Original Articles

Expression of the transcriptional regulator snail1 in kidney transplants displaying epithelial-to-mesenchymal transition features

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ABSTRACT

Background. The epithelial response to injury is stereotypical and reminiscent of epithelial-to-mesenchymal transitions (EMTs), such as those observed during embryogenesis and tumour metastasis. In the context of solid organ transplantation, EMT-like features are often acquired by epithelial cells and are predictive of graft fibrosis. Here, we studied the possible involvement of several major transcriptional regulators, including snail1, phospho-Smad 2/3 and zeb1, in EMT induction in human renal grafts.

Methods. We used immunohistochemistry to detect the presence of these EMT transcriptional regulators along with that of two validated EMT markers (intra-cytoplasmic translocation of β-catenin, de novo expression of vimentin), in 103 renal graft biopsy samples taken for routine surveillance or for a clinical indication.

Results. We observed the nuclear accumulation of snail1 and phospho-smad2/3 in tubular cells displaying EMT. The level of snail1 was significantly correlated with the scores of EMT markers (β-catenin: \( \rho = 0.94, P < 0.0001 \); vimentin: \( \rho = 0.93, P < 0.0001 \)) and with deteriorated graft function and proteinuria at the time of biopsy. Furthermore, intense staining for both snail1 and vimentin in tubular cells (≥10% of tubules) was predictive of graft dysfunction 21 months post-biopsy, independently of the other known risk factors for long-term graft dysfunction. In contrast, in both normal and diseased graft, zeb1 expression was detected exclusively in the endothelial cells of glomeruli and peritubular capillaries.

Conclusion. This study suggests that snail1 is closely related to the fibrogenic, EMT-like response of the tubular epithelium in human renal grafts and predictive of graft function loss.

Keywords: early fibrotic markers, EMT, kidney, transcriptional factors, transplantation

INTRODUCTION

Epithelia respond to injury in adult organs in a stereotypical manner. Under assault, epithelial cells will cease performing the physiological functions for which they are programmed (as in a diapause [1]) and acquire a number of mesenchymal features [2]. The presence of mesenchymal molecules that are usually expressed by fibroblasts are accepted criteria for ‘epithelial-to-mesenchymal transition’ (EMT) [3]. However, we are still ignorant of the real consequences of EMT in adult organs, because the results of lineage-tracing studies are contradictory [4–7]. Since the EMT acronym is often taken to refer to cellular transit (rather than a simple transition from one phenotype to another), the latest report from the BANFF working group has asked for a new term to be coined to designate the epithelial response to injury in the context of solid organ transplantation [8]. Our previous analysis of data from several different renal transplantation centres did show that detecting EMT-like features was useful for renal graft surveillance: the expression in tubular epithelial cells of human renal grafts of these ‘EMT-like’ features was closely associated with
Here, in order to investigate the possible involvement of snail1, phospho-smad 2/3 and Zeb1 in EMT induction in injured human renal grafts, we used immunohistochemistry to detect the expression of these three members of the pro-EMT loop in the tubular epithelial cells of human renal grafts.

**MATERIALS AND METHODS**

**Biopsy samples**

The leftover material from 103 renal graft biopsies taken from 91 adult kidney recipients between 2006 and 2010 was available for the study of the markers. In accordance with the French law for non-interventional studies using leftover human samples (Public Health Code, article L.1121-1, revised in May 2009), all patients were invited to participate in the study after giving oral consent. Most of the patients (88.8%) had received a graft from a deceased donor, and the others (11.2%) from living donors. The mean recipient age was 49 years at the time of biopsy, and 68.3% of recipients were male. The mean donor age was 53.7 years. The mean cold ischemia time was 1111 min (18.5 h). The immunosuppressive regimen consisted essentially of conventional triple-therapy including a calcineurin inhibitor (CNI), corticosteroids, and mycophenolate mofetil. Only four patients were on a CNI-free regimen and treated with rapamycin, an mammalian target of rapamycin inhibitor.

Fourty-four biopsies (43%) were performed for graft surveillance, 84% of them were taken at 3 months post-transplant, and 59 (57%) were carried out for a clinical indication (delayed graft function after transplant surgery, or following acute deterioration of graft function at later time points).

