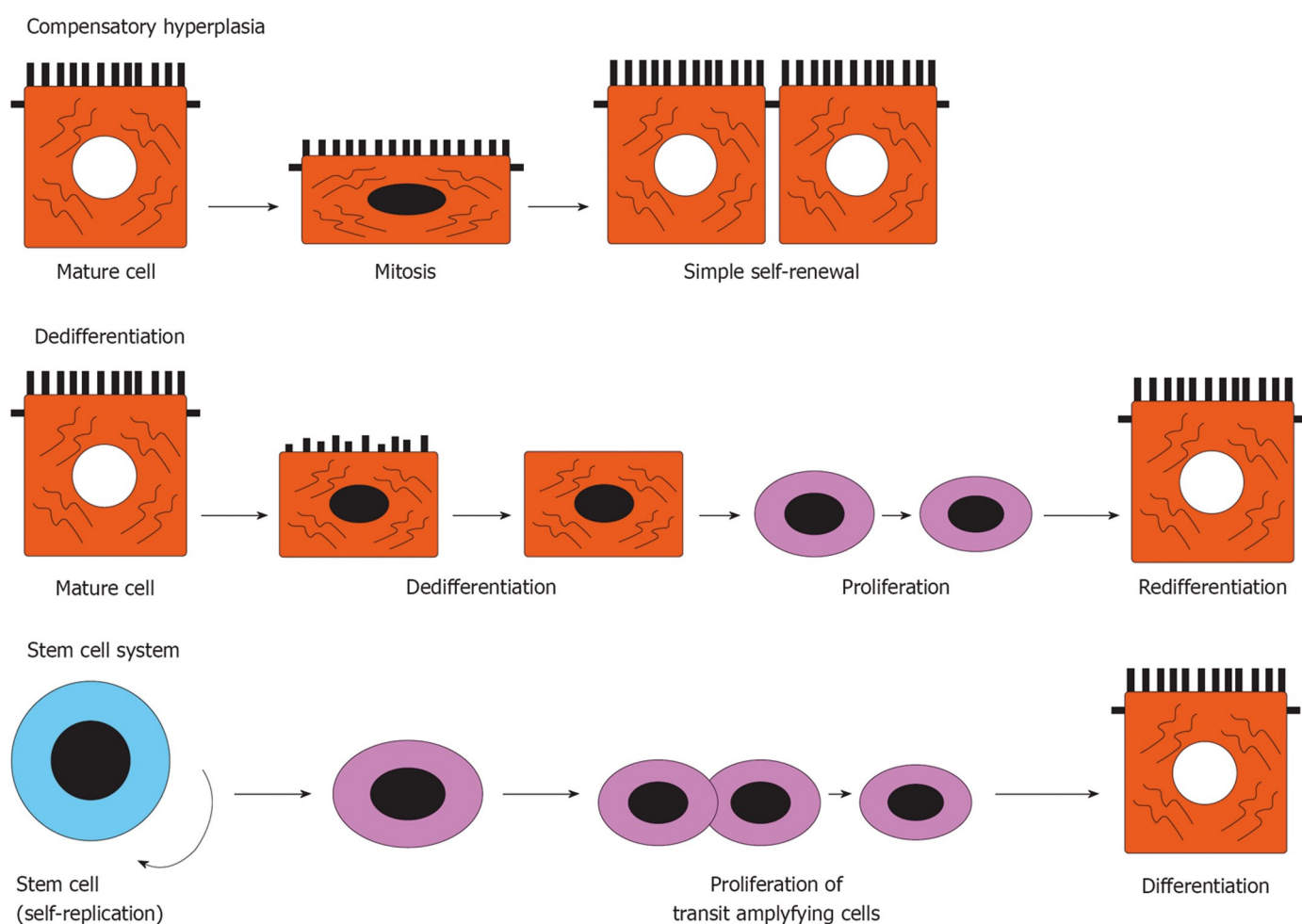


# World Journal of *Nephrology*

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## Different modes of renal proximal tubule regeneration in health and disease

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### Abstract

Tissues are equipped with reasonable strategies for repair and regeneration and the renal proximal tubule (PT) is no exception. New information has become available on the mode of PT regeneration in mammals. Unlike the intestinal epithelium with a high rate of turnover maintained by the stem cell system, the kidney has low turnover under normal physiological conditions. The PT seems to be maintained physiologically by hyperplasia, a regenerating system with self-renewal of mature tubular cells. This mode of regeneration is advantageous for effective replenishment of randomly isolated and eliminated tubular cells by self-renewal of adjacent cells. On the other hand, it has been suggested that dedifferentiation of mature tubular cells plays a role in regeneration after acute kidney injury. Recent studies employing genetic labeling and DNA-labeling techniques have confirmed that the proliferation of preexisting injured mature tubular cells contributes mainly to PT regeneration in ischemic reperfusion injury. This mode of regeneration is beneficial with regard to the rapid reparation of focally injured tubules often induced by ischemic reperfusion injury. What happens, however, when the PT is homogeneously injured with almost no remaining surviving cells? Is the PT equipped with an-

other backup regeneration system, e.g., the stem cell system? Is it possible that certain types of renal injuries evoke a stem cell response whereas others do not? This review focuses on all three possible modes of tissue regeneration (compensatory hyperplasia, dedifferentiation and stem cell system) in mammals and their involvement in PT regeneration in health and disease.

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**Key words:** Proximal tubule; Regeneration; Compensatory hyperplasia; Dedifferentiation; Stem cell; Progenitor cell

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### MECHANISMS OF RENAL TUBULE REGENERATION

There are three mechanisms of tissue regeneration in vertebrates<sup>[1]</sup>, as illustrated in Figure 1: (1) compensatory hyperplasia, where mitosis of cells occurs during the differentiation state (e.g., liver, pancreas<sup>[2,3]</sup>); (2) dedifferentiation of mature cells where stem-like cells are raised by the dedifferentiation of differentiated cells (e.g., myofibers, lens<sup>[4-6]</sup>); and (3) activation of undifferentiated adult stem cells sequestered during tissue development, where the stem cell divides to produce one daughter cell committed to specific lineage differentiation, while another daughter cell is renewed as a stem cell (e.g., skin epidermis, hair follicles, epithelium of the digestive tract<sup>[7-9]</sup>). In general,

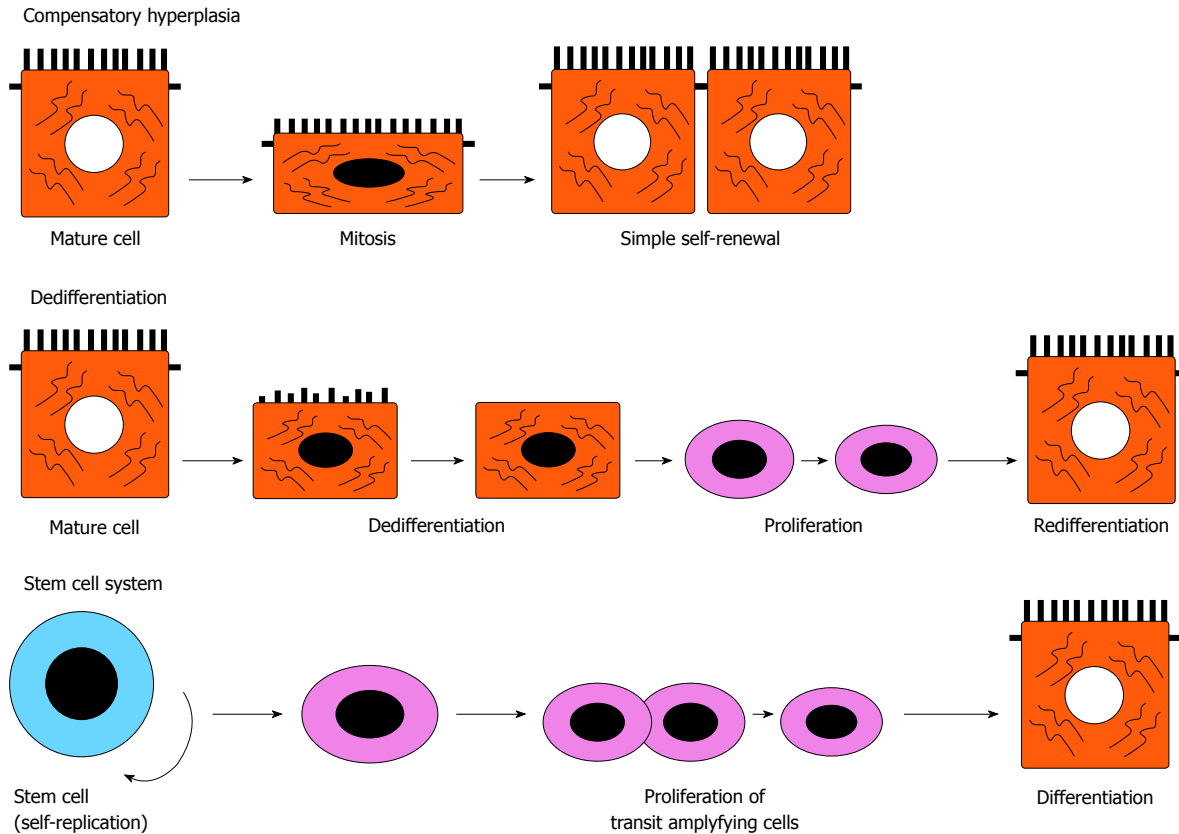


Figure 1 Three mechanisms of tissue regeneration in vertebrates.

epithelial tissues equipped with a stem cell system may use such a system to maintain cell turnover under both physiological and pathological conditions.

Like other organs, the kidney is also known to regenerate completely in lower vertebrates, such as teleost fish, the skate, elasmobranch fish and zebrafish, during which the entire nephron regenerates following injury or partial removal of the kidney<sup>[10-13]</sup>. The source of the new nephrons is a population of stem cells that exist in the special nephrogenic zone<sup>[14]</sup>. On the other hand, the regenerative capacity of the mammalian kidney is limited compared to that of lower vertebrates. However, it is well known that even in mammalian kidney, renal tubules have regenerative capacity, especially after acute kidney injury such as acute tubular necrosis<sup>[15]</sup>, through yet unknown regeneration mechanisms.

Recent interest in stem cell-based therapy led many investigators to study the source of regenerating tubular cells and the role of stem cells after acute kidney injury in mammalian kidneys<sup>[16-33]</sup>. However, accumulating evidence indicated that the main source of regenerating cells is resident kidney cells, not bone marrow-derived cells (e.g., hematopoietic stem cells, mesenchymal stromal cells and endothelial progenitor cells)<sup>[34,35]</sup>. Moreover, resident kidney cells, rather than bone marrow-derived cells, were the main contributors to the tubular repair in the ischemic reperfusion injury model<sup>[34,35]</sup>. However, recent reports have demonstrated that the bone marrow-derived mesenchymal stromal cells play renoprotective roles in

tubular repair and/or recovery by producing various humoral factors not at a cell basis<sup>[36-41]</sup>. More recent studies of rats with ischemic reperfusion injury indicated that the source of tubular regenerating cells that contribute to the repair of renal tubules is limited to resident (pre-existing) tubular cells<sup>[42]</sup> and that the intratubular stem cell system is not involved in tubular regeneration<sup>[43]</sup>. However, it is too early to conclude that there is no intratubular stem cell system in mammalian kidneys.

In this review, we discuss the modes of regeneration of proximal tubules (PTs) and their implication in health and disease.

## REGENERATION OF PT CELL UNDER PHYSIOLOGICAL CONDITIONS

The physiological processes of renal tubular cell turnover play an important role in the maintenance of normal tissue function and architecture, which is achieved by a dynamic balance between the rate of cell elimination and the rate of cell proliferation. In 1959, McCreight and Sulkin counted the number of mitotic figures and calculated the proliferation index of PT cells to be 0.1% in the normal rat kidney, and hence concluded that the kidney has a low cell turnover under physiological conditions<sup>[44]</sup>. In another study, the estimated proliferation indexes of PT cells stained for S-phase markers in paraffin sections (percentages of cells positive for the proliferation



cell nuclear antigen and Ki67 antibodies) were 0.22 and 0.24, respectively<sup>[45]</sup>. In a study from our laboratory<sup>[46]</sup>, about 40% of S3 segment of PT cells were labeled by the S-phase marker, bromodeoxyuridine (BrdU), when adult normal rats were treated with BrdU by osmotic mini-pump for 2 wk. Since the number of eliminated cells should be substituted by the same number of newly regenerating tubular cells to maintain normal tissue function and architecture, at most about 20% of tubular cells in the S3 segment should divide into two cells during a 2-wk period if they divided only once during the period. This suggests that the proliferation index 1 h after BrdU administration may be about 0.06% in the S3 segment in adult rats. Considered together, the above studies indicate that PT cell proliferation or turnover is slow in adults.

Recently, Voetseder *et al*<sup>[47,48]</sup> concluded that the S3 segment of PT is maintained by a physiological regenerating system with self-renewal of mature tubular cells. Their conclusion was based on the finding of numerous cells with proliferative potency (retaining positivity for the proliferation marker, BrdU). Both cycling and non-cycling cells remained morphologically and phenotypically fully differentiated PT cells and cycling cells did not show the characteristics of transit amplifying cells<sup>[47,48]</sup>, which can expand the number of cells by rapid cycling after division from stem cell<sup>[49]</sup>. This does not support the notion that stem cell system ensures turnover of tubular cells under physiological conditions.

In our study, we also found that BrdU+ proliferating PT cells in the S3 segment of normal rat nephron exhibited a mature PT phenotype, such as staining for megalin, aquaporin 1 and Na<sup>+</sup>K<sup>+</sup>-ATPase, and also maintained a mature PT ultrastructure<sup>[50]</sup>. However, these cells did not express vimentin, a marker of mesenchyme or dedifferentiated PT cells<sup>[51]</sup>. These findings suggest that normal PT cells can undergo cell division without dedifferentiation.

In the liver, cell marking studies indicated that during normal liver turnover and after partial hepatectomy, hepatocytes are replaced by compensatory hyperplasia of existing hepatocytes<sup>[52]</sup>. Interestingly, mature hepatocytes can replicate during normal liver growth, but the newly formed cells do not migrate<sup>[53]</sup>. Since cells generated by simple self-renewal through compensatory hyperplasia cannot migrate, it is unlikely that these newly regenerating cells can repair largely damaged areas in a mode of simple self-renewal. This may also be the case in renal PT cells. Thus, compensatory hyperplasia provides effective replenishment of randomly eliminated tubular cells by self-renewal of adjacent cells under physiological conditions. However, other modes of regeneration are required under pathological conditions.

## REPAIR AFTER ACUTE TUBULAR INJURY

The mammalian kidney is classically regarded as an organ that cannot truly regenerate. In the past, it was thought that acutely injured tubular cells slough off the tubular

basement membrane and that the surviving tubular cells undergo migration, dedifferentiation, proliferation and redifferentiation to reline the injured tubules<sup>[54,55]</sup>. Voetseder *et al*<sup>[48]</sup> reported that in rats treated with potent proliferative agents (lead acetate injection<sup>[56]</sup>), PT proliferation did not require stem cells but involved proliferation of preexisting differentiated tubular cells. Interestingly, they concluded that PT cells were probably not quiescent but resting in G1-phase of the cell cycle, i.e., they could divide rapidly in response to injury. Recently, Humphreys *et al*<sup>[42]</sup> used sophisticated technology to demonstrate that tubular cells *per se* are the source of regenerating tubular cells. They prepared transgenic mouse strains in which all cells involved in nephrogenesis were lineage labeled. Using these mice, they tested whether any endogenous cell type entered the tubules and contributed in the repair process of tubules after ischemic reperfusion injury. Their data showed a lack of non-tubular cells in renal tubules before as well as after ischemic reperfusion injury. However, this finding neither excludes the possibility of the existence of intratubular stem cells/progenitor cells nor the proliferation of preexisting differentiated cells within the tubules.

More recently, Humphreys *et al*<sup>[43]</sup> used a DNA analog-labeled approach to chase multiple rounds of cell divisions in mice after ischemic reperfusion injury and demonstrated that PT cell division in the cortex and outer medulla occurred predominantly in injured and dedifferentiated PT cells. PT cell injury was confirmed by Kim-1 expression<sup>[57]</sup> and dedifferentiated cells by both PAX-2 expression<sup>[58]</sup> and reduction in Na<sup>+</sup>K<sup>+</sup>-ATPase expression in proliferating cells labeled with DNA analog. A stochastic kinetics of proliferation was identified, probably reflecting simple self-duplication rather than selective activation of an intratubular progenitor population. The findings of Humphreys *et al*<sup>[43]</sup> strongly suggest that proliferation of preexisting differentiated cells within the tubules is the main event in PT regeneration in ischemic reperfusion injury.

