Transpl Int (2005) 17: 680–686
DOI 10.1007/s00147-004-0792-6

ORIGINAL ARTICLE

Sonia J. Wakelin
John Casey
Amanda Robertson
Peter Friend
Bryon C. Jaques
Hester Yorke
Sue P. Rigden
Xavier F. S. Emmanuel
Lucia Pareja-Cebrian
John L. R. Forsythe
Peter J. Morris

The incidence and importance of bacterial contaminants of cadaveric renal perfusion fluid

Received: 14 June 2003
Revised: 2 August 2004
Accepted: 9 September 2004
Published online: 24 November 2004
© Springer-Verlag 2004

S. J. Wakelin (✉) · J. Casey
J. L. R. Forsythe
Renal Transplant Unit, Royal Infirmary,
51 Little France Crescent,
Edinburgh, EH16 4SA, UK
E-mail: Sonia.Wakelin@btinternet.com
Tel.: +44-131-2421715
Fax: +44-131-2421739

A. Robertson
Renal Transplant Unit, Royal Melbourne Hospital, Parkville, Melbourne, Australia

P. Friend
Oxford Transplant Centre, The Churchill Hospital, Old Road, Headington Oxford, OX3 7LJ, UK

B. C. Jaques
Liver Transplant Unit, Freeman Hospital, Freeman Road, High Heaton Newcastle Upon Tyne, Tyne and Wear, NE7 7DN, UK

H. Yorke · S. P. Rigden
Paediatric Renal Unit, Guy's Hospital, St Thomas Street, London, SE1 9RT, UK

X. F. S. Emmanuel · L. Pareja-Cebrian
Department of Medical Microbiology, Royal Infirmary, 51 Little France Crescent, Edinburgh, EH16 4SA, UK

P. J. Morris
Nuffield Department of Surgery
University of Oxford,
John Radcliffe Hospital,
Headington Oxford,
OX3 9DU, UK

Abstract Infections represent a significant risk in the postoperative transplant recipient. The perfusion fluid used to perfuse and preserve the kidneys prior to transplantation represents a potential medium in which organisms can grow. The aim of this study was to determine the incidence and clinical relevance of bacterial contamination of perfusion fluid. A total of 4 centres participated in the study and 269 perfusion fluid samples were taken for microbiological analysis. Organisms were isolated from 38 out of 218 (17.4%) perfusion fluid samples taken prior to allograft implantation and 23 out of 51 (45%) samples taken at procurement. Low virulence organisms predominated although *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* were also isolated. Although infective complications were not seen in the allograft recipients, given the frequency with which contamination occurs and the variation in unit antibiotic protocols, we recommend the routine culturing of perfusion fluid to ensure that any potentially significant organisms are identified and treated appropriately.

Keywords Renal transplantation · Contamination · Perfusion fluid

Introduction

Infections constitute a significant cause of postoperative morbidity. Renal allograft recipients represent a group of patients at particularly high risk of this complication not least because the immunosuppressive drugs used to promote allograft survival also lower resistance to infection [1]. Infections in the renal recipient may originate from a number of sources including the transmission of organisms from the donor [2]. Serious consequences may result, including wound infections, disruption of the vascular anastomoses requiring transplant nephrectomy [3, 4, 5, 6] and systemic sepsis [7].

Contamination of the kidney with infective organisms may occur at a number of stages during the process of cadaveric transplantation, particularly since the multi-step retrieval process occurs at a centre some distance from the transplanting centre. The fluid used to perfuse the kidney following donor nephrectomy and prior to packing does not, in most instances, contain antibiotics and thus represents a potential medium in which contaminating organisms can grow. This same perfusion fluid is used as a preservation medium for packing, storage and transportation to the recipient centre where implantation occurs, often many hours later. It is generally accepted that the perfusion fluid is sterile. There have, however, been a few reports in the literature over the last 25 years to suggest that in samples of perfusion fluid taken prior to transplantation, contaminating organisms may be present. Contamination rates of 7–24% have been reported [8, 9, 10, 11] and organisms isolated have included both Gram positive and negative organisms. The origin of the organisms is not always clear. Transfer from contaminated surfaces, direct inoculation and importantly airborne transmission represent mechanisms by which bacterial contamination of the perfusion fluid may occur with relative ease.