Histopathological analysis was scored according to the updated Banff working classification for renal graft pathology [10]. Thirty-two of the biopsies indicated acute rejection, including eight that were subclinical and diagnosed by the surveillance biopsy. Twenty-one displayed acute tubular necrosis and 16 CsA toxicity-related lesions. The mean Banff acute scores were 0.75, 0.46, 0.34, and 0.39 for i, t, g, and ptc, respectively, and the chronic scores were 0.14, 0.78, 0.67, and 0.87 for cg, ci, ct, and cv, respectively.

Three normal kidney samples obtained from the healthy parts of kidneys that had been removed because of a malignant tumour, plus three implantation biopsies, were used as controls.

**Immunohistochemistry of transcription factors (snail1, Zeb1, and phospho-smad2/3) and of EMT markers (β-catenin and vimentin)**

Immunohistochemistry was performed on paraffin-embedded tissues as described previously [11,13]. Target retrieval was carried out by heating the tissue in a citrate buffer (Dako cytometry) at pH 6 for snail1, phospho-smad2/3, β-catenin, and vimentin, or at pH 9 for Zeb1. Endogenous peroxidase was inactivated by incubating the specimen for 10 min at room temperature in 0.03% H2O2. The sections were incubated overnight at 4°C with phosphate-buffered saline containing 1/3000 anti-snail1 (rabbit polyclonal antibody; abcam), 1/3000 anti-phospho-smad2/3 (rabbit polyclonal antibody; Santa Cruz...
Biotechnology), 1/2500 anti-Zeb1 (rabbit polyclonal antibody, kindly provided by Dr Darling), 0.1 µg/mL anti-β-catenin (rabbit polyclonal antibody; Santa Cruz Biotechnology), and 1:4000 anti-vimentin (mAb V9; Zymed). The immunoreactive proteins were visualized using the Envision + HRP system (AEC; DakoCytomation). Finally, the tissue sections were counterstained with hematoxylin and mounted using aqueous mounting medium (Dako). The primary antibodies were replaced by equal concentrations of rabbit or mouse IgG (Dako) as negative controls.

Semi-quantitative analysis of immunohistochemical staining

The nuclear accumulation of the transcriptional factor (snail1) and the tubular expression of EMT markers (β-catenin and vimentin) were measured as described previously [11, 20], by the semi-quantitative determination of the proportion of tubules displaying positive staining (0, none; 1, < 10%; 2, 10–24%; 3, 25–50%; 4, >50%). EMT+ or snail1+ graft designates a graft in which at least 10% of tubules displayed both the de novo expression of vimentin and the intracellular translocation of β-catenin or nuclear staining for snail1. All stained tissues were scored three times in a blind fashion by YCXD, who had no knowledge of the clinical, biological, or morphological data, including the result of immunostaining scores. We did not quantitate the tubular nuclear accumulation of phospho-smad2/3, because its basal expression in the normal kidneys was so strong in the distal tubules and in collecting ducts, so the quantification would have been doubtful in diseased conditions. Similarly, Zeb1 was not quantified, since it was not detectable in the tubular epithelial cells of normal or diseased renal grafts.

Statistical analysis

The association between the patient’s clinical and morphological data (eGFR, proteinuria, and Banff scores) at the time of biopsy and the markers studied here was assessed by Spearman’s rank correlation. Results were reported as Spearman’s coefficient, ρ, with the associated P-value. The clinical data were also compared according to the snail1 and/or EMT status (positive versus negative) by the unpaired t-test and Wilcoxon’s rank-sum test. The results were reported as the mean ± SE, with the P-value generated from a t-test for continuous values or from the Wilcoxon’s rank-sum test for ordinal values or to compare groups containing less than 30 biopsies. Logistic regression was used to estimate the adjusted odds ratios (ORs) and 95% confidence intervals (CIs) of potential risk factors for low graft function at 21 months after biopsy. The cutoff point for eGFR (36 mL/min) was determined as the median eGFR at this time. For this analysis, only one biopsy for each patient was included in the model: when a patient had more than one biopsy, the graft surveillance biopsy or the biopsy carried out more than 3 months post-transplant was used. This decision was made before we performed the statistical analysis. Statistical analysis was carried out using STATA 8 (Stata Corp. College Station, TX, USA). The test was defined as being significant if P < 0.05.