We also examined the importance of dedifferentiation in the initiation of cell division of PT cells after acute PT injury induced by uranyl acetate (UA), a nephrotoxic agent<sup>[59]</sup>. High-dose UA induced severe PT injury of the S3 segment and the first proliferating PT cells showed loss of PT cell protein phenotype (megalin, aquaporin 1 and Na<sup>+</sup>K<sup>+</sup>-ATPase) but became positively stained for vimentin. In comparison, low-dose UA induced focal PT injury of the S3 segment, with the first proliferating PT cells still exhibiting the PT phenotype and not staining for vimentin. Subsequently, the proliferating PT cells showed loss of PT cell phenotype and expressed vimentin. Thus, similar to the changes seen under physiological conditions, the PT cells can enter the cell cycle without apparent dedifferentiation after low-dose UA-induced focal PT injury. However, dedifferentiation with vimentin expression may follow after initial cell division. Interestingly, continuously proliferating tubular cells tend to express vimentin unlike regenerating cells under physiologi-



cal conditions<sup>[54,55]</sup>. Since vimentin is a major intermediate filament protein and is associated with the development of migratory capacity<sup>[59,60]</sup>, it is conceivable that proliferating PT cells can acquire vimentin expression to undergo cell division more than once and to migrate to cover the denuded tubular basement membrane. This may not be the case in regenerating PT cells under physiological conditions.

Thus, dedifferentiation must be a beneficial mode of regeneration for rapid reparation of focal areas following focal injury of the tubule, such as after ischemic reperfusion injury<sup>[61]</sup>. However, questions remain on whether all PT cells possess the ability to enter the cell cycle and acquire dedifferentiation property (i.e., is a stem-like cell) and whether the insult of ischemic reperfusion injury is adequate to activate intratubular progenitor cells, if they do exist. It is also possible that certain forms of renal damage can evoke a stem cell response whereas others do not.

## DIFFERENT REPAIR PROCESSES OF PT AFTER ACUTE TUBULAR INJURY

The study of Oliver and colleagues<sup>[61]</sup> indicated that the main site of tubular injury following traumatic and toxic insults is PTs, based on histopathological examinations of cadaver kidneys in patients with severe fatal acute renal failure. They also found two types of tubular injuries. The first was nephrotoxic necrosis limited to that part of the nephron in the PT that is functionally concerned with the handling of poisons; the necrosis was homogeneous in that part of the nephron. The second type of lesion was disruption of the renal tubule due to focal cortical ischemia. It occurs at random among nephrons. The authors suggested that, in the kidney of any case of fatal acute renal failure arising under various clinical circumstances, these two types of lesions appear in varying proportions depending on the nature of the renal insult, whether toxic or circulatory or both. Thus, the number and distribution of surviving tubular cells after acute tubular injury must be highly variable among the different causes of acute tubular injury. It is also conceivable that different repair processes of tubules also occur under different pathological conditions.

In fact, we found two different modes of repair processes of PT after acute tubular injury induced even by the same nephrotoxic agent, low- or high-dose of UA in rats using the <sup>3</sup>H-thymidine pulse/chase approach<sup>[62]</sup> for the detection of early regenerating PT cells<sup>[63]</sup>. In these studies, low-dose UA (0.25 or 0.5 mg/kg) induced mild and focal PT depletion in S3 segment without significant increase in serum creatinine. Some of the surviving PT cells scattered in the proximal three quarters of the S3 segment became thymidine-incorporating (detected by grain on sections) early regenerating PT cells. They were increasingly found in the proximal three quarters of S3 and to a lesser extent in the distal S3 at day 7, and decreased in number by day 42. The number of label-

retaining PT cells increased in the entire S3 and the number of label-diluted PT cells was significantly increased, mainly in the proximal three quarters of S3, and both were decreased in parallel at day 42. Early regenerating cells maintained the differentiated phenotype initially then loss of the phenotype was noted shortly after the initial regeneration<sup>[50,63]</sup>. Taken together, the surviving PT cells contributed to the repair of focal PT injury, suggesting that dedifferentiated PT cells, derived from preexisting mature PT cells are responsible for focal repair of the S3 segment.

On the other hand, high-dose UA (1 or 5 mg/kg) induced a significant increase in serum creatinine and necrotic PT started to appear at the corticomedullary junction as early as day 2 after injection of UA, and then maximally spread in the entire S3 segment with almost complete PT depletion in three-quarters of the S3 segment with less PT depletion in the distal quarter of S3 by day 5<sup>[63,64]</sup>. The BrdU or thymidine-incorporating early regenerating cells were limited to the distal area of the S3 segment from days 2 to 3, remote from the initial site of damage, then upstream proliferation of PT cells occurred along the denuded tubular basement membrane, which was almost completed by day 7<sup>[63,64]</sup>. Thymidine-labeled PT cells were increasingly found in the entire S3 at day 7 during the repair phase. Label retaining PT cells were increased in the entire S3 and to a significantly greater extent in the distal S3. They were rapidly decreased in number in the proximal three quarters of S3 by day 21, but their number remained constant in the distal S3 until day 42. In contrast, the label-diluted PT cell population increased in the entire S3, although to a significantly lesser extent in the distal S3 at day 7, and their numbers decreased markedly in the entire S3 by day 42<sup>[63]</sup>. Early regenerating cells after high-dose UA insult seem to be the cellular source of regenerating tubules with high proliferative properties to repair the entire S3 segment with infrequent cycling after completion of the repair process of PT. Thus, we hypothesized that these cells might be slow cycling cells responsible for the repair of the entire S3. Next, we examined whether they could be designated the "target cells" and have intratubular progenitor-like properties.

## POSSIBLE EXISTENCE OF RENAL TUBULAR PROGENITOR-LIKE CELLS

No specific renal tubular stem/progenitor cell markers are currently available. Therefore, indirect markers of slow cell cycling properties (label retention) have so far been used to search for potential population of intratubular progenitor cells. These include transcription factors and cell surface expression markers. However, once the PT cells are injured *in vivo* or isolated into a culture system, they also express genes and proteins of earlier stages of development<sup>[65]</sup>, which makes it difficult to distinguish dedifferentiated tubular cells from mature differentiated tubular cells and intratubular progenitor cells. Therefore,

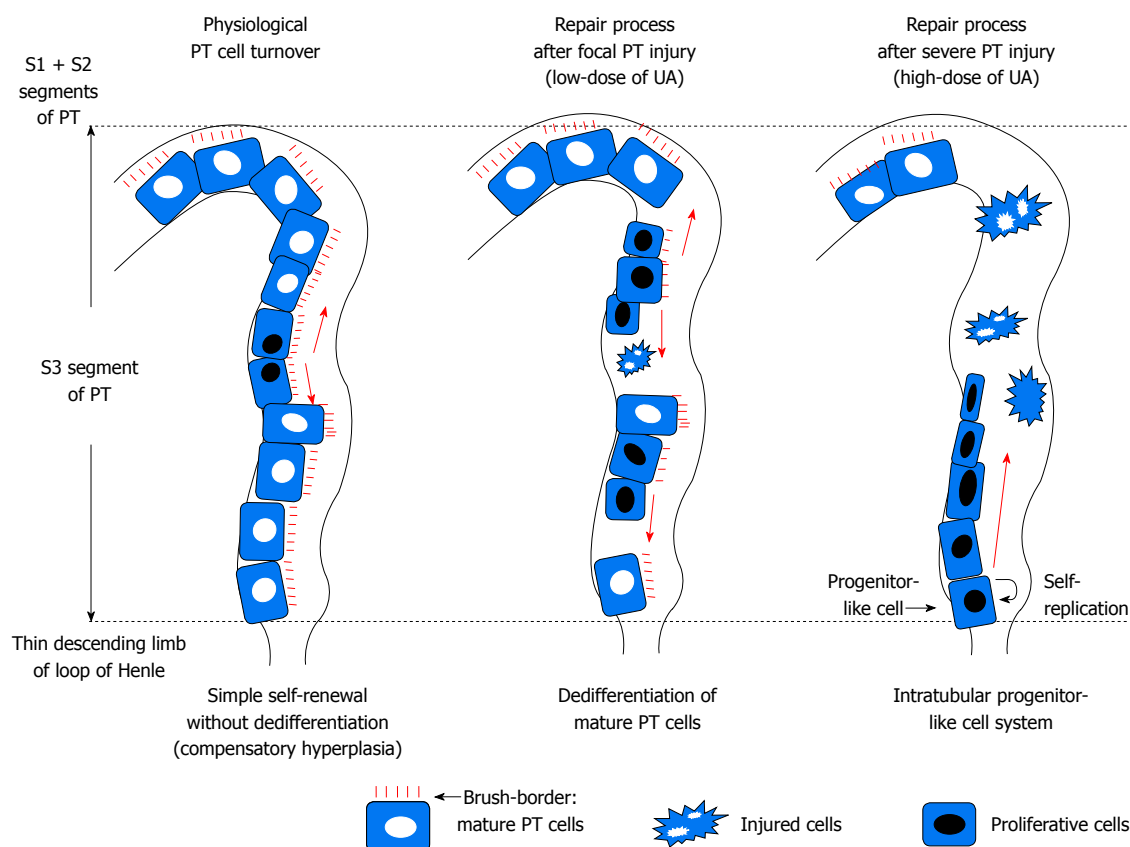


Figure 2 Three modes of regeneration of tubular cells in S3 segment of proximal tubule. PT: Proximal tubule.

at present there seems to be no reliable method to prove the existence of intratubular stem cells, both *in vivo* and *in vitro*.

To examine the specific properties of the early regenerating cells (designated as "target cells") in the S3 segment of rats with high-dose UA-induced acute tubular injury, we searched for possible cell features that could define progenitor-like cells *in vivo* among the different tubular cells in the S3 segment. Our studies yielded the following conclusions regarding target cell characteristics: (1) The target cells (i.e., thymidine-labeled cells) were persistently present in the distal area of the S3 up to and including week 40<sup>[66]</sup>, further suggesting slow-cycling cells; (2) About 60% of PT cells in the S3 segment were "thymidine-labeled cells" at day 7 after high-dose UA-induced acute renal failure<sup>[66]</sup>, suggesting that the majority of the regenerating cells in the S3 were newly synthesized following injury and originated from the target cells in the distal area of S3; (3) Some target cells re-proliferated after a second high-dose UA insult<sup>[66]</sup>; (4) The target cells were resistant to 5-fluorouracil (5-FU) *in vivo* and showed restoration of regenerative property after withdrawal of 5-FU and were also reactivated by the second UA insult<sup>[66]</sup>. Whereas there is substantial information on the response of hematopoietic stem cells to 5-FU<sup>[67]</sup>, there is little information on the response of epithelial stem/progenitor cells to 5-FU. Previous studies reported that 5-FU is cytotoxic to proliferating epithelial cells such as retinal pigment epithelial cells<sup>[68]</sup> and lens epithelial cells<sup>[69]</sup>. Thus,

the findings suggest that the target cells may be in some way unique with possible progenitor-like cell properties; (5) The target cells showed weak or no staining for all three markers of mature PT phenotype (megalin, aquaporin1 and Na<sup>+</sup>K<sup>+</sup>-ATPase) but became positive for a mesenchymal marker (vimentin)<sup>[50]</sup>. On the other hand, following acute tubular injury induced by low-dose UA, the initial proliferating PT cells divided while keeping the mature PT phenotype, but subsequently showed regression of this phenotype<sup>[50]</sup>. Unlike other PT cells, the target cells could undergo cell cycle progression without accumulation of heat shock protein 27<sup>[70]</sup>, which is thought to provide partial protection for PT cells against injury or death by acting as a molecular chaperone and thus promotes the stabilization, repair and/or disposal of denatured proteins<sup>[71]</sup>. The data showed that the PT cell phenotype at the time of initial cell division was different between the target cell and other tubular cells, suggesting that the target cells are probably unique; (6) The target cells exhibited morphological features of dedifferentiated/undifferentiated cells, such as smaller brush-border, large nuclei, fewer cytoplasmic organelles and spindle-like morphology<sup>[66]</sup>, compatible with the features of progenitor cells<sup>[72]</sup>. At present, there are no reports on the existence of morphologically and phenotypically unique cells (e.g., cells lacking brush-border or cells negative for markers of mature PT) among PTs based on histological examination under physiological conditions. However, progenitor cells usually exhibit spindle-like morphology

with small length; thus, it is difficult to detect them when they are sequestered and/or buried among other PT cells without proper labeling such as BrdU; (7) A proportion of the target cells were localized at the transition zone between PT and the thin descending limb of Henle<sup>[66]</sup>. The target cells might have a bipotential differentiation because they exist at a unique location where cells can differentiate into both PT cells and thin descending limb of Henle; and (8) Under physiological conditions, most target cells did not enter the cell cycle based on BrdU-labeling<sup>[66]</sup>, probably being different from the previously reported progenitor-like cells, which can be labeled with BrdU, during a 2 wk observation under physiological conditions<sup>[22,23]</sup>. This also suggests that the target cells do not contribute to the maintenance of cell turnover under physiological conditions but may be activated after severe PT injury in the S3 segment.

As mentioned earlier, we cannot confirm the existence of intratubular progenitor cells due to the lack of definitive markers for these cells, although some recent reports have provided some evidence for the existence of intratubular progenitor-like cells<sup>[22-30,33]</sup>. Therefore, it is not clear at this stage whether our “target cells” are truly progenitor-like cells or merely dedifferentiated PT cells that can acquire progenitor-like properties. However, our findings suggest the presence of a distinct population of tubular cells in the distal area of the S3 segment or at the transition zone between PT and thin descending limb of Henle. This cell population can be activated and stimulated to proliferate for adequate repair of PTs after severe impairment of the replicative capacity of PT cells in S3 segment or upon depletion of surviving PT following acute tubular injury.

## PERSPECTIVES

Based on our data, we conclude that the three modes of regeneration, compensatory hyperplasia, dedifferentiation of mature tubular cells and intratubular progenitor-like cell system, as illustrated in Figure 2, may be involved in PT repair. Compensatory hyperplasia provides effective PT cell turnover by self-renewal of adjacent cells without dedifferentiation under physiological conditions. However, PT cells are vulnerable because they are exposed to a variety of toxins and are susceptible to ischemic injury. This might explain why PT cells can regenerate through dedifferentiation of mature tubular cells, which can result in effective and rapid repair of focal PT lesions. Intratubular progenitor-like cells can play a role as a backup system to repair severely injured PTs. This does not exclude the possibility that both dedifferentiation and intratubular progenitor-like cells also contribute together to repair PTs in certain types of tubular injury. Interestingly, evidence points to the presence of stem cells in the liver of several rat models of liver injury, which promote tissue regeneration as a second backup system for liver regeneration when the proliferative capacity of hepatocytes *via* compensatory hyperplasia is compromised<sup>[52]</sup>. The PTs

also seem to be equipped with the same backup system for PT regeneration, including intratubular progenitor-like cells at different locations. For instance, severe injury in S1 and S2, but not S3 segment, of PT induced by gentamicin<sup>[73]</sup> might evoke different progenitor-like cells than in other intratubular locations.

Unfortunately, there is only a limited knowledge about the modes of regeneration of tubular cells and the factors that induce regeneration. Understanding tubular regeneration in health and disease can potentially allow the design of new therapeutic strategies against various tubular diseases.

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## Vitamin E-derived copolymers continue the challenge to hemodialysis biomaterials

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### Abstract

Improving material biocompatibility has been a continuous effort and remains a major goal of dialysis therapy. In this respect, vitamin E-modified copolymers have been used to produce a generation of biomaterials that has offered new clinical challenges and the chance of further improving the quality of synthetic hemodialyser membranes. This mini review article describes the evolution of these copolymers that only recently have been adopted to develop new vitamin E-modified polysulfone hemodialysers. Biomaterial characteristics and clinical aspects of these membranes are discussed, starting from the most recent contributions that have appeared in the literature that are of interest for the community of nephrology and dialysis specialists, as well as biomaterial scientists.

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**Key words:** Vitamin E; -tocopherol; Copolymer; Biocompatibility; Antioxidant; Hemodialysis; Hemodialyser membranes

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### INTRODUCTION

By definition, a vitamin E-derived copolymer is a macromolecular complex of natural or synthetic origin with repeated subunits connected by covalent chemical bonds that contain vitamin E as a stable modifier. This is a fat-soluble antioxidant vitamin (Figure 1A) with 8 natural vitamers and several synthetic analogues so far produced for nutritional and pharmacological purposes<sup>[1-3]</sup>.