In the past, many transplant centres routinely collected samples of perfusion fluid from the slush fluid surrounding the kidney prior to transplantation. This is a practice carried out now by only a few centres across the UK. Early pilot data from the Oxford Transplant Unit suggested that a significant number of contaminating organisms may be present in samples of perfusion fluid taken just prior to transplantation. Over a 1 year sampling period, 39 samples had been taken from renal perfusion fluid just prior to implantation of the allograft: 7 samples (18%) were positive for contaminating organisms which included *Candida*, *Pseudomonas aeruginosa* and coagulase negative staphylococci. A multicentre study was therefore set up to evaluate the incidence of contamination on a wider scale. Four renal transplant units across the UK contributed data to the study—Oxford, Edinburgh, Newcastle and Guys Hospital, London. The object was to determine the incidence of contamination in pre-implantation samples of perfusion fluid and to assess the contribution of the donor and the procurement process to levels of contamination by evaluating samples of perfusion fluid, peritoneal swabs and blood cultures from a population of organ donors.

Materials and methods

All four transplant centres contributed to the pre-implantation data-set of perfusion fluid samples (collected over the period 1999–2002) and the procurement samples were collected by the Edinburgh transplant team during multi-organ retrievals.

Procurement samples

Following procurement and before the packing of the kidneys for transport, two samples of perfusion fluid were taken from the fluid immediately surrounding the kidneys. One sample was introduced into a sterile universal container for later direct plating. The second sample was delivered into blood culture bottles under aseptic conditions as for routine blood culture analysis and both samples were transported back to the retrieving centre for microbiological processing. In addition, where there was a clinical indication, such as the possibility of donor sepsis, peritoneal swabs and blood cultures (if not already taken during the patient's admission) were also taken from the donor for microbiological analysis. The peritoneal swabs were taken during the initial laparotomy, carried out prior to any formal dissection being performed in the assessment of the patient's suitability for organ donation. Blood cultures, where appropriate, were taken prior to the start of the retrieval process. The results from the perfusion fluid cultures, peritoneal swabs and blood cultures were then retrieved from the microbiological database in Edinburgh.

Pre-implantation samples

Immediately prior to the back-table dissection of the kidney, two samples of perfusion fluid were taken from the bag containing the kidney and, as for the procurement samples, one sample was retained in a universal container for later direct plating and the second sample was delivered into blood culture bottles for broth culturing. These samples were sent to the local microbiology department for analysis. This microbiological processing involved both direct primary and enrichment

broth cultures to dilute out the effect of any perioperative antibiotics given to the donor or into the perfusion fluid prior to donation. The results from the perfusion fluid samples were then retrieved from the microbiology databases of the individual hospitals.

Recipient follow-up

The finding of a perfusion fluid sample positive for any form of growth was followed by a review of the recipient's medical records to determine whether any clinical sequelae arose as a direct result of the contaminating organism.

Review of antibiotic protocols

The perioperative recipient antibiotic regimens used by the UK renal transplant centres were reviewed by contacting the renal transplant unit by telephone and documenting the most commonly used antibiotic regimen.

Results

Together, the 4 transplant units participating in this study performed 307 renal transplants in 2001, cadaveric allografts constituted 78% (private correspondence, UK Transplant): 218 pre-transplant perfusion fluid samples were available for analysis as were 51 perfusion fluid samples taken at the time of organ procurement. In addition, 36 donor peritoneal swabs and 34 blood culture results were available.

Implantation samples

Of the 218 perfusion fluid samples taken during the back-table dissection prior to implantation, 38 (17.4%) were found to be contaminated with 1 or more organisms. The organisms isolated together with the frequency with which they occurred are demonstrated in Table 1.

