Hypertension after kidney transplantation
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CHAPTER 6

KIDNEY TRANSPLANT $^{123}$I-$m$IBG SCINTIGRAPHY AND FUNCTIONAL SYMPATHETIC REINNERVATION

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ABSTRACT

Background
In human kidney allografts there is histological evidence of reinnervation but whether this is functional is unknown. We hypothesized that parameters of $^{123}$I-metaiodobenzylguanidine ($^{123}$I-mIBG) uptake in the human kidney allograft as a measure of organ specific functioning sympathetic innervation, correlate with time after transplantation.

Study design
Prospective observational study in kidney transplant recipients.

Setting and participants
In 12 adult kidney transplant recipients (median graft survival 8 years (range 1 month - 34 years), median creatinine clearance 59 ml/min) planar $^{123}$I-mIBG images were made at 15 min and 4 h after intravenous administration of $^{123}$I-mIBG and a SPECT-CT at 4 h was made.

Outcomes and measures
Regions of interest of the allograft (specific) and muscle (non-specific) were drawn on planar geometric mean images and volumes of interest on SPECT-CT. We calculated $^{123}$I-mIBG uptake as a ratio of specific counts vs. non-specific counts and determined washout between 15 min and 4 h.

Results
Relative uptake measured as allograft-to-reference ratio correlated with transplant vintage for 15 min ($r=0.720, p=0.008$) but not for 4 h images ($r=0.105, p=0.746$). Time after transplantation correlated with $^{123}$I-mIBG washout between 15 min and 4 h ($r=0.608, p=0.036$). There was no correlation between creatinine clearance and either $^{123}$I-mIBG uptake ($r=-0.035, p=0.914$ at 15 min p.i. and $r=-0.315, p=0.319$ at 4 h p.i.) or washout ($r=0.210, p=0.513$).

Limitations
Optimal timing sequences for renal $^{123}$I-mIBG are undefined yet. The study is limited by a small sample size.

Conclusion
Our data suggest that there is functional sympathetic reinnervation in human renal allografts increasing over time after transplantation, independent of allograft function.
INTRODUCTION

Kidney transplantation is the only durable therapy for end stage renal disease, but chronic allograft nephropathy remains an important clinical problem. Two important factors that contribute to allograft nephropathy are hemodynamic stressors (i.e. hypertension) and immunologic factors. It seems that reinnervation of renal allografts does not affect renal hemodynamics. Interestingly, the sympathetic nervous system may be involved in intra-renal inflammatory processes that potentially contribute to allograft nephropathy. However, the time course of functional reinnervation in the allograft in relation to graft function has surprisingly obtained little attention.

During the transplantation procedure the kidney allograft is entirely surgically denervated. Histological evidence from animal studies as well as post mortem human studies show that renal nerves partially restore after transplantation. The evidence for nerve regrowth is as early as one month posttransplantation and histological reinnervation is a process that may take several years. After heart transplantation, the myocardium shows signs of reinnervation over time as assessed with norepinephrine spill-over methods. Cardiac reinnervation after transplantation has shown to be related to better cardiac as well as exercise performance. For kidney allografts, it remains elucidative to what extent the reinnervated nerves are functional and clinically relevant.

A minimally invasive method for assessment of functional sympathetic nerves in human organs is metaiodobenzylguanidine (mIBG) scintigraphy. mIBG is a norepinephrine analogue that accumulates in the presynaptic adrenergic nerve endings. When labeled with Iodide-123, the uptake and washout of mIBG via the presynaptic nerve endings can be visualized. Uptake of mIBG reflects the density and functional intactness of the neural tissue within the organ, whereas washout is thought to reflect sympathetic activity. Cardiac mIBG imaging is routinely used for the measurement of cardiac sympathetic activity in chronic heart failure patients. After heart transplantation there is progressive increase in myocardial uptake of mIBG, proving reinnervation of the myocardium. However, the technique has not been applied for the assessment of sympathetic activity of human kidney allografts.