In the early 1990s, vitamin E copolymers were developed and used for the first time to produce hollow-fiber hemodialyser membranes that soon after were introduced in clinical practice, first in Japan and then in Europe. The aim of that pioneering biomaterial technology was to coat the blood surface of both cellulosic and polysulfone (PS)-based hemodialysis membranes (Figure 2) with a physiological molecule present in cell membranes and circulating lipoproteins. The ultimate goal was that of achieving higher biocompatibility and antioxidant protection in the extracorporeal circulation. Available knowledge cannot disclose if these two features are separated by a functional dichotomy or are just two faces of the same vitamin E-derived function. Actually, vitamin E is a physiological fat-soluble micronutrient with ubiquitous distribution in solid tissues and body fluids and so it is biocompatible by definition. Clinical studies examined in this review paper have provided conclusive recognition of the good level of biocompatibility reached by the last generation of PS-based vitamin E-modified dialysers.



At the same time, vitamin E has well-known antioxidant properties<sup>[4]</sup>, functioning as a hydroperoxyl radical scavenger and chain breaker (i.e., inhibitor of the chain reaction of lipid peroxidation) and as a H-atom or electron donor. Antioxidant effects are due to the hydroxyl group in position 6 of the chroman ring in the vitamin E structure (Figure 1A) and have been conclusively confirmed *in vitro* for the coating of vitamin E of the hollow-fiber dialysers<sup>[5]</sup>. Clinical studies have demonstrated effects of prevention against oxidative stress markers and particularly on lipid oxidation and low-density lipoprotein (LDL) damage<sup>[6-8]</sup>. These pieces of evidence pave the way to the definition of “interactive or functional membrane”, which extends the concept of biocompatibility to include that of (antioxidant) bioactivity. Besides filtration and biocompatibility, antioxidant bioactivity represents a third and original dimension in the functional chart of hemodialyser membranes recently described in<sup>[9]</sup>.

As recently shown by Dahe *et al*<sup>[10]</sup>, other analogues of this vitamin, such as D- $\alpha$ -tocopheryl polyethylene glycol 1000 succinate (TPGS; Figure 1A), could be used to produce biocompatible copolymers that may have future application in hollow-fiber membrane production technology. Several of the synthetic derivatives of vitamin E are, however, “redox-silent” and this is the case of TPGS. Therefore, TPGS-derived copolymers cannot claim to express the “antioxidant bioactivity” of vitamin E. Synthetic derivatives of tocopherols and tocotrienols include promising antioxidant agents, such as amine derivatives, recently characterized in our laboratories<sup>[11]</sup>.

These innovations and a brief historical overview of the literature of vitamin E-modified hemodialysers are presented in this mini review paper.

## HISTORICAL AND CLINICAL OUTLINE OF VITAMIN E-DERIVED COPOLYMERS FOR HEMODIALYSIS THERAPY

Vitamin E-derived copolymers for hemodialysis therapy were originally produced by Terumo Co., Japan, and introduced in clinical practice in the early 1990s as cellulose membranes coated with  $\alpha$ -tocopherol (Figure 1A)<sup>[6,7]</sup>. Synthetic dialyser membranes modified with vitamin E have been developed starting from these prototypal membranes and at the beginning of 2000, Ashai Kasei Medical Co., Japan, launched a composite PS-polyvinylpyrrolidone (PVP) copolymer embedded with  $\alpha$ -tocopherol (Figure 2) that is marketed with the commercial name of VitabranE<sup>TM</sup>.

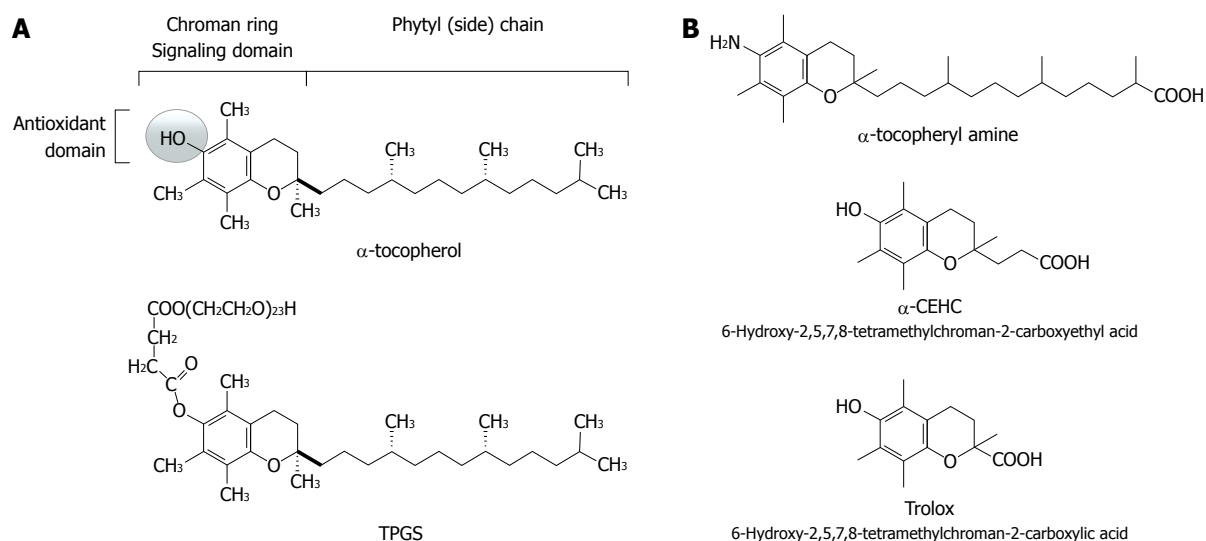
Extensive investigation of these dialyser membranes over more than two decades of clinical practice and *in vitro* studies<sup>[12]</sup>, demonstrated that coating cellulose membranes with vitamin E helps to prevent lipid oxidation and LDL damage as cardiovascular risk factors for chronic kidney disease (CKD) patients treated with cellulose and even synthetic dialyser membranes<sup>[6,7]</sup>. This effect was confirmed in subjects treated with VitabranE<sup>TM</sup>, i.e., PS-

PVP membranes modified with vitamin E<sup>[8]</sup>, and appears to be the consequence of an antioxidant effect of these membranes. Such an antioxidant activity was conclusively identified and quantified in this laboratory<sup>[5]</sup> using a recirculation model system in which the actual antioxidant power of VitabranE minimodule dialysers was measured together with the quota of vitamin E that is active at the blood-biomembrane interface.

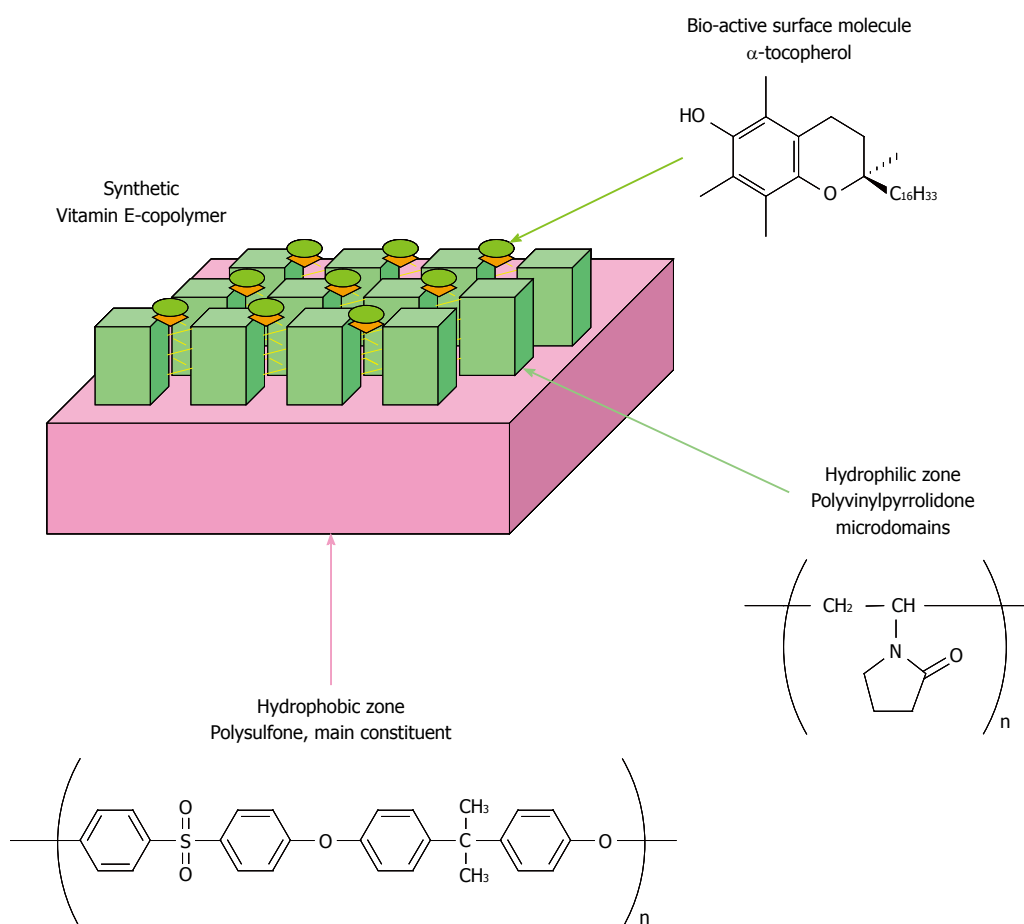
In recent years, the introduction of vitamin E-modified PS-PVP in clinical practice has renewed interest and stimulated further clinical research on these membranes. Several aspects of CKD comorbidity have been investigated and early randomized trials have described that these PS-PVP modified membranes may help to alleviate the resistance to erythropoiesis stimulating agents and to reduce, to some extent, sub-clinical markers of inflammation<sup>[13-15]</sup>, intradialytic hypotension<sup>[16]</sup> and anti-coagulation therapy<sup>[17]</sup>.

## BIOCOMPATIBILITY AND ANTIOXIDANT BIOACTIVITY: THE MECHANISM OF ACTION OF VITAMIN E COPOLYMERS

If this burden of clinical evidence is confirmed in further trials of larger proportions, this copolymer will represent one of the highest examples of biocompatibility in dialysis therapy. As introduced above, vitamin E copolymers can offer a functional extension of the concept of biocompatibility, which deals mainly with a better control of blood cell activation, towards the inclusion of antioxidant bioactivity that may provide protection against lipid peroxidation and other oxidative reactions occurring in the extracorporeal circulation. The functional dichotomy between biocompatibility and antioxidant function could be trivial and available data are not sufficient to define whether these are distinct functions of the same molecule or just two faces of the same vitamin E-derived function. Actually, this fat-soluble vitamin is a physiological, and so biocompatible, component of blood cell membranes and lipoprotein particles, which operates to maintain the physical and chemical characteristics of these lipid micro-environments. If, on one hand, the main function for the vitamin E used as a coating agent is that of masking the structure of other components of the copolymer, thus avoiding their contact with blood components, then on the other hand, vitamin E is a lipophilic antioxidant<sup>[5]</sup> with the importance in the scavenging of hydroperoxyl radicals (Figure 3A) in the plasmalemma and in the body of lipoprotein particles<sup>[4]</sup>. Vitamin E also stabilizes the physical structure of lipid bilayers, showing strategic localization (penetration) and movements that produce key lipid-lipid interaction in actual cell membranes. This function is facilitated by the phytol chain and is strategic to explaining the dynamic activity of vitamin E as an anti-peroxidative molecule in complex lipid structures. Actually, during lipid peroxidation reactions, the antioxidant function of vitamin E produces a tocopheryl radical



**Figure 1** Molecular structure of some forms of vitamin E. A:  $\alpha$ -tocopherol (top) and its redox-silent synthetic analogue TPGS 1000 (bottom); B: Some examples of vitamin E analogues with antioxidant activity: the synthetic forms  $\alpha$ -tocopherylamine (top) and Trolox (middle), and the hepatic short-chain metabolite  $\alpha$ -CEHC (bottom).

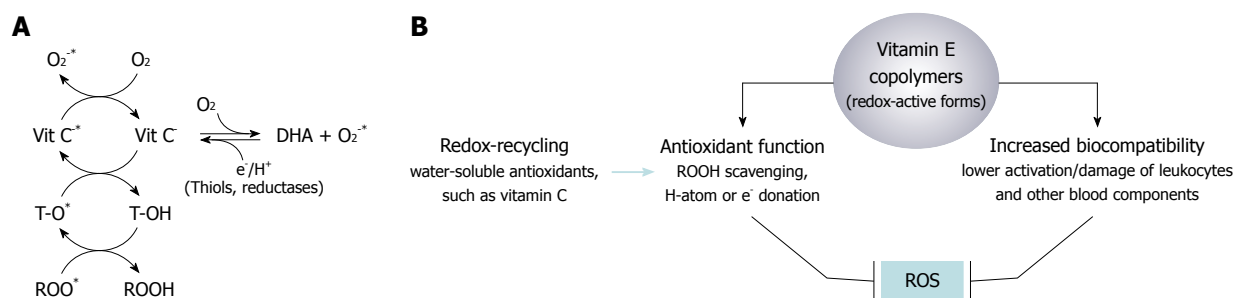


**Figure 2** Schematic structure of the PS-PVP based copolymer functionalized with  $\alpha$ -tocopherol. Kindly provided by the R and D department of Ashai Medical, Tokyo, Japan.

intermediate that has higher stability than hydroperoxyl radicals formed on polyunsaturated lipids. This relatively stable radical intermediate of vitamin E delocalizes from the oxidation sites to the water interface on the outer part of the cell membrane to undergo physiological recycling

by cytosolic antioxidants such as vitamin C (ascorbic acid). These steps ultimately avoid the progression of lipid peroxidation chain reactions.

Moreover, vitamin E has other and so far poorly understood biological properties that appear to arise from



**Figure 3** Coupled redox reaction of vitamin E and vitamin C (co-antioxidants) during the reduction (scavenging) of peroxy radicals (A) and proposed scheme of the mechanisms that may lead vitamin E copolymers to control the flux of reactive oxygen species in the extracorporeal circulation (B). ROS: Reactive oxygen species; Vit C: Ascorbic acid; Vit C•: Ascorbate anion; Vit C••: Ascorbyl radical anion; DHA: Dehydroascorbate; O<sub>2</sub>: Molecular oxygen; O<sub>2</sub><sup>-</sup>: Superoxide anion; e<sup>-</sup>/H<sup>+</sup>: Electron/proton; T-OH: Tocopherol; T-O•: Tocopheryl radical; ROOH: Peroxyde (reduced form); ROO•: Peroxyl radical.

antioxidant-independent mechanisms<sup>[18,19]</sup>. For instance, besides physiological lipid-lipid interactions, the chroman ring and the hydrophobic tail (Figure 1) represent functional domains that may lead vitamin E molecules to interact with the hydrophobic moieties of surface proteins of blood cells, and may provide the physiological environment for the hydrophobic interaction with main plasma proteins and particularly with albumin, i.e., a circulating ligandin operating a low-affinity binding of vitamin E as well as free fatty acids and several other fat-soluble molecules. Worthy of note, this interaction may ultimately affect the antioxidant activity of vitamin E<sup>[20]</sup> and could influence that antioxidant status of serum albumin, a main sacrificial target of oxidative reactions in the uremic blood<sup>[21]</sup>.

Antioxidant activity of natural substances often co-exists with anti-inflammatory properties that have been described both *in vitro* and *in vivo* for this vitamin, as well as for some of the physiological products of its metabolism, such as carboxyethyl-hydroxychromans, long chain metabolites and tocopheryl-phosphate<sup>[22]</sup>.

Although some of these (non-antioxidant) biological effects of vitamin E could be hindered to some extent by the immobilization on the copolymer structure, their existence suggests different biological mechanisms and clinical applications of vitamin E copolymers. Accordingly, clinical and biological evidence of the antioxidant effects of vitamin E copolymers could be also ascribed to the effect that the improved biocompatibility of this type of biomaterials may exert on blood cells (mainly leukocytes), mitigating their activation during the contact with the dialyser membrane. And antioxidant effects of the other vitamin E copolymers could be erroneously ascribed to the indirect effect that an improved biocompatibility exerts on the production of reactive oxygen species (ROS), by a lowered leukocyte activation (Figure 3B). In this respect, NADPH-oxidase, myeloperoxidase and the inducible isoform of nitric oxide synthase, i.e., iNOS, can be involved as the main ROS generating enzymes, and both tocopherols and tocotrienols have been described to produce inhibitory effects on enzymatic and transcriptional events of these inflammatory and ROS generating pathways<sup>[2,3,23]</sup>. Accordingly, cellulosic membranes coated

with vitamin E produce lower leukocyte activation and thus lower generation of ROS than uncoated membranes<sup>[24]</sup>. This is expected also for VitabranE™ dialyser membranes that have been recently suggested to lower inflammatory indices in HD patients<sup>[15]</sup>.