RESULTS

Nuclear accumulation of snail1 and phospho-smad2/3, but not of Zeb1, in tubular epithelial cells of renal graft biopsies

In the normal kidneys and in renal implant biopsies, nuclear snail1 staining was either absent or very faint in the proximal tubules and was weakly detectable in the distal tubules and collecting ducts (Figure 1a). In the diseased graft kidneys, we found intense nuclear snail1 staining in many tubules, including the proximal tubules (Figure 1b and e). In normal kidneys, strong phospho-smad2/3 staining was found in the distal tubules and the collecting ducts, but relatively weaker staining in the proximal tubules (Figure 1c). In the diseased renal grafts, strong nuclear phospho-smad2/3 staining was seen in a large number of tubules (Figure 1d).

Zeb1 was undetectable in tubular epithelial cells, in either normal (Figure 1f) or diseased graft kidneys (Figure 1g), even when EMT markers were present. This was not due to a failure to detect Zeb1 with the antibody that we used since (i) clear nuclear staining of Zeb1 was found in the endothelial cells of glomeruli and peritubular capillaries from both the native (Figure 1f) and the transplanted (Figure 1g) kidneys, and (ii) as a positive control, we used a (uterine) tumour sample, in which Zeb1 was detected in the nuclei from epithelial and stromal cells (Figure 1h). The presence or the absence of EMT in graft kidneys did not affect the intensity of endothelial Zeb1 expression that was observed.

Expression of EMT markers (β-catenin, vimentin) and their co-localization with nuclear snail1 or phospho-smad2/3 expression in tubular epithelial cells

As reported previously [11, 13], in the normal kidneys, we observed β-catenin expression at the basal pole of proximal tubular epithelial cells, at the basolateral surface of distal tubules, and of collecting ducts (Figure 2a). No vimentin expression could be detected in the epithelial cells of the normal kidneys or in implant biopsies (Figure 2b). No vimentin expression was seen in a large number of tubules (Figure 1d).

Nuclear accumulation of snail1 (Figure 2e) was mainly found in tubules displaying the EMT-like phenotypic changes described above (Figure 2f). Similar results were obtained for phospho-smad2/3, the expression of which (Figure 2g) was greater in vimentin+ tubules (Figure 2h). Confocal microscopy was used to confirm that the de novo expression of vimentin was present in the tubules displaying nuclear staining of snail1 (Figure 2i) and phospho-smad2/3 (Figure 2j).

Spearman’s rank correlation analysis showed that the nuclear staining score for snail1 was strongly correlated with that for vimentin (ρ = 0.93, P < 0.0001) and with that for β-catenin (ρ = 0.94, P < 0.0001). This was true whether the biopsies had been taken in response to a clinical indication (ρ = 0.9, P < 0.0001 for vimentin; ρ = 0.94, P < 0.0001 for β-catenin) or for graft
**FIGURE 1:** Snail1 and phospho-smad2/3, but not of Zeb1, accumulate in the nuclei of tubular epithelial cells from human renal grafts. In the normal kidneys, no nuclear snail1 staining was seen in the proximal tubular epithelial cells, and only moderate staining in those of the distal tubules and collecting ducts (a). In contrast, intense nuclear snail1 staining was detectable in numerous tubules in pathological grafts (arrows) (b and e). Strong nuclear phospho-smad2/3 staining was observed in distal tubules and collecting ducts and less intense nuclear staining in the proximal tubules from normal kidney (c). Nuclear phospho-smad2/3 expression was observed in a more diffused pattern in tubules from pathological grafts and in particular it was strong in proximal tubules (arrows) (d). (e) High-magnification image of (b) showing the nuclear accumulation of snail1 in tubular epithelial cells in the pathological graft. Nuclear expression of Zeb1 in glomerular and peritubular endothelial cells was detected in both the normal kidney (f) and diseased renal grafts (g). (h) The positive Zeb1 staining obtained in a tumour sample (uterine tumour) used as a positive control.
surveillance ($\rho = 0.95$, $P < 0.0001$ for vimentin; $\rho = 0.94$, $P < 0.0001$ for $\beta$-catenin).