Against this background we set out to assess the feasibility of mIBG scintigraphy in kidney allografts to quantify functional renal sympathetic reinnervation. We hypothesized that human renal allograft sympathetic reinnervation is a slow process that takes several years to reach functional capacity and we therefore explored the association between time after transplantation assessed and renal mIBG uptake and washout. A patient who underwent a renal autotransplantation for a fibromuscular dysplasia induced renal artery stenosis served as control (i.e. no immunosuppressant use). For additional reference we used the data of mIBG scintigraphies of native kidneys in 21 non-transplant patients with hypertension.
Chapter 6

METHODS

Patients
We studied 12 kidney transplant recipient (KTRs) with a renal allograft in situ with a median time after transplantation of 7.4 years (range 1 month to 34 years; interquartile range (IQR) 0.9-19.6). All patients had a stable graft function with a median creatinine clearance (calculated from 24 h urine collection and plasma creatinine) of 59 ml/min (range (IQR) 54-94 ml/min) without significant proteinuria (0.3 g/L (IQR 0.1-0.4)). Immunosuppressive and antihypertensive medications were continued throughout the study. Classes of antihypertensive agents were evenly distributed among the patients.

Table 1 summarizes the patient characteristics. All patients provided written informed consent and all study procedures were approved by the Medical Ethics Committee of the Academic Medical Center of the University of Amsterdam, the Netherlands. All patients provided written informed consent and all study procedures were approved by the Medical Ethics Committee of the Academic Medical Center of the University of Amsterdam, the Netherlands.

In addition to the KTRs, we studied a 25-year-old male patient who underwent an autotransplantation of his right kidney because of renal artery stenosis caused by fibromuscular dysplasia that caused recurrent hypertensive episodes. We used the post-transplant $^{123}$I-mIBG scintigraphy.

Furthermore, as a reference we included data of $^{123}$I-mIBG scintigraphies that were performed in our center in 21 so called ‘treatment resistant’ hypertensive non-transplant patients. These $^{123}$I-mIBG scintigraphies were performed using the identical scanning protocol as described in this manuscript.

$^{123}$I-mIBG imaging study protocol
To avoid thyroidal uptake of $^{123}$I not labeled to mIBG, patients took a capsule of 100 mg potassium iodide 2 hours prior to the intravenous administration of approximately 185 MBq $^{123}$I-mIBG (5 mCi; ± 10%; AdreView™, GE Healthcare). Patients kept their regular fluid intake and no additional diuretics were given. Anterior and posterior semi-whole-body (i.e. from skull base till upper thighs) planar images were acquired 15 min and 4 h post injection (p.i.). Also, at 4 h p.i. single positron emission computed tomography (SPECT) was performed, combined with a low dose CT of the abdomen (without intravenous contrast) to relate $^{123}$I-mIBG uptake to anatomical structures (Figure 1). A vial with a reference amount of approximately 3.5 MBq Iodide-123 was included in the planar images. The planar and SPECT images were acquired with a 15% energy window centered at 159 keV with low-energy high-resolution collimation. The SPECT images were corrected for attenuation using the low-dose CT. SPECT and CT images were checked for accurate alignment and corrected when necessary. Geometric means (GM) images were created using the anterior and posterior planar semi-whole-body images.
Image analyses

Geometric mean (GM) based image analysis
On the semi-whole body GM images one investigator blinded to clinical information (LCD) manually drew anatomical regions of interest (ROIs), muscle (m. quadriceps femoris), the kidney allograft and the $^{123}$I vial (Figure 2). A predefined ROI for muscle (approximately 100 pixels) was used for all patients. Based on the mean counts per pixel per ROI.