Table 1 Organisms isolated and the frequency with which they occurred in samples of perfusion fluid taken prior to allograft implantation

Organism cultured	Frequency of occurrence
Coagulase negative Staphylococcus	27
Yeasts	6
Stenotrophomonas maltophilia	1
Escherichia coli	3
Pseudomonas aeruginosa	3
Methicillin-resistant *Staphylococcus aureus*	1

In none of the 38 cases did clinical sequelae of an infective nature develop as a consequence of the contaminating organism. In 3 of the 38 cases, however, the transplanting centre modified their pre-existing antibiotic protocol to ensure effective cover against the organisms, namely 2 cases of *Pseudomonas aeruginosa* and 1 case of methicillin-resistant *Staphylococcus aureus* (MRSA).

Procurement samples

A total of 51 samples of perfusion fluid were collected at the time of kidney procurement and of these, 23 samples (45%) were found to be contaminated with 1 or more organisms. The organisms isolated from the perfusion fluid are listed in Table 2 together with the frequencies with which they occurred. For 17 of the 23 positive perfusion fluid samples, peritoneal swab results were also available. In all but 1 case, the peritoneal swab results were negative. Of the 23 positive perfusion fluid results, 13 also had blood culture results available and of these 13, 12 were negative. The single positive blood culture result originated from the same donor as the positive peritoneal swab. All samples taken from this single donor (perfusion fluid, peritoneal swab and blood culture) demonstrated coagulase negative staphylococci.

Of the 23 positive perfusion fluid samples taken at the time of organ procurement, 9 samples were from kidneys subsequently transplanted by the retrieving centre. Of these 9 samples 8 demonstrated coagulase negative staphylococci and in a further case *Acinetobacter* was isolated. Clinically apparent infection due to these organisms was not seen in the recipients of these 8 grafts.

Recipient antibiotic protocols

All centres involved in the study routinely give perioperative broad-spectrum antibiotics to the cadaveric kidney recipients. We have reviewed the protocols used by 28 renal transplant units across the UK and the results are summarised in Tables 3 and 4. Most units gave a single or the first dose of prophylactic broad-spectrum

Table 2 Organisms isolated and the frequency with which they occurred in samples of perfusion fluid taken at organ retrieval

Organism cultured	Frequency of occurrence
Coagulase negative *Staphylococcus*	19
Diphtherioids	2
Acinetobacter	2
Yeasts	1
Escherichia coli	1
Pseudomonas aeruginosa	1

Table 3 Summary of the perioperative antibiotic regimens used by UK transplant centres for renal allograft recipients

Antibiotic regimen	Number of centres
Cephalosporin-based regimen	13
Co-amoxiclav-based regimen	8
Flucloxacillin ± Aztreonam	3
Tazocin	1
No routine broad-spectrum antibiotics	1
No routine broad-spectrum antibiotics but routine co-trimoxazole for PCP prophylaxis	2

Table 4 Length of course of perioperative antibiotics used by UK transplant centres in renal allograft recipients

Length of course	Number of centres
Induction only	13
Up to 24 h	4
24–48 h	3
48 h–1 week	3
Longer term i.e. > 1 week	2

antibiotics at anaesthetic induction or pre-operatively and a number of units continued antibiotics into the postoperative period. Furthermore, most units also gave co-trimoxazole for prophylaxis against *Pneumocystis pneumonia* even if they did not otherwise give broad-spectrum perioperative cover.

Discussion

Several important messages emerge from the findings of this study. Some 30 years on from the earliest studies investigating the incidence of contamination, organisms are still isolated from the solutions used to perfuse and transport cadaveric kidneys. Previous studies have documented the incidence of perfusion fluid contamination in samples taken just prior to transplantation. To our knowledge however, we are the first to also specifically evaluate the incidence of contamination in perfusion fluid samples taken at the time of organ procurement. Samples of perfusion fluid withdrawn directly from the giving set are sterile. However, our finding that 23 out of 51 (45%) of the procurement perfusion fluid samples were contaminated suggests that the procurement process may be a significant source of contaminating organisms. Infection in the donor, however, does not appear to be the source of the contaminants in the most part as evidenced by the finding of only one positive donor blood culture and one positive peritoneal swab culture (both from the same donor) from the set of 23 positive perfusion fluid culture results. This is in keeping with earlier reports [10] suggesting that careful donor screening has a negligible influence on the incidence of perfusion fluid contamination. This finding is not surprising given that intravenous antibiotics, namely gentamicin, benzylpenicillin and cefotaxime, are routinely administered to the donors prior to the start of the procurement process. The effect of these antibiotics, together with further residual cover provided by antibiotics given to the donor during their admission, will inevitably play a role in the number and type of organisms isolated from sampling during the procurement. In particular, renally excreted antibiotics administered to the donor may persist in the renal tissue following nephrectomy, exerting some antibiotic effect within the kidney and diffusing into the perfusion fluid surrounding it. This may help to explain in part why the incidence of contamination prior to implantation is lower (17.4%) than that seen at the time of procurement (45%). Some caution in interpreting this finding must be observed, however, not least because procurement and pretransplant perfusion fluid samples for any one donor-recipient set are not available. Furthermore, the results presented here represent only a small sample population collected by one retrieving centre which may or may not be representative of the situation elsewhere.