**Association between the tubular nuclear expression of snail1 and of the mesenchymal cell marker vimentin with graft histological lesions (Banff scores), graft dysfunction, and proteinuria**

Spearman’s rank correlation showed that both vimentin and snail1 were significantly correlated with the Banff acute (i and ptc) and chronic (ci, ct) scores (Table 1) and with a low eGFR [14] ($\rho = -0.6$, $P < 0.0001$ for snail1; $\rho = -0.645$, $P < 0.0001$ for vimentin, Table 1) and proteinuria ($\rho = 0.38$, $P = 0.0005$ for snail1; $\rho = 0.444$, $P < 0.0001$ for vimentin) at the time of the biopsy. The significant correlation between snail1 or vimentin and eGFR at this time point was observed in both surveillance biopsies ($\rho = -0.4$, $P = 0.008$ for snail1; $\rho = -0.47$, $P = 0.0015$ for vimentin) and biopsies intended for a clinical indication ($\rho = -0.56$, $P < 0.0001$ for snail1; $\rho = -0.58$, $P < 0.0001$ for vimentin). In fact, the correlation was also observed in biopsies both very early during the time course, pre-3 months post-transplant ($\rho = -0.6$, $P = 0.0022$ for snail1; $\rho = -0.58$, $P = 0.0027$ for vimentin), and after more than 3 months ($\rho = -0.6$, $P < 0.0001$ for snail1; $\rho = -0.63$, $P < 0.0001$ for vimentin). However, as shown in Table 2, the Banff scores themselves were not significantly correlated with renal graft injury indicators, such as eGFR at the time of the biopsy.

Nevertheless, unlike the acute scores, Snail or vimentin expression in tubular epithelial cells and the Banff chronic ci, ct,
cg, and cv scores were good predictors of the deterioration of eGFR at 9 and 21 months post-biopsy (Table 2).

Figure 3 shows the graft function, at the time of the biopsy, 9 and 21 months later, according to the fibrotic status (a graft was classified as being fibrotic when ci and ct scores ≥2) and in terms of the level of snail1 expression in the nuclei of tubular cells (a graft was classified as snail1+ when ≥10% of tubules displayed nuclear snail1 staining). As expected, patients with a fibrotic graft and who also expressed snail1 progressively lost their graft function over time (with a mean loss of 10 mL/min over 21 months). In patients with a fibrosis-free graft, nuclear staining for snail1 was associated with poorer graft function at the time of the biopsy (eGFR: m = 26.7 ± 2.7 versus 49.4 ± 2.7 mL/min, P < 0.0001), and despite some recovery after biopsy continued to display persistent significantly lower graft function at 9 (m = 35.7 ± 3.5 versus 50 ± 2.9, P = 0.0031) and 21 months (m = 28 ± 3.8 versus 47.5 ± 3.3, P = 0.0009) after biopsy than those who were free of both significant fibrosis and snail1 expression. In this latter group, graft function remained remarkably stable over time (Figure 3). This was not due to any difference in graft fibrosis (m = 0.5 versus 0.6, P = 0.4; m = 0.35 versus 0.5, P = 0.17 for the comparison of the ci or ct scores in these two groups, respectively).

Logistic regression analysis showed that the tubular expression of snail1 or of vimentin was an independent risk factor for predicting a low eGFR (<36 mL/min) at 21 months post-biopsy, with an OR of 11.8 (95% CI = [2.6–55], P = 0.002) for snail1 and an OR of 4.62 (95% CI = [1.23–17], P = 0.023) for vimentin after adjustment for some known risk factors or events for the long-term loss of graft function, such as donor age, cold ischemia time, graft inflammation i, ptc, or graft fibrosis, ci, and cv scores (Table 3).

**DISCUSSION**

Our study yielded three major findings. First, the expression of snail1 in the nuclei of tubular epithelial cells co-localizes with two major markers of EMT-like features known to predict graft fibrosis and its progression in human kidney recipients, namely the de novo expression of vimentin and the translocation of β-catenin into the cytoplasm. Second, like the EMT marker vimentin, Snail1, but not the conventional morphological lesions scored by the Banff classification, was a sensitive marker of graft injury and fibrosis. Third, quantifying snail1

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**Table 2.** Spearman’s rank correlation of the nuclear accumulation of snail or the expression of the EPC marker vimentin in tubular epithelial cells with renal graft morphological lesions (Banff scores) and renal graft injury indicators (eGFR, proteinuria) at the time of the biopsy