Further investigation of the anti-inflammatory contribution that vitamin E can provide to the biocompatibility of hemodialysis copolymers is awaited. In this respect, future trials should take into account that antioxidant and non-antioxidant responses to vitamin E in humans varies on an individual (genetic) basis<sup>[3]</sup>. This was also recently documented for the anti-inflammatory response to oral vitamin E in individuals assessed for the polymorphic expression of main inflammatory genes<sup>[25]</sup>.

## OTHER VITAMIN E-COPOLYMERS AND THEIR POSSIBLE APPLICATION TO HEMODIALYSIS THERAPY

A recent study by Dahe *et al*<sup>[10]</sup> reported on a new PS-based hollow fiber membrane incorporating from 5% to 20% (w/w) TPGS (Figure 1A). This study demonstrates that synthetic derivatives of vitamin E obtained starting from the backbone of natural vitamers can be used to develop other vitamin E copolymers, thus contributing new generations of biocompatible and possibly functional biomaterials for hemodialysis therapy. Several of these synthetic forms of vitamin E have been prepared and investigated by us and others<sup>[1-3]</sup> as pharmacological agents of possible relevance in cancer and other immuno-inflammatory diseases.

Biocompatibility and separation performance of these new TPGS-PS fibers were assessed with different *in vitro* methods and the reported results suggest the achievement of an “enhanced biocompatibility”. The authors, however, incorrectly claimed this biomaterial as “antioxidative composite PS” and this definition was supported by the definition of TPGS as “biologically active vitamin E”.

TPGS is not an “antioxidative” molecule but rather a redox-inert derivative of vitamin E. Indeed, the succinylation of the chroman ring that serves to produce

TPGS, as in the case of  $\alpha$ -tocopheryl succinate that is the prototypal succinyl ester of  $\alpha$ -tocopherol with well known anti-cancer activity<sup>[1]</sup>, is operated in position 6 by esterification on the hydroxyl moiety that provides the classical antioxidant function to the vitamin E molecule (for further structural and biological details on synthetic derivatives of vitamin E see<sup>[1,2]</sup> and the references therein). This esterification prejudices the radical scavenging and electron donating properties of  $\alpha$ -tocopherol. It is worthy of note that TPGSs (there are ester derivatives of  $\alpha$ -tocopherol with PEG chains from 4 to 136 monomeric units and molecular masses from 200 to 6000 kDa, respectively) have completely different properties when compared with authentic  $\alpha$ -tocopherol (see structural features in Figure 1A). TPGSs are redox-silent forms of vitamin E comprised of a hydrophilic polar (water-soluble) head and a lipophilic (water-insoluble) alkyl tails. These behave as non-ionic surfactants and TPGS100 is the most widely used, from applications in cosmetics and the pharmaceutical industry. Actually, this is a solubilizer and emulsifier that is used as a vehicle for lipid-based drug delivery formulations<sup>[26]</sup>, being recognized as a biocompatible compound with defined molecular stability and pharmacological properties<sup>[27]</sup>. This means that the most likely biological consequence of incorporating TPGS or other synthetic redox-silent derivatives of vitamin E into PS-like copolymers, is that of producing an inert (eventually less bioactive) biomaterial, which may represent an advantage in terms of biocompatibility, but not any direct antioxidant or radical scavenging effect. Structural investigation by Dahe *et al*<sup>[10]</sup> suggested that TPGS is stabilised in the fiber structure, which is key to exclude that the modifier would be released under normal operative conditions in the patient's blood. *In vitro* data<sup>[4]</sup> have demonstrated that this is the case of the vitamin E embedded in the copolymer of VitabranE™ dialyser membranes and covalently bound to the PS-PVP copolymer. Accordingly, the treatment with these membranes does not influence blood levels of vitamin E of the patient<sup>[14]</sup>.

The molecular stability of TPGS copolymers should be assumed as a positive feature. The possibility that TPGS would be exposed or released from the composite PS may offer a chance for untoward biological consequences due to the pharmacological effects of this molecule that include, for instance, the inhibition of metabolic enzymes as the ATP-dependent pump p-glycoprotein<sup>[27]</sup>. Alternatively, TPGS could liberate free vitamin E by enzymatic de-esterification through the activity of endogenous esterases. These enzymes could be released after blood cell damage on the surface of the membrane dialyser. Neither the release of TPGS nor that free (and thus bioactive) vitamin E from TPGS have been investigated in detail by Dahe *et al*<sup>[10]</sup>, which are aspects of possible interest deserving further consideration in the future. Recirculation experiments carried out as described in<sup>[5]</sup> or cell uptake tests could be used to investigate these points dealing with stability and pharmacological activity of vitamin E biomaterials.

This aspect was also preliminarily investigated by means of *in vitro* biocompatibility tests for the TPGS-PS copolymer<sup>[10]</sup>. The production of ROS by cells maintained in culture with TPGS-PS hollow fibers was lower than in unmodified PS but available information cannot conclusively demonstrate if this is due to TPGS activity and bioavailability. At the same time, *in vitro* biocompatibility tests carried out with the approach of fibers maintained in a static cell culture used in the study by Dahe *et al*<sup>[10]</sup> can produce artifactual results, being the cells in static contact with the external surface of the fibers which may have different composition and morphology with respect to the inner surface. Circulation experiments are more appropriate to simulate the type of interaction (or contact) occurring *in vivo* between the plasmalemma of blood cells and the inner surface of the hollow fibers. This aspect suggests the need for further investigation by suitable circulation model systems and appropriate controls to conclusively define biocompatibility characteristics described for TPGS-PS hollow fiber membranes.

Other synthetic derivatives of vitamin E recently investigated in this laboratory may provide functionalised copolymers of interest to dialyser membrane technology. Amine derivatives of tocopherols and tocotrienols, such as  $\alpha$ -tocopheryl amine (Figure 1B), have been demonstrated to be effective antioxidants in different model systems that include radical scavenging and electron transferring reactions<sup>[11]</sup>. Importantly, preliminary data suggest that tocopheramines may provide higher radical scavenging activity than  $\alpha$ -tocopherol in polar solvents (i.e., under reaction conditions mimicking, for instance, the lipid-water interface of plasma lipoproteins), while  $\alpha$ -tocopherol was found to be a superior scavenger in organic solvents (i.e., the conditions of reaction found within the lipid structure of a lipoprotein particle) that is in agreement with other studies comparing this natural form with other vitamin E forms<sup>[4]</sup>.

Short chain metabolites and the pharmacological analogue Trolox (Figure 1B) are also redox-active forms of possible interest in the development of copolymers. These types of derivatives lose most of the phytyl tail of tocopherols and tocotrienols, which ultimately lowers their hydrophobic strength. Prototypal copolymers have been produced in our laboratories using activated cellulose beads as support backbone and preliminary characterization demonstrated that the hydroxylic groups of the cellulose backbone were blocked by the modifiers and these maintained their antioxidant activity (Galli F, unpublished observation).

## CONCLUSION

In conclusion, vitamin E-derived copolymers, already introduced in clinical practice, have clearly shown the potential for providing one of the highest standards of biocompatibility, remaining a unique example of biomaterial with application in the antioxidant therapy of CKD patients on regular HD. In this context, the reference co-



polymer used to produce hollow-fiber membranes for clinical use is vitamin E-modified PS-PVP. New generations of vitamin E copolymers such as the redox-silent TPGS-PS are now approaching the field of biomaterials for HD therapy with promising biocompatibility and filtration performances that need to be confirmed with further pre-clinical and clinical investigation. Many redox-silent and -active derivatives are available to design other functionalized copolymers with anticipated antioxidant and/or biocompatibility properties.

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## Peritoneal dialysis associated infections: An update on diagnosis and management

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### Abstract

Peritoneal dialysis (PD) is associated with a high risk of infection of the peritoneum, subcutaneous tunnel and catheter exit site. Although quality standards demand an infection rate < 0.67 episodes/patient/year on dialysis, the reported overall rate of PD associated infection is 0.24-1.66 episodes/patient/year. It is estimated that for every 0.5-per-year increase in peritonitis rate, the risk of death increases by 4% and 18% of the episodes resulted in removal of the PD catheter and 3.5% resulted in death. Improved diagnosis, increased awareness of causative agents in addition to other measures will facilitate prompt management of PD associated infection and salvage of PD modality. The aims of this review are to determine the magnitude of the infection problem, identify possible risk factors and provide an update on the diagnosis and management of PD associated infection. Gram-positive cocci such as *Staphylococcus epidermidis*, other coagulase negative staphylococci, and *Staphylococcus aureus* (*S. aureus*) are the most frequent aetiological agents of PD-associated peritonitis worldwide. Empiric antibiotic therapy must cover both gram-positive and gram-negative organisms. However, use of systemic vancomycin and ciprofloxacin administration for example, is a simple and

efficient first-line protocol antibiotic therapy for PD peritonitis - success rate of 77%. However, for fungal PD peritonitis, it is now standard practice to remove PD catheters in addition to antifungal treatment for a minimum of 3 wk and subsequent transfer to hemodialysis. To prevent PD associated infections, prophylactic antibiotic administration before catheter placement, adequate patient training, exit-site care, and treatment for *S. aureus* nasal carriage should be employed. Mupirocin treatment can reduce the risk of exit site infection by 46% but it cannot decrease the risk of peritonitis due to all organisms.

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**Key words:** Exit site infection; Peritonitis; Tunnel infection; Polymicrobial infection; Catheter removal; Dialysis modality change; Fungal peritonitis; Sclerosing encapsulating peritonitis; Peritoneal dialysis

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### INTRODUCTION

All dialysis treatments include a certain risk of infection because of the decreased immune defenses of patients in established renal failure (ERF) and because dialysis techniques increase the potential of microbial contamination. Peritoneal dialysis (PD), and in particular continuous ambulatory PD (CAPD), is associated with a high risk of infection of the peritoneum, subcutaneous tunnel and catheter exit site<sup>[1]</sup>. Exit site infection (ESI) and tunnel



infection (TI) *per se* pose little risks but the possibility of developing PD peritonitis demands careful attendance to these problems. It is estimated that 12% of cases of ESI and TI result in PD peritonitis<sup>[2]</sup>. As many as 15%-50% of ERF patients are on PD, but recurrent or prolonged peritonitis may cause technique failure in PD.

The majority of catheter related problems are of an infection nature - mainly represented by peritonitis (61%); ESI and TI (23%); catheter obstruction, dislocation and leakage making up the rest. Peritonitis can be associated with severe pain leading to hospitalisation, catheter loss, and a risk of death; and it therefore continues to be a serious complication for PD patients<sup>[3-6]</sup>. PD peritonitis usually has an excellent prognosis with resolution within days but it can lead occasionally to the much dreaded sclerosing encapsulated peritonitis (SEP).

The incidence of peritonitis has markedly decreased since the late 1980s, but the infection remains a significant complication of chronic PD. Very low rates of peritonitis in a program are possible if close attention is paid to the causes of peritonitis and protocols implemented to reduce the risk of infection<sup>[3]</sup>. Although several organisms are involved in causing PD associated infections (PDAI), coagulase negative staphylococci (CoNS) appear to be the most common<sup>[7]</sup>. However, rare forms of PD infection, for example, rapidly growing non tuberculous mycobacterium are associated with catheter loss (80%) and significant mortality (40%)<sup>[8]</sup>.

Action to decrease the risk of PDAI should start in the pre-catheter insertion phase. In order to obtain a reduction of the complications, achieve prolonged catheter duration and a better quality of life for PD patients, the surgical technique requires strict adherence to a standardised procedure and a dedicated team<sup>[4]</sup>. Improved diagnosis, increased awareness of causative agents in addition to other measures will facilitate prompt management of PDAI and salvage of PD modality. The aims of this review are to determine the magnitude of the infection problem, identify possible risk factors and provide an update on the diagnosis and management of PDAI.

## EPIDEMIOLOGY

Peritonitis continues to be the most frequent cause of PD failure, with an important impact on patient mortality. Peritonitis risk is not evenly spread across the PD population or programs. During a median follow-up of 1.9 years, about 50% a large cohort of 7401 PD patients aged 65-100 years had at least one infection-related hospitalisation<sup>[6]</sup>. In another series, catheter-related peritonitis occurred in about 20% of patients and ESI was responsible for catheter removal in more than one-fifth of cases<sup>[9]</sup>. The infection may be caused by the surgical procedure to insert a PD catheter or the conduct of PD. Over a 4-year period in one institution, the complications seen with 384 catheters inserted into 319 patients (95% were in ERF) by 22 different operators were 24 cases (6.3%) of ESI, 14 (3.6%) of culture-proven wound infection and 11 (2.9%)

post-insertion peritonitis<sup>[10]</sup>. The UK Renal Association standard for peritonitis is one episode per 18 mo in adults (0.67 episodes per patient-year)<sup>[11]</sup>. The overall rate of PDAI is between 0.24 to 1.66 episodes per patient years on dialysis (Table 1)<sup>[8,11-25]</sup>. Even though technological advancements such as use of double-bag, disconnect and other connector systems have continuously reduced peritonitis rates worldwide, the extremely low rates of infection reported in Asia (Table 1) is difficult to explain, prompting the belief that Asian patients are probably different from their Western counterparts. Possible reasons include a relatively younger patient group compared to the West, increased PD education as the modality is more prevalent in Asia than in the West. Though the reasons for the outstanding results reported by Han *et al*<sup>[25]</sup> were not fully explained, reducing PDAI would improve PD technique survival.

Peritonitis remains a leading complication of PD. Around 18% of the infection-related mortality in PD patients is the result of peritonitis. Although less than 4% of peritonitis episodes result in death, peritonitis is a "contributing factor" to death in 16% of deaths on PD. In addition, severe and prolonged peritonitis can lead to peritoneal membrane failure and peritonitis is probably the most common cause of technique failure in PD<sup>[26]</sup>. It is estimated that for every 0.5-per-year increase in peritonitis rate, the risk of death increases by 4%<sup>[27]</sup> and 18% of the episodes resulted in removal of the PD catheter and 3.5% resulted in death<sup>[28,29]</sup>.

## Organisms

Gram-positive cocci such as *Staphylococcus epidermidis* (*S. epidermidis*), other CoNS, and *Staphylococcus aureus* (*S. aureus*) are the most frequent aetiological agents of PD-associated peritonitis worldwide<sup>[1,2,8,30]</sup>. The spectrum of organisms associated with PD peritonitis varies geographically as does the rate of culture negative episodes<sup>[30,31]</sup>. For example, Gram-negative (G-ve) PD peritonitis is more frequent than G+ve peritonitis in the CAPD population in India and is associated with worse outcome<sup>[19]</sup>. There was no significant difference in causative agents between home and hospital acquired peritonitis<sup>[32]</sup>. Though a wide spectrum of organisms are responsible for PDAI (Table 2)<sup>[8,12,16,31,33-45]</sup>, it must be borne in mind that a significant proportion of the infections are culture negative - about 20% to 32.5%<sup>[12,31]</sup>. As there is a culture-negative rate of 20%, it is recommended by the Renal Association that appropriate laboratory samples are obtained before commencement of antibiotics<sup>[11]</sup>.