SPECT-CT derived volumes of interest
To optimize the contour tracking of the kidneys on the geometrical mean images an additional method provided the renal $^{123}$I-mIBG uptake from the SPECT-CT images. The main advantage of this method is the availability of anatomical information for a better delineation of kidneys and a subsequently better estimation of the renal $^{123}$I-mIBG uptake. Regions of interest (ROIs) were manually drawn in transverse CT images following contours of the allograft kidneys and excluding the calyces. The separate renal ROIs were then fused into volumes of interest (VOIs) and copied to the co-registered SPECT (Figure 3) (Hybrid Viewer™, Hermes Medical Solutions, Stockholm, Sweden). Mean counts/voxel expressed $^{123}$I-mIBG uptake. Uptake in skeletal muscle (quadriceps femoris muscle) served as reference for background activity.

Background uptake of $^{123}$I-mIBG
In analogy to the heart-to-mediastinum ratio as used in cardiology, we chose for a kidney allograft-to-skeletal muscle ratio. Skeletal muscle was chosen as background since this was applicable for both GM images analysis as well as the SPECT CT analysis.
Study variables

The relative uptake between allograft (specific) versus muscle (nonspecific) quantifies neural uptake of $^{123}$I-mlBG and reflects neuron function that results from $^{123}$I-mlBG uptake, storage and release. These can be derived using mean counts from the 15 min and 4 h p.i. GM images and the 4 h p.i. $^{123}$I-mlBG SPECT-CT images:

$$^{123}I - mlBG \text{ relative uptake} = \frac{\text{allograft (specific)} - \text{muscle (non-specific)}}{\text{muscle (non-specific)}}$$

Semi quantitative uptake in the allograft at 15 min and 4 h p.i. was also calculated as a percentage of the injected dose of $^{123}$I-mlBG using the vial with a reference amount of $^{123}$I-mlBG in GM images.

Washout (WO) between 15 min and 4 h p.i. based on GM images reflects sympathetic activity and was calculated from the kidney-to-muscle ratio between 15 min and 4 h p.i:

$$\text{Washout} = \left( \frac{\text{uptake kidney 15 min}}{\text{uptake muscle 15 min}} \right) - \left( \frac{\text{uptake kidney 4 hr}}{\text{uptake muscle 4 hr}} \right) \times 100$$

We emphasize that washout can either have negative or a positive value: a positive WO percentage reflects decrease (by removal or washout) and a negative WO percentage reflects an increase of the radioactivity.
Statistical analysis
Data are presented as medians and interquartile ranges (IQR) for 25 and 75%. Regression analyses by determination of a Spearman rho correlation coefficient were performed to identify correlations between transplant vintage and $^{123}$I-mIBG uptake and washout between the 15 min and 4 h acquisitions. $P$-values below 0.05 were considered statistically significant. All analyses were performed using IBM SPSS Statistics® software for Windows version 21.0 (IBM, USA). All data on the renal autotransplantation patient are presented separately and they were not included in the analysis of the allograft patient group.19

RESULTS
The relative uptake of $^{123}$I-mIBG in the kidney allograft relative to muscle in GM images at 15 min was 1.9 (IQR 1.0-3.3) and 0.8 (IQR 0.4-1.4) at 4 h p.i. The percentage injected dosage in the allograft at 15 min p.i. was 8.8% (IQR 5.9-11.4), at 4 h p.i. 5.7% (IQR 4.5-8.0). Washout based on allograft-to-muscle ratio for GM images between 15 min and 4 h p.i. was 33.9% (IQR 15.2-56.7). The washout corrected for background was 50.3% (IQR 28.7-71.4). SPECT-CT image relative uptake with muscle as background at 4 h p.i. was 0.6 (IQR 0.2-3.1). In all patients a baseline renal content of $^{123}$I-mIBG was detected independent of allograft vintage (Figure 4a). No correlation between allograft function (creatinine clearance) and relative uptake was present ($r=-0.035$, $p=0.914$ at 15 min p.i. and $r=-0.315$, $p=0.319$ at 4 h p.i., Figure 4a) nor between allograft function and washout of $^{123}$I-mIBG was found ($r=0.210$, $p=0.513$) (Figure 4b). Table 2 shows the results of the regression analyses.