The finding in this study that 17.4% of pre-implantation samples are contaminated is consistent with the results of some of these earlier studies although significantly higher than reported by most other authors (see Table 5). This apparent increase may simply reflect advances in microbiological detection. Centres employing a combination of direct plating and broth culture techniques, as in the present study, to identify contaminating organisms will yield more "positive" cultures than those using direct plating methodologies alone. Indeed, the omission from analysis of organisms isolated by broth culture in this study's "procurement-alone" samples would drop the incidence of contaminating organisms from 45% to 20%, indicating that, in most samples, the number of organisms were so small that detection by solid agar cultures was not possible. The possibility exists, however, that the incidence of positive cultures at best remains unchanged and at worst may have increased over the last quarter of a century. Possible sources of contaminating organisms include handling of the organs and exposure to contaminants during the retrieval process, an issue of particular significance given that multi-organ retrievals, rather than kidney-alone procedures, are now more commonplace. It might then be speculated that longer operations would be associated with a higher incidence of perfusion fluid contamination. Interestingly, on review of the donor operations, there was not a marked difference between the lengths of the operations in those donors with positive cultures compared to those with negative cultures. Of the 51 donor operations (from initial laparotomy to start of

Table 5 Summary of reports in the literature investigating contamination of renal allografts correlated with clinical outcome

Authors	No. of positive samples	Incidence of clinically important sequelae	Organism responsible and outcome
Spees et al. [4]	23/177 (13%)	4	*Ps. aeruginosa*: arterial anastomosis complications (2 cases) *Candida albicans*: arterial anastomosis complications (2 cases)
Weber et al. [5]	10	6	3 Graft losses 3 Deaths
McCoy et al. [7]	14/81 (17.3%)	5	*Ps. aeruginosa*: death (1 case), loss of allograft (2 cases) *E. Coli*: UTI *E. Coli*: Blood cultures/wound infection
Anderson et al. [8]	19/83 (22.9%)	1	*Candida albicans*: wound infection
Benoit et al. [9]	35/500 (7%)	0	
Mora et al. [10]	48/446 (10.7%)	2	*E. coli*: wound infection *S. aureus:* wound infection
Bijnen et al. [11]	39/200 (19.5%)	9	*S. aureus*: perinephric abscess-nephrectomy (1 case), mycotic aneurysm-nephrectomy (1 case), isolated from wound/wound infection (2 cases) *S. epidermidis:* isolated from wound/wound infection (4 cases) *Ps. aeruginosa*: sepsis
Kyriakides et al. [12]	25/107 (23.3%)	0	
Häyry et al. [18]	3/84 (4%)	1	*E. Coli*: UTI
Wilson et al. [13]	2	2	*Ps aeruginosa:* Arterial anastomosis complications, nephrectomy (1 case), death (1 case)
Bore et al. [19]	1/40 (2.5%)	1	*Bacteroides*: UTI, wound infection
Majeski et al. [20]	22/514 (4.2%)	0	

nephrectomy), those with positive perfusion fluid cultures took 182 min (range 117–320) compared to 175 min (range 120–250 min) for those with negative perfusion fluid cultures. These results would suggest that the length of the operation alone is not sufficient to predict the incidence of positive perfusion fluid cultures. Other factors worthy of consideration include the possible direct contamination of the preservation/transport medium and the possibility that contaminants may be introduced during the microbiological processing procedure itself.