<table>
<thead>
<tr>
<th>Spearman’s rank correlation of</th>
<th>Snail1</th>
<th>Vimentin</th>
</tr>
</thead>
<tbody>
<tr>
<td>i</td>
<td>0.285</td>
<td>0.218</td>
</tr>
<tr>
<td>t</td>
<td>0.11</td>
<td>0.07</td>
</tr>
<tr>
<td>g</td>
<td>0.16</td>
<td>0.17</td>
</tr>
<tr>
<td>ptc</td>
<td>0.4</td>
<td>0.354</td>
</tr>
<tr>
<td>cg</td>
<td>0.2</td>
<td>0.166</td>
</tr>
<tr>
<td>ci</td>
<td>0.36</td>
<td>0.33</td>
</tr>
<tr>
<td>ct</td>
<td>0.384</td>
<td>0.363</td>
</tr>
<tr>
<td>cv or ah</td>
<td>0.123</td>
<td>0.136</td>
</tr>
<tr>
<td>eGFR</td>
<td>−0.6</td>
<td>−0.645</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>0.38</td>
<td>0.444</td>
</tr>
<tr>
<td>Vimentin</td>
<td>0.93</td>
<td>&lt;0.0001</td>
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<tr>
<td>β-Catenin</td>
<td>0.94</td>
<td>&lt;0.0001</td>
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"FIGURE 2: Continued"
and vimentin staining in tubules by immunohistochemistry of a biopsy sample provides prognostic information about long-term renal graft function loss, and this prognostic value was independent of existing graft fibrosis. During kidney development, the mesenchyme adjacent to the nephrogenic mesenchyme expresses snail1 genes, which are critical in tissue formation [18]. Once the differentiation of the kidney is complete, these genes are repressed, but they can be induced again, for example, in the rat model of ureteral obstruction [24] or in rapidly fibrotic myeloma cast nephropathy in humans [20]. Recently, the increased expression of snail1 has also been reported in tubular epithelial cells from human kidneys displaying diabetic nephropathy lesions or IgA nephropathy [21]. Snail1 probably plays a major role in renal fibrogenesis, since the reactivation of its expression is sufficient to induce renal fibrosis in adult mice, even in the absence of any epithelial injury [18]. The general view is that it promotes fibrosis by activating an EMT program, as in tumours, where the nuclear accumulation of snail1 is associated with E-cadherin repression, tumour recurrence, and metastasis.

So far, very little was known about snail1 expression in human transplanted kidneys, which are very prone to fibrosis. In this study, we observed that snail1 was expressed in tubules showing phenotypic alterations known to predict graft fibrosis [13], which suggests—although it does not prove—that snail1 is involved in activating an EMT program in the kidney. More importantly, though, we found that snail1 expression in grafts that were free of significant fibrosis reflected graft dysfunction (as attested by lower eGFR and higher proteinuria at the time of the biopsy), and the graft did not go on to recover completely from this dysfunction (even if the snail1+ grafts did not display fibrotic lesions, they did not perform as well as snail1- grafts in the long term). One possible explanation is that the reactivation of snail1 is followed by an excessive repair response and/or by the initiation of a self-perpetuating fibrotic process. Fibrosis of the graft leads to loss of function. We do not have a second biopsy to prove it in this cohort, but in our previous studies we investigated this issue and found that

<table>
<thead>
<tr>
<th>Spearman’s rank correlation of snail1 at 0.6 cm of biopsy</th>
<th>eGFR at time of biopsy</th>
<th>Spearman’s rank correlation of vimentin at 0.6 cm of biopsy</th>
<th>eGFR 9M after biopsy</th>
<th>Spearman’s rank correlation of vimentin at 0.6 cm of biopsy</th>
<th>eGFR 21M after biopsy</th>
</tr>
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<tbody>
<tr>
<td>rho: 0.0001 P-value: 0.003</td>
<td>rho: 0.0001 P-value: 0.0003</td>
<td>rho: 0.0001 P-value: 0.0003</td>
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**Figure 3**: Evolution of graft function, estimated using the MDRD equation (eGFR), based on the IF/TA score (a graft with ci/ct scores of ≥2 was classified as an IF/TA+ graft) and the snail1 status (a graft with at least 10% of tubules staining positive for snail1 in the nuclei was defined as a snail+ graft). Positive snail1 staining was closely associated with significantly poorer graft function even in non-significantly fibrotic grafts compared with those without snail1 expression both at the time of biopsy (P < 0.0001) and at later time points (at 9 months post-biopsy, P = 0.0031; at 21 months, P = 0.0009).