Renal $^{123}$I-mIBG uptake at 15 min p.i. showed a significant correlation with time after transplantation: 15 min p.i.: $r=0.720$, $p=0.008$, 4 h p.i.: $r=0.105$, $p=0.746$ (Figure 5). The percentage of injected dosage in the allograft at 15 min p.i. did not have a significant correlation with transplant vintage ($r=-0.014$, $p=0.966$ at 15 min), nor at 4 h p.i. ($r=-0.406$, $p=0.191$ at 4 h).

Washout based on the GM images using the kidney allograft-to-muscle ratio was significantly correlated with transplant vintage (Figure 5, $r=0.608$, $p=0.036$).

For the SPECT-CT based calculations, the relative uptake showed no correlation with time after transplantation ($r=-0.217$, $p=0.499$).

In the pre-and posttransplant $^{123}$I-mIBG scintigraphies of the autotransplant patient, the relative uptake at 15 min p.i. in the transplanted kidney decreased from 0.78 to 0.55.
pretransplant to 0.62 (-21%) posttransplantation and at 4 h p.i. from 0.66 to 0.47 (-29%). Washout between 15 min and 4 h decreased from 36% to 29% in the autograft.

Data of $^{123}$I-mIBG scintigraphies from 21 patients with so called ‘treatment resistant’ hypertension served as controls. Scintigraphies were performed similar to the scanning protocol of this study and patients received 20 mg of oral furosemide mimicking the furosemide effect in the KTRs. In these patients (age 60.0 years (IQR 53.0-70.0), 15 males (72%), eGFR 60.7 (48.5-101.9 ml/min/1.73 m$^2$), mean uptake of $^{123}$I-mIBG GM images at 15 min p.i. was 3.08, at 4 h 1.64 and washout was 41.53%. For SPECT-CT at 4 h p.i. mean uptake of $^{123}$I-mIBG was 1.41. Since the control group showed overall lower levels of uptake and washout (3.08 vs. 1.9 for 15 min; 1.64 vs. 0.8 and 41.5% vs. 33.9%), we conclude that these differences suggest that uptake in the allografts is less than in native innervated kidneys with a normal kidney function.

**DISCUSSION**

This is the first report on renal $^{123}$I-mIBG scintigraphy for the assessment of functional renal sympathetic nerves in vivo in kidney transplant recipients. We show that $^{123}$I-mIBG is feasible in kidney allografts. The clear relationship between time after transplantation and $^{123}$I-mIBG-washout rates supports the hypothesis that restoration of sympathetic function during and after histological reinnervation is an ongoing process that may last for decades. Our data also indicate that reinnervation of kidney allograft occurs independent of allograft function.

Our findings are relevant for two reasons: They provide preliminary proof-of-concept of $^{123}$I-mIBG scintigraphy of renal sympathetic tissue and secondly, they may open a new window on graft biology.
Kidney transplant $^{123}$I-mIBG scintigraphy and functional reinnervation

Up to date, no study has been performed about $^{123}$I-mIBG scintigraphy of kidney tissue. We have experience with one case that suggested that the technique could indeed be used to assess activity of the renal sympathetic system.\textsuperscript{20} In view of an absent comparison to a gold standard, the current study is not a formal validation. Norepinephrine spillover as a gold standard method is currently not feasible since the compound is not distributed in Europe. However, our study has revealed some important aspects of the technique that justify its further development.