The current observation that the majority of organisms isolated were coagulase negative staphylococci is consistent with other studies [7, 8, 10, 11, 12]. These low virulence organisms are unlikely to be significantly pathogenic even in the immunosuppressed patient and indeed, in the literature, there have been few reports of such low virulence organisms giving rise to significant pathology. The high incidence of coagulase negative staphylococci in the procurement samples lends further support to breaches of sterility during the harvesting procedure being responsible for the contaminants seen, as previously suggested [4, 11]. This may be contributed to by a number of factors. In this study, all of the kidney retrievals were part of a multi-organ retrieval process. In 51 cases, the liver was retrieved together with, in many cases, the heart and lungs and, in 1 case, the pancreas also. Such a multi-step, multi-specialty approach has the inevitable consequence of prolonging what is already a lengthy procedure. Invariably, the kidneys are the last organs to be retrieved, by which time organisms from a variety of sources (notably from the air, skin and bowel) have had time to colonise the exposed peritoneal cavity and its contents. Consistent with this is the finding that all but one of the peritoneal swabs were negative despite positive perfusion fluid culture results. The peritoneal swabs were all taken early in the retrieval process, at a time when donor antibiotics are likely to be still circulating and when the abdomen has been open for only a short time. Whether swabs taken from the abdominal cavity are still negative just prior to the kidneys being removed at the end of the procurement is unknown.

Whilst low virulence organisms may give little cause for concern, it is possible that high virulence and/or drug-resistant organisms may also contaminate the perfusion fluid. The isolation of *Staphylococcus aureus* and *Pseudomonas aeruginosa* in the current study is potentially significant as all have previously been associated with sepsis (local and general), anastomotic failure and graft loss [3, 6, 8, 13, 14]. Furthermore, *Escherichia coli* was isolated from one procurement and three pretransplant samples. This may reflect direct breach of the bowel as may occur during the opening of the gallbladder or division of the bile duct. It may also result from the translocation of gut-derived bacteria from the gut to the bloodstream and thereon to other organs as previously reported [15]. Interestingly, in 1979 Weber et al. reported on the effect of transplanting canine kidneys which had previously been perfused with *Escherichia coli*-contaminated perfusion fluid [5]. The untreated recipients died from either sepsis or anastomotic rupture whereas antibiotic prophylaxis was found to be protective. However, when antibiotics were commenced 1 day postoperatively, 60% of the canine recipients died.

That serious infection-related sequelae did not develop in any of the patients in the current study is in keeping with a number of previous studies [8, 9, 10] and probably reflects the administration of broad-spectrum antibiotics to renal recipients by the centres involved in the study. Nevertheless, organisms such as MRSA and resistant coliforms may evade the perioperative antibiotics given to both the donor and recipient and potentially will lead to serious complications. Serious infection did not result from contamination with MRSA and *Pseudomonas aeruginosa* in this study. However, in each case, the recipient centre was alerted to the results of the perfusion fluid culture and the recipient antibiotic protocol adjusted accordingly to ensure that potentially serious infective complications were avoided.

Conclusion

The central message emerging from this study is that the contamination of perfusion fluid occurs frequently and that this contamination is most likely to occur during the procurement process. Given that this is the case lends us to speculate whether changes should be introduced to the procurement process. In particular, procurement is often a lengthy process and organisms can gain access to the peritoneal cavity with relative ease.

Measures to reduce contamination should be optimised with attention to a vigorous aseptic technique and indeed the use of incision drapes as used for a number of other lengthy or high risk operations may be justified [16, 17]. Furthermore, the frequency with which contamination occurs justifies routine culturing of the perfusion fluid to ensure that potentially high virulence organisms are not missed and treatment is instituted appropriately. The fact that culture results are invariably not available in time for transplantation further emphasises the importance of perioperative antibiotic prophylaxis, both for the donor and for the recipient. Most, but not all, renal transplant units give recipients perioperative antibiotics routinely. However, protocols vary both in terms of the agents used and the duration of the course. Antibiotics are chosen to minimise the risk of infection acquired from many endogenous and exogenous sources, not just the transplanted kidney, in the perioperative period. In this regard, broad-spectrum betalactam antibiotics are the first line choice for most units and, in view of the data presented in this study, these should probably remain the first choice of antibiotic prophylaxis, not least because antibiotics active against the more virulent and resistant pathogens, such as vancomycin and gentamicin, are precluded by potential nephrotoxicity. In view of the variation in protocols and the possibility that virulent organisms requiring extended or modified antibiotic regimens may be necessary, alerting recipient centres to the results of procurement perfusion fluid samples would be useful to ensure that potentially serious infective complications are prevented.