| Table 3. (a and b) Risk factors for a low eGFR (<36 mL/min) 21 months after biopsy by logistic regression analysis in renally transplanted patients |
|-------------------------------------------------|----------------------|----------------------|----------------------|
| OR                                             | 95% CI               | P-value              |
| a C1                                             | 1.0004               | [0.998–1.002]       | 0.7                  |
| C2                                             | 1.01                | [0.97–1.06]         | 0.65                |
| C3                                             | 1.23                | [0.54–2.8]          | 0.62                |
| C4                                             | 1.57                | [0.64–6.7]          | 0.54                |
| C5                                             | 0.99                | [0.4–2.5]           | 0.98                |
| C6                                             | 1.86                | [0.83–4.2]          | 0.13                |
| b C1                                             | 1.0004               | [0.999–1.002]       | 0.635               |
| C2                                             | 1.002               | [0.96–1.04]        | 0.94                |
| C3                                             | 1.29                | [0.58–2.8]         | 0.53                |
| C4                                             | 1.58                | [0.43–5.9]         | 0.5                 |
| C5                                             | 1.3                 | [0.56–3]            | 0.54                |
| C6                                             | 1.7                 | [0.815–3.6]         | 0.155               |
significant renal graft fibrosis progression was observed in grafts expressing an EMT marker at an early stage (at the 3-month surveillance biopsy) [13]. A possible alternative explanation is that this reactivation reflects an uncontrolled epithelial injury, which leads to the subsequent loss of graft function. This is of interest, because if this is true, the detection of snail1 as well as of the EMT marker, vimentin, could provide a useful early marker for assessing transplant injuries that may not always be detectible by conventional morphologic analysis. This early and sensitive marker could help us to improve our graft surveillance.

Zeb factors are important transcription repressors that induce EMT by suppressing the expression of various epithelial genes in different kinds of human cancers. Zeb1 also suppresses the expression of tubular basement membrane components, which could facilitate the migration of epithelial cells. To the best of our knowledge, Zeb1 expression has never been reported in the kidneys. Here, we used a validated antibody against Zeb1 that in our hands produced positive nuclear staining in epithelial and stromal cells from a malignant tumour, as previously reported by others [25]. In the native as well as the transplant kidneys, the nuclear expression of Zeb1 was found exclusively in the endothelial cells from glomeruli and peritubular capillaries, whether or not histological lesions were detected. Zeb1 expression was not found in tubules showing phenotypic changes suggestive of EMT. This finding is in keeping with the absence of Zeb1 expression that has been reported even in a CCl4-induced model of liver fibrosis (an acute model of liver fibrosis during which snail1 is induced) [19]. Taken together, these data suggest that in contrast to tumour-associated EMT, Zeb1 does not play a critical role in fibrosis-associated tubular EMT.

In conclusion, the reactivation of snail1 expression in human renal grafts could be essential to switch on a fibrogenic, EMT programme in tubular epithelial cells. In grafts that are not yet fibrotic, the presence of snail1 reflects an ongoing injury and may predict the loss of renal function in the long term.

ACKNOWLEDGEMENTS

We would like to thank Douglas S. Darling (Department of Oral Health and Rehabilitation, and Center for Genetics and Molecular Medicine, Louisville, Kentucky) for providing us with the anti-zeb1 antibody. We are grateful to Monika Ghosh for revising the English text.

CONFLICT OF INTEREST STATEMENT

None declared.

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Patient attitudes towards kidney transplant listing: qualitative findings from the ATTOM study

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ABSTRACT

Background. There is variation in time to listing and rates of listing for transplantation between renal units in the UK. While research has mainly focused on healthcare organization, little is known about patient perspectives of entry onto the transplant waiting list. This qualitative study aimed to explore patients’ views and experiences of kidney transplant listing.

Methods. Semi-structured interviews were conducted with patients aged under 75, who were on dialysis and on the transplant waiting list, not on the waiting list, undergoing assessment for listing or who had received a transplant. Patients were recruited from a purposive sample of nine UK renal units, which included transplanting and non-transplanting units and units with high and low wait-listing patterns. Interviews were transcribed verbatim and analysed using thematic analysis.

Results. Fifty-three patients (5–7 per renal unit) were interviewed. Patients reported that they had received little information about the listing process. Some patients did not know if they were listed or had found they were not listed when they had thought they were on the list. Others expressed distress when they felt they had been excluded from potential listing based on age and/or comorbidity and felt the process was unfair. Many patients were not aware of pre-emptive transplantation and believed they had to be on dialysis before being able to be listed. There was some indication that pre-emptive transplantation was discussed more often in transplant than non-transplant units. Lastly, some patients were reluctant to consider