Primarily, the data show that even in fully denervated kidneys, i.e. shortly after transplantation both in autologous as well as in allogeneic kidney transplantation, there remains some renal $^{123}$I-mIBG content. This can in part be explained by $^{123}$I-mIBG in pre-urine since the majority of $^{123}$I-mIBG (< 2% protein bound, 275 kDa) is excreted unmetabolized by glomerular filtration.\textsuperscript{20,21}

Secondly, the washout rate of $^{123}$I-mIBG from the renal tissue among the whole group of allografts appears higher than that reported in cardiac tissue, in which physiological

\textbf{Figure 4a and 4b.} Creatinine clearance and uptake and washout of renal $^{123}$I-mIBG. Figure 4a (upper): creatinine clearance and uptake of renal $^{123}$I-mIBG, Figure 4b (lower): creatinine clearance and washout of renal $^{123}$I-mIBG.
values lie below 20%.\textsuperscript{22} This is in agreement to a study by Kopin et al, who showed a 10-fold norepinephrine spill-over rate in kidney as compared to the myocardium.\textsuperscript{23} This high washout leads to a faster decline of $^{123}$I-mIBG content in the kidneys and mandates a different timing of the scintigraphic sequence. We took the acquisition times of 15 min and 4 h after injection from the myocardial protocol. There is no information in the original publications that introduced cardiac $^{123}$I-mIBG on the rationale for these time points as a best reflection of cardiac sympathetic activity. We observed a significant correlation with transplant vintage only in the 15 min p.i. images and not in 4 h p.i. GM images and 4 h SPECT-CT images. Therefore, we assume that the optimal timing for sympathetic activity.

We observed a significant correlation with transplant vintage only in the 15 min p.i. images and not in 4 h p.i. GM images and 4 h SPECT-CT images. Therefore, we assume that the optimal timing for scintigraphy to determine uptake, may be somewhere in between 15 min and 4 h and rather closer to 15 min p.i. Since we performed SPECT-CTs at 4 h p.i., this may account for the absent correlation in SPECT-CT images. This should be further investigated in larger samples.

Thirdly, because we did not have complete mediastinal scintigraphic data in SPECT-CT images, we took skeletal muscle tissue as a reference for background $^{123}$I-mIBG uptake without a formal comparison to other reference tissue. Adipose tissue would have been favorable over muscle for having less sympathetic innervation, but adequately distinguishing adipose tissue on the planar GM images was not feasible. We emphasize that these results can only be interpreted for patients with well-functioning allografts since GFR impacts clearance rates of $^{123}$I-mIBG and may therefore affect organ retention.\textsuperscript{24} These aspects should be taken into account when further developing the technique.

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### Table 2. $^{123}$I-mIBG quantifications

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<thead>
<tr>
<th></th>
<th>Kidney transplant recipients</th>
<th>Control group</th>
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<tbody>
<tr>
<td></td>
<td>$n=12$</td>
<td>$n=21$</td>
</tr>
<tr>
<td><strong>Uptake 15 min (GM)</strong></td>
<td>1.9 [1.0-3.3]</td>
<td>3.1 [2.8-5.0]</td>
</tr>
<tr>
<td>Correlation with time after Tx</td>
<td>$r=0.720$, $p=0.008$</td>
<td></td>
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<tr>
<td><strong>Uptake 4 h (GM)</strong></td>
<td>0.8 [0.4-1.4]</td>
<td>1.6 [1.4-2.0]</td>
</tr>
<tr>
<td>Correlation with time after Tx</td>
<td>$r=0.105$, $p=0.746$</td>
<td></td>
</tr>
<tr>
<td><strong>Uptake 4 h (SPECT-CT)</strong></td>
<td>0.6 [0.2-3.1]</td>
<td>1.4 [1.0-1.9]</td>
</tr>
<tr>
<td>Correlation with time after Tx</td>
<td>$r=-0.217$, $p=0.499$</td>
<td></td>
</tr>
<tr>
<td><strong>Injected dose 4 h (%) (GM)</strong></td>
<td>8.8 [6.0-11.4]</td>
<td>17.9 [17.9-21.8]</td>
</tr>
<tr>
<td>Correlation with time after Tx</td>
<td>$r=-0.014$, $p=0.966$</td>
<td></td>
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<tr>
<td><strong>Injected dose 4 h (%) (GM)</strong></td>
<td>5.7 [4.5-8.0]</td>
<td>8.9 [8.9-13.5]</td>
</tr>
<tr>
<td>Correlation with time after Tx</td>
<td>$r=-0.406$, $p=0.191$</td>
<td></td>
</tr>
<tr>
<td><strong>Washout 15 min-4 h (%) (GM)</strong></td>
<td>33.9 [15.2-56.7]</td>
<td>41.5 [28.4-56.4]</td>
</tr>
<tr>
<td>Correlation with time after Tx</td>
<td>$r=0.608$, $p=0.036$</td>
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**Abbreviations:** Tx = transplantation; GM = geometric mean.
For fundamental research use, especially extending SPECT-CT derived parameters may provide additional insight into the spatial differentiation of renal sympathetic tissue. One of the possible applications in clinical research of the technique would be to assess the effectiveness of catheter based renal denervation, which is currently under scrutiny after the negative results of the sham-intervention controlled Symplicity HTN-3 trial.25