Acknowledgements We would like to thank the transplant coordinators and theatre staff from the participating hospitals for their cooperation in collecting perfusion fluid samples.

References

1. Rubin RH, Tolkoff-Rubin NE. Infection: The new problem. Transplant Proc 1989; 21:1440.
2. Gottesdiener KM. Transplanted infections: donor-to-host transmission with the allograft. Ann Intern Med 1989; 110:1001.
3. Nelson PW, Delmonico FL, Tolkoff-Rubin NE et al. Unsuspected donor pseudomonas infection causing arterial disruption after renal transplantation. Transplantation 1984; 37:313.
4. Spees EK, Light JA, Oakes DD, Reinmuth B. Experiences with cadaver renal allograft contamination before transplantation. Br J Surg 1982; 69:482.
5. Weber TR, Freier DT, Turcotte JG. Transplantation of infected kidneys. Transplantation 1979; 27:63.
6. Harrington JC, Bradley JW, Zalneraitis B, Cho SI. Relevance of urine cultures in the evaluation of potential cadaver kidney donors. Transplant Proc 1984; 16:29.
7. McCoy GC, Loening S, Braun WE, Magnusson MO, Banowsky LH, McHenry MC. The fate of cadaver renal allografts contaminated before transplantation. Transplantation 1975; 20:467.
8. Anderson CB, Haid SD, Hruska KA, Etheredge EA. Significance of microbial contamination of stored cadaver kidneys. Arch Surg 1978; 113:269.
9. Benoit G, Tiguert R, Bensadoun H et al. Incidence of transport medium contamination in cadaver kidney procurement. Transplant Proc 1988; 20:895.
10. Mora M, Wilms H, Kirste G. Significance of bacterial contamination of cadaver donor renal allografts before transplantation. Transplant Proc 1991; 23:2648.
11. Bijnen AB, Weimar W, Dik P, Oberop H, Jeekel J. The hazard of transplanting contaminated kidneys. Transplant Proc 1984; 16:27.
12. Kyriakides GK, Simmons RL, Najarian JS. Wound infections in renal transplant wounds: pathogenetic and prognostic factors. Ann Surg 1975; 182:770.
13. Wilson CH, Gregory RT, Wheeler JR, Hurwitz RL, Vansant JH, Thomas FT. Complications of end-to-side renal artery anastomosis in renal transplant infection. Vasc Surg 1979; 13:207.
14. Doig RL, Boyd PJR, Eykyn S. *Staphylococcus aureus* transmitted in transplanted kidneys. Lancet 1975; 9:243.

15. Redan JA, Rush BJ, Lysz TW, Smith S, Machiedo GW. Organ distribution of gut-derived bacteria caused by bowel manipulation or ischaemia. Am J Surg 1990; 159:85.
16. French ML, Eitzen HE, Ritter MA. The plastic surgical adhesive drape: an evaluation of its efficacy as a microbial barrier. Ann Surg 1976; 184:46.
17. Whyte W, Hambraeus A, Laurell G, Hoborn J. The relative importance of routes and sources of wound contamination during general surgery. J Hosp Infect 1991; 18:93.
18. Häyry P, Renkonen O-V. Frequency and fate of human renal allografts contaminated prior to transplantation. Surgery 1979; 85:404.
19. Bore PJ, Basu PK, Rudge CJ, Sells RA. Contaminated renal allografts. Arch Surg 1980; 115:755.
20. Majeski JA, Alexander JW, First MR, Munda R, Fidler JP, Craycraft TK. Transplantation of microbially contaminated cadaver kidneys. Arch Surg 1982; 117:221.