Enterococcal peritonitis though uncommon, is a serious complication of PD. A review of 116 episodes of enterococcal peritonitis in 103 individuals showed its tendency to be associated with older age, renovascular disease and coronary artery disease. Polymicrobial peritonitis was significantly more common when an enterococcus species was isolated than when it was not (45% *vs* 5%, respectively)<sup>[18]</sup>. It is also associated with increased risk of catheter loss, change in dialysis modality and death. In

**Table 1 Incidence and spectrum of peritonitis in peritoneal dialysis patients**

Ref.	No.	Infection rate (episodes/ patient-year)	Organisms	Comments
Lobo <i>et al</i> <sup>[12]</sup> 2003-2007	330	0.42	<i>S. aureus</i> 27.5% <i>E. coli</i> 13.4% Culture negative 32.5%	Brazil Hypoalbuminaemia a risk factor
Shyr <i>et al</i> <sup>[13]</sup> 1990-1993	55	0.56 0.36 (ESI)		Experience surgeon may be a factor preventing infection
Cleper <i>et al</i> <sup>[14]</sup> 1997-2007	29	1.66	<i>S. aureus</i> 32.5% Pseudomonas 16%	Children; modality change in 18%
Shigidi <i>et al</i> <sup>[15]</sup> 2003-2007	241	0.24 ± 0.1	<i>S. aureus</i> 21% <i>E. coli</i> 9% Culture negative 28%	Qatar; Catheter loss 19%; Mortality 3% due to candida and pseudomonas peritonitis
Kofteridis <i>et al</i> <sup>[16]</sup> 1990-2007	82	0.89	G+ve 42% G-ve 19% Polymicrobial 5% Fungal 4%	Greece
Freitas <i>et al</i> <sup>[17]</sup> 2005-2008	137	0.31 (ESI)	G+ve 56% G-ve 27% Pseudomonas Fungi	Cure rate 96% Catheter loss in 3 patients; peritonitis in two
Edey <i>et al</i> <sup>[18]</sup> 2003-2006	103	116 episodes in 103 pts	Polymicrobial	Enterococci peritonitis is associated with catheter loss
Prasad <i>et al</i> <sup>[19]</sup> 1993-2001	168	0.63	G-ve 60% G+ 40% Polymicrobial Fungal	G-ve peritonitis has worse outcome than G+ve
Boehm <i>et al</i> <sup>[20]</sup> 1994-2003	30	0.82		USA and European data
Goffin <i>et al</i> <sup>[21]</sup> 1991-2000	101	0.41	G+ve 51.5% G-ve 27.7% Polymicrobial 13% Culture negative 7.9%	
Nessim <i>et al</i> <sup>[22]</sup> 1996-2005	4247	0.36		Double cuff catheters had better results
Tan <i>et al</i> <sup>[23]</sup>	64	0.23		Singapore
Li <i>et al</i> <sup>[24]</sup>	110	0.29		Hong Kong
Han <i>et al</i> <sup>[25]</sup> 1981-2005	2301	0.38	G+ve 42.6% G-ve 17.0% Fungal 2.1% Culture negative 37.3%	Korea Peritonitis rates fell from 0.57 in earlier to 0.29 in latter period

*S. aureus*: *Staphylococcus aureus*; *E. coli*: *Escherichia coli*.

a 5-year retrospective series from a single-centre multi-ethnic Asian population, rapidly growing non tuberculous mycobacterium constituted 3% of all culture-positive ESI and PD peritonitis<sup>[8]</sup>. Mycobacterial peritonitis can be caused by *Mycobacterium tuberculosis* or non tuberculous mycobacteria, such as *Mycobacterium fortuitum*, *Mycobacterium avium*, *Mycobacterium abscessus*, and *Mycobacterium chelonae*. The incidence of tuberculous peritonitis is higher in Asia than elsewhere probably because the infection is endemic in their population. It is important to differentiate patients with miliary tuberculosis, whose peritonitis is part of the disseminated disease, from those with isolated tuberculous peritonitis without extraperitoneal infection.

Fungal peritonitis is usually preceded by multiple episodes of bacterial peritonitis and poses a significant risk of dropout of the patient from the PD program<sup>[37]</sup>. Fungal peritonitis is relatively uncommon and is caused mainly by *Candida albicans*, entering the peritoneal cavity via the catheter lumen or vagina in females. Kazancioglu *et al*<sup>[38]</sup> reported 15 cases in a 10-year period with a mean

duration of dialysis from the initiation of treatment until the development of fungal peritonitis of 41 mo<sup>[38]</sup>. *Candida* species were the most common pathogens and *Candida albicans* was the most frequent, but high prevalence of *Candida parapsilosis* has been observed in the last decade<sup>[36,46]</sup>.

### **Risk factors for PDAI**

The incidence of chronic PD-associated peritonitis has decreased largely due to technical advances and the identification and control of risk factors such as ESI, poor technique of catheter insertion and use, colonisation with *S. aureus* and lack of patient motivation. Treatment of bacterial colonisation by current antimicrobial protocols may not permit adequate dosing to penetrate bacterial biofilm and be a reason for recurrent or repeat episodes of peritonitis<sup>[11]</sup>. The risk factors for infection include extremes of age, female sex, diabetes, heart failure, pulmonary disease, anaemia and low serum albumin level<sup>[6,47,48]</sup>. A retrospective analysis of 330 patients over

**Table 2** Organisms causing peritoneal dialysis associated infections

Organism	Comments	Ref.
Gram-positive cocci	Commonest cause of PDAI	Gupta <i>et al</i> <sup>[30]</sup> ; Fedorowsky <i>et al</i> <sup>[33]</sup> , Renaud <i>et al</i> <sup>[8]</sup>
<i>Staphylococcus epidermidis</i>		
<i>Staphylococcus aureus</i>		
<i>A and β-haemolytic Streptococcus</i>		
<i>Micrococci</i>		
Gram-negative	Recent change from HD to PD	Gupta <i>et al</i> <sup>[30]</sup> ; Chang <i>et al</i> <sup>[34]</sup> , Lobo <i>et al</i> <sup>[12]</sup> ; Koffleridis <i>et al</i> <sup>[16]</sup> , Krishnan <i>et al</i> <sup>[35]</sup>
<i>Enterobacteriaceae</i>		
<i>Pseudomonas aeruginosa</i>		
VRE		
<i>Escherichia coli</i>		
<i>Klebsiella oxytoca</i>	Polymicrobial/catheter loss/transfer to HD	García-Agudo <i>et al</i> <sup>[36]</sup> , Predari <i>et al</i> <sup>[37]</sup> ; Kazancioglu <i>et al</i> <sup>[38]</sup> , Troidle <i>et al</i> <sup>[31]</sup>
<i>Acinetobacter sp</i>		
<i>Serratia marcescens</i>		
<i>Enterococci</i>		
Fungi		
<i>Candida albicans</i>	India and mainly developing economies	Troidle <i>et al</i> <sup>[31]</sup>
<i>Candida parapsilosis</i>		
<i>Candida glabrata</i>		
<i>Neosartorya hirsutiae</i>		
<i>Aspergillus fumigatus</i>		
Anaerobes	More common in immunosuppressed patients	Lunde <i>et al</i> <sup>[39]</sup> ; Chan <i>et al</i> <sup>[40]</sup> , Vera <i>et al</i> <sup>[41]</sup> ; Renaud <i>et al</i> <sup>[8]</sup> , Mendoza-Guevara <i>et al</i> <sup>[42]</sup> , Byrd <i>et al</i> <sup>[43]</sup> ; Kimura <i>et al</i> <sup>[44]</sup>
Unusual		
<i>Mycobacteria sp</i>		
Rapidly growing nontuberculous <i>Mycobacteria</i>		
<i>Listeria monocytogenes</i>		
<i>Serratia marcescens</i>	May be confused with peritoneal macrophages or lymphocytes	Tilak <i>et al</i> <sup>[45]</sup>
<i>Bordetella bronchiseptica</i>		
<i>Corynebacterium ulcerans</i>		
<i>Acanthamoeba</i>		

PD: Peritoneal dialysis PDAI: PD associated infections.

a 5-year period identified hypoalbuminaemia, inadequate education and ESI as significant risk factors for PD associated peritonitis but failed to confirm gender, age, family income, diabetes mellitus, type of PD treatment, type of catheter and its surgical implant as risk factors for PDAI<sup>[12]</sup>. In another series of 141 PD patients in which 8 patients died and 40 patients had major cardiovascular or infection events, the malnutrition-inflammation score (closely associated with the Charlson comorbidity index) was shown to be an independent predictor of cardiovascular and infection events<sup>[49]</sup>.

### Age

The pattern of PDAI in children is different from adults. Yinnon *et al*<sup>[50]</sup> isolated 481 organisms from 378 peritoneal fluid specimens collected from 135 patients (45 children, 90 adults). The number of different organisms as well as the total number of isolates per patient were significantly greater in children than in adults. After *S. epidermidis*, *S. aureus* was the most frequently isolated organism, occurring in 18% of episodes in adults and 12% in children ( $P < 0.01$ ). CAPD-associated peritonitis occurs significantly more often in children than adults<sup>[50]</sup>. Recent US registry data and a European multicenter study described the increased risk of peritonitis in young children on PD. Boehm *et al*<sup>[20]</sup> identified six risk factors in a univariate analysis (age, APD treatment, ESI, low urinary volume, low residual GFR and low normalised protein

catabolic rate), which were significantly correlated with two or more of the outcome indices, but only ESI and residual urine volume were strong independent predictors of PDAI on multivariate analysis. Other risk factors for peritonitis in children include: first infection within less than 6 mo from starting treatment, *Pseudomonas* exit-site colonisation, and contaminating conditions (gastrostomies, diaper use, enuresis) and age  $< 5$  years<sup>[14]</sup>.

### Type of PD catheter

A review of 298 patients from 49 European centres showed that the type of catheter and the frequency of dressing changes were associated with a high infection risk<sup>[51]</sup>. Though it has been hypothesized that double-cuff catheters might be superior to single-cuff catheters in preventing peritonitis caused by periluminal entry of organisms, no catheter type has consistently been shown to reduce the peritonitis risk. The association between the number of catheter cuffs and peritonitis was tested using data collected in the multicentre Canadian Baxter Peritonitis Organism Exit-Sites Tunnel Infections project. There were 2555 peritonitis episodes in 4247 incident patients (0.364 per dialysis year at risk) with double-cuff catheter use being associated with a lower peritonitis rate ratio (RR) = 0.90, 95% CI: 0.80-1.01,  $P = 0.08$ . This trend was largely due to a decreased *S. aureus* peritonitis rate in those with a double-cuff catheter (RR = 0.46, 95% CI: 0.33-0.64,  $P < 0.001$ )<sup>[22]</sup>.

Lo *et al*<sup>[52]</sup> compared outcomes for catheters with different configurations: conventional straight, swan-neck straight tip, and swan-neck curled tip. They randomized 93 new CAPD patients without prior PD catheter insertion to receive a conventional straight, double-cuffed catheter, a swan-neck straight catheter, or a swan-neck curled tip catheter in 2:1:1 ratio. Swan-neck catheters were associated with a slightly better ESI rate, but had a high migration rate. The Cochrane review of 17 trials (1089 patients) did not find any significant difference in the risk of peritonitis, peritonitis rate, ESI or TI, or catheter removal/replacement between straight *vs* coiled intraperitoneal portion catheters<sup>[53]</sup>.

### Method of PD catheter insertion

Effective immobilisation of the peritoneal catheter has repeatedly been associated with positive catheter-related outcomes. A single-center retrospective community study compared infectious complication rates for peritoneal catheters that exit from a highly mobile structure (the abdomen) with rates for catheters exiting from a structure with minimal associated motion (the chest). Patients undergoing catheter implantation were divided into two groups: 22 patients with 23 abdominal catheters; 21 patients with 22 presternal catheters. For abdominal and presternal catheters respectively, the rates of exit-site infection were 0.22 episodes/patient-year and 0.11 episodes/patient-year, and the incidences of peritonitis were 0.41 episodes/patient-year and 0.27 episodes/patient-year with removal of two abdominal catheters. Though the rates were not significantly different, the more effective catheter immobilisation on the chest may lower the frequency of infectious complications<sup>[54]</sup>.

An alternative peritoneal catheter exit-site location is sometimes needed in patients with obesity, floppy skin folds, intestinal stomas, urinary and fecal incontinence, and chronic yeast intertrigo. Two-piece extended catheters permit remote exit-site locations away from problematic abdominal conditions. The effect on clinical outcomes by remotely locating catheter exit sites to the upper abdomen or chest was compared to conventional lower abdominal sites. In a non randomised design, peritoneal access was established with 158 extended catheters and 270 conventional catheters based upon body habitus and special clinical needs. Time until first ESI was longer for extended catheters ( $P = 0.03$ ) but there was no difference in ESI, TI and peritonitis rates<sup>[55]</sup>. Guidelines for optimal PD access support both downward and lateral exit-site directions. Crabtree and co-workers<sup>[56]</sup> conducted a prospective study comparing infectious and mechanical complications between 85 catheters with a preformed arcuate bend to produce a downward exit site and 93 catheters with a straight intercuff segment configured to create a lateral exit site. There were no differences in rates (episodes/patient-year) of ESI, TI, peritonitis, or catheter loss for downward and lateral exit sites. Several PD catheter-related interventions (catheter designs, surgical insertion approaches, and connection methods)

have been purported to reduce the risk of peritonitis in PD. Strippoli *et al*<sup>[57]</sup> conducted a systematic review of randomised trials (37 eligible trials and 2822 patients) of catheter types and related interventions in PD using The Cochrane CENTRAL Registry, MEDLINE, EMBASE, and reference lists. Their review demonstrates that of all catheter-related interventions designed to prevent peritonitis in PD, only disconnect (twin-bag and Y-set) systems have been proved to be effective.

### Type of PD Solution

The acidity and high glucose degradation product concentration of standard dialysates is thought to inhibit the function of polymorphonuclear leucocytes and macrophages within the peritoneal cavity<sup>[58]</sup>. The newer, more biocompatible solutions such as bicarbonate/lactate (have a neutral pH and a low concentration of glucose degradation products) are thought to be less cytotoxic to mesothelial cells and to improve the function and viability of peritoneal membrane and cells associated with host defence<sup>[59]</sup>. Ahmad *et al*<sup>[58]</sup> reported a significantly lower peritonitis rate of 1 per 52.5 patient-months (0.29 episodes per patient-year) in patients using biocompatible solutions compared to those using standard lactate (1 per 26.9 patient-months or 0.47 episodes per patient-year) -  $P = 0.0179$ . However, the results from clinical trials are conflicting. Kim *et al*<sup>[60]</sup> reported a higher peritonitis rate in the group using biocompatible solutions compared to those using conventional solutions (0.24 episodes per patient-year *vs* 0.09 episodes per patient-year) but others demonstrated no significant difference in rates<sup>[61,62]</sup>. Srivastava and co-workers<sup>[63]</sup> conducted a prospective randomised controlled open label trial of incident patients starting PD comparing the use of biocompatible and conventional solutions. Of a total of 267 patients entered into their study, 139 used biocompatible whereas 128 used conventional solutions. Neither the peritonitis-free survival (23.1 mo *vs* 26.7 mo) nor the peritonitis rates (1 per 34.7 patient-months *vs* 1 per 31.5 patient-months) between those using biocompatible and conventional solutions respectively were significantly different ( $P = 0.61$ ). This study though representing the largest randomised study to date comparing different PD solutions is probably limited by statistical power in producing conclusive evidence of the beneficial effect of biocompatible dialysates on PD peritonitis.

### Nasal carriage of bacteria

Surveillance for nasal methicillin resistant *S. aureus* (MRSA) carriage and infection among dialysis patients, healthcare workers and their family members in a dialysis centre was prospectively undertaken using molecular typing to determine epidemiological relationship. Among 1687 samples collected, MRSA colonisation rates were 2.41% (2/83) for PD patients and 2.36% (12/509) for haemodialysis patients. Five (5/14) subjects subsequently had MRSA infection. The clinical MRSA isolates had the same molecular type as the colonized strains of the same person,



indicating MRSA colonisation preceded clinical infection. Monitoring and eradication of MRSA from patients, health care workers and their family members should be considered to prevent continuous spread between health-care facilities and the community<sup>[64]</sup>. Luzar *et al*<sup>[65]</sup> studied 140 consecutive patients beginning CAPD at one of seven hospitals to assess the relation of the nasal carriage of *S. aureus* to subsequent catheter-exit-site infection or peritonitis. The carriers of *S. aureus* had a significantly higher rate of exit-site infection than the noncarriers (0.40 vs 0.10 episode per year,  $P = 0.012$ ).

Amato *et al*<sup>[66]</sup> found 17 of 27 patients (63%) carried an identical strain of staphylococcus species causing peritonitis being present in the exit site, nose, or nails. The most frequently colonised site with strains identical to that causing the peritonitis episode was the catheter exit site, followed by nose and nails. Aktaş *et al*<sup>[67]</sup> demonstrated a clear association between *S. aureus* carriage and *S. aureus* infection in PD patients. Determining the *S. aureus* carriage state of patients undergoing dialysis can help guide infection prevention measures and treatment strategies<sup>[65,67]</sup>. A Cochrane review of randomised controlled trials (19 trials and 1949 patients) to evaluate what evidence supports the use of different antimicrobial approaches to prevent peritonitis in PD patients demonstrates that nasal mupirocin reduces ESI/TI but not peritonitis<sup>[68]</sup>.

### Developing economy

PD is eminently suited to developing countries due to its relative cheapness, lack of HD facilities and unsatisfactory road network making access to HD centres problematic. However, the wide application of PD is hampered by infection. Hot tropical climate and poor hygiene among patients is thought to be responsible for the high rate of peritonitis<sup>[48]</sup>. The spectrum of bacterial peritonitis in patients on CAPD in India may be different from that seen in developed countries because of differences in culture and in social, environmental, financial, and educational status<sup>[19]</sup>.

## DIAGNOSIS OF INFECTION

### ESI and TI

An exit-site infection is defined by the presence of purulent drainage, with or without erythema of the skin at the catheter-skin interface. Pericatheter erythema without purulent discharge is sometimes an early indication of infection but can also be a simple skin reaction, particularly in a recently placed catheter or after trauma to the catheter<sup>[26]</sup>.