Our data provide new insights in the function of reinnervated neural tissue after transplantation in humans. Up to date, only one study systematically investigated the function of the renal sympathetic system after transplantation. This well-designed study by Hansen et al. showed that inducing systemic sympathetic activation by means of applying lower body negative pressure did not affect tubular markers of sympathetic activation in renal allografts as compared to native kidneys in control subjects.3 They also showed that the absence of this reflex was independent of graft age (median of 58 months in the long-term transplants, median of 1.5 month in the newly transplanted patients). In contrast, our data suggest that sympathetic reinnervation does have
some level of functionality expressed as norepinephrine washout and uptake that may
go undetected when using a relatively crude method such as lower body negative
pressure. Moreover, the transplant vintage of Hansen et al. is shorter than the KTRs
studied in our study, presuming that functional innervation in Hansen’s study might
have remained undetected because of a too short timeframe after transplantation.3

There is growing evidence that sympathetic neural activity may be a driving, chemotactic
factor in (low grade) inflammatory processes in visceral organs, including the kidney.4,5,26,27
We speculate that the sympathetic function that we found in long surviving kidney grafts
may yield an immunomodulatory factor. An additional interesting possibility that arises
from our data is that reinnervation would be affected by differences in immunosuppressive
treatment regimens between patients who were recently transplanted and those who
were transplanted many years ago. Whereas the regimens within the ‘older’ group mainly
consisted of azathioprine and prednisolone, all recently transplanted patients used the
potentially neurotoxic calcineurin inhibitors.28 The interaction between reinnervation
and graft biology and the effects of immunosuppressive agents should be subject to
further studies.

CONCLUSION

We have shown that renal 123I-mIBG scintigraphy is feasible in human kidney allografts.
The technique should be further developed. The current data suggest that functional
sympathetic reinnervation in renal allografts is a slow process and independent of
allograft function.

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Contributions: research idea and study design: LCD, HJV, FJB, IJMtb, CTPK. Data
acquisition: LCD, HJV, FJB, CTPK; data analysis/interpretation: LCD, HJV, JB, IJMtb,
BJvdB, CTPK; statistical analysis: LCD, HJV, BJvdB, CTPK; supervision or mentorship:
HJV, FJB, IJMtb, CTPK. Each author contributed important intellectual content during
manuscript drafting or revision and accepts accountability for the overall work by
ensuring that questions pertaining to the accuracy or integrity of any portion of the
work are appropriately investigated and resolved. HJV and CTPK take responsibility
that this study has been reported honestly, accurately, and transparently; that no
important aspects of the study have been omitted; and that any discrepancies from
the study as planned (and, if relevant, registered) have been explained.
REFERENCES


Chapter 6