division 5 of the Food and Drug Regulations of Health Canada.

The above REB File Number has been assigned to your project. Please use this number on <u>all</u> future correspondence.

Yours sincerely,

S. Salkauskis

Stan Salkauskis, M.D.
Non-Oncology Research Ethics Board Chair

Forward all correspondence to the attention of the Research Ethics Board Coordinator

APPENDIX [B]: COPYRIGHT RELEASE OF PUBLISHED MATERIAL

From: Byers Sally [mailto:Sally.Byers@oxon.blackwellpublishing.com] On Behalf Of Journals Rights
Sent: Friday, August 24, 2007 6:55 AM
To: Pauly, Robert
Subject: RE: Permission to use text in MSc thesis

Dear Robert Pauly,

Thank you for your email request. Permission is granted for you to use the material below for your thesis, subject to the usual acknowledgements and on the understanding that you will reapply for permission if you wish to distribute or publish your thesis commercially.

With best wishes,
Sally

Sally Byers
Permissions Assistant
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UK
Tel.01865 476149
Fax. 01865 471149

-----Original Message-----
From: Pauly, Robert [mailto:Robert.Pauly@uhn.on.ca]
Sent: 23 August 2007 23:39
To: Journals Rights
Subject: Permission to use text in MSc thesis

I am writing with regard to the following article:

Robert P. Pauly MD FRCPC and Christopher T. Chan MD FRCPC. Reversing the risk factor paradox: is daily nocturnal hemodialysis the solution?

From: Department of Medicine, Division of Nephrology, Toronto General Hospital - University Health Network, Toronto, ON, Canada

200 Elizabeth Street,
8N - Rm 842
Toronto, ON, Canada
M5G-2C4
Tel.: 416-340-3073
Fax.: 416-340-4999

I am the primary author this article which will be published in Seminars in Dialysis in the next few months.

I must submit an MSc thesis to McGill University of which the aforementioned article will be a part. As such, I am seeking permission to include this text (or parts thereof) as part of my thesis for the sole purpose of this dissertation.

Thank you.

Robert P. Pauly MD, MSc, FRCPC
Research Fellow, Nocturnal Hemodialysis
University Health Network, University of Toronto
tel. 416.340.3073
fax. 416.340.4999