TI may present as erythema, edema, or tenderness over the subcutaneous pathway but is often clinically occult, as shown by ultrasound studies<sup>[69]</sup>. TI usually occurs in the presence of an ESI but rarely occurs alone. *S. aureus* and *Pseudomonas aeruginosa* ESI are often associated with concomitant TI and are the organisms that most often result in catheter infection-related peritonitis.

Any purulent discharge from the exit site should be swabbed for culture and Gram-stain in addition to culture of the peritoneal dialysate. The extent of involvement of the subcutaneous PD catheter tract is of major importance in the management of PD peritonitis. The diagnosis of these infections as well as the more sinister TI is based mainly on clinical signs. Korzets *et al*<sup>[70]</sup> examined the usefulness of ultrasound examination (US) of the catheter tract in delineating catheter-related (exit-site and tunnel) infections, and their relationship to each other and to peritonitis. They regarded the findings as positive if an area of hypoechoogenicity (indicative of fluid collection) > 2 mm in width along any portion of the catheter tract. They performed a total of 56 US (26 episodes of peritonitis, four TI, 13 ESI and 13 controls) and reported that majority of the collections (13/16 in episodes of peritonitis and 5/8 ESI) were localised to the internal cuff region<sup>[70]</sup>. Other imaging techniques like positron emission tomography scanning and scintigraphy, may be useful for diagnosing and managing PD catheter infections<sup>[71]</sup>.

### PD peritonitis

PD patients presenting with cloudy effluent should be presumed to have peritonitis and confirmed by obtaining effluent cell count > 100 WBC/mL, differential count, culture and Gram staining<sup>[72-74]</sup>. Peritonitis should always be included in the differential diagnosis of any PD patient with abdominal pain, even if the effluent is clear, as a small percentage of patients present in this fashion. Other causes, such as constipation, renal or biliary colic, peptic ulcer disease, pancreatitis, and acute intestinal perforation, should also be investigated in the PD patient with abdominal pain and clear fluid. The degree of pain is somewhat organism specific (e.g., generally less with CoNS and greater with streptococcus, G-ve rods, *S. aureus*) and can help guide the clinician in the decision to admit or treat as an outpatient<sup>[26]</sup>.

The diagnosis of peritonitis in patients on automated PD with night dwell (dry daytime) is slightly more cumbersome than in those on CAPD. The International Society for Peritoneal Dialysis recommends using the proportion of polymorphonuclear cells rather than the absolute numbers (> 50% is diagnostic even if the cell count is < 100/ $\mu$ L). Alternatively, the clinician is advised to instil one litre of dialysate, draining it after 1-2 h to check for turbidity, cell count/differential count and culture. Sometimes a second exchange with a dwell time of at least 2 h is required to clinch the diagnosis<sup>[26]</sup>.

Given the immunocompromised state of most patients on PD, a high index of suspicion is required for making a timely diagnosis. Any patient on PD presenting with evidence of infection (fever, peripheral leucocytosis) without an obvious cause should have aspirate cultures done even if the aspirate is clear and abdominal pain is absent<sup>[75]</sup>. Other causes of abdominal pain should be investigated in PD patients with clear fluid. A cloudy effluent does not always equate to PD peritonitis as this may

be caused by chemical inflammation, haemoperitoneum, eosinophilia of the effluent, malignancy, chylous effluent or specimen taken from “dry” abdomen<sup>[26,74]</sup>.

Correct microbiological culturing of peritoneal effluent is of great importance in establishing the micro-organism(s) responsible. Identification of the organism and subsequent antibiotic sensitivities will not only help guide antibiotic selection but, in addition, the type of organism can indicate the possible source of infection<sup>[30,37]</sup>. Concentration methods not only facilitate correct microbial identification but also reduce the time necessary for bacteriological cultures. However, rapid blood-culture techniques (e.g., BACTEC, Septi-Chek, BacT/Alert; Becton Dickinson) may further speed up isolation and identification and are probably the best approach. Two recent prospective studies also support the routine use of the broth culture technique<sup>[76,77]</sup>, while the lysis-centrifugation technique needs further evaluation. Yoon *et al*<sup>[78]</sup> evaluated 112 dialysates from 43 patients suspected of CAPD peritonitis between 2000 and 2008 by inoculating 5 to 10 mL of dialysate into a pair of BacT/Alert blood culture bottles, and comparing it with 50 mL of centrifuged dialysate simultaneously inoculated into a solid culture media for conventional culture. The blood culture method was positive in 78.6% (88/112) of dialysate specimens and the conventional culture method in 50% (56/112,  $P < 0.001$ ). They showed that the blood culture method using the BacT/Alert system is useful for culturing dialysates and improves the positive culture rate in patients with suspected peritonitis compared to the conventional culture method.

A number of novel diagnostic techniques have been explored for the early diagnosis of peritonitis including leucocyte esterase reagent strip, broad-spectrum PCR with RNA sequencing, quantitative bacterial DNA PCR assays (especially in patients with previous or current antibiotic use), matrix metalloproteinase-9 test kit and *in situ* hybridization have been summarised by Li and co-workers<sup>[26]</sup>. The lysozyme (muramidase) content of peritoneal fluid samples has been found to be an early indicator of the onset of infection in the course of PD. A level of 10.0 mug/mL indicates peritoneal infection and one of 7.5 mug/mL is highly suspicious<sup>[79]</sup>.

Mycobacteria are an infrequent cause of peritonitis and can be difficult to diagnose. While the classic symptoms of fever, abdominal pain, and cloudy effluent may occur with mycobacterial peritonitis, the diagnosis should be considered in any patient with prolonged failure to thrive, prolonged symptoms despite antibiotic therapy, and relapsing peritonitis with negative bacterial cultures. There should be a high index of suspicion of tuberculosis as cause of PD peritonitis in endemic areas. Mycobacterial infections should be suspected in the presence of persistently elevated mononuclear cell counts in the presence of negative cultures. Acid-fast bacilli may be negative in 90% of cases but formal cultures are likely to be positive<sup>[80]</sup>. When under clinical consideration, special attention must be paid to culture techniques<sup>[26]</sup>.

## PATHOLOGICAL CONSIDERATIONS

### Pathophysiology

Infection of the peritoneal cavity occurs *via* the catheter lumen, bacterial migration *via* the tract or in females, through the vagina. The localisation of infection to the internal cuff region in cases of ESI probably occurs as a result of downward migration of bacteria along the catheter tract. This supports the notion that the exit site should be pointing caudally or that the peritoneal catheter have a swan-neck configuration. Though staphylococci cannot grow in commercial peritoneal dialysate solutions, these fluids are modified during dialysis and become enriched by a plasma ultrafiltrate which can support bacterial growth<sup>[7]</sup>. With regard to peritonitis, infection within the peritoneal cavity appears to extend and involve the internal cuff region challenging the traditional thinking that both the internal and external cuffs offer an effective barrier against the spread of infection<sup>[70]</sup>.

Peritonitis is associated with peritoneal inflammation leading to hyperaemia and changes in peritoneal transport. The changes of impaired ultrafiltration<sup>[81]</sup>, increased peritoneal transport of low-molecular-weight solutes and increased rates of glucose absorption, are usually transient and typically resolve within a month after resolution of the peritonitis<sup>[82,83]</sup>.

### Fungal infection

Fungal infection is rare but it is associated with high morbidity, PD modality change and mortality. Its incidence varies from 4% to 10% of all peritonitis episodes in children and from 1% to 23% in adults<sup>[36,38]</sup>. Risk factors include a history of multiple episodes of bacterial peritonitis and treatment with broad-spectrum antibiotics<sup>[84]</sup>. Fungal peritonitis is very closely associated with polymicrobial infections. Barraclough *et al*<sup>[46]</sup> examined the frequency, predictors, treatment, and clinical outcomes of PD-associated polymicrobial peritonitis in a large observational cohort study using The Australia and New Zealand Dialysis and Transplant Registry data. They reported 359 episodes of polymicrobial peritonitis in 324 individuals, representing 10% of all peritonitis episodes during 6002 patient-years. The organisms isolated included mixed G+ve and G-ve organisms, and mixed bacteria and fungi. There were no significant independent predictors of polymicrobial peritonitis except for the presence of chronic lung disease<sup>[46]</sup>. But fungal peritonitis can be the primary episode of infection.

### Sclerosing encapsulating peritonitis

SEP is a serious complication of PD characterized by thickened peritoneal membrane, which lead to decreased ultrafiltration and intestinal obstruction. Its early clinical features are nonspecific, and it is often diagnosed late following laparotomy and peritoneal biopsy, when the patient develops small bowel obstruction. However, this is changing with increasing awareness of computed tomography (CT) findings in SEP. CT can yield an early, non-invasive diagnosis that may improve patient outcome<sup>[85]</sup>.



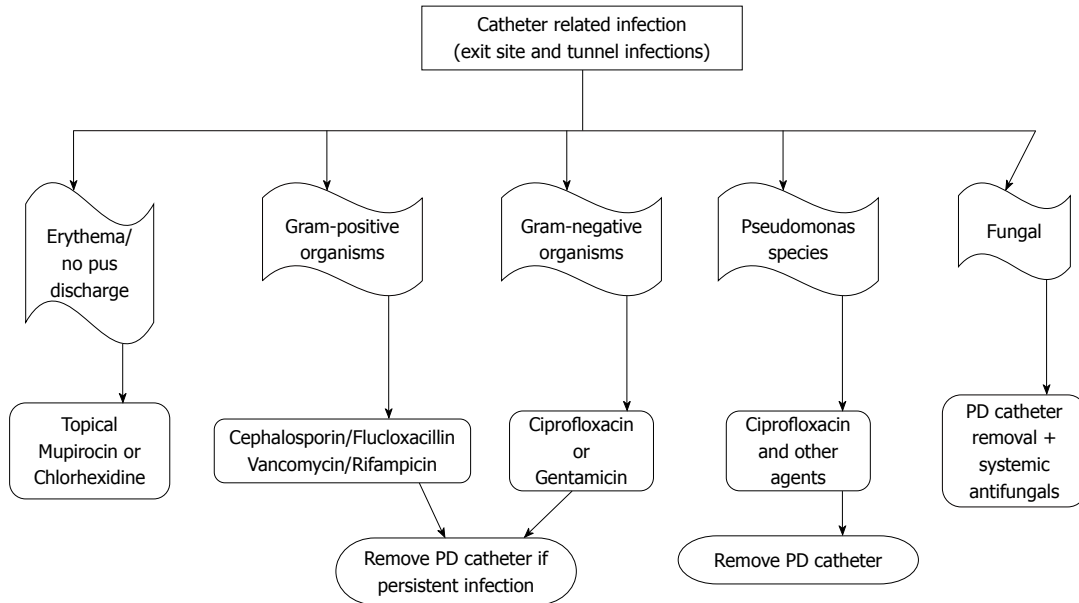


Figure 1 Treatment of Peritoneal dialysis catheter related infections. PD: Peritoneal dialysis.

Ultrasonography can also be useful in diagnosis, the main features being: increased small bowel peristalsis, tethering of the bowel to the posterior abdominal wall, intraperitoneal echogenic strands and, in the late stages of the disease, membrane formation<sup>[86]</sup>.

SEP is thought to be due to a persisting expression of *TGF $\beta$ 1* gene on peritoneal mesothelial cells<sup>[87]</sup>. Predisposing factors include recurrent peritonitis, presence of acetate in the dialysate, antiseptics used during bag exchanges, chlorhexidine gluconate in alcohol, glucose-based dialysis solutions, plasticizers and particles<sup>[88,89]</sup>. Sclerosing encapsulating peritonitis frequently leads to intestinal obstruction, small-bowel necrosis, enterocutaneous fistulas, and malnutrition<sup>[90,91]</sup>. Patients are typically seriously ill, with evidence of infection and requirement for parenteral nutrition. A mortality rate of 60%-73% has been reported<sup>[91,92]</sup>. A high index of clinical suspicion for sclerosing peritonitis is desirable, perhaps facilitated by routine screening of at-risk patients<sup>[92]</sup>.

## TREATMENT OF ESI AND TI

Antiseptic and non antiseptic agents have both been used for exit-site cleansing. An ideal cleansing agent should reduce the number of microorganisms, be harmless to the body's defenses and not interfere with wound healing. Antimicrobial soap is recommended for cleansing a healed exit site, but biocompatible solution is preferred for the postoperative, infected, or traumatised exit site. *In vivo* studies on the effectiveness of some cleansing agents are still lacking, and clinical study of exit-site cleansing is needed to determine the most effective agents for the task<sup>[93]</sup>.

Appropriate care of the exit site will avoid loss of catheter and unnecessary dialysis modality change. Reports from Italy show the efficacy of treating ESI caused by *Pseudomonas* with sodium hypochlorite packs as well

as systemic and local antibiotic therapy. Considering the encouraging results obtained on *Pseudomonas* infection, the same schedule for the treatment of ESI caused by other organisms which are generally difficult to eradicate was used. Sodium hypochlorite (50% packs) has a wide antimicrobial spectrum and a rapid onset of action by creating a protective barrier on the exit-site<sup>[9]</sup>.

Antibiotic therapy must be continued until the exit site appears entirely normal. Two weeks is the minimum length of treatment time; treatment for 3 wk is probably necessary for ESI caused by *P. aeruginosa*. If prolonged therapy (longer than 3 wk) with appropriate antibiotics fails to resolve the infection, the catheter can be replaced as a single procedure under antibiotic coverage<sup>[94]</sup>. If the cuffs are not involved, revision of the tunnel may be performed in conjunction with continued antibiotic therapy. This procedure, however, may result in peritonitis, in which case the catheter should be removed. Ultrasound examination of the tunnel has been shown to be useful in evaluating the extent of infection along the tunnel and the response to therapy and may be used to decide on tunnel revision, replacement of the catheter, or continued antibiotic therapy<sup>[95]</sup>. In general, catheter removal should be considered earlier for ESI caused by *P. aeruginosa* or if there is TI<sup>[26]</sup>.

The strategy for managing ESI and TI is shown in Figure 1. Every effort must be made to make a diagnosis including determining the presence of peritonitis before antibiotic treatment is commenced. If there is no improvement within 1 wk, and for G+ve infections, catheter or catheter salvage measures - cuff shaving<sup>[96]</sup>; simultaneous removal and reinsertion of PD catheter with a new exit site (especially in cases with *Pseudomonas*) may be applied. A patient with an ESI that progresses to peritonitis, or who presents with an ESI in conjunction with peritonitis with the same organism, will usually re-

quire catheter removal. Catheter removal should be done promptly rather than submitting the patient to prolonged peritonitis or relapsing peritonitis. Antibiotics are usually continued for about 2 wk<sup>[2,26]</sup>.

## TREATMENT OF PD PERITONITIS

### Empirical therapy

Peritonitis due to PD is best treated empirically while waiting for the results of dialysate culture. Empirical treatment is based on the organisms that are most frequently isolated and their susceptibilities. Antibiotics are preferentially delivered *via* the peritoneal route to ensure maximal concentrations are delivered at the site of infection. It must be borne in mind however, that drugs administered intraperitoneally can be absorbed into the systemic circulation. Drugs excreted by the kidneys accumulate in PD patients, increasing the risk of toxicity<sup>[97]</sup>. The optimal treatment strategy for peritonitis caused by CoNS species remains controversial. A 3-wk course of antibiotic can probably achieve a higher cure rate in relapse or repeat episodes<sup>[98]</sup>. Gentamicin should be considered over other agents for empiric G-ve coverage as it also provides synergy in the setting of *S. aureus*. Also, the newer anti-staphylococcal drugs should be tested for their performance in a biofilm using the MBEC method<sup>[99]</sup>.

Rapid exchanges in automated PD may lead to inadequate time to achieve intraperitoneal levels of antibiotics. It is therefore necessary to administer vancomycin or teicoplanin intermittently and monitor the serum levels when treating such patients<sup>[26]</sup>. It is still not clear whether to lengthen the dwell times on the cyclor or convert automated PD to CAPD.

The duration of antibiotics treatment depends on clinical improvement of the patient and the organisms responsible for the infection. Therapy must be adequate but not too long as to precipitate fungal infections or resistance to antibiotics. It has been suggested that, if sensitivity testing shows resistance to cephalosporin but the patient is improving on intraperitoneal cephalosporin, then there may be no need to change the antibiotic as the intraperitoneal concentration of cephalosporin is higher than concentrations used in the microbiology laboratory to determine sensitivity or resistance. However, Heywood *et al*<sup>[100]</sup> advice caution due to the increased risk of relapsing peritonitis with such an approach. They conducted a retrospective review looking at the incidence and treatment of CoNS peritonitis reported as resistant to cephalosporins. Of the 200 new cases of peritonitis, 65 (32.5%) were identified as CoNS. All were treated empirically with cefazolin (or vancomycin if allergic) for G+ve coverage and either tobramycin or ceftazidime for G-ve coverage. Of the 38 episodes of CoNS reported as resistant, 10 were treated throughout with cephalosporin (with four relapsing) whereas 28 either started with or were changed to vancomycin (with two relapsing). Their study suggests that, although cephalosporin-resistant cases of CoNS initially resolve with cephalosporin treatment, they are

indeed associated with a greater risk of relapse. Patients with CoNS peritonitis reported resistant to cefazolin may benefit from a change to vancomycin to reduce the risk of relapse<sup>[100]</sup>. A high degree of resistance to third generation cephalosporins (66.7%) was noted amongst the G-ve bacilli. Also, all the G-ve bacilli isolated from patients who had prior empirical antibiotic therapy with ceftazidime, were resistant to third generation cephalosporins<sup>[30]</sup>.

Goffin *et al*<sup>[21]</sup> evaluated the efficiency of a vancomycin and ciprofloxacin combination given as the first-line treatment for PD peritonitis as these covered all CoNS and 96% of G-ve bacilli. They explored a systemic route of administration of the antibiotics as an alternative to the usually cumbersome intraperitoneal drug administration. Intravenous vancomycin 15 mg/kg body weight, intravenous, and oral ciprofloxacin 250 mg two times per day (500 mg twice per day if residual creatinine clearance was above 3 mL/min) were prescribed at diagnosis of peritonitis. Vancomycin injections were repeated (when blood trough level was expected to be below 12 µg/mL) in cases of G+ve organisms for a total duration of 3 wk. Ciprofloxacin was given for a total of 3 wk in cases of G-ve and a total of 10 d for susceptible G+ve infections. The overall treatment success rate was 77.2% (78 of the 101 peritonitis episodes): 61.4% at first intention and 15.8% after optimization of the antibiotic therapy (second intention). Systemic vancomycin and ciprofloxacin administration is a simple and efficient first-line protocol antibiotic therapy for PD peritonitis. Oral ciprofloxacin provides satisfactory results in G-ve infections, comparable to those obtained with intraperitoneal ceftazidime or aminoglycosides<sup>[21]</sup>. Shigidi *et al*<sup>[15]</sup> reported a 79% response rate to antibacterial therapy (cefazoline and ceftazidime or vancomycin and gentamicin if allergic to β-lactams).

### Fungal peritonitis

An earlier report<sup>[101]</sup> indicated the possibility of retaining catheter use by low dose intravenous amphotericin B but in more recent series the PD catheter was removed in seven out of eight cases of fungal peritonitis<sup>[37]</sup>. It is now standard practice to remove PD catheters in all cases in addition to antifungal treatment for a minimum of 3 wk<sup>[36]</sup> and subsequent transfer to hemodialysis. Fluconazole and amphotericin B are the recommended antifungal agents but newer drugs such as voriconazole and caspofungin are effective<sup>[36,84]</sup>. The 2010 International Society of Peritoneal Dialysis update on the treatment of fungal peritonitis advocates catheter removal immediately after fungi are identified by microscopy or culture<sup>[26]</sup>. Use of intraperitoneal taurolidine (a non-antibiotic antimicrobial, with broad bactericidal and fungicidal properties) solution did not prevent PD catheter removal<sup>[102]</sup>.

### SEP

Bearing in mind that several factors/mechanisms are involved in producing SEP, it is not surprising that several

therapies have been applied. The initial step in therapy should be the cessation of PD<sup>[103]</sup> and removal of the PD catheter. Removal of the PD catheter is controversial as some leave it in to allow peritoneal lavage as a way of discouraging adhesion formation between loops of bowel<sup>[104]</sup>. Additional treatment options include: steroid therapy<sup>[105]</sup>; anti-inflammatory and immunosuppressive drugs<sup>[87,103,106-108]</sup>; and use of tamoxifen<sup>[87,103]</sup>. The management of SEP can be summarised as follows: (1) painstaking resection of the membrane when feasible; (2) in case of inadvertent intestinal wound(s), the most proximal one should be brought out as a stoma, and partial resections should not be anastomosed primarily; and (3) no surgical treatment is required in ascites, asymptomatic SEP or subacute intestinal obstruction<sup>[90,109]</sup>.

## TISSUE PLASMINOGEN ACTIVATOR

There have been anecdotal reports of the use of tissue plasminogen activator for obstructed PD catheters in both adults and children. Tissue plasminogen activator was also administered to 5 patients with relapsing peritonitis; 3 patients, all with *S. epidermidis*, recovered and did not experience further recurrence<sup>[110]</sup>.

## REMOVAL OF PD CATHETER

This is to prevent further damage to the peritoneal membrane in order to salvage PD modality where possible. The indications for PD catheter removal in PDAI include: ESI/TI with peritonitis; ESI/TI due to gram-negative organisms not responding to antibiotics; fungal peritonitis; lack of improvement by 5 d on appropriate antibiotics (irrespective of causative organisms); relapsing peritonitis; and refractory catheter related infection (ESI/TI)<sup>[2]</sup>. The duration of antibiotics varies depending on clinical course and the organisms involved but this is generally for 2 to 3 wk.

## OUTCOME OF PDAI

### Infection related hospitalisation

Infection remains a major problem for the ERF patient whether he/she is managed by HD or PD. Williams *et al*<sup>[111]</sup> studied the effect of infection related hospitalisation between 97 HD and 71 PD patients and showed no difference between PD and HD in the risk of access loss (28% *vs* 35%), modality change (22% *vs* 0%), or death (17% *vs* 6%) following hospitalisation for infection.

### PD catheter removal and modality change

PD catheter survival ranges from 80%-93% at 1 year to 58%-91% at 3 years<sup>[4,112]</sup>. Severe and prolonged PD peritonitis can lead to peritoneal membrane failure and is the most common cause of technique failure in PD<sup>[15,26]</sup>. In a large retrospective study of 315 patients, PD catheter survival was not significantly linked to factors such as age, body mass index, diabetic status, previous abdominal

surgery or infections<sup>[102]</sup>. PD catheters were removed in 19% of episodes of PD peritonitis<sup>[113]</sup>. Change in dialysis modality is reported in up to 42% due to peritonitis and access-related infections<sup>[19,114]</sup>. The outcome of PD peritonitis depends on the type of sepsis and the offending organism. Catheter loss (17/45 *vs* 5/20,  $P = 0.04$ ), hospitalization (31/45 *vs* 13/30,  $P = 0.03$ ), death [9/45 *vs* 3/30,  $P =$  non significant (NS)], switch to hemodialysis (8.9% *vs* 3.3%,  $P =$  NS), and reimplantation of the catheter (6.6% *vs* 3.3%,  $P =$  NS) were all more frequent in G-ve episodes than in G+ve episodes<sup>[19]</sup>.

### PD function

A single, isolated episode of peritonitis ( $n = 86$ ) had no significant effect on longitudinal peritoneal function, whereas recurrences or clusters of infection ( $n = 70$ ) caused increases in dialysate/plasma ratio of creatinine and reductions in ultrafiltration, the significance of which increased with the number of episodes. Davies *et al*<sup>[115]</sup> demonstrated that solute transfer increases and ultrafiltration declines with time on PD. This process is exacerbated and accelerated by peritonitis, and appears to be proportional to the degree of associated inflammation and number of infections in close proximity.

### Mortality

Less than 4% of PD peritonitis results in death, but peritonitis is a contributing factor to death in 16% in<sup>[26]</sup>. Mortality is more likely in patients with PD peritonitis due to *Candida* species and *Pseudomonas aeruginosa*. Sepsis (42%) and cardiac related causes (31%) were the two major causes of death<sup>[113]</sup>. Fungal peritonitis is associated with high morbidity and mortality.

### Factors influencing outcome

Several factors act together or alone to influence the outcome of PDAI. Krishnan and co-workers<sup>[35]</sup> after analysing 399 episodes of bacterial peritonitis in 191 patients on dialysis, did not find the number of peritonitis episodes (before the episode in question), vancomycin-based initial empiric treatment, serum albumin level, total lymphocyte and initial dialysate white blood cell count, age, sex, diabetes, previous renal transplantation, and use of steroids to have a significant effect on the outcome of peritonitis. Not all agree on the role of various factors. In a multivariate analysis of 247 episodes of PD peritonitis in 82 patients, Kofteridis *et al*<sup>[116]</sup> found the presence of a purulent ESI, more than 5 d PD effluent cell count  $> 100 \times 10^6/L$ , use of antimicrobials during the preceding 3 mo, and low serum total protein level on admission were independent predictors of a complicated course of PDAI<sup>[116]</sup>.

### Patient related factors

Patients receiving enhanced training (mean training time of 29 h) had significantly fewer ESI (0.38 episodes per patient year) compared with patients receiving standard training (mean training time of 22.6 h; ESI rate of 0.67

episodes per patient year,  $P = 0.003$ ). They also had a reduced rate of peritonitis (0.33 per patient year *vs* 0.43 episodes per patient year,  $P = 0.098$ )<sup>[116]</sup>.

### Species/virulence

Bacterial species and virulence factors rather than antibiotic resistance have more important influence on the outcome of staphylococcal peritonitis<sup>[117]</sup>. G+ve peritonitis has a significantly higher resolution rate than either polymicrobial peritonitis or G-ve peritonitis. *S. aureus* episodes have poorer resolution than other G+ve infections. Non pseudomonal peritonitis has a better outcome than *Pseudomonas aeruginosa* episodes<sup>[32]</sup>. Barretti *et al*<sup>[117]</sup> studied 86 new episodes of staphylococcal peritonitis in a single university hospital (35 due to *S. aureus*, 24 to *S. epidermidis* and 27 to other CoNS). The oxacillin susceptibility rate was 85.7% for *S. aureus*, 41.6% for *S. epidermidis*, and 51.8% for other CoNS. Production of toxins and enzymes, except for enterotoxin A and  $\alpha$ -hemolysin, was associated with *S. aureus* episodes, whereas slime production was positive in 23.5% of CoNS and 8.6% of *S. aureus* strains. The resolution odds were 68 times higher for non-slime producers and were not influenced by oxacillin resistance among vancomycin-treated cases. Also slime and  $\alpha$ -haemolysin production were independent predictors of non-resolution.

Compared with single-organism infections, polymicrobial peritonitis are associated with higher rates of hospitalisation, catheter removal, permanent haemodialysis transfer, and death<sup>[18,46]</sup>. In a study by Barraclough and co-workers<sup>[46]</sup>, isolation of fungus or G-ve bacteria was the primary predictor of adverse clinical outcomes. Patients who had their catheters removed > 1 wk after polymicrobial peritonitis onset were significantly more likely to be permanently transferred to hemodialysis therapy than those who had earlier catheter removal (92% *vs* 81%,  $P = 0.05$ ). Isolation of G-ve bacteria (with or without G+ve bacteria) or fungi carries a worse prognosis and generally should be treated with early catheter removal and appropriate antimicrobial therapy<sup>[46]</sup>.

### Dialysate cell count and duration of PD

For those peritonitis episodes in which the PD fluid cell count was > 100/ $\mu$ L for more than 5 d, the non resolution rate was 45.6%, compared to a 4.2% non resolution rate when the cell count returned to 100/ $\mu$ L or less in less than 5 d. Those patients that had a successful outcome had been on CAPD for a significantly shorter period of time than those patients that had nonresolution. The non resolution rate for those patients that had been on PD for more than 2.4 years was 24.4%, compared to 16.5% for those that had been on PD for less than 2.4 years ( $P = 0.05$ )<sup>[35]</sup>.

### Relapse

The largest multicenter, prospective study on relapsing peritonitis (specifically the relationship of postempiric antibiotic treatment regimens to the subsequent risk of

relapse in children) was produced recently by the International Pediatric Peritonitis Registry<sup>[118]</sup>. An online, prospective data entry on peritonitis cases by participating centers including 490 episodes of non fungal peritonitis, 52 (11%) of which were followed by a relapse was analysed. There was no significant difference between relapsing and non-relapsing peritonitis in the distribution of causative organisms and antibiotic sensitivities. Switching to monotherapy with a first-generation cephalosporin on the basis of culture results was associated with a higher relapse rate (23%) than other final antibiotic therapies. Other risk factors included young age, single-cuff catheter, downward-pointing exit site, and chronic systemic antibiotic prophylaxis were additional independent risk factors for relapsing peritonitis in the multivariate analysis. Compared with non-relapsing, relapsing peritonitis was associated with a lower rate of full functional recovery, higher ultrafiltration problems, and higher rate of permanent PD discontinuation<sup>[118]</sup>.

## PREVENTION OF PD PERITONITIS

Despite advances in technology, prevention of peritonitis remains one of the major challenges in PD patients. Several innovative developments like antimicrobial coating of PD catheters, flushing before fill, avoiding spiking of solution bags, connectology and double-bag systems have shown an impact on peritonitis rates. New PD solutions with neutral pH and low concentrations of glucose degradation products have also shown beneficial effects on cell viability and have improved peritoneal host defenses but without any difference in peritonitis rates<sup>[119]</sup>. Nasso<sup>[120]</sup> initiated a continuous quality improvement project to address the problem of PD peritonitis involving: analysis of data to ensure accuracy about causative organisms; education for the home dialysis nurses; creation of a home visit form, revisions to routine doctors' orders, revision of PD education tools; use of specialty materials for high-risk patients; one-time use for all drain equipment; change to peritonitis treatments; and group education for patients. These measures did not reduce their peritonitis rates after a 12-mo period. Further actions including making changes to patient training and developing a home visit protocol; partnership with local Community Care Access Centre and teaching of community nurses on how to help patients with their PD were required to significantly improve peritonitis rates<sup>[120]</sup>. This indicates that intensive patient training with careful attention to their home environment is critical in achieving good PD outcomes.

The Kidney Disease Outcomes Quality Initiative guidelines for PD emphasize the need for quality improvement interventions to improve outcomes in PD. Qamar *et al*<sup>[121]</sup> reported their 17 years experience of initiatives focused on lowering peritonitis rates in a single PD program. The peritonitis rate declined from 0.5 episodes per year at risk in 1990-1991 to 0.25 episodes per year at risk in 2005-2007 ( $P < 0.004$ ). The ESI rate de-



**Table 3** Exit site management

Ref.	No.	Trial/protocol	Results	Comments
Mahaldar <i>et al</i> <sup>[132]</sup>	100	Mupirocin <i>vs</i> Gentamicin	No difference in ESI rates	Trend to higher peritonitis in gentamicin group. Retrospective study
Wong <i>et al</i> <sup>[133]</sup>	154	Mupirocin <i>vs</i> Control	Mupirocin effective in preventing G+ve peritonitis	Randomised controlled trial. No adverse effects with mupirocin
Fong <sup>[122]</sup>	69	Providone-iodine <i>vs</i> Control	PVI 2.9% Control 8.8%	Nasal carriers high in PVI group! Randomised controlled trial
Bernardini <i>et al</i> <sup>[134]</sup>	133	Gentamicin (67) <i>vs</i> Mupirocin (66)	0.23 peritonitis episodes per patient-year (gentamicin) <i>vs</i> 0.54 for mupirocin	Time to first infection longer with Gentamicin
McQuillan <i>et al</i> <sup>[135]</sup>	201	Polysporin Triple Ointment (P3) <i>vs</i> Mupirocin	No difference in time to ESI or peritonitis but higher rate of fungal infections and more redness of exit site in P3 group	Multicentre randomised controlled trial

ESI: Exit site infection.

clined from 0.72 episodes per year at risk to 0.1 episodes per year at risk over the same period ( $P < 0.0001$ ) clearly showing that quality improvement initiatives can reduce infection rates in PD patients<sup>[121-123]</sup>.

Protocols to decrease infection risk in PD patients include proper catheter placement<sup>[13]</sup>, exit-site care that includes *S. aureus* prophylaxis, careful training of patients and periodic retraining, treatment of contamination, and prevention of procedure-related peritonitis. Quality improvement programs with continuous monitoring of infections, both of the catheter exit site and peritonitis, are important to decrease PDAI. Continuous review of every episode of infection to determine the root cause of the event should be routine in PD programs<sup>[3]</sup>. The efficacy of silver-ion treated catheters in reducing PDAI was tested in prospective, randomised controlled trial. Patients were implanted with either a silver-treated study catheter (67) or a control catheter (72). ESI rates for the study group and control group (0.52 and 0.45 episodes/patient-year of dialysis respectively) were not different by Poisson regression analysis ( $P = 0.4$ ) and peritonitis rates were identical for the two groups (0.37 episodes/patient-year)<sup>[124]</sup>.

### Nasal carriers

Eradication of *S. aureus* colonising the catheter exit site may be more important and have a greater likelihood of success than maneuvers directed to more distant locations<sup>[66]</sup>. However, nasal carriage status should be routinely identified in all patients entering PD programme and the carriers properly treated<sup>[125]</sup>. The nasal carriage of *S. aureus* is associated with an increased risk of catheter-ESI and that the performance of nasal cultures before the implantation of the catheter can identify patients at high risk of subsequent morbidity<sup>[65]</sup>.

### Antimicrobial prophylaxis

Perioperative intravenous antibiotics compared with no treatment significantly reduced the risk of early peritonitis (four trials, 335 patients, RR = 0.35, 95% CI: 0.15-0.80) but not ESI and TI (three trials, 114 patients, RR = 0.32, 95% CI: 0.02-4.81)<sup>[68]</sup>. A single dose of an intravenous

antibiotic (first-generation cephalosporin or vancomycin) should be given at the time of catheter insertion<sup>[126,127]</sup>.

Majority of fungal peritonitis episodes are preceded by courses of antibiotics<sup>[84]</sup>. The International Society for Peritoneal Dialysis recommends fungal prophylaxis in patients undergoing prolonged antibiotic therapy as a way to decreasing the incidence of fungal peritonitis in programs characterised by high fungal infection rates<sup>[26]</sup>.

### ESI

Meticulous exit-site care is vital in preventing ESI. Avoiding trauma to the exit-site and daily cleaning of the exit-site with a dedicated antimicrobial soap is essential for the longevity of the PD catheter. Antibiotics cream and disinfectant agents including povidone-iodine, chlorhexidine, electrolytic chloroxidizing solutions (Amuchina 10% - ExSept Plus, Amuchina 5% - ExSept) are useful to keep the resident micro-organisms inhibited. ESI rates in PD patients treated with Amuchina 10% (ExSept Plus) and Amuchina 5% (ExSept) for the exit-site care are similar or lower compared to povidone-iodine or chlorhexidine<sup>[128]</sup> or pH neutral soap<sup>[42]</sup>. Amuchina 10% solution is effective in preventing infection on the exit-site, without any secondary topical reaction.

Topical application of antimicrobial agents such as mupirocin, gentamicin and polysporin triple ointment to prevent exit-site infections has been successfully used. Mupirocin application at the exit site significantly lowers the incidence of ESI and peritonitis caused by *S. aureus* without any significant side effects<sup>[17,129]</sup>. Strong support for the use of mupirocin in preventing ESI and peritonitis comes from a systemic analysis of 13 articles (1233 patients *vs* 1217 controls). Based on the six non randomised trials, the reduced risk rate for mupirocin therapy was found to be 80% (95% CI: 0.39-0.93,  $P = 0.004$ ) in ESI and 91% (95% CI: 0.72-0.97,  $P < 0.0001$ ) in peritonitis due to *S. aureus*; 70% (95% CI: 0.47-0.82,  $P < 0.0001$ ) in ESI and 42% (95% CI: 0.25-0.55,  $P < 0.0001$ ) in peritonitis due to all organisms among mupirocin-treated and non treated subjects. Based on three randomised controlled trials, ESI and peritonitis due to *S. aureus* were reduced by 73% (95% CI: 0.63-0.80,  $P < 0.0001$ ) and

40% (95% CI: 0.17-0.56,  $P = 0.002$ ), respectively. The randomised controlled trial evidence is that mupirocin treatment can reduce the risk rate of ESI by 46% (95% CI: 0.35-0.55,  $P < 0.00001$ ) but it cannot decrease the risk rate of peritonitis due to all organisms ( $P = 0.56$ )<sup>[130]</sup>. Lobbedez *et al*<sup>[131]</sup> found no significant increase in the mupirocin resistant *S. aureus* prevalence in PD patients who routinely apply mupirocin ointment at the catheter exit site. Studies involving comparison of topical cleaning solutions or agents are shown in Table 3<sup>[122,132-135]</sup>.

Bacteria hiding within biofilms are known to be responsible for chronic PDAI. Branger *et al*<sup>[136]</sup> developed a new approach in the prevention of chronic PD-related infection by regular injection of specific formulations containing detachment-promoting agents. Compared to daily treatment with taurolidine which left 48% of the biomass, weekly treatment with these agents led to a 97% reduction of surface coverage. Weekly treatment with such agents is recommended to reduce the frequency of chronic PDAI.

## LIMITATIONS

There was a dearth of randomised controlled trials on the big questions regarding PDAI. Most reports were retrospective, describing small series from single units. There were a number of large studies, for example Cochrane reviews but the studies reviewed were not always optimal. There was a general lack of details of social care and the dependency status of PD patients.

## CONCLUSION

PDAI is a significant reason for removal of PD catheter, loss of PD function, modality change and death.

Nasal and nail carriage status should be routinely identified in all patients entering PD programme and the carriers properly treated.

The surgical technique requires a strict adherence to a standardized procedure and a dedicated team, in order to obtain a reduction of the complications, prolonged catheter duration and a better quality of life.

Every effort must be made to identify the causative organism(s) responsible for PDAI.

Isolation of fungus or G-ve bacteria is a strong predictor of adverse clinical outcomes. Pure G+ve peritonitis are associated with the best clinical outcomes while delay in PD catheter removal of > 1 wk after polymicrobial peritonitis onset is significantly associated with dialysis modality change and increased morbidity. Isolation of G-ve bacteria (with or without G+ve bacteria) or fungi carries a worse prognosis and generally should be treated with early catheter removal and appropriate antimicrobial therapy.

Recurrent episodes of PD peritonitis must be followed by careful monitoring of PD function and surveillance for complications of PD like SEP.

Intensive patient training is a key to successful infec-

tion-free PD. All patients must be trained in aseptic techniques in order to avoid contamination of the PD fluid.

Given the large number of patients on PD and the importance of peritonitis, the lack of adequately powered RCTs to inform decision making about strategies to prevent peritonitis needs to be addressed.

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## Events Calendar 2012

January 3-7, 2012

UK Renal Association-Advanced Nephrology Course  
 Corpus Christi College,  
 Oxford, United Kingdom

January 13-15, 2012

NATCO Symposium for Advanced Transplant Professional  
 Loews Miami Beach Hotel,  
 Miami, FL, United States

January 14-16, 2012

American Association of Tissue Banks (AATB) Tissue Donor Suitability Workshop  
 Ritz Carlton Tysons Corner  
 McLean, VA, United States

February 7-9, 2012

From Novice to Specialist-Empowering and Leading Families Donation Discussions  
 Gift of Life Institute  
 Philadelphia, PA, United States

February 13-15, 2012

Consensus Conference on Transplant Program Quality and Surveillance  
 Key Bridge Marriott  
 Arlington, VA, United States

February 14-17, 2012

17th Continuous Renal Replacement Therapies Conference  
 San Diego, CA, United States

February 17-19, 2012

Transplant Nursing Across the Lifespan...Sharing Best Practices  
 DoubleTree Atlanta Buckhead  
 Atlanta, GA, United States

February 26-28, 2012

32nd Annual Dialysis Conference (18th International Symposium on Hemodialysis-23rd Annual Symposium on Pediatric Dialysis)  
 Henry B. Gonzalez Convention Center,  
 San Antonio, TX, United States

March 7-8, 2012

Family Empowerment through Effective Advocacy: Averting and Overcoming Obstacles to Donation  
 Today's Healthcare Environment  
 Gift of Life Institute,  
 Philadelphia, PA, United States

March 13, 2012

The Cutting Edge in Transplantation: New Insights  
 University Hotel,

Minneapolis, MN, United States

March 13-15, 2012

AOPO Quality Improvement Council  
 Embassy Suites San Diego Bay,  
 San Diego, CA, United States

March 15, 2012

Renal Physicians Association (RPA) East Coast Regional Nephrology Coding and Billing Seminar  
 Marriott Wardman Park,  
 Washington, DC, United States

March 15-18, 2012

2012 Renal Physicians Association (RPA) Annual Meeting  
 Marriott Wardman Park,  
 Washington, DC, United States

March 24-27, 2012

American Association of Tissue Banks (AATB) 16th Annual Spring Meeting  
 Caribe Hilton, San Juan, Puerto Rico

March 28-30, 2012

The Consensus Conference on Kidney Paired Donation (KPD)  
 Hyatt Dulles, Herndon, VA, United States

March 28-31, 2012

American Society of Extracorporeal Technology (AmSECT) 40th International Conference  
 Sheraton New Orleans,  
 New Orleans, LA, United States

April 13, 2012

Challenges and Innovations in Pediatric Transplantation  
 Boston Children Hospital - Folkman Auditorium,  
 Boston, MA, United States

April 26-29, 2012

American Association of Tissue Banks (AATB) CTBS Training & Review Course  
 Ritz Carlton Tysons Corner,  
 McLean, VA, United States

April 29-May 2, 2012

ANNA 43rd National Symposium  
 Walt Disney World Dolphin,  
 Orlando, FL, United States

May 2-3, 2012

Building Strong Hospital Partnerships: The Architecture of Strategic Hospital Development  
 Gift of Life Institute,  
 Philadelphia, PA, United States

May 4, 2012

Renal Physicians Association (RPA) West Coast Regional Nephrology Coding and Billing Seminar  
 The Wyndham Phoenix,  
 Phoenix, AZ, United States

May 9-11, 2012

Vascular Access for Hemodialysis XIII Symposium  
 Orlando, FL, United States

May 9-13, 2012

National Kidney Foundation (NKF) 2012 Spring Clinical Nephrology Meetings  
 Gaylord National,  
 Washington, DC, United States

May 20-24, 2012

Transplant Donation Global Leadership Symposium (GLS)  
 Gift of Life Institute,  
 L'Auberge Del Mar,  
 Del Mar, CA, United States

May 23-25, 2012

National Patient Safety Foundation (NPSF) 14th Annual Patient Safety Congress  
 Gaylord National Hotel and Conference Center,  
 Washington, DC, United States

May 24-27, 2012

IL ERA-EDTA Congress  
 Palais des Congrès, Paris, France

June 2-6, 2012

American Society of Nephrology (ASN) Kidney Week  
 Boston, MA, United States

June 6-9, 2012

40th Annual Renal Society of Australasia (RSA) Conference: Celebrating our Culture and Diversity in Renal Care  
 The Sebel Albert Park Melbourne,  
 Melbourne, Australia

June 15, 2012

Renal Physicians Association (RPA) MidWest Regional Nephrology Coding and Billing Seminar  
 Renaissance St. Louis Grand Hotel,  
 St. Louis, MO, United States

June 19-22, 2012

Association of Organ Procurement Organization (AOPO) Annual Meeting  
 Fairmont Chicago,  
 Chicago, IL, United States

July 11-13, 2012

Renal Physicians Association (RPA) 2012 Advanced Practitioner Conference  
 The Hilton Minneapolis,  
 Minneapolis, MN, United States

August 12-15, 2012

NATCO 37th Annual Meeting  
 Grand Hyatt Washington DC,  
 Washington, DC, United States

August 25-31, 2012

American Society of Nephrology (ASN) Review Course & Update  
 The Palace Hotel,  
 San Francisco, CA, United States

September 9-12, 2012

American Association of Tissue Banks (AATB) 36th Annual Meeting  
 Keystone Resort and Conference Center,  
 Keystone, CO, United States

September 9-12, 2012

14th Congress of the International Society of Peritoneal Dialysis  
 Kuala Lumpur, Malaysia

September 28, 2012

Renal Physicians Association (RPA) Southeast Regional Nephrology Coding and Billing Seminar,  
 Charlotte, NC, United States

October 4-5, 2012

National Learning Congress 2012  
 Gaylord Texan,  
 Grapevine, TX, United States

October 4-5, 2012

Central Manchester University Hospitals NHS Foundation Trust Hospital's 5th Annual Home Dialysis Conference  
 The Lowry Hotel, Dearmans Place,  
 Manchester, United Kingdom

October 4-7, 2012

International Association for the History of Nephrology 2012 Conference  
 8th Congress of the IAHN  
 Paestum, Salerno, Italy

October 30-November 4, 2012

American Society of Nephrology (ASN) Kidney Week 2012  
 San Diego Convention Center,  
 San Diego, CA, United States

November 7-8, 2012

Building Strong Hospital Partnerships: The Architecture of Strategic Hospital Development  
 Gift of Life Institute,  
 Philadelphia, PA, United States

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*World Journal of Nephrology* (*World J Nephrol*, *WJN*, online ISSN 2220-6124, DOI: 10.5527) is a bimonthly peer-reviewed, online, open-access (OA), journal supported by an editorial board consisting of 103 experts in nephrology from 30 countries.

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There are unstructured abstracts (no less than 256 words) and

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An informative, structured abstracts of no less than 480 words should accompany each manuscript. Abstracts for original contributions should be structured into the following sections. AIM (no more than 20 words): Only the purpose should be included. Please write the aim as the form of "To investigate/study/...; MATERIALS AND METHODS (no less than 140 words); RESULTS (no less than 294 words): You should present *P* values where appropriate and must provide relevant data to illustrate how they were obtained, e.g.  $6.92 \pm 3.86$  vs  $3.61 \pm 1.67$ ,  $P < 0.001$ ; CONCLUSION (no more than 26 words).

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Please list 5-10 key words, selected mainly from *Index Medicus*, which reflect the content of the study.

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Data that are not statistically significant should not be noted. <sup>a</sup>*P* < 0.05, <sup>b</sup>*P* < 0.01 should be noted (*P* > 0.05 should not be noted). If there are other series of *P* values, <sup>c</sup>*P* < 0.05 and <sup>d</sup>*P* < 0.01 are used. A third series of *P* values can be expressed as <sup>e</sup>*P* < 0.05 and <sup>f</sup>*P* < 0.01. Other notes in tables or under illustrations should be expressed as <sup>1</sup>E, <sup>2</sup>F, <sup>3</sup>F; or sometimes as other symbols with a superscript (Arabic numerals) in the upper left corner. In a multi-curve illustration, each curve should be labeled with ●, ○, ■, □, ▲, △, *etc.*, in a certain sequence.

### Acknowledgments

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### Format

#### Journals

*English journal article (list all authors and include the PMID where applicable)*

- 1 **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World J Gastroenterol* 2007; **13**: 6356-6364 [PMID: 18081224 DOI: 10.3748/wjg.13.6356]

*Chinese journal article (list all authors and include the PMID where applicable)*

- 2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diar-rhoea. *Shijie Huaren Xiaobua Zazhi* 1999; **7**: 285-287

*In press*

- 3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

*Organization as author*

- 4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMID: 2516377 DOI: 10.1161/01.HYP.0000035706.28494.09]

*Both personal authors and an organization as author*

- 5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol*

2003; **169**: 2257-2261 [PMID: 12771764 DOI: 10.1097/01.ju.0000067940.76090.73]

*No author given*

- 6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI: 10.1136/bmj.325.7357.184]

*Volume with supplement*

- 7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI: 10.1046/j.1526-4610.42.s2.7.x]

*Issue with no volume*

- 8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI: 10.1097/00003086-200208000-00026]

*No volume or issue*

- 9 Outreach: Bringing HIV-positive individuals into care. *HRS A Careaction* 2002; 1-6 [PMID: 12154804]

### Books

*Personal author(s)*

- 10 **Sherlock S**, Dooley J. Diseases of the liver and biliary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

*Chapter in a book (list all authors)*

- 11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

*Author(s) and editor(s)*

- 12 **Breedlove GK**, Schorheide AM. Adolescent pregnancy. 2nd ed. Wiczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

*Conference proceedings*

- 13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

*Conference paper*

- 14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

**Electronic journal** (list all authors)

- 15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: <http://www.cdc.gov/ncidod/eid/index.htm>

**Patent** (list all authors)

- 16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

### Statistical data

Write as mean  $\pm$  SD or mean  $\pm$  SE.

### Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as  $\chi^2$  (in Greek), related coefficient as *r* (in italics), degree of freedom as *v* (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

### Units

Use SI units. For example: body mass, *m* (B) = 78 kg; blood pressure, *p* (B) = 16.2/12.3 kPa; incubation time, *t* (incubation) = 96 h; blood glucose concentration, *c* (glucose)  $6.4 \pm 2.1$  mmol/L; blood CEA mass concentration, *p* (CEA) =  $8.6 \pm 24.5$   $\mu$ g/L; CO<sub>2</sub> volume fraction, 50 mL/L CO<sub>2</sub>, not 5% CO<sub>2</sub>; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, etc. Arabic



numerals such as 23, 243, 641 should be read 23 243 641.

The format for how to accurately write common units and quantums can be found at: [http://www.wjgnet.com/2220-6124/g\\_info\\_20100725073806.htm](http://www.wjgnet.com/2220-6124/g_info_20100725073806.htm).

### Abbreviations

Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further explanation.

### Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.

Genotypes: *gyrA*, *arg 1*, *c myc*, *c fos*, etc.

Restriction enzymes: *EcoRI*, *HindI*, *BamHI*, *Kho I*, *Kpn I*, etc.

Biology: *H. pylori*, *E. coli*, etc.

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